Understanding the effects of ocean warming and acidification on mangrove communities

Submitted for Master of Philosophy

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Abstract

The quality of living habitat provided by foundation species is tied closely to the composition and physical complexity of their structure. Global climate stressors, like ocean warming and acidification, contribute to the erosion of structural complexity in marine foundation habitats by promoting the growth of low-relief turf, increasing grazing pressure on marine vegetation, and by directly affecting the growth and survival of foundation species. Mangroves are a unique foundation species in that their structural complexity is created not only by their submerged woody roots but also by the fouling species (epibionts) that occupy their surfaces. As epibiont communities consist of diverse collections of sessile animals and plants which show varied sensitivities to climate stressors; their composition – and therefore their structural complexity – is subject to change with ocean warming and acidification. As foundation habitats are affected by climate stressors, so too are their inhabitants. Ocean acidification is known to affect neurotransmitter functioning in marine fish and warming can directly impact activity levels and species distributions. These will impact how fish interact with others, how they locate and select habitats and how they utilise and value resources. In this thesis, I used a large scale mesocosm experiment to investigate how a conservative carbon emission scenario affects the composition and structural complexity of mangrove epibiont communities and the macro- and microhabitat choice patterns of juvenile fish. I demonstrate that even a modest increase in seawater temperature of 1.2 °C leads to the homogenisation and flattening of mangrove root epibiont communities. Warming led to a 24% increase in the overall cover of algal epibionts on roots while the diversity of the epibiont species decreased by 33%. Epibiont structural complexity decreased owing to the shorter stature of weedy algal turfs which prospered under elevated temperature. Juvenile fish showed species-specific patterns of macrohabitat choice, but these were unaffected by the climate treatments. In contrast, the climate stressors did alter the microhabitat choices of fish and these were independent of changes to the root epibiont community. My results reveal that the quality of mangrove habitats and their perceived value for associated fauna are still vulnerable under a globally reduced carbon emission scenario, and therefore warrant urgent global actions to reduce anthropogenic climate warming.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Introduction

Mangroves are predominantly an intertidal habitat that occupies sheltered, shallow waters in the warm tropics and subtropics (Nagelkerken *et al.,* 2008). Often revered for their value as a nursery habitat, submerged mangrove roots act as a refuge for juveniles from predators, provide an abundance of food and provide shelter from physical disturbance (Robertson & Blaber, 1992; Blaber, 2000). Critically, each of these services are not provided just by the presence of mangrove roots but are aided greatly by the attachment of epibionts (Laegdsgaard & Johnson, 2001). Epibionts are plants and animals that attach themselves to hard structures. Some epibionts interact with their host substrates (to positive or negative effect), whilst many simply occupy space (Farnsworth & Twilley, 2008; Ellison & Farnsworth, 1992). In mangrove ecosystems, epibiont communities are generally very diverse and greatly enhance the structural complexity of underwater mangrove habitats (MacDonald & Weis, 2013). The epibionts themselves also act as a food source for juvenile fish and host increased numbers of food items like copepods and other small crustaceans (Lubbers, Boynton, & Kemp, 1990). By enhancing structural complexity and providing more food options, epibionts increase the suitability of the mangrove habitat for a greater collection of species. In this way, they are an incredibly important contributor to the value of mangrove habitats.

Whilst mangroves are not immune to global change stressors, the most significant risks being local sea-level rise and erosion (Jennerjahn *et al.,* 2017), they have exhibited a high degree of ecological stability and persistence throughout times of environmental change (Alongi, 2015). This may depend, in part, on the fact that most of their photosynthetic biomass sits above the water line, decreasing their sensitivity to changes in water quality (Kathiresan & Bingham, 2001). Because of their unique nature and hardiness, mangroves may provide climate refugia for a range of generalist coastal species that require structurally complex habitats, as other more vulnerable foundation species are lost from coastal environments (Saintilan & Williams, 1999; Orth *et al.,* 2016). Critically, their ability to continue to act as a valuable habitat depends heavily on the persistence of their epibiont communities, which may be much more sensitive to changing environmental conditions than mangroves themselves (Gazeau, Quiblier & Jansen, 2007; Vermeij, Moorselaar & Engelhard, 2010). Adding another layer of complexity, their continued value is also dependent upon what will be perceived as “valuable” by future mangrove occupants, given that fish behaviour is sensitive to changing ocean chemistry (Nagelkerken & Munday, 2016).

In this study, we constructed a complex, multi-species experimental set-up in which I tested the effects of ocean warming and acidification on mangrove fish and plant communities. The experiment involved 12 large mesocosms, containing realistic mangrove root mimics with real epibionts and a community of juvenile mangrove fish. The study centred around two key aims:

Aim 1: To investigate the effects of ocean warming and acidification on the macrohabitat choices of juvenile fish.

Aim 2: To investigate the effects of ocean warming and acidification on the structural complexity and diversity of mangrove root epibiont communities and examine whether changes to the epibiont communities affect microhabitat selection by juvenile fish.

This thesis begins with a contextualising overview of the existing literature on the importance of mangrove habitats and the contribution of epibionts. The literature overview is followed by two manuscripts that explore the central aims given above, written for scientific journal submission. The manuscripts are followed by a detailed description of the mesocosm technical design and finally, a general discussion of the findings of this body of work. This thesis represents an important contribution to the existing literature on mangrove ecology, being the first to holistically examine the effects of ocean warming and acidification on a near-complete mangrove community.

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Chapter One

Literature Overview

Mangroves

Mangroves are a tropical and subtropical intertidal habitat comprised of woody trees and shrubs (Alongi, 2002). Mangroves occupy ~81,849 km^2 globally, consisting of around 70 species, spread across nine orders, 20 families and 27 genera (Hamilton & Casey, 2016). Because of their unique morphological and physiological adaptations, mangroves are able to tolerate salt water submersion, high salinities, extreme tides, strong winds, high temperatures and anaerobic, muddy soils (Kathiresan & Bingham, 2001). Few other groups of plants show such an extensive set of adaptations to extreme environments. Mangroves act as an important habitat for a wide variety of species (Nagelkerken *et al.,* 2008). Above the water, mangrove canopies host populations of birds, mammals, reptiles and insects. Below the water level, submerged mangrove parts provide substratum for epibionts like algae, bivalves, tunicates and sponges. The spaces between roots are an important refuge for fish and prawn species and the soft sediment below hosts rich assemblages of infaunal and epifaunal species (Nagelkerken *et al.*, 2008).

Mangroves are a key nursery habitat in coastal environments. The term 'nursery' can be defined in different ways, each outlining a different criterion for the inclusion or exclusion of a habitat. The most common definition cited in the literature defines nurseries as habitats that, on average, contribute a greater number of recruits to an adult population per unit area compared with other habitats where juveniles may reside (Beck *et al.*, 2001). This definition requires not only that a habitat is abundant with juveniles, but also that those juveniles survive and successfully recruit into adult populations. Mangroves are commonly described as an important nursery habitat, hosting many species of commercial value. Because the performance of a nursery is intrinsically difficult to measure, this conclusion is most often based on the high incidence of juvenile fish within these habitats, and less often on observations of increased growth and survival (Saenger, Gartside & Funge-Smith, 2013). Mangroves are probably effective nurseries because 1) they act as a refuge for juveniles from predators, 2) they provide an abundance of food and 3) they provide shelter from physical disturbance (Robertson & Blaber, 1992; Blaber, 2000). The relative contribution of each of these qualities is unknown.

The functioning of mangroves as nursery habitats is based upon the unique environment provided by submerged roots, branches and trunks. Where mangroves extend into the intertidal and subtidal, they provide hard substrate, otherwise absent in muddy, soft-bottom habitats (Ellison & Farnsowrth, 1992). As such, they attract rich epifaunal communities including bacteria, fungi, macroalgae and invertebrates (Kathiresan & Bingham, 2001). Growth on submerged mangrove parts, and the invertebrates taking refuge within the growth and amongst the substrata, act as important food sources for juvenile fish (Verweij, Nagelkerken & de Graaff, 2006; Blaber, 2000). Submerged mangroves and their associated epibionts also provide a structurally complex habitat, offering shelter and protection for juvenile fish from larger predators who are less able to negotiate through the mangrove matrix (Laegdsgaard & Johnson, 2001). Protection from predators is also enhanced by reduced underwater visibility. Shading and increased turbidity are often found to correlate with increased survival within these habitats (Blaber & Blaber, 1980; Blaber & Cyrus, 1981). Where mangroves occupy extensive sections of coastline, they enhance the rate of intercept and entrapment of planktonic fish and invertebrates. Characterised by low current speeds and low wave action, mangroves also promote the retention of larvae and as such, increase rates of settlement (Chong, 1995).

Pneumatophores are specialised mangrove roots that provide oxygen to the main root system of mangroves. When pneumatophores are submerged, they produce oxygen internally via photosynthesis, primarily utilising the CO_2 produced by the respiration of the main root system (Yabuki, Kitaya & Sugi, 1990; Aiga *et al.,* 1995: Kitaya *et al.,* 2002). Pneumatophore gas exchange can have a significant impact on water chemistry, depending on the shape, size, depth and refreshment rate of the areas where mangroves grow (Gedan *et al.,* 2017). Areas with low refreshment can experience large variation in pH and other water parameters, with highly acidic conditions often recorded at night (Gedan *et al.,* 2017). In contrast, the dissolved inorganic carbon released by mangroves in open coastal zones can result in a net increase in pH (Sippo, *et al.,* 2016).

Despite a growing recognition of their economic and environmental significance, coastal marine and estuarine ecosystems remain as some of the most highly threatened environments worldwide. Between 2000 and 2012, mangrove cover declined globally by 1646 km^2 at an average rate of 0.16% per year (Hamilton & Casey, 2016). Aquaculture expansion, particularly for shrimp farming, has contributed significantly to mangrove deforestation in recent years. Aquaculture accounts for 52% of mangrove loss globally, with 26% attributed just to shrimp farming expansion (Valiela, Bowen & York, 2001). Harvesting for industrial lumber and woodchip (26%), freshwater diversion (11%) and land reclamation for other purposes (5%) are some of the other key sources of loss (Valiela, Bowen & York, 2001).

Epibionts

Epibionts are algae, plants and sessile animals that settle on biotic or abiotic substrates. Where sufficient light is available, macroalgae usually form a key component of epibiont communities (Wahl & Mark, 1999). Sponges, corals, ascidians, bryozoans, tube building annelids, and some cnidarians, bivalves, sessile gastropods and boring urchins are animal constituents of epibiont communities. With the exception of corals, these groups exclusively occupy hard substrate where light is too limiting for algal growth (Wahl & Mark, 1999). The species that utilise and create hard-substrate together form an extremely important part of marine communities. Filter feeders improve water quality (Ribes *et al.,* 2005), molluscs, bivalves and other invertebrates serve as reef builders (Hutchings *et al.,* 2007; Barbosa *et al.,* 2008) and all sessile constituents act as shelter and provide microhabitats for a suite of other organisms (Puce *et al.,* 2005). Many epibiont species are also harvested or cultured for food and other human uses (Wood, 2001).

Despite the requirement for some mangrove roots to photosynthesise, they are able to accommodate an extensive coverage of epibionts. Where they attach to mangrove roots, epibionts can affect the growth of their host, both directly and indirectly. Examples of each of these mechanisms have been observed in Belizean mangrove communities. In one study, transplants of live sponges onto naked mangrove roots increased the growth rate of roots two- to fourfold compared to controls (Farnsworth &

Twilley, 2008). A closer look into the structure of naturally fouled mangrove roots found that mangroves produce adventitious fine rootlets throughout sponge tissue and that these rootlets closely resemble the kinds of structures that mangroves deploy below ground for nutrient uptake (Farnsworth & Twilley, 2008). In a second Belizean study, mangroves further from the mainland were found to have a greater coverage of sponges and ascidians and so suffered less damage by boring isopods. In mainland estuaries where the epibionts were less common, boring isopods reduced relative root growth by 55 % (Ellison & Farnsworth, 1992). For epibionts, the advantages (or disadvantages) of settling on mangrove roots versus other substrates are poorly understood. In terms of the effects of pneumatophore gas exchange, the impact on epibiont composition is probably unique to the dynamics of individual mangrove creeks.

We know that mangrove epibionts influence the assemblages of fish that utilise mangrove habitats. Several studies have examined the effect of altering epibiont characteristics on fish habitat use. One such study altered the length, vertical orientation and three-dimensional structural complexity of root mimics to explore how they affected a variety of fish community variables (Nagelkerken *et al.*, 2010). It was found that fish abundances decreased significantly with increasing distances between individual mimic roots, suggesting that distance to nearest refuge may be an important determinant of habitat quality (Nagelkerken *et al.*, 2010). The study noted that adding epibionts greatly reduced the interstitial distances between roots and therefore led to increases in fish abundance. In another study, a visual census of natural mangrove roots where epibionts were either left intact or removed by hand showed that epibiont diversity and abundance were the most important determinants of fish diversity and biomass (MacDonald & Weis, 2013). Similar findings were recorded in Laegdsgaard and Johnson (2001), who found that fish abundances increased four-fold when mimic mangrove roots were left to naturally accrue epibionts.

Habitat complexity

The habitat heterogeneity concept is well established in ecology (e.g. Simpson, 1949; MacArthur & Wilson, 1967; Lack, 1969). Essentially, habitats that are structurally complex provide a greater number of niches and opportunities for resource exploitation, thus increasing species diversity (Bazzaz, 1975). In marine habitats, diverse collections of plant and animal species occupy coral reefs where they take advantage of the variety of microhabitats and food sources on offer (Huston, 1985). When structure enhances species diversity, it can, in turn, promote ecosystem stability by increasing the chance that a community will contain species that are resilient to environmental change (McCann, 2000). Higher species diversity also increases ecosystem productivity by decreasing the loss of unused resources (Hooper *et al.,* 2005). In most environments, it is plant communities that generate physical structure (Tews *et al.,* 2003).

The habitat heterogeneity hypothesis outlines how structural diversity is often a more important determinant of species richness than the diversity of species providing the habitat (MacArthur & MacArthur, 1961). For example, MacArthur & MacArthur (1961) identified that for bird species richness, the structural complexity of forest habitats (specifically how the foliage is distributed vertically) is more important than forest composition. The physical structure provided by epibionts in mangrove communities may fulfil a similar role for fish communities in coastal environments. A study by McDonald *et al.* (2008) looked at the role of physical structure versus epibiont type in attracting fish and found that artificial epibionts with a high degree of structural complexity attracted a greater abundance and richness of fish, compared to roots with living, diverse epibiont communities. Notably, while structural diversity may initially attract fish, it may not be conducive to a resource rich habitat. For example, large areas of macroalgae may hold less prey items for juvenile fish than low-profile turf algae (Heldt *et al.,* 2016). When attempting to elucidate the effects of structure on habitat use, these are all important (and confounding) factors that are likely to influence community composition.

Climate change and mangrove habitats

In the last 200 years, the world's oceans have taken up approximately one third of the anthropogenic CO_2 produced (Sabine *et al.*, 2004). Although oceanic uptake of CO_2 has lessened the effect of global warming, it has resulted in changes to ocean chemistry. By the end of the century, if the global community can contain CO_2 emissions to the conservative targets stipulated in the COP21 Agreement, the oceans will experience an average decrease in pH of -0.10 pH units, and an increase in sea surface temperature of 1.2°C (Magnan *et al.,* 2016). Warming and elevated CO_2 concentrations are also expected to affect marine ecosystems via alterations in sea-level, carbonate availability, storm intensity and frequency, oxygen dynamics, upwelling and ocean currents (Hobday *et al.*, 2006; Barange, 2010).

Predicting the fate of mangroves is complicated by the integrative manner in which plants respond to multiple, simultaneous environmental changes. Mangroves are sensitive to a number of changing environmental conditions, including temperature, CO_2, sea-level and rainfall. Warming is expected to result in range extensions at higher latitudes, alongside marginal increases in photosynthesis, respiration, litter fall, microbial decomposition, growth and reproduction (UNEP, 1994). Elevated CO_2 enhances the growth of some species of mangroves, however, this is dependent upon on salinity, humidity and other local environmental parameters (Ball & Cochrane, 1997). Sea level rise and enhanced erosion are the greatest climate related stressors facing mangrove communities (Alongi, 2002). Where landward migration is restricted, evidence suggests that mangroves are able to cope with moderate increases in sea-level, however, some species will experience reduced growth in deeper waters (Ellison & Farnsworth, 1997). Compared with seagrasses, which are highly sensitive to changes in coastal water quality (Orth *et al.,* 2016), and saltmarshes, which are likely to experience range-contractions as mangroves extend into higher latitudes (Vanderzee, 1988; Saintilan & Williams, 1999), mangroves are likely to be one of few resilient coastal habitats moving forwards.

Several studies have established the effects of climate change on natural epibiont communities, although climate experimentation on mangrove epibionts is rare. To investigate the effects of elevated CO_2 on epibionts, Kroeker *et al.* (2012) deployed recruitment tiles at varying distances from a natural CO_2 vent off the coast of Ischia Island, Italy. After 14 months, the tiles from each location differed significantly in their assemblages. The tiles residing in ambient pH harboured the same species as those placed in a lower pH, however, the relative abundance of each species varied significantly (Kroeker *et al.,* 2012). Calcareous species, such as erect and crustose algae, barnacles and calcareous tubeworms, had a higher percentage cover in ambient pH, whereas non-calcareous, fleshy algae dominated in low pH (Kroeker *et al.,* 2012).

It could be expected that fleshy algae will come to dominate mangrove epibiont communities with warming and acidification. Carbonate ions are used by a broad range of epibiont calcifiers (corals, molluscs, echinoderms, crustaceans, sponges, polychaetes etc.) to build their calcium carbonate ($CaCO_3$) shells and skeletons (Fabry *et al.*, 2008). Owing to a decrease in the availability of carbonate ions, and an increase in the concentration of carbonate ions needed to maintain saturation, ocean acidification may interfere with shell and skeleton formation and also act to weaken existing carbonate structures (Kleypas, Feely & Fabry, 2005; Gazeau, Quiblier & Jansen, 2007). The resulting increase in total alkalinity will in turn increase the capacity of the ocean to absorb CO_2, further intensifying these affects over the coming centuries (Tyrrell, 2008). Calcifying species may play a decreasing role in mangrove root communities where they are unable to adapt to elevated CO_2 concentrations. Macroalgal species are likely to benefit to some degree from elevated CO_2, however, when combined with elevated temperature, ephemeral algae tend to outcompete long-lived, slower growing species (Vermeij, Moorselaar & Engelhard, 2010).

An increased presence of algae in mangrove root epibiont communities may alter the functioning of these habitats for the animals that utilise it. Although an increase in fast growing algae may be at the demise of other epibiont species, it may provide the same level, or even an enhanced level of structural complexity for its occupants. Enhanced physical structure may decrease predation risks for juvenile fish and harbour elevated numbers of amphipods and other food items. The idea that climate related changes could improve the functionality of mangrove root habitats is in stark contrast with the predicted outcomes for other important nursery habitats like seagrass and saltmarsh.

Climate change and marine fish

Future CO_2 levels have been found to interfere with the functioning of vertebrate brain neurotransmitters, altering the sensory responses and behaviours of marine fish (Nilsson *et al.,* 2012). Some of the observed changes include increased boldness and activity, loss of behavioural lateralization, altered auditory preferences and impaired olfactory function (including attraction to predators and towards unfavourable habitats) (Munday *et al.* 2009; Dixson, Munday & Jones, 2010; Munday *et al.,* 2010; Domenici, McCormick & Munday, 2011; Simpson *et al.,* 2011). Rossi *et al.* (2015) showed that barramundi larvae, who rely heavily on the protection of mangroves during early life stages, were repelled from mangrove auditory cues like snapping shrimp clicks, showed reduced swimming speeds and exhibited heightened anxiety levels with elevated CO_2. Barramundi are a commercially important species in Queensland, Australia, contributing ~$20 million to the state economy each year (Australian Bureau of Agricultural and Resource Economics, 2009). Alterations to behaviours of this nature will affect mortality rates of some species and therefore have significant implications for community dynamics and ecosystem functioning (Nilsson *et al.,* 2012).

Ocean warming also influences fish behaviour (Nagelkerken & Munday, 2016). Warming induces higher metabolic rates which can lead to altered activity levels, risk-taking behaviour, foraging requirements and habitat use (Pörtner, Langenbuch & Reipschlager, 2004; Biro, Post & Booth, 2007; Pörtner & Peck, 2010). It can also affect the timing of reproductive and migration events, drive changes in range sizes and alter the distribution of species (Pankhurst & Munday, 2011; Martins *et al.,* 2010). Importantly, both ocean warming and acidification can alter species interactions, either by directly effecting one species involved in the interaction or indirectly by forcing novel interactions between species who may not have shared a range or resource previously (Edwards & Richardson, 2004; Harley, 2011; Tunney *et al.,* 2014; Nagelkerken *et al.,* 2015). The impacts of climate stressors on animal behaviour are likely to be far reaching, and difficult in their nature to predict.

Summary

Mangroves and their epibionts create a valuable habitat for a diverse collection of marine species. Epibionts add structural complexity and an increased abundance of food in an already resource-rich habitat. With ocean warming and acidification, the composition of the epibiont community may change, potentially altering the value of the habitat for its animal inhabitants. Meanwhile, marine fish will also experience behavioural changes due to ocean warming and acidification, which are likely to affect how they will interact with mangrove habitats in the future.

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Chapter Two

Fish habitat choices in mangroves and adjacent habitat zones are unaffected by moderate ocean warming and acidification

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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

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Abstract

Individuals make habitat choices at all scales, from selecting a geographic area and associated climate to selecting a specific spot in which to nest. There are many considerations involved in selecting a habitat, including density dependence, the proximity of resources and mates and the threat of predation. Understanding the drivers of habitat choice therefore provide a great deal of insight into a species' life history. Like the quality of available habitats, for some species the internal processes that govern habitat choices may be affected with environmental change. Ocean acidification is of particular significance for marine fish, as it is known to interfere with brain neurotransmitters, resulting in significant and often deleterious behavioural changes. Here, we undertook a large scale, community based mesocosm experiment to understand how exposure to moderate levels of ocean warming and acidification affect how mangrove fish utilise available macrohabitats. We looked specifically at five macrohabitat zones; 1) the mangrove matrix (directly amongst mangrove roots), 2) the mangrove undercut (beneath mangrove roots), 3) the mangrove benthic (on the sediment beneath mangrove roots), 4) the unstructured pelagic zone (representing the middle of a mangrove channel), and 5) the unstructured benthic zone (on the sediment in the middle of a mangrove channel). Remarkably, we found that ocean warming and acidification had no effect on the macrohabitat choices of the fish. Regardless of the climate stressors, all species utilised the mangrove root zone as their primary habitat, however, they showed varied preferences for their secondary and tertiary choices, where in some cases, ~15 % of the population would reside at any one time. Here, we demonstrate that if the global community is able to contain CO_2 emissions to the level of conservative emission scenarios, it is likely that current patterns of mangrove use by marine fish will remain intact in the near future.

Introduction

Habitat choice is at the core of key ecological processes such as population control, species interactions, the organisation of ecological communities and the origin and maintenance of biodiversity (Morris, 2003). The selection of a habitat is not independent from density dependence (Bowler & Benton, 2007), resource requirements and availability (Gregory & Gaston, 2003), or predation and reproduction (Magnhagen, 1991). When we observe habitat choices, we are therefore provided with a unique insight into a species' life history.

Habitat choice occurs at many spatial scales (Resetarits, 2005). Migratory birds regularly travel thousands of kilometres to access new or better resources, or to reproduce (Alerstam, Hendenstöm & Akesson, 2003). At this scale, habitat choice is occurring at its broadest level, with some species shifting into unfamiliar biomes and crossing climatic boundaries. For other species, their primary habitat may be predetermined based on their ability, or lack-there-of, to travel large distances. In this case, we still see patterns of microhabitat choice at local scales (Fulton *et al.,* 2016). Among species living in proximity, the difficulty of finding an available space that satisfies the most important habitat requirements can result in spatial or temporal niche segregation. For example, tropical cricket species display significant vertical stratification in rainforest environments (Schmidt, Römer & Riede, 2013). Even more remarkably, their calls vary in sound frequency depending on how many other species are residing in the same area (Schmidt, Römer & Riede, 2013). In species-rich areas, the crickets use narrower bands of frequencies to call to conspecifics, and also call at specific times during the day to minimise the masking of their calls by other species (Schmidt, Römer & Riede, 2013). For most species, the scales at which habitat selection takes place and driving factors behind their choices are yet to be elucidated.

Whilst the advantages of habitat selectivity are clear, we are still understanding the mechanisms used by species to detect, assess and select their habitats. In the case of marine fish, we know that several senses are involved in choosing a suitable settlement location. Auditory, olfactory and visual cues are used to locate suitable habitats and the development of operational fins and strong musculature allow individuals to move towards those cues once recognised (Leis, 2006; Montgomery *et al.,* 2006: Dixson, Albrego & Hay, 2014). Different habitat cues are used at varying distances from ideal habitats. For example, electrophysiological assessments of larval fish show that they can gather both qualitative (e.g. type of habitat) and quantitative (e.g. distance to habitat) information about their surroundings (Wright, Higgs & Leis, 2011). Auditory information is more important than visual cues at greater distances from habitats, and for fish that settle at night, visual cues may not play a role in macrohabitat choices at all. In terms of detecting and selecting suitable microhabitats within the matrix of their desired macrohabitat, it is likely that the same cues are utilised, although the relative importance of each is likely to be highly species specific (Huijbers *et al.,* 2012).

Environmental change could be expected to lead to a re-organisation of communities where macrohabitat choice is at play. Degraded habitats are typically characterised by reduced resource availability. As such, they are unable to support the same diversity of species as healthy habitats, where the availability of a range of macrohabitats and resources enable niche segregation. In the marine realm, changing habitat quality may also be accompanied by alterations in the ability of organisms to orientate themselves towards suitable habitats (Rossi *et al.*, 2016). It is well established that ocean acidification interferes with fish neurotransmitters, which has been linked to striking behavioural abnormalities (Nagelkerken & Munday, 2016). For example, fish who are continuously exposed to elevated CO_2 at natural vents show attraction towards predator odours, as well as increased boldness overall (Munday *et al.,* 2014; Nagelkerken *et al.,* 2015). Where environmental change alters the quality of a habitat and simultaneously alters the sensory abilities of its inhabitants, it becomes extremely difficult to predict the shape of key marine communities moving forward.

Mangroves are an excellent study case for examining the effects of climate change on macrohabitat choice. Among other ecological services, mangroves are an important nursery ground for commercially significant fish and crustacean species (Nagelkerken *et al.,* 2008). Part of their capacity to host a diverse range of species is the variety of macrohabitats available within and around the mangrove root matrix. Mangrove roots create spaces for smaller individuals to hide and nooks for prey items to occupy (Laegdsgaard & Johnson, 2001; Verweij et al., 2006). Mangrove epibionts like algae and calcifying animals, increase the structural complexity of mangrove roots and provide more food, both by hosting small prey items and as a primary source (Lubbers, Boynton, & Kemp, 1990). Below the region where mangrove roots penetrate the sediments, erosion by water movement along mangrove lined channels often creates an “undercut”, where a second key macrohabitat is created. This under-cut space is often darker and lined with silty sediments where prey items are found in enormous numbers (Nagelkerken *et al.*, 2008). Moving outwards from the mangrove matrix, shallow, slow moving waters and vast areas lined with silty sediments creates a third macrohabitat where larger or less vulnerable species are often found to occupy.

Here, we examine how ocean warming and acidification alter mangrove macrohabitat choices. We created realistic mangrove communities in large mesocosms and exposed them to elevated temperature and CO_2. Our mesocosms incorporated five macrohabitat zones, including mangrove roots and their associated epibionts, a mangrove undercut habitat, shaded and exposed benthic habitats and a pelagic, unsheltered habitat. We surveilled the behaviour of five mangrove fish species after four weeks of exposure to the climate treatments and tested the hypothesis that future conditions would induce changes in the macrohabitat choices of at least some of our study species.

Materials and Methods

Mesocosms and climate treatments

Our mesocosms were designed to simulate shallow, permanently inundated mangrove root habitats. In the natural environment, these habitats are found in coastal areas experiencing relatively low tidal amplitudes, or in permanently inundated tidal channels in areas with large tidal amplitudes. The mangrove communities in these environments are typically occupied by trees within the genera *Rhizophora* and *Avicennia*. The set-up consisted of 12 circular mesocosms, each with a capacity of 2,300 litres (1.20 m high, 1.6 m diameter). We administered current and predicted concentrations of CO_2 (350-400 and ~500 ppm, respectively) in a crossed combination with ambient and elevated temperatures (ambient +1.2 °C), equating to three replicates for each of the four treatments (control, elevated CO_2, elevated temperature, elevated CO_2 and elevated temperature). The climate treatments were based on an aggregation of intended nationally determined contributions (INDCs) towards carbon emission reductions for the year 2100 (see http://climateactiontracker.org/). These intended contributions were updated following the COP21 agreement and equate to sea surface temperature and pH changes of 1.2°C and -0.10 pH units respectively (Magnan *et al.,* 2016). See supplements (page 47) for a detailed recount of the mesocosm methodology.

Constructing the mangrove community

Mimic mangrove roots were placed in two natural mangrove creeks in June 2016. At these sites, natural *A. marina* roots hosted algae-dominated epibiont communities, common among temperate mangroves (Gwyther & Fairweather, 2005), consisting primarily of *Enteromorpha* spp. and *Ulva* spp. The mimic roots consisted of untreated, smooth pine stakes, measuring 17 × 17 × 1200 mm. The stakes were hammered into the banks of inundated channels, mimicking the position and orientation of natural roots. The stakes were retrieved after approximately four months of colonisation time. To create the mangrove habitat in the mesocosms, wooden frames were constructed to hold 10 artificial mangrove roots along a length of 1.2 m. Shade cloth was fitted over the mangrove root habitat removing 90% of incoming light. The number of density of roots, angle of orientation and the light conditions used in the experiment were based on the results of field surveys conducted prior to the experiment (see supplements).

The mesocosms were stocked with common juvenile fish found in local mangrove creeks and estuaries. Two pelagic species (*Liza argentea* and *Aldrichetta forsteri*) and three benthic species (*Neoodax balteatus*, *Tetractenos glaber* and *Pseudaphritis urvillii*) were chosen of which there were seven individuals per species allocated to each mesocosm. Fish were caught across a variety of coastal sites in South Australia using a seine net. All the selected species are common across South Australian estuaries, bays and coastal regions. While none of the species are exclusively found in mangrove habitats, they were the most common species observed at our mangrove sampling sites.

A. forsteri is the most common mullet species in southern Australia. Along with *L. argentea*, they are found in coastal marine regions, entering freshwater zones (Bray, 2018(b); Bray & Gomon, 2018). *T. glaber* is abundant in bays and estuaries throughout South Eastern Australia, favouring sandy, muddy habitats, often seen in mangroves and adjacent seagrass beds (Bray, 2018(c)) and *P. urvillii* inhabits slow moving waters in estuaries, rivers and creeks, easily moving between brackish and freshwater habitats (Bray & Thompson, 2017(b)). Little information is available on the ecology of *N. balteatus*, however, we found an abundance of these individuals in amongst seagrass beds and adjacent mangroves. We elected to limit the number of individuals of each species to seven in each mesocosm. This was based on a need to have sufficient replicates whilst avoiding overcrowding. We acknowledge that this may influence fish behaviours, particularly for species like *A. forsteri* and *L. argentea* who typically school, and *N. balteatus* who is used to less densely populated environments.

All fish were between 4 and 7 cm in length at the beginning of the experiment. Upon their capture, fish were first habituated to captivity for three weeks in smaller bins and then introduced into the mesocosms. One week after their addition to the mesocosms, the climate treatments were applied progressively over seven days until the treatment targets were achieved. The fish were fed a combination of blended sardines, prawn and squid *ad libitum*.

Habitat zone definition

For the purpose of the habitat behavioural analyses, we defined three different zones within the mesocosms 1) the mangrove zone, 2) a buffer zone and 3) an unstructured zone (Fig. 1). Each zone occupied one third of the mesocosm area. The mangrove zone was further divided into three subzones (Fig. 1); the mangrove matrix was defined as the area into which the mangrove roots protruded and the spaces between the roots, the mangrove undercut was defined as the open area directly beneath the roots and the mangrove benthic zone was defined as the base of the mesocosm in the mangrove zone third. The base of all mesocosms was lined with sandy, silty sediments.

The unstructured zone was divided into two subzones; the unstructured pelagic, defined as the open water inside the unstructured third of the mesocosm, and the unstructured benthic zone, defined as the base of the mesocosm in the unstructured zone third. The buffer zone in the middle of each mesocosm was not surveyed, and served to create a distinction between the two experimental zones, which was especially useful in making observations in video recordings.

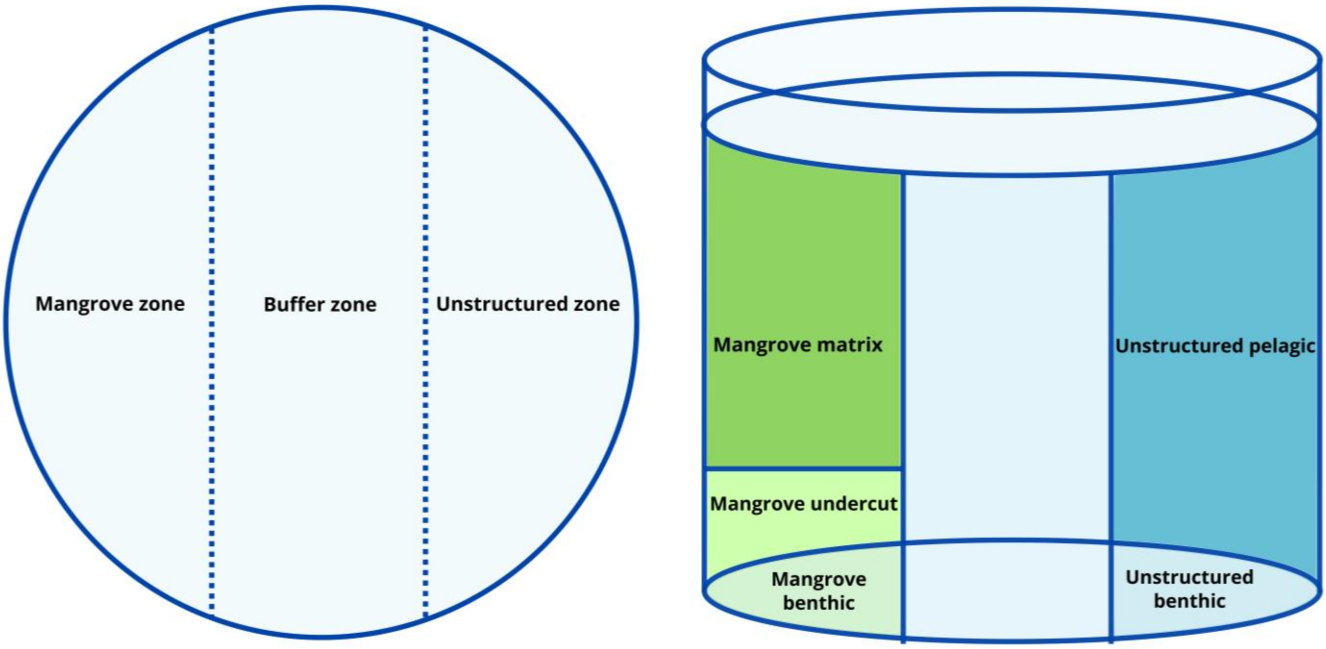

Figure 1: Left: diagram showing the mesocosms from an aerial view. The mesocosms were divided into three zones, the mangrove zone, an unstructured zone and a buffer zone between the two. Note that there is no physical barrier separating the zones. Right: diagram showing the definition of five macrohabitat sub-zones.

Video recordings

Using three high-resolution submersible cameras (GoPro Hero4 Silver, 60fps 1080p), fish macrohabitat use was recorded in each mesocosm after four weeks of exposure to the climate treatments. One camera was positioned 30 cm below the surface of the water at a 90° angle and 1.4 m from the mangrove habitat, such that all the roots were in view. A second camera was positioned 50 cm below the surface at a 135° angle, such that the mangrove undercut and the mangrove benthic zone were in view. The third camera pointed in the opposing direction, 40 cm below the surface at a 120° angle, capturing the entire unstructured zone. In each mesocosm, the cameras recorded for 10 minutes at midday. To account for the disturbance created by the introduction of the camera frame, the fish were always allowed to acclimate to the frame for 3.5 min before observations were recorded.

The video footage was analysed using the program VLC, which allowed the viewer to zoom in as required and slow or pause the footage at their leisure. To quantify the habitat choices of each study species, we calculated the average proportion of each species' population within the five zones, over six minutes of recording time. At intervals of 20 seconds, we paused the footage and counted the number of fish from each species residing in each of the zones. Because it was difficult to quantify the number of surviving fish four weeks into the experiment, the maximum number of fish belonging to each population observed in any single interval was used to calculate proportions for the remaining intervals.

Ethics

This research was carried out under the approval of The University of Adelaide Animal Ethics Committee (project: S-2016-087). Fish collections were permitted by the Minister for Transport and Infrastructure and the Government Department of Primary Industry and Regions SA (exemption: 9902844).

Statistical analysis

A PERMANOVA analysis was conducted to assess the level of macrohabitat segregation between the fish species. This was completed using the function "adonis" in the R package "Vegan" (Oksanen *et al.,* 2018). A non-metric multi-dimensional scaling plot was also produced using the "Vegan" R package.

Results

The fish species showed a significant segregation (MANOVA, p = 0.0001) in their use of the five macrohabitat zones (Fig. 2). The nature of this segregation was unaffected by the climate treatments (Table 1).

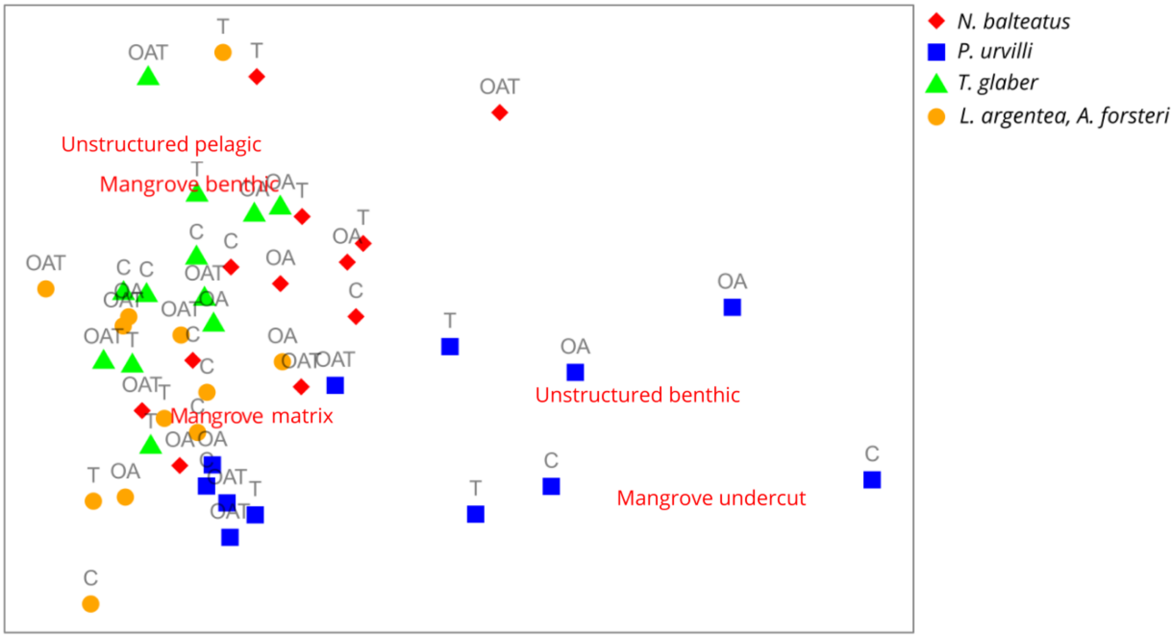

Figure 2: A non-metric multi-dimensional scaling plot constructed from the proportion of individuals of each fish species occupying each of five mangrove macrohabitat zones. Each marker represents a population of one fish species from a single mesocosm. The grey text outlines the climate treatment each population was subjected to. The macrohabitat zones are superimposed in red. Markers clustered around the macrohabitat zone labels indicate populations who use those zones frequently. Markers that are close together indicate similar macrohabitat decisions amongst the populations. (C = control, T = elevated temperature, OA = elevated $p\text{CO}_2$, OAT = combined elevated temperature and $p\text{CO}_2$)

Table 1: PERMANOVA analysis for macrohabitat use.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
T	1	88.961	88.961	0.87607	0.4491	9001
OA	1	37.864	37.864	0.37288	0.734	8963
Species	**3**	**2151.2**	**717.08**	**9.5683**	**0.0001**	**9930**
TxOA	1	62.421	62.421	0.61471	0.5734	8958
TxSpecies	3	275.03	91.676	1.2233	0.2995	9946
OAxSpecies	3	357.98	119.33	1.5922	0.1354	9941
Mesocosm(TxOA)	8	812.37	101.55	1.355	0.1581	9907
Pooled	27	2023.5	74.943			
Total	47	5809.3				

The mangrove matrix formed the primary macrohabitat for all species of fish (Fig. 3). The macrohabitat segregation detected in the PERMANOVA represented the varied preferences for secondary and tertiary habitats amongst the fish species (Fig. 3). When not occupying the mangrove matrix, *P. urvilli* took refuge in the two benthic zones, whilst *T. glaber* utilised the mangrove benthic zone and unstructured pelagic zone. The mangrove benthic zone was the second preference for *N. balteatus*, *L. argentea* and *A. forsteri*.

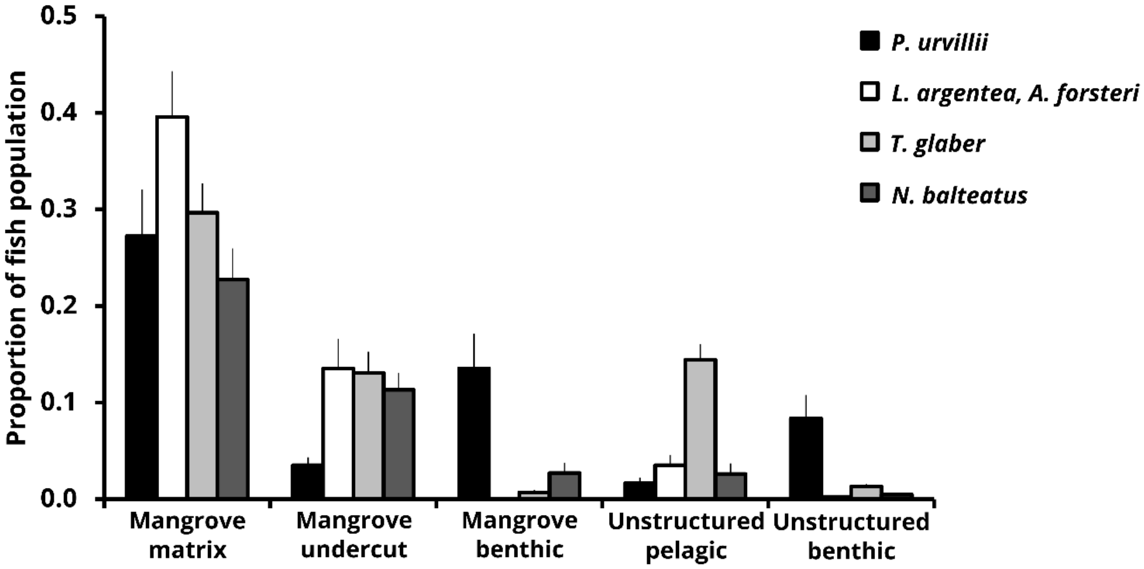

Figure 3: The average proportion of each fish population occupying five mangrove macrohabitat zones at any one time.

Discussion

Climate stressors have been reported to affect habitat choices via a number of direct and indirect pathways (Nagelkerken & Munday, 2016). Ocean acidification has been found to alter the behaviours of fish by interfering in the function of neurotransmitters in the brain, leading to increased boldness and attraction towards predators and unsuitable habitats (Nilsson *et al.,* 2012; Watson *et al.,* 2014). These affects can be detected within 24-96 hours of exposure, depending on the CO_2 level experienced (Munday et al., 2010) Secondly, by altering the type of quality of available habitats and their resources, both warming and acidification can illicit changes in habitat choice (Lönnstedt *et al.,* 2014; Ockendon *et al.,* 2014; Nagelkerken *et al.,* 2015). Thirdly, they can alter the nature of physical and biological cues that fish use to locate and choose habitats (Arnold *et al.,* 2012; Leduc *et al.,* 2013; Chivers *et al.,* 2014) and finally, warming and acidification can modify the abundance, distribution or behaviours of another species with whom one interacts (Vergés *et al.,* 2014).

In contrast to our hypothesis, we found no effect of ocean warming or acidification on the macrohabitat choices of any of the study species. In terms of the direct effects of acidification on neurotransmitter function, it is entirely possible that the conservative emission scenario tested here was not significant enough to illicit an effect. Moreover, where there may have been changes to the structure of the epibiont habitat and the behaviours of some of the study species due to the climate stressors, these were not significant enough to drive changes in the general macrohabitat choices made by the fish. Notably, here, we have only investigated habitat choice at the macrohabitat scale. It is possible that the climate stressors affected the behaviours of the study species at the microhabitat level, within one of the habitat zones. It is little surprise that the mangrove matrix was utilised most by all of the study species. Experimental efforts to elucidate why mangroves are densely populated by juvenile fish have shown strong ties to physical structure. Laegdsgaard and Johnson (2001) found that artificial mangrove structures in the field attracted slightly more juvenile fish than unstructured sites and that artificial structures left to accumulate epibionts attracted four times the number of juvenile fish than cleaned structure. Interestingly, they also found that the fish communities attracted to the three levels of structure differed in their species composition. They found that food availability, shelter and reduced predation pressure had differing levels of importance for the various species they studied (Laegdsgaard & Johnson, 2001). It is likely that whilst the mangrove matrix was the primary habitat choice for all of our study species, the differences in secondary and tertiary choices reflect a similar pattern to the Laegdsgaard and Johnson (2001) findings.

It is likely that shelter played an important role in the habitat choices of *N. balteatus*, *L. argentea* and *A. forsteri*, for example, as these species were scarcly observed in either of the two unstructured zones. In comparison, *P. urvillii* and *T. glaber* were relatively well represented in at least three zones, including one of the two unstructured. *P. urvillii* is a benthic species, often observed resting or feeding in the sediment along shallow, calm shore lines (Bray & Thompson, 2017). Similar behaviours were noted during informal observations of *P. urvillii* over the experiment. Even within the mangrove matrix where the species resided most, they were most often seen laying upon a mangrove root, rather than swimming between the roots, as was common for the remaining study species. Whilst *N. balteatus*, *L. argentea, A. forsteri* adnd *T. glaber* are all pelagic species, the tendency of *T. glaber* to utilise not only the mangrove matrix but also the unstructured zone is most likely representative of their lack of predation risk and general boldness (Bray, 2018(c)). *N. balteatus*, who often occupies dense seagrass in addition to mangroves (McGrouther, 2019), showed a consistent attraction to stucture, staying within the mangrove matrix and mangrove undercut almost exclusively.

It is important to note that the species included in this study are not found exclusively in mangrove habitats. Had our chosen species had more specialist habitat requirements or lower tolerances to variations in water chemistry, ocean warming and acidification may have had a greater influence on their habitat choices. Nevertheless, being the most abundant species within South Australian mangroves, the results are representative of the level of impact expected in the region

Here, we demonstrate that by reducing CO_2 emissions to reach the moderate targets stipulated by the COP21 Agreement (Magnan *et al.,* 2016), the habitat choices of juvenile fish will likley be unaffected. However, as ecological communities are complex and community organisation occurs at all levels, further work is required to determine how the climate stressors affected our study species at other scales and across other aspects of their life histories. We note that our observations of secondary and tertiary habitat choices closely followed the known behaviours of our study species, instilling confidence that our experiment provided a good frame work for the accuate study of the effects of climate stressors on community dynamics and further supporting the need for complex, realisitc experiments where study species are allowed to behave in ways in which they would in their natural environments.

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Supplementary tables and figures

Mesocosms

The set-up consisted of 12 circular mesocosms, each with a capacity of 2,300 litres. Each mesocosm contained a mangrove root habitat, comprised of artificial mangrove roots, colonised by natural mangrove epibionts. Two additional header tanks received a constant inflow of fresh, unfiltered seawater from an offshore pipeline, located 1.5 km off the coast at 8 m depth. Each header tank held six submersible pumps (~ 1.8 m^3 h^{-1}) that delivered water to eight treatment mesocosms. Four control mesocosms received inflowing seawater directly from the offshore pipeline. The inflow rate of seawater in all mesocosms was set at two litres per minute, equating to a full refreshment of each mesocosm every 15 hours. Excess water drained from the mesocosms through a 2 mm mesh filter head. The set-up was established outdoors under full sunlight.

Climate treatments

The climate treatments were based on an aggregation of intended nationally determined contributions (INDCs) towards carbon emission reductions for the year 2100 (see http://climateactiontracker.org/). These intended contributions were updated following the COP21 agreement and equate to sea surface temperature and pH changes of 1.2°C and -0.10 pH units respectively (Magnan *et al.,* 2016). We administered current and predicted concentrations of CO_2 (350-400 and ~500 ppm, respectively) in a crossed combination with ambient and elevated temperatures (ambient +1.2 °C), equating to three replicates for each of the four treatments (C: control, OA: elevated pCO_2, T: elevated temperature, OAT: elevated pCO_2 and elevated temperature). Treatment levels were maintained for four weeks.

The temperature and pH treatment targets were achieved by pre-treating inflowing seawater and by directly administering treatments within the mesocosms. We used one header tank to distribute pre-heated seawater to all elevated temperature mesocosms. The mesocosms subject to elevated temperature were also each equipped with two supplementary heaters. Field measurements indicate that the water temperature of permanently inundated mangrove creeks are largely determined by the air temperature

(Fig. S1). As the experiment was conducted outdoors in uninsulated tanks, the temperatures of our control mesocosms fluctuated in a very similar way (Fig. S1). We measured temperature three times per day and adjusted the heating accordingly to achieve an average temperature difference of 1.21 ± 0.09 °C throughout the experimental period.

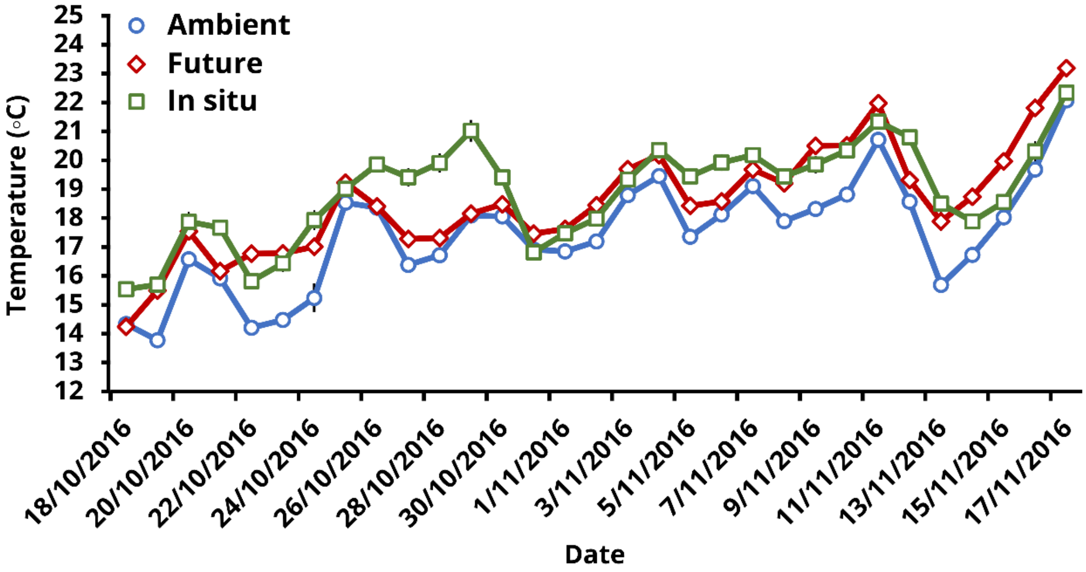

Figure S1: Daily average temperature in future temperature mesocosms (T and OAT), ambient temperature mesocosms (C and OA), and in a natural, permanently inundated mangrove creek at Saint Kilda (*in situ*), South Australia. Saint Kilda functioned as an arbitrary mangrove creek to show the pattern of variability in seawater temperature rather than absolute temperatures. Neither fishes nor algal epibiont assemblages were collected from St. Kilda. Mesocosm averages are based on 3 measurements taken per mesocosm at 9:00 am, 12:00 pm and 3:30 pm each day using two probes (913 Metrohm and Mettler Toledo SG2 SevenGo™ probes, 18 measurements per time point). Field measurements at Saint Kilda, SA, were taken every 30 minutes using a HOBO underwater temperature logger. Error bars are ± standard error.

A second header tank was used to distribute pre-acidified seawater to all elevated $p\mathrm{CO}_2$ mesocosms. The pH was lowered in the CO_2 header tank by bubbling pure CO_2 directly into the water at a constant rate. The header tank pH varied between 8.08 and 8.10 (0.13–0.15 pH units below ambient seawater). A gas mixer was also used to deliver enriched air at 500 ppm $p\mathrm{CO}_2$ to the elevated $p\mathrm{CO}_2$ mesocosms. Non-acidified mesocosms received ambient air at the same rate (pH = 8.23 ± 0.01). The combination of delivering pre-treated water and enriched air lowered the pH in OA and OAT tanks to 8.13 ± 0.01 and 8.14 ± 0.01, respectively, an average reduction of -0.10 pH units compared to the controls (Fig. S2). Temperature and pH were measured three times per day using two handheld meters (913 Metrohm and Mettler Toledo SG2 SevenGo™) calibrated once daily. Alkalinity and pCO_2 were calculated weekly (Table S1) using CO2SYS (Pierrot, Lewis & Wallace, 2006) for Excel with constants from Mehrbach *et al.*(1973) refit by Dickson and Millero (1987).

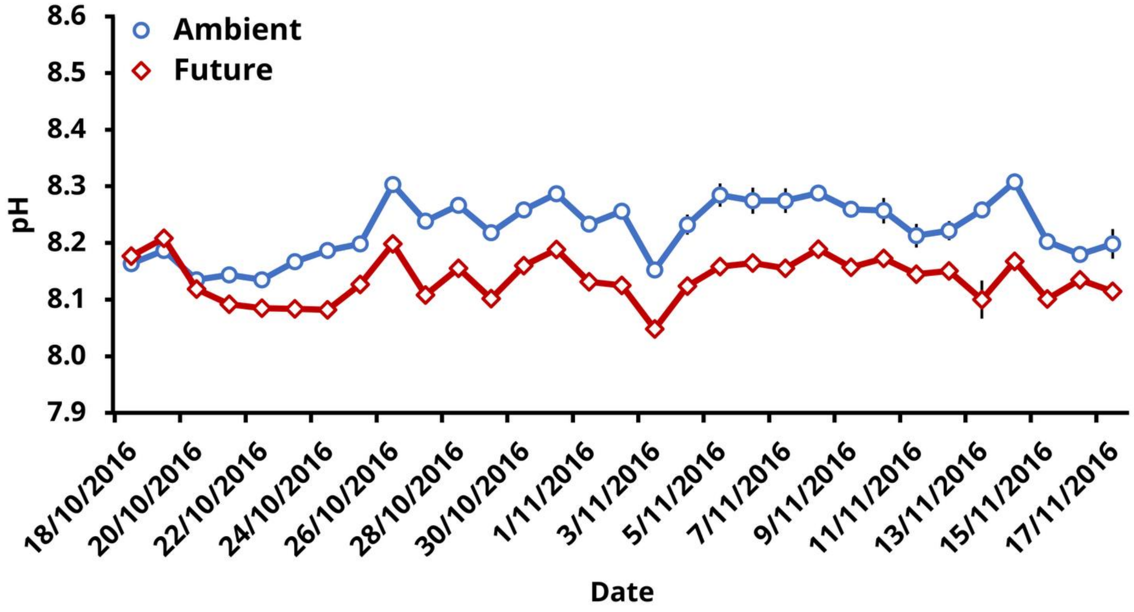

Figure S2: Daily average pH in future pCO_2 (OA and OAT) and ambient pCO_2 (C and T) mesocosms. Averages are based on 3 measurements taken at 9:00 am, 12:00 pm and 3:30 pm each day using two probes (913 Metrohm and Mettler Toledo SG2 SevenGo™ probes, 18 measurements per time point). Error bars are ± standard error.

The salinity of the incoming seawater measured consistently at 36 ppt. In-field measurements varied between 10 and 36 ppt.

Table S1: Alkalinity and pCO_2 treatment averages (± standard error). Alkalinity and pCO_2 were measured weekly over the duration of the experiment in each mesocosm) using CO2SYS (Pierrot, Lewis, & Wallace, 2006) for Excel with constants from (Mehrbach, Culberson, Hawley, & Pytkowitz, 1973) refit by (Dickson & Millero, 1987).

	C	OA	T	OAT
Alkalinity (μmol kg^{-1})	2258.56 ± 28.66	2315.29 ± 5.76	2309.53 ± 40.49	2259.96 ± 26.56
pCO_2 (ppm)	353.53 ± 36.09	530.97 ± 5.16	375.40 ± 14.87	518.93 ± 19.35

Constructing the mangrove habitat

Prior to the experiment, a series of field surveys were conducted to characterise the structural complexity of mangrove prop roots. Permanently inundated channels, lined by *Avicennia marina*, were studied at Port Gawler, Middle beach (two sites), Chinaman Creek and Weerona Island, South Australia. At each site, three 10-m transects were set along the creek banks, each comprising of ten 1 m x 1 m quadrats. Within each quadrat, the number of submerged roots was counted to provide a measure of root density per metre of bank. The length of each root and the angle at which it protruded through the bank were also measured (n = 1126 individual roots). Across sites, prop roots occurred at an average density of 8.8 ± 0.5 roots m-1. The majority of the roots protruded through the creek banks at ~90° from vertical (55.3 ± 3.4% of all roots measured). The remainders were ~135° (36.8 ± 3.2%) and ~180° (7.9 ± 1.6%) from vertical. The average length of the roots was 29.0 ± 0.5 cm. To characterise the light environment in the root habitat, one light measurement was taken in each quadrat using a light meter held just above the surface of the water alongside the bank. The mangrove canopy allowed ~6% of incoming light to reach the prop root habitat at midday, with an average light intensity across sites of 135.3 ± 22.3 μmol m^{-2} s^{-1}.

Following the site surveys, mimic mangrove roots were placed in two natural mangrove creeks (Middle Beach and Port Gawler) in June 2016. At these sites, natural *A. marina* prop roots hosted algae-dominated epibiont communities, common among temperate mangroves (Gwyther & Fairweather, 2005), consisting primarily of *Enteromorpha* spp. and *Ulva* spp. The mimic roots consisted of untreated, smooth pine stakes, measuring

17 × 17 × 1200 mm. Whilst their shape and surface simulated mangrove roots well, the pine stakes were not living structures and therefore could not respire and photosynthesise like real mangrove pneumatophores. We acknowledge that the assumption that root gas exchange will have no effect on the epibiont community (and vice versa) is somewhat limiting; however, previous studies have found that with sufficient time, wooden mimics can host the same composition of algal epibionts as real roots (Gwyther & Fairweather, 2002). The stakes were hammered into the banks of inundated channels, mimicking the position and orientation of natural prop roots. The stakes were retrieved after approximately four months of colonisation time. At the time of retrieval, all the collected stakes had been colonised to some degree by turf and macroalgae.

Based on the density of mangrove roots observed in field surveys (see above), wooden frames were constructed to hold 10 artificial mangrove roots along a length of 1.2 m. To approximate the natural positioning of mangrove roots, six stakes were positioned 90° from vertical and four were positioned 135° from vertical. Likewise, the stakes were cut to lengths between 8 and 31 cm to match field observations. Care was taken to ensure that the artificial root habitats in each mesocosm started the experiment with approximately the same composition and biomass of the most abundant algal species. Shade cloth was fitted over the mangrove root habitat (Figs. S3, S4) removing 90% of incoming light, closely approximating the light climate created by natural mangrove canopies.

Figure S3: top left: a typical temperate mangrove creek. Top right: protruding mangrove roots hosting algae, sponges and trapped debris. Bottom: wooden stakes placed amongst mangrove roots to seed (Port Gawler, South Australia).

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Chapter Three

A future 1.2 °C increase in ocean temperature alters mangrove habitats for marine plants and animals

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Statement of Authorship

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Name of Principal Author (Candidate)	Georgia Walden		
Contribution to the Paper	Study design, data collection, data analysis, writing		
Overall percentage (%)	80		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	28/03/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Christelle Noirot		
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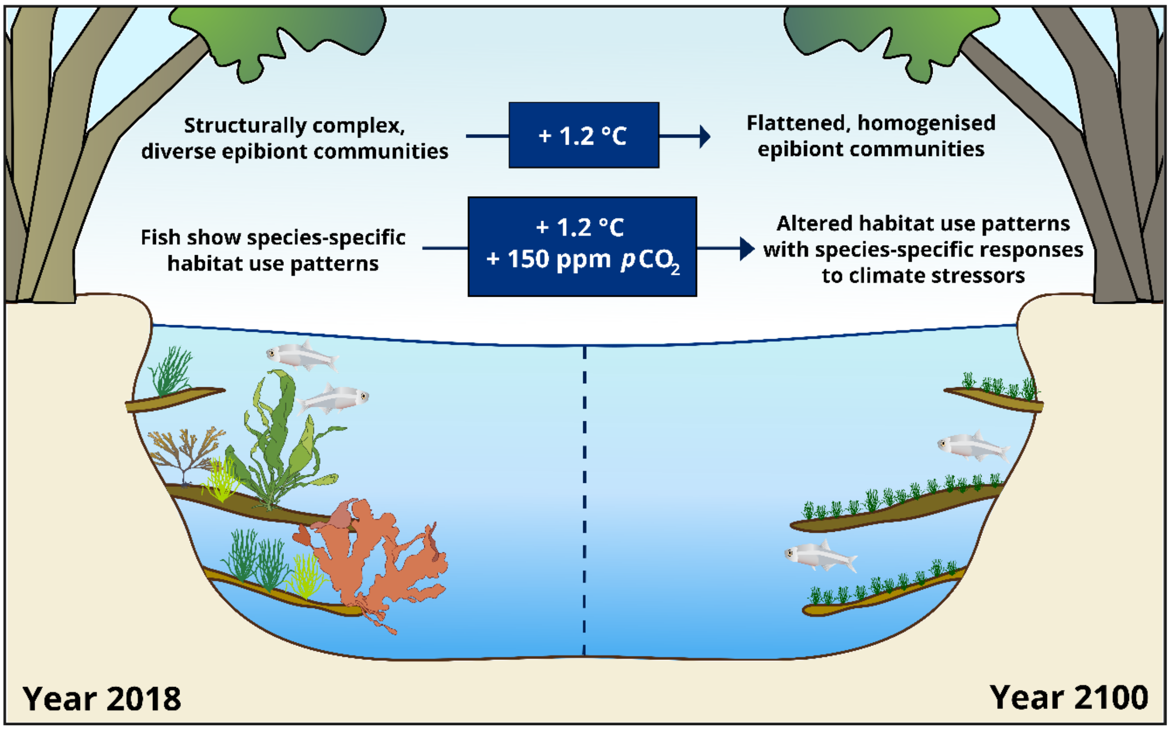
Contribution to the Paper	Study design, writing		
Signature		Date	28/03/2019

Graphic Abstract



Graphic abstract: A conceptual diagram showing how ocean warming can modify the structure and composition of mangrove epibionts, and how the combination of ocean warming and acidification can alter the habitat use patterns of juvenile fish.

Abstract

The quality of living habitat provided by foundation species is tied closely to the composition and physical complexity of their structure. Global climate stressors, like ocean warming and acidification, contribute to the erosion of structural complexity in marine foundation habitats by promoting the growth of low-relief turf, increasing grazing pressure on marine vegetation, and by directly affecting the growth and survival of foundation species. Mangroves are a unique foundation species in that their structural complexity is created not only by their submerged woody roots but also by the fouling species (epibionts) that occupy their surfaces. Because mangrove roots are woody and their epibionts are used to ever-changing conditions in highly variable environments, mangrove habitats may be more resilient to global change stressors than other marine foundation species. Using a large-scale mesocosm experiment, we examined how ocean warming and acidification, under a reduced carbon emission scenario, affect the composition and structural complexity of mangrove epibiont communities and the use of mangrove habitat by juvenile fish. We demonstrate that even a modest increase in seawater temperature of 1.2 °C leads to the homogenisation and flattening of mangrove root epibiont communities. Warming led to a 24% increase in the overall cover of algal epibionts on roots while the diversity of the epibiont species decreased by 33%. Epibiont structural complexity decreased owing to the shorter stature of weedy algal turfs which prospered under elevated temperature. Juvenile fish showed species-specific alterations in mangrove habitat use with ocean warming and acidification, but these were independent of changes to the root epibiont community. Our results reveal that the quality of mangrove habitats and their perceived value for associated fauna are still vulnerable under a globally reduced carbon emission scenario.

Introduction

Habitat structural complexity is an important mediator of species niche partitioning that sustains biodiversity (Tews *et al.,* 2004). Structure enhances the surface area of available habitat, creates more physical refugia in a variety of shapes and sizes, increases the abundance of limiting resources (MacArthur & MacArthur, 1961) and moderates predator efficiency by reducing prey capture rates (Crowder & Cooper, 1982). Across nearly every land- and seascape, organisms are attracted in some way towards physical structure. In arid landscapes, scattered trees provide refugia and create a more favourable microclimate for plants and animals beneath their canopies, resulting in a greater diversity of organisms relative to surrounding plains (Manning, Fischer, & Lindenmayer, 2006). Likewise, diverse collections of plant and animal species occupy coral reefs where they take advantage of the variety of microhabitats and food sources on offer (Huston, 1985). When structure enhances species diversity, it can, in turn, promote ecosystem stability by increasing the chance that a community will contain species that are resilient to environmental change (McCann, 2000). Higher species diversity also increases ecosystem productivity by decreasing the loss of unused resources (Hooper *et al.,* 2005).

Foundation species are those that have a strong influence on community dynamics and ecosystem processes, often modifying the abiotic environment in ways that are beneficial and depended upon by a range of other species. More often than not, they are also living providers of physical structure (Ellison *et al.,* 2005). Anthropogenic activities have led to the loss of foundation species in a variety of environments, having broad consequences for associated biota, biodiversity, ecosystem function and stability (Ellison *et al.,* 2005).

Perhaps the most widely felt losses of foundation species occur where they are replaced with a flat, homogeneous habitat. In the marine environment, the value and scarcity of physical structure are evidenced by the rapid colonisation of manmade additions like jetty posts and wave breaks (Bohnsack, 1989; Rilov & Benayahu, 1998). Mangroves, oysters, kelps, corals and seagrasses are key foundation species and often the only natural providers of physical structure in coastal habitats. Each of these habitats has their own unique set of vulnerabilities to global change stressors and local anthropogenic impacts. Ocean warming has facilitated the loss of temperate kelp forests, for example, by enabling the spread of the herbivorous long-spined urchin (Ling *et al.,* 2009). Likewise, seagrasses and corals are experiencing significant and widespread declines linked to coastal eutrophication, and the overfishing and spread of disease amongst key herbivore species (Hughes *et al.,* 2007; Waycott *et al.,* 2009). In each of these cases, the removal of foundation species and the physical structure they provide has been associated with documented losses in community biodiversity (Hughes *et al.,* 2009; Ling, 2008; Reed & Hovel, 2006). Moreover, the increase in climate-related stressors is expected to exacerbate each of these processes, leading to further loss of critical habitat structure (Diaz-Pulido *et al.,* 2011; Ling *et al.,* 2009; Nagelkerken *et al.,* 2016; Orth *et al.,* 2006).

Mangroves are a key foundation species in coastal environments and are unique to corals, kelps and seagrasses in that the majority of their photosynthetic biomass usually sits above water. Via a number of unique morphological and physiological adaptations, mangroves can tolerate submersion, high salinities, extreme tides, strong winds, highly variable temperatures, and anaerobic, muddy soils (Kathiresan & Bingham, 2001). Few other groups of plants are able to adapt to such an extensive list of stressors, some of which vary along their full axes within a single day (Sippo *et al.,* 2016). Mangroves are not immune to global change stressors, the most significant risk being local sea-level rise (Jennerjahn *et al.,* 2017); however, they have exhibited a high degree of ecological stability and persistence throughout times of environmental change (Alongi, 2015). Because of their unique nature and hardiness, mangroves may provide climate refugia for a range of generalist coastal species that require structurally complex habitat, as other more vulnerable foundation species are increasingly lost from coastal environments.

Submerged mangrove prop roots and pneumatophores are a major provider of three-dimensional structure in what are otherwise soft-bottomed, homogeneous environments. They are used by an extensive range of species for shelter and food, and play a particularly important role as a nursery habitat, promoting the growth and survival of juvenile fish (see review by Nagelkerken *et al.,* 2008). Pneumatophores are specialised roots that provide oxygen to the main root system of mangroves. When pneumatophores are submerged, they produce oxygen internally via photosynthesis, primarily utilising the CO_2 produced by the respiration of the main root system (Yabuki, Kitaya & Sugi, 1990; Aiga *et al.,* 1995: Kitaya *et al.,* 2002). Pneumatophore gas exchange can have a significant impact on water chemistry, depending on the shape, size, depth and refreshment rate of the areas where mangroves grow (Gedan *et al.,* 2017). Areas with low refreshment can experience large variation in pH and other water parameters, with highly acidic conditions often recorded at night (Gedan *et al.,* 2017). In contrast, the dissolved inorganic carbon released by mangroves in open coastal zones can result in a net increase in pH (Sippo, *et al.* 2016). Despite their requirement for photosynthesis, pneumatophores are able to accommodate an extensive coverage of epibionts (i.e. fouling species like fleshy algae, sponges, oysters and tunicates). The effects of pneumatophore gas exchange on epibiont community composition, and vice versa, are poorly understood and are probably unique to individual mangrove creeks.

Unlike seagrasses and corals, the excessive growth of epibionts will not typically compromise the survival of mangroves but can actually enhance the value of the mangrove habitat. Experimental studies have shown that larger numbers of juvenile fish will occupy mangroves when they are fouled by epibionts, compared with un-fouled sites (Laegdsgaard & Johnson, 2001; Verweij *et al.,* 2006). Algae, in particular, host elevated numbers of small prey items for juvenile fish and other fauna (Lubbers, Boynton, & Kemp, 1990) and all epibionts enhance the structural complexity of the mangrove habitat, creating a more diverse collection of shelters (MacDonald & Weis, 2013). In temperate Australia, *Avicennia marina* is the only widespread mangrove species, and is typically fouled by barnacles, the rhodophytes *Caloglossa* spp. and *Catenella* spp., and the chlorophytes *Ulva* spp. and *Enteromorpha* spp. (Gwyther, 2000: Gwyther & Fairweather, 2002). The assemblages of meiofauna found on pneumatophores are unique to their epibiont communities but have been shown to be more diverse and abundant on algal epibionts (Gwyther & Fairweather, 2002), as well as distinct from the meiofauna found in surrounding sediments (Gwyther, 2000).

There is growing evidence that ocean acidification and increasing ocean temperature will lead to an increase in the growth of turf algae on kelp beds and coral reefs (Connell & Russell, 2010; Kroeker *et al.,* 2011; Nagelkerken *et al.,* 2016; Ober, Diaz-Pulido, & Thornber, 2016), and it is yet to be determined if a similar response is seen in mangrove root epibiont communities. Turfs are an excellent food source for amphipods and other small invertebrates, which additionally benefit from relaxed constraints on reproduction under the combination of acidification and warming (Heldt *et al.,* 2016). Where previous studies have examined the attraction of fish to various epibiont characteristics, the composition of the growth usually takes second place to the size and shape of the epibiont community (MacDonald, Glover & Weis, 2008; Nagelkerken *et al.,* 2010). As such, an increased dominance of turf, driven by climate change, and perhaps even at the expense of other epibiont species, may enhance the level of food and shelter supplied by the mangrove root habitat, increasing its value under future climates.

Here, we tested how future ocean warming and acidification modify the composition and physical structure of mangrove epibionts and evaluated whether any such changes altered the use of the mangrove root habitat by juvenile fish. We mimicked a mangrove prop root environment in large 2,300 L mesocosms using artificial roots, colonised with naturally accrued epibionts, and introduced juveniles of a variety of common mangrove fish species. The mangrove communities were subjected to modest increases in temperature (+ 1.2 °C) and $p\text{CO}_2$ (+ ~150 ppm) to understand the impacts of a low greenhouse gas emission scenario (as per the COP21 Paris Climate Agreement for the year 2100 (Magnan *et al.,* 2016), or alternatively, nearer-term predictions if the global community is unable to reduce current emissions (RCP 8.5 for the year 2070 (Reisinger *et al.,* 2014)). Both of these scenarios are heavily under-studied. We hypothesised that warming and acidification could lead to an increase, rather than decrease, in the structural complexity of epibiont communities and thus increase the value of these habitats for associated fauna, a scenario that would stand in stark contrast to the grim predictions put forward for many other key marine habitats.

Materials and methods

Mesocosms

Our mesocosms were designed to simulate shallow, permanently inundated mangrove prop root habitats. In the natural environment, these habitats are found in coastal areas experiencing relatively low tidal amplitudes, or in permanently inundated tidal channels in areas with large tidal amplitudes. The mangrove communities in these environments are typically occupied by trees within the genera *Rhizophora* and *Avicennia*. The set-up consisted of 12 circular mesocosms, each with a capacity of 2,300 litres. Each mesocosm contained a mangrove root habitat, comprised of artificial mangrove roots, colonised by natural mangrove epibionts. Two additional header tanks received a constant inflow of fresh, unfiltered seawater from an offshore pipeline, located 1.5 km off the coast at 8 m depth. Each header tank held six submersible pumps (~ 1.8 m^3 h^{-1}) that delivered water to eight treatment mesocosms. Four control mesocosms received inflowing seawater directly from the offshore pipeline. The inflow rate of seawater in all mesocosms was set at two litres per minute, equating to a full refreshment of each mesocosm every 15 hours. Excess water drained from the mesocosms through a 2 mm mesh filter head. The set-up was established outdoors under full sunlight.

Climate treatments

The climate treatments were based on an aggregation of intended nationally determined contributions (INDCs) towards carbon emission reductions for the year 2100 (see http://climateactiontracker.org/). These intended contributions were updated following the COP21 agreement and equate to sea surface temperature and pH changes of 1.2°C and -0.10 pH units respectively (Magnan *et al.,* 2016). We administered current and predicted concentrations of CO_2 (350-400 and ~500 ppm, respectively) in a crossed combination with ambient and elevated temperatures (ambient +1.2 °C), equating to three replicates for each of the four treatments (C: control, OA: elevated pCO_2, T: elevated temperature, OAT: elevated pCO_2 and elevated temperature). Treatment levels were maintained for four weeks.

The temperature and pH treatment targets were achieved by pre-treating inflowing seawater and by directly administering treatments within the mesocosms. We used one header tank to distribute pre-heated seawater to all elevated temperature mesocosms. The mesocosms subject to elevated temperature were also each equipped with two supplementary heaters. Field measurements indicate that the water temperature of permanently inundated mangrove creeks are largely determined by the air temperature (Fig. S1). As the experiment was conducted outdoors in uninsulated tanks, the temperatures of our control mesocosms fluctuated in a very similar way (Fig. S1). We measured temperature three times per day and adjusted the heating accordingly to achieve an average temperature difference of 1.21 ± 0.09 °C throughout the experimental period.

A second header tank was used to distribute pre-acidified seawater to all elevated pCO_2 mesocosms. The pH was lowered in the CO_2 header tank by bubbling pure CO_2 directly into the water at a constant rate. The header tank pH varied between 8.08 and 8.10 (0.13–0.15 pH units below ambient seawater). A gas mixer was also used to deliver enriched air at 500 ppm pCO_2 to the elevated pCO_2 mesocosms. Non-acidified mesocosms received ambient air at the same rate (pH = 8.23 ± 0.01). The combination of delivering pre-treated water and enriched air lowered the pH in OA and OAT tanks to 8.13 ± 0.01 and 8.14 ± 0.01, respectively, an average reduction of -0.10 pH units compared to the controls (Fig. S2). Temperature and pH were measured three times per day using two handheld meters (913 Metrohm and Mettler Toledo SG2 SevenGo™) calibrated once daily. Alkalinity and pCO_2 were calculated weekly (Table S1) using CO2SYS (Pierrot, Lewis & Wallace, 2006) for Excel with constants from Mehrbach *et al.* (1973) refit by Dickson and Millero (1987).

Constructing the mangrove habitat

Prior to the experiment, a series of field surveys were conducted to characterise the structural complexity of mangrove prop roots. Permanently inundated channels, lined by *Avicennia marina,* were studied at Port Gawler, Middle beach (two sites), Chinaman Creek and Weerona Island, South Australia. At each site, three 10-m transects were set along the creek banks, each comprising of ten 1 m x 1 m quadrats. Within each quadrat, the number of submerged roots was counted to provide a measure of root density per metre of bank. The length of each root and the angle at which it protruded through the bank were also measured (n = 1126 individual roots). Across sites, prop roots occurred at an average density of 8.8 ± 0.5 roots m^{-1}. The majority of the roots protruded through the creek banks at ~90° from vertical (55.3 ± 3.4% of all roots measured). The remainders were ~135° (36.8 ± 3.2%) and ~180° (7.9 ± 1.6%) from vertical. The average length of the roots was 29.0 ± 0.5 cm. To characterise the light environment in the root habitat, one light measurement was taken in each quadrat using a light meter held just above the surface of the water alongside the bank. The mangrove canopy allowed ~6% of incoming light to reach the prop root habitat at midday, with an average light intensity across sites of 135.3 ± 22.3 μmol m^{-2} s^{-1}.

Following the site surveys, mimic mangrove roots were placed in two natural mangrove creeks (Middle Beach and Port Gawler) in June 2016. At these sites, natural *A. marina* prop roots hosted algae-dominated epibiont communities, common among temperate mangroves (Gwyther & Fairweather, 2005), consisting primarily of *Enteromorpha* spp. and *Ulva* spp. The mimic roots consisted of untreated, smooth pine stakes, measuring 17 × 17 × 1200 mm. Whilst their shape and surface simulated mangrove roots well, the pine stakes were not living structures and therefore could not respire and photosynthesise like real mangrove pneumatophores. We acknowledge that the assumption that root gas exchange will have no effect on the epibiont community (and vice versa) is somewhat limiting; however, previous studies have found that with sufficient time, wooden mimics can host the same composition of algal epibionts as real roots (Gwyther & Fairweather, 2002). The stakes were hammered into the banks of inundated channels, mimicking the position and orientation of natural prop roots. The stakes were retrieved after approximately four months of colonisation time. At the time of retrieval, all the collected stakes had been colonised to some degree by turf and macroalgae.

Based on the density of mangrove roots observed in field surveys (see above), wooden frames were constructed to hold 10 artificial mangrove roots along a length of 1.2 m (Fig. S3). To approximate the natural positioning of mangrove roots, six stakes were positioned 90° from vertical and four were positioned 135° from vertical. Likewise, the stakes were cut to lengths between 8 and 31 cm to match field observations. Care was taken to ensure that the artificial root habitats in each mesocosm started the experiment with approximately the same composition and biomass of the most abundant algal species. Shade cloth was fitted over the mangrove root habitat (Figs. S3, S4) removing 90% of incoming light, closely approximating the light climate created by natural mangrove canopies.

The mesocosms were stocked with common juvenile fish found in local mangrove creeks and estuaries. Four pelagic species (*Atherinosoma microstoma, Atherinosoma elongata*, *Liza argentea* and *Aldrichetta forsteri*) and three benthic species (*Neoodax balteatus*, *Tetractenos glaber* and *Pseudaphritis urvillii*) were chosen of which there were seven individuals per species allocated to each mesocosm. Fish were caught across a variety of coastal sites in South Australia using a seine net. All the selected species are common across South Australian estuaries, bays and coastal regions. While none of the species are exclusively found in mangrove habitats, they frequently occupy the habitat and were the most common species observed at our sampling sites. *A. microstoma* and *A. elongata* are both endemic to southern Australia and are often seen swimming in schools, especially in brackish waters (Bray & Thompson, 2017(a); Bray, 2018(a)). *A. forsteri* is the most common mullet species in southern Australia. Along with *L.* argentea, they are found in coastal marine regions, entering freshwater zones (Bray, 2018(b); Bray & Gomon, 2018). *T. glaber* is abundant in bays and estuaries throughout South Eastern Australia, favouring sandy, muddy habitats, often seen in mangroves and adjacent seagrass beds (Bray, 2018(c)) and *P. urvillii* inhabits slow moving waters in estuaries, rivers and creeks, easily moving between brackish and freshwater habitats (Bray & Thompson, 2017(b)). Little information is available on the ecology of *N. balteatus*, however, we found an abundance of these individuals in amongst seagrass beds and adjacent mangroves. All fish were between 4 and 7 cm in length at the beginning of the experiment. Upon their capture, fish were first habituated to captivity for three weeks in smaller bins and then introduced into the mesocosms. One week after their addition to the mesocosms, the climate treatments were applied progressively over seven days until the treatment targets were achieved. The fish were fed a combination of blended sardines, prawn and squid *ad libitum*.

Quantifying epibiont composition and structure

After four weeks of exposure to the target climate treatments, the epibionts occupying the artificial roots in each mesocosm were differentiated (16 taxa identified in total) using underwater photographs and categorised into the broader groups of turf, macroalgae, calcareous algae and sessile animals. Here, 'turf' was defined as low growing, filamentous or branching taxa. 'Macroalgae' included only the species *Ulva lactuca,* and 'sessile animals' included one species of crust forming bryozoa. Calcareous algae were encountered very infrequently in all treatments. The 16 taxa were amalgamated into a code for input into 'Coral Point Count with Excel' (CPCe), a tool originally developed for the determination of coral cover using transect photos (Kohler & Gill, 2006). In CPCe, digital makers were randomly placed over the epibiont growth at a density of two markers for every one cm^2 of mangrove root. The species growing directly beneath each marker was then selected from the encoded list and finally expressed as percent cover. The Shannon Index (Shannon, 1948), which takes into account both the diversity and abundance of each epibiont species, was calculated for each root using the percent cover data for individual taxa.

Epibiont structural complexity was measured as an indicator of the sheltering capacity of the artificial mangrove roots. Using ImageJ (Schindelin *et al.,* 2012), turf height was estimated from side-view underwater photographs by measuring from the base of the root to the top of the turf at haphazardly chosen points along the length of the root. We calculated the average height of turf and also multiplied the average height by the area covered by turf to calculate turf microhabitat volume. The length, width and height of each individual macroalgal specimen were also measured and volume calculated. We also calculated the average height and range of heights (max. height minus min. height) across all epibionts.

Habitat use

Using a high-resolution submersible camera (GoPro Hero4 Silver, 60fps 1080p), fish behaviour was recorded in each mesocosm after four weeks of exposure to the climate treatments. The camera was positioned 30 cm below the surface of the water at a 35° angle, 1.4 m from the mangrove habitat such that all the roots were in view. In each mesocosm, the camera recorded for 10 minutes at midday. To account for the disturbance created by the introduction of the camera frame, the fish were always allowed to acclimate to the frame for 3.5 min before observations were recorded. We used VLC Media Player for the habitat use analysis and zoomed in to focus on a single mangrove root at a time. The number of visits by fish to an individual mangrove root was recorded. It was difficult in the low-light conditions of the mangrove habitat to visually distinguish *A. microstoma* from *A. elongate*, and *L. argentea* from *A. forsteri*; therefore, these pairings were analysed together. Because the analysis was time intensive, six of the ten mangrove roots in each mesocosm were analysed using this technique.

Statistical analysis

To assess the effect of future climate on the extent of cover, height, volume and diversity of the epibiont community, 3-way ANOVAs were conducted using the "ezANOVA" package (Lawrence, 2013) in R (R Development Core Team, 2017) with temperature and ocean acidification as fixed factors and mesocosm as a nested factor. Where an interaction was detected, ANOVAs were followed by pairwise comparisons with Bonferroni corrections. To assess the effect of future climate on the composition of the epibiont communities, a non-parametric MANOVA was conducted, along with non-metric multidimensional scaling.

Generalised linear mixed models with the R packages "glmmADMB" (Skaug *et al.,* 2016) and "lme4" (Bates *et al.,* 2015) were used to assess the relationships between the visitation of fish to individual prop roots and the climate treatments, algal epibiont diversity, epibiont habitat volume, epibiont average height, epibiont height range, prop root length and species of fish. Here, the response variable was treated as a binomial variable where fish either visited or did not visit individual roots. The models were fitted with a logit link function. The total epibiont habitat volume and average height were $\log_{10}(x + 1)$ transformed. The mesocosm and a unique identifier for each mangrove root were included initially as nested random effects to account for individual fish using the same roots within the same mesocosm, but this did not improve the model. We used a likelihood ratio test and AICc (Akaike's Information Criteria with corrections for small sample sizes) to compare various sub-models to the global model.

Based on the output of the model selection, we analysed the effects of future treatments and fish species on the frequency of visitation by fish to the mangrove prop roots using 3-way ANOVAs. Temperature and ocean acidification were set as fixed factors and mesocosm was included as a nested factor. Where an interaction was detected, ANOVAs were followed by pairwise comparisons of means with Bonferroni corrections.

Ethics

This research was carried out under the approval of The University of Adelaide Animal Ethics Committee (project: S-2016-087). Fish collections were permitted by the Minister for Transport and Infrastructure and the Government Department of Primary Industry and Regions SA (exemption: 9902844).

Results

Epibiont composition and structural complexity

The total area of colonised space on the mangrove prop roots increased by 22.5% and 24.9% under the elevated temperature (T) and the combined elevated temperature and $p\mathrm{CO}_2$ (OAT) treatments (T and OAT; $F = 21.70$, $p < 0.001$), respectively (Fig. 1). This was primarily due to an increase in the cover of algal turf from an average of 56% in controls to ~83% under elevated temperature (T and OAT; $F = 13.10$ $p < 0.001$, Fig. 1). The cover of fleshy macroalgae remained the same between treatments and ranged between 6.2% and 8.8% (Fig. 1), as did the cover of bryozoans, which ranged between 0.1% and 1.1% (Table S4). Acidification had no effect on total epibiont cover, nor did it alter the relative cover of turf or macroalgae (Fig. 1).

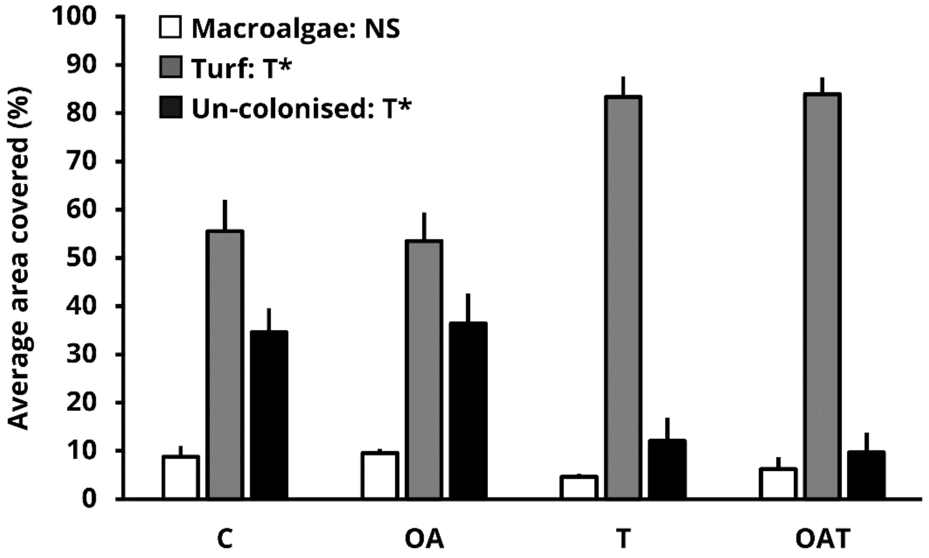


Figure 1: The average percent area occupied by macroalgae and turf algae, and the average percent area remaining un-colonised on artificial mangrove roots exposed to one of four climate treatments over four weeks (C = control, OA = elevated $p\text{CO}_2$, T = elevated temperature, OAT = combined elevated $p\text{CO}_2$ and temperature). Error bars are ± standard error. * marks significant effects, 'NS' indicates no significant effect.

Under elevated temperature (T and OAT), the average height of the epibiont community decreased by 0.4 cm ($F = 4.32$, $P = 0.037$). Epibiont height range (maximum height minus minimum height) also decreased in T and OAT mesocosms to 2.7 cm ± 0.2 and 3.3 cm ± 0.4 respectively, compared with 4.3 cm ± 0.2 in controls (near significant T×OA interaction: $F = 4.44$, $p = 0.052$). The height of turf decreased with elevated temperature (T and OAT) by 0.5 cm relative to controls ($F = 5.86$, $p = 0.017$, Fig. 2a), equating to an average height reduction of 25%. There was no difference in the average height of macroalgae across treatments (Fig. 2b). Despite the greater cover of epibionts under elevated temperature, the flattening of the turf communities meant that total algal habitat volume did not change, nor were there differences in the volume of turf or macroalgae between treatments (Fig. S5).

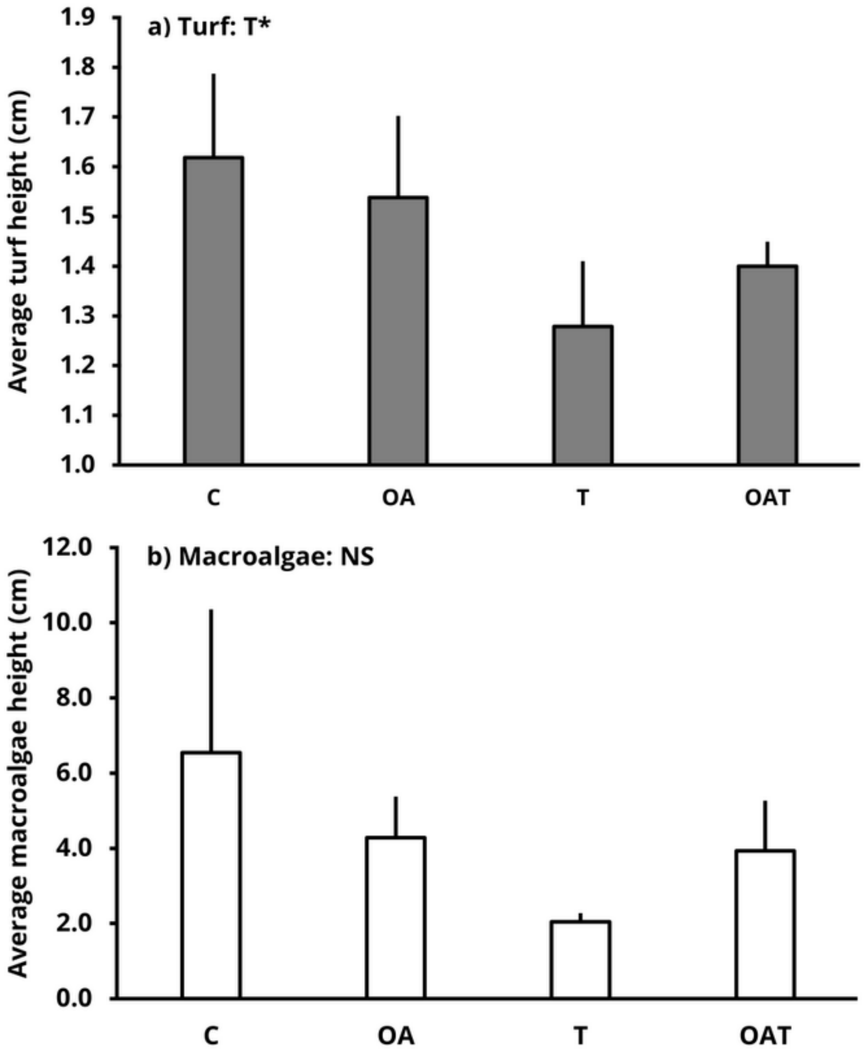


Figure 2: The average height of a) turf epibionts and b) macroalgae epibionts on mangrove prop roots exposed to one of four climate treatments over four weeks (C = control, OA = elevated $p\mathrm{CO}_2$, T = elevated temperature, OAT = combined elevated $p\mathrm{CO}_2$ and temperature). Error bars are ± standard error. * marks significant effects, 'NS' indicates no significant effects.

Elevated temperature (T and OAT) also altered the taxonomic composition of the epibiont communities ($F = 3.31$, $p = 0.013$; Table S2, Fig. S6). Of the 16 morphologically distinct taxa identified across all prop roots, one type of weedy turf (Turf sp. 2) occupied an average of 49.7% of the available root space under elevated temperature (Table S3). In comparison, the most abundant taxa in ambient temperature treatments (Turf sp. 1) occupied an average of only 18.5% of the root area available (Table S3). Elevated temperature reduced the overall diversity of algal epibiont communities by 33% compared to ambient conditions ($F = 6.29$, $p = 0.013$, Fig. 3). Moreover, bryozoans, calcareous algae and three species of turf were completely lost from epibiont communities exposed to elevated temperatures (Table S4).

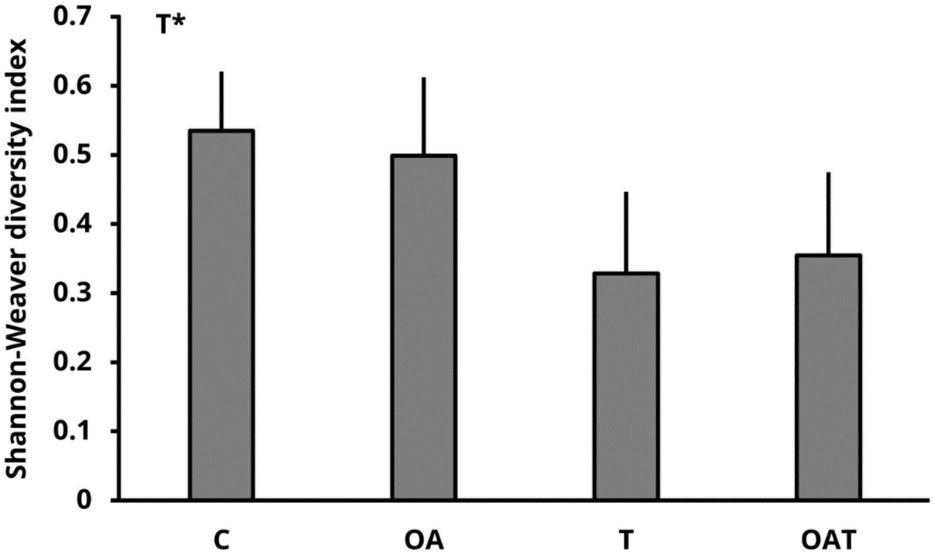


Figure 3: The Shannon Index for epibiont community diversity on artificial mangrove prop roots after four weeks exposure to one of four climate treatments (C = control, OA = elevated $p\text{CO}_2$, T = elevated temperature, OAT = combined elevated $p\text{CO}_2$ and temperature). Error bars are ± standard error. * marks significant effects, 'NS' indicates no significant effects.

Fish habitat use

The most highly ranked model identified in the model selection process was '*visits ~ T × OA × Species*' (Table 1), referencing temperature, ocean acidification and fish species as the most important factors determining the habitat use patterns of juvenile fish in the mangrove habitat. The interaction between these variables suggests that the effects of temperature and acidification on habitat choice manifest differently, depending on the species of fish considered. None of the predictor variables related to the composition or structure of the algal epibiont communities (i.e. total habitat volume, average epibiont height, epibiont height range, epibiont diversity or root length) were included in the top-

3 ranked models (Table 1), suggesting that alterations to fish behaviour were not a response to the flattening or homogenisation of the prop root epibionts.

Table 1: The top three models for explaining the occurrence of visits by juvenile fishes to artificial mangrove prop roots after four weeks exposure to one of four climate treatments. In addition to the parameters selected for in the top-ranked models, the global model included parameters for the average height of epibionts, total epibiont habitat volume, epibiont height range, Shannon Index, root length and the percent cover of the prop roots colonised by epibionts. All models used a binomial distribution with a log-it link. The table includes degrees of freedom (df), log-likelihood values (LL), the difference in Akaike's Information Criteria corrected for small sample size relative to the top-ranked model ($\triangle AIC_c$) and their weights ($wAIC_c$).

Rank	**Formula**	**df**	**LL**	**$\triangle AIC_c$**	**$wAIC_c$**
1	~ *T* x *OA* x *Species*	20	-187.66	0	0.53
2	~ *Species* + *T* x *OA*	8	-201.72	2.01	0.19
3	~ *T* x *Species* + *OA*	11	-198.67	2.26	0.17

Two fish species, *T. glaber* and *N. balteatus*, visited the mangrove roots less with elevated temperature (T and OAT) compared to ambient temperature conditions (F = 13.87, p = <0.001; F = 11.44, p = 0.010, Fig. 4). Conversely, ocean acidification alone increased the frequency of visitation by *N. balteatus* (F = 17.25, p = <0.001), as well as by *P. urvillii* (F = 6.67, p = 0.033, Fig. 4). None of the climate treatments altered the number of visits by the pelagic species (*A. microstoma, A. elongata*, *L. argentea* and *A. forsteri*).

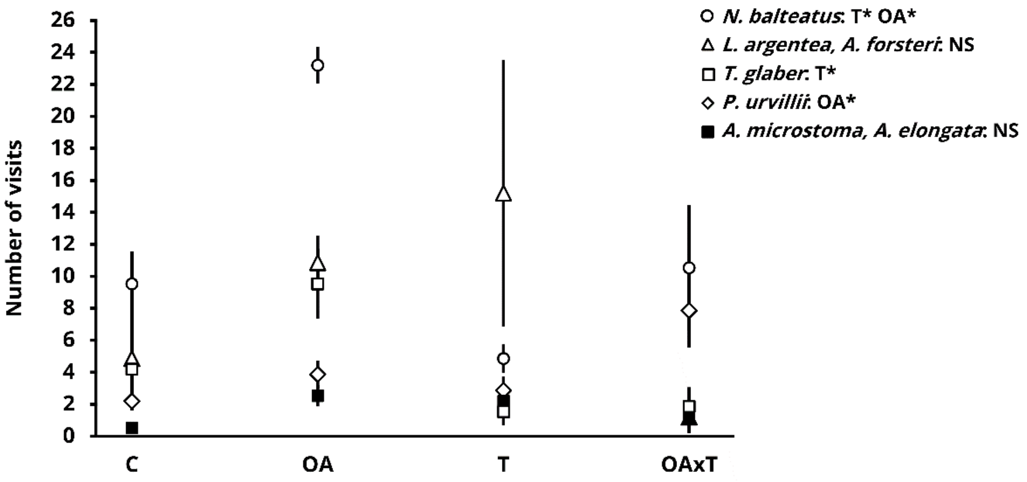


Figure 4: Number of visits to the mangrove prop roots by juveniles of various species of fish after four weeks of exposure to one of four climate treatments (C = control, OA = elevated $p\mathrm{CO}_2$, T = elevated temperature, OAT = combined elevated $p\mathrm{CO}_2$ and temperature). Error bars are ± standard error. * marks significant effects, 'NS' indicates no significant effects.

Discussion

The loss of structural complexity in foundation habitats decreases refuge availability and ultimately reduces the richness of associated fauna (Graham *et al.,* 2007; Gratwicke & Speight, 2005). We found that temperature reduced the average height of mangrove root algal epibionts, as well as the range of epibiont heights. The presence of mangrove epibionts with high and variable elevation are important assets in submerged mangrove habitats because they create a larger number of micro-habitats, as well as reduce the interstitial distances between roots and therefore the distance to shelter for resident fish. Studies have shown that the interstitial distances between roots can have a measurable impact on the richness and abundance of fish that occupy mangrove habitats in the natural environment (Nagelkerken *et al.,* 2010). The flattening of mangrove epibionts is likely to limit the suitability of the mangrove habitat to a smaller collection of species, with fewer appropriate shelters, especially for small-bodied animals that associate with the microstructure of the epibionts (MacDonald & Weis, 2013). Our results indicate that relatively small elevations in mean seawater temperature can drive a reduction of three-dimensional microhabitat structure in favour of a two-dimensional habitat extension, which can have direct consequences for its occupants.

Elevated temperature not only flattened the algal epibiont habitat but also reduced the epibiont species diversity. Calcareous algae and some species of fleshy algae were lost from elevated temperature communities entirely, the outcome being a simplified, more homogenised community, dominated by a single algal taxon. Epibiont diversity provides a greater number of resource axes for fish species, including prey types, camouflage opportunities, refuge spaces and breeding sites (Gratwicke & Speight, 2005). As such, a number of studies have found positive correlations between fish species richness and substratum species diversity (See Gratwicke & Speight, 2005). Reduced epibiont diversity was the main driver of the observed decreases in average epibiont height and height range in this study. Naturally, diversity in epibiont species leads to variation in epibiont height, explicitly linking epibiont diversity to niche partitioning in the mangrove habitat. Community homogenisation of epibionts under climate change is therefore likely to lead to a reduction in the richness of associated plants and animals.

We hypothesised that ocean warming and acidification would increase the structural complexity of mangrove root epibionts, strengthening its potential to act as refugia for species whose habitats are compromised by changing environmental conditions. In the last 40 years, the architectural complexity of Caribbean reefs has undergone major declines, partly due to disease outbreaks and major bleaching events (Alvarez-Filip *et al.,* 2009) and these reefs now host less diverse assemblages of fish (Newman *et al.,* 2015). Likewise, regime shifts from kelp forests to urchin barrens or turf-dominated systems have resulted in significant losses of associated species diversity (Nagelkerken *et al.,* 2017; Nagelkerken *et al.,* 2016; Wernberg *et al.,* 2016). Within persisting mangrove forests, the modification of mangrove epibiont communities could be expected to be less catastrophic for mangrove occupants than the structural losses documented in compromised kelp, coral and seagrass beds, as mangrove prop roots themselves still provide at least a constant base level of physical structure (Pratchett *et al.,* 2008).

In contrast to our predictions, changes to the epibiont structural complexity and composition were not functionally linked to fish behaviour. It is possible that alterations to the epibiont community were simply not substantial enough to induce an altered habitat use. The baseline structural complexity offered by the mangrove roots may have provided sufficient shelter for fish, minimising the impact of changes to the epibionts. Coral reef studies have shown that fish abundance and diversity remain consistent between high and intermediate levels of reef complexity. It is only between intermediate and low levels of complexity that fish numbers fall, supporting the notion of a critical complexity threshold (Newman *et al.,* 2015). It is important to note, however, that the loss of Caribbean fish diversity on even low complexity sites did not peak until years after major degradation events (Alvarez-Filip *et al.,* 2009). It was proposed that this lag in time was due to a reduction in suitable settlement sites, thus lowering recruitment in the years following the loss of habitat structure, while adult fish occupying the reefs were largely unaffected (Graham *et al.,* 2007). It is possible that the full effects of epibiont community flattening and homogenisation cannot be captured by a relatively short-term experiment.

Alternatively, changes to the epibiont habitat might have indirectly elicited altered fish habitat use, but these were superimposed by direct climate-driven changes to the behavioural choices of the fish. Elevated temperature, ocean acidification and fish species were identified in the model selection process as the only factors highly related to the habitat choices of the fish in our experiment. Ocean acidification is known to interfere with brain neurotransmitters in marine animals (Nilsson *et al.,* 2012), in some cases completely reversing how they respond to predator scents, relevant habitat sounds and food cues (Cripps, Munday & McCormick, 2011; Munday *et al.,* 2014; Rossi *et al.,* 2015). Compared to ocean acidification, there is less evidence for direct effects of increased temperature on choice behaviour in marine fish (Nagelkerken & Munday, 2016). In our study, elevated $p\mathrm{CO}_2$ increased visitation to the mangrove roots by two species while elevated temperature decreased visitation by two others. The four pelagic species showed little change in their patterns of use in the mangrove habitat. Alterations to habitat use patterns by some species, but not all, are likely to bring about changes in species interactions as communities reshuffle to restore a level of niche segregation.

We acknowledge that our study has certain limitations that warrant further investigation. Ideally, our experiment would have been conducted over a longer duration. The duration of all climate change studies impacts the ability of the researcher to understand the adaptive capacity of the species under study, the effects of seasonality and, especially in this case, the composition of the community once equilibrium has been reached. Likewise, our experiment would have ideally included a longer acclimation period. Additionally, we were unable to include the effects of mangrove root respiration on the water chemistry in the area surrounding the roots, and therefore the impact of this respiration on future epibiont communities. This is further confounded by the possible effects of climate change stressors on root respiration itself. While this is an important limitation, we believe that we are still able to show important differences between current and future mangrove epibionts. There is significant evidence to suggest that the effects of acidification at moderate levels are only exacerbated when further acidification is applied (e.g. Wittman & Portner, 2013; Gattuso *et al.,* 2015). Additionally, ocean acidification has been shown to affect ecosystems that are already exposed to low pH levels (Kroeker *et al.,* 2010; Kroeker *et al.,* 2013). Any additional reduction in pH brought about by pneumatophore gas exchange could be expected to exacerbate the effects of climate change on fish behaviour and algal growth that we have recorded here. Notably, many of the changes in epibiont community and fish behaviour that we observed were induced by ocean warming, in isolation from acidification.

Here, we provide evidence for changes to fish behaviour, habitat structure and habitat composition with moderate increases in seawater temperature and $p\mathrm{CO}_2$. Moreover, we used a multi-species, multi-stressor design in an attempt to encapsulate the effects of climate change on the complex relationships that exist amongst species, and between species and their habitats. It is imperative that we understand how communities will respond to moderate global temperature changes if we are to contribute to discussions around the effectiveness of the climate agreements in a meaningful way. We show that a key coastal foundation habitat, whose underwater habitat structure is postulated to be one of the least sensitive to climate change, is still sensitive to a relatively small increase in average seawater temperature. This has implications for the use of this habitat by coastal fauna as a refuge under future climates, as well as their renowned function as juvenile habitat for various commercial and ecologically important species.

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Supplementary tables and figures

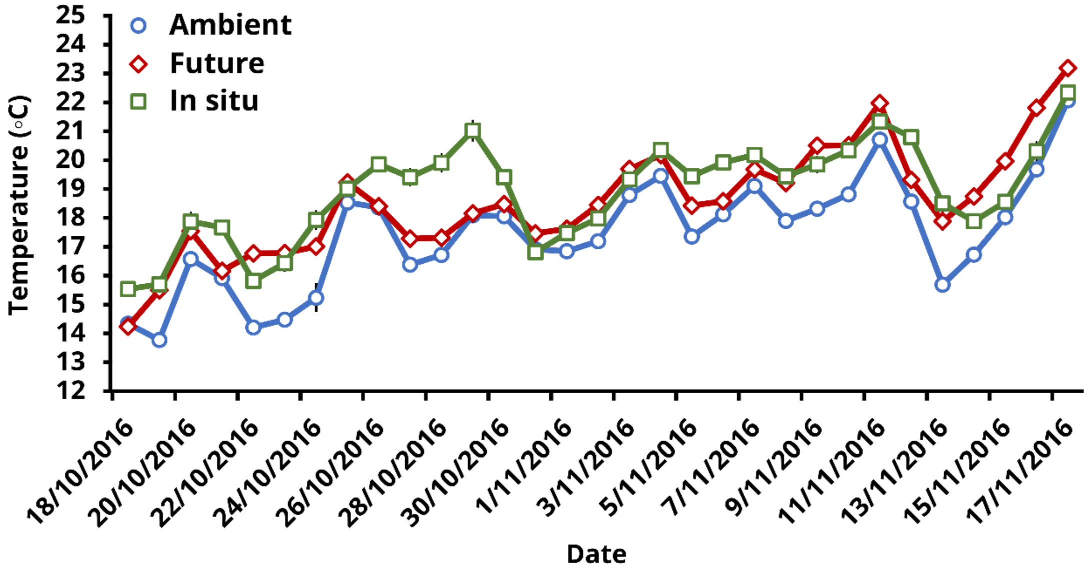

Figure S1: Daily average temperature in future temperature mesocosms (T and OAT), ambient temperature mesocosms (C and OA), and in a natural, permanently inundated mangrove creek at Saint Kilda (*in situ*), South Australia. Saint Kilda functioned as an arbitrary mangrove creek to show the pattern of variability in seawater temperature rather than absolute temperatures. Neither fishes nor algal epibiont assemblages were collected from St. Kilda. Mesocosm averages are based on 3 measurements taken per mesocosm at 9:00 am, 12:00 pm and 3:30 pm each day using two probes (913 Metrohm and Mettler Toledo SG2 SevenGo™ probes, 18 measurements per time point). Field measurements at Saint Kilda, SA, were taken every 30 minutes using a HOBO underwater temperature logger. Error bars are ± standard error.

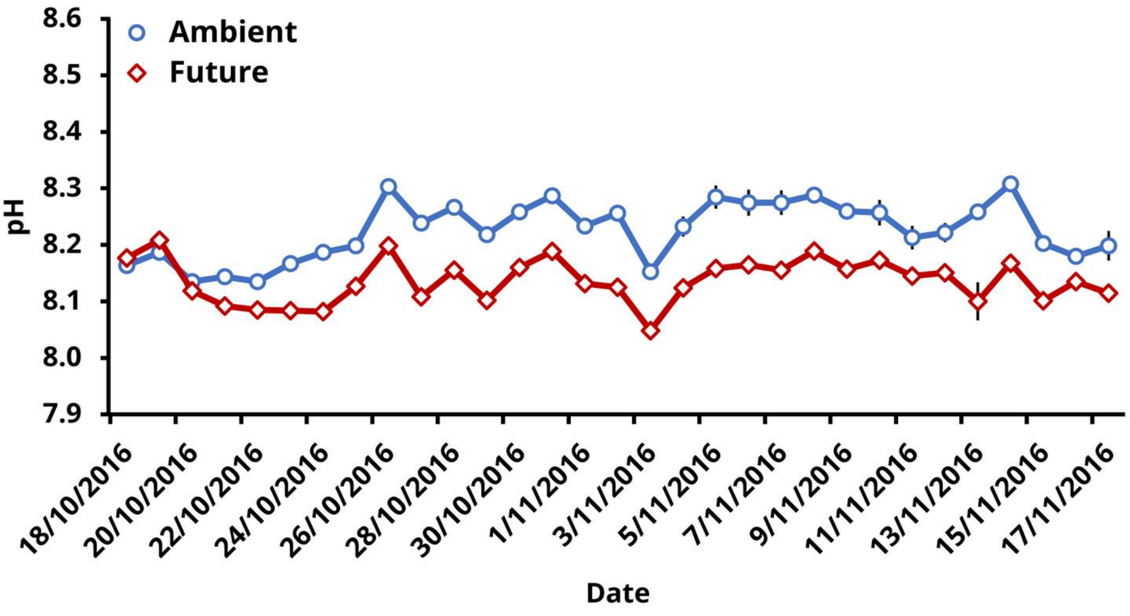

Figure S2: Daily average pH in future *p*CO_2 (OA and OAT) and ambient *p*CO_2 (C and T) mesocosms. Averages are based on 3 measurements taken at 9:00 am, 12:00 pm and 3:30 pm each day using two probes (913 Metrohm and Mettler Toledo SG2 SevenGo™ probes, 18 measurements per time point). Error bars are ± standard error.

The salinity of the incoming seawater measured consistently at 36 ppt. In field measurements varied between 10 and 36 ppt.

Table S1: Alkalinity and *p*CO_2 treatment averages (± standard error). Alkalinity and *p*CO_2 were measured weekly over the duration of the experiment in each mesocosm) using CO2SYS (Pierrot, Lewis, & Wallace, 2006) for Excel with constants from (Mehrbach, Culberson, Hawley, & Pytkowitz, 1973) refit by (Dickson & Millero, 1987).

	C	**OA**	**T**	**OAT**
Alkalinity (μmol kg^{-1})	2258.56 ± 28.66	2315.29 ± 5.76	2309.53 ± 40.49	2259.96 ± 26.56
***p*CO_2 (ppm)**	353.53 ± 36.09	530.97 ± 5.16	375.40 ± 14.87	518.93 ± 19.35

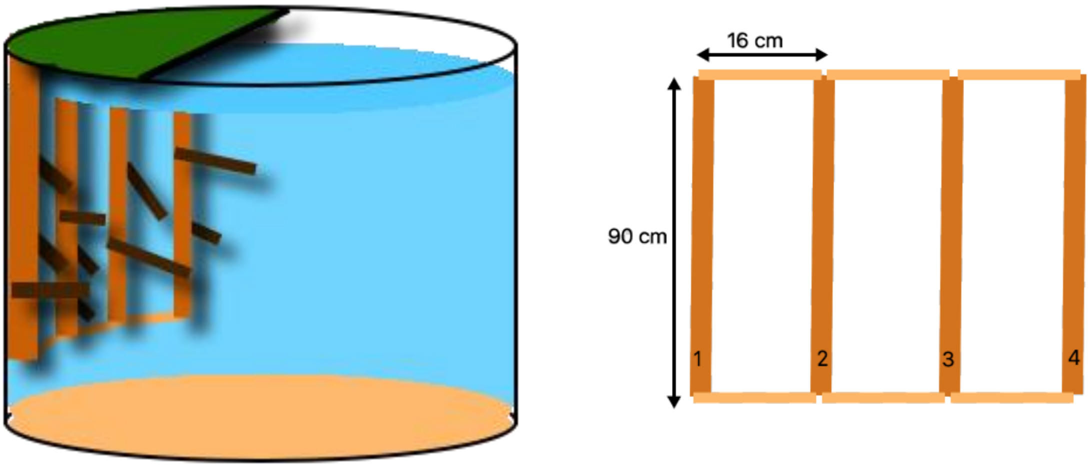

Figure S3: A schematic showing the mangrove root frame design. The artificial roots in the mangrove zone were attached to four weighted vertical wooden planks.

Figure S4: Top: View of the experimental mesocosms. The shade cloth was sprayed green on the underside where visible to the fish. Bottom: A close up photograph of one artificial mangrove root (OAT mesocosm, week one), covered with *Ulva lactuca*. The white pole in the centre of the photo is attached to a metal ruler and scribe plate used for photo analysis (not a permanent structure). Several other roots are visible in the background attached to wooden posts as shown in the schematic in Fig. S3.

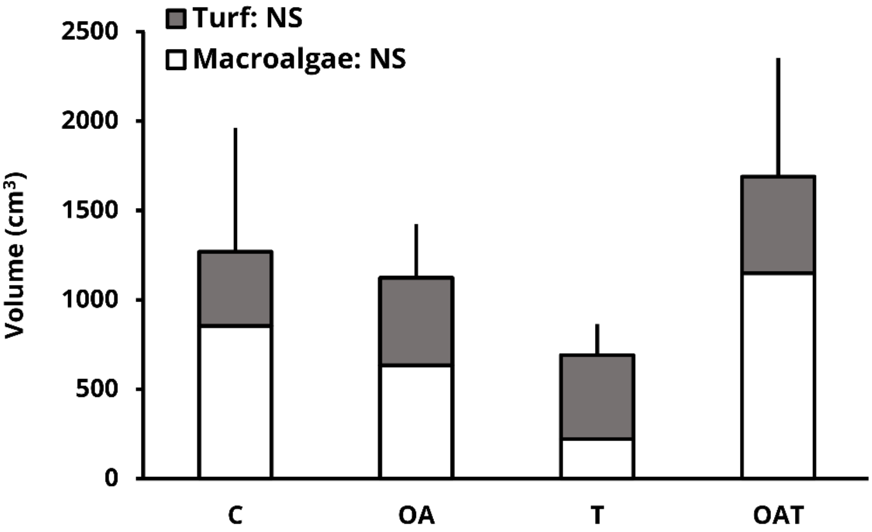

Figure S5: The total volume of macroalgae and turf algae occupying artificial mangrove roots exposed to four climate treatments over four weeks (C = control, OA = elevated pCO_2, T = elevated temperature, OAT = combined elevated pCO_2 and temperature). Error bars are ± standard error given for the total volume of algae across groups. 'NS' indicates no significant effect of any treatment.

Table S2: PERMANOVA analysis for the effect of the climate treatments on the composition of prop root epibiont communities.

Source	**df**	**MS**	**Pseudo F**	***P* value**
T	1	38691	3.3094	**0.0128**
OA	1	19475	1.6657	0.1270
T x *OA*	1	24200	2.0699	0.0725
Tank (T x *OA)*	8	11699	5.2349	0.0001
Residual	108	2234		

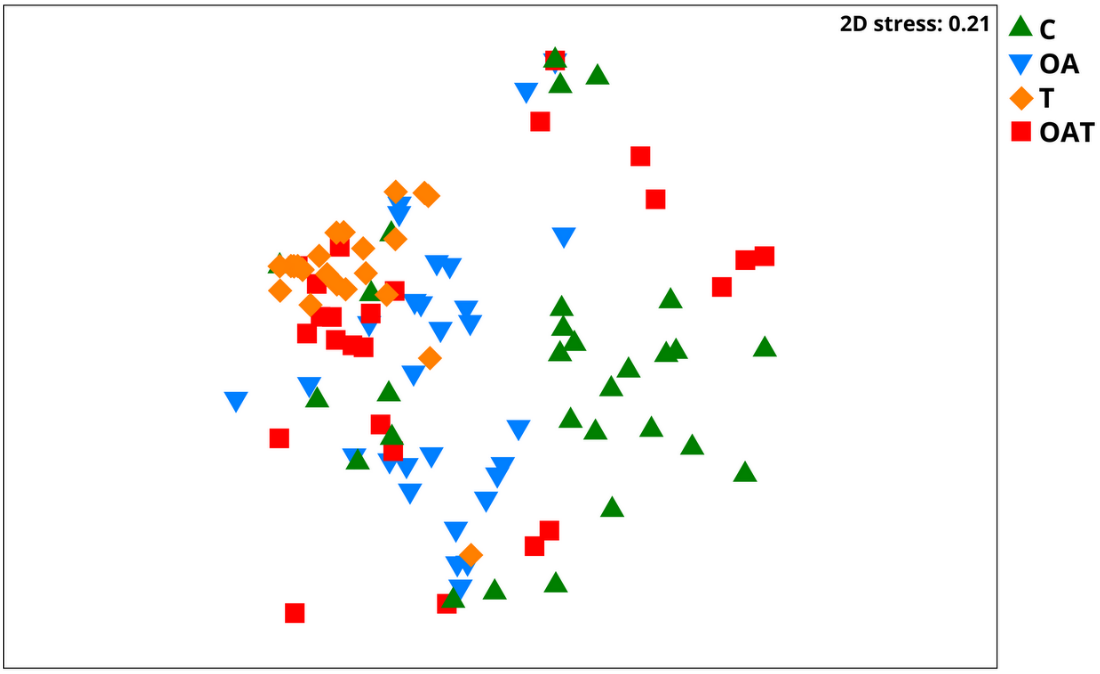

Figure S6: Non-metric multi-dimensional scaling plot for epibiont community composition on artificial mangrove prop roots. The plot shows the ordination of the epibiont community for 120 artificial prop roots (n = 10 per tank) after four weeks exposure to one of four climate treatments (C = control, OA = elevated $p\text{CO}_2$, T = elevated temperature, OAT = combined elevated $p\text{CO}_2$ and temperature).

Table S3: A SIMPER analysis showing the average cover of the most prevalent algal taxa and the percent similarity contribution (% SC) of each of those taxa for each of the main treatment combinations.

	Ambient temperature (C & OA)		**Future temperature (T & OAT)**		**Ambient CO_2 (C & T)**		**Elevated CO_2 (OA & OAT)**	
Species	**Mean cover**	**% SC**	**Mean cover**	**% SC**	**Mean cover**	**% SC**	**Mean cover**	**% SC**
Turf sp. 1	18.5	42.4	-	-	17.3	32.5	-	-
Turf sp. 2	13.0	30.4	49.7	84.8	20.3	41.0	42.5	85.9
Turf sp. 3	-	-	14.8	9.7	14.8	17.4	-	-
Turf sp. 8	4.4	5.6	-	-	-	-	-	-
Turf sp. 9	4.9	9.8	-	-	-	-	4.9	4.8
Ulva lactuca	9.2	6.0	-	-	-	-	-	-

Table S4: The average percent cover (± standard error) of each taxa identified on artificial mangrove roots after four weeks of exposure to one of four treatments (C = control, OA = elevated pCO_2, T = elevated temperature, OAT = combined elevated pCO_2 and temperature). Dash (-) refers to 0 % cover.

	C	**OA**	**T**	**OAT**
Turf				
Turf sp. 1	10.9 ± 5.6	26.2 ± 7.3	2.9 ± 2.9	8.4 ± 4.3
Turf sp. 2	12.6 ± 11.6	13.4 ± 3.4	72.3 ± 9.0	27.1 ± 16.9
Turf sp. 3	2.4 ± 2.4	-	-	29.5 ± 29.5
Turf sp. 4	-	2.8 ± 2.5	4.9 ± 2.6	5.7 ± 2.3
Turf sp. 5	2.0 ± 2.0	4.0 ± 4.0	-	0.6 ± 0.6
Turf sp. 6	-	1.3 ± 1.3	-	0.4 ± 0.4
Turf sp. 7	0.3 ± 0.3	-	-	0.1 ± 0.1
Turf sp. 8	7.6 ± 4.1	1.2 ± 1.2	-	-
Turf sp. 9	9.9 ± 8.9	-	-	-
Turf sp. 10	-	-	0.6 ± 0.6	0.8 ± 0.8
Filamentous sp. 1	6.3 ± 6.3	-	-	-
Brown cyanobacteria	0.6 ± 0.3	2.1 ± 1.7	1.8 ± 1.3	1.5 ± 1.0
Green cyanobacteria	1.0 ± 1.0	2.7 ± 1.0	0.8 ± 0.8	9.8 ± 9.7
Turf total	53.5 ± 6.6	53.5 ± 5.9	83.3 ± 4.3	84.0 ± 3.5
Macroalgae				
Ulva lactuca	8.8 ± 2.2	9.6 ± 0.9	4.6 ± 0.6	6.2 ± 2.5
Calcareous species				
Calcareous algae sp. 1	2.0 ± 2.0	-	-	-
Bryozoan				
Bryozoan sp. 1	1.1 ± 1.1	0.5 ± 0.4	-	0.1 ± 0.1

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Chapter Four

Mesocosm technical design

Introduction

The term mesocosm refers to an experimental enclosure ranging from one to several thousand litres in size. Inside these enclosures, a population, community or ecosystem is maintained and then made subject to a set of treatments. Mesocosms are emerging as an excellent approach for understanding the ecological impacts of global change. Small scale laboratory experiments have been used frequently to relate components of global change to a physiological state or population response, however, their limited realism can make any extrapolations to natural ecosystems difficult to justify (Stewart *et al.,* 2013). Because mesocosms are large experimental units, they can encapsulate greater biological complexity than smaller, more traditional laboratory set-ups.

This mesocosm experiment was the third iteration in a series of similar designs carried out by the Southern Seas Laboratory at The University of Adelaide. Whilst these earlier experiments focused on subtidal habitats, here we aimed to re-create current and future conditions expected in permanently inundated mangrove channels in South Australia. We achieved this by adapting the design used by Falkenberg, Russell and Connell (2016) and Goldenberg *et al.* (2017). Here, we provide a more detailed overview of the technical design of our experimental set-up. As with other large and complex experimental designs, we faced many technical challenges in the design and construction of our set-up and we hope that by recording these here, this thesis will aid others who undertake similar efforts.

Location

The experiment was run at The South Australian Research and Development Institute (SARDI), a marine research facility in Adelaide, South Australia. As the facility is purpose built for marine research, there were many advantages associated with hosting our experiment at SARDI. Onsite, fresh, unfiltered seawater was continually pumped from an offshore pipeline (1.5 km off the coast at ~8 metres deep) into storage tanks for experimental use. From the storage tanks, seawater is plumbed to multiple experimental plots where electricity and compressed air are also available. After examining the various indoor and outdoor plots available at the research facility, we selected an outdoor courtyard where all the above amenities were already installed and operational. An outdoor site was selected as we felt that the exposure to natural sunlight was more important than the temperature control advantages gained by housing the experiment indoors, given that we would be examining the effects of climate change on photosynthesising organisms. The same plot was used by Falkenberg, Russell and Connell (2016).

Experimental layout

Our setup consisted of 14 rainwater tanks with the tops removed to create open cylinders. Each had a capacity of 2,300 litres. Twelve tanks were used as the experimental units and two were used as 'header tanks' for administering the treatments. At the beginning of the set-up phase, we evenly spaced the tanks in a gravelled courtyard and used a random number generator to assign one of the four treatments to the tanks to ensure that no treatment group was affected by greater sunlight, wind exposure, more attention from observers or any other unforeseeable influence (Fig. 1). The two most central tanks were assigned as the header tanks prior to treatment allocations. All tanks were placed within close proximity to the pre-installed electrical outlets, compressed airlines and seawater pipelines.

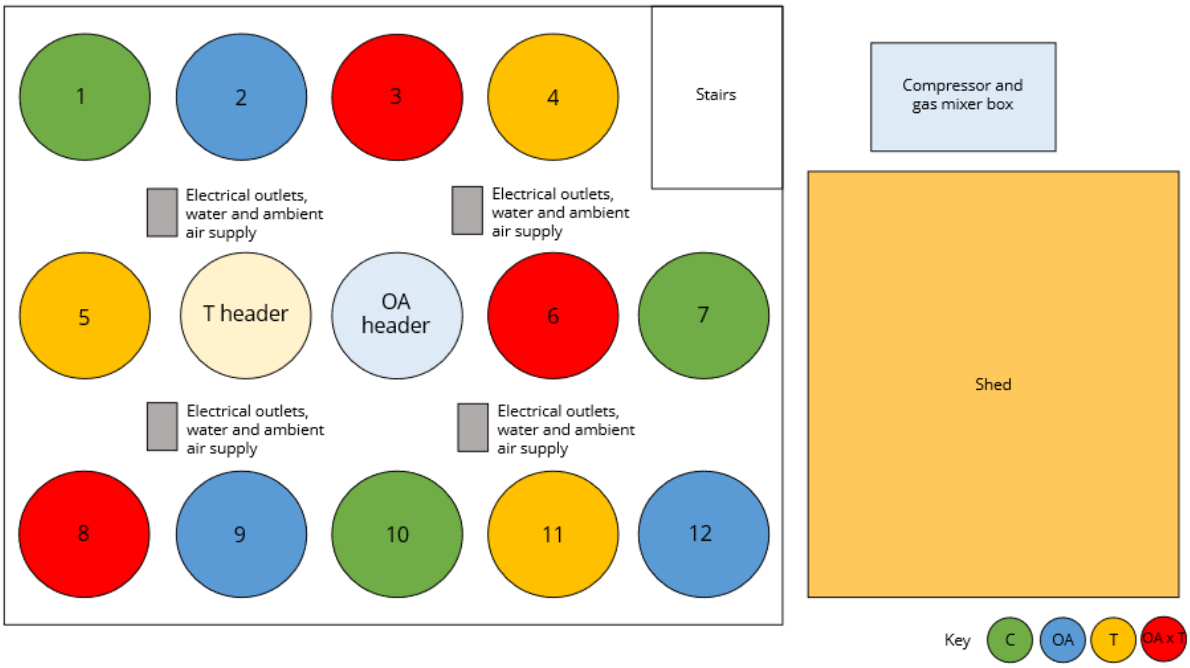

Figure 1: The layout of the experimental site at The South Australian Research and Development Institute (SARDI).

The header tanks were each connected to six treatment mesocosms via 25 mm PVC piping (Fig 2). Inside the header tanks, the plumbing was connected to six submersible pumps (~ 1.8 m^3 h^{-1}) set up to pump treated water from each header tank to one of eight treatment mesocosms. The pumps were attached to metal frames inside the header tanks using zip ties so they were positioned just below the surface of the water. This minimised the height that the water had to be pumped out of the header tank and therefore gave more leeway for the length of plumbing we could install. This meant, however, that we needed to be extra mindful of the water level dropping in the header tanks at any time during the experiment, in order to avoid damaging the pumps

Three mesocosms received inflowing seawater from both header tanks to create the combined treatment conditions of elevated temperature and CO_2. Six others received water from only one header tank and three control mesocosms received inflowing seawater directly from the offshore pipeline, bypassing the header tanks entirely. The inflow rate of seawater in all mesocosms was set at two litres per minute, equating to a full refreshment of each mesocosm every 15 hours. The flow rate from each pump was controlled by a tap which was fitted at the outlet of every pipe that led to a mesocosm. We used a stop watch and measuring jug to check the flow rate regularly and make fine-scale adjustments to the taps when it had changed. Excess water drained from the mesocosms through a cylindrical filter head. The cylinders were five cm long and the openings had a diameter of 15 cm. Both ends were fitted with two mm mesh. The top edge of the filter heads were positioned just below the rim of the mesocosms so that the water level in each tank sat 15 cm below the rim, draining from just the lower part of the filter head. This meant that when the bottom of the filter head inevitably became blocked, the water level would rise slightly to a new layer of mesh and there was a buffer remaining before water would begin overflowing from the mesocosm. Water flowed in through the mesh and out of the bottom of the filter head through connected PVC piping which ran down the inside of the mesocosm and was connected to an outlet at the bottom of the tanks. This pipeline was connected to a tap and more piping that led to seawater safe drains.

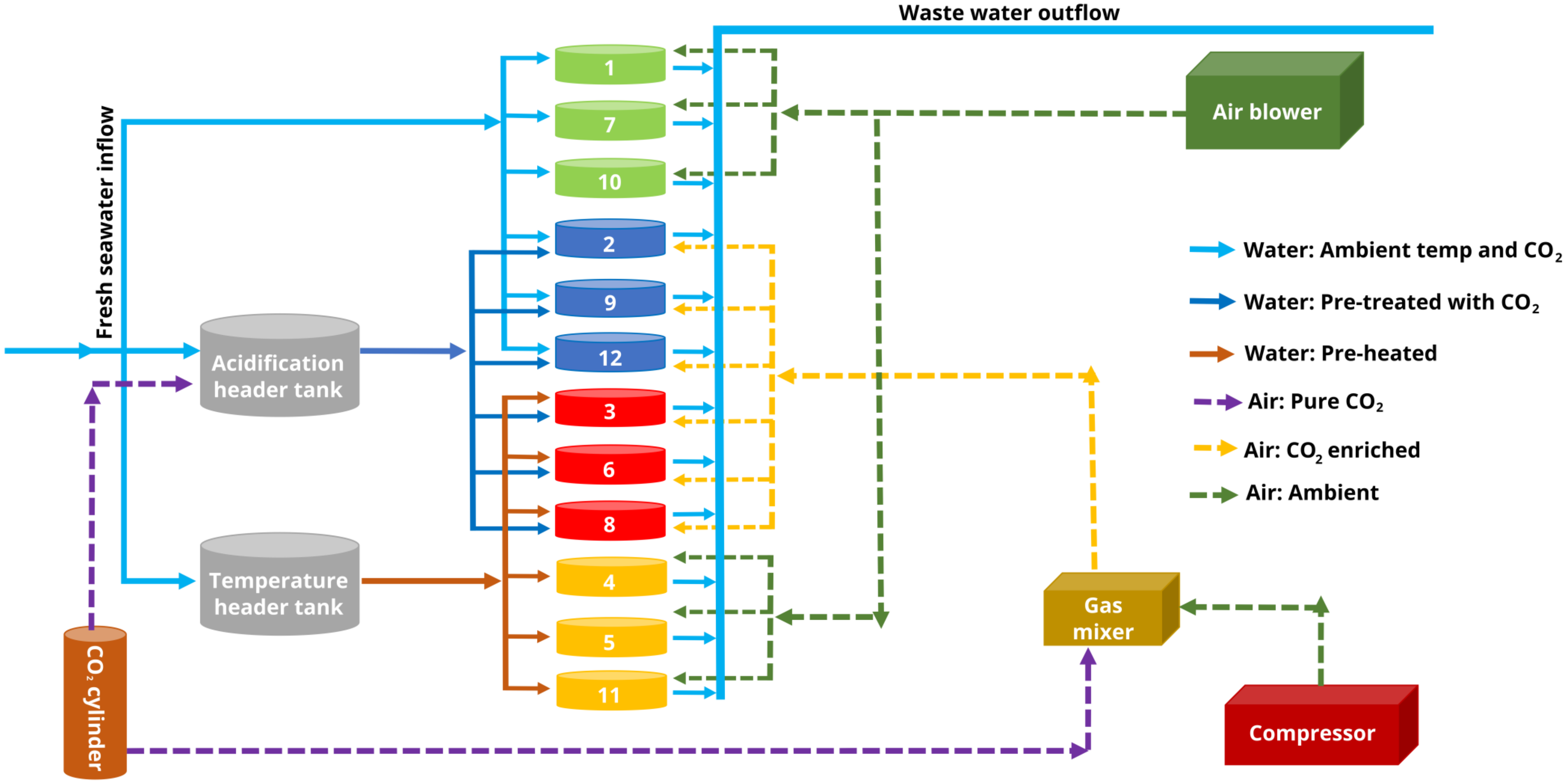

Figure 2: A diagram showing how each of the mesocosms were plumbed to the various header tanks and air supplies (Diagram by Regtein)

Electrical considerations

The electrical set-up for the experiment took some consideration. Because of the dangers of using electricity in proximity to water, it was essential that our electrical devices were installed safely and according to the policies of SARDI. Our electrical devices included the header tank pumps and heaters. We had more electrical devices than available outlets, so we needed to utilise power boards. SARDI required that all electrical plugs be kept at least 800 mm from the edge of the mesocosms. Additionally, the power boards needed to sit higher than the devices that were plugged in so that rain water and condensation would drip down the cord towards the device, rather than downwards into the electrical outlets. We also needed to utilise grounding rods which were connected to the water and plugged into the same power board that ran all the electrical devices in any one tank. To meet these standards, we hammered metal star droppers into the compacted earth to which we attached IP64 weatherproof rated outdoor safety boxes to hold our power boards. The star droppers were installed after ensuring we were clear of electrical lines. They had to be hammered at least 40 cm into the dirt to ensure there was no chance of them blowing down in windy weather. We used large, eight outlet power boxes. At some stations, the boxes were tightly packed with heater plugs and control units, as well as pump plugs and grounding rods. We colour coded the power boxes to the actual electrical outlets so that the entire box could be turned off with one switch before any observer interacted with a mesocosm (Fig. 3). All observers were inducted to the site and were required to wear rubber boots at all times and put on rubber gloves whenever putting their hands or something they were holding into the water.

Figure 3: Top: The electrical power boards can be seen in the middle of the image, attached to star droppers and plugged into outdoor electrical outlets. Bottom: The colour coded electrical outlets for easy on/off procedures.

Manipulating temperature

Because our experiment aimed to recreate shallow mangrove habitats, our temperature manipulation was distinct from Falkenberg, Russell and Connell (2016) and Goldenberg *et al.* (2017) who both modelled subtidal environments.

To increase the temperature in the treatment tanks relative to the naturally fluctuating control tanks, we took a two-part approach. Firstly, we increased the temperature in the temperature header tank by 1.5°C relative to the ambient water temperature. This was achieved using four 500-watt titanium aquarium heaters that could be set in increments of 0.5°C. This meant that the inflowing seawater that reached elevated temperature tanks was preheated. This was obviously more effective in tanks that only received inflowing seawater from the temperature header. For tanks that had the combined treatment and also received water from the CO_2 header, half of their incoming water was at a control temperature. For this reason, all temperature tanks were also equipped with two additional 500-watt titanium aquarium heaters that heated the water *in situ*.

These were also set at 1.5 °C above ambient. Using this two-part strategy, we created an average temperature difference of 1.21 ± 0.09 °C.

We measured the temperature of the mesocosms at 8:30 am, 12:00 pm and 3:30 pm daily and adjusted the heaters accordingly. For these measurements, we used two probes, the 913 Metrohm and Mettler Toledo SG2 SevenGo™. These provided fine scale, accurate measurements of both temperature and pH. The heaters were turned down at 5:00 pm so that as the temperature dropped in control tanks overnight, the temperature difference between treatments did not become extreme. This took some trial and error, as we had no access to sites overnight and relied on the effectiveness of whatever action we had taken before leaving the site the previous night to maintain our treatments. So that we could observe how the treatment differences at night, we installed an automatic temperature and pH monitoring system by 'Aquatronica' (ACQ110). We had an Aquatronica temperature and pH probe installed in every mesocosm, and these were connected by USB to an interface unit. The system could be programmed to alert the user by SMS if the temperature or pH shifted outside of a pre-set range. It also logged temperature and pH data at intervals of the users' choice which could be later downloaded for viewing on a computer. Overall, we found the system to be extremely unreliable. It served its purpose in that we almost always had temperature data from a subset of the tanks overnight, giving as insight into how effective our overnight heating strategy was, however, the pH probes often showed wild figures, far outside of what was realistically occurring in the tanks (and what was reflected when comparing the automatic probe to our hand held probe measurements) and many of the temperature probes would not work at all. The downloading of the data from the interface was also very temperamental. Whilst automatic logging of water parameters is extremely useful, we are yet to find an effective system that can be accessed readily for real-time viewing.

We collected temperature data over two months in a natural mangrove creek in Saint Kilda, South Australia, using a continuously logging 'HOBO' logger. The data showed that the shallow water of permanently inundated channels largely varies with the air temperature. Whilst we had previously planned to insulate the mesocosms to try and reduce the temperature variation, we decided that it would be more realistic if we allowed the baseline temperature of the control mesocosms to shift naturally and to adjust the treatment mesocosms accordingly to maintain our treatment targets. At the conclusion of the experiment, we compared our data to temperature data collected at Saint Kilda (Fig. 4). From this comparison, it is clear that our strategy followed natural temperature fluctuations well (Fig. 4) and did not compromise our ability to create a treatment difference.

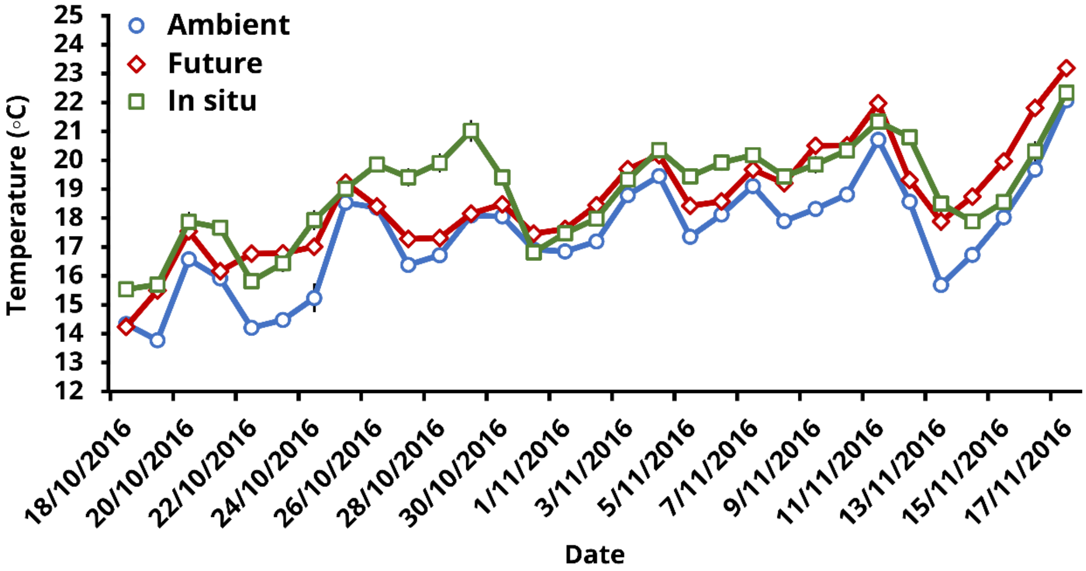

Figure 4: Daily average temperature in future temperature mesocosms (T and OAT), ambient temperature mesocosms (C and OA), and in a natural, permanently inundated mangrove creek at Saint Kilda (*in situ*), South Australia. Mesocosm averages are based on 3 measurements taken per mesocosm at 9:00 am, 12:00 pm and 3:30 pm each day using two probes (913 Metrohm and Mettler Toledo SG2 SevenGo™ probes, 18 measurements per time point). Field measurements at Saint Kilda, SA, were taken every 30 minutes using a HOBO underwater temperature logger. Error bars are ± standard error.

Manipulating pH

Like the temperature manipulation, we took a two-part approach to the acidification of the treatment mesocosms. A gas mixer was used to deliver air with a higher concentration of CO_2 to the elevated CO_2 mesocosms. Compressed ambient air and pure CO_2 were fed into a gas mixer (PEGAS 4000MF, Columbus Instruments, USA), producing air with a concentration of CO_2 of ~700 ppm. This air was delivered at a rate of 20 L per minute to the acidified mesocosms. The non-acidified mesocosms received ambient air at the same rate from the pre-installed SARDI compressed airlines. We had some difficulty with the initial set-up of the gas mixer. We used 8-mm pneumatic hosing to transport the acidified air from the gas mixer to the mesocosms. We initially installed lines that were too long and made up of joined pieces of hose. The joiners themselves were enough to reduce the pressure of air that reached the air stones, resulting in a lack of bubbling. We found that installing shorter, continuous stretches of hosing solved this problem.

As well as delivering CO_2 enriched air to the elevated CO_2 mesocosms, we also acidified the water delivered to these mesocosms via the CO_2 header tank. To acidify the CO_2 header tank, we set up a cylinder of pure CO_2 next to the tank and fed pneumatic hosing from the cylinder into the inlet of a very small pump. When the pump ran, it drew CO_2 in from the cylinder and bubbled CO_2 into the water around it, slightly reducing the pH in the header tank over time. Throughout the experimental period, the pH varied between 8.08 and 8.10 (0.8 – 1 pH units below ambient seawater) in the header tank.

Controlling the amount of CO_2 entering the tank via the bubbler took some trial and error. We installed a regulator to the cylinder and set it to release the CO_2 at a very slow rate. We found that we had to tape the tap on the regulator in place as even fine scale adjustments would dramatically affect the pH of the header tank. We also attached a valve to the hosing leaving the cylinder. The valve restricted the size of the hose opening, the size of which could be adjusted using a tiny key. This aiding in reducing the pressure of air reaching the blower and gave us the ability to control the flow of air at a finer scale.

The result of this double approach was a relatively consistent treatment difference between acidified and ambient pH mesocosms (8.13 ± 0.01 and 8.14 ± 0.01 in OA and OAxT tanks respectively, an average reduction of -0.10 pH units compared to the controls). We also were able to capture natural metabolic fluctuations in pH throughout the experimental period (Fig. 5). Like temperature, pH was measured twice daily using both the 913 Metrohm and Mettler Toledo SG2 SevenGo™ probes.

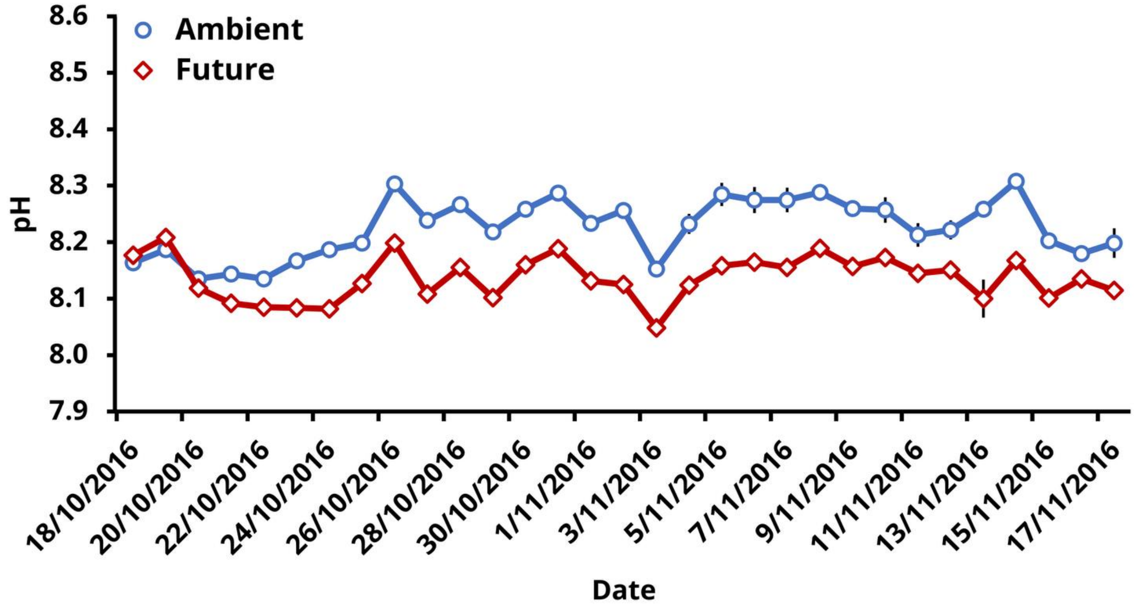

Figure 5: Daily average pH in future pCO_2 (OA and OAT) and ambient pCO_2 (C and T) mesocosms. Averages are based on 3 measurements taken at 9:00 am, 12:00 pm and 3:30 pm each day using two probes (913 Metrohm and Mettler Toledo SG2 SevenGo™ probes, 18 measurements per time point). Error bars are ± standard error.

Alkalinity and pCO_2

Throughout the experimental period we measured salinity and alkalinity weekly and used these metrics to calculate CO_2 partial pressure. We managed to achieve relatively consistent and target appropriate pCO_2 levels (Table 1).

Table 1: Alkalinity and pCO_2 treatment averages (± standard error). Alkalinity and pCO_2 were measured weekly over the duration of the experiment in each mesocosm) using CO2SYS (Pierrot, Lewis, & Wallace, 2006) for Excel with constants from (Mehrbach, Culberson, Hawley, & Pytkowitz, 1973) refit by (Dickson & Millero, 1987).

	C	OA	T	OAT
Alkalinity (µmol kg^{-1})	2258.56 ± 28.66	2315.29 ± 5.76	2309.53 ± 40.49	2259.96 ± 26.56
pCO_2 (ppm)	353.53 ± 36.09	530.97 ± 5.16	375.40 ± 14.87	518.93 ± 19.35

Summary

Despite the difficulty in designing and establishing a large, working experimental set-up, we were able to create a system that met our needs and allowed for some interesting experimentation. We found that there were many benefits to running a larger scale, multi-species experiment, as is expressed in the above two data chapters. Where finances allow, we hope that more ecologists will attempt to create multi-species experimental set-ups and that this chapter will help to ease some of the challenges that are likely to arise.

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General Discussion

Summary and significance

The primary aim of this thesis was to understand how ocean warming and acidification affects the structure and composition of mangrove epibiont communities and examine the interactions between changing epibiont communities and mangrove fish. We know that epibionts are, by nature, sensitive to climate stressors, most being either calcareous, and therefore sensitive to ocean acidification (Fabry *et al.,* 2008), or species of algae, who respond at different levels to the increased availability of CO_2 (Connell *et al.,* 2013). We also know that fish brain neurotransmitters are particularly sensitive to acidification and that this often results in changes to a variety of behaviours (Nilsson *et al.,* 2012). Prior to this study however, there was a lack of research examining what future mangrove communities would look like when the effects of ocean warming and acidification are felt by a whole community simultaneously.

Studying multiple stressors and multiple species at one time raises certain logistical challenges and consequently, it is not often attempted. This is despite a general recognition amongst ecologists of the need for more complex, holistic experimentation where community effects are concerned. Here, we undertook the third iteration in a series of mesocosm experiments attempted by the Southern Seas Laboratory at The University of Adelaide. Although the lab had some existing expertise, we faced many new challenges and pioneered the replication of realistic mangrove habitats in a closed, ex-situ setup. In this thesis, I provided a chapter on the technical set-up used to conduct the experiments outlined in chapters two and three. This is an important contribution, in that it may help solve some of the challenges likely to be faced by another student or scientist attempting to recreate a similar set-up. Only by attempting complicated methodology and reporting on it, will our ability to conduct better science improve.

Here, I presented two manuscripts, each reporting on novel findings in the field of mangrove ecology. Both studies looked at the effects of moderate levels of ocean warming and acidification on different aspects of mangrove communities. We chose to employ a moderate projection for warming and acidification because we felt there was an excess of ecological research looking exclusively at the more extreme and longer-term scenarios proposed by the IPCC. Although the general lack of action by the global community to reduce carbon admissions makes more extreme scenarios more and more likely to eventuate, our approach was to test whether the conservative targets proposed in the COP21 Agreement would be effective, should carbon admissions be contained in the foreseeable future. Looking at more conservative admission projections had the double advantage of also acting as near term predictions for more extreme scenarios, should carbon admissions continue to steer towards higher levels by 2100. In this respect, the manuscripts presented in this thesis contribute towards a smaller body of climate change research, one that we feel needs to grow. Afterall, future climate agreements should aim to set targets likely to reduce the impacts to important and vulnerable ecosystems. It is only by testing those targets that more accurate ones can be set in the future.

In chapter two, we examined the effects of ocean warming and acidification on the macrohabitat choices of mangrove fish, who were subjected, along with their epibiont habitats, to elevated temperature and CO_2. In contrast to our hypothesis, we found that the climate stressors had no effect on the macrohabitat choices of any of the five species tested. We were intrigued by this result, mostly because there were visible differences in the composition and structure of the epibionts between treatments. Despite this, all species made choices consistent with those made under control conditions. Importantly, the nature of their habitat choices matched the behaviours we had personally observed in the field and what was recorded about their life-histories in existing literature. This was an important finding, in that it showed we had created a set-up that was realistic enough to convince our specimens to behave as they would in their natural environments.

Following the completion of the macrohabitat study, we were interested in undertaking a comprehensive analysis of changes to the epibiont communities, as well as investigating whether fish habitat choice behaviour was affected by the epibionts, or by the climate stressors at any other scale. We report on this study in chapter three. In chapter three, we found that ocean warming had a particularly strong effect on the diversity and structural complexity of the epibiont communities. When temperature was elevated, a small collection of algal species came to dominate, increasing the cover of algae on the mimic mangrove roots, but leading to a reduced structural complexity overall, owing to the shorter stature of the dominating species. We then used a generalised linear modelling technique to investigate how changes to the epibionts affected the selection of individual mangrove roots by the mangrove fish. We were surprised to see that according to our model, the changes to the epibionts had not affected the microhabitat choices of the fish, however the climate stressors had. Here, we showed that the effects of climate change on habitat choice can manifest at one scale, whilst showing no effect at another.

Future research

There are important compromises associated with conducting a multi-species, multi-stressor experiment. Most importantly, it is difficult to elucidate the exact mechanisms behind each phenomenon overserved, when, as with the real world, there are many interacting changes occurring at once. Whilst we believe that undertaking a multi-species, multi-stressor experiment is the best way to get an over all picture of community level responses to climate change, there is certainly a place for simpler experiments targeting individual observations.

A next step to this study would be to further investigate the mechanisms behind changes to the microhabitat choices of the fish. Our best guess, as reported in chapter three, is that the climate change stressors had a direct effect on the behaviour of the mangrove fish. The possibility remains that parameters not included in our model could have affected the choices of the fish. For example, it could be that the fish sought out *in situ* prey items on top of their supplied fish food, and this may have been a driving factor in their habitat choices. And whilst we did investigate species interactions (and found very few interactions occurring at all), it could be that the fish established an early hierarchy, missed during our observational periods, and that this led a treatment dependent structuring of habitat use. For example, it could be that increased aggression in one species caused others to occupy roots furthest from the aggressive individuals. By designing smaller experiments with these mechanisms in mind, we may have more insight into the factors determining microhabitat use.

Conclusion

Here, I present two years of work examining the effects of moderate ocean warming and acidification on mangrove habitat and community dynamics. I attempted to study mangrove systems in a large scale, complex experiment, involving real epibionts and whole communities of fish. The results presented here, and the lessons learnt in designing and constructing a complex experimental setup, represent important contributions to the fields of mangrove ecology and climate change research. This thesis demonstrates that just moderate levels of ocean warming and acidification are enough to evoke changes in habitat structure and habitat use. It also highlights the benefits of utilising multi-species, multi-stressor systems in detecting changes in different layers of community functioning. It is hoped that further ecological climate change research is undertaken using this approach and that this will lead to a better understanding of community wide effects and more informed global emission scenario targets.

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