Coastal pH variability and the eco-physiological and behavioural response of a coastal fish species in light of future ocean acidification

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Abstract

Ocean acidification (OA) is a global phenomenon referring to a decrease in ocean pH and a perturbation of the seawater carbonate system due to ever-increasing atmospheric CO_2 concentrations. In coastal environments, identifying the impacts of OA is complex due to the multiple contributors to pH variability by coastal processes, such as freshwater inflow, upwelling, hydrodynamic processes, and biological activity. The aim of this PhD study was to quantify the local processes occurring in a temperate coastal embayment, Algoa Bay in South Africa, that contribute to pH and carbonate chemistry variability over time (monthly and 24-hour) and space (~10 km) and examine how this variability impacts a local fish species, *Diplodus capensis*, also commonly known as 'blacktail'.

Algoa Bay, known for its complex oceanography, is an interesting location in which to quantify carbonate chemistry variability. To assess this variability, monitoring sites were selected to coincide with the Algoa Bay Sentinel Site long-term ecological research (LTER) and continuous monitoring (CMP) programmes. The average pH at offshore sites in the bay was 8.03 ± 0.07 and at inshore sites was 8.04 ± 0.15. High pH variability (~0.55–0.61 pH units) was recorded at both offshore (>10 m depth) and inshore sites (intertidal surf zones). Many sites in the bay, especially the atypical site at Cape Recife, exhibit higher than the average pH levels (>8.04), suggesting that pH variability may be biologically driven. This is further evidenced by high diurnal variability in pH (~0.55 pH units). Although the specific drivers of the high pH variability in Algoa Bay could not be identified, baseline carbonate chemistry conditions were identified, which is necessary information to design and interpret biological experiments. Long-term, continuous monitoring is required to improve understanding of the drivers of pH variability in understudied coastal regions, like Algoa Bay.

A local fisheries species, *D. capensis*, was selected as a model species to assess the impacts of future OA scenarios in Algoa Bay. It was hypothesized that this temperate, coastally distributed species would be adapted to naturally variable pH conditions and thus show some tolerance to low pH, considering that they are exposed to minimum pH levels of 7.77 and fluctuations of up to 0.55 pH units. Laboratory perturbation experiments were used to expose early postflexion stage of *D. capensis* to a range of pH treatments that were selected based on the measured local variability (~8.0–7.7 pH), as well as future projected OA scenarios (7.6–7.2 pH). Physiological responses were estimated using intermittent flow respirometry by quantifying routine and active metabolic rates as well as relative aerobic scope at each pH treatment. The behavioural responses of the larvae were also assessed at each pH treatment, as activity levels, by measuring swimming distance and speed in video-recording experiments, as well as feeding rates.

D. capensis had sufficient physiological capacity to maintain metabolic performance at pH levels as low as 7.27, as evidenced by no changes in any of the measured metabolic rates (routine metabolic rate, active metabolic rate, and relative aerobic scope) after exposure to the range of pH treatments (8.02–7.27). Feeding rates of *D. capensis* were similarly unaffected by pH treatment. However, it appears that subtle increases in activity level (measured by swimming distance and swimming speed experiments) occur with a decrease in pH. These changes in activity level were a consequence of a change in behaviour rather than metabolic constraints. This study concludes, however, that based on the parameters measured, there is no evidence for survival or fitness related consequences of near future OA on *D. capensis*.

OA research is still in its infancy in South Africa, and the potential impacts of OA to local marine resources has not yet been considered in local policy and resource management strategies. Integrating field monitoring and laboratory perturbation experiments is emerging

as best practice in OA research. This is the first known study on the temperate south coast of South Africa to quantify local pH variability and to use this information to evaluate the biological response of a local species using relevant local OA scenarios as treatment levels for current and near future conditions. Research on local conditions *in situ* and the potential impacts of future OA scenarios on socio-economically valuable species, following the model developed in this study, is necessary to provide national policy makers with relevant scientific data to inform climate change management policies for local resources.

Declaration

I, Carla Edworthy, hereby declare that the work in this thesis is entirely my own under the supervision of Dr NC James (primary supervisor), Professor WM Potts and Professor S Dupont, with the South African Institute for Aquatic Biodiversity and the Department of Ichthyology and Fisheries Science, Rhodes University as the host institutions. This thesis contains the original work of the author and has not been submitted to any other university.

Signed  on this day 4th of December 2020.

Ethical clearance

The collection of fish from the wild and experimental protocols for the purpose of this research were approved by the animal ethics committee at Rhodes University. Additional permits for animal collection were obtained from the Department of Environmental Affairs with permit number RES2018/26. All animals were treated and euthanized ethically according to standard, regulated practice.

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Finally, thanks to all the fish!

"The proper use of science is not to conquer nature but to live in it."

—Barry Commoner

This thesis is dedicated to the influential women in my life

Mom & Omi

I would never have started this without your encouragement

I would never have continued without your creative guidance, motivation, and input in my life

And

I would never have **finished** this without knowing how proud it would make you both

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Chapter 1

General introduction and literature review: local ocean acidification scenarios and impacts

The multiple stressors of global change in oceans and coasts

Marine ecosystems are increasingly being threatened both directly and indirectly by human activities. The combined impacts of global climate change, land use and development, pollution and habitat degradation are threatening biodiversity and ultimately ecosystem health and resilience (Harley *et al*., 2006). The responses of organisms to changes in their environments are variable and complex depending on a number of interacting mechanisms (Cheung *et al.*, 2009), which ultimately determines whether species are 'winners' or 'losers' when faced with future changes (O'Brien and Leichenko, 2003).

Human activity is primarily driving dramatic changes in global climates, both on land and at sea. The ever-increasing atmospheric carbon dioxide (CO_2) concentrations in the earth's atmosphere is the largest contributor to global climate changes (Vitousek, 1994). Carbon dioxide (CO_2) gas occurs naturally in the earth's atmosphere. However, the concentration of this gas is increasing rapidly as a result of human activity (Fig 1.1) (IPCC, 201). Since the industrial revolution, human activities such as burning fossil fuels for energy and changes in land use practices have resulted in a cumulative increase of atmospheric CO_2, which has now exceeded 410 ppm (McGee, 2019). This is an exponential increase from the preindustrial level of 280 ppm, and more than 40% of this increase has occurred in the last few decades (Feely *et al.*, 2009). Global models suggest that under the business-as-usual scenario (Representative Concentration Pathway - RCP 8.5), atmospheric CO_2 is projected to continue increasing at an exponential rate to reach levels of almost 1000 ppm by the end of the century (Fig 1.1). This projection assumes relatively high population growth and high energy demands, and therefore high greenhouse gas emissions (Riahi *et al*., 2011). The more conservative projection is the RCP 2.6 scenario, which assumes a decline in CO_2 emissions due to stringent mitigation measures (Van Vuuren *et al*., 2011*;* IPCC, 2014). For the purpose of this thesis, all future reference to climate projections will be related to the RCP 8.5 'business-as-usual' emission scenario.

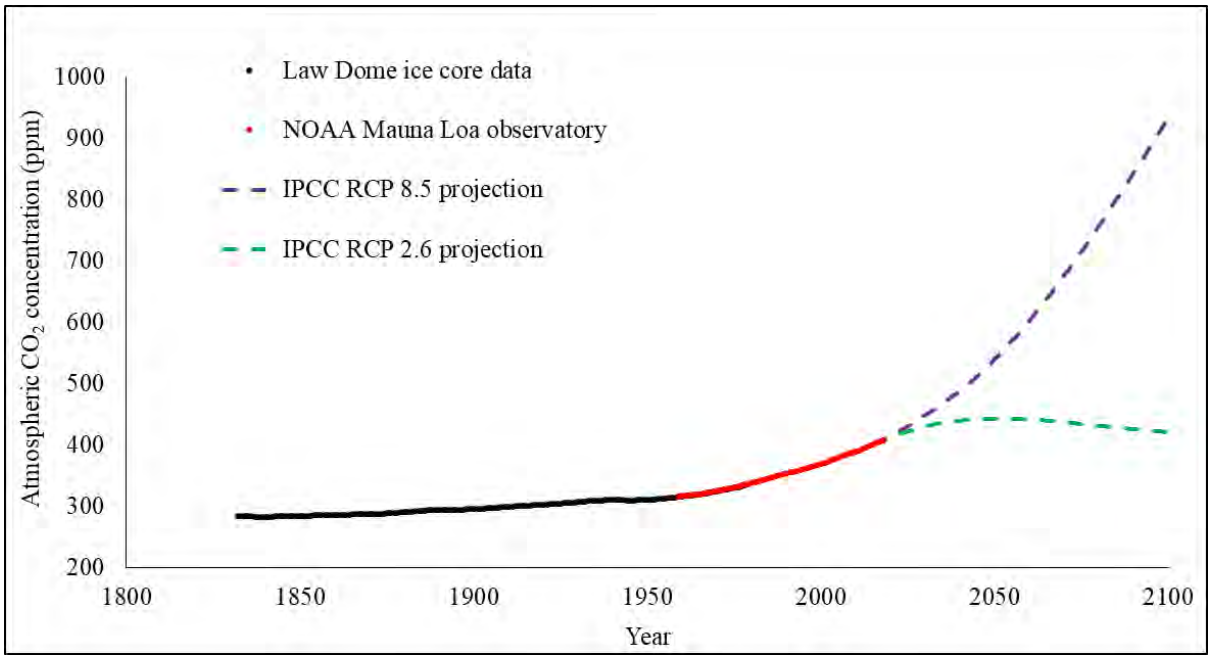

Figure 1.1: Records of annual atmospheric mean CO_2 (ppm) from a) ice core data from the Law Dome ice cores 1987–1993 (Etheridge *et al.*, 1998), b) the Mauna Loa observatory, Hawaii as reported by the National Oceanic and Atmospheric Administration (NOAA) (Tans and Keeling, 2018) and c) the IPCC's projected concentrations according to the RCP 8.5 (Riahi *et al.*, 2011) and 2.6 (Van Vuuren *et al.*, 2011) emission scenarios

Increasing atmospheric CO_2 has many consequences, the most notable and commonly reported being global warming: the increase in global average temperature resulting from the greenhouse effect (Bindoff *et al.*, 2019). Global mean atmospheric temperatures have been rising linearly at a rate faster than ever before in history and the last five years have been the warmest on record (Bindoff *et al.*, 2019). The world's oceans are similarly being affected by global warming (Hoegh-Guldberg and Bruno, 2010). Because of its equilibrium with the atmosphere, the mean global surface temperature of the ocean has been increasing (Levitus *et al.*, 2005; Domingues *et al.*, 2008), and the heat accumulated by the ocean accounts for 90% of the earth's stored energy (Bindoff *et al.*, 2019). Data have shown that the surface temperatures of the oceans have increased by 0.13°C each decade for the last 100 years (NOAA National Centres for Environmental Information, 2020) and that an additional increase of up to 4°C is expected by the end of the century (Bindoff *et al.*, 2019).

Although there are several reports on the impacts of ocean warming on marine life (e.g., Perry *et al*.. 2005, Pörtner and Knust 2007a, Sunday *et al*., 2012), this is not the only change

occurring in oceans because of increasing anthropogenic CO_2 emissions. Aside from global atmospheric and ocean temperature increases, increasing atmospheric CO_2 concentrations are also causing a phenomenon called ocean acidification (hereafter referred to as OA). Oceans are not only absorbing heat, but also more than a quarter of the CO_2 emitted by human activities. Ocean acidification refers to the gradual decrease in pH of seawater because of a disruption in the balance of its carbonate chemistry by the addition of dissolved CO_2 (Doney *et al.*, 2009b; Feely *et al.*, 2009). This affects animals directly by disrupting their internal pH balance and increasing their exposure to pCO_2, which has been shown to incur physiological and behavioural responses (Doney *et al.*, 2020). A review assessing the sensitivity of five marine taxa (corals, echinoderms, molluscs, crustaceans, and fishes) to ocean acidification suggested that at least 50% of all tested species are threatened by OA (Wittmann and Pörtner, 2013). Despite the recent significant increase in OA research and published literature, our understanding of the impacts of this phenomenon is in its infancy, with studies showing variable species responses, such that making generalizations about patterns in sensitivity has become impossible (Doney *et al.*, 2020).

Ocean acidification chemistry

The term OA is used to describe the change in the chemical composition of seawater, which occurs when atmospheric CO_2 from the earth's atmosphere is accumulated and dissolved in seawater. This accumulation of atmospheric CO_2 occurs because seawater readily absorbs this gas to maintain an air-ocean equilibrium (Feely *et al.*, 2009; Rhein *et al.*, 2013; Howes *et al.*, 2015). The ocean has been responsible for absorbing up to 30% of anthropogenic CO_2 emissions since the beginning of the industrial revolution (Sabine *et al.*, 2004; Doney *et al.*, 2009b; Rhein *et al.*, 2013), which has resulted in dramatic shifts in the carbonate chemistry of seawater on a global scale.

The chemistry involving the reaction of CO_2 with seawater is complex and depends on various interacting physical and chemical factors. Accumulation of dissolved CO_2 gas in seawater ultimately catalyses a series of complex equilibrium reactions according to the following equation:

$$CO_{2(atmos)} \leftrightarrow CO_{2\,(aq)} + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + CO_3^{2-}$$

(Dickson *et al.*, 2007).

In summary, CO_2 gas dissolves rapidly in seawater, which forces the reaction to produce carbonic acid (H_2CO_3) that then immediately dissociates to form bicarbonate (HCO_3^-) and hydrogen ions (H+). Bicarbonate then dissociates further to form carbonate ions and additional hydrogen ions. The equilibrium of these reactions, and resultant speciation of dissolved inorganic carbon compounds ($H_2CO_3^-$; HCO_3^-; CO_3^{2-}), is dependent on factors such as temperature, salinity, and pressure (Dickson, 2010). At pH 8.1 (current global average surface seawater pH), a salinity of 35 and temperature of 15°C, the majority of dissolved CO_2 is in the form of HCO_3^-, and not as actual CO_2 gas (Gattuso and Hansson, 2011).

The outcome of adding excess CO_2 to this reaction is an increase in dissolved CO_2 and bicarbonate ion (HCO_3^-) concentration and a reduction in carbonate ions (CO_3^{2-}) and pH ($-\log_{10}[H^+]$), resulting in more acidic conditions (Guinotte and Fabry, 2008; Doney *et al.*, 2009b; Field *et al.*, 2014; Hoegh-Guldberg *et al.*, 2014). It is the increase in hydrogen ion concentration at this state of equilibrium that ultimately results in a decrease in pH. Because the average pH of surface seawater in most cases is naturally alkaline (pH >7), a reduction of pH resulting from anthropogenic carbon dioxide is not likely to result in acidic conditions (pH <7), rather it creates more acidic conditions through an *acidification*, which is why this phenomenon is termed OA.

Current global knowledge of ocean acidification

The 30% of anthropogenically produced CO_2 that has been absorbed by the ocean has already resulted in a decline in mean surface seawater pH by 0.1 units since the industrial revolution (Fig 1.2) (Sabine *et al.*, 2004; Orr *et al.*, 2005; Feely *et al.*, 2009; Hoegh-Guldberg *et al.*, 2014). If anthropogenic CO_2 emissions continue unabated, a further decline of 0.3–0.4 pH units is expected for the end of the century under RCP scenario 8.5 representing a 150% increase in acidity (Caldeira and Wickett, 2003; Guinotte and Fabry, 2008; Doney *et al.*, 2009b; Feely *et al.*, 2009) (Fig 1.2). This amounts to a decline of pH by 0.0015 and 0.0024 annually at the current rate of CO_2 emissions (Field *et al.*, 2014).

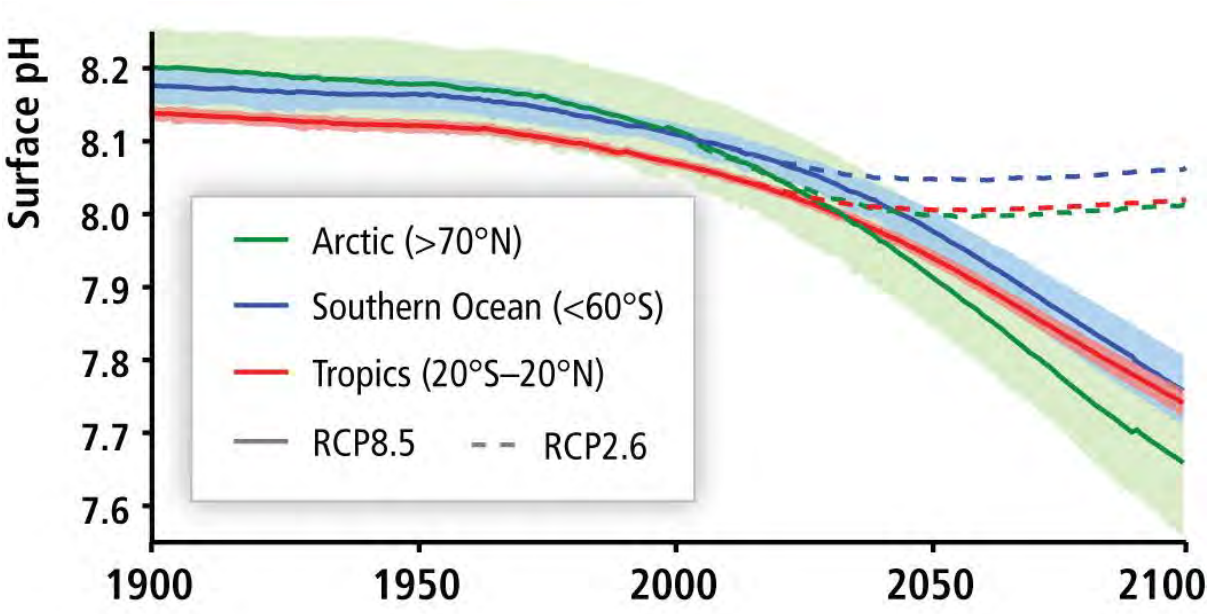

Figure 1.2: Historical and projected decline in surface pH for the global oceans under the RCP 8.5 and RCP 2.6 emission scenarios (from Field *et al.*, 2014)

There has been an increased effort to monitor ongoing OA in the world's oceans. This has been achieved by constructing long time-series of carbonate chemistry data over the last few decades, mostly from long term moorings and ship cruises (Bates *et al.*, 2018; Bushinsky *et al.*, 2019) and the well-established network for monitoring of OA in open oceans (Newton *et al.*, 2015). The findings from this monitoring network have been incorporated into models, and data-based extrapolations have been used to estimate earlier conditions and predict future trends in OA (Tanhua *et al.*, 2015). This has only been possible because of the generally consistent nature of open ocean chemistry, where OA resulting from anthropogenic CO_2 is occurring gradually at a steady rate over time (Doney, 2010).

Characterizing variability in coastal and nearshore ecosystems

Anthropogenic CO_2 is not the only source of acidification in the world's oceans leading to high spatial (site-wide, regional, or even between ocean basins) variability in carbonate chemistry conditions. For example, areas immediately surrounding naturally occurring, deep-sea hydrothermal vent fluids are rich in CO_2 and acids creating low pH environments (~5.0 pH units) that differ from the surrounding seawater (Le Bris *et al.*, 2001; Barry *et al.*, 2010). Deeper oceanic waters also generally show different pH and carbonate chemistry conditions to surface waters due to remineralization of organic matter at depth resulting in CO_2

enrichment (Lauvset *et al.*, 2020). Conversely, pH in surface waters is higher and generally modulated to some extent by biological activity in the photic zone together with atmospheric inputs of CO_2 (Lauvset *et al.*, 2020).

In coastal environments, atmospheric CO_2 is also not the only source of acidification making the contribution of true OA difficult to identify (Strong *et al.*, 2014). Shallower coastal habitats are characteristically more variable than the open ocean and a host of local processes, such as upwelling (Feely *et al.*, 2008; Vargas *et al.*, 2016; Schulz *et al.*, 2019), freshwater inflow (Salisbury *et al.*, 2008; Chierici and Fransson, 2009), cyclical changes in temperature, high biological activity (Cai *et al.*, 2011; Sunda and Cai, 2012) and other human activities contribute to variability in carbonate chemistry and pH over various scales of space and time. While the term OA in the open ocean refers to long-term and large-scale changes in pH on a scale of decades or centuries and longer (Doney *et al.*, 2020), OA is harder to identify with short-term temporal (days, hours, and minutes) and fine-scale spatial (metres and centimetres) variability in pH in coastal areas (Crain *et al.*, 2009; Hofmann *et al.*, 2011).

It has been shown that pH can vary by more than 1 unit in many coastal environments, (Hofmann *et al.*, 2011; Sunda and Cai, 2012; Dorey *et al.,* 2013; Carstensen and Duarte, 2019; Dziergwa *et al.*, 2019) and can have much higher means than the open ocean in environments such as tidal pools or CO_2 vents (Morris and Taylor, 1983; Le Bris *et al.*, 2001). These levels of variability exceed the projected decrease in pH in the open ocean for, at least, the next few centuries. Together with ongoing atmospherically driven OA, which acts to continuously decrease mean pH in seawater, coastal areas are likely to experience an increase in the frequency, magnitude, and duration of low pH events in the future (Hofmann *et al.*, 2011; Duarte *et al.*, 2013; Strong *et al.*, 2014; Waldbusser and Salisbury, 2014) (Fig 1.3). Coastal species are therefore exposed to pCO_2 and pH levels that not only vary constantly but also exceed the range of pH expected for the open ocean within the next 100 years (Hofmann *et al.*, 2011). As organisms tend to be adapted to their environment, quantifying coastal carbonate chemistry and pH variability is essential when assessing the impact of OA on coastal marine organisms.

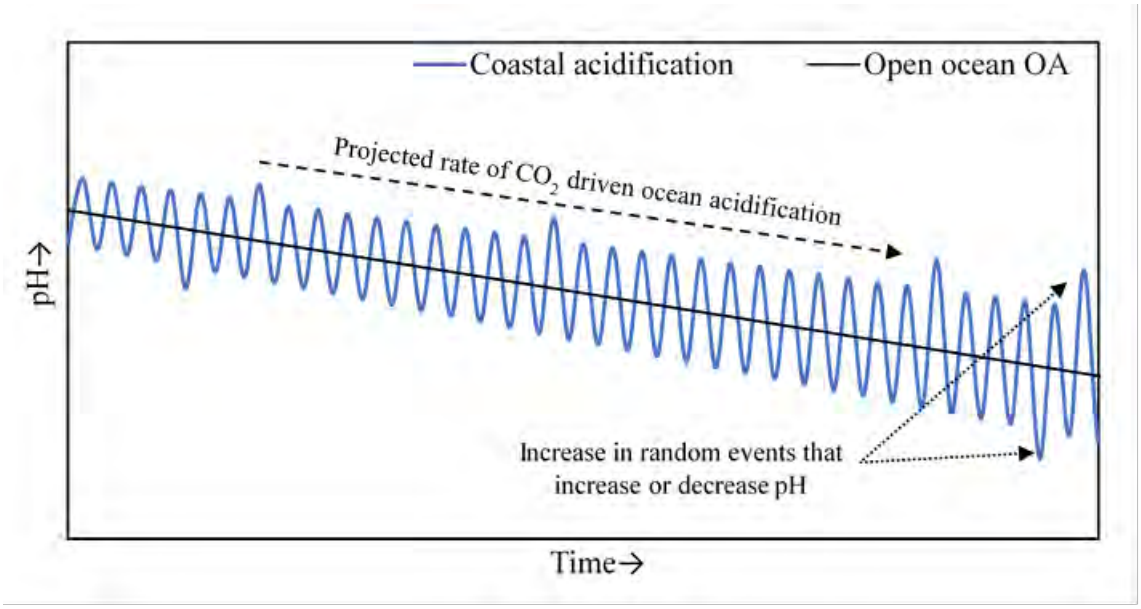

Figure 1.3: Theoretical projected coastal acidification highlighting the steady decline of pH over time as well as increased coastal variability in pH expected in future

The impacts of ocean acidification on marine organisms

The impacts of OA on marine organisms have been found to vary among taxonomic groups and species (Wittmann and Pörtner, 2013; Kroeker *et al.*, 2013a). There are many simultaneous chemical changes that occur with OA that each can have different effects on organisms (Hurd *et al.*, 2020). For example, during OA, there are not only changes occurring in seawater pH but also in other inorganic carbon parameters such as concentrations of total dissolved inorganic carbon (DIC), dissolved CO_2, ion concentrations of HCO_3^-, CO_3^{2-} and H^+ and $CaCO_3$ saturation states (Hurd *et al*., 2020). Each of these components of the carbonate system may affect the physiology of marine organisms differently (Hurd *et al*., 2020). For example, in invertebrates, changes in the calcification process have been linked to imbalances in availability of HCO_3^-, which provides a source of carbonate for calcification, and $CaCO_3$ saturation state, which when low, increases carbonate dissolution (Melzner *et al.,* 2020). However, imbalance in extra-cellular pH in invertebrates, which requires energetically costly regulation, also plays an indirect role on the calcification process, by reducing energy availability for the maintenance of calcification (Melzner *et al*., 2020). In fishes, the primary influence of the carbonate system on fishes is increasing pCO_2, which ultimately disrupts their internal acid-base balance (Heuer and Grosell 2014; Hurd *et al*., 2020). Increasing internal ion concentrations resulting from compensatory acid-base regulation mechanisms

have also shown consequent impacts on fish otolith development through hypermineralization (Heuer and Grosell, 2014; Melzner *et al*., 2020). The variety of direct and indirect physiological impacts of the various components of the carbonate system on species is complex, making the changing carbonate system during OA a multiple driver itself.

Organism responses to these changes in the carbonate chemistry system are species-specific because of the range of physiological and behavioural strategies that have evolved to allow species to survive in their respective environments (Doney *et al.*, 2020). Although several negative responses to OA have been documented for many species, from single celled organisms to invertebrates and fishes (Doney *et al.*, 2020), many have shown neutral and even positive responses to acidification (reviewed by Hendriks *et al*., 2010; Harvey *et al*., 2013; Kroeker *et al.,* 2013a; Clements and Hunt 2015; Cattano *et al*. 2018).

There are several factors that contribute to the sensitivity of marine taxa to OA. For example, it is well documented that calcifying organisms are generally negatively impacted by OA due to extra energy costs to produce and maintain their calcified structures (Orr *et al.*, 2005; Kroeker *et al.*, 2010; Harvey *et al.*, 2013). As a result, this group of organisms has received considerable attention and it is now common knowledge that calcifiers (*e.g*., calcifying algae, corals, molluscs, and echinoderms) are generally sensitive to acidification, particularly in their early developmental stages (Harvey *et al.*, 2013; Kroeker *et al.*, 2013a). Alternatively, fish and crustaceans appear to be more tolerant (Kroeker *et al.*, 2010). However, the sensitivity of a species to OA also varies within different life-stages of the same species, with the early life-stages often being more sensitive than the adult stages (Talmage and Gobler, 2010; Crim *et al.*, 2011). In addition to these intraspecific factors, other factors can contribute to species, population, and community level sensitivity, including food supply, ecological interactions, adaptation potential or other drivers (Hofmann *et al.*, 2011; Strong *et al.*, 2014).

Fishes have generally been considered tolerant of high levels of pCO_2. This is attributed to their ability to efficiently regulate internal acid-base balance (Claiborne *et al.*, 2002; Melzner *et al.*, 2009b) particularly in the adult stages (Ishimatsu *et al.*, 2004). Indeed, many studies have shown physiological tolerance in fishes (survival, growth, metabolic rate) at relatively high pCO_2 levels (Cattano *et al.*, 2018). However, as research in this field has expanded to the earlier life stages, which may have underdeveloped acid-base regulatory abilities, there has been increasing evidence for OA sensitivity in fishes (Cattano *et al.*, 2018). Wittmann and Pörtner (2013b) concluded that the early stages of fishes are among the most sensitive of

all tested groups to OA. Despite the increase in number of experimental studies assessing the impact of OA on the various species and life-stages of fishes, there remains considerable uncertainty on the overall impacts on this taxonomic group (Esbaugh, 2018; Cattano *et al.*, 2018).

Physiological performance is a commonly used criterion for assessing sensitivity to sub-optimal environmental conditions (Pörtner *et al.*, 2004). Physiological performance in marine organisms can be inferred from measures such as metabolic rates, growth rates, morphological changes, calcification rates, regulatory efficiency, swimming performance, fertilization, development, and survival/mortality (Fry, 1971; Ishimatsu *et al.*, 2004; Heuer and Grosell, 2014). In principle, when organisms are exposed to non-optimal environmental conditions, they are likely to respond with a decrease in physiological performance, and this, in turn, may negatively affect these processes with ultimate consequences on fitness (Clark *et al.*, 2013; Horodysky *et al.*, 2015). The physiological response of fishes to changes in sea-water chemistry and low pH has been well studied (Killen *et al.*, 2013; Heuer and Grosell, 2014; Esbaugh, 2018). Measurements of metabolic rate, by estimation of individual oxygen consumption rates, are a commonly used physiological measure when assessing the energetic demands of changing environmental conditions.

Despite this physiological tolerance to OA, there is evidence suggesting that OA elicits a range of behavioural responses in marine fish (Munday *et al.*, 2009b; Jutfelt *et al.*, 2013; Nagelkerken and Munday, 2016; Rossi *et al.*, 2016), particularly in their early life-stages (late larval and early juvenile). Current research on this is biased towards coral reef species, which have generally showed deleterious behavioural responses to OA predicted for the end of the century (e.g., Dixson *et al*., 2010; Simpson *et al*., 2011; Munday *et al*., 2014). However, in an attempt to replicate several published studies on coral reef fishes, Clark *et al*. (2020) did not observe the same negative impacts of OA on behaviours. This has led to discussions on the importance of experimental design (Munday *et al.,* 2020). There is therefore currently a lot of uncertainty around the true impacts of OA on fish behaviours.

There is evidence to suggest that species living in naturally variable environments may be less sensitive to low pH (Vargas *et al.*, 2017; Doney *et al.*, 2020). This is facilitated by high levels of local adaptation and phenotypic plasticity that exist within populations inhabiting variable environments (Vargas *et al.*, 2017; Guscelli *et al.*, 2019), such as nearshore coastal areas. Often organisms can tolerate pH values that are within the present range of their

natural variability and stress only occurs when pH drops below present lows (Dorey *et al.*, 2013; Thor and Dupont, 2015; De Wit *et al.*, 2016; Ventura *et al.*, 2016). Thus, species that are living at the limits of their physiological tolerance may be affected by small changes in their environment (Pörtner and Farrell, 2008; Doney *et al.*, 2020).

The variability in carbonate chemistry observed in coastal waters and associated biological adaptation has consequences for experimental design and the interpretation of experimental results. Many articles in the literature are using scenarios based on open ocean projections (e.g., present pH 8.1 vs. future scenario of 7.7). However, these scenarios are often within the present range of variability and thus not appropriate to use as future OA scenarios when testing the response of local species (Vargas *et al.*, 2017). Aside from not being representative of coastal variability, this may result in poor estimations of the tolerance/vulnerability of species which are naturally exposed to the tested pH conditions. Testing the impact of future OA conditions on coastal marine organisms, particularly in regions with known variability, should start with field carbonate chemistry monitoring to inform laboratory experiments (Hofmann *et al.*, 2011; Cornwall and Hurd, 2016). This allows the selection of relevant OA treatments, deviating from the present variability to which the organism is adapted (Vargas *et al.*, 2017; Doney *et al.*, 2020).

Bates *et al.* (2018), in a paper encouraging biologists to consider how species respond to local conditions rather than using overall global averages, highlighted that due to the variable nature of coastal oceans it is important to collect data representative of the 'ocean weather' rather than long-term climate related changes. In order to address this in the context of OA, the Global Ocean Acidification Observing Network (GOA-ON) developed a framework whereby they defined the data precision and measurement requirements to collect data to the standard of identifying the 'ocean weather' (Newton *et al.*, 2015). In their document, they highlight that the measurement standards required for 'ocean weather' are suitable for identifying short-term variation and spatial patterns in carbonate chemistry, which is essential when interpreting species and ecosystem responses to local OA (Newton *et al.*, 2015).

Ocean acidification in the oceans and coastlines off South Africa

The African continent is behind in its efforts to monitor and assess the impacts of OA on marine species. A review of 37 OA studies by Dupont and Pörtner (2013) highlight the limited research on OA in Africa. More recently Tilbrook *et al*. (2019) also highlighted the

limited OA publications on the African continent (Fig 1.4). This is concerning, especially when one considers the biological, social, and economic vulnerability of many countries in Africa, which rely heavily on marine resources. It is essential to understand the impacts of future OA in these data-poor regions to inform local management strategies (Kelly *et al.*, 2011).

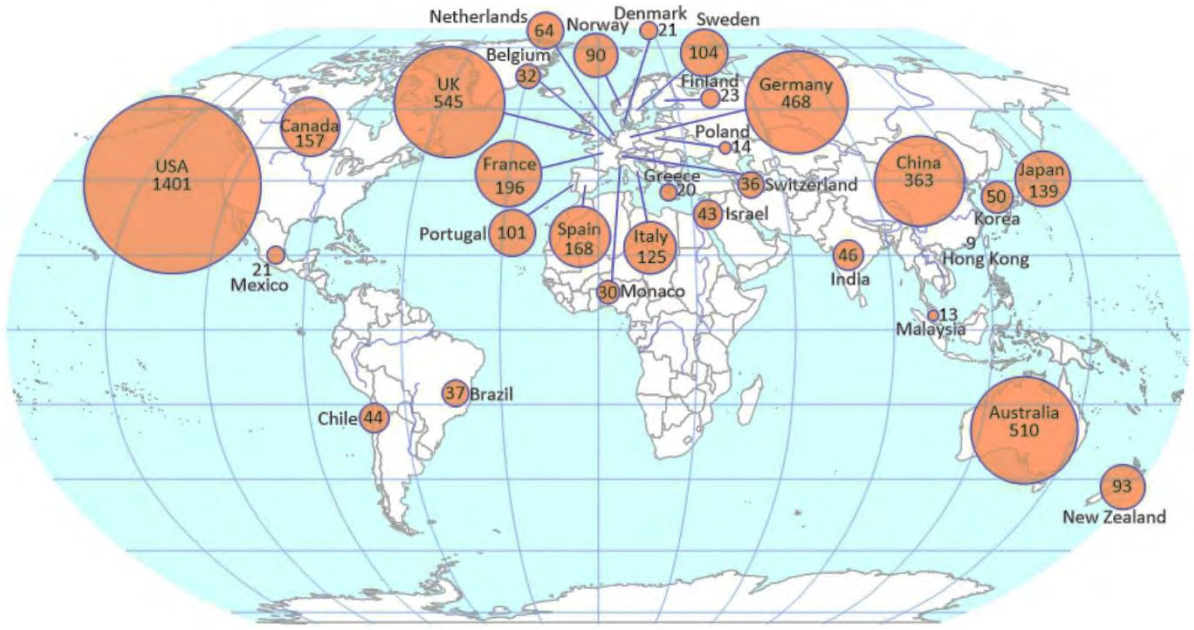

Figure 1.4: Figure from Tilbrook *et al*. (2019) showing OA publications by country based on data obtained by the IAEA Ocean Acidification International Coordination Centre (OA-ICC) that indicate the significant lack of published data in Africa

Ocean acidification research has been similarly limited in South Africa (Moloney *et al.*, 2013). Published studies are restricted to the southern Benguela region on the far west coast of the country (González-Dávila *et al.*, 2009; Santana-Casiano *et al.*, 2009; Gregor and Monteiro, 2013). These studies have assessed the carbonate chemistry, mostly in the open ocean, along the eastern boundary upwelling system (Benguela Current Upwelling System) in the Atlantic Ocean influenced region of the coast. This is an extremely active seasonal, wind-driven upwelling system, which drives the high biological activity observed on the shelf (Gregor and Monteiro, 2013). Deep-source seawater, particularly near the coast, is rich in CO_2 due to remineralization of organic matter and this CO_2 rich and low pH water is brought to the surface through coastal upwelling (Feely *et al.*, 2008). This upwelled seawater is similarly rich in nutrients and promotes high biological activity (Bailey, 1991; Pitcher *et al.*, 1992). In terms of local carbonate chemistry variability, studies in the Benguela Current

Upwelling Region found that this region exhibits complex carbonate chemistry dynamics, which are typically associated with the dynamic occurrence of upwelling and biological activity. For example, González-Dávila *et al*. (2009) identified that the greatest variability in CO_2 and temperature was associated with the largest upwelling cell in the region. The high biological activity observed in this region often results in the drawdown of CO_2, which identifies the region as a net CO_2 sink (Santana-Casiano *et al.*, 2009; Gregor and Monteiro, 2013). However, with continuous upwelling serving as a source of CO_2, flux dynamics are complex over various temporal and spatial scales. These studies show that although upwelling regions typically would be associated with a net source of CO_2, due to the high productivity they promote, they can serve as CO_2 sinks like those on the west coast of South Africa (Santana-Casiano *et al.*, 2009).

A biological study, also on the west coast of South Africa, assessed the impact of projected future OA on the puffadder shyshark (*Haploblepharus edwardsii*). This study found that this species is physiologically well-adapted to low pH conditions (7.4–7.6) resulting from the frequent occurrence of upwelling in its natural habitat (Dziergwa *et al.*, 2019). However, structural, and compositional changes in denticles (structures that form shark skin) were observed after 9 weeks of exposure to low pH treatments (Dziergwa *et al.*, 2019). Another study experimentally tested the impact of low pH (7.78) on the early development (hatching to settlement) of dusky kob (*Argyrosomus japonicus*), a tropical marine fish species. This study found significant effects of low pH on the metabolism, growth, and skeletal development of this species with ultimate survival consequences (Edworthy *et al.*, *in prep.*).

Rationale and motivation

In a recent publication, Duncan *et al.* (2019) highlighted the increasing variability in local climate conditions on the south coast of South Africa. The authors found that upwelling events are increasing with time on this coast (associated with El Niño Southern Oscillations), and this results in more variable local temperature conditions. Since local pH can be modulated by upwelling events, it is expected that pH variability will increase similarly in future. Coastal pH and carbonate chemistry conditions have not yet been quantified along this region of the coastline and there is a need to understand the variability that occurs here, currently and in the future, to inform local climate patterns and species responses.

Algoa Bay, which is situated in the temperate south coast of South Africa (33° 50' S; 25° 50' E), is an ideal site for monitoring the spatial and temporal variability in coastal pH and the associated impacts of relevant OA treatments for several reasons. The bay is known for its variability in local biogeophysical processes such as active wind-driven upwelling (Goschen and Schuman, 1995; Goschen and Schumann, 2011), high nearshore and surf zone biological productivity (Campbell and Bate, 1988; Lemley *et al*., 2019), a large subtidal macroalgal reef (Knoop, 1988) and freshwater inflow via estuaries, aquifers or anthropogenic inputs (Campbell and Bate, 1998; Lemley *et al*., 2019). The bay is divided into two sections with the eastern coastline being described as the 'windward' side of the bay characterized by high current and wave action (Roberts, 2010; Goschen and Schumann, 2011) whereas the 'leeward' side of the bay on the western coastline exhibits calmer conditions.

As a result of these unique biogeophysical conditions, Algoa bay has been identified as a sentinel site for long term oceanographic and ecological research. As a result, there already exists an extensive database for both physical and biological data at the sites selected for the carbonate chemistry monitoring in this study that could provide baseline information (Fig 2.2). Furthermore, by partnering with a current monitoring program, logistic difficulties and cost-related constraints could be mitigated. The study site is also serviced by two weather stations that were suitably located to provide detailed weather data for all areas of the bay. Finally, the study species was easily accessible in the required numbers at the selected site, which was information that could be obtained from the numerous ichthyological studies that have been conducted in this region for many years. This provided the information needed to locate and catch the study species. Finally, being a small coastal area, Algoa Bay and the species that occur there still hold social and economic relevance to local communities, making it an essential region to identify the impacts of future OA. Identifying site suitability, logistical requirements, as well as the impact and relevance of the work locally, is necessary when developing an OA monitoring program in a new region. Algoa Bay suited all these criteria and can therefore be considered a suitable region to initiate OA monitoring in the country.

Overall aims of this research and thesis structure

The overarching aim of this thesis was to understand the impacts of coastal pH and associated carbonate chemistry variability on *Diplodus capensis* in the context of OA. This included the monitoring of local carbonate chemistry conditions in Algoa Bay, South Africa to the

standard of identifying 'ocean weather' and local variability. Information on the local variability in pH (and associated parameters) was then used to identify suitable treatment levels to evaluate the physiological (metabolic) and behavioural response (swimming activity and feeding rates) of *D. capensis* exposed to present and future pH conditions.

To do this, the thesis is divided into six chapters.

Chapter One introduces the study and provides an overview of the current literature and the relevance of this work in an international and South African context.

The first data chapter, **Chapter Two**, describes the study site, Algoa Bay, and relevant weather processes that may have influenced carbonate chemistry dynamics during the study period, including sea surface temperature, wind, and rainfall. The study species is also described in this chapter.

Chapter Three presents results from discrete monthly and continuous 24-hour monitoring of carbonate chemistry parameters (including pH, pCO_2, total alkalinity, Ω Ar, Ω Ca) and associated physico-chemical parameters. This chapter aimed to identify the local variability in carbonate chemistry at offshore and inshore sites in the study area as well as identify the potential drivers of the observed variability in pH. These pH data were then used to identify relevant treatment levels for the following laboratory experiments.

Chapter Four reports on the physiological response (metabolic rates and relative aerobic scope) of early post-flexion stage *D. capensis* to a range of pH levels (8.0–7.2 pH units) which represent the extremes of the measured local variability and pH levels associated with future coastal OA.

The final data chapter, **Chapter Five**, used the same treatment levels and experimentally assessed the impact of pH on typical swimming behaviours.

Chapter Six synthesises and discusses the overall findings of the study and their relevance to global and local initiatives. It also covers the limitations of the study and makes recommendations for future research.

Chapter 2

Description of the study site, relevant local weather processes and the study species

Coastal carbonate chemistry may be influenced by several drivers in coastal areas. Weather associated processes, such as wind driven current and wave dynamics (Saderne *et al.*, 2013), upwelling (Feely *et al.*, 2008), rainfall and freshwater inflow (Manzello, 2010) may all play a role in modulating carbonate chemistry and pH in local environments. Understanding the potential contribution of these processes to coastal pH variability is essential, as they may drive dynamic changes over scales of space and time. The coastline of South Africa is known for its dynamic nature due to the interaction of several coastal and weather processes. The complexity of the South African coastline is likely reflected by equally complex carbonate chemistry dynamics and therefore pH variability.

The coastline of South Africa

The South African coastline is uniquely positioned at the meeting point of two oceans and at the confluence of two vastly different ocean currents. This contributes to its dynamic oceanography and biogeography, and a richness in diversity that cannot be equalled by any other coastline of similar size in the world (Mead *et al.*, 2013; Whitfield *et al.*, 2016). The South African coastline is some 3 650 km long and is bordered by the Indian Ocean on the east and south coast and the Atlantic Ocean on the west coast (Fig 2.1). The warm Agulhas Current flows in a south-westerly direction down the east and south coast of the coastline in close proximity to the shore, following the narrow continental shelf until deflecting southward forming a large retroflexion when reaching the broad Agulhas Bank on the south coast (Fig 2.1) (Lutjeharms, 2006). On the west coast, the cold Atlantic Ocean Benguela Current flows in a northerly direction up the coast, which brings cold, nutrient rich waters close to the shore through frequent upwelling events (Hutchings *et al.*, 2009). The transition from warmer seawater conditions on the east coast to cold on the west coast has resulted in the classification of three broad biogeographic regions namely, the sub-tropical zone on the east coast, the warm-temperate on the south coast and cool-temperate west coast (Turpie *et al.*, 2000; Harrison, 2004).

South Africa's coastal habitats are diverse and comprise rocky shores, sandy intertidal beaches, shallow reefs, estuaries, mangrove forests, salt marsh habitats, stromatolite pools,

kelp forests and areas of mixed shoreline (Bally *et al.*, 1984; Whitfield *et al.*, 2016). A total of 290 estuaries occur along the coast of South Africa, the majority of which occur along the warm-temperate south (124) and subtropical east (130) coasts (Van Niekerk *et al.*, 2020). This composition of diverse habitats, together with different biogeographic conditions and unique biogeography, has contributed to the incomparably high coastal species diversity in South Africa (Whitfield *et al.*, 2016).

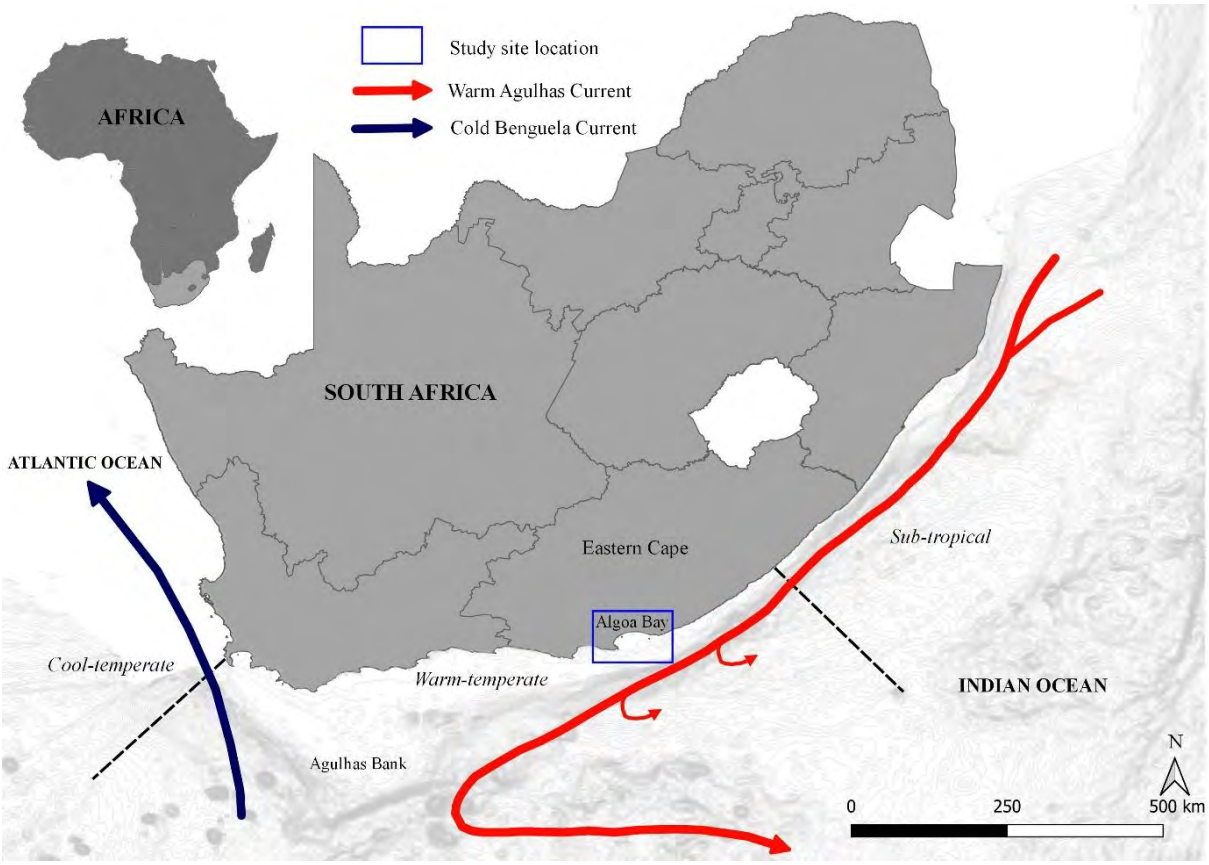

Figure 2.1: Map of South Africa showing the complex coastline bordered by two oceans and major currents, the warm Agulhas (red line) and cold Benguela (blue line). The divisions into three major biogeographic regions, sub-tropical (east), warm-temperate (south), and cool-temperate (west) are indicated by the dashed lines.

Algoa Bay

Algoa Bay is the largest of several embayments along the warm-temperate south-eastern coastline of South Africa (Roberts, 2010). The bay is bordered by the Cape Recife headland on the western boundary and Cape Padrone on the eastern boundary (Fig 2.2). The mouth of the bay is approximately 80 km wide, eastward facing into the Agulhas dominated region of

the Indian Ocean (Fig 2.1) (Goschen and Schumann, 2011). Algoa Bay is known for its dynamic oceanography due to the interaction between nearshore coastal and deep-water oceanic processes (Goschen and Schumann, 2011) as well as anthropogenic influences as a consequence of its proximity to the large, urbanized Nelson Mandela Bay municipal area (Fig -2.2) (Lemley *et al.*, 2019).

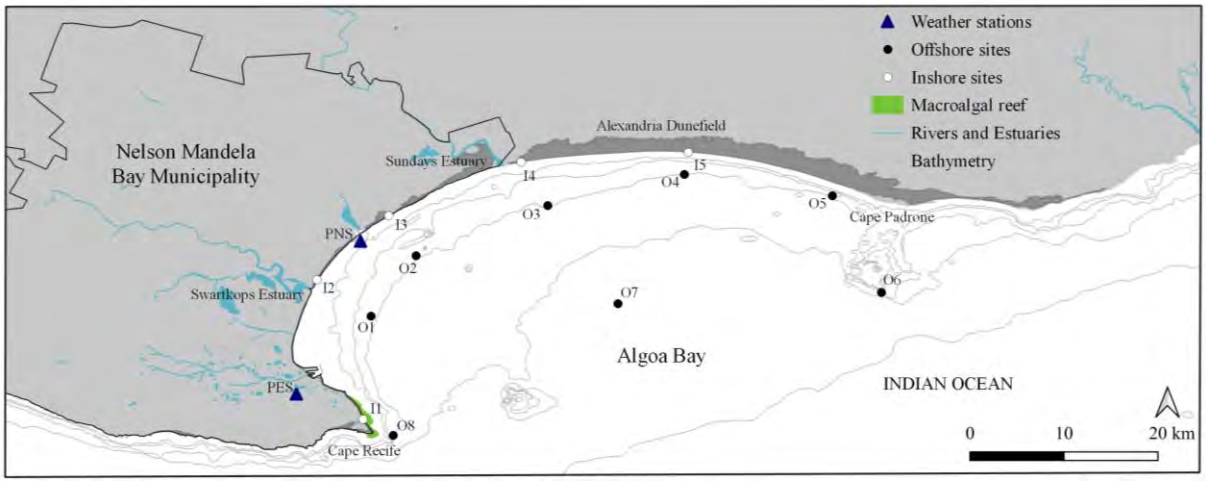

Figure 2.2: Map of the study site, Algoa Bay, indicating the offshore (O1–O8) and inshore (I1–I5) sampling sites for carbonate chemistry monitoring, which took place monthly from June 2018 – January 2020, as well as the two weather stations (Port Elizabeth Station – PES and the Port of Ngqura station – PNS) where the supporting weather data were collected

The primary current influence is from the Agulhas Current, which occurs approximately 60 km offshore and flows in a south-westerly direction along the continental shelf edge (Goschen *et al.*, 2012). South-westerly winds dominate throughout the year in the region and are known to increase in frequency and intensity during the winter months (Schumann and Martin, 1991; Goschen and Schumann, 2011). In summer, alternating westerly and easterly winds are common (Roberts, 2010). Surface currents in the bay are wind driven and with the south-westerly winds being dominant, currents typically follow a similar direction parallel to the coastline (Roberts, 2010; Pattrick *et al.*, 2013). Wind dynamics, responsible for current dynamics and coastal mixing, also drive upwelling events in the bay.

In the western sector of the bay especially, currents can vary significantly over space and time (Schumann *et al.*, 2005). Due to the log-spiral shape and orientation of Algoa Bay, the western sector of the bay is more exposed to wind, current and wave dynamics and is therefore regarded as the ‘windward’ sector of the bay (Pattrick, 2013; Pattrick and Strydom,

2014a). On the eastern sector, conditions are more sheltered behind the protruding headland at Cape Recife, and this is regarded as the 'leeward' sector of the bay (Fig 2.2).

Sea temperatures in Algoa Bay typically range between 16 and 22°C. In the deeper areas of the bay, summer thermoclines to a depth of ~20 metres over the shelf are common with isothermal conditions occurring in winter (Schumann *et al.*, 2005). No thermoclines occur in the shallower (<50 m depth) areas of the bay (Beckley, 1983a). Sporadic processes occur that contribute to temporary variability in temperature. Occasionally, large Agulhas current meanders, called 'Natal Pulses', pass into the bay and this is driven by strong south-westerly winds (Goschen and Schumann, 2011; Goschen *et al.*, 2015). The intrusion of these Agulhas Current waters into the bay results in patches of warm seawater reaching temperatures as high as 27°C (Beckley, 1983a; Schumann *et al.*, 1988) (an example of an Agulhas intrusion event is shown in Fig 2.3 a). Conversely, upwelling events can drop the sea temperature to 10°C (Goschen and Schumann, 2011; Goschen *et al.*, 2015) (an example of an upwelling event is shown in Fig 2.3 b). Closer to shore, high-energy wave action as well as wind-driven, tidal, and inertial currents are responsible for circulation and mixing (Roberts, 2010; Goschen and Schumann, 2011). This results in high temperature variability throughout the year.

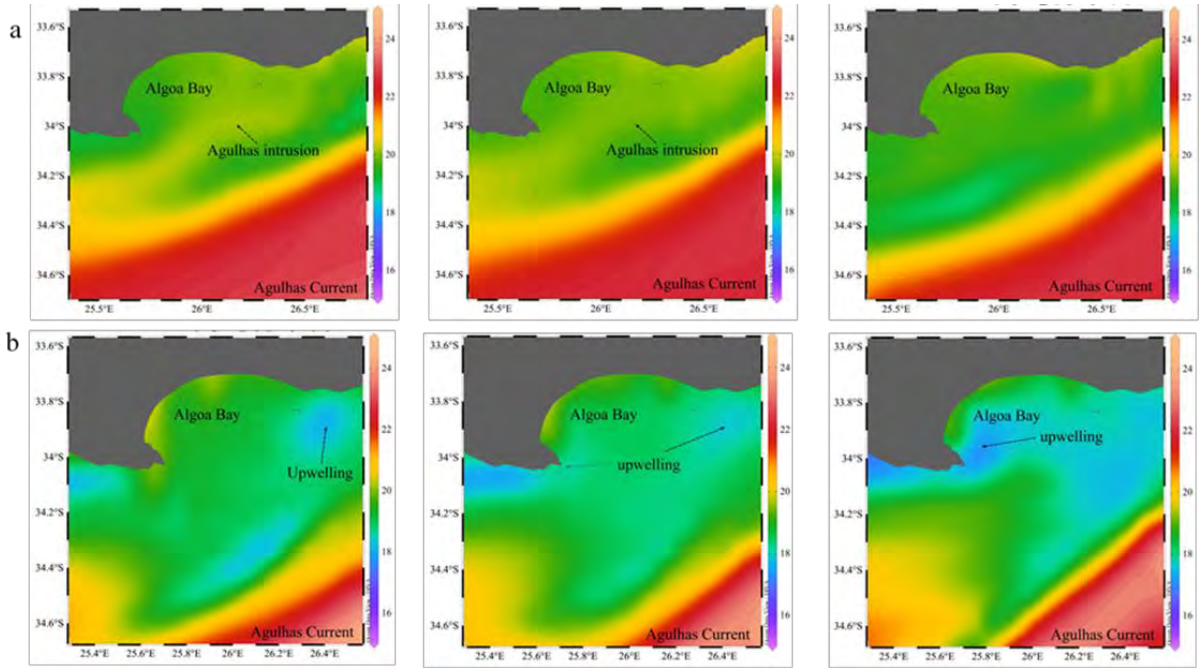

Figure 2.3: Examples of a) an Agulhas Current intrusion over the days (from left to right) 10, 12 and 14 October 2018 and b) an upwelling event that occurred over the days (from left to right) 29, 30 and 31 October 2018. The temperature scale (°C) is shown on the right of each

plot. Temperature data were obtained from the South African Environmental Observation Network's (SAEON) Continuous Monitoring Program (CMP).

Upwelling in Algoa Bay commonly occurs on the eastern and western capes of the bay. On the eastern boundary of the bay these events can be driven by the movement of warm Agulhas Current waters following an intrusion (Goschen and Schumann, 2011). However, most upwelling events are predominantly wind driven, and occur when easterly winds persist over a few days (Goschen and Schumann, 2011). Resulting upwelled cold water can then be driven into the bay if strong westerly winds occur after the upwelling event (Schumann *et al.*, 1988; Goschen and Schumann, 1995). Easterly winds, and their associated upwelling events, are most common in summer in this region (Walker, 1986; Goschen and Schumann, 1995). Wind-driven upwelling events are common on both the eastern and western capes of the bay (Goschen and Schumann, 1995; Goschen *et al.*, 2015) (Fig 2.2 b). Upwelled water from the deep is enriched with CO_2 and nutrients and has been associated with low pH conditions along the west coast of South Africa (7.4–7.6 pH) (e.g., Dziergwa *et al.*, 2019). Movement of these water sources to the surface can create patches of low pH waters that differ from the surrounding surface water (Raven *et al.*, 2020). Upwelling events also drive coastal productivity due to the surfacing of nutrient rich conditions required by many marine phytoplankton (Lutjeharms *et al.*, 1996; Pitcher and Probyn, 2011; Raven *et al.*, 2020). This productivity following upwelling events can then further influence local carbonate chemistry conditions (Pitcher and Calder, 2000; Pitcher and Probyn, 2011).

There is no pronounced seasonality in rainfall patterns in this area since this region falls between typical summer and winter rainfall regions (Blamey *et al.*, 2018; Mahlalela *et al.*, 2020). Algoa Bay is situated in a water scarce area of South Africa and is often subjected to periods of prolonged drought (Jury and Levey, 1993). In 2019, the Nelson Mandela Bay Municipality (Fig 2.1) was declared a 'disaster area' because of a drought that has gripped the area since 2015 (Mahlalela *et al.*, 2020).

Two large permanently open estuaries, the Swartkops and Sundays, together with input from groundwater discharge from the Alexandria dune field on the eastern shore of the bay, constitute the largest source of freshwater into the bay (Campbell and Bate, 1998; Lemley *et al.*, 2019). Due to the lack of recent rainfall, however, the freshwater runoff into estuaries and the bay has been limited. Two wastewater treatment facilities (serviced by the Nelson Mandela Bay Municipality) discharge directly into the nearshore coastal environment in

Algoa Bay and are a point source for pollution. These are the Cape Recife Wastewater Treatment Plant (WWTP) and the Fish Water Flats Domestic and Industrial WWTP, which are both situated near the developed Nelson Mandela Bay municipal area (Lemley *et al.*, 2019). These WWTPs intermittently discharge wastewater effluent directly into the surf-zone along the western sector of the bay. The wastewater varies in composition, but it is known to contribute to anthropogenic nutrient loading at these discharge locations (Lemley *et al.*, 2019).

The occurrence of frequent upwelling as well as inflow of freshwater through estuaries, wastewater treatment works and groundwater into the bay results in nutrient rich conditions in certain areas of the bay, which are conducive to high productivity (Campbell and Bate, 1988, 1998; Lemley *et al.*, 2019). This can drive high phytoplankton productivity, which is common in the surf zone on the western sector of the bay. These patches of high productivity are typically associated with the algal surf zone diatom species *Anaulus australis,* which often form dense localized accumulations and discolour the water at these sites (Campbell and Bate, 1988).

Harmful algal blooms, or 'red tides' occur more frequently in the Benguela upwelling region of the west coast of South Africa than other parts of the coastline (Pitcher and Calder, 2000). These blooms are associated with various phytoplankton species, which can form dense aggregations that create hypoxic conditions when they decay, which is harmful to many marine species (Pitcher and Probyn, 2011; Blamey *et al.*, 2015). Fewer harmful algal bloom events have occurred on the southern and eastern coastlines of the country (Whitfield *et al.*, 2016). In 2014, however, an atypical harmful bloom of the dinoflagellate species *Lingulodinium polyedra* occurred all along the southern cape coastline for over 500 km, causing long term ecological consequences for the region (Whitfield *et al.*, 2016).

Algal bloom events are known to influence the pH of surrounding seawater. Depending on the species, the general changes that occur are an increase in pH during the productive phase of the bloom followed by a rapid drop in pH during the decomposition phase (Wootton *et al.*, 2008; Raven *et al.*, 2020). Conditions of dense algal aggregations or blooms will also likely amplify the diurnal cycle of pH variability as higher pH levels may be reached during the photosynthetic phase in the day and lower values will be reached during the respiration phase at night (Wootton *et al*., 2008; Raven *et al*., 2020). The changes in the frequency, magnitude and seasonality of algal bloom events associated with global change in coastal areas, such as

Algoa Bay (Whitfield *et al.*, 2016; Lemley *et al.*, 2019), are therefore likely to influence the local carbonate chemistry dynamics in the future.

Study sites

Algoa Bay has been identified as a National Sentinel Site for long-term ecological monitoring, which is carried out by the South African Environmental Observing Network (SAEON) Elwandle Node. The sites selected for the monitoring conducted in this study (Chapter Three) are the same sites that are regularly monitored by the Algoa Bay Sentinel Site long-term ecological research (LTER) and continuous monitoring (CMP) programmes. Due to the complex nature of the oceanography in this area, this Sentinel Site represents many common coastal processes, presenting it as an interesting area in which to assess carbon chemistry dynamics. The study sites for carbonate chemistry and associated physico-chemical monitoring (Chapter Three) and fish collection for the laboratory experiments (Chapters Four and Five) were situated across offshore and inshore areas in Algoa Bay (Fig 2.2).

The study sites are situated in areas that regularly or sporadically experience the various coastal processes that occur in the bay, including sites influenced by upwelling, the two large estuaries, areas that typically experience high algal productivity, bloom events and freshwater inflow from various sources. The site for fish collection was in an intertidal gulley at Cape Recife (Fig 2.2). This site is known to support high numbers of coastal fish larvae from various species (Pattrick and Strydom, 2014a) and similarly the study species, post-flexion stage *Diplodus capensis* (Chapters Four and Five), occurred here in high numbers. In order to quantify the variability in carbonate chemistry that occurs at the fish collection site over the short-term, this site was also used for the *in situ* deployment of an iSAMI-pH sensor that measured pH at half hourly intervals over a period of five days (Chapter Three).

Quantifying local weather processes during the study period

In order to describe the local weather processes that occurred during the study period, data on sea temperature, wind speed and direction, and rainfall were sourced from the SAEON Elwandle Node's continuous monitoring program (CMP) and the South African Weather Service (SAWS). Hourly sea temperature data were obtained for the sites corresponding to the offshore monitoring in Algoa Bay (Fig 2.2) from June 2018 to January 2020. Weather

data (wind and rainfall) were obtained from two weather stations namely, the Port Elizabeth Station (PES, - 33.9860, 25.6160) situated in the western sector of the bay in the city of Port Elizabeth and the Port of Ngqura Station (PNS, -33.8090, 25.6900) situated closer to the centre of the bay between the Swartkops and Sundays estuaries from June 2018 to January 2020 (Fig 2.2).

Sea temperature

Continuous sea temperature data for the study period were recorded by SAEON's CMP by seven Underwater Temperature Recorders (UTRs) moored at a depth of 10 m at the offshore stations shown in Fig 2.1 (excepting Site O1). Based on hourly data during the study period (June 2018 – January 2020), sea temperature ranged from a minimum of 11.49 to a maximum of 23.16°C with an average of 17.70 ± 1.70°C. Average temperatures from each station showed that temperature generally increased slightly from the eastern (windward) to western (leeward) side of the bay (Table 2.1). The coldest average temperature was recorded at Site O6 near Bird Island, and the warmest average temperature was recorded at Site O2 near the Swartkops Estuary mouth (Fig 2.1). A temperature time series for the study period is presented in Fig 2.4. The missing data for the period of May 2019 to October 2019 resulted from a missing UTR that did not resurface after deployment.

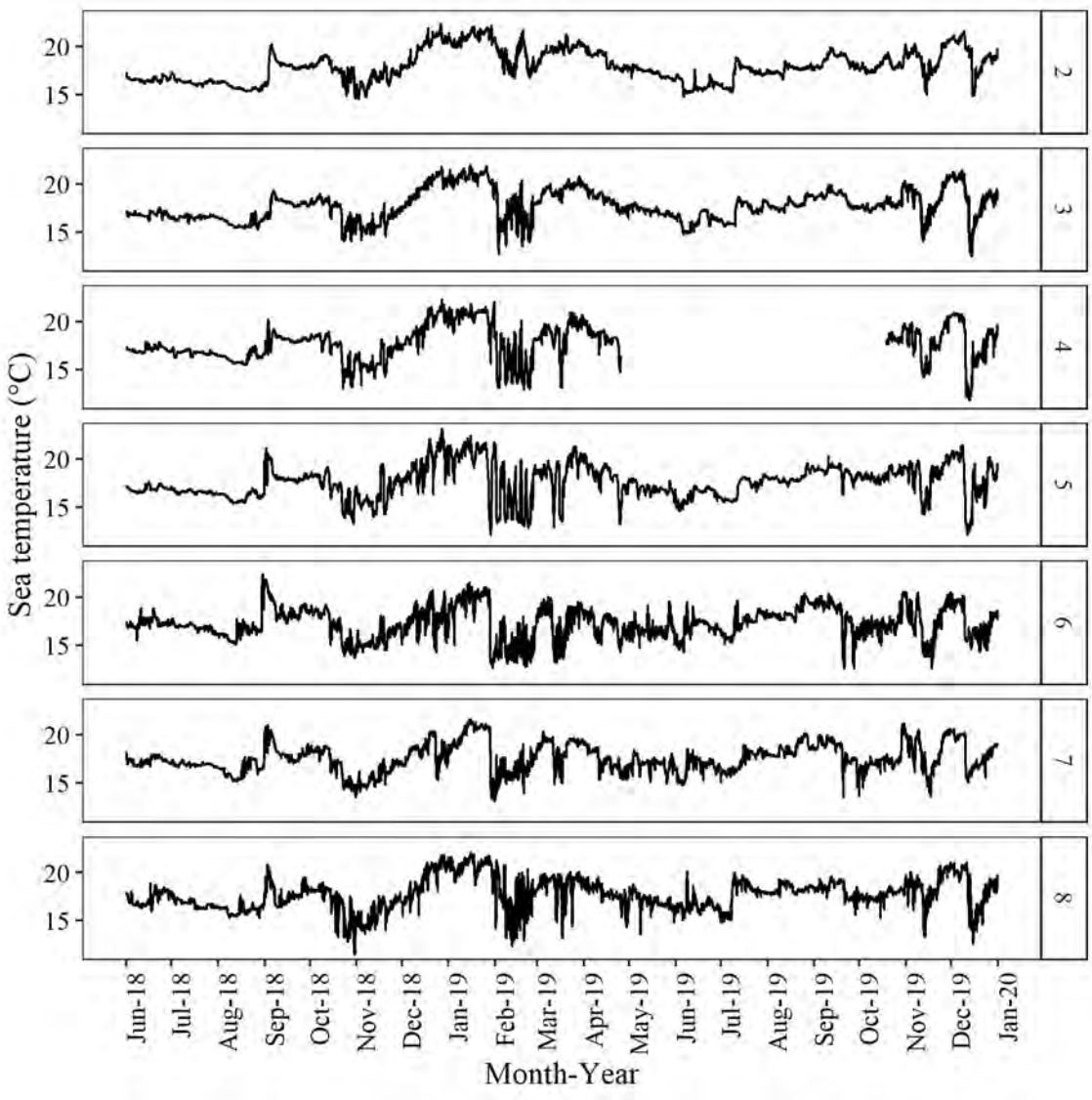

Figure 2.4: Sea temperature (10 m) time series from hourly temperature measurements at seven offshore sites (O2–O8) in Algoa Bay from the period of June 2018 to January 2020. Data supplied by the South African Environmental Observation Network's (SAEON) Continuous Monitoring Program (CMP)

The sites that experienced the highest variability in sea temperature during the study period were located on the eastern and western capes of the bay: sites O4 and O5 near Cape Padrone in the east and Site O8 near Cape Recife in the west, all showing ranges in sea temperature that exceeded 10°C (Table 2.1). The coldest temperature was recorded at Site O8 near Cape Recife. These are the sites known to experience wind driven upwelling, which propagates cold water into these nearshore areas when favourable winds (north-easterlies) persist over a few days (Schumann *et al.*, 1988; Goschen and Schumann, 2011).

Table 2.1: Summary statistics of sea temperature (°C) at the seven offshore sites (O2–O8) in Algoa Bay from the period of June 2018 – January 2020. Data obtained from the South African Environmental Observation Network's Continuous Monitoring Program.

Site	*Sea temperature (°C) mean ± SD*	*Min, Max*	*Range*
2	18.0 ± 1.66	14.5, 22.4	7.9
3	17.8 ± 1.63	12.5, 21.9	9.4
4	17.7 ± 1.90	11.8, 22.3	10.5
5	17.6 ± 1.80	12.0, 23.2	11.2
6	17.3 ± 1.65	12.6, 22.4	9.8
7	17.6 ± 1.53	13.1, 21.6	8.5
8	17.8 ± 1.67	11.5, 22.5	11.0

During the study period, temperature varied the most in January (range = 10.5°C) and February (range = 9.4°C) at all sites (Fig 2.4). The most variability in sea temperature occurred in summer (range = 11.4), with a minimum temperature of 11.8°C recorded. This was likely due to upwelling events that are common during this season in Algoa Bay. The highest recorded sea temperature was a maximum of 23.16°C, also recorded during summer in December 2018, which is a typical high summer temperature for this region. Much less variability was observed in autumn (range = 8.5) and winter (range = 8.1).

Wind patterns

Data on hourly wind direction and speed (m s^{-1}) for the study period were obtained for the two weather stations in the bay from June 2019 to January 2020 (Fig 2.1). The PES station showed a lower average wind speed (5.30 ± 3.22 m s^{-1}) than the PNS station (5.66 ± 2.98 m s^{-1}). However, a higher maximum wind speed of 22.5 m s^{-1} was recorded at the PES compared to 17.5 m s^{-1} at the PNS. Wind speeds at the PES were higher during the spring and summer months, with the highest average wind speed being recorded in November (6.06 ± 3.25). The lowest wind speed was recorded during May (4.21 ± 2.91). Similarly, higher average wind speeds were recorded in summer and spring months at the PNS, with the highest average wind speed recorded in January (6.40 ± 3.20) and the lowest in May (4.71 ± 2.31).

West-south-westerly winds were most prevalent at the PES during the study period (Fig 2.5), as is typical for this area (Goschen and Schumann, 2011). In contrast, westerly winds dominated at the PNS (Fig 2.5). Easterly winds occurred more frequently at the PES than the PNS (Fig 2.5).

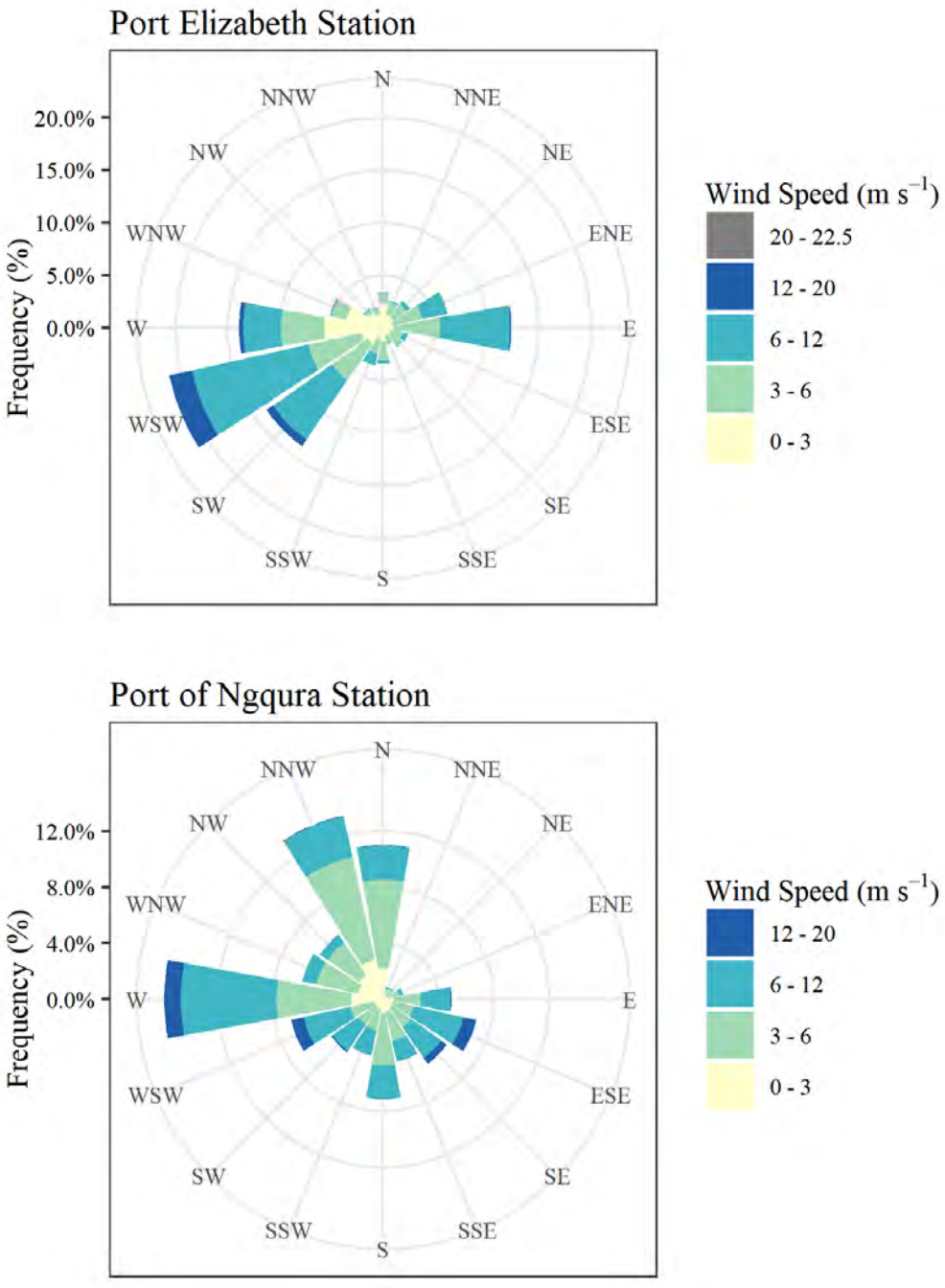

Figure 2.5: Rose diagrams showing the prevalent wind direction and speed (m s^{-1}) at each weather station during the study period from June 2018 – January 2020

Figures A.1 and A.2 in the appendix show monthly wind roses for each station to visualise the seasonality in wind speed and direction. Easterly winds were most common during the summer (December – February) at the PES but were less common in spring (September – November), which was dominated by west-south-westerly winds. In contrast, the wind direction at the PNS was generally mixed in summer, alternating between westerly and east-south-easterly winds, while north-north-westerly winds dominated during the winter months.

Coastal rainfall and freshwater inflow

Average daily precipitation data were obtained for both the PES and the PNS from January 2003 until January 2020. The average daily precipitation recorded during the study period (May 2018 – January 2020) was 1.71 ± 3.91 mm at the PES (Fig 2.6) and 0.68 ± 2.71 mm at the PNS (Fig 2.7). The total cumulative rainfall recorded over the study period was 722.8 at the PES and 436.8 mm at the PNS. No rainfall was recorded on 455 and 497 of the 641 days of the study period representing 70.9% at the PES and 77.5% at the PNS, respectively. Rainfall measured throughout the study period was low as a result of the drought (Fig 2.6 and 2.7).

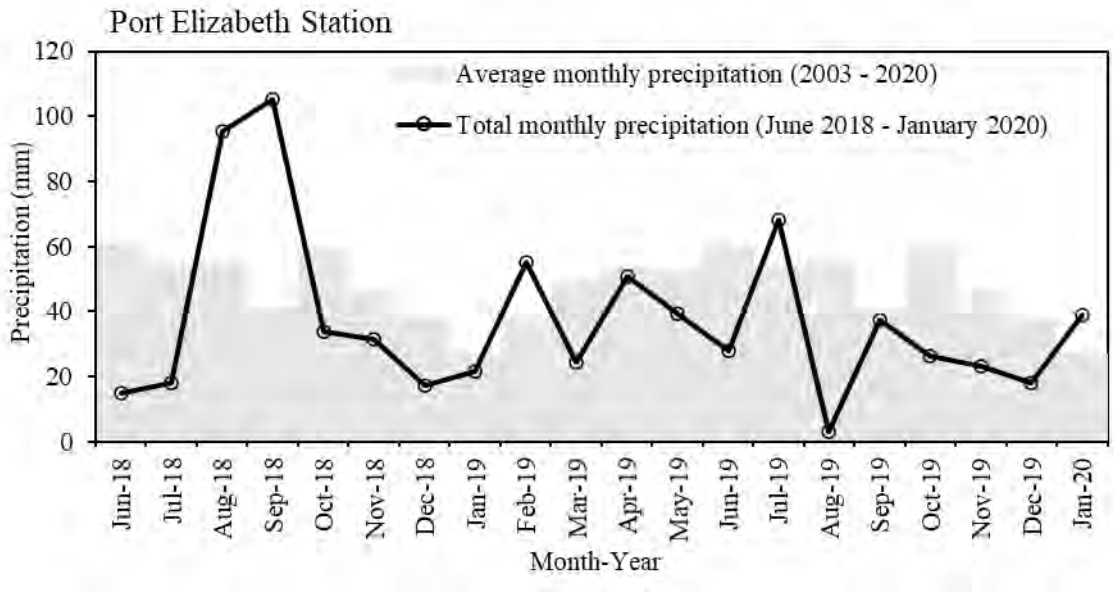

Figure 2.6: Average monthly precipitation (mm) at the Port Elizabeth Station (PES) from 2003 to 2020 and total monthly precipitation during the study period from June 2018 to January 2020. Data were obtained from the South African Weather Service (SAWS).

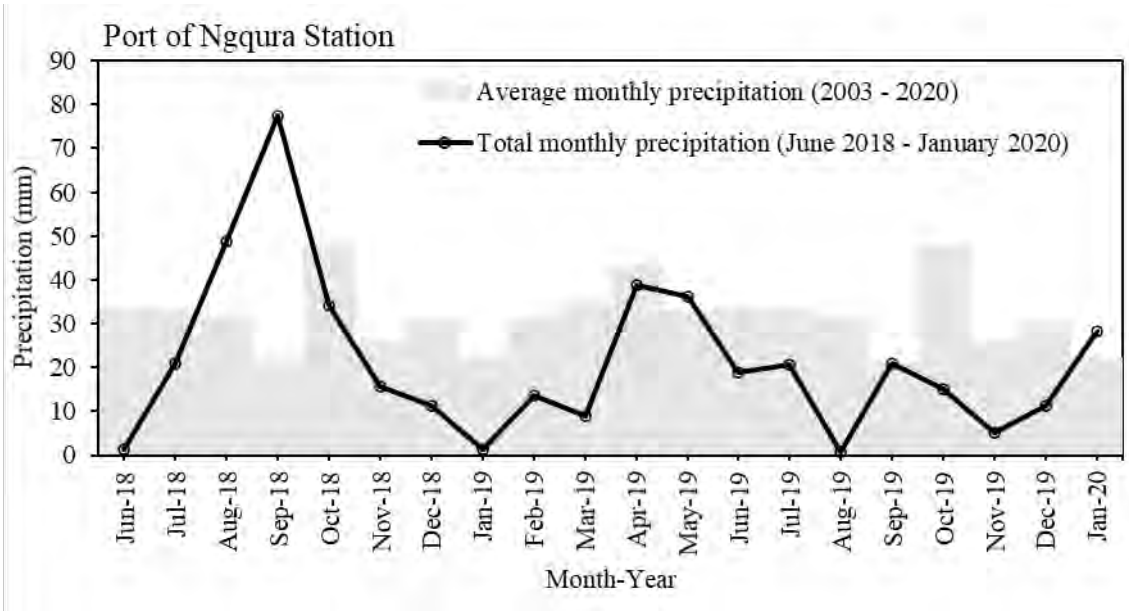

Figure 2.7: Average monthly precipitation (mm) at the Port of Ngqura Station (PNS) from 2003 to 2020 and total monthly precipitation during the study period from June 2018 to January 2020. Data were obtained from the South African Weather Service (SAWS)

Summary statistics for precipitation (mm) for data grouped by month and season at each station are presented in Table A.1. The highest average rainfall was recorded in September at both the PES (2.41 ± 6.44) and the PNS (1.64 ± 4.76), with rainfall in September 2018 higher than the monthly long-term average for this month (Table A.1). The lowest rainfalls were observed in April at the PES and in December in the PNS. In terms of seasonal patterns, rainfall was highest in autumn at both sites (Table A.1). This is atypical for this region in which spring usually contributes between 25 and 35% of the total annual rainfall (Mahlalela *et al.*, 2020).

Diplodus capensis as a model species

The Sparidae family, known as 'seabreams' or 'porgies', contains several species which are endemic to the South African coastline. More than one third of the world's Sparidae occur along the southern African coastline (Parenti, 2019). These species, although found in deeper offshore habitats (up to 450 m), are more commonly associated with shallow coastal, intertidal, and estuarine habitats (Beckley, 1985a; Smale and Buxton, 1989; Parenti, 2019). They are often found in large schools or smaller aggregations particularly in the juvenile stages, whereas the adults tend to be solitary (Parenti, 2019). The eggs and larvae of most sparid species are pelagic (Beckley, 1986; Pattrick, 2013), with juveniles recruiting into

settlement habitats, after which most species are territorial and maintain small home ranges (Watt-Pringle *et al.*, 2013).

Diplodus capensis, commonly known as 'cape white seabream' or 'blacktail', are small temperate to subtropical marine seabreams that are common residents in South African coastal waters (Smith and Heemstra, 2012). Like many other sparids, this species is considered slow-growing, long-lived and late to mature, reaching maximum sizes of ~45 cm fork length (approximately 3kg) (Mann and Buxton, 1997). They have a broad distribution that covers all the biogeographic regions of South Africa and extends to southern Mozambique on the east coast and Cape Point to the west (Mann, 1992, 2013). A genetically separated sub-species occurs on the western distribution from Namibia to southern Angola (Richardson, 2010).

Many Sparidae are targeted in the commercial, recreational, and small-scale hook and line-fisheries in South Africa (Potts *et al.*, 2020). *D. capensis*, are targeted in all three fishing sectors in South Africa (Pradervand, 2004; Richardson *et al.*, 2011). This species is commonly targeted by the shore-based angling fishery where it is ranked as the third most important fishery species in terms of catch (Brouwer and Buxton, 2002; Mann *et al.*, 2003; Watt-Pringle, 2009). Currently, this species is listed as 'least concern' on the IUCN Red List of Threatened Species; however, their stock status was last assessed in 2009 (Mann, 2013).

Due to their occurrence in dynamic coastal habitats and wide distribution, it is likely that this species is adapted to withstand high variability in environmental conditions. In rock pools and the lower reaches of estuaries especially, high variability in temperature, salinity and pH is common (Morris and Taylor, 1983; Huggett and Griffiths, 1986). Species, such as *D. capensis*, that occur in these habitats therefore require physiological tolerance to fluctuating environmental conditions throughout their development.

Although some research has been conducted on the response of juvenile and adult *D. capensis* to climate warming (Kemp, 2009; van der Walt, 2019), little is known about the response of the early life stages of this species to global changes. Potts *et al.* (2014) found that a sub-species of the *Diplodus sargus* complex shows reproductive sensitivity (i.e., reduced spawning) at high temperatures (>20°C), suggesting that this sub-species complex will be sensitive to ocean warming, and this was attributed to cold-water stenothermy of the early life stages. The larval stages of *D. sargus* (from hatching to three days post hatch) were

also found to be sensitive to ocean acidification with larvae raised in 7.8 pH treatments showing reduced hatching and growth rates as well as larger otoliths (Faria *et al.*, 2017). This highlights the need to identify the sensitivity of different processes and different life stages to various global related changes to infer true species sensitivity.

The distribution of *Diplodus capensis* in Algoa Bay

The eggs and larvae (hatchlings and preflexion stage) of the study species *D. capensis* have a pelagic distribution and occur inshore of the Agulhas Current across their distribution range (Connell, 2012). Within Algoa Bay, newly hatched and preflexion stage *D. capensis* larvae (~3mm and five days old) have been found in high numbers in the offshore areas of Algoa Bay (Pattrick, 2013). Spawning peaks appear to occur in spring and summer, but larvae of this species have been found year-round (Pattrick, 2013). Mid- to late-stage larvae recruit into coastal habitats and are typically associated with shallow subtidal bays and rocky shores, which appear to be a preferred nursery habitat for this species (Beckley, 1985a; Strydom, 2008). Flexion, postflexion and juvenile stage *D. capensis* have also been found to occur in high numbers in the lower reaches of the Sundays (Beckley, 1984) and Swartkops estuaries (Whitfield, 1999; Nel *et al.*, 2018; Kisten *et al.*, 2020), generally associated with vegetated habitats (Edworthy and Strydom, 2016; Nel *et al.*, 2018; Kisten *et al.*, 2020). This species has been identified as an estuarine opportunist (as defined by Potter *et al*. 2015), whereby they occur opportunistically in estuaries in high numbers but also utilize coastal marine waters as nursery areas. Adults (>150 mm) commonly associate with shallow rocky substrates and shallow reefs in the coastal surf zone (up to 40 m depth) and are highly resident (Attwood and Bennett, 1995; Cowley *et al.*, 2002). The life-history of this species, their value to local fisheries, coastal distribution and their accessibility in shallow coastal habitats during their early post-flexion stages makes them ideal model species for the research proposed in this thesis.

Table 2.2: Summary of studies that have recorded *Diplodus capensis* in the wild including size class, developmental stage and habitat association. Developmental stages are abbreviated as follows: Pr = preflexion, F = flexion, Po = postflexion, EJ = early juvenile, J = juvenile

Size class (mm)	Life stage	Habitat	Reference
2.1–15.0	Pr, F, Po	Surf zone	Lasiak, 1981; Rishworth *et al.*, 2015
2.5–9.9	Pr, F, Po	Nearshore	Pattrick and Strydom, 2008
10–134	Po, EJ, J	Estuary	Beckley, 1983b, 1984; Edworthy and Strydom, 2016; Nel *et al.*, 2018; Kisten *et al.*, 2020
21–89	EJ, J	Tidal rock pools	Beckley, 1985b

Chapter 3

Spatial and temporal variability in carbonate chemistry in Algoa Bay

Introduction

Concern about the impact of ocean acidification (OA) on marine organisms has resulted in an increase in carbonate chemistry monitoring in marine environments (Tilbrook *et al.*, 2019). These data are needed to establish global patterns of carbonate chemistry variability and rates of change, which inform projection modelling of future scenarios. Furthermore, understanding changes in carbonate chemistry is needed to understand the risk to ecologically and economically important marine species and resources (Vargas *et al.*, 2017).

Monitoring of carbonate chemistry in marine environments has largely been applied in the open ocean, with a focus on informing global climate projection models (Tanhua *et al.*, 2015). These data are collected during ship cruises, by long-term moorings or floats (Bates *et al.*, 2018; Bushinsky *et al.*, 2019). This 'climate' level monitoring has generally focused on modelling longer term changes over periods of years and over a macroscale (100s of km) (Bates *et al.*, 2018). As such, the current understanding of contemporary conditions and future acidification is relatively comprehensive for the open ocean on a global scale. It has become clear, however, that acidification does not occur uniformly across regions (Friedrich *et al.*, 2012) and marine habitats (Hofmann *et al.*, 2011; Waldbusser and Salisbury, 2014).

Coastal environments are known to be more dynamic and variable in nature and therefore likely to show different carbonate system dynamics compared to open oceans (Miller *et al.*, 2009; Duarte *et al.*, 2013). Since the major concern regarding OA is its impact on marine life, it is essential that monitoring efforts focus on coastal areas where most marine organisms occur and at the spatio-temporal scale relevant for biological response (short term variation; 'weather'). In coastal waters, carbonate chemistry equilibrium is collectively driven by both chemical and biological processes, which together with anthropogenic atmospheric inputs of CO_2 gas can result in very different scenarios compared to typically stable open ocean conditions (Provoost *et al.*, 2010; Hofmann *et al.*, 2011; Lowe *et al.*, 2019). These include riverine inflow (e.g., Vargas *et al*., 2016), upwelling (e.g. Saderne *et al*., 2013; Schulz *et al*., 2019), eutrophication (e.g*.,* Sunda and Cai, 2012), algal bloom events (e.g*.,* Hinga, 1992) and biological activity (e.g., Chan *et al*., 2016). Ultimately, these interacting processes determine

the carbonate chemistry conditions and acidification of coastal areas over temporal and spatial scales (Hendriks *et al.*, 2015; Cattano *et al.*, 2018).

Changes in carbonate chemistry equilibria, as indicated by an increase or decrease in pH, in coastal areas can occur over annual, seasonal, monthly, daily, or hourly scales (Hofmann *et al.*, 2011; Zongo and Schmitt, 2011; Carstensen and Duarte, 2019). In a review of trends in pH from 11 coastal regions between 1950 and 2016, Carstensen and Duarte (2019) found that ~55% (46 of the 83 sites) showed decreasing annual pH by up to -0.023 units per year, which exceeds the ~ -0.0004 to 0.0026 annual decrease predicted for open ocean (Bindoff *et al.*, 2019). Conversely, annual pH increased over time by up to 0.023 yr^{-1} in ~45 % of the sites, which led to the authors concluding that there is no clear directional trend in the pH of coastal ecosystems (Carstensen and Duarte, 2019). Over shorter time scales, pH was found to vary by up to 1.4 units seasonally and by up to 1.6 units inter-annually (Carstensen and Duarte, 2019). In areas influenced by high levels of auto- or heterotrophic biological activity, for example, near dense macroalgal aggregations or phytoplankton blooms, pH can vary over daily and hourly timeframes (Hendriks *et al.*, 2015).

In addition to large temporal variability, a mosaic of different carbonate chemistry conditions can occur over relatively small spatial scales in coastal areas (Hofmann *et al.*, 2011; Cattano *et al.*, 2018). For example, in regions that experience seasonal upwelling, cold bottom waters, which are typically more acidic (pH <7.8) than surface waters, are brought to the surface over areas of the continental shelf (Feely *et al.*, 2008; Hauri *et al.*, 2013). Biologically driven temporal and spatial variability is also common in shallow bays or estuaries that frequently experience algal bloom events or eutrophic conditions (Sunda and Cai, 2012). It has been reported that in areas of dense algal blooms, particularly when they are concentrated in shallow areas, pH may vary by up to ± 0.25–1.1 units over relatively small spatial scales (metres) (Sunda and Cai, 2012; Hendriks *et al.*, 2015). Habitats with dense macroalgal vegetation growth have also been shown to exhibit variability in pH, particularly over diurnal cycles (Middelboe and Hansen, 2007; Krause-Jensen and Duarte, 2016). Finally, freshwater inflow from rivers and groundwater seeps may also influence carbonate chemistry and pH in adjacent coastal areas (Feely *et al.*, 2009; Vargas *et al.*, 2016; Carstensen and Duarte, 2019).

Together with the spatial and temporal pH fluctuations in coastal waters, acidification from increasing atmospheric CO_2 concentrations continues to influence marine and coastal carbonate chemistry (Raven *et al.*, 2005; Doney *et al.*, 2009b, 2009a). The contributions of

these various coastal processes to carbonate chemistry variability can create positive and negative feedback loops to existing anthropogenic OA (Duarte *et al.*, 2013). However, it is likely that future increases in anthropogenic CO_2 emissions and resultant OA will ultimately further augment variability in coastal areas (Duarte *et al.*, 2013). This is because coastal processes, when interacting with increased uptake of CO_2 from the atmosphere may result in acidified conditions that far exceed the OA scenarios predicted by the atmospheric CO_2 uptake alone (Duarte *et al.*, 2013).

It is important to consider the required spatial and temporal resolution when monitoring carbonate chemistry to capture the variability required (IOC, 2019). 'Weather' quality OA data are defined by Newton *et al*. (2015) as "measurements of quality sufficient to identify relative spatial patterns and short-term variation, particularly in nearshore regions where variability is higher". These data are suitable to interpret the ecosystem responses to OA. These 'weather' quality data differ to the 'climate' level data that are applied to projection modelling, which are used to detect long-term trends in OA (Newton *et al.* 2015). In coastal areas, more data points are required because these areas experience higher temporal and spatial variability in carbonate chemistry compared to the open ocean (IOC, 2019). Surprisingly, many studies assessing the impacts of climate change and OA on coastal organisms have focused on larger-scale averages, which are not appropriate when variability is high over much smaller temporal and spatial scales (Bates *et al.*, 2018). Identifying the pH extremes, as well as the temporal and spatial scales of variability is necessary when assessing the impact of pH and associated parameters on organisms inhabiting these variable environments. Capturing local variability, however, is often hindered by practical limitations such as time, weather, and equipment resources.

The overall aim of this chapter was to produce a baseline overview of the carbonate chemistry processes occurring in Algoa Bay, a complex and productive coastal embayment. This was done to identify temporal and spatial variability in pH experienced by coastal organisms in the bay, in order to inform the laboratory perturbation experiments in chapters 4 and 5. Since carbonate chemistry dynamics have not previously been quantified and described in this region, a second aim of this chapter was to identify the potential drivers of pH variability in this coastal area in order to better understand these processes. The sampling design aimed to capture at least some of the pH variability in the bay over broad spatial

(~10 km) and temporal (monthly, and half-hourly for 24 hours) scales within the practical sampling and analysis constraints.

Methods

Discrete offshore and inshore monthly sampling

Carbonate chemistry and associated physico-chemical parameters (temperature, salinity, and dissolved oxygen) were monitored monthly over a total period of ~20 months (June 2018 – January 2020) at eight offshore (O1–O8) and five inshore (I1–I5) sites in Algoa Bay, South Africa (Fig 2.1). The offshore sites were defined as areas with a depth of >10 m and inshore sites were in the surf zones of coastal intertidal areas at a depth of <1 m. The offshore sites were accessed by boat and the inshore sites were accessed from the land by wading into the surf.

Offshore sampling trips were purely weather dependent and were generally conducted during a good weather period with little wind or swell. The offshore sites were always sampled during the day in the same order (starting with site O8 in the morning and completing site O1 by late afternoon). The inshore sampling was also always conducted during the day and during a spring tide period. Sampling was always started at the peak of the first low tide for the day and sites were also sampled in the same order starting from site I5 to site I1. The sampling sites were selected to correspond with the South African Environmental Observation Network's (SAEON) existing long-term ecological research (LTER) monitoring program in Algoa Bay. The selected sites represented areas exhibiting typical coastal processes, such as seasonal upwelling, riverine and groundwater inflow (near the mouths of the Sundays and Swartkops estuaries and the Alexandria dune field) and biological processes such as algal blooms (eastern coastline) and macroalgal reef stands (Cape Recife).

Discrete measurements of carbonate chemistry parameters were obtained by collecting a surface water sample at each offshore and inshore site using the standard operating procedure (SOP 1) outlined in the "Best Practice Guide for Ocean Acidification Research and Monitoring" (Dickson *et al.*, 2007). Care was taken to ensure minimal air exposure when collecting seawater samples in sterilized 500 mL boro-silicate glass bottles (Pyrex) with ground glass stoppers. At offshore sites, water was collected from the surface using a manually deployed Niskin bottle and sample bottles were filled with the Tygon © tube directly from the Niskin bottle. At inshore sites, samples were collected by submerging the

sample bottle containing a Tygon© air outlet pipe to minimize bubbling. Sample bottles were rinsed at least three times before filling with the sample. Once collected, seawater samples were poisoned with 200 µl saturated mercury (II) chloride solution, sealed with Apezion© grease and the stoppers were secured with a rubber band, after which they were stored in a dark container until later analysis. Measurements of pH and total alkalinity (T_A) were conducted in the laboratory within 24 hours of sample collection, where possible.

The pH of each sample was measured (Dickson *et al*., 2007, SOP 6b) using a double-beam spectrophotometer (Hiatchi UH5300) with 1 x 1 cm glass cuvettes and metacresol purple (mCP) (ACROS Organics) indicator dye solution (2 mmol dm^{-3}) corrected to a pH of 8.0 by adding NaOH (1 M). 50 µl of mCP solution was added to the spectrophotometer cell containing the seawater sample and the cuvettes were sealed with a teflon cap with no headspace. Absorbance (λ) was measured at wavelengths of 434 and 578 nm, which are the absorbance maxima for the acid and base forms of mCP. Three measurements per sample were taken and absorbance values were corrected with the blank measurements. The pH was then calculated using the following equation (Dickson *et al*., 2007):

$$pH = pK_2 + log_{10}\left(\frac{\frac{A_1}{A_2} - \varepsilon_1(HI^-)/\varepsilon_2(HI^-)}{\frac{\epsilon_1(I^{-2})}{\epsilon_2(HI^-)} - (A_1/A_2)\,\epsilon_2\,(I^{2-})/\epsilon_2\,(HI^-)}\right)$$

where pK_2 is the acid dissociation constant for the species HI^- (mol kg-$soln^{-1}$), A_1 and A_2 are the corrected absorbances measured at the wavelengths corresponding to the absorbance maxima of the acid and base forms. The remaining parameters are the extinction coefficient ratios for m-cresol purple at the specified wavelengths.

Total alkalinity (T_A, µmol kg^{-1}) was measured using an open cell potentiometric GRAN titration (Dickson *et al*., 2007, SOP 3b). A manual titration was performed on 50 mL of the seawater sample in a jacketed beaker (for temperature control) containing a magnetic stir bar. An initial dose of 1.2 mL of hydrochloric acid (HCl) alkalinity titrant (Scripps Institute for Oceanography, corrected to a salinity of 35 for seawater with sodium hydroxide (NaOH)) was added to the sample to reduce the pH to approximately 3.5–4.0. The sample was then left for 10 minutes with the magnetic stirrer on 500 rpm to allow the CO_2 to degas. Following this, the titration was continued by adding HCl in 0.5 mL increments until a pH of

approximately 3.0 was reached. Following each addition of HCl, a calibrated glass pH electrode (Metrohm) was used to measure the emf to 0.0001 V by means of a digital voltmeter (Keysight U3402A dual display meter). A non-linear least-squares approach using titrant volume and change in e.m.f. was used to calculate T_A (μmol kg^{-1}).

Supplemental physico-chemical metadata, including temperature (°C), salinity and dissolved oxygen, were obtained from the surface measurements (<1 m depth) of a CTD cast that took place at each offshore site. At the inshore sites, temperature and salinity measurements were collected manually using a digital thermometer and refractometer.

The concentrations of minor nutrient species, silicate, and phosphate were determined to account for their contribution to alkalinity and ultimately acid-base processes in the offshore sites only. Concentrations of silicates and phosphates (μmol L^{-1}) were determined from surface samples collected at each site (Sites O1–O8) on the monthly sampling trips. Samples of seawater were collected on board the sampling vessel and gravity filtered through 0.45 μm Whatman (GF/C) glass fibre filter. A sample of the filtered water was stored frozen (at -30°C) in 30 mL acid washed urine jars. Prior to analysis, samples were thawed at room temperature and shaken to minimize any concentration gradients. The samples were then prepared for nutrient analysis by an auto-analyser (Multitest MT19, Seal Analytical), which uses automated spectrochemical colorimetric methods to determine phosphate (Grasshoff *et al*., 1983) and silicate concentrations (Murphy and Riley, 1962).

Non-measured carbonate speciation parameters pCO_2, as well as aragonite (Ω Ar) and calcite (Ω Ca) saturation states were calculated using the software CO_2SYS (Lewis and Wallace, 1998) and using the measured pH and T_A as input parameters. Temperature, salinity, silicate and phosphate concentration were included in the calculations as supplemental data which influence carbonate speciation. The calculations used equilibrium constants from Mehrbach *et al*. (1973), refit by Dickson and Millero (1987) and KSO_4 from Dickson (1990).

Biological sampling

Chlorophyll-*a* concentration (μg L^{-1}) was determined from surface samples from both offshore and inshore sites to identify the contribution of biological activity to pH variability. At each site, a sample of 500 mL seawater was collected from the surface and gravity filtered through a 0.45 μm Whatman (GF/C) glass fibre filter on board the sampling vessel. The filter

papers were stored in tinfoil and frozen until later extraction. Prior to analysis, the samples were extracted in 95 % ethanol after 24 hours in a refrigerator. The samples were re-filtered and prepared for measurement in 10 cm glass cuvettes on a double beam spectrophotometer (Hiatchi UH5300). Light absorbance was measured on the samples before and after the addition of three drops of 0.1 N HCl. Chlorophyll-*a* concentration was then calculated using the following equation based on the methods of Nusch (1980): $\text{Chlorophyll-}a\text{ concentration } (\mu g\ L^{-1}) = (E_{b665} - E_{a665}) \times 29.6 \times (v/(V \times I))$ where: E_{b665} is the absorbance at 665 nm before acidification; E_{a665} is the absorbance at 665 nm after acidification with 0.1 N HCl; v is the volume of solvent used for extraction (mL); V is the volume of sample filtered (L); I is the path length of the cuvette (cm); and 26.6 is a constant calculated from the maximum acid ratio (1.7) and the absorption coefficient of chlorophyll-*a* in ethanol (82 g L^{-1} cm^3).

Diurnal pH measurements

During the spring/summer sampling period (October – November 2019), 24-hour variability in pH was assessed near coastal Site I1 (Cape Recife). An iSAMI-pH logger (Sunburst Sensors) was deployed at the site for a total period of five days. The iSAMI-pH logger was housed in a custom-built stainless-steel cage secured onto two steel railways and deployed in a hollow of a deep (1 ± 0.8 m) gully (Fig 3.1). The gully received consistent free flow of seawater during all tidal cycles. The deployment and retrieval of the iSAMI-pH logger took place during low tide during a neap tide cycle so that it remained submerged throughout the deployment period.

Figure 3.1: Map of the location of the iSAMI-pH deployment site at Cape Recife (34°01'36.9"S 25°42'03.2"E) and image of the iSAMI-pH logger housed in the stainless-steel cage

The iSAMI-pH logger was set to measure pH every 30 minutes. The logger measures pH to the total scale using a spectrophotometric method, whereby a sample of seawater is drawn through an inlet tube, and pre-calibrated quantities of indicator dye are added in order to measure the pH of the sample using the spectrophotometric method.

An index for potential upwelling events

Hourly, *in situ* sea temperature data were sourced from the South African Environmental Observation Network's (SAEON) continuous monitoring program (CMP) database. These data were collected using temperature loggers (Onset HOBO Water Temperature Pro v2 data loggers) attached to an array of seven strings of underwater temperature recorders (UTRs), which are permanently moored at each of the offshore sites O2–O8, with the exception of Site O1. Measurements of sea temperature (at 10 m depth) were collected hourly from the period of June 2018 until January 2020. Hourly temperature measurements for each day, at each site, were pooled to get an average daily temperature for the calculation of upwelling indices.

An index for the intensity and duration of potential local upwelling events was estimated based on the methods used by Tapia *et al*. (2009) and later by Duncan *et al*. (2019). A 30-day moving average was fitted through the daily average sea temperature time-series to identify a baseline temperature estimate for each day. The difference between the 30-day moving average baseline and observed sea temperature recordings was used to calculate a daily temperature anomaly (moving average less actual daily sea temperature) (Fig A.3 in appendix). Only temperature anomalies of less than -2°C were used to identify potential upwelling events during the time-series to discount any diurnal or random temperature variability that may not be associated with true upwelling. Integrated anomaly (IA) events were calculated from the area between the anomaly curve and the lowest temperature during a potential upwelling event (Fig A.3). The IA value is an index that signifies the duration and intensity of the upwelling event (Tapia *et al.*, 2009). An IA value was calculated from the hourly temperature measurements for the two days preceding, the day of, and two days following the monthly offshore pH sampling events.

Statistical analyses

Statistical tests, graphs and plots were conducted and produced in Microsoft Office Excel 2013, R Studio and ArcMap (version 10.1) (geospatial processing program by ArcGIS ® by ESRI).

Temperature and pH data produced by the iSAMI-pH sensor, during the short-term, high frequency pH measurements were correlated (Pearson correlation) to identify whether diurnal

variability in temperature drives pH variability. Pairwise correlations (Pearson's correlation) were conducted with all the monthly measured and calculated parameters including temperature, salinity, DO, pH, phosphate, silicate, pCO_2, Ω Ca, ΩAr and Chlorophyll-*a*.

Principal Component Analysis (PCA) in R using the package 'factoextra' (Kassambara and Mundt, 2017) was used to visualize the major environmental parameters responsible for spatial and temporal differences during the sampling period. Monthly measured parameters of temperature, pH, DO, TA and Chlorophyll-*a* were included in the PCA analysis and were ordinated by site and month. pCO_2 was excluded from the analysis due to its strong correlation with pH. All environmental data were standardized prior to the PCA analysis to standardize the weight of the measurement units.

Distance based linear modelling (DISTLM) of the relationship between pH and predictor variables including temperature, TA, DO and chlorophyll-*a* concentration was explored using the DISTLM procedure in Primer 7. The model was constructed based on a Euclidean distance similarity matrix for pH and normalized predictor variables. An average contribution was estimated for missing values. Basic data diagnostics such as distribution of residuals and identification of outliers were conducted to test for multicollinearity to ensure the suitability of the data for PCA analysis and DISTLM.

The calculated upwelling index, integrated anomaly (IA) values were correlated (Pearson correlation) with pH to identify whether upwelling drives pH variability in Algoa Bay.

Results

The dataset produced from the thirteen offshore and inshore monthly time-series stations consists of ~20 months of measured carbonate chemistry data from a total of 129 offshore samples and 69 inshore samples for the period of June (offshore) and August (inshore) 2018 to January 2020. Missing data corresponds to those months where sampling was not possible due to adverse weather conditions or other reasons.

Spatial variability

Among the offshore sites, temperatures were generally warmer at the sites closer to shore (O1–O5).The lowest average temperatures were recorded at site O7 at the mouth of the bay and sites O6 and O8 at the eastern and western boundaries of the bay. Among the inshore

sites the highest average temperature was recorded at site I1 at Cape Recife on the western side of the bay decreasing in an easterly direction along the coastline towards the eastern side of the bay (Fig 3.2). Warmer average temperatures were recorded at the inshore sites (I1–I5) than at the offshore sites (O1–O8), and average surface temperature generally decreased when moving offshore (Fig 3.2).

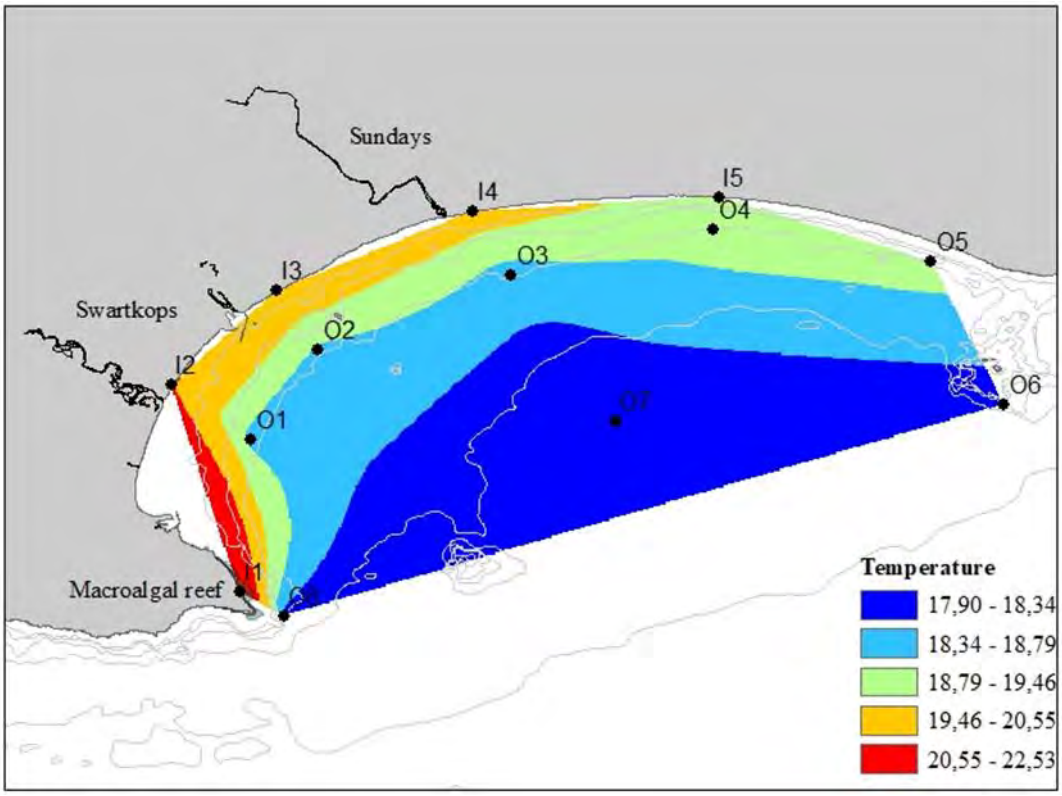

Figure 3.2: Average surface temperature (°C) among the offshore (O1–O8) and inshore (I1–I5) sites in Algoa Bay from the period of June/August 2018 – January 2020

The overall spatial difference in average pH recorded among all the sites (offshore and inshore) was 0.34 units and the total pH range was 0.69 units. At the offshore sites (O1–O8), the average pH was 8.03 ± 0.07 SD, and the range was 0.55 units. The highest average pH among the offshore sites was recorded at Site O2 (8.06 ± 0.08) and the lowest at Site O6 (8.00 ± 0.04) (Fig 3.3a, Table A.2). The sites showing the highest pH variability were sites O2, O3, O5 and O8 (Fig 3.3b). The highest variability in pH throughout amongst inshore and offshore sites was recorded at Site O5 (range = 0.45) and Site O8 (range = 0.42) (Table A.2). At the inshore sites, the average pH was 8.04 ± 0.15 SD, and the range was 0.61 pH units. Similar average pH levels (7.96–7.99) were recorded at inshore sites I2 to I5. In contrast, consistently higher pH values were recorded at Site I1 at Cape Recife (8.30 ± 0.08) (Fig

3.3a,b). The inshore sites showing the highest variability were sites I1, I4 and I5 (0.31–0.35) (Fig 3.3b). A decline in pH was observed when moving in an eastward direction from Site 1 along the coastline of Algoa Bay (Fig 3.3).

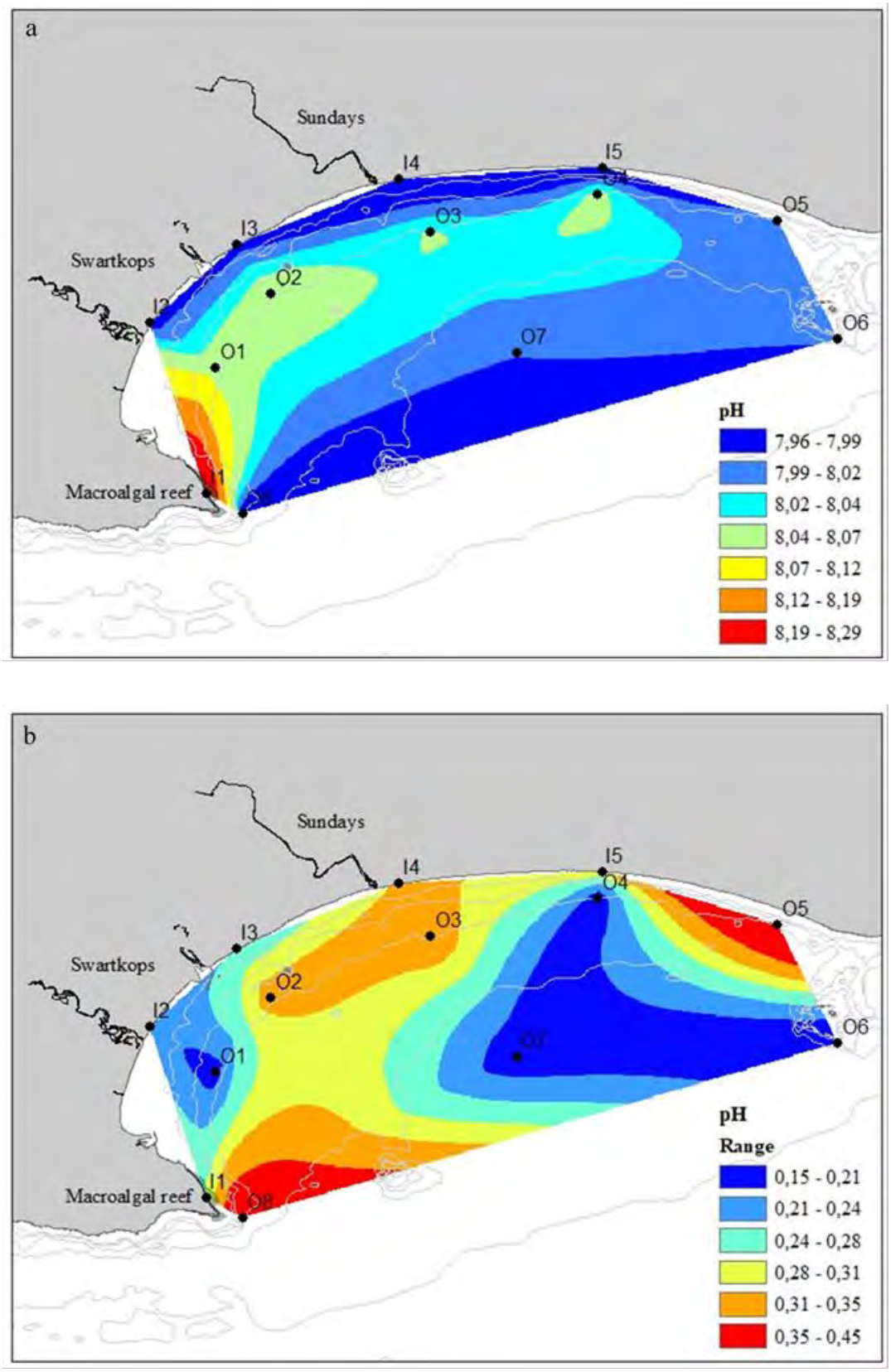

Figure 3.3: a) Average spatial pH and b) pH variability (range) among the offshore (O1–O8) and inshore (I1–I5) sites in Algoa Bay from the period of June/August 2018 – January 2020

Among the offshore sites, the lowest average chlorophyll-*a* concentration was recorded at Site O8 (2.9 ± 4.7) on the western boundary of the bay and the highest was recorded at Site O3 (5.0 ± 7.9). The offshore sites closer to the shore (O1–O5) showed higher average chlorophyll-*a* concentrations than the sites at the eastern and western boundaries (sites O6 and O8), as well as the centre of the bay (site O7) (Fig 3.4). Spatial variability among the inshore sites showed that the eastern boundary of the bay (sites I4 and I5) had higher average chlorophyll-*a* concentrations (> 9.41 µg L^{-1}) than the western boundary of the bay (sites I1 and I2) (< 9.41 µg L^{-1}) (Fig 3.4). The highest range in chlorophyll-*a* concentration was recorded at Site I5 (range = 52.15). Average chlorophyll-*a* concentrations decreased when moving offshore (Fig 3.4). Table A.3 in the appendix compares the summary statistics of the offshore and inshore sites.

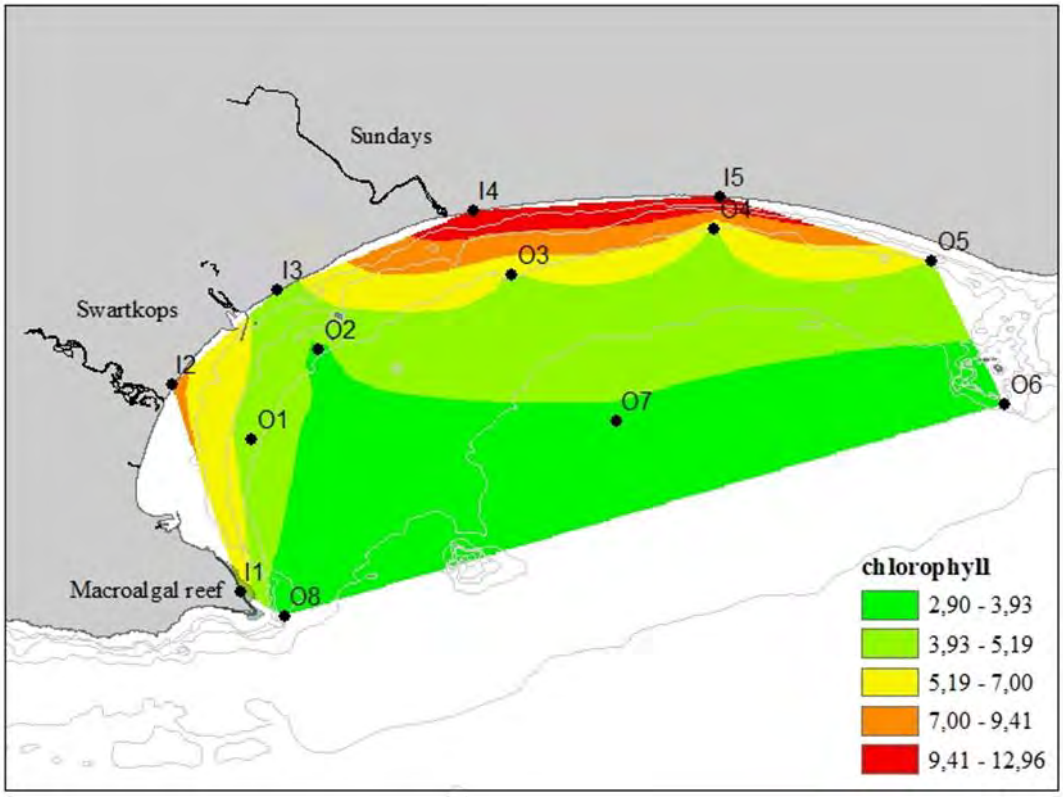

Figure 3.4: Spatial variability in chlorophyll-*a* concentration (µg L^{-1}) among the offshore (O1–O8) and inshore sites (I1–I5) in Algoa Bay from the period of June/August 2018 – January 2020

Monthly temporal variability

Offshore sites

Temperature, pH, pCO_2 and TA data from ~129 offshore observations over the 20-month sampling period are plotted in Fig 3.5.

At the offshore sites, temperature varied from the lowest average of 16.47°C in August 2018 to the highest average of 21.20°C during December 2020 (Fig 3.5 a). Average temperatures were higher in the summer and autumn months and were lower during the winter and spring months (Fig 3.5 a). Temperature varied more between October and March 2019 than any other time during the sampling period (Fig 3.5 a).

The pH at the offshore sites varied between an absolute minimum (lowest recorded value) of 7.72 and a maximum (highest recorded value) of 8.27 showing a total range in pH of 0.55 pH units throughout the sampling period. The overall average pH was 8.03 ± 0.07. The lowest average pH was 7.96 recorded during July 2018 and the highest average pH was 8.14 recorded during March 2019 (Fig 3.2 b). Similar to temperature, pH varied most during the period of October 2018 and March 2019 (Fig 3.2 b). During this period, average pH varied by up to 0.12 pH units, whereas during the remaining months of the study variability did not exceed 0.06 pH units.

The pCO_2 showed similar variability to pH (Fig 3.5 c). The lowest average pCO_2 (309 µatm) was recorded in March 2019 which, as expected, coincided with the highest pH. Conversely, the highest average pCO_2 (508 µatm) was recorded in July 2018 coinciding with the lowest recorded pH.

Total alkalinity did not appear to show any distinct pattern of variability throughout the sampling period at the offshore sites. Higher average TA was recorded at the beginning of the sampling period from June to August 2018, after which it declined and remained relatively stable from November 2018 to January 2020 (Fig 3.5 d). The highest TA was recorded during June 2018, which coincided with the highest recorded pCO_2 of 952 µatm (Fig 3.5 d).

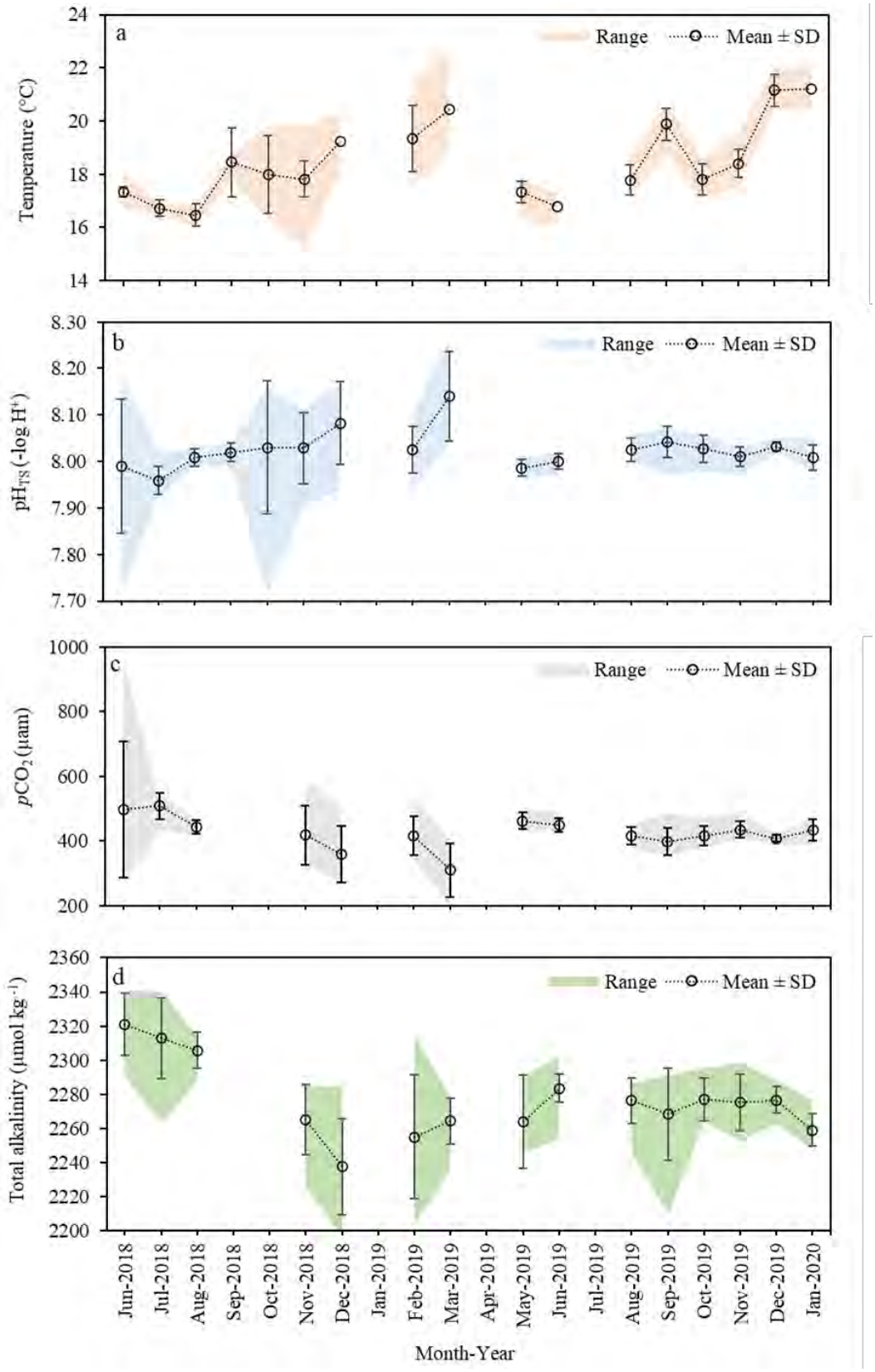

Figure 3.5: Monthly variability (mean ± SD) in: a) temperature (°C); b) pH_{TS} (-log H^+, spectrophotometer); c) pCO_2 (µatm); and d) TA (µmol kg^{-1}); at offshore sites (O1–O8) in Algoa Bay from June 2018 – January 2020

Dissolved oxygen and chlorophyll-*a* concentration were variable over the 20-month sampling period at the offshore sites (Fig 3.6 a, b). Dissolved oxygen was most variable during December 2018 and March 2019 (Fig 3.6 a). The lowest average DO concentration of 4.50 mg L^{-1} was recorded during August 2019. Dissolved oxygen did not reach hypoxic levels (< 2 mg L^{-1}) at any stage during the study period.

There was a peak in chlorophyll-*a* concentration during March and May 2019 (Fig 3.6 b). During these months, chlorophyll-*a* concentration reached average levels of 14.6 and 9.6 µg L^{-1}, and maximum levels were recorded in May 2019 reaching 34.2 and 28.1 µg L^{-1} at site O3 and O4, respectively. These recorded concentrations were much higher than those recorded during the other months of the year which typically ranged between ~0.00 and 10.0 µg L^{-1}. The highest average chlorophyll-*a* concentration was recorded during March 2019, and this coincided with the highest recorded average pH levels.

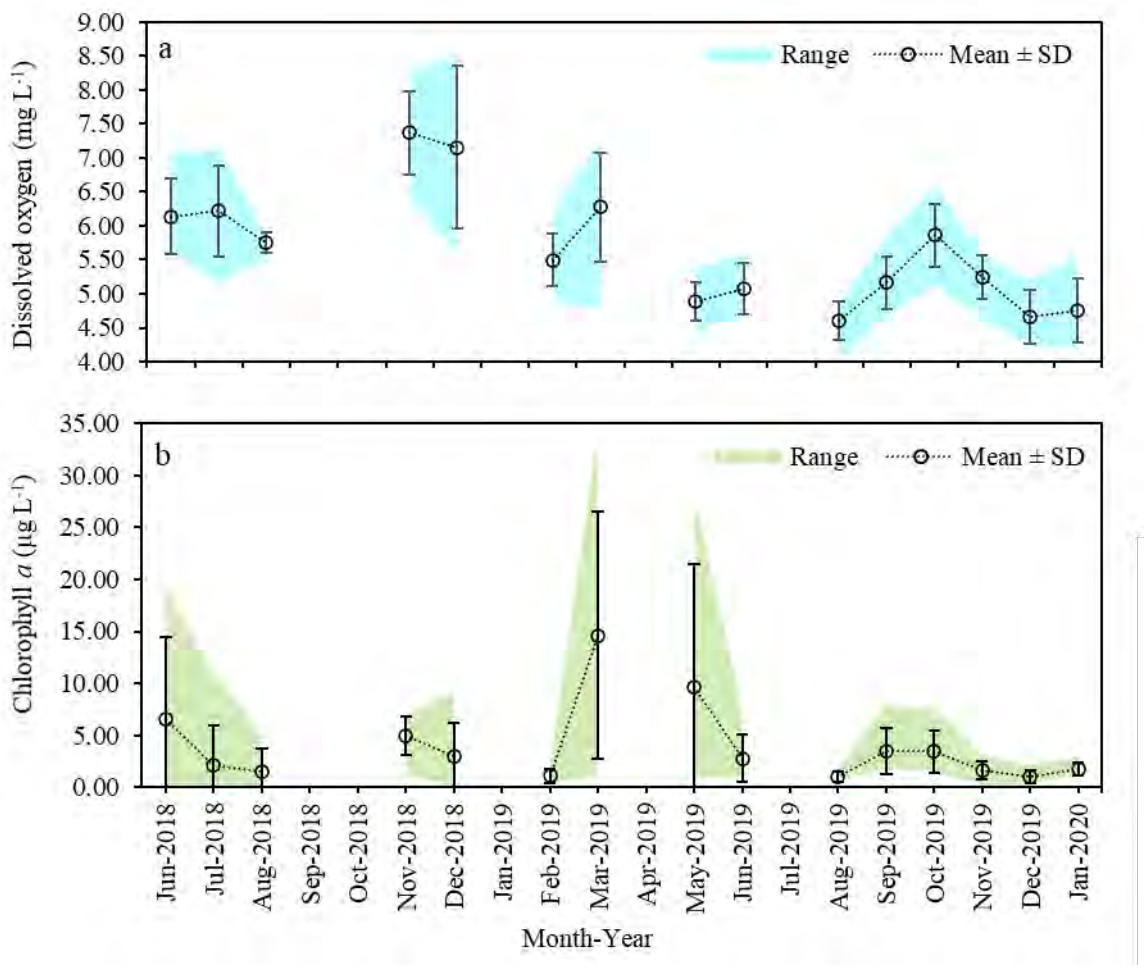

Figure 3.6: Monthly variability in a) dissolved oxygen (mg L^{-1}) and b) chlorophyll-*a* concentration (µg L^{-1}) (mean ± SD) at offshore sites (O1–O8) in Algoa Bay from June 2018 – January 2020

Inshore sites

Temperature, pH, pCO_2 and TA data from ~69 inshore observations over the 18-month sampling period are plotted in Fig 3.7. Temperature, pH and pCO_2 showed high variability at inshore sites throughout the sampling period (Fig 3.7 a, b, c). The inshore sites generally showed higher variability in temperature, pH and pCO_2 than the offshore sites (see Table A.2 in the appendix for a more detailed comparison).

At the inshore sites, temperature varied from the lowest average of 17.1°C in June 2019 and highest average of 23.6°C during January 2020 (Fig 3.7 a). Temperature showed similar seasonal patterns as described for the offshore sites, with higher temperatures being recorded in the typically summer and autumn months and lower temperatures in the winter and spring

months (Fig 3.7 a). The highest average temperatures were recorded in January 2019 (23.60°C) and January 2020 (21.82°C) (Fig 3.7 a).

The pH varied between an absolute minimum (lowest recorded value) of 7.80 and a maximum (highest recorded value) of 8.41 showing a total range in pH of 0.61 pH units throughout the sampling period. The overall average pH for the inshore sites was 8.04 ± 0.15. The inshore sites showed slightly higher average pH levels than offshore sites (see Table A.2). The lowest average monthly pH at inshore sites was recorded during January 2020 (7.95) and the highest average pH occurred during March 2019 (8.16) (Fig 3.7 b).

The pCO_2 showed similar variability to pH during the sampling period (Fig 3.7 c) with the lowest pCO_2 levels recorded (278.4 μatm) coinciding with the highest pH (March 2019).

Total Alkalinity appeared to be less variable at the inshore sites than the offshore sites (See Table A.2). TA did not appear to change significantly during the sampling period except for an outlier (higher than average TA) in August 2018 (Fig 3.7 d), which may be ascribed to measurement error.

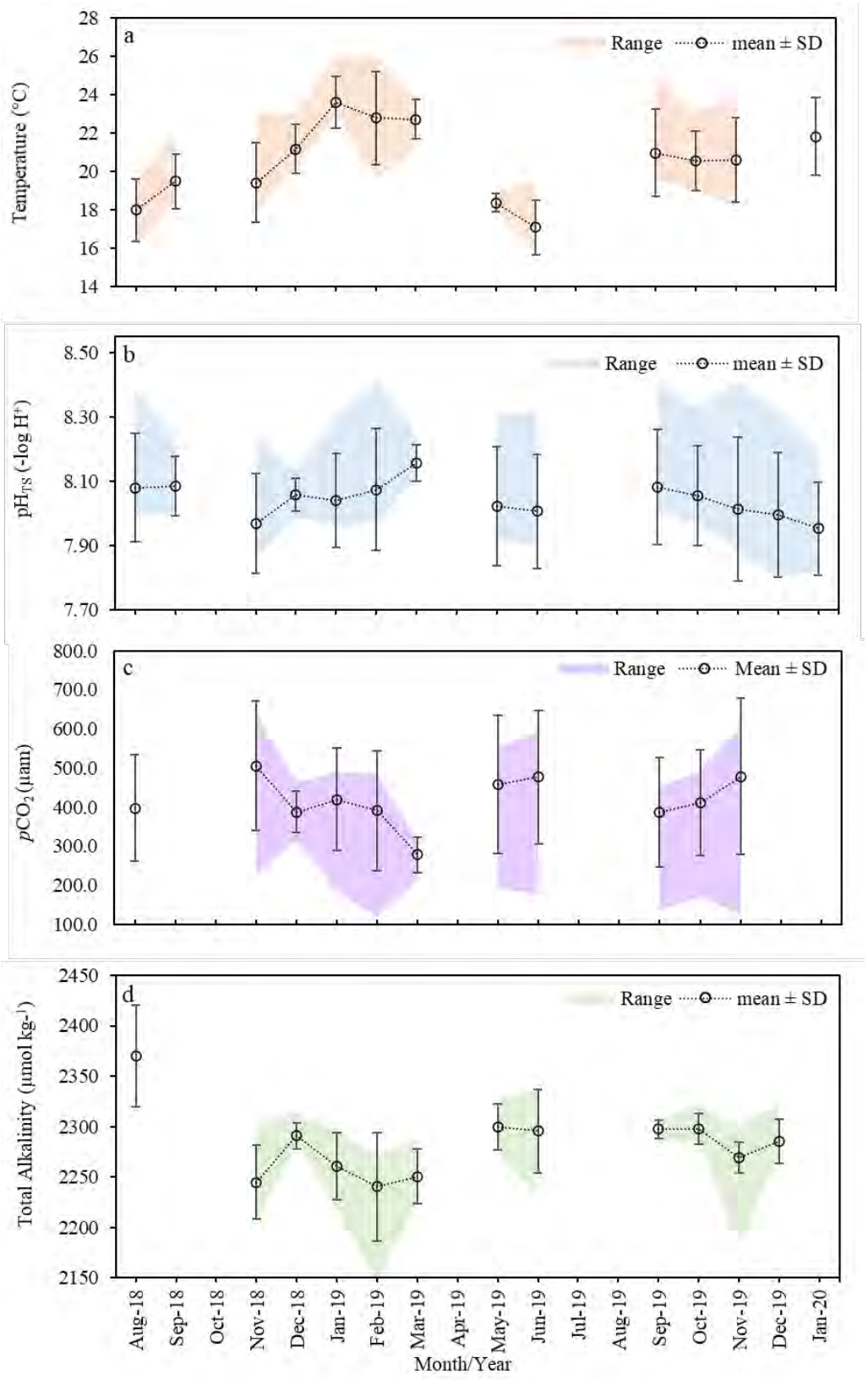

Figure 3.7: Monthly variability in a) temperature (°C) b) pH_{TS} (-log H^+, spectrophotometer), c) pCO_2 (µatm) and d) total alkalinity (µmol kg^{-1}) among the inshore sites (I1–I5) in Algoa Bay from August 2018 – January 2020

Chlorophyll-*a* concentration was variable during the sampling period at the inshore sites (Fig 3.8). There was a peak in chlorophyll-*a* concentration during September 2018 during which the highest average chlorophyll-*a* concentration (14.15 ± 23.22) as well as the largest range (54.17) was recorded (Fig 3.8). The high average and range in chlorophyll-*a* concentration during this month was attributed to a high chlorophyll-*a* reading of 55.57 µg L^{-1} at site I5. Variability in chlorophyll-*a* concentration was higher in the typical summer and spring months. There was less variability during the winter months of May and June 2019. Dissolved oxygen concentrations were not measured at the inshore sites.

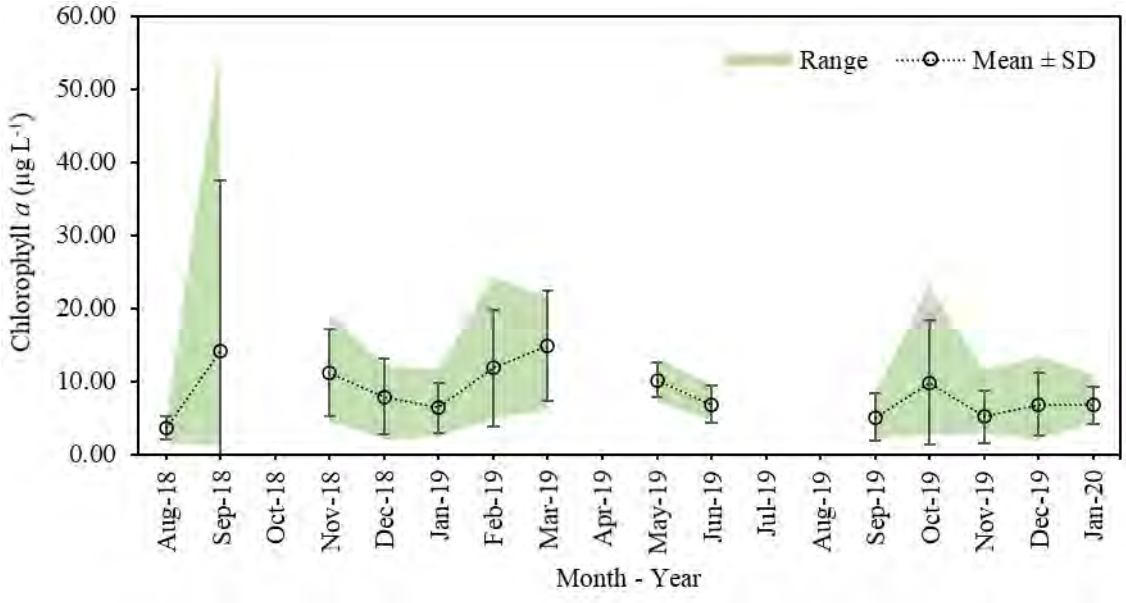

Figure 3.8: Monthly variability in chlorophyll-*a* concentration (µg L^{-1}) (mean ± SD) at inshore sites in Algoa Bay from August 2018 – January 2020

Diurnal pH and temperature variability

The half hourly measurements of pH over the five-day sampling period resulted in a total number of 180 pH readings over 90 hours. The average pH measured during this period was 8.07 ± 0.16). A cyclical pattern in pH occurred over the five-day sampling period (Fig 3.9). The pH rose consistently each morning to peak daily around midday after which it declined in the afternoon to reach a low around midnight. A maximum pH of 8.35 and a minimum of 7.79 was recorded during the five-day sampling period. This resulted in a 24-hour range of 0.56 units. These maximum and minimum readings were recorded during the day and night, respectively. The average daytime pH was 8.13 and night-time pH was 7.98. Temperature showed similar variability to pH, with cyclical increases in temperature during the day and lower temperatures at night (Fig 3.9).

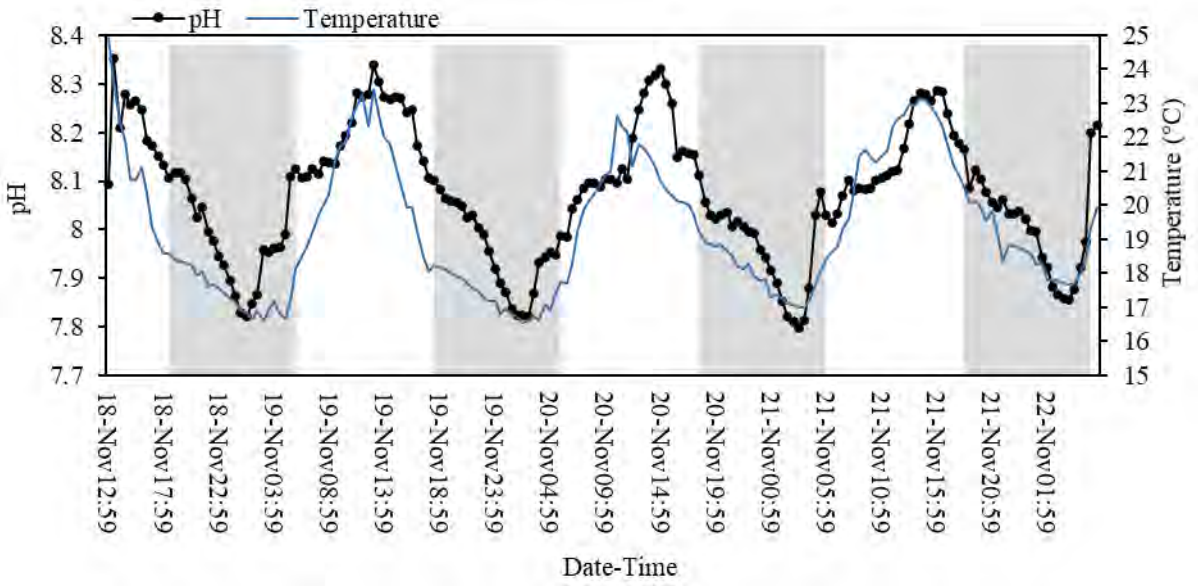

Figure 3.9: Variability in pH and temperature (°C) over 24 hours over a period of five days (18–22 November 2019) at the site near Cape Recife. Night-time measurements are indicated by the grey area.

Temperature (°C) and pH from the half-hourly iSAMI measurements showed a significant positive correlation (r = 0.83, P <0.0001) (Fig 3.10).

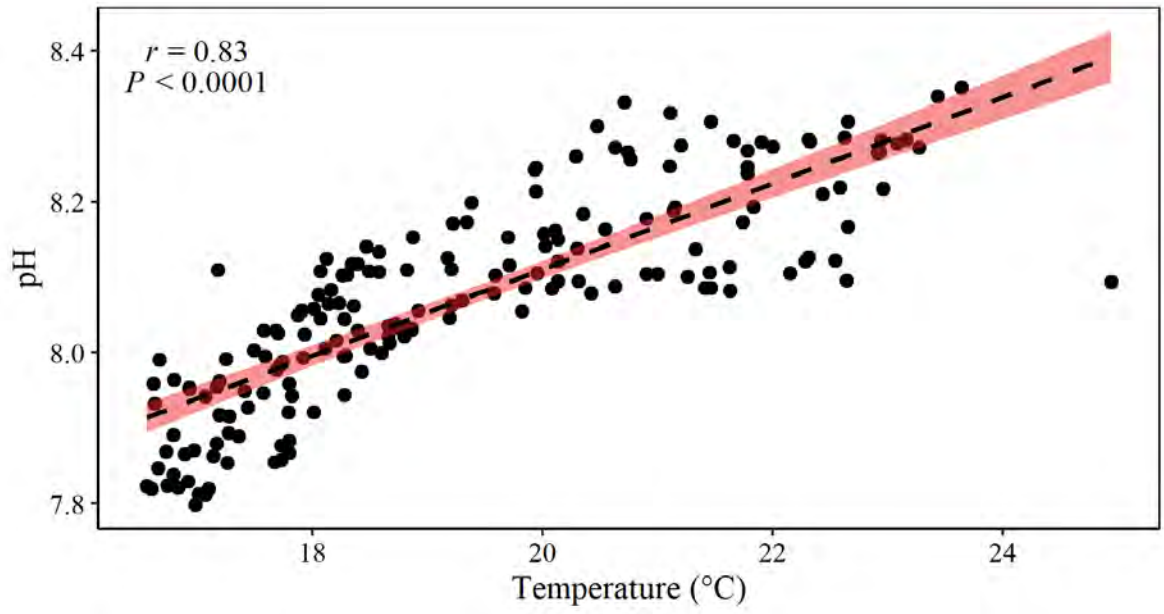

Figure 3.10: Linear correlation between half-hourly measurements of temperature (°C) and pH during the five-day iSAMI-pH sensor deployment at the site near Cape Recife. The 95% confidence interval is indicated by the red area

Correlations and multivariate analyses

Offshore sites

Results of the pairwise correlation analyses (Pearson's correlation) are presented in the correlation matrix in Fig 3.11. As expected, the measured and calculated carbonate speciation parameters (pH, pCO_2, Ω Ar and Ω Ca) were all significantly correlated (Fig 3.11). pH showed a significant positive correlation with Ω Ar and Ω Ca (R = 0.94 and 0.95) and a negative correlation with pCO_2 (R = - 0.97). These parameters, together with pH also showed a strong significant correlation with temperature. Other significant, but weaker, positive correlations occurred between pH and DO (R = 0.29) and pH and chlorophyll-*a* concentration (R = 0.28) (Fig 3.11). There was also a strong positive correlation between chlorophyll-*a* concentration and Ω Ca (R = 0.309) and Ω Ar (R = 0.306) (Fig 3.11).

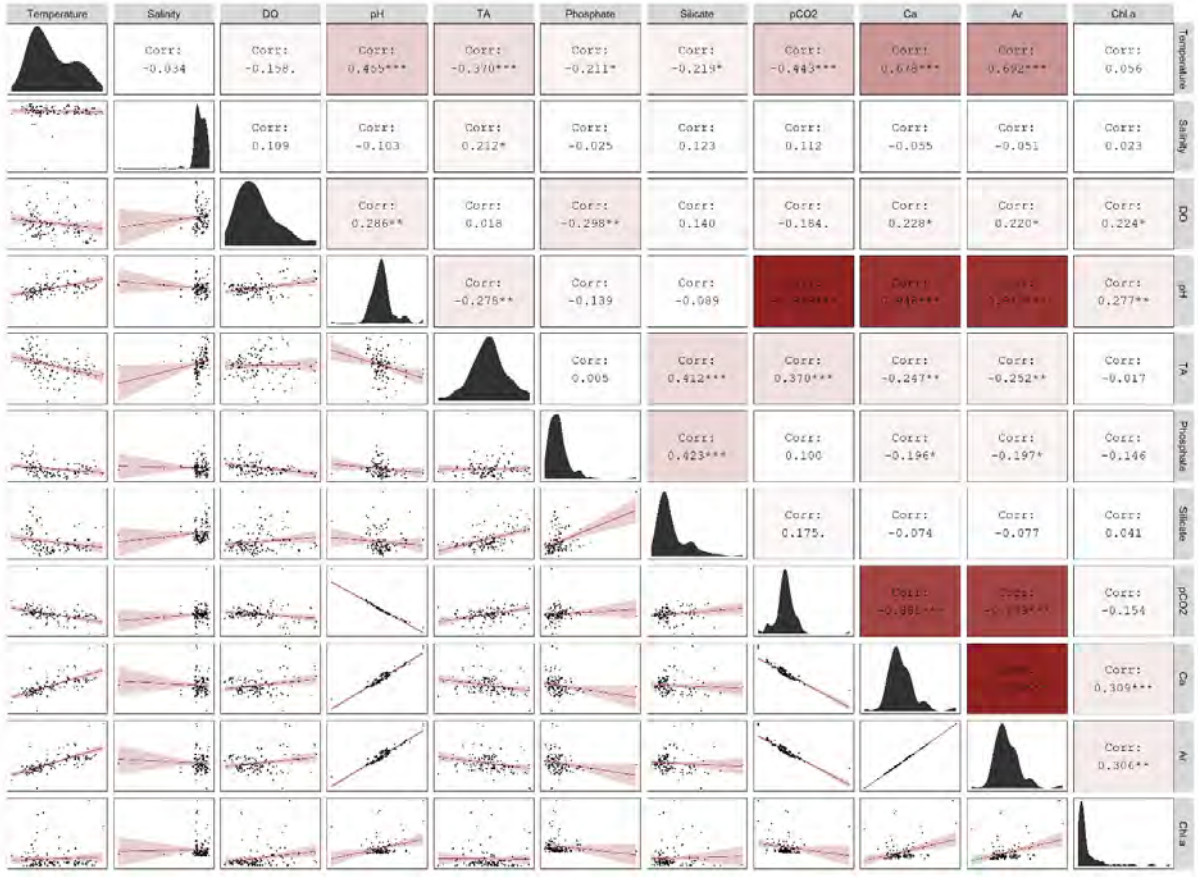

Figure 3.11: Correlation matrix presenting the linear relationships with correlation coefficients (R, Pearson's method) for pairwise combinations of all the parameters measured at the offshore sites. The strength of the significance of the relationship between parameters is indicated by the number of * with *** indicating a highly significant relationship, ** a moderately significant relationship and * a weak significant relationship. Values presented with no * indicate that the correlation was not significant.

The PCA results showed some clustering of the sampling months along PC1, which was primarily driven by temperature and pH (Fig 3.12 a). Less clustering occurred along PC2 which was associated with DO, TA and chlorophyll-*a* concentration (Fig 3.12 a). Clustering of sites on both PC1 and PC2 was less pronounced (Fig 3.12 b). Temperature and pH were the primary drivers of monthly and spatial variability in environmental conditions at offshore sites (Fig 3.12 a,b).

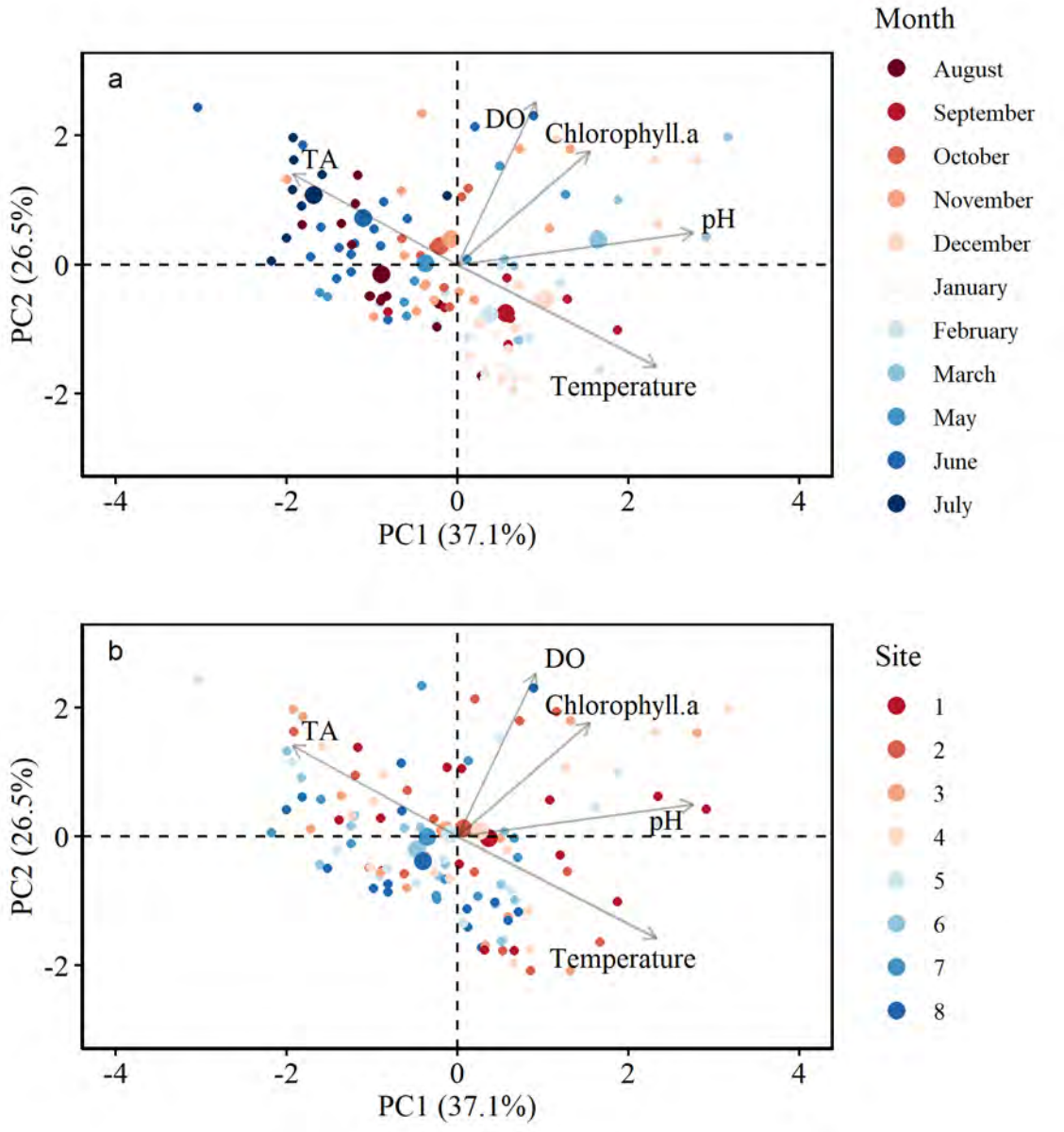

Figure 3.12: Principal component analysis (PCA) of the environmental conditions at offshore sites in Algoa Bay with a) monthly ordination and b) spatial ordination. Together the clustering along the primary (PC1 – 37.1 %) and secondary (PC2 – 26.5 %) axes account for 63.6 % of the total variance.

Summary statistics of the DISTLM model results are presented in Table A.4 in the appendix. DISTLM analysis revealed that all the predictor variables (temperature, TA, DO and chlorophyll-*a* concentration) significantly contributed (P <0.01) to the explained variance in pH. Temperature accounted for 20% of the variance in pH (R^2 = 0.20), with DO and chlorophyll-*a* similarly accounting for only 8% (R^2 = 0.08) and 7% (R^2 = 0.07) of the variation, respectively. Together, the predictor variables explain 36% (R^2 = 0.36) of the variance in pH at the offshore sites.

Inshore sites

Results of the pairwise correlation analyses are presented in the correlation matrix in Fig 3.13. Similarly to the results for the offshore data, expected correlations occurred among pH and the calculated carbonate chemistry speciation parameters pCO_2, Ω Ar and Ω Ca. Temperature showed a strong positive correlation with pH (R = 0.60), Ω Ar (r = 0.70) and Ω Ca (R = 0.70) and a negative relationship with TA (R = 0.52) (Fig 3.13). pH showed a weaker relationship with salinity (R = -0.30) and no significant correlation with chlorophyll-*a* concentration (R = -0.09), unlike in the offshore sites (Fig 3.11).

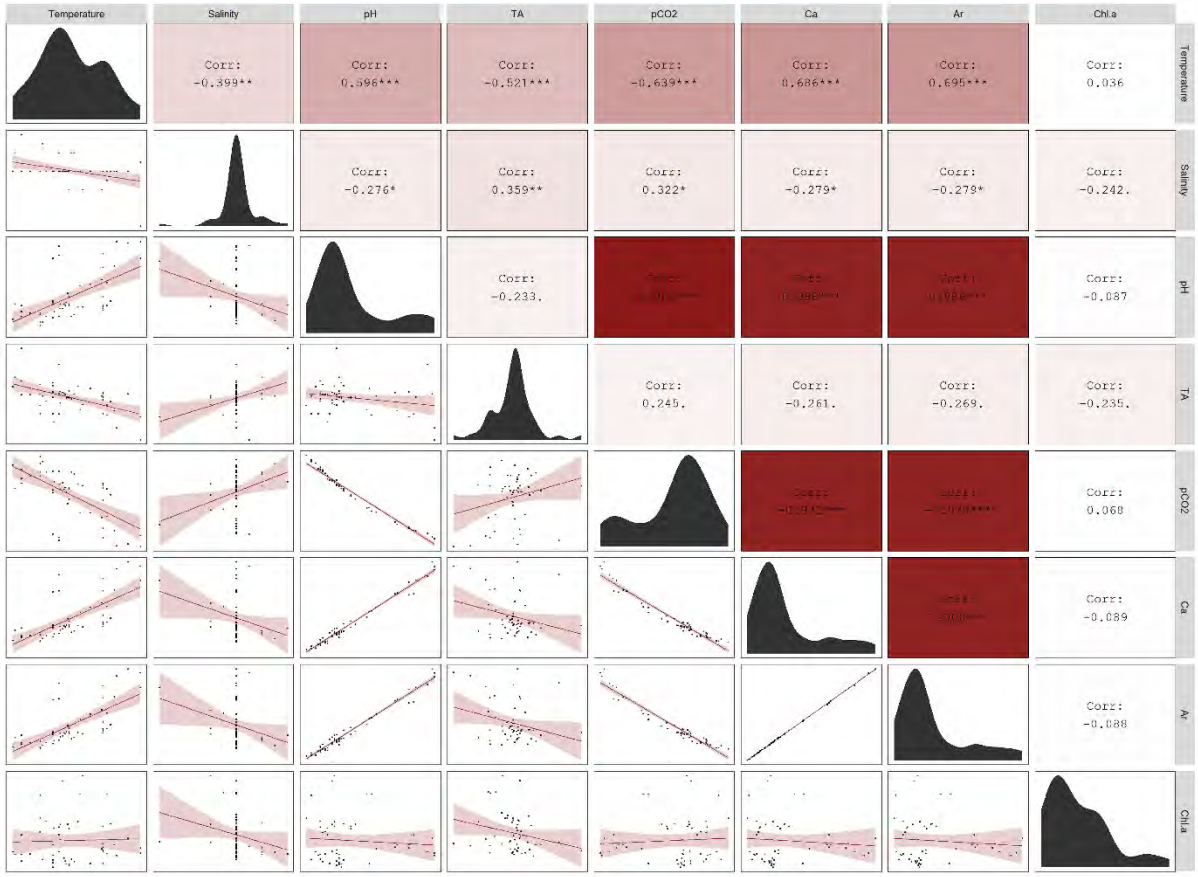

Figure 3.13: Correlation matrix presenting the linear relationships with correlation coefficients (*R*, Pearson's method) for pairwise combinations of all the parameters measured at the inshore sites. The strength of the significance of the relationship between parameters is indicated by the number of * with *** indicating a highly significant relationship, ** a moderately significant relationship and * a weak significant relationship. Values presented without a * indicate that the correlation was not significant

The PCA analysis revealed some clustering of sampling months along PC1 and PC2 (Fig 3.14 a). There was clear clustering of sampling sites along PC1 (Fig 3.14 b) and this clustering was primarily driven by pH and temperature. The PCA results revealed that in inshore sites, temperature and pH were similarly responsible for driving environmental variability on both monthly and spatial scales (Fig 3.14 a, b).

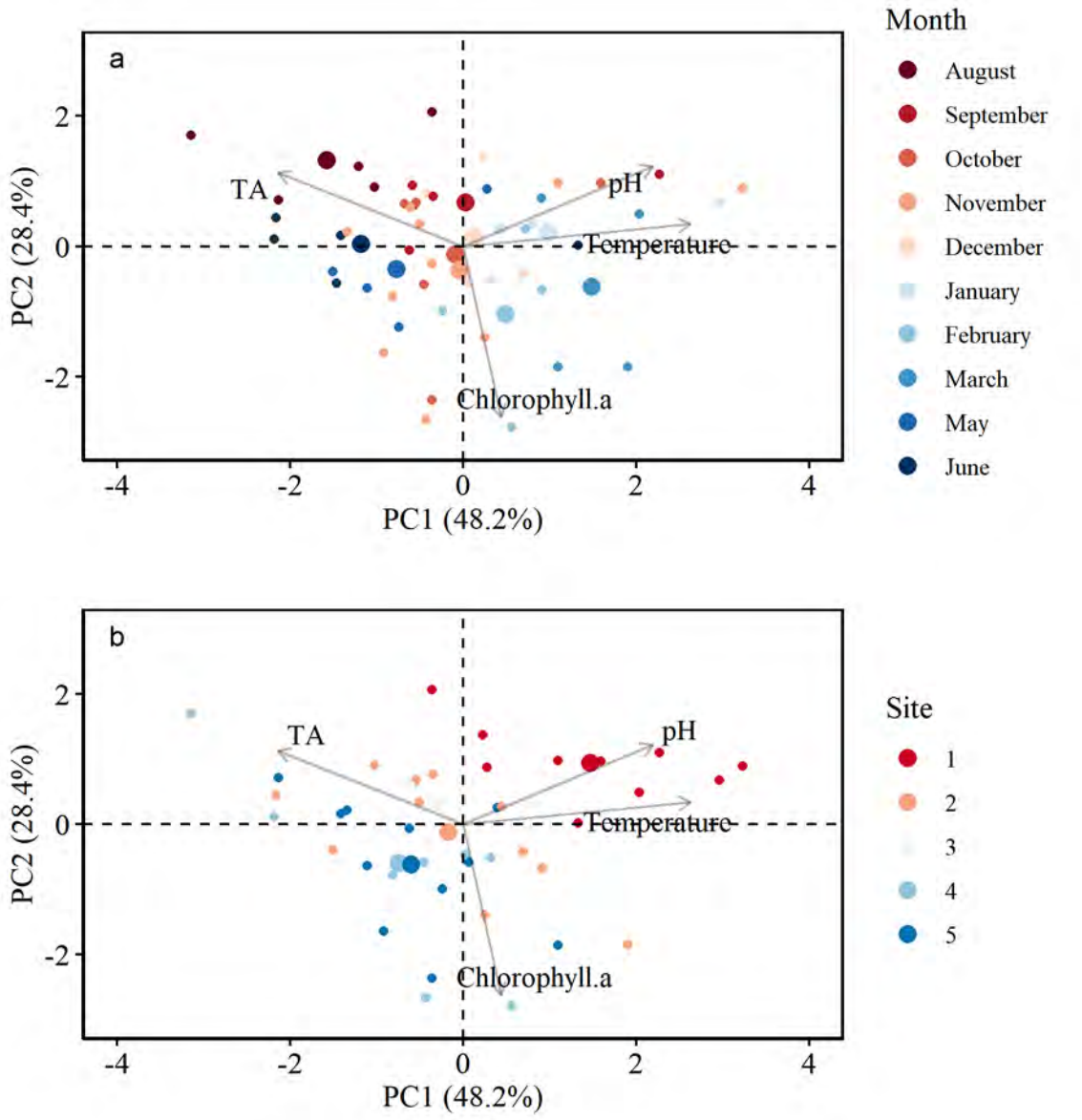

Figure 3.14: Principal component analysis (PCA) of the environmental conditions at inshore sites in Algoa Bay with a) monthly ordination and b) spatial ordination. Together the clustering along the primary (PC1 – 48.2 %) and secondary (PC2 – 28.4 %) axes account for 76.6 % of the total variance

Summary statistics of the DISTLM model results are presented in Table A.4 in the appendix. DISTLM analysis revealed that only temperature contributed significantly ($p < 0.01$) to the explained variance in pH. Temperature accounted for 25% of the variance in pH ($R^2 = 0.25$), whereas TA only explain 3% ($R^2 = 0.03$) of the variance in pH. Chlorophyll-*a* did not explain any of the variance in pH and contributed negatively to the combined model ($R^2 = -0.01$).

Together, the predictor variables explain 25 % (R^2 = 0.25) of the variance in pH at the inshore sites.

Upwelling event-scale variability

Average integrated anomaly values (IA) as an index for upwelling intensity and duration were highest during October 2018 (5.13 ± 1.41) (Fig 3.15). The maximum IA was recorded during October 2018 (IA = 7.33) at Site O8 at Cape Recife. March 2019 also showed a high average IA value of which the highest values were recorded at sites O6 (IA = 6.89) and O8 (IA = 5.87). The highest variability in IA was during the typically spring and summer months (September – February). Lower average IA values were recorded in the winter months (June – August).

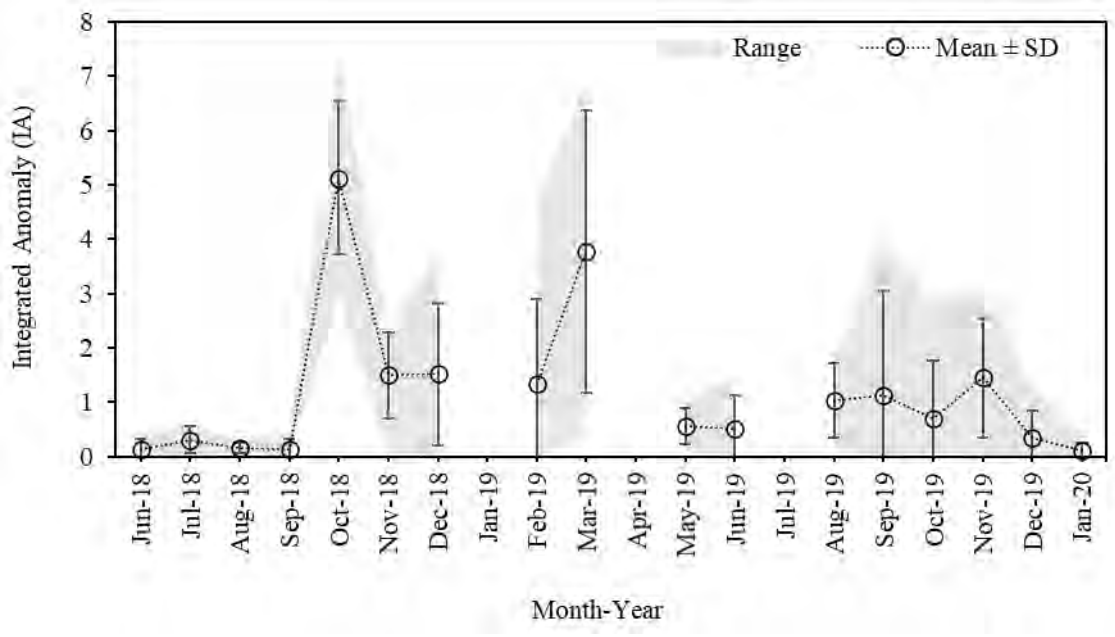

Figure 3.15: Integrated anomaly (IA) values as an index for potential upwelling events at seven offshore sites (sites O2–O8) during the study period June 2018 – January 2020

The highest average IA values during the study period were recorded at sites O4 (IA = 1.57 ± 2.17), O8 (IA = 1.46 ± 2.21) and O6 (1.26 ± 1.98) (Fig 3.16). Within-site variability in IA was high throughout the study period (Fig 3.16).

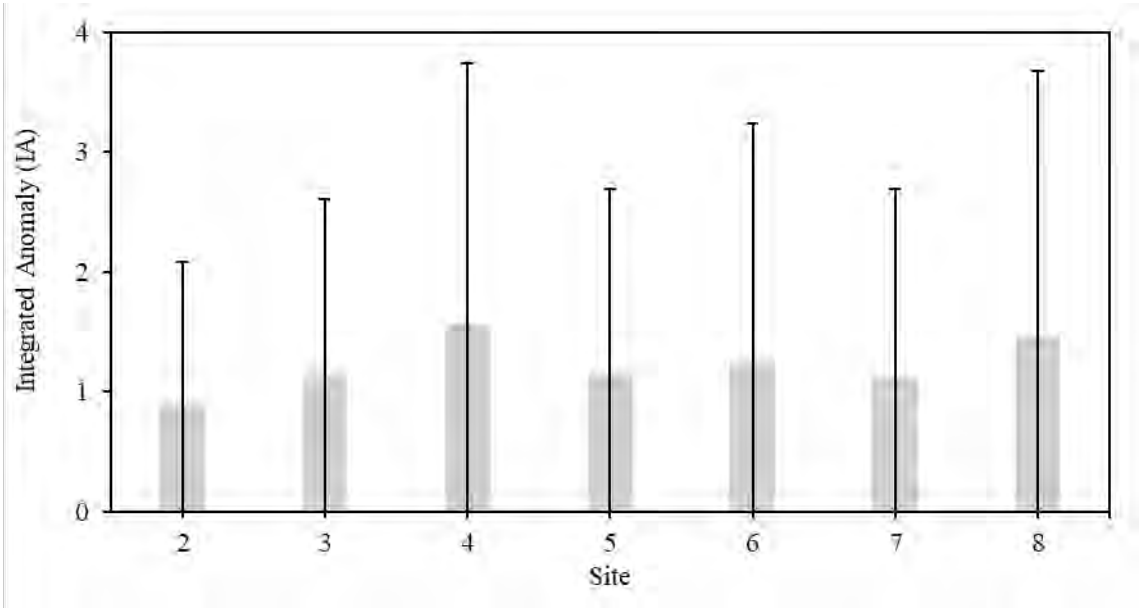

Figure 3.16: Integrated anomaly (IA) values as an index for potential upwelling events at each offshore site (O2–O8) during the study period June 2018 – January 2020

Correlation analysis (Pearson's correlation) revealed no relationship (R^2 = 0.068, P = 0.48) between pH and upwelling index (IA).

Discussion

This study quantified monthly, diel (24-hour) and spatial (at a resolution of ~10 km) variability in pH and associated biogeophysical parameters across offshore and shallow inshore sites in a productive coastal embayment. The results showed that pH and related physical and biological parameters in the coastal area of Algoa Bay vary over space and time. The average pH recorded at inshore sites (8.03 ± 0.07 SD) and offshore sites (8.04 ± 0.15 SD), throughout the bay are typical for coastal areas. Similar average pH levels have been recorded in other coastal ecosystems, such as in the Californian La Jolla kelp forests (8.05–8.1) (Frieder *et al.*, 2012), two Antarctic coastal habitats (8.02–8.05) and a Pacific coral reef (8.03) (reviewed by Hofmann *et al*., 2011). Overall pH varied among the offshore and inshore sites by ~0.69 pH units (range = 7.72–8.41). It is common in many coastal areas for pH to vary by more than Δ 0.50 units (Hofmann *et al.*, 2011; Kapsenberg *et al.*, 2015), which aligns with the temporal and spatial variability observed in this study. The levels of variability in pH in the bay suggests that present and future pH conditions will differ from those currently observed in, and projected for open oceans, where variability is typically low (Hofmann *et al.*, 2011).

Spatial and temporal variability

Spatial variability in pH over various scales (from km to cm) is common in coastal areas (Chan *et al.*, 2017; Lowe *et al.*, 2019). The sites showing the highest pH variability were sites O2, O3, O5 and O8 (Fig 3.3b). Variability at these sites is likely driven by either biological processes (high algal productivity events) at sites O2 and O3, and/or event scale processes (upwelling) at sites O5 and O8. These events are likely to drive spatial variability in carbonate chemistry conditions by either increasing pH (recorded maximums of >8.20) due to high photosynthetic activity during the day (as evidenced by the correlation between chlorophyll-*a* and pH) or reducing pH as a result of cold upwelled water entering the bay typically at site O5 and O8 (where minimum pH levels <7.80 were recorded) (Fig 2.3 b).

Interestingly, the apparent association between pH and chlorophyll-*a* concentration was not evident in the surf zone (inshore sites), where the sites with the highest chlorophyll-*a* concentrations (I4 and I5) had lower average pH levels (Fig 3.3 and 3.4). These high

chlorophyll-*a* concentrations are common in this area of the bay due to the high productivity of the surf zone diatom *Annalus australis* (Campbell and Bate, 1988). The intertidal areas along this section of the coastline, which is named the 'windward' side of the bay, are heavily influenced by wave action, rip currents and the predominant south-westerly swell direction (Branch and Branch, 1981). This section of the bay's coastline is therefore considered highly dynamic and is subject to more intertidal mixing (McLachlan *et al.*, 1981; Talbot and Bate, 1987) than the western, 'leeward' section of the bay (see description in Chapter Two). This continuous mixing and water movement may be buffering the pH changes in these surf zones. This could explain why the pH levels recorded along this section of the coast are comparable to those at the mouth and boundaries of the bay, which likely most closely represent areas that are at an equilibrium with atmospheric conditions.

The inshore sites experiencing lower average pH (I2–I5) are likely also influenced by the discharge of the two large estuaries (Swartkops Estuary and Sundays Estuary). The influence of riverine inflow in creating patches of low pH conditions is well documented in many other coastal areas (e.g., Vargas *et al*., 2016). Furthermore, the sites on the eastern boundary of the bay (I4 and I5) experience fresh groundwater seeping from the Alexandria dune field (Campbell and Bate, 1998), which may further contribute to the lower pH levels observed at these sites. This groundwater is rich in ammonia and nitrate levels, which has been suggested to contribute to the productivity of surf zone phytoplankton at these sites (Campbell and Bate, 1988; Lemley *et al.*, 2019). Campbell and Bate (1998) found the average rate of discharge into the surf zone occurred at a rate of 1000 L per metre of beach. However, it is likely that this discharge rate from both rivers and groundwater sources is limited due to the ongoing drought currently experienced by this region.

A clearly atypical inshore site at Cape Recife (Site I1) consistently showed different carbonate chemistry and physical conditions when compared to other sites in the bay. This site showed consistent above average pH (8.13–8.41) as well as higher temperatures throughout the sampling period. The sub-tidal section of this coastline consists of a mosaic of rocky reef and sand dominated by macroalgae, particularly red seaweeds *Plocamium corallorhiza* and *Amphiroa ephedrae* (Knoop, 1988). This site is different from the other inshore sites, which are located along a sandy shoreline. Wahl *et al.* (2017) also found that sites located in macrophyte (brown alga and seagrass) habitats in the western Baltic had a higher mean pH (by up to 0.3 units) than similar habitats that had no macrophytes. Other

studies have also found that macroalgal photosynthesis may raise mean pH in the surrounding water, with this effect increasing during the daily photoperiod (e.g., Krause-Jensen *et al.,* 2016). Diurnal pH measurements taken at this site varied by 0.56 units over a 24-hour period (Fig 3.12), with higher pH ~8.13 being recorded during the day and decreasing to a low ~7.98 at night. Middelboe and Hansen (2007) studied sites densely covered in *Fucus* species and found that pH reached almost 9.0 during the day and dropped to 7.80 at night.

Although biological modulation by macroalgae at the site at Cape Recife is likely, other anthropogenic processes at this site may be further contributing to the observed variability in pH and temperature. At this site, the inflow of wastewater from a nearby Wastewater Treatment Plant (WWTP), which sporadically releases wastewater effluent into the area (Lemley *et al.*, 2019), may be influencing the conditions adjacent to the outflow. This effluent is rich in inorganic nutrients (dissolved inorganic nitrogen and phosphate), is also high in ammonium and has been linked to causing biological bloom events, which are often harmful algal blooms (Lemley *et al.*, 2019). However, the source and composition of the wastewater is likely variable and not known, and therefore it may be responsible for the higher than average pH levels occurring at this site.

At a monthly scale, pH varied in the bay by Δ 0.55 and Δ 0.61 pH units at offshore and inshore sites, respectively. This aligns with findings from other coastal areas where it is common for pH to vary seasonally by up to 0.6 units (e.g., Manzello 2010, Provoost *et al*., 2010, Gray *et al*., 2012). In some coastal areas, this variability may be more extreme with seasonal and inter-annual variability in pH that can exceed 1 pH unit (Semesi *et al.*, 2009; Carstensen and Duarte, 2019). This differs significantly to the variability of approximately ~0.02 units observed in the more stable open ocean (Hofmann *et al.*, 2011). At the offshore sites, pH was more variable during the typically productive spring and summer months and was generally less variable in the winter (Fig 3.2). At the offshore sites, the most variable pH conditions during the study occurred in the period from October 2018 to March 2019, which also coincided with a period of high temperature variability (Fig 3.2). After this, variability in pH and temperature was much lower for the remainder of the study period (May 2019 – January 2020). In contrast, the inshore sites showed no obvious seasonality in pH variability (Fig 3.7).

Although studies have shown that upwelling of low pH water, and the resulting biological activity, modulates pH conditions on the west coast of South Africa in the eastern boundary

upwelling system (Santana-Casiano *et al*., 2009; Gregor and Monteiro, 2013), the upwelling indices calculated during this study did not show a significant correlation with pH. A similar finding was reported in a study in the coastal region of Washington where an upwelling signal was expected, but the authors found that biological activity played a stronger role in modulating variability despite the occurrence of upwelling in these areas (Lowe *et al*., 2019). The lack of correlation between IA and pH may also be an artefact of the sampling design, as sampling by boat at sea could not take place during periods of high wind speed that upwelling in Algoa Bay is associated with (as discussed in Chapter Two).

Drivers of variability

The patterns of variability observed in this study suggest that the drivers of pH and carbonate chemistry are complex and variable over space and time, as would be expected for a dynamic coastal area (Dore *et al*., 2009). Temperature appeared to vary concurrently with pH in this study over both temporal and spatial scales. It did not, however, give a clear indication of the driving process behind this variability. Autotrophic biological activity may be a potential contributor to pH and carbonate chemistry variability over spatial as well as medium- and short-term temporal scales in Algoa Bay. This is evidenced by the observed spatial and temporal alignment of pH and chlorophyll-a concentrations during the monthly day-time measurements taken at the offshore sites. However, this relationship did not occur in the inshore sites (Fig 3.12) except for Site I1 at Cape Recife where some biological modulation from macroalgae is likely. This confirms the complexity of localized pH and carbonate chemistry modulation in shallow coastal habitats.

Several other physical processes such as rainfall and associated freshwater inflow from estuaries and groundwater may be contributing to variability in pH in Algoa Bay. These influences may result in patches of carbonate chemistry and pH variability that occur both over space and time making this coastal area highly dynamic, like many others (Hofmann *et al.*, 2011). However, as highlighted in Chapter Two, the study area occurs in a rainfall scarce area (Mahlalela *et al.*, 2020). Although the quantity of freshwater entering the marine environment from estuaries and groundwater is has not been quantified, it is not likely that this would play a role in the observed variability in pH in this study since rainfall was generally low (on average <2 mm, see Chapter Two) throughout the study period.

Limitations in capturing local variability

This study does not allow for the detection of long-term climate trends in pH related to OA. This would require extensive time series data over multiple years with high precision (Newton *et al.*, 2015). This data set to some extent captures the 'ocean weather' and local variability. However, this study was designed around several practical and logistic limitations that influenced the resolution of the physico-chemical data and associated temporal and spatial variability that likely led to an under-estimation of the natural variability and several potential artefacts. Furthermore, it is likely that the sampling design may have introduced some confounding factors that influenced the observed spatial and temporal patterns reported in this study.

It is important to consider that the monthly sampling only took place during the day when the most photosynthetic activity is expected, which may have resulted in the higher pH readings during this time. At night, pH may drop below average levels when the biological aggregations are in the respiration state, resulting in increased CO_2 in the surrounding seawater. This pattern of diel variability was visible in the 24-hour cyclical change in pH observed at the coastal site at Cape Recife where pH drops at night and rises during the day (Fig 3.14). Algal blooms are known to result in low pH levels during the decomposition phase when respiration from bacterial decomposition is high. The spatial patterns in pH variability similarly could have been influenced by the sampling strategy as sites were always sampled in the same order on each trip (over 6–9 hours during daylight). Since photosynthetic activity has been shown to modulate pH, and changes in a cyclical manner throughout the day, this could have influenced the observed spatial differences among the sites. Similarly, the site at Cape Recife was always sampled just after midday when peak photosynthetic activity could be expected (as shown by the 24-h pH measurements) which may explain why the pH was always highest at this site compared to the other inshore sites. The timing of sampling can therefore influence the observed patterns in pH produced by this study, which further accentuates the advantages of continuous monitoring at capturing local variability in coastal areas.

Consideration of the resolution of the presented data is essential when interpreting the response of species as the variability experienced by the organisms in question may not be fully captured. For example, the results from the short-term, intensive pH monitoring reported in this chapter reveal distinct diel variability in pH. However, the monthly

monitoring only captured pH variability during the day. This makes it difficult to interpret the true variability experienced by organisms over the long-term. In this case, the short-term high-frequency data produced by the iSAMI-pH sensor in this study was more useful at capturing the local variability in this coastal area. Future research could consider this in the experimental design to fully capture the 'ocean weather' by focusing on higher resolution (hourly) monitoring over a relevant timeframe (e.g., by *in situ* sensor deployment with continuous recording). Such information would be more useful when designing biological response experiments as it fully captures the variability in local conditions experienced over a relevant scale, and over the full duration experienced by the organism, particularly when focusing on a specific life-stage as in this study (chapters Four and Five).

The findings of this chapter highlight that the atmospheric equilibrium model applied to open oceans is not a suitable approach to select OA scenarios for the coastal area of Algoa Bay. This is clearly demonstrated by the high spatial and temporal heterogeneity in carbonate chemistry conditions over both temporal and spatial scales in Algoa Bay. It appears that several interacting coastal processes, which may include biological activity, freshwater inflow and potentially upwelling are likely modulating pH variability depending on seasonal and spatial considerations.

The contribution of local processes to pH variability in Algoa Bay is complex, suggesting that future processes related to OA will be similarly dynamic and variable. This variability in pH and associated carbonate chemistry over multiple scales of time and space will have an influence on organisms living in Algoa Bay and these findings will provide a useful benchmark for the design of experiments to examine the impact of changing pH conditions on the marine fauna in the bay (see chapters Four and Five).

Chapter 4

The metabolic physiology of Diplodus capensis *over a range of pH*

Introduction

Whole organism physiology is a useful tool for understanding the mechanistic interactions between organisms and their physico-chemical environment (Clarke, 1993; Pörtner, 2010; Svendsen *et al.*, 2017). This interaction is based on the fact that many coastal organisms, including fish, strive to regulate internal homeostasis in changing environmental conditions in order to maintain conditions for optimal fitness (Claiborne *et al.*, 2002; Svendsen *et al.*, 2017). Disturbances in any of an organism's internally regulated physiological processes resulting from environmental stress will require additional energy to counteract any negative effects (Baker and Brauner, 2012; Esbaugh, 2018). The resulting trade-offs in energy allocation, which affect overall metabolic function, are often detectible in physiological performance measures, such as ventilation rates (Ishimatsu *et al.*, 2008; Ern and Esbaugh, 2016) and whole organism metabolic rates (Fabry *et al.*, 2008; Heuer and Grosell, 2014). Assessing organism physiology, by measuring oxygen consumption rates, provides insight to the metabolic response of an organism when under environmental stress (Rodgers *et al.*, 2016).

Metabolic rate has become a commonly measured physiological parameter for determining the performance of species in response to environmental stress and is normally estimated by measuring the oxygen consumption rate of an organism (Clark *et al.*, 2013; Rodgers *et al.*, 2016; Svendsen *et al.*, 2016). Measuring oxygen consumption rates under different experimental conditions have been used for aquatic organisms, and these can give insight to an organism's resting (SMR) and maximum metabolic rates (MMR), which reflect the energy requirements of survival processes and maximum activity, respectively. Metabolic scope (or aerobic scope) is commonly reported and is a useful metric as it translates directly to individual fitness and performance (Heuer and Grosell, 2014). Metabolic scope (MS = MMR - SMR), is the aerobic capacity to perform metabolic tasks over and above maintaining baseline survival processes (Fry, 1971). Routine metabolic rate is a general measure of energy expenditure during regular spontaneous activity (Fry, 1971). These metrics give insight into various components of the metabolic capacity of an organism and are useful in

detecting the metabolic trade-offs that occur as a result of stress from changes in the external environment (Pörtner and Knust, 2007b; Bozinovic and Pörtner, 2015).

Metabolic rates have been used to assess the impact of stressors in marine environments associated with global change, including increased sea surface temperature (e.g., Munday *et al.,* 2009*;* Anestis *et al.* 2010*;* Slesinger *et al.* 2019*)*, hypoxia (e.g., Pan *et al.*, 2017; Stevens and Gobler, 2018), exposure to toxicants (e.g., Rowe *et al.*, 2001; Lannig *et al.*, 2006) and reduced seawater pH as a result of ocean acidification (OA) (e.g., Heuer and Grosell 2014; Pope *et al*., 2014; Esbaugh *et al.,* 2016; Crespel *et al*., 2019). Changes in seawater pH have a significant effect on the energy budget of marine organisms due to disturbance of the organism's internal acid-base homeostasis (Fabry *et al.*, 2008; Heuer and Grosell, 2014). Many coastal organisms normally respond to this disturbance by increasing their energy allocation to processes responsible for maintaining internal pH balance (Claiborne *et al.*, 2002). This is usually detectible by increases in SMR and is described as 'loading stress' (Esbaugh, 2018). Alternatively, 'limiting stress' is detected by changes in MMR and is related to some other metabolic constraint related to activity (Esbaugh, 2018).

In fishes, a decrease in pH in their seawater environment causes intra- and extra-cellular hypercapnia, which requires internal regulation to maintain optimal pH for physiological functioning (Fabry *et al.*, 2008; Heuer and Grosell, 2014). Fishes manage the internal accumulation of pCO_2 and reduced internal pH caused by acidification by active ion transport, metabolic buffering, CO_2 transport (Fabry *et al.*, 2008; Heuer and Grosell, 2014; Esbaugh, 2018) or, in extreme cases, the suppression of their metabolism to conserve energy during periods of stress (Pimentel *et al.*, 2014). Irrespective of the mechanism, the maintenance of homeostasis and management of internal pH is energetically costly, which when sustained over long periods may impact important processes such as growth, behaviour, or reproduction, with ultimate long-term fitness consequences (Heuer and Grosell, 2016; Esbaugh, 2018).

In general, fishes are thought to be somewhat physiologically tolerant to pH changes due to relatively large metabolic capacities and advanced acid-base regulation strategies compared to invertebrates giving them the ability to compensate for internal pH changes (Esbaugh, 2018). For example, some fish species appear tolerant even to very low pH conditions as

shown by aquaculture settings where pH can reach levels <7.0 (up to 10 000 μatm CO_2) for extended periods (Heuer and Grosell, 2014; Ellis *et al.*, 2017).

The regulatory response of fishes to low pH is not necessarily common to all species. Since research has been expanded to a larger variety of species from various families, life stages, biogeographical regions, life-history strategies and habitat associations, it has become clear that the vulnerability or tolerance of fishes to OA is more complex than previously thought, and the capacity for internal pH regulation varies both among and within species (Cattano *et al.*, 2018), with the metabolic response of fishes to OA being highly variable (Lefevre, 2016; Cattano *et al.*, 2018; Esbaugh, 2018).

The general consensus is that species that have adapted to highly variable environments (e.g., the pH gradients in hydrothermal vents, seasonal upwelling areas, tidal pools, or estuarine habitats) have likely evolved a higher aerobic capacity and efficient acid-base regulation (Fabry *et al.*, 2008; Munday *et al.*, 2009a; Hofmann *et al.*, 2011). On a broader biogeographic scale, tropical species may be more vulnerable to OA than temperate species as temperate species are exposed to higher environmental variability (Cattano *et al.*, 2018), with many temperate species showing metabolic tolerance to a wide range of pH (Ern and Esbaugh, 2016; Esbaugh *et al.*, 2016; Kwan *et al.*, 2017; Lonthair *et al.*, 2017).

Although some species may show tolerance to low pH conditions, all species have a finite metabolic capacity, which will ultimately limit their stress response when pushed to the extreme limits of their tolerance (Fry, 1971; Nelson and Chabot, 2011). Coastal environments are known for their variability, and species inhabiting coastal habitats require the metabolic capacity to compensate for the dynamic variation in physico-chemical parameters (Melzner *et al.*, 2009b; Couturier *et al.*, 2013). Furthermore, OA is also occurring at a different rate in coastal environments compared to the open ocean (Duarte *et al.*, 2013; Baumann *et al.*, 2015). Many species may already be exposed to pH levels at or below their metabolic limits over various scales of space and time in coastal habitats (Hofmann *et al.*, 2011). Although coastal species may be tolerant of low pH conditions over the short term, the increased frequency and duration of low pH events associated with future OA in coastal areas may exceed their abilities for long-term physiological compensation. Furthermore, efficient physiological functioning requires energy assimilation from food resources (Cominassi *et al*., 2020), which may be indirectly affected when OA impacts the prey species, habitat quality

(Nagelkerken *et al.*, 2016) or the ability of the predator to identify and capture prey (Ashur *et al*., 2017). Ocean acidification can therefore also have indirect effects on the physiological management of low pH conditions through ecological and trophic interactions.

In addition to varying among species, metabolic capacity, tolerance, and adaptive ability also varies within species during ontogenetic development. Consideration of life-stage when assessing species responses to environmental conditions is important as vulnerability or exposure to low pH conditions may change during ontogenetic development (Kikkawa *et al.*, 2003; Ishimatsu *et al.*, 2004). Although most fishes are fairly tolerant of low pH during their adult stages, recent research that has focused on the early (larval and early juvenile) life stages has suggested that they may not yet have developed sufficient metabolic capacity and acid-base regulation mechanisms to manage low pH conditions (Lonthair *et al.*, 2017).

Tolerance may even change from day-to-day during early development of fishes. For example, Ishimatsu *et al*. (2004) found that the early stages (pre-flexion and flexion) of the silver seabream (*Pagrus major*) and Japanese sillago (*Sillago japonica*) had a high tolerance to pCO_2 acidified seawater but their tolerance decreased in the cleavage and early juvenile stages. This suggests that tolerance to acidification may not be consistent throughout development, which may affect survival at later life stages. Edworthy (2017) identified that the preflexion and flexion stages of a temperate, estuarine-dependent marine species *Argyrosomus japonicus*, were less sensitive to CO_2 acidified seawater than the postflexion stages (specifically on day 21 and 22 post hatching). Although egg and larval development occurs in the more stable marine environment, this species recruits into estuarine habitats (which have more variable pH) from the postflexion stage. The post-flexion stages also have high energetic demands due to the extensive bone and cartilage development (Erasmus, 2017) and increased activity (Edworthy, 2017), which occur during this developmental phase. These post-flexion life stages were therefore considered to be a crucial bottleneck in development and ultimately recruitment to juvenile habitats (Edworthy 2017, Erasmus 2017).

Most studies assessing the impact of low pH on fishes have used scenarios based on open ocean OA projections. Another approach is to use a pH gradient covering the present and future natural range experienced by the respective organism (Dorey *et al.*, 2013; Thor and Dupont, 2015; Jager *et al.*, 2016; Ventura *et al.*, 2016). This can be achieved by testing the response of organisms to a range of stable treatments at regular increments. Using a gradient approach allows for the identification of tipping points in the physiological response curve,

which can project the response of species to low pH and potential future OA conditions (Dorey *et al.*, 2013; Ventura *et al.*, 2016).

The aim of this chapter was to experimentally assess the physiological response, by the estimation of metabolic rates, of post-flexion stage *Diplodus capensis* to a range of low pH levels which cover and exceed the natural variability measured in their wild habitats, which ranged from 8.41 to 7.72 (see Chapter Three). The pH treatments used for the experiments in this chapter ranged from 8.02 (seawater untreated by CO_2) to a low of 7.27 at approximately 0.2 pH unit increments. It was hypothesized that deviations in standard metabolic rate (SMR) and relative aerobic scope (RAS) would only occur at pH treatments below the minimum pH recorded in their natural habitat.

Methods

Experimental animals

Approximately 200 post-flexion stage *D. capensis* larvae (~12.00 ± 1.15 mm TL, mean ± SD) were collected from the wild at Cape Recife (see Fig 2.2 in Chapter Two) on 21 November 2019 using a larval seine net (3 m wide by 1.5 m high with a mesh size of 500 µm) in the shallow intertidal area (1–2 m depth). The 200 larvae were transferred into two 25 L buckets containing approximately 100 larvae each and containing an oxygen supply for transport. The collected fish were transported to the laboratory at the Ocean Science Campus, Nelson Mandela University. In the laboratory, the larvae were transferred to five 25 L static experimental holding tanks (black sided buckets with a white bottom).

The seawater in the tanks was pumped from an open intake situated in a deep rock pool on the boundary of the subtidal zone in Algoa Bay (facilitated by Bayworld Oceanarium, Port Elizabeth). The water was treated with 0.2 ppm chlorine to reduce biofouling. It was then passed through a sedimentation system, followed by a mechanical filter and an activated carbon filter. Finally, the water was passed through a 5 and 1 µm filter after which it was collected in 250 L tanks and transported to the laboratory. The seawater had a salinity of 35 when it was filled into the experimental tanks. The seawater in each 25 L tank was aerated with air pumps and air stones, which also facilitated water movement and mixing within the buckets. The seawater in all the tanks was temperature controlled at 23 ± 0.5°C by means of aquarium heaters each connected to a digital temperature controller (STC-1000). This temperature was selected based on the average coastal sea temperature at the collection site

during this period (identified from data from Chapter Three). The light cycle in the experimental laboratory was maintained at 16L:8D, which corresponded to the natural light cycle at the time of the experiments.

To stock the larvae into the tanks, they were gradually acclimated to the experimental temperatures by suspending them in their transport seawater in bags before releasing them into the tanks after 10 to 15 minutes. Approximately 25–30 individuals were stocked into each tank. Mortality from handling was common during the first day of the experiments and 2–7 larvae across all treatments died within the first 12 hours, after which no more mortality was observed.

The fish were fed twice daily to satiation with flash frozen *Artemia* nauplii (*Artemia salina*). Daily siphoning of debris from the bottom of the tank and 10% water changes in the tanks ensured that waste build up (ammonia and nitrate) remained negligible (<1 mg L^{-1} NH_4 and NO_2, measured using TETRA test kits).

All the methods for the collection, handling, and housing of the larvae in the experimental system were based on a pilot study with different individuals which was conducted before the experiments. This was to ensure that the methods used were suitable for the study species.

Experimental design and seawater chemistry

The experiment was designed to create a gradient of pH conditions to be tested, so that the larvae could be exposed to one of five treatment levels during the experiment. To do this, each tank was randomly allocated one of the five pH treatments, which ranged from 7.27 to 8.03, at increments of ~0.2 pH (Table 3.1). This approach was based on the experimental design used by Dorey *et al*., 2013. The five treatment levels were selected based on the measured range of pH in the inshore intertidal sites (I1–I5) as discussed in Chapter Three. The high pH treatment, which was seawater untreated by CO_2, was similar to the average pH measured at the coastal sites in the bay. Finally, the two lowest pH treatments simulated a 0.2- and 0.4-unit decline in pH from the lowest pH value recorded in this study (Chapter 3) as anticipated for 2050 and the end of the century, respectively (RCP 8.5, business-as-usual scenario). The tanks were filled with 1 µm filtered seawater sourced from a shallow coastal site in the bay. The remaining pH treatments were selected to include the lowest recorded pH at the coastal sites (7.80 pH) as well an outlier pH (~0.76 units lower). To meet the targeted

pH treatments in each tank, carbon dioxide gas (CO_2) was added by means of bubbling the gas into each tank (with exception of the control tank). The addition of the CO_2 was controlled by a pH control system (Tunze 7074) fitted to each tank, which recorded pH with a calibrated electrode (NBS) and controlled a solenoid valve which was connected to the CO_2 supply (Technical grade CO_2, 30kg cylinder, AFROX).

At the time of stocking, all tanks were at pH 8.03 and, after adding the fish, pH was gradually reduced (by 0.2 pH units every hour) in each tank until the correct treatment level was reached. After reaching the relevant pH treatments in all six tanks, the fish were allowed to acclimate to their experimental treatments for five days before experiments were started. This exposure time was selected based on previous studies that have shown that the short-term effects of low pH are evident in fishes within four days of exposure (Munday *et al.*, 2010; Ferrari *et al.*, 2011a; Nilsson *et al.*, 2012).

Temperature and pH were measured daily in the experimental tanks using a handheld pH and temperature meter (Hannah Instruments HI 8424), which was calibrated daily using a TRIS buffer solution (Tris/HCl) in synthetic seawater (salinity = 35) (provided by A. Dickson of the Scripps Institution for Oceanography, California, USA) in order to express treatment pH on the total scale (pH_{TS}). The daily measured pH values in the tanks were used to adjust the pH controller settings to reach true total scale target pH values by the controlled addition of the CO_2. On the fifth day of the experiment, at the end of the acclimation period, samples for the measurement of total alkalinity (TA) were taken from each tank and measured using a manual GRAN titration (method described in detail in Chapter Three). Associated parameters of the carbonate system (pCO_2, Ω Ar, Ω Ca) were calculated using the CO_2SYS program (Lewis and Wallace, 1998) with dissociation constants reported by Mehrbach *et al*. (1973) refitted by Dickson and Millero (1987).

Respirometry

Following the five-day acclimation to the experimental treatments, metabolic rates of the fishes kept in each pH treatment were measured within four days. This timeframe was selected to minimize the influence of development and growth on metabolic rates. All metabolic measurements were taken during daylight as this species is diurnal and feeds during the day (Mann, 1992). As such, daytime metabolic measurements complement the activity and feeding experiments conducted in Chapter Five.

Small volume, intermittent flow respirometry chambers were used to measure oxygen consumption rates in the larvae. The design of the respirometry system were based on the chamber design and testing recommendations made by Clark *et al*. (2013), Peck and Moyano (2016), Rodgers *et al*. (2016) and Svendsen *et al.* (2016). The chambers, which had a volume of 17.3 mL including the volume added by the tubing system, were constructed from clear Plexiglass (Fig 4.1b). Chamber dimensions and volume were suited to accommodate the size, shape and activity level of the fish being tested to avoid unnatural movement or swimming while still allowing sufficient space for routine activity. The chamber design also allowed for adequate mixing and limited oxygen ingress and drift from the surrounding water bath into or out of the respirometry chamber. Two different loops of Tygon© tubing cycled water through the chambers (Fig 4.1a), one facilitated the flushing of the chambers with fresh, oxygen replenished seawater from the water bath and the other cycled water through the chamber during the measurement phase. The system was designed in a way to allow the oxygen sensors to measure both the water being circulated during the measurement phase as well as water entering the chamber during the flush phase by using the same inlet into the chamber for both of these loops. A 4-channel oxygen reading system (Firesting, Pyroscience GmbH, Germany) measured the oxygen concentration in the water passing through the measurement cells containing optical oxygen sensors using REDFLASH dye technology. Water flow through the system was propelled by a peristaltic pump set to a flow rate of 2 mL min^{-1} and cycling of water in either the flush or measurement phases was controlled with valves (Fig 4.1a).

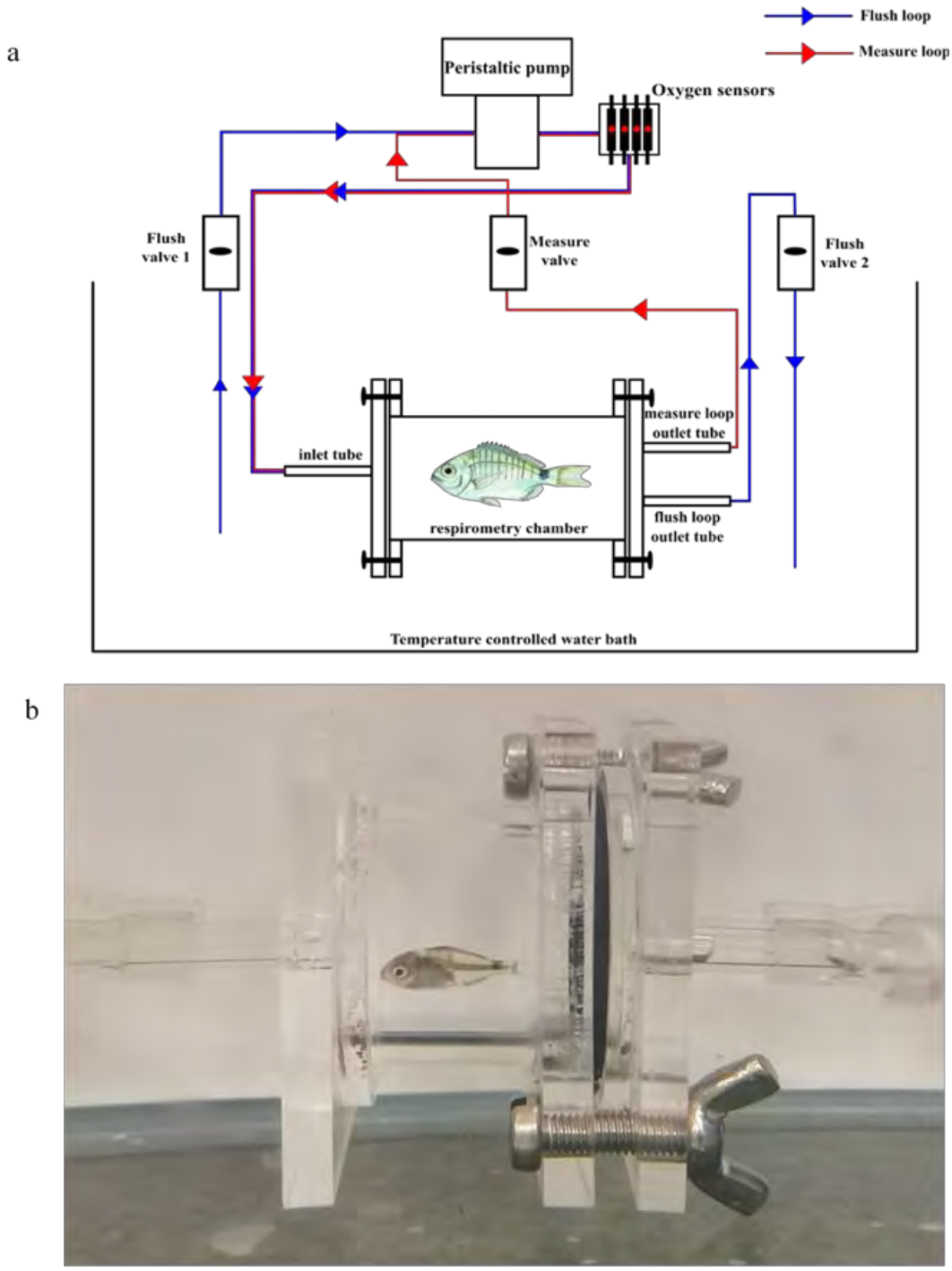

Figure 4.1: a) Schematic diagram of the respirometry system showing the flow circulation within one of the four respirometry chambers used for each trial and b) image of a post-flexion stage *Diplodus capensis* housed in a custom made flow through respirometry chamber

A total of 20 respirometry trials were conducted over the four days (one respirometry trial per treatment per day), each lasting approximately 90 minutes. Each respirometry trial measured four individuals from one treatment at a time allowing for the measurement of ~16 fish per

treatment over the four days. A single individual was placed into each of the four respirometry chambers. The chambers were housed in a 50 L water bath containing seawater adjusted to the specific pH treatment being measured at the time and regulated to the experimental temperature of 23 + 0.05°C with an automated heating system.

Once the fish were placed into the respirometry chambers they were allowed to habituate for ten minutes. The ten-minute habituation period was based on pilot studies that assessed the time required for oxygen consumption rates and activity levels to stabilise after handling. Oxygen consumption rate measurements were recorded for the calculation of routine metabolic rate (RMR) and maximum metabolic rate (MMR). Each respirometry trial consisted of three ten-minute measurements for the determination of RMR (Fig 4.2). Each RMR measurement phase was followed by a ten-minute flush phase to replenish oxygen concentration in the chambers, which was adequate time to replace the full volume of the chamber. After the measurements of RMR, fish were removed from the respirometry chambers and placed into a beaker containing 250 mL of the water from the water bath. A stir bar and stir plate were used to create a circular water current (at 580–600 rpm) in the beaker, and fish were exercised to exhaustion by allowing them to swim against the current in the beaker. The fish were considered exhausted when they could no longer maintain their position in the current. They were then immediately returned to the respirometers and a measurement phase was initiated immediately, which lasted for five minutes for the determination of maximum metabolic rate (MMR) (Fig 4.2). At the end of each trial, the fish were removed from the chambers, euthanized with an overdose of clove oil and then measured to the nearest 0.01 mm total length and weighed to the nearest 0.0001 g.

A blank measurement was conducted in all four chambers for ten minutes at the beginning and end of each trial to account for background bacterial respiration rates not related to the respiration rates of the fish (Fig 4.2). These measurements accounted for both the bacteria present in the water used for the measurements as well as potential bacteria introduced into the chambers by the fish.

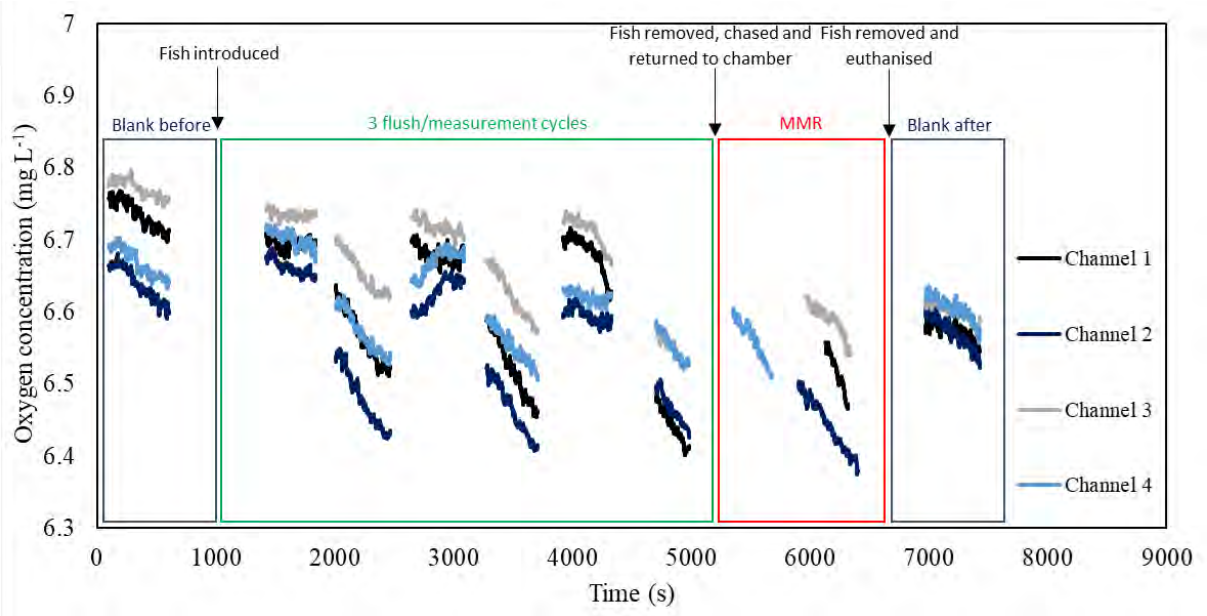

Figure 4.2: Hypothetical visual representation of data obtained during an entire respirometry trial, including a blank chamber measurement, an acclimation period, three intermittent RMR measurements with flush periods in between, an MMR measurement after the fish were chased to exhaustion and finally another blank chamber measurement at the end of the trial

Metabolic rate calculations

The slopes of each measurement phase were used to determine the rate of oxygen consumption over time by means of a linear regression. For each measurement phase, a suitable linear period of decline in oxygen concentration was selected visually. All the regressions were performed over a minimum period of five minutes. The initial two minutes of each measurement period were always excluded to account for any disturbance of the larvae induced by switching valves and changes in water flow within the chambers during the initiation of the measurement phase. SMR was not determined in this study due to the difficulty in quantifying the sporadic swimming activity of the larvae during the trials.

The slopes of the significant regression models were used to determine $\Delta[O_2]$, which is the difference in oxygen concentration in the chambers at the beginning and the end of each measurement period. RMR and MMR were calculated for each individual using the following equation: $Oxygen\ consumption\ rate\ (MO_2) = \frac{[\Delta O_2] \times vol}{t}$ where $\Delta[O_2]$ is the decrease in

the oxygen concentration in the chamber ($\Delta\ mg\ O_2$) during t, the total recording time (h) of the trial and vol is the volume of the respirometer – volume of the fish (L).

Oxygen consumption rates for the blank measurements were calculated from the entire trial and included both the before blank measurement, the three flush phases and the after blank measurement in order to accurately account for any background respiration occurring in the chambers, tubing, and seawater in the water bath. Oxygen consumption rates calculated from the linear slope of decline in oxygen concentration during these periods combined were subtracted from individual oxygen consumption rates prior to further metabolic rate calculations. After correcting the individual metabolic rates by subtracting background respiration rates, all metabolic rates were divided by individual mass (g) to determine mass specific metabolic rates (mg O_2 g^{-1} h^{-1})

Individual RMR was estimated as the average of the calculated oxygen consumption rates from the three RMR measurement periods and MMR was the rate from the single measurement, which was taken after the fish were chased (Boucher *et al.,* 2018). Relative aerobic scope (RAS) was calculated by subtracting the average RMR per individual from MMR: $RAS = MMR - RMR$ as per the method used by Boucher *et al.,* (2018).

Background bacterial respiration rates

Although care was taken to limit bacterial respiration rates as much as possible, relatively high background respiration rates (on average 6% of the respiration rate signal) were measured during this study (see Fig A.4 in the appendix). This is likely due to the small volume of the respirometry system, the warm measurement temperatures and high surface area to volume ratio introduced by the tubing system. This was evident as background metabolic rates appeared to increase throughout the day, although new filtered seawater was used each day. This was most likely attributed to bacterial build-up in the water bath, chambers, and tubing throughout the day. However, background respiration rates were strictly accounted for in each trial by subtracting the overall background respiration rates from the respiration rates recorded in each chamber.

Statistical analyses

All statistical analyses were conducted using Microsoft Office Excel 2013 and R Studio (Version 1.3.1056). All statistical analyses were based on a significance level of 0.05 and confidence interval of 95%. All summary statistics were reported as mean ± standard deviation (SD). All data were tested for normality using normal probability plots with a Chi-Square and Kolmogorov–Smirnov goodness-of-fit tests. Homogeneity of variance was tested using Bartlett's tests.

Tank pH and temperature data were not normally distributed and did not meet the assumption of homogeneity of variance (Temperature: K^2 (4) = 216.28, P <0.0001; pH: K^2 (4) = 216.28, P <0.0001). Therefore, a one-way analysis of means, that does not assume equal variances, was used to compare the difference in pH and temperature among the pH treatment tanks. A pairwise t-test with non-pooled SD and P values adjusted by the Bonferroni method was used to compare the difference in temperature and pH between each pairwise combination of the pH treatment tanks. The results from these analyses are summarized in Table 3.2.

Residuals were plotted for the biological (total length mm, wet mass g and condition factor mg mm^{-3} x 100) and metabolic data (RMR, MMR and RAS) and checked for homoscedasticity to ensure the data met the assumptions for the application of linear models. All the biological and metabolic data met the assumptions for normal distribution except for wet mass (g), which had to be $\log_{10}$ transformed.

Prior to testing for pH treatment effect, the relationships between the metabolic rates (RMR, MMR and RAS) with potential covariates including wet mass (g), total length (mm) and exposure time (measurement day) were assessed by means of forward stepwise regression analysis. As a result of the potential influence of measurement day on the overall metabolic rates, this parameter was included as a random intercept in all analyses. As fish wet mass ($\log_{10}$ g) showed a significant relationship with RMR and MMR, but not with RAS, all calculated metabolic rates were divided by mass before their inclusion in further analyses and were expressed as mass specific metabolic rates (mg O_2 g^{-1} h^{-1}). The relationships between the pH treatments and each of the metabolic rates (RMR, MMR and RAS) were then modelled using linear mixed effects models with measurement day as a random intercept.

Results

Seawater chemistry

Carbonate chemistry and associated physico-chemical parameters measured in the experimental tanks are summarized in Table 4.1. Although significant differences in temperature were observed among the treatment tanks (F (4) = 10.19, P <0.0003) this was likely attributed to temperature drops (to 21.7°C and 22.4°C) overnight on the first day of the experiment in the 7.2 and 8.03 treatment tanks, respectively, due to faulty temperature controllers. The following morning this was immediately rectified and after this temperature returned to the target of 23°C. Following the second day of the acclimation period, and throughout the measurement days, temperature in all the tanks remained within the range of ± 0.5°C of the target temperature of 23°C. Measured pH differed significantly among the experimental treatment tanks (F (4) = 2291.1, P <0.0001) and pairwise t-tests revealed that each pairwise combination differed significantly, indicating that there was no significant overlap in the pH treatments as expected (Table 4.2). Variability in TA and calculated parameters (pCO_2, ΩCa, ΩAr) could not be statistically tested as these parameters were only measured on a single occasion. TA did not vary among the treatments by more than 50 µmol kg^{-1}. pCO_2 increased as expected in accordance with the declining pH in the treatments.

Table 4.1: Carbonate chemistry and associated parameters for each treatment tank where pH_{TS} (electrode, mean ± SD), temperature (°C, mean ± SD) and salinity are the averages of daily measurements over the nine days of the experiment. Total alkalinity (TA, µmol kg^{-1}) was measured once during the experimental period (on day five) and pCO_2 (µatm), Ω Ca and Ω Ar were calculated in CO_2SYS.

Tank ID	Target pH	Temp	pH_{TS}	TA	pCO_2	ΩCa	ΩAr
		Measured *Daily*		Measured *Day 5*	Calculated in CO_2SYS *Day 5*		
4	8.00	22.6 ± 0.1	8.02 ± 0.02	2346.3	418.59	4.77	3.12
5	7.80	23.2 ± 0.3	7.75 ± 0.05	2315.6	853.23	2.91	1.91
3	7.60	23.1 ± 0.2	7.60 ± 0.04	2355.6	1180.17	2.26	1.48
1	7.40	23.0 ± 0.1	7.46 ± 0.01	2376.6	1959.76	1.50	0.98
2	7.20	22.5 ± 0.5	7.27 ± 0.01	2373.0	2953.27	1.02	0.67

Table 4.2: Summary of the results of the one-way analyses of means (not assuming equal variances) and pairwise t-tests with Bonferroni correction comparing variability in temperature and pH among the experimental treatment tanks. Significant values indicated in bold

One-way analysis of means (Temperature)	F (4) = 10.19, P = 0.0003			
Temperature	**8.02**	**7.75**	**7.60**	**7.46**
7.75	**0.012**			
7.60	**0.003**	1.000		
7.46	**0.005**	0.990	1.000	
7.27	1.000	**0.038**	0.115	0.205

One-way analysis of means (pH)	F (4) = 2291.1, P <**0.0001**			
pH	**8.02**	**7.75**	**7.60**	**7.46**
7.75	**2.2×10^{-5}**			
7.60	**5.0×10^{-8}**	**0.0004**		
7.46	**3.2×10^{-14}**	**2.5×10^{-5}**	**0.0004**	
7.27	**2.0×10^{-16}**	**5.0×10^{-7}**	**5.4×10^{-7}**	**1.2×10^{-10}**

Biological measurements

A total of 74 fish were used during the experiment. The average total length of the larvae was 12.04 ± 5.40 and mass (g) was 0.0143 ± 0.238 (see Table A.5 in the appendix for summary statistics of the biological measurements). The variability in size and mass was because the larvae were wild caught, and individuals from different cohorts may have been included. None of the biological parameters (length, mass, condition factor) showed a significant relationship with pH treatment (see Table A.6).

RMR, MMR and relative aerobic scope

Routine metabolic rate (RMR), maximum metabolic rate (MMR) and relative metabolic scope (RAS) were plotted against pH treatment and their relationships were assessed by means of a linear mixed effects model (LMEM), with measurement day (as an indicator of exposure time) as a random intercept (Fig 4.3 a, b, c). Regression analyses to further explore whether the increase in metabolic rates over the measurement days was equal across treatments revealed that only the highest pH treatment showed a significant increase in all three metabolic rates over the four measurement days. None of the fish in any of low pH tanks (7.75–7.27) showed a significant increase in their metabolic rates over time.

Linear mixed effects models revealed that pH treatment did not show a significant linear relationship with RMR ($P = 0.96$, Fig 4.3 a), MMR ($P = 0.88$, Fig 4.3 b) or RAS ($P = 0.69$, Fig 4.3 c). Metabolic rates showed neither an increase nor decrease in response to a decline in pH to levels as low as 7.27. Measurement day (when included as a random intercept in the LMEM) did not significantly influence the intercept or significance of the relationship between RMR ($F = 0.006$; $P = 0.94$), MMR ($F = 0.00003$; $P = 0.96$) and RAS ($F = 0.20$; $P = 0.67$) with pH treatment (as a fixed intercept).

The lowest individual RMR (3.47 mg O_2 g^{-1} h^{-1}) was recorded in the pH 7.75 treatment. Variability in metabolic measurements was generally high and similar across all pH treatments, except for RMR which showed slightly higher variability (5.02–30.84 mg O_2 g^{-1} h^{-1}) in the pH 7.27 treatment (Fig 4.3 a). However, size variability was also the greatest in this treatment. The highest individual MMR recorded (98.61 mg O_2 g^{-1} h^{-1}), which also resulted in the highest individual RAS (81.11 mg O_2 g^{-1} h^{-1}), was recorded in the pH 7.60 treatment. The lowest average RAS (20.30 mg O_2 g^{-1} h-1) was observed in the pH 8.03 treatment. Subsequently, the pH 7.75 treatment showed the most individual variability in

RAS compared to the other treatments. However, all these reported differences in metabolic rates were minor and no statistically significant difference in any of the metabolic rates among treatments were identified.

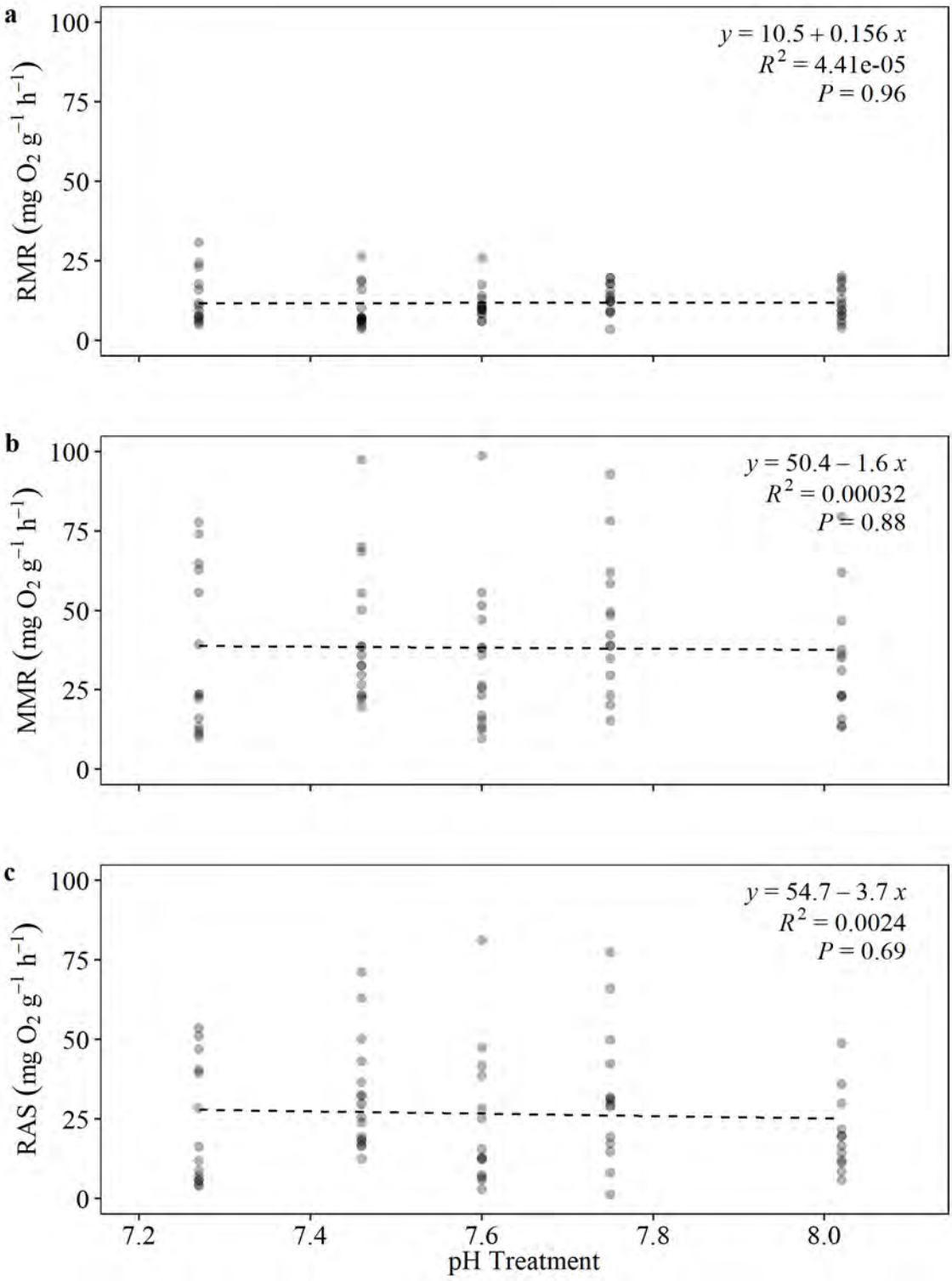

Figure 4.3: a) Routine metabolic rate (RMR), b) maximum metabolic rate (MMR) and c) relative aerobic scope (RAS) (mg O2 g^{-1} h^{-1}) at each pH treatment

Discussion

The physiological response of the post-flexion larval stages *of D. capensis* to low pH conditions was tested by exposing them to a gradient of pH conditions from 8.03 to 7.27 and measuring routine and maximum metabolic rates in order to calculate relative aerobic scope, which gives an indication of metabolic performance (Fry, 1971; Heuer and Grosell, 2014). Metabolic rates are a useful physiological parameter to identify a stress response as they indicate whether changes in environmental conditions elicit any basal (survival) or active (behavioural) responses (Claireaux and Lefrançois, 2007; Roche *et al.*, 2013). The results from the experimental study showed no effect of pH treatments as low as 7.2 on any of the measured metabolic rates (RMR, MMR and RAS). This suggests that post-flexion stage *D. capensis* appear physiologically adapted to coping with short-term exposure to low pH levels that may occur in coastal environments in the future due to coastal OA.

There appear to be no basal (SMR) or active (MMR) metabolic constraints in *D. capensis* larvae as the pH in their environment decreases. This means that the energy requirements for acid-base regulation and routine swimming activities are maintained within the metabolic capacity of the larvae. As a result of no changes in SMR and MMR, there is also no effect of pH on relative aerobic scope, suggesting that low pH likely would not impact other survival processes, such as growth or foraging activity (Pörtner and Farrell, 2008). Although it has long been assumed that low pH will increase SMR in fishes (Munday *et al.*, 2009a; Ern and Esbaugh, 2016) due to the apparent energetic demands of acid-base regulation, many recent studies have reported no effect of low pH on resting metabolic rates in many species (Couturier *et al.*, 2013; Esbaugh *et al.*, 2016; Lefevre, 2016). Since the emergence of these recent findings, it has been suggested that OA does not appear to be a consistent loading stress on fishes (Esbaugh, 2018).

In terms of activity-related metabolic rates (MMR) and metabolic scope, results have been more ambiguous and show variable responses among species (Esbaugh, 2018). Some species have shown increasing (e.g., *Hippoglossus hippoglossus*, Gräns *et al.*, 2014), and others show decreasing (e.g., *Ostorhinchus cyanosoma* and *Ostorhinchus doederleini,* Munday *et al.*, 2009a), aerobic scope at low pH levels (<7.8 pH) even when SMR is unaffected (e.g., *Dicentrarchus labrax*, Crespel *et al.*, 2019). However, it is also not uncommon for low pH treatments to elicit no response of either resting or active metabolic rates, even at pH levels that exceed projected OA scenarios. For example, Melzner *et al.* (2009a) found no effect of

pH levels as low as 7.01 on either SMR, MMR or metabolic scope on juvenile Atlantic Cod (*Gadus morhua*). The temperate juvenile blue rockfish (*Sebastes mystinus*) similarly showed no effect of low pH levels (7.32 pH) on SMR, MMR or aerobic scope (Hamilton *et al.*, 2017). This species also occurs in an eastern boundary upwelling system in habitats such as shallow rocky areas and kelp forests, which frequently experience high pH variability on a variety of temporal scales (Frieder *et al.*, 2012; Hamilton *et al.*, 2017).

The results from this study support the consensus in the literature that species inhabiting highly variable, coastal environments are expected to have sufficient metabolic capacity for regulatory processes (Cattano *et al.*, 2018). Studies on coastal fish, that are known to occur in dynamic coastal habitats, have similarly shown no metabolic response to low pH treatments (Ern and Esbaugh, 2016). For example, the estuarine red drum (*Sciaenops occelatus*) shows no effect of low pH ~7.22 on SMR in its juvenile stages, which has been attributed to its frequent exposure to natural pH variability in estuaries and ability to energetically manage the required regulatory processes (Ern and Esbaugh, 2016; Lonthair *et al.*, 2017). Another study, which assessed the medium-term exposure (~69 days) of juvenile European seabass (*D. labrax*) to projected OA conditions (7.82 pH) similarly found that there was no effect of pH treatment on RMR or MMR (Pope *et al.*, 2014). These temperate species also inhabit variable coastal environments and estuary mouths as juveniles and adults, which may explain their tolerance to the OA treatments over medium-term exposures.

The hypothesis, based on the concept of local adaptation (Vargas *et al.,* 2017), was that *D. capensis* would experience physiological stress at pH values deviating from the extreme of the present measured range of variability. However, the sufficient metabolic capacity in *D. capensis* appears to allow for additional investment of energy into acid-base regulation, at pH levels lower than the range measured in the shallow coastal environment in this study, without compromising activity. This may be a consequence of an under-estimation of the pH range experienced by the species, both at the sampling site (see Chapter Three) or within the total geographical range of the species. Similar to the red drum and European seabass, *D. capensis* are known to occur in highly variable coastal environments, such as the lower reaches of estuaries, tidal rock pools, surf zones and intertidal gullies (Beckley, 1985a; Heemstra and Heemstra, 2004). All of these habitats are known for their variability and show large differences in not only pH (up to 1 pH unit) but also in temperature, salinity, and dissolved oxygen (Ganning, 1971; Gibson, 1986; Hofmann *et al.*, 2011; Carstensen and

Duarte, 2019). It is therefore expected that postflexion stage *D. capensis* would show a high tolerance to the pH range tested in this study.

Diplodus capensis also utilize different habitats during their development. Although the early larval stages (hatching to pre-flexion) are associated with the pelagic marine environment (Pattrick and Strydom, 2008) a large number of postflexion larvae and early juveniles have been found to associate with shallow coastal gullies, subtidal reefs, the lower reaches of estuaries and intertidal surf zones (Lasiak, 1986; Strydom, 2008; Rishworth *et al.*, 2015; Edworthy and Strydom, 2016). The results from this study suggest that short-term tolerance to low pH conditions is evident even in the young post-flexion stages of *D. capensis*, which may explain their early occurrence in these dynamic shallow coastal areas, like the shallow gulley where they were collected for this study. A study by Kisten *et al.* (2020) in a permanently open estuary (Swartkops Estuary) opening into Algoa Bay, found that a small number of *D. capensis* larvae, as early as the flexion stage, also occur in the lower reaches of this estuary where pH is typically even lower than in other coastal areas. As such, tolerance to low pH even in the early stages can be expected. A better understanding of the changes in vulnerability to pH throughout ontogenetic development, coupled with information on habitat association throughout ontogenetic development would be required to investigate this further.

The larvae collected for this study were in their early post-flexion stage, and collected from a dynamic intertidal gully, where physico-chemical conditions, including pH, vary over various scales (See Chapter Three). Treatment levels were selected based on the *in situ* measured variability at this site to identify relevant current and future (below the current range) pH treatment levels (See Chapter Three). This intertidal site is situated in a macroalgal dominated habitat, characterized by high pH and high variability (up to ~0.55 units). The pH at this site was often higher than the highest tested pH level, especially during the day, which is typical of sites influenced by high biomass macroalgal aggregations (Wahl *et al.*, 2018). The presence of extensive macroalgae at this site may mitigate the effects of OA by increasing pH as well as variability. Wahl *et al.* (2018), similarly found that macroalgae, by increasing pH and its fluctuations, mitigated the effects of OA on blue mussel *Mytilus edulis*. Considering that young *D. capensis* generally associate with vegetated habitats (e.g., seagrass habitats in estuaries) (Edworthy and Strydom, 2016; Nel *et al.*, 2018), the occurrence of high pH levels in these habitats may similarly be influencing their response to pH.

Although short-term tolerance to low pH in *D. capensis* is likely, this study could not infer the long-term effects of continued regulation costs which may be associated with future OA. For example, a long-term study (1.5 years) on European Seabass showed a significant long-term increase in MMR at low pH conditions (~7.6), while SMR remained unaffected (Crespel *et al.*, 2019). Short term exposure of the same species to slightly higher pH levels of ~7.8, had no effect (Pope *et al.*, 2014). This indicates that although there appears to be no change in maintenance costs associated with pH regulation, there may be evidence for long term changes in metabolic pathways, particularly related to activity (Crespel *et al.*, 2019). The timeframes assessed in this study, however, were not long enough to test these long-term effects, which may be useful to assess in future research. Furthermore, it is difficult to identify whether short-term physiological plasticity will be beneficial in a coastal setting when the current and future variability in these habitats is largely unknown.

A further consideration is that metabolic rates can vary significantly (threefold) among individuals of the same species and age (Auer *et al.*, 2015; Norin and Metcalfe, 2019). This variability ultimately increases the intra-specific potential for metabolic adaptation in populations, which is likely contributing to the population-level tolerance observed in this study. It would be useful, in future studies, to maximize individual sample sizes to get the best possible population estimate of vulnerability/tolerance. This is a general limitation in physiological studies such as this one, due to the number of logistical constraints involved in experimental design and execution.

Another factor that may have influenced the lack of metabolic response in this study could have been the access to sufficient food during the experimental period. Food availability is known to modulate the physiological response of fishes to OA treatments. For example, a study by Cominassi *et al*. (2020) found that the effect of low pH treatments (7.6 pH) on juvenile European seabass (*D. labrax*) was not significant when allowed to feed *ad libitum*, but when feeding was restricted (to 25 % *ad libitum*), the effects of low pH on growth were enhanced. Since food provides energy for metabolic compensation at low pH conditions, it is possible that the effects of low pH were underestimated in this study due to the *ad libitum* feeding regime used during the experimental housing. Under natural conditions in the wild, food resources may be more limited, which may increase the sensitivity of *D. capensis* to low pH conditions. This highlights the need to consider restricted feeding when assessing the impacts of OA experimentally.

With so many factors influencing species responses to acidification, experimental laboratory studies remain limiting due to the difficulty in truly replicating natural conditions. However, there is value in understanding the response of species to low pH, particularly in the context of OA conditions predicted for the future to identify sensitive and tolerant species. The conclusion from this study is that post-flexion stage *D. capensis* appear tolerant of low pH conditions and have the metabolic capacity to persist in shallow coastal environments with relatively high pH variability. This aligns with the conclusions drawn in recent research that suggests that fishes, particularly those that associate with dynamic habitats, are generally physiologically tolerant to the low pH conditions that may occur because of OA. Further research, however, should focus on including other life stages and additional coastal habitats that this species associates with to fully understand the impact of future OA on this species.

Chapter 5

Swimming activity and feeding behaviour of Diplodus capensis *over a range of pH*

Introduction

Animals typically modify their behaviours as a first response to changes in their environment (Nagelkerken and Munday, 2016b). Marine organisms, including early-stage fishes, respond to their external environment through various sensory stimuli such as chemical, visual, or auditory cues (Ashur *et al.*, 2017). Low pH conditions associated with ocean acidification (OA) are known to influence the olfactory (e.g*.,* Munday *et al*., 2009b; Dixson *et al*. 2010; Cripps *et al.* 2011; Sundin and Jutfelt 2016), auditory (e.g., Simpson *et al.* 2011; Rossi *et al.* 2018) and visual (e.g*.,* Ferrari *et al.*, 2012; Chung *et al.*, 2014) sensory processes in the early life stages of some fish species. This has ultimate consequences for behaviours such as settlement (Munday *et al.*, 2009c), predator avoidance (Dixson *et al.*, 2010), behavioural lateralization (Jutfelt *et al.*, 2013a; Lopes *et al.*, 2016), boldness (Jutfelt *et al.*, 2013; Munday *et al.*, 2014), anxiety (Hamilton *et al.*, 2014) and feeding rates (Cripps *et al.*, 2011; Nowicki *et al.*, 2012). Changes in these behaviours may increase susceptibility to predation, affect food intake or alter the ability to orientate towards and recruit into settlement habitats, with ultimate downstream consequences (Nagelkerken and Munday, 2016a).

There are various reported mechanisms that may elicit sensory and behavioural responses in fishes during OA conditions. For example, a study by Rossi *et al.* (2016) linked disruptions in auditory orientation to increased otolith size in future OA conditions. Similarly, Bignami *et al*. (2013) reported that change in otolith structure (size and density) influenced auditory perception and associated behaviours in a tropical species. Possibly the most frequently reported mechanism for behavioural changes associated with OA is the disruption of neural functioning, specifically of the GABA-A receptor, during low pH conditions (Nilsson *et al.*, 2012; Tresguerres and Hamilton, 2017; Jiahuan *et al.*, 2018a). This disruption has been linked to changes in olfactory sensory ability in fishes (Nilsson *et al.*, 2012; Lai *et al.*, 2015; Jiahuan *et al.*, 2018b), which ultimately alters behaviour (Heuer *et al.*, 2016). In addition to the direct changes in behaviour associated with neural and sensory organs, changes in development and growth may have indirect effects on behaviours.

Laboratory methods to identify behavioural changes associated with swimming at low pH have included assessments of swimming performance (e.g., Melzner *et al*., 2009; Cominassi

et al., 2019) and swimming activity (e.g., Nowicki *et al*., 2012; McCormick *et al*., 2013). Although OA does not appear to affect the swimming performance (by measures of critical or endurance swimming ability) in the early life stages of many species, changes in swimming activity have been reported (e.g*.,* in juvenile Atlantic cod *Gadus morhua* (Melzner *et al.*, 2009a; Maneja *et al.*, 2013), larval dolphinfish *Coryphaena hippurus* (Bignami *et al.*, 2014) and larval Atlantic herring *Clupea harengus* (Maneja *et al.*, 2015). For example, increased swimming activity at low pH was reported in the juveniles of coral trout *Plectropomus leopardus* (Munday *et al.*, 2013), hardyheads *Atherinosoma microstoma* and southern longfin gobies *Favonigobius lateralis* (Rodriguez-Dominguez *et al.*, 2019). These findings suggest that swimming ability may not be affected by a metabolic or developmental constraint but rather through changes in behaviour. However, in one study on the larval stages of dolphinfish, reduced activity levels co-occurred with metabolic depression, suggesting that metabolic capacity limited activity levels in this species (Pimentel *et al.*, 2014).

Changes in behaviour may also have consequences for feeding as prey capture is intrinsically linked to changes in swimming activity (Cripps *et al.*, 2011). In a recent review, Cattano *et al*. (2018) reviewed the effects of moderate (~8.0) to low (~7.8) pH treatments on fish behaviour and foraging activity, especially in the larval stages, with several studies finding that swimming activity increases and feeding activity decreases at moderate and low pH levels. This may be a result of the reduced ability of fishes to use olfactory mechanisms to detect prey in low pH treatments, resulting in increased swimming activity and visual searching for prey (Cripps *et al.*, 2011; Bignami *et al.*, 2014).

The aim of experiments presented in this chapter was to examine whether baseline swimming activity and feeding rate in the post-flexion stages of the study species, Diplodus capensis, would be modified when exposed to pH treatment levels that exceed the levels that they are currently exposed to in the wild. Similar to the hypothesis for the metabolic response to low pH (in Chapter 4), it was expected that changes in routine swimming activity and feeding rate in D. capensis would only occur at pH treatment levels below the minimum pH recorded in their natural habitat.

Methods

Swimming activity video recordings

Post-flexion stage *D. capensis* were collected from the wild and exposed to five pH treatments (ranging from 8.03 to 7.27) for five days as described in Chapter Four. Experiments (swimming activity trials) to quantify baseline swimming activity were conducted within four days following the five days of exposure to the pH treatments. This involved four to five video recorded swimming activity trials (each lasting approximately five minutes) for each pH treatment over the four days that the measurements took place. Each swimming activity trial used different larvae from the experimental tanks.

Pilot trials were conducted to identify the best approach for the swimming activity trials. These trials revealed that individual larvae showed signs of stress (swimming along the edge of the tank and not feeding) when alone in the experimental chamber. As this is a shoaling species, we opted to measure the swimming activity of *D. capensis* in a group of five larvae at a time. Therefore, for each swimming activity trial, five larvae, from one treatment tank at a time, were introduced into a small 12 X 12 cm square glass tank with a white bottom containing seawater with the appropriate treatment pH, temperature, and salinity at 4 cm depth. The water used for the experiments was aerated until the recordings commenced, however, there was no aeration during the recordings as this would obscure the video image. The order of the experiments followed the order of treatments from 8.02 to 7.27, and the order was reversed on alternate days. To minimize disturbance, the tank was placed in an opaque container, which contained a lamp that was orientated to ensure uniform lighting and minimise shadows for the video recordings. A 2 cm scale bar was drawn onto the bottom of the tank as a size reference for image scaling (Fig 5.1).

Figure 5.1: Image of the test arena containing five larval *Diplodus capensis* and a 2 cm scale bar for size reference

A Huawei p20 pro smartphone with a 40-megapixel triple sensor Leica camera was fixed above the tank to record swimming activity. The video period lasted for a total of five minutes where only the final one minute of the recording was used for analysis. The first four minutes allowed for habituation to the test tank. Recording quality was set to 1080 pixels at 30 frames per second (fps). VLC media player software was used to crop the last minute of the recorded video for swimming activity analysis. The automated video tracking software idTracker (Romero-Ferrero *et al.*, 2019) was used to process the videos and extract swimming data for each individual.

Trajectories

Trajectory data (x, y position in the test tank) for each individual were obtained through the idTracker software using an algorithm that identifies individuals and detects their position

throughout the time series. These trajectories were first scaled and visualized to ensure their reliability (example of a suitable individual trajectory shown in Fig 5.2). Only reliable trajectories with few individual identification errors (>70 % detection accuracy) were used for further data extraction. The accurate individual trajectories were used to calculate total swimming distance (cm) (which was the total distance covered by each individual during the 1 min video recording) and average swimming speed (cm s^{-1}) (the average swimming speed of each individual during the 1 min video recording). All trajectory visualizations and parameter calculations were conducted in R Studio version 1.2.5042 using the package Trajr for trajectory analysis (McLean and Skowron Volponi, 2018).

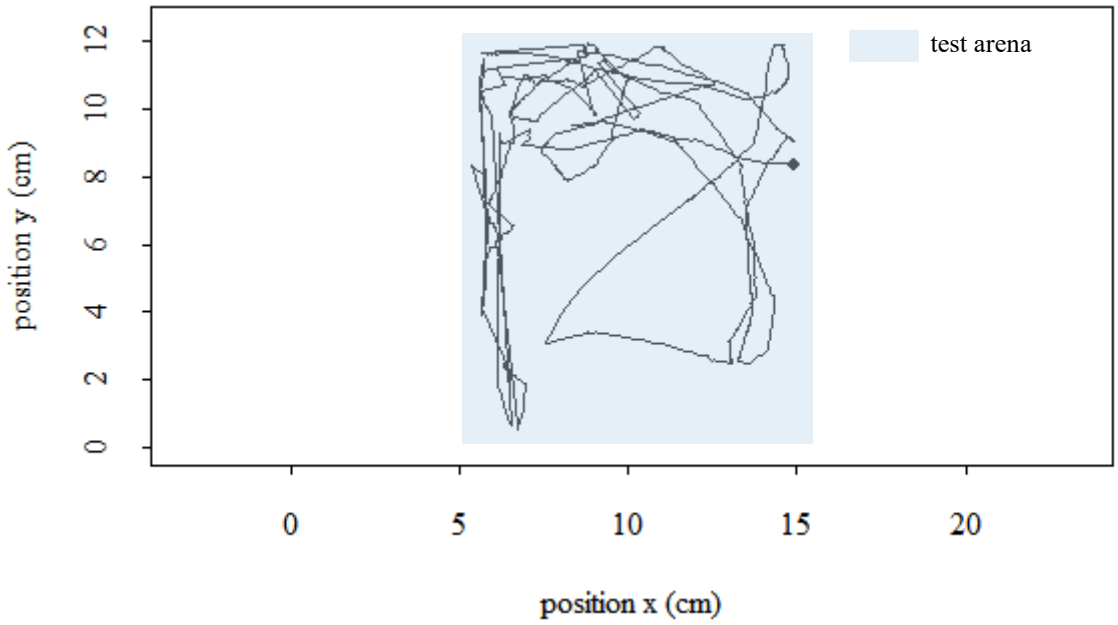

Figure 5.2: Example of an accurate individual trajectory from an individual from the pH 7.60 treatment on Day 4 (for an animated example of moving trajectories (GIF) see https://twitter.com/fishseaology/status/1194525880992882688)

Regression analysis was used to examine the relationship between pH and average swimming speed, and pH and swimming distance. The effect of pH treatment on these two parameters was compared using separate linear regression models. Measurement day was first included in the models to identify if acclimation to experimental treatment conditions and experimental methods potentially affected swimming activity as well as to account for trial related influences. Since the larvae were tested in groups, individuals could not be identified and assigned to individual trajectories after being euthanised. As such, individual size or

mass could also not be assigned to the trajectories and therefore size-related differences in swimming activity could not be accounted for.

The distribution of residuals (residual diagnostic plots) for swimming distance and swimming speed data were visually assessed to ensure the data were suitable for the application of linear models by ensuring the normal distribution of residuals and homoscedasticity. Homogeneity of variance was tested using a Bartlett's test.

The feeding experiments directly followed each of the five-minute video recordings for swimming activity assessments. The larvae were not fed for 12 - 15 hours prior to the feeding experiments. Seawater (fixed volume of 2 mL) containing a fixed density (~350–400 individuals mL^{-1}) of flash frozen artemia nauplii (*Artemia salina*) was added to the glass experimental tank containing the same five larvae used in the swimming activity trials. The density of the food supplied was based on pilot studies that revealed that the larvae ate between 30 and 50 artemia within a minute. Therefore, a higher density of food was used so that there was no need for the five larvae in the experimental chamber to compete for food. The food was introduced to the experimental tank using a long tube attached to a pipette so that the food could be introduced without opening the opaque container and disturbing the larvae. The larvae were allowed to feed on the artemia for one minute before being immediately euthanized in the test tank with an overdose of clove oil, which was administered directly into the experimental tank using a pipette. The larvae were then removed from the tank, weighed to the nearest 0.0001 g and total length was measured using a digital calliper to the nearest 0.01 mm. The stomachs of the larvae were then immediately extracted by dissection at 5x magnification under a dissection microscope (Zeiss Stemi508) and stored in 10% formalin for later analysis. The contents of the fish stomachs were later extracted and the number of individual artemia consumed by each individual were counted under the dissection microscope.

Feeding rate was divided by individual mass (mg) to account for size-related differences in feeding prior to further analysis. The relationship between individual feeding rate (no. prey consumed mg^{-1} min^{-1}) and pH treatment was assessed by fitting a simple linear regression model.

The distribution of the data (Shapiro–Wilk tests) and residuals (residual diagnostic plots) were visually assessed to ensure the data were suitable for the application of linear model

analysis by ensuring the normal distribution of residuals and homoscedasticity. Homogeneity of variance was assessed using a Bartlett's test.

Results

Swimming distance and swimming speed

Swimming distance (cm) and average swimming speed (cm s^{-1}) were measured in a total of 64 individuals. The 7.27 pH treatment had the least replication (n = 8 individuals from only two experiments) due to poor individual detection during video analysis (Table 5.1).

Preliminary data assessments revealed that swimming distance and swimming speed were significantly correlated (Pearson correlation: R (62) = 0.91, P<0.0001) (Fig A.5 in appendix), which is why the two models were developed separately. Measurement day significantly influenced the relationship between swimming distance and pH treatment level (t = -2.45; P = 0.02) and was retained in the final swimming distance model. However, measurement day did not significantly influence the relationship between swimming speed and pH treatment (t = -1.39; P = 0.17) and as such this parameter was excluded from the final swimming speed model.

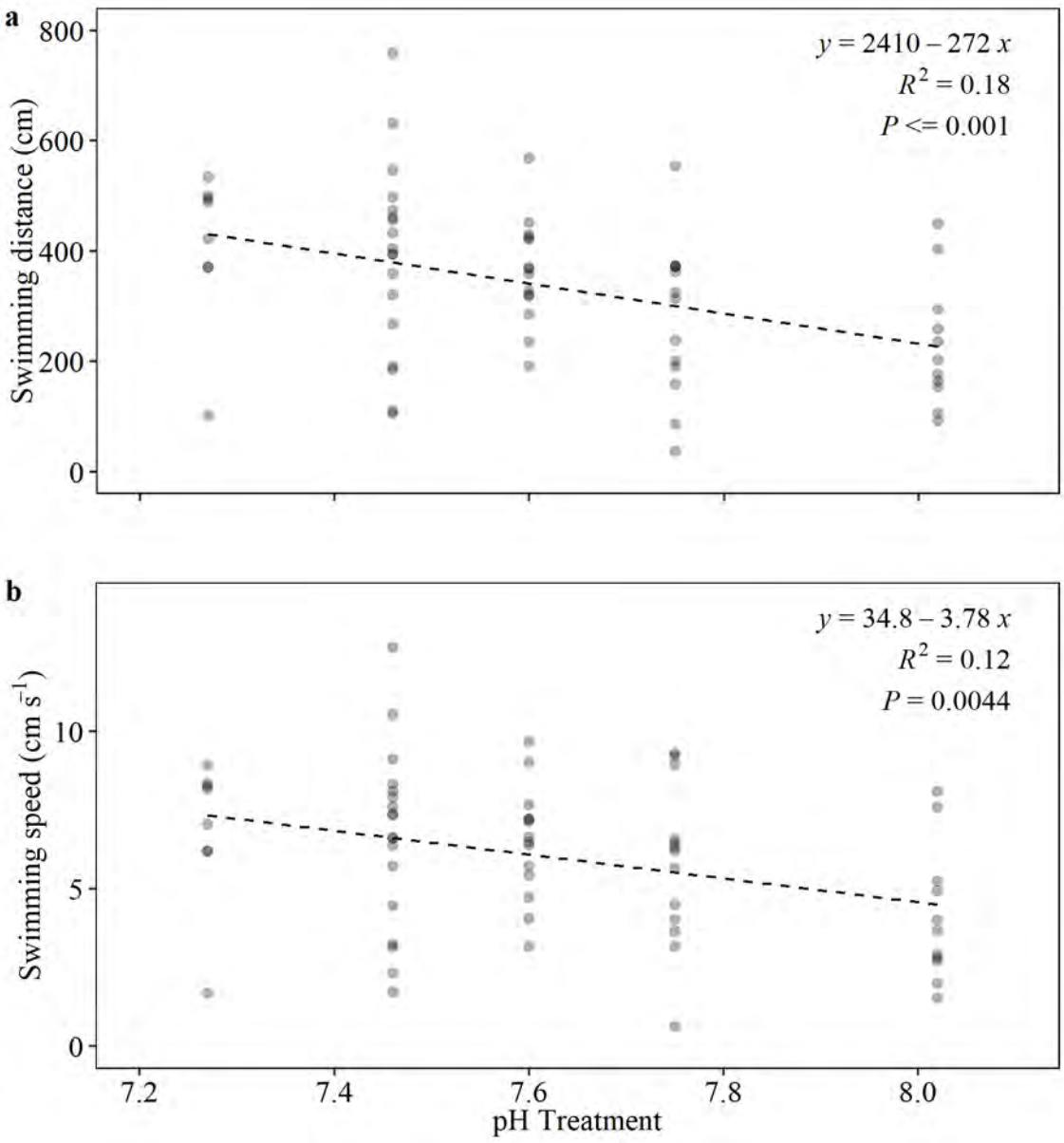

Figure 5.3: a) Total swimming distance (cm) and b) average swimming speed (cm s^{-1}) of *Diplodus capensis* larvae during experimental activity trials at each pH treatment

The shortest average swimming distance was observed for larvae in the 8.02 pH treatment (231 ± 115 cm) as well as the slowest average swimming speed (4.13 ± 2.15 cm s^{-1}). The longest average swimming distance was observed for larvae in the in the 7.27 pH treatment (410 ± 139 cm), which also showed the fastest (6.84 ± 2.32 cm s^{-1}) average swimming speed (Table 5.1). Regression models revealed a negative linear relationship between both swimming distance (F (63) = 13.64, P = 0.0005) and average swimming speed (F(63) = 8.73, P = 0.004) with increasing pH (Fig 5.3 a, b).

Variability in swimming distance and swimming speed was high across all treatment levels (See Fig A.6 in the appendix). The 7.46 pH treatment showed the most variability (Table 5.1). The least variability was in the 7.27 pH treatment, except for one individual that showed a noticeably short swimming distance and low average swimming speed (Table 5.1, Fig 5.3).

Table 5.1: Summary statistics of *Diplodus capensis* swimming activity

			Swimming distance (cm)	**Swimming speed (cm s^{-1})**
pH Treatment	*N (individuals)*	*No. of trials*	*Mean ± SD*	*Mean ± SD*
8.0	11	3	231 ± 115	4.13 ± 2.15
7.75	13	3	276 ± 141	5.74 ± 2.55
7.60	14	4	362 ± 95	6.46 ± 1.78
7.46	18	4	388 ± 173	6.61 ± 2.85
7.27	8	2	410 ±139	6.84 ± 2.32

Although individual lengths for the swimming experiments could not be measured, the mean size of experimental animals did not increase from the beginning of the experiment (see Fig A.7 in appendix). Conversely, the size of the larvae measured in the experiments decreased over the four day experimental period. The inclusion of measurement day in the regression model significantly influenced the slopes of the relationship between swimming distance and treatment (t (62) = -2.45, P = 0.02). Swimming distance declined significantly (y = -39.11x + 434.90, r^2 = 0.10, P = 0.009) with time during the four day experimental period. There was a significant relationship between swimming distance and pH treatment on the first day of the experiments, after which pH treatment had no effect on swimming distance in the following three days. However, the number of pH treatments tested on each day were not even due to failed experiments (Table 5.2).

Table 5.2: Regression model results for swimming distance (cm) with pH treatment on each day of the experiments. Significant results are indicated in **bold**

Measurement day	pH treatments represented	Coefficient	*t*-value	*P*	R^2
Day 1	7.46, 7.60, 7.75, 8.02	**-4.43**	**-2.96**	**0.009**	**0.34**
Day 2	7.27, 7.46, 7.60	6.88	1.33	0.21	0.15
Day 3	7.27, 7.46, 7.75	-3.62	-1.08	0.31	0.10
Day 4	7.46, 7.60, 7.75, 8.02	0.28	0.10	0.92	< 0.01

The distribution of the swimming distance (Shapiro–Wilk: W = 0.98, P = 0.42) and swimming speed data (Shapiro–Wilk: W = 0.98, P = 0.42) and residuals met the assumptions for the application of linear models. Bartlett's tests revealed that there were no significant differences in the variances of swimming distance (Bartlett's: B (4) = 5.29, P = 0.26) and swimming speed (Bartlett's: B(4) = 3.28, P = 0.51) among pH treatments.

Feeding rate

Individual feeding rate was measured in a total of 99 *D. capensis* larvae over the four-day measurement period.

Preliminary data assessment revealed that individual feeding rate, expressed as number of artemia consumed in one minute, showed a significant correlation with fish mass (mg) (Pearson correlation: R = 0.50, df = 97, P <0.0001) (Fig A.8 in appendix). Therefore, individual feeding rate was divided by individual mass prior to further analyses.

The highest average feeding rate (no. prey consumed mg^{-1} min^{-1}) occurred in the 7.75 pH treatment (2.62 ± 0.82) and the lowest in the 7.2 pH treatment (1.99 ± 1.02) (Fig 5.4, Table 5.3). Summary statistics are presented in Table 5.3. Average feeding rate showed no relationship with pH treatment (F (97) = 0.68, P = 0.41) (Fig 5.4) indicating that pH treatment did not influence individual feeding rates.

Table 5.3: Summary statistics of *Diplodus capensis* feeding rate (no prey consumed mg^{-1} min^{-1})

		Feeding rate (no. prey consumed mg^{-1} min^{-1})	**Mass (g)**
pH Treatment	*N*	*Mean ± SD*	*Mean ± SD*
8.02	21	2.16 ± 1.02	10.77 ± 2.96
7.75	21	2.62 ± 0.82	13.94 ± 3.58
7.60	20	2.30 ± 0.94	11.78 ± 2.61
7.46	20	2.41 ± 0.98	13.50 ± 2.62
7.27	20	1.99 ± 1.02	12.29 ± 3.33

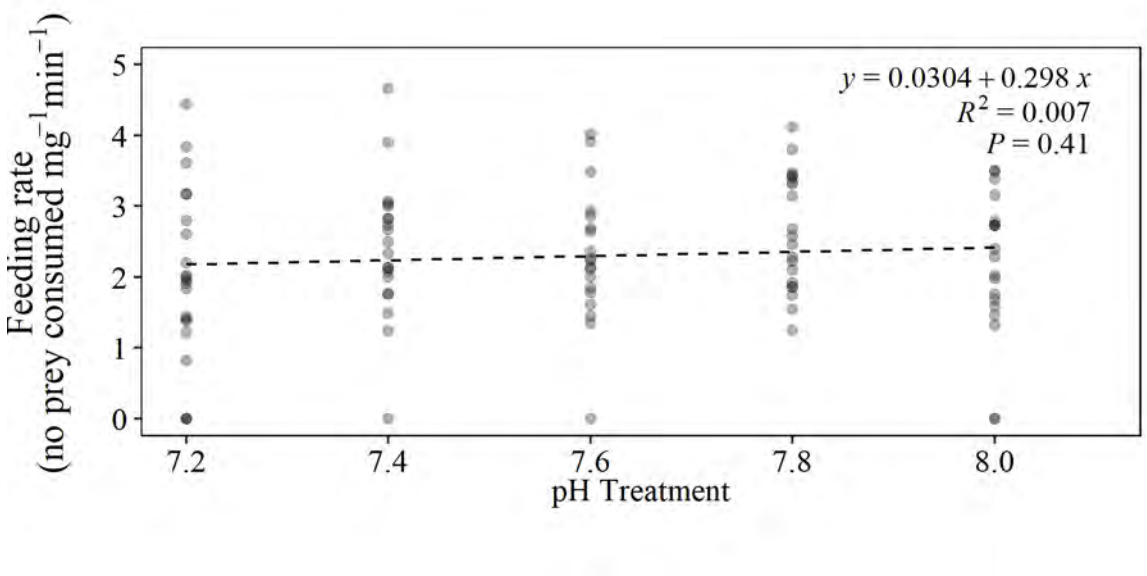

Figure 5.4: Individual feeding rate (no. prey consumed min^{-1} mg^{-1}) of *Diplodus capensis* during experimental trials at each pH treatment

The distribution of the feeding rate data residuals met the assumption for the application of a linear model. A Bartlett's test revealed that there were no significant differences in the variance among pH treatments (K^2 (4) = 3.46, P = 0.48).

Discussion

There was an increase in the swimming activity (both swimming distance and speed) of *D. capensis* post-flexion larvae with a decrease in pH in the experimental environment. As the increased swimming activity was not reflected by a metabolic response (see Chapter Three), it appears that potential behavioural changes, rather than metabolic constraints, are likely responsible for the observed differences in swimming activity. Although swimming activity increased, there was no change in feeding behaviour among treatments.

A few studies have examined activity levels at low pH treatments. The majority of these have found no impact of low pH on swimming activity, such as in larval cobia (*Coryphaena hippurus*) at pH 7.54 (Bignami *et al.*, 2014), temperate juvenile goldsinny wrasse (*Ctenolabrus rupestris*) at pH 7.68 (Sundin and Jutfelt, 2016) and even in young newly hatched sharks (*Heterodontus portusjacksoni*) at pH ~7.8 (Pistevos *et al.*, 2017). In the tropical environment, however, Munday *et al*. (2013) found significantly higher levels of activity in juvenile coral trout *Plectropomus leopardus* at low pH (7.85 pH) compared to control conditions (8.1 pH). They attributed this to bolder behaviour at low pH despite this species usually being naturally cryptic (Munday *et al.,* 2013). Similar increased activity levels and boldness were also reported for other tropical species such as adult five-lined cardinalfish, *Cheilodipterus quinquelineatus* at 7.86 pH (Devine *et al.*, 2012). While increased activity levels and boldness may not affect juvenile fishes (such as *D. capensis*) directly, it is likely that these behaviours may substantially increase the risk of predation (Munday *et al.,* 2013). This was the case in coral trout, which also appeared to be attracted to the odour of predators in low pH treatments (Munday *et al*., 2013).

Up to now, there has been limited understanding of the mechanism driving changes in activity in low pH conditions. Cripps *et al.* (2011) suggested that changes in activity levels in coastal species at low pH may be a result of increased reliance on visual prey detection when olfactory abilities are reduced. Reduced olfactory abilities in low pH conditions have been reported in many juvenile coral reef species (Munday *et al.*, 2009c; Dixson *et al.*, 2010; Cripps *et al.*, 2011) and in some temperate coastal species, such as the larval stages of black seabream (*Acanthopgrus schlegelii*) (Jiahuan *et al.*, 2018b) and juvenile seabass (*Dicentrarchus labrax*) (Porteus *et al.*, 2018). These studies attribute reduced olfactory abilities to interference with neurotransmitter functioning of the GABA-A receptor, which ultimately disturbs olfactory detection of chemical cues (Nilsson *et al.*, 2012; Regan *et al.*,

2016; Ashur *et al.*, 2017). This occurs due to changes in extracellular ion concentrations (HCO_3^- and Cl^-) because of acid-base regulation processes employed when internal pH is lowered (Nilsson *et al.*, 2012; Tresguerres and Hamilton, 2017). This can inhibit proper neural functioning, which has been mechanistically linked to alteration of a number of fish behaviours such as lateralization, activity levels, boldness, and olfactory signal detection (Heuer *et al.*, 2016; Schunter *et al.*, 2019). The effects of OA on behaviour are reversible when applying a GABA antagonist (gabazine) treatment. Treating OA exposed fish with gabazine has shown a reversal of the effects of OA on behaviours such as lateralization (Lai *et al.*, 2015; Lopes *et al.*, 2016), anxiety (Hamilton *et al.*, 2014) and olfactory identification of predators (Chivers *et al.*, 2014). These studies have suggested that change in behaviour is not driven by other mechanistic drivers such as otolith structure. Similarly, in this study, since the fish were only exposed to low OA conditions for a short period (5–9 days), it is unlikely that skeletal defects or otolith structure would be responsible for the changes in behaviour at low pH.

Despite changes in swimming activity, low pH (7.27) did not affect feeding rates of *D. capensis* larvae. This is similar to the findings of Nowicki *et al.* (2012) and Rodriguez-Dominguez *et al.* (2019) in their OA research on feeding behaviours of juvenile anemonefish *Amphiprion melanopus* and hardyheads *Atherinosoma microstoma* where no effect of low pH (7.9–8.1) was observed. Other studies, which have typically used choice chambers to assess feeding have found that low pH treatments may have a negative impact on olfactory prey detection (e.g., brown dottyback *Pseudochromis fuscus*, Cripps *et al*., 2011), foraging behaviour (e.g., black sea bream, Jiahuan *et al*., 2018) and feeding rates (Cripps *et al.*, 2011; Chivers *et al.*, 2014; Pimentel *et al.*, 2016). However, fishes do not only rely only on olfaction to detect prey and use visual and other sensory mechanisms for foraging and feeding (Cripps *et al.*, 2011). The methods used in the feeding experiments in this study allowed for both olfactory and visual detection of prey during the experiments, which may explain why feeding was not impaired by pH treatment: fish relied on all sensory mechanisms to detect prey rather than limiting them to olfaction only. Considering that activity levels and feeding are inherently linked (Cripps *et al.*, 2011; Pimentel *et al.*, 2016), the increased activity levels at lower pH levels in *D. capensis* may be indicative of their increased reliance on visual searching for prey due to olfactory abilities being inhibited. However, this would need to be assessed with further olfactory experiments to correctly identify the trade-offs in these mechanisms.

It is also necessary to consider that high prey densities were used in the feeding experiments, allowing the larvae to feed *ad libitum* during the 1 min feeding experiments. This may have influenced the experimental results showing no effect of low pH on feeding, as feeding was not limited in any way. It has been suggested, however, that at lower prey densities when *ad libitum* feeding is not possible, fishes may be more susceptible to the effects of environmental stress such as OA (Cominassi *et al.*, 2020). This has been shown in juvenile sea bass (*D. labrax*) where the impacts of OA and warming on feeding efficiency were exacerbated when feeding was restricted, which ultimately affected growth (Cominassi *et al.*, 2020). Therefore, although increased swimming activities in *D. capensis* may have occurred to increase feeding under stressful low pH conditions, this experimental design may not have been sensitive enough to detect this. There are other potential reasons for increased swimming activity not related to feeding, such as anxiety or an adaptive escape response, but these were not assessed in this study.

It is important to consider the limitations of laboratory experiments when comparing studies (Clark *et al.* 2020; Munday *et al.* 2020) or inferring the response of animal behaviour to scenarios typical of their wild environments. It cannot be discounted that the change in activity levels shown in this study may have been influenced by the laboratory environment or experimental design. An observation during this study was that *D. capensis* larvae are extremely quick to adapt to captivity and the laboratory environment. For example, larvae held in captivity for more than five days quickly became accustomed to accepting food. Although effort was made to minimize regularities in feeding (by randomizing feeding times, see methods in Chapter Four) to avoid learned behaviour, it is impossible to discount the effect of this on the experimental process.

Despite the numerous limitations involved in interpolating laboratory experiments to real world scenarios (see general discussion), simple experiments to quantify the basic behaviours of early-stage fishes is important when identifying their response to various environmental stressors. Many studies have focused on the physiological impacts of OA when most fishes are rather impacted at a behavioural level (Cattano *et al.* 2018, Clements and Hunt 2015). Even though early-stage *D. capensis* appear physiologically tolerant of low pH (Chapter 4), routine swimming behaviours may be influenced, which could have subsequent consequences in the long-term. This research provides the first insight to the behavioural response of *D. capensis* larvae to acidification, in the context of the current measured pH conditions. Future

research should expand this understanding by assessing the mechanisms behind the observed behavioural response to truly capture the response of this species to future OA.

Chapter 6

General discussion and conclusion of results

Overview of findings

The threat of anthropogenic global changes on marine ecosystems and the services they provide is well established. Ocean acidification (OA), resulting from increased anthropogenic CO_2 emissions, has come to the forefront as a potentially concerning driver of change in marine environments. However, in order to fully understand the impacts of OA there is a need to quantify local-scale variability in carbonate chemistry and pH, particularly in coastal areas in regions where data are lacking, and then to identify the vulnerability of important local species and resources to pH values outside of this range.

This study addressed these research gaps by 1) quantifying spatial and temporal variability in pH and carbonate chemistry in a coastal bay situated in the warm-temperate region of South Africa and 2) quantifying the vulnerability of a local fisheries species under the hypothesis that *Diplodus capensis* is adapted to local variability and that it is only sensitive to low pH at levels below its natural exposure. The research presented in this thesis is in line with international research initiatives but also has significant local relevance and presents a framework for future OA monitoring.

Local variability in pH and driving processes

This study is the first to monitor inshore variability in pH and carbonate chemistry in the southern, warm-temperate bioregion of South Africa, expanding the understanding of local variability from previous work that focused on the Benguela Current Large Marine Ecosystem (BCLME) in the Atlantic Ocean region on the west coast of the country (Santana-Casiano *et al.*, 2009; Gregor and Monteiro, 2013). Considering that the South African coastline has been identified as a climate change 'hotspot', based on the fact that the coastline experiences higher than global average temperatures, there is a need to expand this understanding to include other physical drivers of change, such as pH (Hobday and Pecl, 2014; Popova *et al.*, 2016). This work has not only provided important baseline information of coastal pH variability (see Chapter 3) but has also provided recommendations for monitoring strategies for future coastal OA research in Algoa Bay and other parts of South Africa.

Although the discrete monthly sampling strategy used in this study was useful in capturing long-term trends, in coastal environments this type of sampling design may not fully encapsulate the rapid, short-term fluctuations in carbonate chemistry conditions that occur over short time scales. The iSAMI-pH sensor used to capture diurnal trends proved to be the ideal equipment to collect high-frequency, continuous measurements and capture the 'ocean weather'. This was illustrated by the short-term (five day) deployment of the autonomous iSAMI-pH sensor that measured pH half-hourly and revealed a ~0.55-unit diurnal variability in pH. The deployment of autonomous sensors would also increase the possibility of capturing daily variations and upwelling events. It is for these reasons that continuous measurement systems (such as iSAMI-, SAMI-pH or similar sensors) rather than discrete sampling methods are recommended for future pH monitoring in Algoa Bay.

The results from the *in situ* monitoring conducted in this study revealed considerable spatial and temporal variability in pH and associated carbonate chemistry (Chapter 3). Variability among sites appeared to be driven by typical coastal processes such as biological activity and upwelling events. The overall pH varied among sites (offshore and inshore) by ~0.69 pH units (range = 7.27–8.41). Based on these spatial and temporal differences in pH conditions, sites that capture the full variability of the bay and that represent the coastal processes driving variability are recommended for future monitoring efforts.

Among the offshore sites, the highest variability occurred at sites O8 and O5 (Fig 3.2b, Chapter 3). Variability at the sites appeared to be primarily driven by event scale processes, driving lower than average pH conditions, where pH levels <7.80 were recorded. These offshore sites should be considered for future continuous monitoring (e.g., by the *in situ* continuous monitoring systems such as iSAMI-pH sensors) in order to capture the variability in pH that potentially occurs as a result of upwelling in Algoa Bay (Fig 6.1). Conversely, the lowest variability was observed at site O7 at the centre of the bay (Fig 6.1), which is likely the most representative of the expected adjacent 'open ocean' conditions. This site would serve as a good 'control' site to establish baseline conditions (Fig 6.1). These suggested offshore sites are currently monitored by the South African Environmental Observation Network's Continuous Monitoring Program, where continuous temperature measurements are being recorded. This means that offshore monitoring infrastructure is already available and the addition of continuous pH monitoring at these suggested sites, to capture spatial and temporal pH variability, would add value to an existing program.

The average pH recorded at the inshore sites was 8.04 ± 0.15 SD, and the range was 0.61 pH units. The clearly atypical site at Cape Recife is responsible for most of this variability, with the remaining inshore sites showing relatively stable pH levels (Fig 3.2b, Chapter 3). The site at Cape Recife, contrary to any other sites in the bay, consistently showed high pH conditions (8.30 ± 8.41). This site is situated in a habitat dominated by red algae (Fig 6.1), which is likely contributing to the unique conditions that occur here. Both the offshore and inshore site at Cape Recife would be valuable sites for future continuous monitoring to represent the interaction of key processes, such as autotrophic biological activity and upwelling that drive the highest pH variability in the bay over various spatial and temporal scales (Fig 6.1). The conditions in the intertidal areas at Cape Recife, on the calmer leeward side of the bay, are conducive to the long-term deployment of a sensor. However, at the other inshore sites in the bay (I2–I5) it is unlikely that the deployment of a sensor would be possible, due to rough conditions and high wave action.

Although estuarine habitats were not monitored this study, it might be useful to consider monitoring pH conditions in the two large permanently open estuaries in Algoa Bay. Freshwater influx is known to alter pH in adjacent coastal waters due to its lower pH and alkalinity conditions compared to marine seawater (Vargas *et al*., 2016; Huang *et al*., 2015). The flow of freshwater into Algoa Bay from these two large estuaries, although not measured during this study, may be altering the carbonate chemistry of adjacent sites depending on the flow of freshwater. These two estuarine systems also differ in physical and biological conditions. The Swartkops Estuary has a strong tidal marine influence in the lower reaches and supports extensive stands of submerged aquatic vegetation (*Zostera capensis*) which are known nursery areas for many larval and juvenile fishes, including *D. capensis* (Edworthy and Strydom, 2016; Kisten *et al*., 2020). Conversely, the Sundays Estuary, which has a smaller mouth and steep banks with less tidal influence has less vegetation than the Swartkops (Sutherland *et al.,* 2012). In these estuaries, pH can vary significantly between surface (8.0–8.5) and bottom waters (7.8–8.5) (James and Harrison, 2010). A comparison of pH conditions in these two systems would be useful in identifying their contribution as refugia from coastal acidification and serve as focus areas for future research.

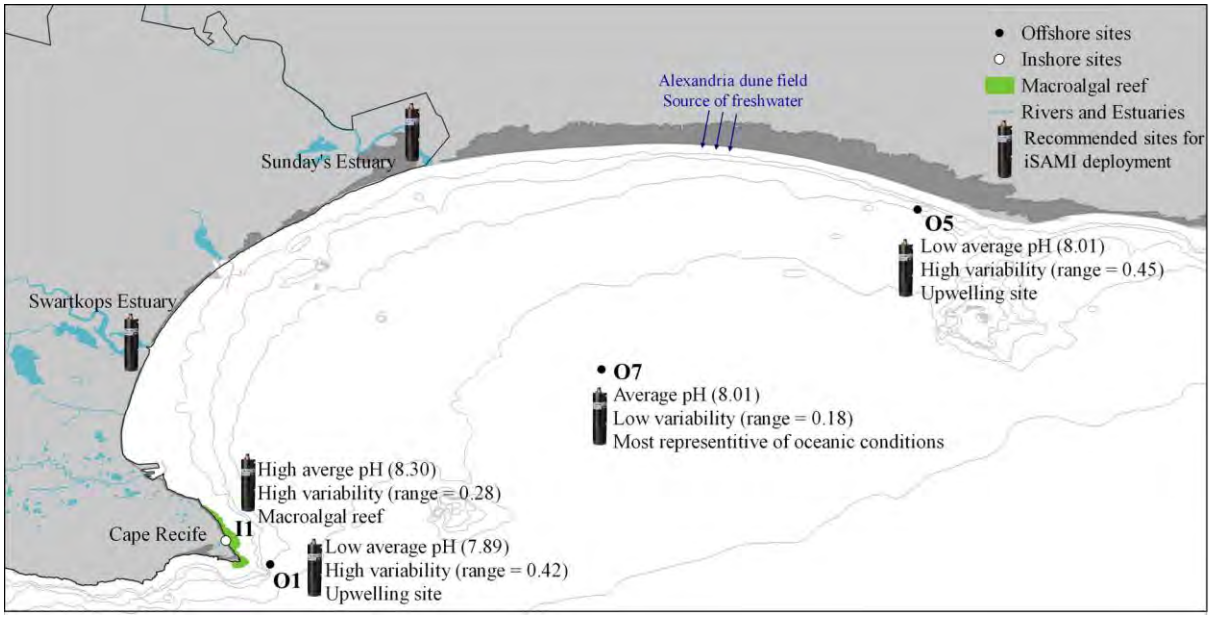

Figure 6.1: Recommended sites for the deployment of continous pH monitoring sensors (iSAMI-pH/SAMI-pH), based on the criteria contributing to their relevance for future monitoring efforts in Algoa Bay

In a biological context, the findings from this study highlight the importance of considering microhabitat variability and habitat association by species when assessing their response to future OA. For example, the high pH and variability recorded at the site at Cape Recife is particularly pertinent to the study species, *D. capensis*, which occurs in the intertidal gullies adjacent to this site in high numbers during their postflexion stages. This species also associates with other vegetated habitats in Algoa Bay, such as seagrass beds in the lower reaches of the permanently open Swartkops Estuary (Edworthy and Strydom, 2016; Nel *et al*., 2018) where pH levels are expected to vary similarly due to photosynthetic and respiratory biological activity. The occurrence of high pH levels at these sites during the day may mitigate the effects of future OA on this species, by acting as a temporal refuge of higher pH conditions (Kapsenberg *et al*., 2015; Wahl *et al*., 2017; Wolfe *et al*., 2020). These sites that are modulated by high autotrophic biological activity may therefore act as refugia for coastal fishes (Koweek *et al*., 2017; Kapsenberg and Cyronak, 2018), particularly in their early life stages, and should be a focus in future research. This information would add to global knowledge of how coastal microhabitats may serve as refuge habitats (Wolfe *et al*., 2020), which will allow for effective mitigation of the effects of OA on coastal species, by identifying sites that should be managed and protected (Kapsenberg and Cyronak, 2018).

Future studies here, and in other parts of the world would do well to consider the extent of pH variability over multiple scales of space and time and use these findings to design appropriate controls and treatments for the selected study species (Pespeni *et al*., 2013; Kapsenberg and Cyronak, 2018). To ensure that appropriate treatments are selected for biological response experiments, a continuous monitoring approach may provide better representation of pH variability. To maintain biological realism, this monitoring should also take place at sites that are relevant to the selected study species. Besides placing autonomous sensors in a broad range of coastal habitats, future research in variable coastal areas, such as Algoa Bay, should also consider concurrently measuring parameters such as chlorophyll-*a* concentration or DO. This will provide us with a better understanding of the mechanisms driving variability in pH in the coastal zone and confirm the hypothesis that biological processes are contributing to pH variability. Furthermore, this information would be useful when moving to a multi-stressor approach in assessing species sensitivity to future conditions.

Species response in the context of local variability

The results reported in chapters 4 and 5 suggest that the early post-flexion stages of a coastal fish species, *Diplodus capensis*, appear generally tolerant to pH levels as low as 7.27, which is far lower than the recorded minimum pH in this study. These results suggest that this species appears unlikely to suffer any immediate survival or fitness consequences under near future OA conditions, with post-flexion larvae exhibiting no changes in metabolic rates (SMR, MMR, RAS) in any of the experimental treatments. However, it is possible that the full extent of natural variability in pH was not captured in Algoa Bay (see Chapter 3). Nevertheless, these findings suggest that *D. capensis* have relatively well-developed acid-base regulation mechanisms, even in the early stages, which may be expected of a species with a coastal distribution. This agrees with the theory, commonly mentioned in the literature, that coastal and temperate species that live in dynamic environments show higher tolerance to future OA (Munday *et al.*, 2009a; Esbaugh, 2018; Cattano *et al.*, 2018).

Despite the apparently broad tolerance to OA, subtle differences in activity levels were observed in *D. capensis* that were subjected to different pH treatments. Here fish appeared to become slightly more active (increased swimming distance and swimming speed) with a decrease in pH in their experimental environment. These changes did not however influence feeding as the larvae fed equally well across all pH treatments. Although the mechanism behind this slight change in behaviour was not apparent, the lack of a concurrent

physiological response suggests that it was not driven by physiological limitation. Further experimentation would be required to identify the mechanism driving the differences in activity between pH treatments.

The tolerance of *D. capensis* to low pH may be attributed to the wide distribution range and dynamic range of coastal habitats the species inhabits, including surf zones (Lasiak, 1981; Rishworth *et al*., 2015), the lower reaches of estuaries (Edworthy and Strydom, 2016; Nel *et al*., 2018), rock pools (Beckley, 1985b), and shallow intertidal gullies (Strydom, 2008) that the species inhabits during its early stages (from flexion). As these environments are highly dynamic, larval *D. capensis* will require well developed regulatory processes such as osmo- and acid-base regulation.

The changes in behaviour that were observed in Chapter 4 may, however, have consequences for *D. capensis*. Changes in behaviour may lead to mortality through an increased risk of predation, or reduced abilities to detect settlement habitats (Munday *et al*., 2009b). For example, early-stage clownfish (*Amphiprion percula*) and damselfish (*Pomacentrus wardi*) had reduced abilities to detect predator olfactory cues, exhibited bolder behaviours and increased their activity under OA (850 μatm CO_2) (Munday *et al.*, 2010). The ecological impact of this change in behaviour was then tested in the field where the authors found that the larvae kept in the high CO_2 OA treatment exhibited significantly higher natural predation than those from the present-day treatment in clownfish and wild caught damselfish (Munday *et al.*, 2010). This decreased survival due to increased predation in future OA scenarios suggests potential recruitment failure into adult populations. Another study on a catadromous species, barramundi (*Lates calcarifer*), showed that the crucial step of recruitment into settlement habitats is impaired by the reduced ability of this species to detect auditory cues from their settlement habitats in OA treatments (7.7 pH) (Rossi *et al.*, 2015). Further research on the behavioural consequences of low pH conditions on *D. capensis* is required to identify if OA has any long-term consequences on recruitment.

Several research considerations were not incorporated into this study yet may have relevance for the examination of the relationship between OA and recruitment success. Recruitment failure may occur when behavioural or sensory processes that affect homing, predator avoidance, feeding, or recruitment to settlement habitats are disrupted (Munday *et al.*, 2010; Stiasny *et al.*, 2016). In addition, several interacting and indirect factors may impact

individual fitness. For example, OA may have an indirect impact on an individual by affecting the food web, or their prey (Kroeker *et al.*, 2014). Future research on *D. capensis* should therefore also endeavour to include the impacts of OA on the senses and should be conducted in food abundant and food limited scenarios.

Ontogenetic and life-history considerations

Consideration of life stage is essential when interpreting the impacts of low pH and future OA on fishes, especially for species that transition between environments during their development, like many marine coastal fishes (Beckley, 1986; Whitfield and Pattrick, 2015). Due to this transition, many species can experience different levels of pH variability at different life stages. In addition, some species may be vulnerable to low pH only during specific life stages (Ishimatsu *et al.*, 2004) or even on very specific days within their ontogenetic development (Edworthy, 2018). There are examples of fish species that show tolerance to low pH levels in their juvenile and adult stages but in their earlier larval stages are much more vulnerable to the same treatments (Ishimatsu *et al.*, 2004). Edworthy (2018) and Erasmus (2017) assessed the sensitivity of the early-life stages of dusky kob (*Argyrosomus japonicus*) to ocean acidification treatments and found that the post-flexion stage of this species, when the most structural development occurs, was the most vulnerable to acidification. This was evidenced by reduced growth, skeletal development, metabolism and ultimately survival in the post-flexion stages when exposed to pH treatments of 7.78 (Edworthy, 2018; Erasmus, 2018).

Although possible physiological bottlenecks in the earlier life stages of *D. capensis* were not assessed in this study, the earlier larval life stages have been reported to occur in surf zones (Rishworth *et al.*, 2015), coinciding with the inshore sites in this study where variability in pH was high. This suggests that the earlier larval life-stages of this species may be similarly tolerant to low pH as the later larval life stages.

Co-occurring stressors

There has been an increase in research that considers the impact of multiple climate stressors on the response of fishes. Some studies have assessed the impact of co-occurring global changes such as warming, hypoxia and OA on fishes (e.g., Munday *et al*. 2009a, Di Santo 2015, Flynn *et al.* 2015, Cominassi *et al*. 2019). These studies found complex species responses to different combinations of stressors. For example, in a study on larval yellowtail

kingfish (*Seriola lalandi*), Laubenstein *et al.* (2018) found that elevated pCO_2 increased resting metabolic rates and this response was strongly modulated by temperature. There is evidence to suggest that temperature may have an impact on juvenile *D. capensis,* with metabolic rates increasing significantly with an increase in temperature (14–28°C) (Kemp 2008). This may suggest that the metabolic costs associated with warming may influence the ability of *D. capensis* to compensate for changes in pH. Therefore, a multi-stressor approach that combines temperature and pH treatments would further our understanding of the response to *D. capensis* to future conditions, where temperature and pH conditions vary concurrently. Since changes in pH and parameters associated with other global changes do not occur in isolation in the wild, it is possible that the metabolic capacity of *D. capensis* is large enough to accommodate a wide range of pH, but when paired with another metabolically expensive regulatory process, such as temperature or osmoregulation, pH tolerance may decrease.

Limitations of the study

Laboratory perturbation studies present several practical and theoretical limitations in simulating the natural environment. For example, full replication of natural pH and other driver variability as experienced in the wild by organisms is not possible. The duration of the experiment is another factor that may influence the effect of reduced pH during an experiment. The exposure time in this study (five days) was based on the potential variability one would expect for a coastal, intertidal area where pH is influenced by drivers such as upwelling, biological bloom events or freshwater inflow. During these events, pH may increase and/or decrease below the natural average for a few days at a time (Feely *et al.*, 2008; Hofmann *et al.*, 2011; Reum *et al.*, 2016). Despite this, the diurnal and other short-term temporal pH variability (see Chapter 3) could not be replicated in the laboratory experiments due to practical and logistical constraints.

The consistent exposure to constant pH treatments (Fig 6.1) in the laboratory may modulate the species' response (e.g., extra costs of coping with fluctuation). Considering variability over all scales that occurs in the natural environment is essential as it may drive the species response in the long term by facilitating selection of more tolerant individuals. The alternative to this is that fluctuating conditions may dampen the effects of low pH on organisms by providing temporary relief from stressful conditions as shown in experimental

studies by Jarrold *et al*. (2017) and Hannan *et al*. (2020). Testing the response to fluctuating conditions would provide vital information for the design of future OA experiments.

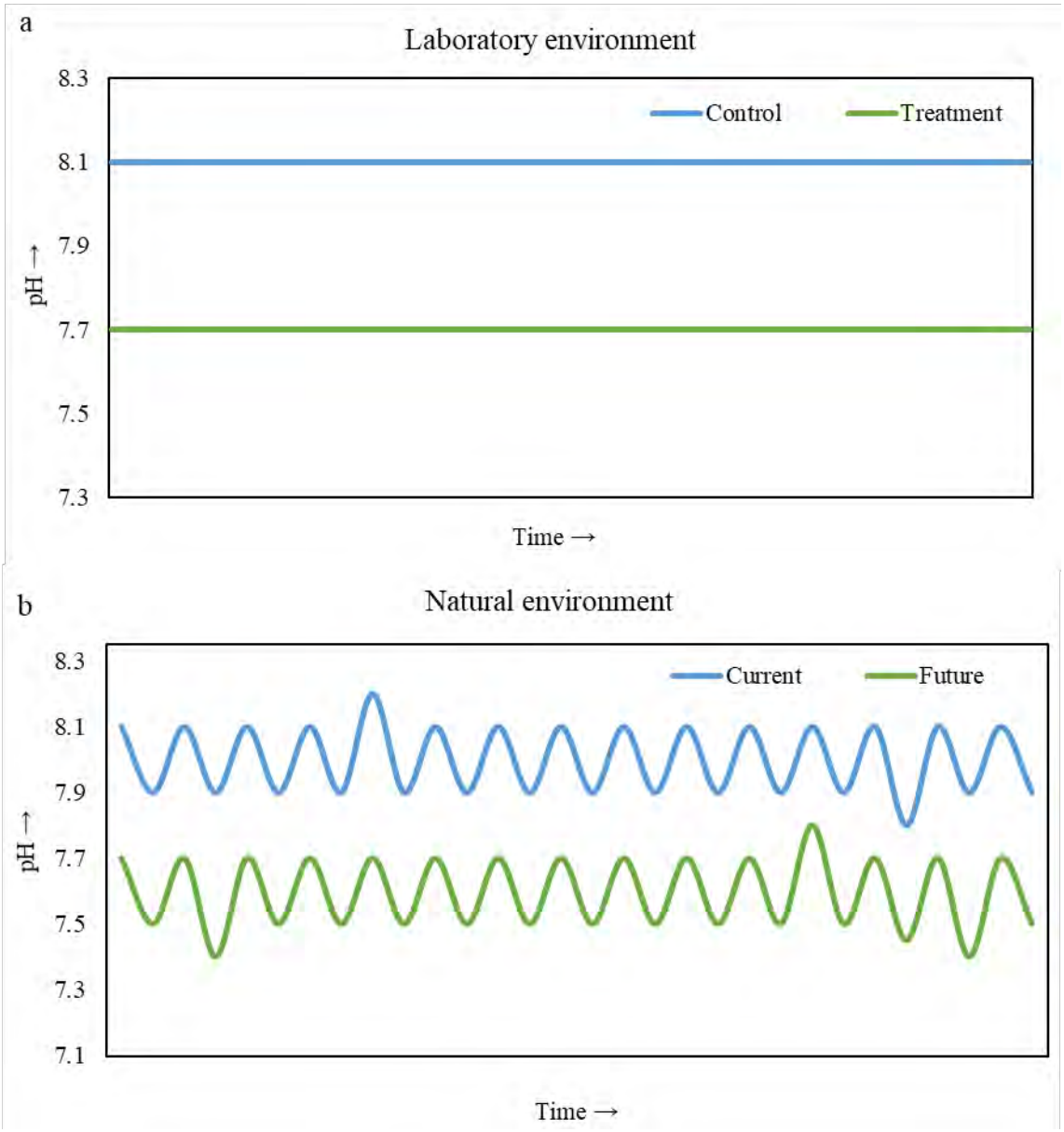

Figure 6.2: Theoretical representation of a) the consistent laboratory pH treatment levels compared to b) the randomly fluctuating current and future conditions in the natural environment

Just as it is impossible to measure the sensitivity of all species and life-stages to OA, it is difficult to measure the sensitivity of all processes. For example, this study aimed to assess the behavioural response of *D. capensis* to OA by measuring activity levels and feeding rates. Although these methods may identify the overall behavioural changes resulting from OA,

they did not identify underlying mechanisms, such as the perturbation of the GABA pathway. It is relatively well established that OA is likely to disrupt the olfactory abilities of many fishes through a disruption of the functioning of the GABA-A neurotransmitter (Dixson *et al.*, 2010; Nilsson *et al.*, 2012; Sundin and Jutfelt, 2016). In some species this influences their ability to detect the chemical cues from predators (Dixson *et al.*, 2010), from conspecifics (Laubenstein *et al.*, 2019) or for the detection of their prey (Cripps *et al.*, 2011). An experiment assessing the olfactory ability of *D. capensis* in low pH conditions will provide further insight to the impacts of OA on this species, as olfactory ability was not assessed in this study.

Single species assessments in a laboratory environment also do not account for interactions with other species (predators, competitors, or prey) or conspecifics that occur in the wild. Whereas single species tested in the laboratory may show a particular response to OA, the fitness consequences may be dependent on the response of other species (Pörtner, 2008; Kroeker *et al.*, 2013b). This may be due to competition for resources (McCormick *et al.*, 2013), changes in predator-prey interactions (Ferrari *et al.*, 2011b; Poore *et al.*, 2013) or interactions with other individuals of the same, or other, species (Johnston and Dixson, 2017). For example, one species of damselfish, *Pomacentrus amboinensis*, generally outcompetes another, *Pomacentrus molluccensis*, under present pH conditions. However, the dominant species is more vulnerable to OA and the competitive outcome between the two species is reversed under future OA scenarios (McCormick *et al.*, 2013). Finally, OA may influence trophic interactions within food webs, which may have cascading indirect consequences on species depending on each other (Guinotte and Fabry, 2008). Moving towards a multi-species response model is therefore essential in identifying the ecosystem level response to future OA. This would require a combination of laboratory and field experiment including multiple species and ecological interactions.

Relevance of the study for local and global initiatives

Local relevance and impact

Ocean acidification is often referred to as a global problem that has local impacts (Cooley *et al.*, 2016). Identifying the consequences of future OA on socio-economically important coastal species is essential in a country, like South Africa, where ocean resources and fisheries play an important role in the coastal economy. South Africa's current legislation

places a strong focus on fisheries resource management, due to reliance of many social and economic sectors on seafood (Branch and Clark, 2006). Global change is a threat to these resources, and the South African Government is committed to mitigating the impacts of global change and climate change on its resources. South Africa is also a signatory of the Paris Agreement whereby the country is committed to acting against global climate change and reducing its contribution to global CO_2 emissions on a national level. In 2019, a National Climate Change Adaption Policy draft document was gazetted, and this document confirms the country's commitment to climate change mitigation and adaption across sectors (DEA, 2019).

South Africa currently has a policy applicable to addressing climate change in marine environments, namely the Climate Change Adaption and Mitigation Plan (second draft, DEA 2017). The focus of this policy is on developing resource resilience and adaptive measures against the impacts of global climate change on fisheries resources. The policy recognizes the impact of future changes in sea surface temperature, frequency of storm events, freshwater inflow, nutrient levels, oxygen levels and weather patterns associated with global climate change on local fisheries (DEA 2017) but makes no mention of OA, which is just as likely to contribute to fisheries resource vulnerability (Branch *et al.*, 2013; Haigh *et al.*, 2015; Potts *et al.*, 2015).

The ultimate solution to OA is mitigation through a reduction in global CO_2 emissions (Gattuso and Hansson, 2011). However, as this is not achievable at a local level and may take several decades, adaptation by managing and reducing the local impacts of OA is necessary (Cooley *et al.*, 2016). To ensure that the threat of OA is considered in future policies, it is necessary to advance OA research in each country and ensure that this research is appropriate for and communicated to policy makers. Research, such as that undertaken in this study should be continued to identify focal species and areas which may be relevant for fisheries management (e.g., marine reserves in areas that serve as CO_2 sinks for sensitive species and fisheries) or species that serve as keystone species that indirectly facilitate ecosystem health and resulting ecosystem services.

At a regional level, there has been an increased effort in the Western Indian Ocean (WIO) region to monitor OA and its impacts. This has been driven by the Western Indian Ocean Marine Science Association (WIOMSA), an organization that co-ordinates regional coastal and marine science research in the WIO region. In 2019, a first draft of a White Paper was

prepared by a collaboration of researchers from various African countries in the WIO region. The aim was to highlight the commitment of WIOMSA to develop OA research in the region and to contribute data to global directives to address the 'knowledge gap' that exists in Africa and surrounding oceans (Ramessur *et al. in prep*). At the same time, an objective of this White Paper is to focus on research that minimizes the impacts of OA in the WIO region, where dependence on marine resources and services is high. Several international partners are involved in advising and funding this research, for the purpose of developing capacity for OA research in these understudied areas. The work presented in this study contributed to the White Paper by summarizing the contribution of research from South Africa in the effort to improve OA research in the WIO region.

The approach used in this study serves as a departure point for OA research in South Africa and the greater WIO region and should be expanded to other countries, ecosystems, and species to motivate consideration for OA in policy and to inform future adaptation plans. Bridging the gap between chemistry and biology and designing a relevant study that quantifies local conditions and assesses locally important species are essential to develop information that can be used to inform and enforce effective management and adaptation under future conditions. This study approached the development of relevant OA monitoring by:

1) formulating a suitable research question, which was centred around the concept of local adaptation to variable coastal environments modulating species sensitivity to OA,

2) identifying a suitable study species that had some socio-economic relevance as a common coastal fisheries species that was also accessible in the required numbers for experiments,

3) identifying a suitable study site and method for monitoring that could capture the variability that the study species is naturally exposed to and,

4) identifying suitable experimental treatment levels to test the hypothesis that the study species is adapted to manage the pH levels within the range it is naturally exposed to.

This approach combines field monitoring and laboratory experiments which is a useful model for future work. Furthermore, some of the limitations that emerged from this research, such

as the difficulty in capturing the true extent of pH variability without continuous monitoring for example, further inform the development of suitable methods for future monitoring and experiments. Localized OA research initiatives should aim to consider a similar approach, perhaps on more sensitive and/or other ecologically and economically important species, in developing their response to OA.

Contribution to the sustainable development goals and global climate action

Ocean acidification research is globally co-ordinated by the Global Ocean Acidification Observing Network (GOA-ON). This network develops and distributes frameworks for OA research that include regional plans, measurement accuracy targets and methodologies (see Newton *et al*., 2015). The GOA-ON contributed to the research presented in this thesis both financially and in an advisory capacity. This research, which worked closely within the framework and guidelines of the GOA-ON, aimed to meet the requirements for data quality and resolution in order to be included in their international OA data platform (that can be found at *http://portal.goa-on.org/*). The aim of this platform is to make regional OA data accessible to the global community with the intention of co-ordinating interaction among OA research efforts. The monitoring data from this study were submitted to the GOA-ON data portal, where previous data from South Africa was limited. The methods for site selection, identification of a suitable socio-economically important species and the combined field and laboratory approach used in this study provides a starting point for the development of a best-practice framework for ocean acidification research in Africa.

Recommendations for future research

This study serves as a baseline for future work to develop and improve OA research in South Africa. The findings from this study clearly suggest that short term, high-frequency pH data are best to capture relevant coastal variability. The addition of concurrent, continuous measurements of other parameters would strengthen this data by aiming to identify the physical and biological drivers responsible for short-term localized pH variability. In terms of species response research, it is suggested that future work continues to focus on commercially, socially, or economically important coastal species in South Africa. Furthermore, it would be useful to move towards achieving an ecosystem view of OA impacts, accounting for important ecological interactions. Experimental studies should therefore take a holistic approach at understanding species and ecosystem responses, by

including various response variables, species, and life-stages, to truly capture the vulnerability of critical coastal ecosystems. For example, further research on *D. capensis*, specifically, should focus on assessing sensitivity during other life-stages and consider the olfactory response of the species to low pH. Although single species and single stressor studies are a suitable starting point in global change research, the eventual goal should be to move towards multiple stressor and multiple response studies in order to truly capture the impacts of global change on marine biodiversity.

Although aiming to understand the full ecosystem level response to OA is ideal, it is important to recognize the limitations of resources for research (human, infrastructure, financial), particularly in developing countries. This limits the extent of the research that can be conducted. There is a need to develop priorities in OA research for developing countries that are best suited to inform adaptation and management of the impacts associated with future OA. Research on OA in South Africa and other countries in Africa should therefore focus on establishing the effects of OA on species that have strong commercial, social, economic, conservation or ecological significance. Furthermore, sites with high ecological relevance (e.g., marine protected areas as refuges), process-related variability (e.g., upwelling zones) or ecosystems with high sensitivity (e.g., coral reefs) should be prioritized for monitoring. Most importantly, sites that could serve as refuge habitats should be identified and prioritized to inform an adaptive response. This recognition of high priority research areas will allow for suitable data to be produced quickly to be recommended for policy and ultimately drive the response to future OA.

Conclusion

This research provided the first known study in the warm-temperate region of South Africa that quantified coastal pH variability to inform a biological response experiment on a coastal fish species. It is clear from this research that the temporal and spatial coastal variability in pH and associated carbonate chemistry parameters reported in this study cannot be compared to open ocean scenarios. This suggests that OA treatments inferred for the open ocean (based on emission scenarios) would not be suitable treatments for experimental assessments of coastal species that occur in Algoa Bay. Microhabitat variability in pH conditions in the coastal area of Algoa Bay suggests that sites that serve as refuges (higher pH levels) from coastal acidification should be prioritized for an adaptive management response.

The postflexion stage of the study species, *D. capensis,* appears tolerant to both the current high variability (Δ ~0.5 pH) in carbonate chemistry conditions as well as potential future coastal acidification (~7.2 pH). This is evidenced by this species' robust metabolic tolerance to low pH treatments as shown by the laboratory experiments in Chapter 4. Feeding behaviour was similarly unaffected by pH with only a slight increase in activity levels being the only response of this species to decreasing pH (Chapter 5). These results do not support significant direct consequences on fitness and may only have some ecological consequences (such as potentially higher predation rates on bolder, more active individuals) but this will require further study. This approach to OA research, that combines local monitoring and species response experiments is a useful model for future approaches and future studies should focus on expanding this understanding to other species, and ultimately to ecosystems in South Africa and surrounding regions in Africa, where information is lacking.

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Appendix

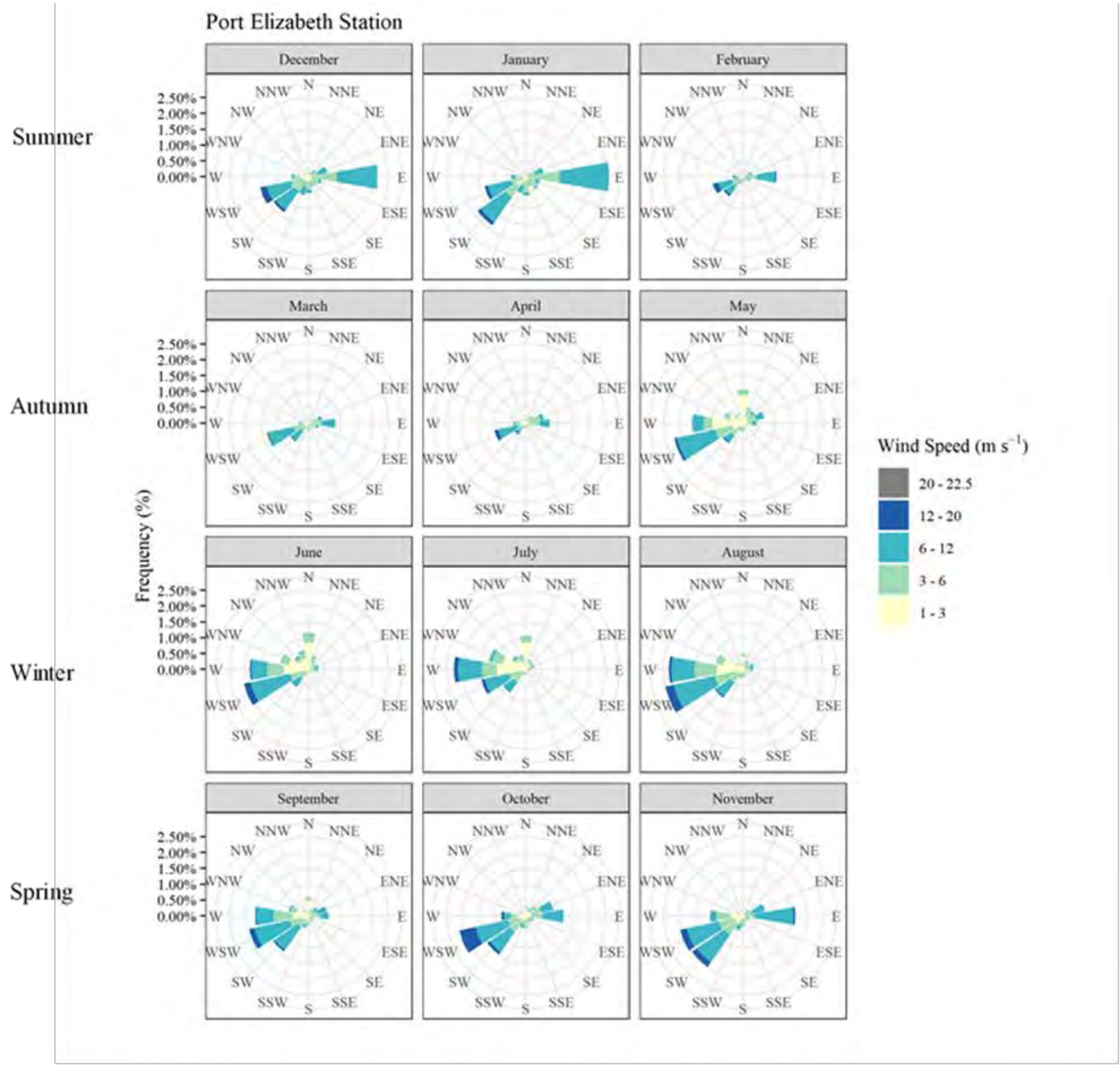

Figure A.1: Monthly wind roses of wind direction and speed (m s^{-1}) for the Port Elizabeth (PES) weather station during the study period (June 2018 – January 2020)

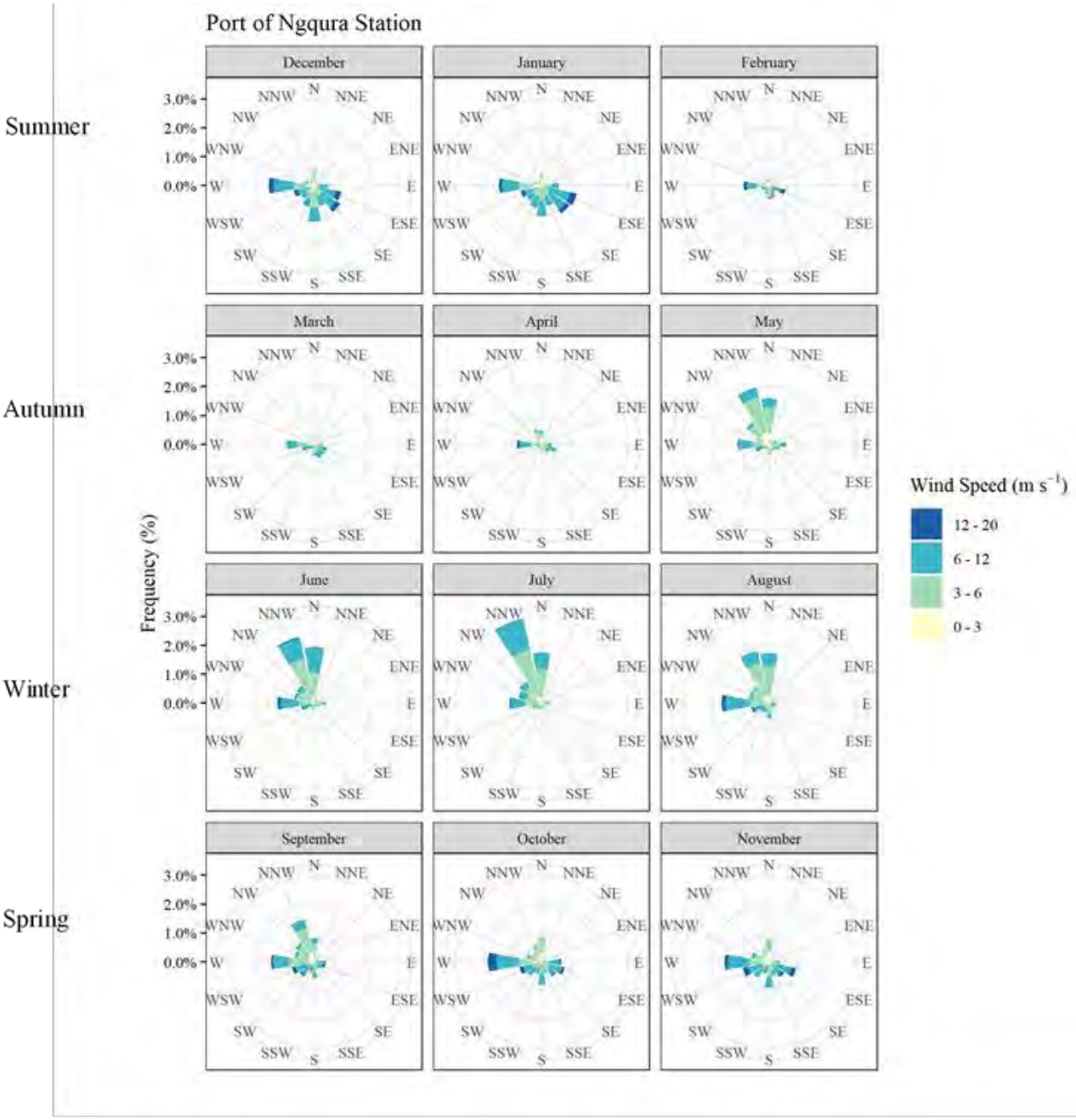

Figure A.2: Monthly wind roses of wind direction and speed (m s^{-1}) for the Port of Ngqura (PNS) weather station during the study period (June 2018 – January 2020)

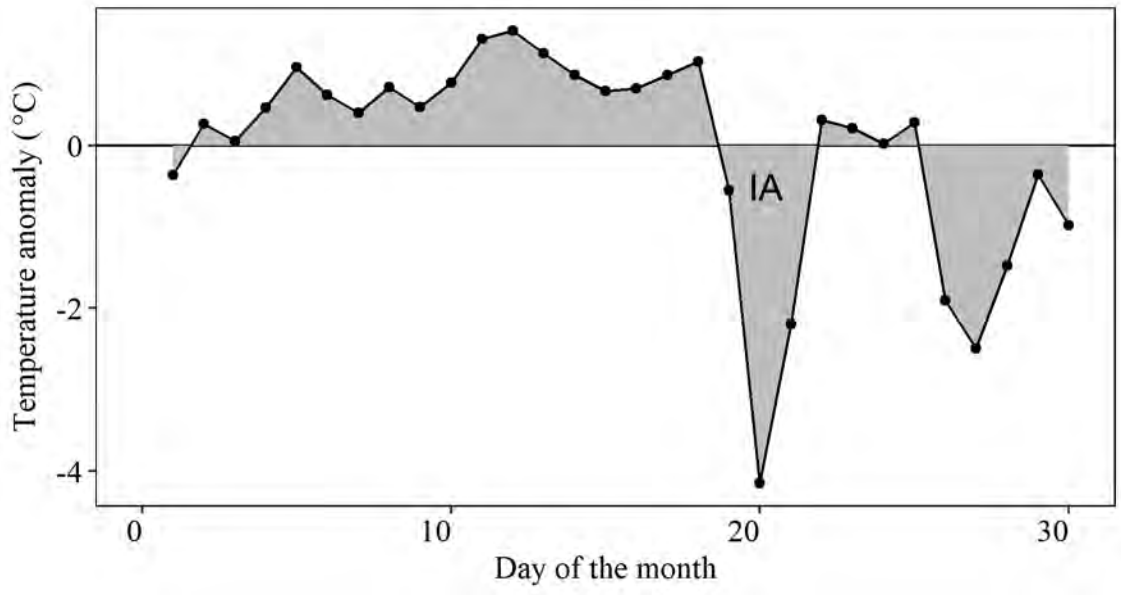

Figure A.3: Example of daily sea temperature anomalies (calculated from the difference between the 30-day moving average temperature and observed sea temperature) for the month of September 2019. Negative values indicate anomalously cold events and indicate potential upwelling. The integrated anomaly as calculated by the area between the zero line and the temperature anomaly indicates the intensity and duration of the potential upwelling event.

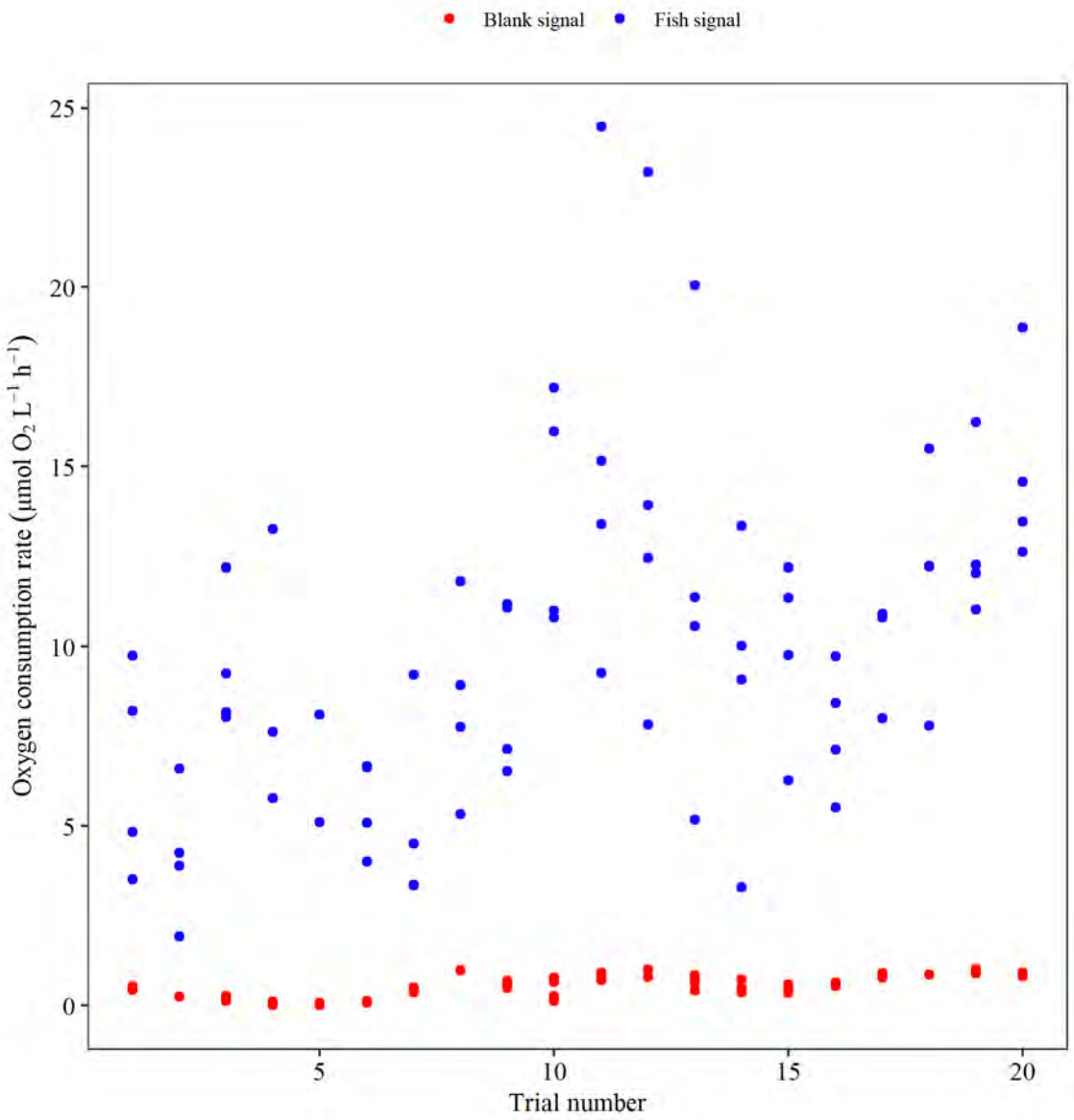

Figure A.4: Oxygen consumption rates of the blank measurements (red) when compared to the measurements containing fish (blue). Blank measurements represented an average of 6.2% of the signal of oxygen consumption rates in the measurements containing the fish

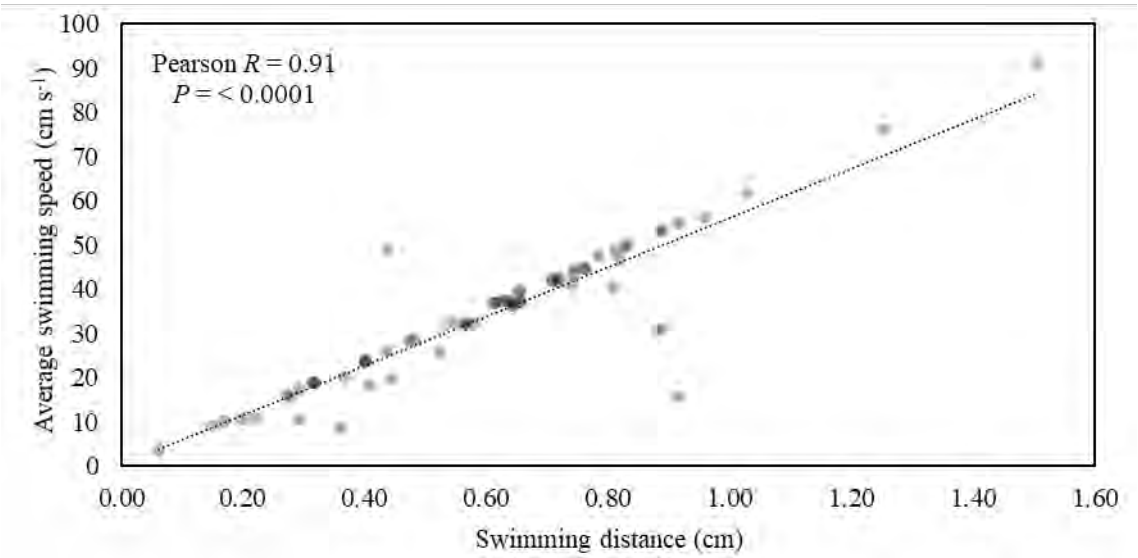

Figure A.5: Significant positive correlation between swimming distance (cm) and swimming speed (cm s^{-1})

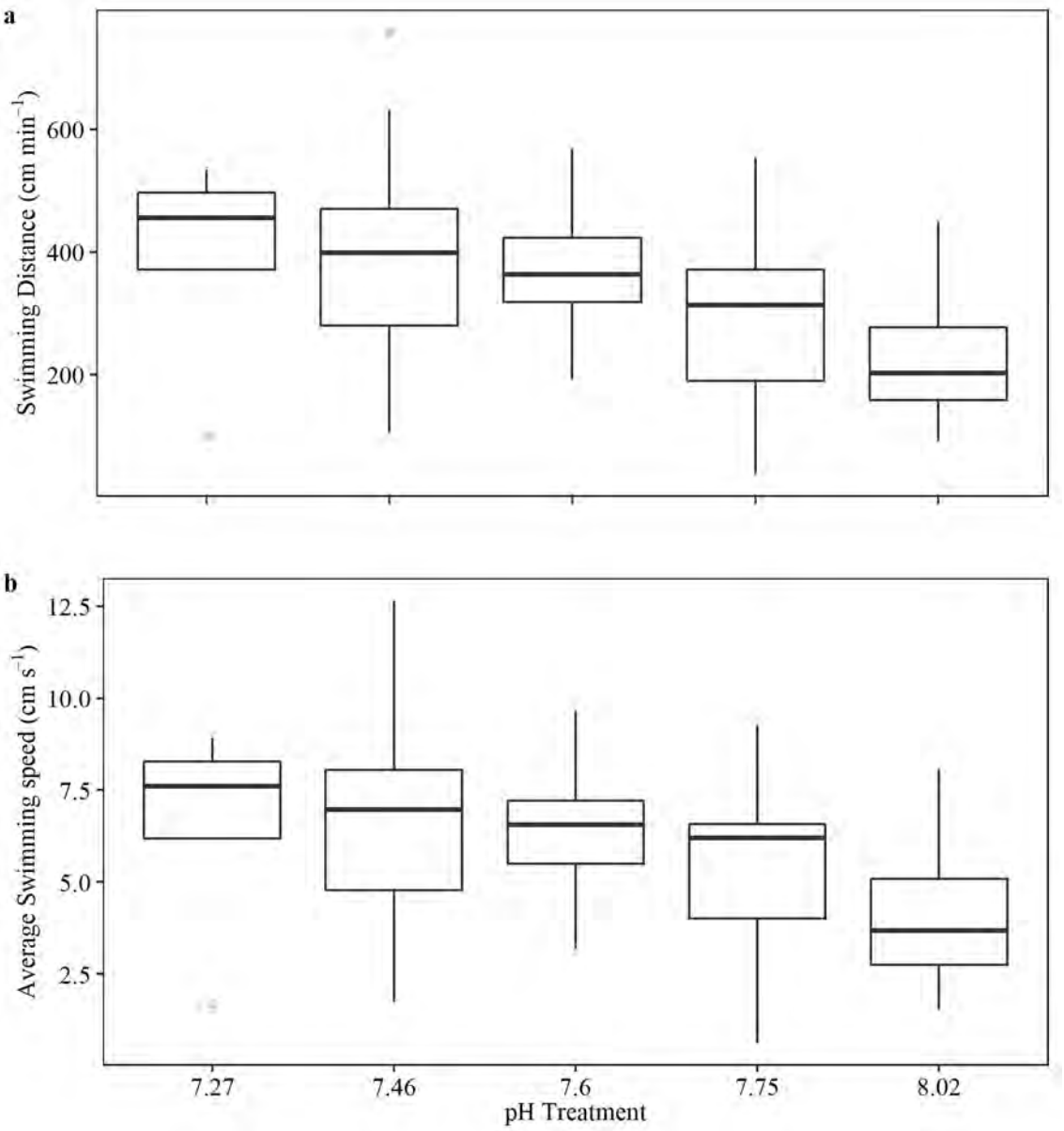

Figure A.6: Boxplots showing variability in a) swimming distance (cm) and b) swimming speed (cm s^{-1}) during the swimming activity experiments

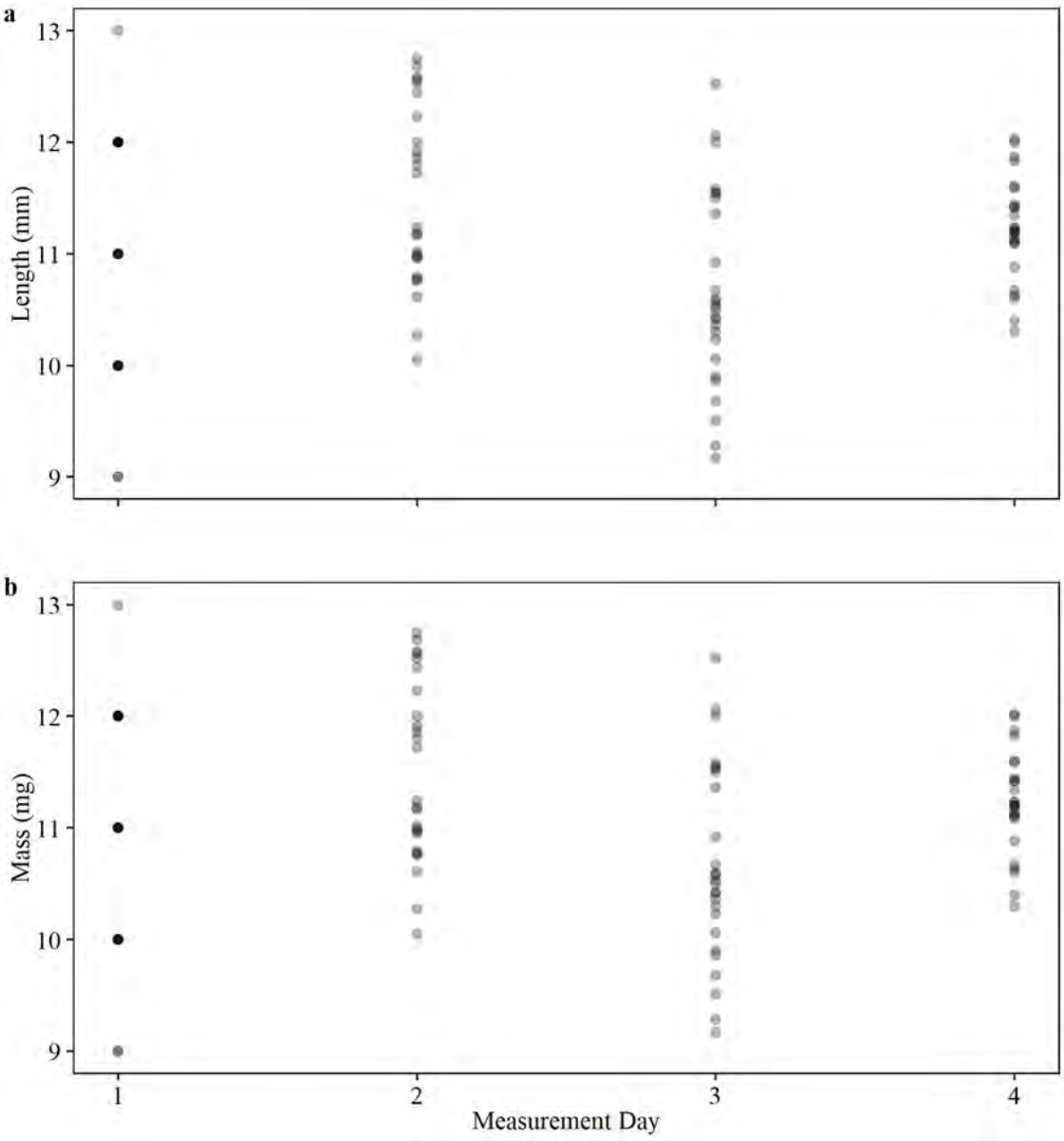

Figure A.7: a) Length (mm) and b) mass (mg) of the larval *D. capensis* used for the behavioural experiments (Chapter Five) over the four measurement days

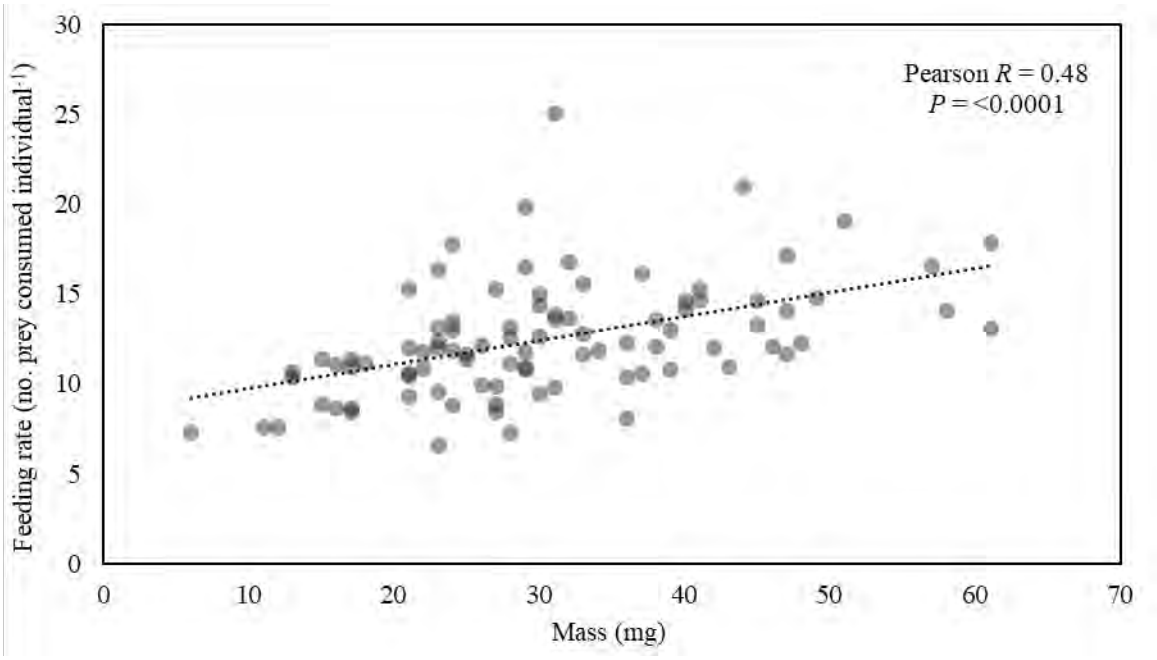

Figure A.8: Significant positive correlation between feeding rate (no. prey consumed indv^{-1}) and body mass (mg)

Table A.1: Summary statistics of precipitation (mm) grouped by month and season at each weather station during the study period (June 2018 – January 2020)

Port Elizabeth Station (PES)				
Month	*N*	*Mean ± SD*	*Min, Max*	*Range*
January	62	0.84 ± 2.0	0, 12.2	12.2
February	28	1.99 ± 5.86	0, 26.4	26.4
March	31	0.89 ± 2.25	0, 10.0	10.0
April	30	1.61 ± 3.69	0, 14.4	14.4
May	62	1.02 ± 3.66	0, 22.4	22.4
June	60	0.70 ± 2.88	0, 19.2	19.2
July	62	1.36 ± 4.47	0, 22.6	22.6
August	62	1.58 ± 5.92	0, 43.0	43.0
September	60	2.41± 6.44	0, 32.6	32.6
October	62	0.89 ± 2.49	0, 14.8	14.8
November	60	0.91 ± 2.03	0, 9.4	9.4
December	62	0.41 ± 1.35	0, 9.2	9.2
Season	*N*	*Mean ± SD*	*Min, Max*	*Range*
Summer	123	1.12 ± 3.35	0, 22.4	22.4
Autumn	182	1.41 ± 4.20	0, 32.6	32.6
Winter	158	0.88 ± 2.95	0, 26.4	26.4
Spring	184	1.21 ± 4.61	0, 43.0	43.0
Port of Ngqura Station (PNS)				
Month	*N*	*Mean ± SD*	*Min, Max*	*Range*
January	62	0.48 ± 1.76	0, 13.0	13.0
February	28	0.49 ± 1.64	0, 8.0	8.0
March	31	0.28 ± 0.85	0, 4.6	4.6
April	30	1.29 ± 3.40	0, 13.8	13.8
May	62	0.76 ± 3.11	0, 21.0	21.0
June	60	0.33 ± 1.40	0, 10.0	10.0
July	62	0.67 ± 2.70	0, 16.8	16.8
August	62	0.80 ± 4.23	0, 31.0	31.0
September	60	1.64 ± 4.76	0, 23.8	23.8
October	62	0.79 ± 2.02	0, 10.6	10.6
November	60	0.34 ± 1.01	0, 7.0	7.0
December	62	0.36 ± 1.37	0, 7.6	7.6
Season	*N*	*Mean ± SD*	*Min, Max*	*Range*
Summer	123	0.77 ± 2.80	0, 21.0	21.0
Autumn	182	0.92 ± 3.06	0, 23.8	23.8
Winter	152	0.43 ± 1.58	0, 13.0	13.0
Spring	184	0.60 ± 3.01	0, 31.0	31.0

Table A.2: Summary statistics (mean ± SD, min, max, range) for physico-chemical and carbonate speciation parameters at both offshore and inshore sites in Algoa Bay

	Offshore sites (1–8) June 2018 – January 2020				**Inshore sites (1–5)** August 2018 – January 2020			
Parameter	***Site***	***Mean ± SD***	***min; max***	***Range***	***Site***	***Mean ± SD***	***min, max***	***Range***
Temperature	1	18.6 ± 1.71	16.1; 21.4	5.3	1	22.6 ± 2.58	19.0; 26.0	7.0
	2	18.7 ± 1.91	16.3; 22.7	6.4	2	20.6 ± 2.31	16.5; 23.6	7.1
	3	18.5 ± 1.85	16.1; 22.1	6.0	3	20.3 ± 2.11	16.5; 23.3	6.8
	4	19.0 ± 1.60	16.8; 22.0	5.2	4	19.9 ± 2.31	15.8; 23.0	7.2
	5	19.0 ± 1.52	16.7; 21.8	5.1	5	19.1 ± 1.80	16.0; 23.0	7.0
	6	18.1 ± 1.70	15.2; 21.0	5.8				
	7	17.9 ± 1.29	16.5; 20.4	3.9				
	8	18.2 ± 1.38	16.2; 20.7	4.5				
pH	1	8.05 ± 0.06	7.97; 8.16	0.19	1	8.30 ± 0.08	8.13; 8.41	0.28
	2	8.06 ± 0.08	7.94; 8.27	0.33	2	7.99 ± 0.06	7.92; 8.14	0.22
	3	8.04 ± 0.08	7.93; 8.27	0.34	3	7.99 ± 0.07	7.91; 8.16	0.25
	4	8.05 ± 0.05	7.99; 8.17	0.18	4	7.96 ± 0.08	7.80; 8.11	0.31
	5	8.01 ± 0.11	7.72; 8.17	0.45	5	7.96 ± 0.08	7.82; 8.12	0.30
	6	8.00 ± 0.04	7.90; 8.05	0.15				
	7	8.01 ± 0.05	7.93; 8.11	0.18				
	8	7.98 ± 0.08	7.72; 8.14	0.42				
***p*CO_2**	1	401 ± 57.2	280; 487	207	1	195 ± 66	120; 318	198
	2	389 ± 77.6	203; 534	331	2	487 ± 118	296; 770	474
	3	406 ± 89.5	205; 552	347	3	495 ± 100	289; 716	427
	4	398 ± 53.8	276; 461	185	4	564 ± 205	395; 1118	723
	5	459 ± 162.0	274; 952	678	5	516 ± 129	316; 809	493
	6	450 ± 55.1	396; 583	187				
	7	447 ± 49.9	393; 544	151				
	8	449 ± 57.3	311; 522	211				
TA	1	2264 ± 30.1	2210; 2315	105	1	2266 ± 66.1	2144; 2394	250
	2	2276 ± 31.6	2204; 2328	124	2	2282 ± 39.0	2203; 2339	136
	3	2280 ± 31.0	2236; 2338	102	3	2280 ± 29.6	2231; 2334	103
	4	2281 ± 24.9	2249; 2341	92	4	2308 ± 56.8	2231; 2447	216
	5	2285 ± 26.3	2246; 2342	96	5	2287 ± 26.8	2244; 2351	107
	6	2278 ± 24.4	2224; 2313	89				
	7	2273 ± 27.5	2224; 2312	88				
	8	2274 ± 34.1	2196; 2334	138				

Table A.3: Summary statistics (mean ± SD, min, max, range) chlorophyll-*a* concentration at both offshore and inshore sites in Algoa Bay

		Offshore sites (1–8) June 2018 – January 2020			**Inshore sites (1–5)** August 2018 – January 2020			
Parameter	***Site***	***Mean ± SD***	***min; max***	***Range***	***Site***	***Mean***	***min,***	***Range***
Chlorophyll-*a*	1	4.4 ± 3.2	0.00, 11.1	11.1	1	6.14	2.42,	7.21
	2	3.8 ± 5.2	0.00, 21.0	21.0	2	7.55	2.30,	17.50
	3	5.0 ± 7.9	0.01, 34.3	34.2	3	4.36	1.40,	11.60
	4	4.9 ± 8.7	0.00, 28.1	28.1	4	11.9	2.99,	21.41
	5	5.2 ± 8.1	0.00, 27.0	27.0	5	13.1	3.45,	52.15
	6	2.9 ± 4.2	0.00, 17.0	17.0				
	7	3.8 ± 4.2	0.00, 13.5	13..5				
	8	2.9 ± 4.7	0.00, 19.4	19.4				

Table A.4: Results of distance based linear model (DISTLM) marginal tests assessing the contribution of each predictor variable (temperature, total alkalinity (TA), dissolved oxygen (DO) and chlorophyll-*a* concentration) to the observed variance in pH. SS = sum of squares, prop. = proportion of explained variance

	Offshore sites				**Inshore sites**			
Variable	*SS (trace)*	*Pseudo- F*	*P*	*Prop.*	*SS (trace)*	*Pseudo- F*	*P*	*Prop.*
Temperature	0.14	32.49	0.001	0.20	0.39	22.80	0.001	0.25
TA	0.05	10.11	0.001	0.07	0.040	1.81	0.20	0.03
DO	0.06	11.79	0.001	0.085				
Chlorophyll-*a*	0.05	9.71	0.009	0.071	-0.03	-1.18	1	-0.02

Table A.5: Summary statistics of the measured biological parameters of individuals from each pH treatment measured during the physiology study (Chapter Four)

Treatment	*Parameter*	*n*	*Mean*	*SD*	*Range*
All	Total length (mm)	74	12.04	1.15	5.40
	Wet mass (g)	74	0.0143	0.01	0.0238
	Condition factor ($mg.mm^{-3}$*100)	74	8.02	1.68	8.27
8.03	Total length (mm)	15	12.49	1.21	3.97
	Wet mass (g)	15	0.0169	0.01	0.0219
	Condition factor ($mg.mm^{-3}$*100)	15	8.21	1.74	5.62
7.75	Total length (mm)	14	11.33	0.79	3.12
	Wet mass (g)	14	0.0125	0.01	
	Condition factor ($mg.mm^{-3}$*100)	14	8.65	2.37	0.0117
7.60	Total length (mm)	15	12.16	1.32	4.41
	Wet mass (g)	15	0.0155	0.01	0.0201
	Condition factor ($mg.mm^{-3}$*100)	15	8.39	1.36	4.809
7.46	Total length (mm)	16	12.13	0.91	3.16
	Wet mass (g)	16	0.0141	0.01	0.0196
	Condition factor ($mg.mm^{-3}$*100)	16	7.64	1.33	4.53
7.27	Total length (mm)	14	12.06	1.26	4.56
	Wet mass (g)	14	0.0129	0.01	0.0166
	Condition factor ($mg.mm^{-3}$*100)	14	7.23	1.22	4.00

Table A.6: **S**ummary statistics of simple linear regression models showing the relationships between the biological parameters and pH treatment

	Linear regression equation	*R^2*	*F statistic*	*P value*
Total Length (mm)	y = 0.22x + 10.40	0.002	0.17	0.68
Mass (mg)	y = 3.44x + -11.93	0.030	2.22	0.14
Condition factor ($mg\ mm^{-3}$*100)	y = 1.48x + -3.21	0.050	3.08	0.05