2020

Tropicalisation of temperate seagrass meadows in Western Australia: Predicting the impact of tropical herbivorous fishes on temperate seagrass meadows

Maria Samsonova
Edith Cowan University

Edith Cowan University

Copyright Warning

Tropicalisation of temperate seagrass meadows in Western Australia:
Predicting the impact of tropical herbivorous fishes on temperate seagrass meadows

This thesis is presented in partial fulfilment of the degree of
Master of Science (Biological Sciences)

Maria Samsonova

Edith Cowan University
School of Science

2020

Declaration of Authorship

I certify that:
   i.   *This thesis is entirely my own work except where stated specifically;*
  ii.   *This thesis does not, to the best of my knowledge and belief, incorporate without acknowledgment any material previously submitted for a degree in any institution of higher education;*
 iii.   *This thesis does not contain any material previously published or written by another person except where due reference is made in the text.*

Maria Samsonova
November 2019

Acknowledgements

Contents

Abstract

Rising sea temperatures through climate change produce shifts in the distribution of tropical species to temperate regions, a process termed "tropicalisation". The poleward expansion of tropical herbivores into temperate seagrass meadows is predicted to increase grazing pressure and alter ecosystem services and processes in these seagrass systems. This study attempted to examine the effects of tropicalisation on temperate seagrass meadows along the western coast of Australia, where the increasing abundance of tropical consumers such as the herbivorous *Siganus fuscescens* has already been documented. Through the assessment of fish assemblages in seagrass meadows and the grazing levels on seagrass in 2001 and 2016/17, as well as *in situ* and mesocosm feeding preference experiments, this study attempted to estimate the grazing rates and impact that the growing abundance of *S. fuscescens* may have in temperate seagrass meadows.

Shifts in the grazing rates on seagrass between 2001 and 2016/17 were inconsistent, varying between seagrass species and location. Based on observational data on the bites on seagrass leaves, rates of consumption increased for *Posidonia sinuosa* while no similar pattern was found for *Posidonia australis*. This was despite an apparent greater consumption on *P. australis* compared to *P. sinuosa* in 2001, and the minimal amount of grazing on tethered seagrass. The higher observed level of *P. sinuosa* consumption in 2016/17 is likely explained by the changed herbivorous fish species composition, even though no fish were clearly observed feeding on seagrass in the current study. The tropical herbivore *S. fuscescens* was more abundant in 2016/17 that 2001, although abundances were patchy and no fish was observed feeding on seagrass. The higher level of *P. sinuosa* consumption in 2016/17, compared to 2001, supports the prediction that with increasing abundances in temperate seagrass ecosystems, tropical herbivores will enhance the consumption of seagrass. However, seagrass consumption is likely to be strongly influenced by the availability of macroalgae which were shown as the preferred food sources.

Feeding trials in mesocosms were compromised by the large number of deaths and the limited grazing on natural food sources by *S. fuscescens*, suggesting that the population in the Perth region is susceptible to adverse handling and husbandry effects. To maximise the survival rate of captured fish, the fishing and handling procedures were altered to adapt to the ongoing observations in the response of fish to handling in the field or in the mesocosm facilities. Lesson learned from the capture, handling and husbandry of *S. fuscescens* in feeding trials in the current study will hopefully provide greater success for feeding preference experiments in the future.

The sampling program initiated in 2001 and repeated in 2016/17 provides base-line data and the opportunity to monitor and track the shift in abundances of tropical herbivores and resultant increases in grazing rates to test the above predictions. The consequences of tropicalisation will depend on the variety of abiotic and biotic factors, including the fish assemblages in the area, the abundance of tropical species, the availability of food, and the feeding preferences that invading species will develop in response to the changed environmental conditions.

1. Introduction

1.1 Background

1.1.1. Climate-driven changes in terrestrial and marine environments

The rising water and air temperatures in temperate and polar environments worldwide can affect a broad range of terrestrial and aquatic species (Walter et al., 2002; Parmesan, 2006; Bellard et al., 2012; Vergés et al., 2014a; Heck et al., 2015). In response, plant and animal species either: tolerate temperature changes; genetically or physiologically adapt to the changed conditions; or shift their geographical distributions (Bates et al., 2014). Numerous examples of recent climate-driven changes in species composition and the resultant ecological responses of a wide variety of organisms exist in land and marine ecosystems (Parmesan, 2006; Bellard et al., 2012; Poloczanska et al., 2013; Vergés et al., 2014a; Heck et al., 2015).

For marine ecosystems, a major observed and predicted impact of climate change is the warming-related poleward shift in the distribution of marine plant and animal populations (Perry et al., 2005; Cheung et al., 2012). Range shifts can be defined as any change in the distribution of species beyond their previously recorded ranges (Sorte et al., 2010; Bates et al., 2014), and can involve geographical expansion or contraction. The expansion of range edges occurs when new locations become suitable for colonists, and species move into those areas (Sorte et al., 2010). The contraction occurs as a result of population decline in the areas historically populated by a species but environmental conditions become unsuitable for sustaining populations (Helmuth et al., 2006).

The poleward range expansions of marine plants and animals are of special interest. They result in the increasing dominance of warmer-water species in cool temperate environments where they were previously absent (Wernberg et al., 2011). By shifting polewards, tropical species begin to compete and interact with native temperate species. This convergence launches changes to various ecological processes, which influence directly and indirectly native species and tropical invaders (Thomson et al., 2015), cause dramatic modifications in faunal assemblages, and impact function and community structure of existing ecosystems (Dawson et al., 2011; Doney et al., 2012). As the global temperatures continue to increase, centres of distribution of many tropical species are expected to shift significantly poleward over the next 100 years (Cheung et al., 2012), where they will influence the productivity and abundance of plants in temperate marine environments and most probably rapidly shift the existing temperate assemblages to alternative states (Vergés et al., 2014a; Hyndes et al., 2016). However, the ecological consequences of these climate-mediated range shifts are not completely understood (Gericke et al., 2014).

1.1.2. Tropicalisation of temperate marine ecosystems

Tropicalisation can be defined as an increase in the ratio of tropical to temperate taxa in a given temperate region (Wernberg et al., 2013), resulting from rising water temperatures and poleward migration of tropical species. Tropicalisation describes the entire suite of observed or anticipated changes in species composition, distribution, abundance and interactions in coastal

ecosystems (Vergés et al., 2014a). Clear examples of tropicalisation are transitions from lush algal forests to barren unvegetated substrates in the Mediterranean Sea (Vergés et al., 2014b), southern Japan (Serisawa et al., 2004), and eastern (Wernberg et al., 2013) and western Australia (Vergés et al., 2014a; Zarco-Perello et al., 2017). Studies of tropicalisation in the USA, Europe and Japan indicate that, while the local rates of warming differ considerably, the mechanisms of ecosystem alteration appear to be similar (Vergés et al., 2014a).

In the Mediterranean Sea, tropical herbivorous rabbitfishes (*Siganus rivulatus, Siganus luridus*) from the Red Sea have colonised the basin via the Suez Canal (Azzurro & Andaloro, 2004; Sala et al., 2011; Vergés et al., 2014b). In the recent decades, this artificial path allowed them to increase their abundance in the south-eastern part of the warmed Mediterranean Sea and become dominant there. This intrusion has profoundly transformed structurally complex benthic communities from productive algal forests to denuded 'barrens' (Vergés et al., 2014a). As the Mediterranean Sea continues to warm (Nykjaer, 2009), rabbitfishes will expand their distribution westwards, threatening algal ecosystems of the northern and western Mediterranean Sea. The impact of the intrusion of tropical species in south-western Japan is analogous to the Mediterranean Sea precedent: increased herbivory and elevated water temperatures have resulted in 'isoyake', which is the replacement of temperate kelp forests (*Ecklonia cava*) by deforested rocky barrens and then coral reef communities through the decline of seaweed beds (Fujita, 2010; Vergés et al., 2014a). The overgrazing of kelp beds and creation of denuded substrate is largely caused by tropical parrotfish (*Calotomus japonicus*), rabbitfish (*Siganus fuscescens*) and various kyphosid fishes, which significantly increased their annual grazing rate as water temperature rose (Yamaguchi, 2010).

In North America, a significant number of tropical plant and animal species have become established among the native temperate fauna and flora in the northern Gulf of Mexico (Fodrie et al., 2010; Heck et al., 2015), including tropical parrotfishes (*Nicholsina usta, Sparisoma radians*), manatees (*Trichechus manatorum*), green turtles (*Chelonia mydas*), warm-water corals (*Acropora palmata*), and black mangroves (*Avicennia germinans*). The impact of herbivore invaders in the region could be profound and unpredictable, as they transform the ecosystems through disturbing the positive balance between seagrass biomass and the diversity and abundance of associated marine organisms. In addition, the geographical ranges of mangrove forests are shifting towards higher latitudes along the northeast coast of Florida (Cavanaugh et al., 2014). These forests are ecologically and economically important for the coastal ecosystems, providing food, nursery grounds and habitat to a great range of terrestrial and marine species (Hoegh-Guldberg & Bruno, 2010). Generally, global warming facilitates increases in mangrove abundance near tropical–temperate transition zones (Cavanaugh et al., 2014).

1.1.3. Seagrass meadows and their functional role

Seagrass meadows are particularly important in shallow coastal water habitats around all continents except Antarctica (Costanza et al., 1997; Burkholder et al., 2013), although vast areas of seagrass meadows have been lost during the past century due to human disturbances (Waycott et al., 2009). Seagrass-dominated habitats enhance the structural complexity of coastal marine waters and provide a range of critical ecosystem services (Duarte, 2002; Barbier et al., 2011).

They are extremely important primary producers which provide food for a wide range of organisms from both the seagrass and the associated algae (Kaiser et al., 2011). The organic seagrass material is either recycled within the ecosystem or transported to adjacent land and marine complexes (Duarte & Cebrian, 1996; Hyndes et al., 2014). Seagrass meadows sequester carbon as organic matter (Duarte et al., 2005; Lavery et al., 2013; Hyndes et al., 2014), and therefore store large amounts of carbon (Fourqurean et al., 2012). However, they also export carbon to other marine and terrestrial ecosystems through the carbon and energy transfer by oceanic currents (Duarte et al., 2005; Fourqurean et al., 2012; Lavery et al., 2013; Hyndes et al., 2014) and its cycling is closely connected to this energy flow. In addition, consumers of seagrass transfer this organic carbon across neighbouring ecosystems (Valentine & Heck, 1999) or transport it offshore, a process particularly driven by some macrograzers (manatees, dugongs, green turtles), which consume large quantities of seagrass (Fourqurean et al., 2010). Carnivores also drive carbon across ecosystem boundaries, as they transfer it from their feeding to shelter habitats (Nagelkerken, 2007). Seagrasses provide other key ecological services, such as filtering the overlaying seawater and improving water quality by intercepting the nutrients and organic matter. They enhance stabilisation of sediments and the sandy sea floor, protecting the shoreline, and preventing resuspension and erosion of sediments (Barbier et al., 2011; Kaiser et al., 2011).

Structurally complex seagrass beds play a significant role, serving as important habitats for different marine invertebrate and vertebrate consumers. The nursery function of seagrass meadows is extremely important. They provide protection from predators and high level of food availability to juvenile consumers, including commercially and recreationally valued finfish and shellfish (Jackson et al., 2001; Heck et al., 2003). The lower rate of predation on seagrasses allows juveniles to survive and grow quickly (Gillanders, 2007), resulting in a greater biomass of fish moving into their spawning habitats.

Many omnivorous, herbivorous and carnivorous organisms, particularly fish and invertebrate species are associated with these communities, becoming both obligate and facultative inhabitants of seagrass meadows (Heck & Valentine, 1995; Jernakoff & Nielsen, 1997; Hyndes et al., 2018; York et al., 2018). Compared to unvegetated areas, diversity and abundance in seagrass beds are very high (Jackson et al., 2001; Gillanders, 2007). Moreover, they provide food sources and shelter from predators, for a wide range of juvenile and adult organisms (Kaiser et al., 2011), preying on the small fish and vertebrates sheltering in the bed. The lost leaves with epiphytes on them contribute to the nearshore food webs, providing feeding material for a large suite of detrivores and then carnivores (Kirkman, 1997), and contribute to the productivity of other ecosystems via export of seagrass detritus (White et al., 2011).

1.1.4. Herbivory on seagrass meadows

Tropical seagrass meadows often support a high diversity and abundance of herbivorous species, compared to the analogous complexes in temperate regions (Horn, 1989; Floeter et al., 2005; Baskett & Schemske, 2018; Longo et al., 2018), although latitudinal patterns of density and biomass remain unclear (Pennings et al., 2009; Poore et al., 2012). Megagrazers, such as manatees (*Trichechus manatus* and *T. senegalensis*), dugongs (*Dugong dugon*) and green turtles (*Chelonia mydas*), are often abundant in the tropics where they consume large amounts of

seagrass (Aragones & Marsh, 2000; Chilvers et al., 2005; Fourqurean et al., 2010; Lal et al., 2010; Marsh et al., 2011; Heithaus et al., 2014). Furthermore, the diversity and abundance of species grazing on seagrass can be high, with species belonging to *Acanthuridae, Siganidae, Scaridae* and *Kyphosidae* consuming seagrasses in tropical regions (Unsworth et al., 2007; Wilson et al., 2010; Vergés et al., 2012). However, the abundance of herbivorous species in temperate regions can still be high. For example, marine waterfowl (ducks, geese, and swans) can be highly abundant and graze significantly on seagrass, removing above- and below-ground material (Dos Santos et al., 2012). In temperate systems, many small herbivorous invertebrates and vertebrates generally feed on the epiphytic algae within seagrass beds, rather than directly grazing the living seagrass material (Jernakoff & Nielsen, 1997; Kaiser et al., 2011; White et al., 2011). Targeting epiphytic flora and fauna, they only incidentally consume small amounts of seagrass (Valentine & Duffy, 2006; MacArthur & Hyndes, 2007; Heck et al., 2015). For fishes, the diversity of omnivores is relatively high, but their consumption of seagrass is limited. Species known to consume seagrass material, albeit in small quantities, or epiphytic algae in temperate seagrass meadows belong to the families of Monacanthidae, Labridae (e.g. *Odax acroptilus, Haletta semifasciata*), Terapontidae (*Pelates sexlineatus*) (MacArthur & Hyndes, 2007; Hyndes et al., 2018). The only large invertebrates that demonstrate a major grazing impact on the seagrass themselves are sea urchins (*Tripneusetes gratilla, Lytechinus variegatus, Diadema antillarum*), but similar to fish, few species appear to solely feed on seagrasses (Heck & Valentine, 1995; Ling et al., 2009).

1.1.5. Tropicalisation affecting seagrass meadows and associated organisms

Tropicalisation in the transitional zones between the tropics and temperate regions has been shown to significantly affect the structure and function of seagrass complexes (Fraser et al., 2014). Over the next century, the tropically associated seagrasses and tropical herbivores associated with them are predicted to respond drastically by moving their ranges southwards in the southern hemisphere, while temperate seagrass species and herbivores inhabiting them will most probably contract southwards (Hyndes et al., 2016). The impacts of such shifts in distribution of tropical flora and fauna could be complex and profound for the habitat-forming ecosystems.

Temperature rises along the west coast of Australia are predicted to affect the composition and abundance of herbivorous species in seagrass ecosystems, forcing tropical fishes to move to higher latitudes. For example, the distribution of marbled parrotfish (*Leptoscarus vaigiensis)*, which is common in tropical north-western Australia and is known to have an impact on seagrasses in the tropics (Vergés et al., 2012), is likely to expand 500 km poleward over the next century (Hyndes et al., 2016). However, the tropical rabbitfish *Siganus* appears to already have a viable breeding population in the temperate coastal waters near Perth (Hyndes et al., 2016), while the tropical range-shifting *Siganus luridus* has also been observed decimating temperate *Posidonia oceanica* meadows in the eastern Mediterranean Sea (Ozvarol et al., 2011).

Poleward expansion of tropical herbivorous fish to higher latitudes will increase the number of species that rely on seagrass as an essential food source in temperate systems (Hyndes et al., 2016). These predicted increases in tropical herbivores in temperate zones will most

probably increase consumption rates and decrease the standing biomass of temperate seagrasses, as herbivores will potentially consume the great amount of above-ground seagrass biomass, reducing the habitat complexity of seagrasses and creating a considerable impact on their role as a nursery habitat. Concurrently, the food web structures in altered temperate seagrass ecosystems are likely to change significantly. In the detritus-based meadows of temperate regions, great quantities are exported to other ecosystems (Hyndes et al., 2014). The intrusion of tropical herbivores is likely to cause shifts from a detritus-based system to a more grazing-based system, although it is not clear at this stage whether tropical invaders will feed on temperate seagrass species in the transition period prior to the establishment of tropical seagrasses (Hyndes et al., 2016). This will probably result in overgrazing of temperate seagrasses by tropical fishes, reduced canopy height and meadow biomass, and large-scale negative impacts on a range of important ecosystem services that seagrass meadows provide (Hyndes et al., 2016). Consumption of plants by tropical herbivores will alter the flow of decomposing seagrass material into adjacent ecosystems thus impacting on habitat, food and nutrient availability in those connected ecosystems (Hyndes et al., 2014).

Temperate seagrass-associated fish assemblages are predicted to be altered, not only through the influx of tropical species, but also the contraction of subtropical and temperate species, thereby significantly altering the biodiversity of seagrass ecosystems in those regions. The distribution of some native temperate species might become restricted to the south-western corner of Western Australia. However, some species endemic to temperate regions may become extinct, especially if they have specific habitat requirements or if they are co-dependent on other species (Hyndes et al., 2016).

1.1.6. Range expansion of species in Australia

With the continued ocean warming, hundreds of species retreat towards and beyond the edge of the Australian continent and the temperate marine communities progressively come to resemble northern ecosystems (Wernberg et al., 2011). The southward extent of distribution along the eastern coast of Australia is documented for several tropical reef fishes (Booth et al., 2007; Last et al., 2011), including Pomacentridae (*Abudefduf vaigiensis, A. sexfasciatus*) and Chaetodontidae (*Chaetodon auriga, C. flavirostris*). Despite strong evidence for the role of the warm poleward East Australian Current in transporting these species, the range extension for many coastal temperate consumers is correlated with increases in water temperature. The eastern coast of Australia is experiencing the decline of seagrass meadows due to a complex of reasons, including invasive species and global climate change (Vergés et al., 2014a).

Emerging evidence suggests that diversity and abundance of tropical and subtropical herbivorous species increases in the temperate waters along the western coast of Australia (Cheung et al., 2012), including damselfish (*Abudefduf sexfasciatus, Abudefduf vaigiensis*), rabbitfish (*Siganus spp.*), western scalyfin (*Parma occidentalis*), butterflyfish (*Chaetodon assarius*), convict surgeonfish (*Acanthurus triostegus*), and lined dottyback (*Labracinus lineatus*) (Hutchins & Pearce, 1994; Pearce & Hutchins, 2009; Wernberg et al., 2013). In addition, there has been a widespread loss of kelp (*Ecklonia radiata*) forests along the temperate western coast of Australia as a result of an extreme marine 'heat wave' event during 2011

(Pearce & Feng, 2013; Smale & Wernberg, 2013; Wernberg et al., 2016). This heat wave was followed by the high levels of grazing by tropical and subtropical herbivorous fishes that prevented the recovery of kelp (Wernberg et al., 2013; Fraser et al., 2014). Similarly, the heat wave caused a range contraction of approximate 100 km for the canopy-forming seaweed *Scytothalia dorycarpa* along the temperate coast of Western Australia (Smale & Wernberg, 2013; Caputi et al., 2014). In both cases, this caused structural changes both at the community and ecosystem levels where canopy-forming seaweed was lost.

1.1.7. Oceanographic processes on the west coast of Australia

There is evidence that intrusion of tropical species into temperate waters in all regions is influenced by poleward flowing boundary currents that create ocean warming hotspots around the globe (Hutchins, 1991; Vergés et al., 2014a). Thus, the abundance and distribution of marine plant and animal species along the western coast of Australia are strongly influenced by the warm poleward flowing Leeuwin Current (Ayvazian & Hyndes, 1995; Watson & Harvey, 2009; Feng et al., 2012). That current has been present for millennia, and has influenced the marine biological assemblages down the south-west coast of Australia over that period (Hutchins & Pearce, 1994; Cresswell & Domingues, 2009). It transports warm, relatively low-saline tropical waters towards temperate regions and then eastwards into the Great Australian Bight, far further south than would otherwise be expected. It makes possible the presence of tropical fauna in the subtropical and temperate waters of the south-western coast (Sen Gupta et al., 2015; Wernberg et al., 2016), which can be seen through a variety of tropical fishes and corals already present in temperate regions (Hutchins, 1994; Pearce & Hutchins, 2009). Moreover, it enhances dispersal and range expansion of marine biota as far as the Great Australian Bight (Maxwell & Cresswell, 1981) and is predicted to continue to drive poleward shifts of marine communities (Wernberg et al., 2013).

Water temperatures have been increasing particularly in the south-eastern sector of the Indian Ocean (Pearce & Feng, 2007). They are projected to increase by up to 2^0C in south-western Australian waters by the end of this century (Lough, 2008). These predictions intersect with extreme thermal events, such as the 'heat wave' in 2011, which produced record nearshore temperatures rising to 5^0C above the average (Caputi et al., 2014). Such events are predicted to increase in frequency and intensity in the future (Wernberg et al., 2013; Wernberg et al., 2016). All these will facilitate faster tropicalisation of marine complexes in the Western Australian waters, with increasing dominance of warmer-water species (Cheung et al., 2012). This is likely to lead to wide range expansions of tropical fauna and flora and unexpected trophic effects in the communities receiving poleward moving species.

1.2 Significance and aim

The study of tropicalisation, which causes dramatic changes in terrestrial, marine and estuarine ecosystems, is a very topical research challenge. This knowledge allows researchers to monitor the impact of global warming on ecosystems and to predict the dynamics of species and

habitat changes. Tropicalisation of marine species as a result of global warming is likely to have important impacts on Western Australian coastal ecosystems and clearly requires further study.

Western Australia is a recognised terrestrial and marine biodiversity hotspot with a high number of endemic species (Hopper, 2009; Hobday & Pecl, 2014). The increasing warming of coastal waters along Western Australia is predicted to continue during the rest of the current century and affect seagrass complexes. It is now necessary to test these predictions of southward expansion of some tropical species' ranges and the contraction of temperate species' ranges down the western coast of Australia. While there are only a few cases where these range shifts have been demonstrated, the impact of tropical species moving south into seagrass ecosystems along the Western Australian coast could be dramatic (Bennett et al., 2015). The indirect effects of tropical species range shifts can be significant, including large alterations in food-web structure and extinction of some endemic species from their native habitats (Cheung et al., 2012). It is therefore imperative that we gain an understanding of the effects of tropicalisation on the important temperate seagrass meadows in the region to underpin future management approaches to adapt to these changing conditions.

This thesis has focused on the tropical herbivorous fish species *S. fuscescens* that has expanded its range southwards and has become established in temperate seagrass communities. Its aim was to test the predictions by Hyndes et al. (2016) by determining the effects of this tropicalisation through this tropical herbivorous fish species on the structure and functioning of seagrass meadows in temperate Western Australia. This has been achieved through the following objectives:

1. Determine the levels of grazing on temperate seagrasses and compare them to grazing levels in 2001.
2. Determine the feeding preferences and grazing rates of tropical consumers in temperate seagrass meadows *in situ* and in mesocosm conditions.

2. Materials and methods

2.1 Objective 1 – Determine the levels of grazing on temperate seagrass and compare them to grazing levels in 2001

The level of grazing on temperate seagrass was determined using a combination of: (1) counting bite marks on seagrass leaves; (2) estimating grazing rates using the tethering technique; and (3) identifying grazers using underwater cameras. The approach and procedures followed those of a study by Fiona Tomas (unpublished data) in 2001, when *Siganus* was presumably absent from the temperate region, or its abundance was very low. Using tethering experiments, the 2001 study showed that the seagrass consumption was always significantly lower than the actual production rate of seagrass at the same time. Video censuses did not show any *Siganus* present in the region of study. The current study intended to revisit the 2001 sampling regime and compare grazing rates to those estimated in 2001. Video censuses were undertaken to determine if abundances of tropical herbivores correspond to any predicted increases in bite marks and grazing rates in temperate waters over that period.

2.1.1 Study region

The study was performed in Marine Protected Areas at Marmion Marine Park (MMP) and Rottnest Island Marine Reserve (RIMR), located along the temperate coast of south-western Australia. Sampling was conducted during November/ December 2001 (Tomas, unpublished data) and again in December 2016/ January 2017. At both MMP and RIMR, sampling was conducted in seagrass meadows situated close to limestone rocky reefs at three sites: Whitford's Rock (31^0 47' S, 115^0 43' E), Wreck Rock (31^0 48' S, 115^0 43' E) and Boy in a Boat (31^0 49' S, 115^0 44' E) at MMP and Parker Point (32^0 02' S, 115^0 52' E), Abraham Point (32^0 01' S, 115^0 46' E) and Stark Bay (32^0 00' S, 115^0 48' E) at RIMR (Fig. 2.1, 2.2). In each location, the seagrass meadows on the shoreward side of the reef were sampled at depths of 2 to 5 m at MMP and RIMR. In Marmion Lagoon, sampling was conducted in the meadows of *P. sinuosa*, mostly monospecific or with the presence of algae (*Sargassum sp., Ulva sp., Ecklonia radiata*), other species of seagrass (*Amphibolis antarctica, Halophila ovalis, Syringodium sp.*) and sandy patches. *P. australis* was absent from all MMP sites except at Whitford's Rock where a small patch was found next to the reef. At RIMR, sampling was conducted in both *P. sinuosa* and *P. australis*, mostly monospecific meadows, sometimes with the presence of the other species of seagrass (*Amphibolis antarctica, Halophila ovalis*), algae (*Sargassum sp.*) and sandy patches.
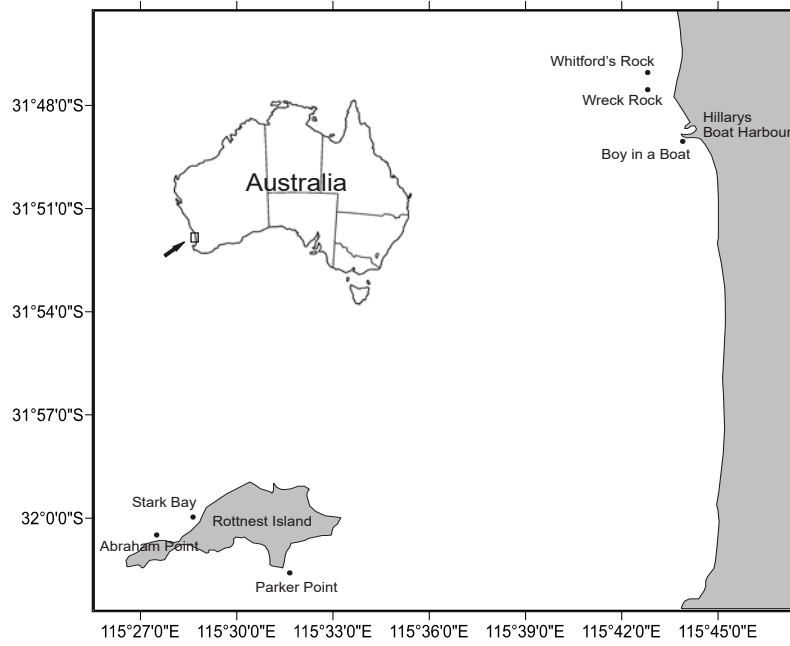
Fig. 2.1. Sampling locations within Marmion Marine Park (Whitford's Rock, Wreck Rock and Boy in a Boat) and Rottnest Island Marine Reserve (Parker Point, Abraham Point, Stark Bay) in south-western Australia.
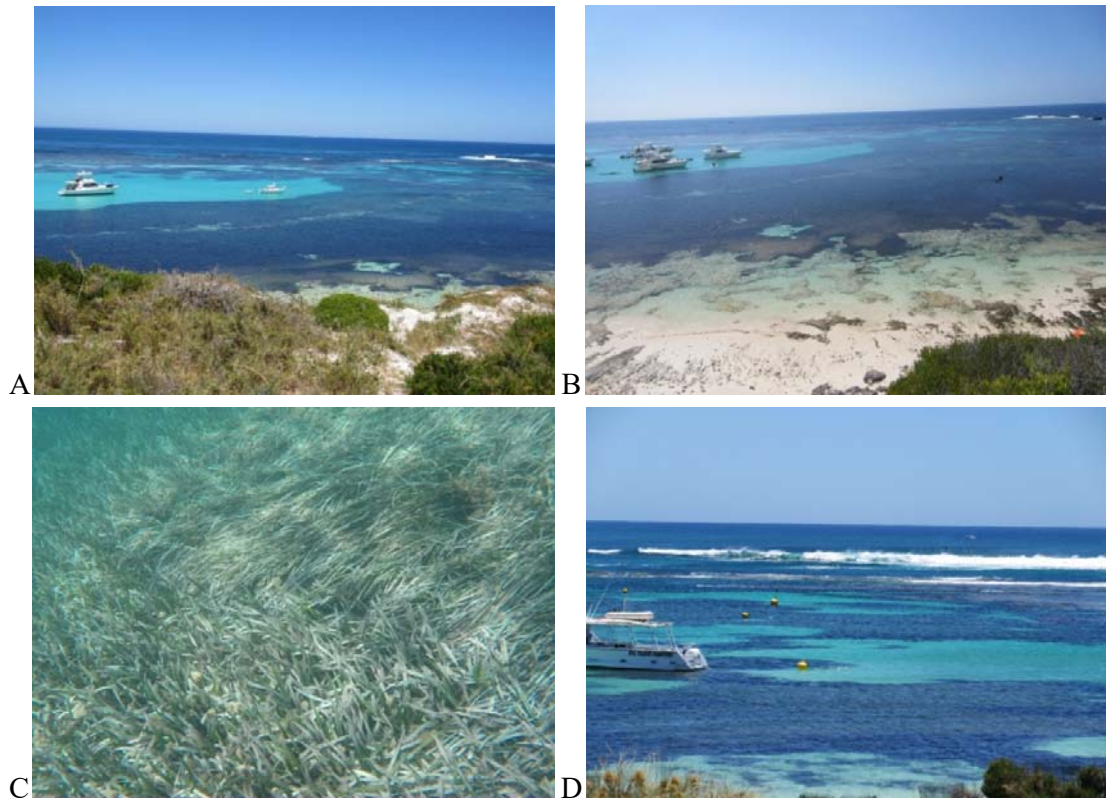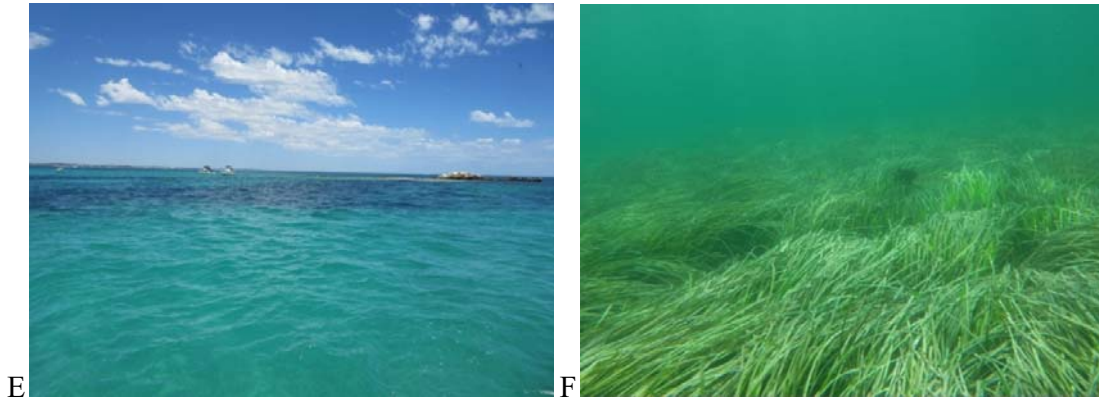
Fig. 2.2. Sampling locations and *P. sinuosa* meadows at Rottnest Island Marine Reserve (A, B – Abraham Point; C, D – Parker Point) and Marmion Marine Park (Whitford's Rock).

2.1.2   Sampling methods

Indirect measurements of herbivory on temperate seagrasses

Herbivory by fish on *P. sinuosa* and *P. australis* was firstly assessed through indirect measurements of grazing by recording existing bite marks on seagrass leaves (White et al., 2011). At each site and year, 50 shoots of *P. sinuosa* and *P. australis* (where possible) were randomly collected within three replicate belt transects (40 x 10 m in 2001 and 35 x 5 m in 2016/2017) at different distances from the rocky reef (0-15 m, 20-30 and 40-60 m). Distinctive semi-circular bite marks left by herbivorous fish in seagrass leaves were counted (Fig. 2.3). Such semi-circular bite marks are known to be specific to herbivorous fish (Mariani & Alcoverro, 1999; Alcoverro & Mariani, 2002; Kirsch et al., 2002; Tomas et al., 2005; Prado et al., 2007; White et al., 2011). The irregular or non-semi-circular shaped marks were not counted to reduce the risk of mistaking marks left on the seagrass leaves by invertebrate grazers, or as a result of leaf abrasion or necrosis (Heck & Valentine, 2006). The number of bite marks per shoot was recorded and the proportion of seagrass shoots with fish bites was determined.

Fig. 2.3. Semi-circular fish bite marks on seagrass leaves.

All collected leaves with identified fish bite marks were photographed in the laboratory over a 1 x 1 cm grid to obtain an estimate of bite mark area, and the number of bite marks and leaves with marks were counted for each transect. The average biomass of seagrass removed per square meter was calculated using this estimate. The lengths of seagrass leaves collected at each site were compared to the expected lengths of undamaged leaves. The difference between the actual and expected lengths of leaves was considered an amount of leaf biomass lost to grazing (Heck & Valentine, 2006).

In 2001, the percentage of shoots with fish bite marks was counted only for *P. sinuosa* for all three sites at MMP and one site at RIMR (Abraham Point), and therefore, no data for fish bite marks on *P. australis* is available for 2001. In 2016/ 17, herbivory by fish was measured both for *P. sinuosa* and *P. australis* (where available) at different distances from the rocky reef. *P. sinuosa* was sampled at all sites and along almost all transects, except 20-30 m and 40-60 m from reef at Stark Bay. *P. australis* was absent from MMP, except 0-15 m to reef at Whitford's Rock, and at RIMR, except 0-15 m to reef at Stark Bay.

Seagrass leaves were collected and the fish bites on seagrass leaves were counted at three distances from the rocky reef. Temperate rocky reefs in near-shore waters around Perth are characterised by high biomass and diversity of macroalgae, which attracts herbivorous and omnivorous fish. Seagrass meadows in the next to reef zone, therefore, are most likely to experience certain grazing pressure from the reef-associated herbivorous fish species. They may prefer to target seagrass in the next proximity to the reef or move further from the reef, especially due to reduced availability of macroalgae communities. The data collected at different distances from the reef allowed to compare the proportions of seagrass consumed in seagrass communities where the preferred food source is easily available to fish herbivores. It also allowed predicting of fish species responsible for the grazing on seagrass.

Tethering experiments

Using bite marks on seagrass leaves can underestimate the grazing intensity, because partly consumed leaves can be weakened and broken off by waves and currents (Prado et al., 2007). To supplement the above indirect measurements, a tethering experiment was used to determine direct grazing rates by herbivorous fishes (Kirsch et al., 2002; Prado et al., 2007). Procedures generally followed those of Verges et al. (2014). Seagrass shoots were collected from each site to construct tethers. All shoots used in the assays had no evident signs of herbivory initially (i.e., no bite marks on tethered leaves) (Vergés et al., 2014b). Eighteen similar, medium-sized shoots of *P. sinuosa*, and where possible, 18 shoots of *P. australis* were tied to a rope and embedded independently and haphazardly in the meadow with metal pegs (between 2.0 and 4.5 m depth) within the 0-15 m of reef at each site. A minimum distance of at least 0.5 m was kept between tethers. Photos were taken for each shoot before deployment (Fig. 2.4). At MMP, tethering was performed for *P. sinuosa* only, and at RIMR both *P. sinuosa* and *P. australis* were deployed.

Fig. 2.4. Tethers of *P. sinuosa* and *P. australis* were photographed before deployment.

The deployed tethers were collected after one week and transported back to the laboratory, where they were visually assessed and photographed. The rate of seagrass consumed by herbivorous fishes in seven days was determined by comparing the before and after digital photographs, and fresh bite marks were counted (Fig. 2.5). Leaves that appeared to be torn (through wave action), rather than being grazed, were excluded from statistical analyses (Kirsch et al., 2002; Fox & Bellwood, 2008). The initial leaf area was compared to the remaining leaf area. The area (cm^2) of tissue consumed by herbivores was measured with metric paper, and the rate of consumption was calculated in cm^2 $shoot^{-1}$ day^{-1} and then transformed to leaf grazing per unit area per day (g dry weight m^{-2} d^{-1}) based on the data collected.

Fig. 2.5. Rates of seagrass consumption were assessed from seagrass tethering assays: the photos show seagrass leaves before and after deployment.

Seagrass production measurement

The rate of consumption (cm^2 shoot^{-1} day^{-1} removed) was compared to the rate of seagrass production (mm day^{-1}). Seagrass production was measured through the hole punch method (Zieman, 1974; Cambridge & McComb, 1984; Cambridge & Hocking, 1997; Westera & Lavery, 2006). Seagrass production was measured only for *P. sinuosa* and only for MMP sites in 2001, whereas it was measured for *P. sinuosa* at all sites except Boy in a Boat (situated within the sanctuary zone) in 2016/17, and *P. australis* at all three sites at RIMR but not at MMP where this seagrass was absent. At each site, 20 shoots per seagrass species were punched *in situ* through the upper section of the leaf sheath near the leaf/sheath junction (Fig. 2.6), producing a lesion on each seagrass leaf (Short & Duarte, 2001; Chiu et al., 2013). Tags were placed on punched shoots to allow for later collection. After seven days, the shoots were collected and transported to the laboratory, where the new growth from the scar (leaf elongation) was measured for each shoot to the nearest 0.1 mm (Fig. 2.6). The epiphytic growth was removed from the leaves with a razor blade. The newly produced parts of the shoots including any new unmarked leaves were removed and dried at 60° C for 44 hours. This allowed the proportion of biomass of *P. sinuosa* and *P. australis* removed by herbivorous/omnivorous species to be compared to the productivity of the seagrass (Lee et al., 2016).

A   B
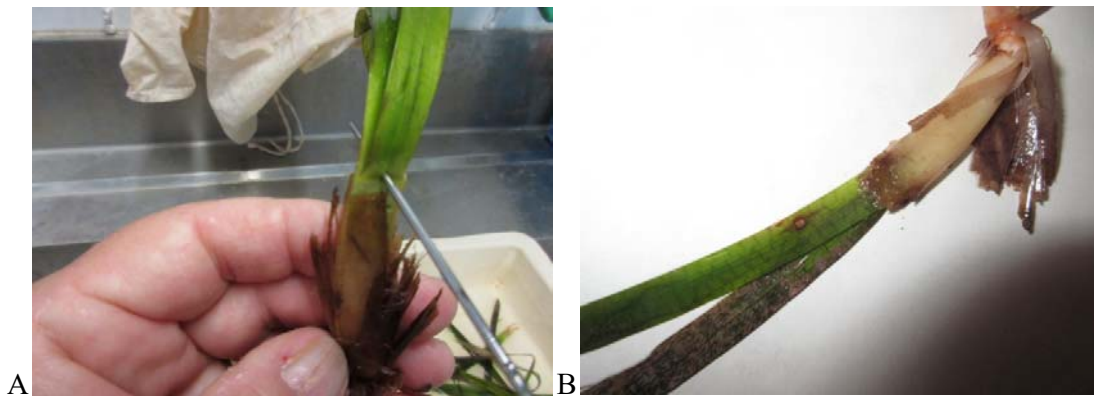
Fig 2.6. The whole punch method (A) was used to measure seagrass production *in situ* in the study area, with the collected seagrass showing growth between the scar and sheath (B).

Underwater video cameras

To determine the grazing species that were responsible for any bite marks on the tethers deployed in 2016/17, two stationary remote underwater video cameras (Sony-HDR SR12) in waterproof housings were attached to concrete blocks and deployed on the sea floor, focusing on tethered seagrass shoots at each site (Michael et al., 2013) (Fig. 2.7). Fishes biting tethered seagrass were recorded over a 2 h period at the same time of the day (between 10.00 and 14.00 h). The procedure was repeated for three consecutive days within each site (Hoey & Bellwood, 2009), yielding an average 10 h of filming for each site (2 h x 3 days x 2 cameras recording each time). Each fish recorded feeding on seagrass tethers was identified, and the number of bites and

the fish size (total length, mm) were recorded from video footage (Rasher et al., 2013). Additional attractive assays of seagrass were placed at the edge of meadows and in bare sandy patches. This was used to attract more herbivorous fish to the seagrass meadow and facilitate their identification. At the beginning of the filming, the camera focal length was calibrated using a scale bar of known length (Michael et al., 2013). A bite was considered every time a fish strokes the seagrass leaf with its jaws opened, and subsequently closing its mouth, regardless of ingestion (Longo & Floeter, 2012). In the case of indiscernible multiple rapid bites, they were considered as one bite (Bellwood & Choat, 1990).
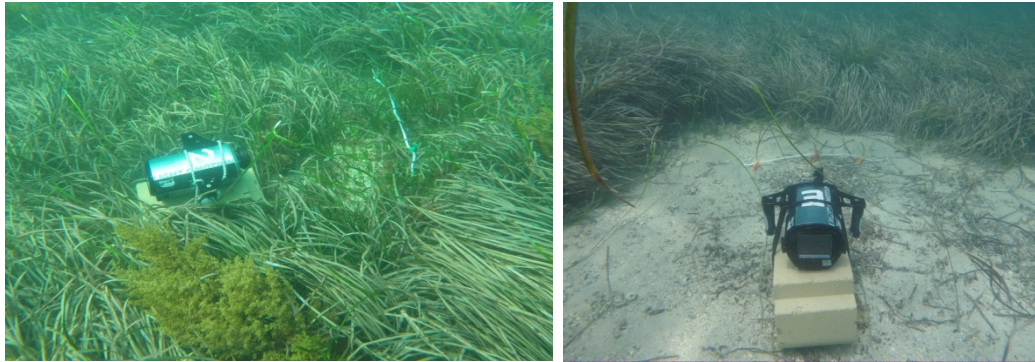
Fig. 2.7. Underwater video camera to estimate fish diversity and abundance in seagrass meadows.

The maximum number of grazing fish observed in one frame over the whole duration of filming (MaxN) was recorded (Watson et al., 2005; Watson et al., 2010). The MaxN was also used as a supplement to UVC in 2016/17 and provided a more comprehensive suite of herbivores/omnivores in the seagrass meadows, since the method produces a larger number of species and a greater number of large-bodied species compared to standard UVC technique (Watson et al., 2010).

Herbivory censuses

Fish abundances were estimated using underwater visual censuses (UVC) over seagrass at different distances from the reef (0-15m, 20-30, 40-60) at each of the three sites in both regions. The number of fish of each species was counted at each site along three replicate belt 40 x 10 m transects. In 2001, fish counts were performed at the same sites, except Parker Point. Surveys were conducted between 9:30 and 12:30 h at all sites. The distance covered for each transect was measured by trailing an underwater measuring tape, which was fixed to the substratum at the starting point of each transect (Dickens et al., 2011; Mallet et al., 2014). The diver swam one way at a constant speed along each survey location recording all the fish encountered (Fig. 2.8). Fish counts from UVC were converted into densities per unit area (individuals per 100 square meters).

Fig. 2.8. Underwater video censuses (conducted by A. Turco).

Statistical analyses

Since MMP was the only region where *P. sinuosa* was present across all distances from the reef, and at all sites, analyses to examine differences in percentage of *P. sinuosa* shoots with bite marks across years were restricted to this region. Variation in rates of consumption of *P. sinuosa* shoots (expressed as the percentage of shoots with fish bite marks from each distance from reef within seagrass meadows) was analysed with three-way analyses of variance (ANOVA), testing for differences between three fixed factors: site, year and proximity to the rocky reef. Post-hoc Turkey's test examined the difference between the percentages of *P. sinuosa* shoots with fish bite marks for year-site-distance combinations.

Due to a three-way interaction for the above test, a two-way ANOVA was performed for each site at MMP to assess the difference in the percentage of *P. sinuosa* shoots with fish bite marks between the two years (2001 and 2016/17) and among all three distances away from the reef (fixed factors). This was followed by post-hoc Turkey's test, examining the difference between the percentages of *P. sinuosa* shoots with fish bite marks for each year-distance combination at MMP.

All data were checked for normality and homogeneity of variances. Levene's test (SPSS Statistics 23) was used to test for homogeneity of variance. When assumptions of homogeneity of variance were violated, square root transformation was applied to meet the homogeneity. The significance was accepted at probability level $P < 0.01$ when data were not homogenous or normal after transformation (Underwood, 1981). These procedures apply to all ANOVA analyses described below.

Two-way ANOVA was applied to assess the differences in the percentage of *P. australis* shoots with fish bite marks as well as the number of bites per shoot nearest to the reef between the two years (2001 vs 2016/17) and across the three sites at RIMR. The post hoc Turkey's test further examined the combinations between years and sites to find where the difference lay.

Consumption rates of *P. sinuosa* tethers nearest to the reef (0-15 m) at MMP, where *P. sinuosa* was found in both years, was analysed with two-way ANOVA to test for differences across the years and sites. The post-hoc Turkey's test was further applied to compare the combinations between years and sites, to find out where the difference lay.

2.2 Objective 2 – Determine the feeding preferences and grazing rates on temperate seagrass and macroalgae

To achieve the objective, the grazing rates, dietary preferences and composition of herbivorous fishes were determined *in situ* in the study region. Secondly, through laboratory experiments, the study intended to determine the effects of increasing sea temperature on grazing rates and preferences by the tropical herbivore *S. fuscescens*.

2.2.1 *In situ* grazing experiments

In situ consumption rate experiments were conducted to determine the current level of grazing and feeding preferences on seagrass and macroalgae in temperate seagrass meadows, using the tethering technique and underwater video cameras to identify the grazers. The study took place in December 2017 in temperate seagrass meadows situated close to limestone rocky reefs in Marmion Marine Park, where tethers comprising a range of potential food sources were placed in inshore seagrass meadows (at depths between 3 and 5 m) at three sites: Whitford's Rock (31^0 47' S, 115^0 43' E), Wreck Rock (31^0 48' S, 115^0 43' E) and Wanneroo Rock (31^0 48' S, 115^0 43' E) (Fig. 2.1, 2.2 E, F). Tethers were located in mostly monospecific meadows of *P. sinuosa*, with the presence of algae (*Sargassum sp., Ulva sp., Ecklonia radiata*), other species of seagrass (*P. australis, Amphibolis antarctica, Halophila ovalis, Syringodium sp.*) and sandy patches. For tethers, seagrass shoots (*P. australis*, *Zostera nigricaulis*) were collected from Woodman point, Fremantle (32^0 13' S, 115^0 74' E). The macroalgae (*Sargassum sp.*, *Ecklonia radiata*) were collected from the Marmion Marine Park, Iluka Beach (31^0 73' S, 115^0 71' E). The water temperature was $19\text{-}20^0$ C.

Shoots of the two seagrass species and thalli of the two macroalgae species were tethered together to form 10 replicate seagrass / macroalgae tethers, which were deployed randomly within the 0-15 m of reef at each site to test for feeding preferences by herbivores (Mariani & Alcoverro, 1999; Alcoverro & Mariani, 2002; Kirsch et al., 2002; Tomas et al., 2005; Prado et al., 2007; White et al., 2011). Thirty seagrass shoots of similar medium size per each species and 30 macroalgae thalli were used to construct tethers for deployment (Vergés et al., 2012). Each tether consisted of 5-6 shoots of *P. australis*, a number of shoots of *Z. nigricaulis*, one thallus of *Sargassum sp.*, and one thallus of *E. radiata* (Fig. 2.9). Multiple shoots of seagrass were provided to keep biomass consistent across the species and similar to those of macroalgae. Prior to deployment, shoots and thalli were weighed to the nearest 0.1 mg, photographed, tied to sisal rope, and then embedded independently in seagrass beds (at 3-5 m depth) next to reef at each site (Mantyka & Bellwood, 2007). Out of 10 tethers deployed at each site, 5 were uncaged (treatment replicates) and 5 were caged (control), with a mesh size (0.6 cm^2) that would exclude fish grazers (Fig. 2.10). The caged tethers were used as a control for losses from herbivorous fish.

Fig. 2.9. Seagrass (*P. australis*, *Z. nigricaulis*) and macroalgae tethers (*Sargassum sp.*, *E. radiata*) prepared in the laboratory prior deployment.

Fig. 2.10. The caged tethers of seagrass and macroalgae (control replicates) deployed in the study sites and covered by the net to exclude fish herbivory.

The deployed tethers were retrieved in 24 hrs. The number of fresh fish bite marks on each species on each tether was recorded. In addition, the initial fresh weight of freshly collected seagrass and macroalgae appearing similar in size and without epiphytes was recorded to the nearest 0.1 mg after the removal of excess moisture by blotting on paper towel (Bennett & Bellwood, 2011; Vergés et al., 2012). The number of blots was standardized for each seagrass and macroalgae species depending on its water retention capacity (Capper et al., 2006). All

procedures were performed by the same person to ensure consistency across samples. The area of plant tissue consumed was measured in cm^2 shoot^{-1} (or thallus) day^{-1} and converted to gDW m^{-2} day^{-1} (Kirsch et al., 2002), based on the measurements of this study.

Underwater video cameras

RUVs in underwater housings were used to film the species feeding on the assays *in situ*, without the presence of an observer. This was to ensure the plant material loss was due to herbivory and not any leaf / thallus damage caused by environmental factors or the conditions of deployment (Mallet et al., 2014; Lee et al., 2016). Two video cameras were attached to concrete blocks and placed on the seabed at each site, focusing on the deployed tethers (Michael et al., 2013; Bell et al., 2019) (Fig. 2.11). Fishes near the tethers were recorded at each site for 2 – 3 hrs straight after deployment (Hoey & Bellwood, 2009), between 09.00 and 13.00 h when the feeding rates are considered to be more intense (Zemke-White et al., 2002). From video footage, each fish recorded feeding on tethers was identified.

Fig. 2.11. Photos taken from underwater video cameras filming seagrass (*P. australis*, *Z. nigricaulis*) and macroalgae tethers (*Sargassum sp.*, *E. radiata*) deployed at study site.

Statistical analyses

Statistical analyses were performed to determine the difference in the seagrass and macroalgae biomass removed by fish from the tethered *P. australis*, *Z. nigricaulis, E. radiata* and *Sargassum sp.* replicates deployed *in situ* within the three seagrass meadows. Since treatment in choice assays lack independence, a Friedman non-parametric test of ranks was run (Suarez-Jimenez et al., 2017). The loss of biomass data for four types of seagrass and macroalgae species offered in consumption rate assays *in situ* was analysed separately for each location to check if there was any significant difference in loss of biomass depending on the location. Friedman test was followed by a post hoc Wilcoxon signed-rank test to identify pairs of seagrass and macroalgae assays that differed significantly within each location.

2.2.2 Mesocosm trials

Experimental design

Choice and no-choice experiments were planned to be conducted for *S. fuscescens* in the laboratory to study their dietary preferences and grazing rates under the current sea surface temperature conditions and those predicted in 100 years, based on:
- o the existing mean winter sea surface temperature in temperate waters of the western Australian coast (19^0C) (Pearce & Feng, 2007; Pearce & Feng, 2013; Smale & Wernberg, 2013);
- o the existing mean summer ocean surface temperature in temperate waters of the western Australian coast (22^0C) (Bureau of Meteorology, http://www.bom.gov.au); and
- o the mean summer SST predicted for the temperate region in 100 years (25^0C) (Lough, 2008).

Both experiments were based on using the herbivore *S. fuscescens*, which has been shown to consume seagrass and be suitable for aquaria experiments (Pillans et al., 2004). Also, the approach planned to use the two temperate seagrass species (*P. australis* and *Z. nigricaulis*), which are abundant in the temperate waters of the western coast of Australia (Kirkman & Walker, 1989; Kirkman, 1997; Carruthers et al., 2007), and two brown algal species (*Sargassum sp*. and *E. radiata*).

Choice experiments were planned to generally follow those of Pillans et al. (2004) and Bryan (1975). Three fish were randomly chosen from the holding tanks and placed into each replicate aquarium for the treatment and temperature. The food (4 choices) attached to metal grids was then randomly placed in the treatment aquaria, and likewise, the paired control aquaria. The fish were left in the treatment aquaria for 48 hrs. It was assumed that the similar environmental conditions affected all replicates of both control and treatment. Biomass consumption was estimated as [(Hi x Cf/Ci) - Hf], where Hi and Hf were initial and final wet masses of tissue exposed to herbivores, and Ci and Cf were initial and final masses in control assays (Parker & Hay, 2005; Tomas et al., 2011).

No-choice food experiments were initially planned to be conducted to compare the grazing rates of *S. fuscescens* on the four food types under the three temperatures. Compared to the

above choice experiment, it was planned to offer fish a single seagrass or macroalgal species, with macrophyte species and temperature forming the factors in the experiment (Bryan, 1975).

Collection of food sources

Seagrass and seaweed species were collected from Woodman Point and Marmion Marine Park and maintained in flowing seawater tanks and used in feeding trials within 48 hrs of collection. Epiphytes were removed, where possible, to eliminate any confounding factors that could be introduced by variance in the type and abundance of epiphytes on the macrophytes. Seaweed and seagrass would be offered to fish under different temperatures simultaneously for feeding preference trials and separately for grazing rates trials, paired with controls with the same food choice but no consumer to account for autogenic responses of food sources independent of the action of the consumers (Peterson & Renaud, 1989; Roa, 1992).

Adult *S. fuscescens* were captured live from reef habitats and seagrass beds in Marmion Lagoon using hook and line, and corn for bait. Fish were handled firmly and carefully, taking care not to drop fish. Many fish were able to be classified as adults by visual estimate of length. Those classified as juveniles were released immediately into the water. To avoid any stress due to entanglement in the net, the net had a relatively small mesh size compared to the size of the target fish. Fish were retained in the water whilst in the net to minimise damage prior to handling them and placing them in transport containers. Barbless hooks as a form of capture were attempted, as they are considered to be one of the best forms of minimising damage to fish during collection (Dr. Roennfeldt, TAFE, personal communication). However, barbed hooks were mostly used, as they allowed to have a stronger hold on the fish and reduced the chance of losing it. To minimize the stress on the fish, they were taken immediately to an aerated holding tank (400 L) on board the vessel. Fish were then observed for changes in behaviour. Not more than two fish at a time were caught to avoid them waiting for too long before being placed into tanks on board.

Fish were transported to the laboratory in an aerated plastic container (400 L, max 10 fish per collection) filled with sea water, supplied by bilge pump to allow constant change of oxygenated sea water in the tank (Fig. 2.12), and transferred to the aquarium facilities within 6 hrs. It was initially planned to capture 250 fish in total (n = 45 fish for multiple choice experiments and n = 180 for no-choice experiments). However, these numbers were not achieved due to health issues experienced in the pilot study. In total, 22 fish were captured and used for the pilot study. The fish of the same size (TL > 20 cm) were used to obviate any feeding differences associated with size dominance (Capper et al., 2006).

Fig. 2.12. Transporting tank with an aerating system and *S. fuscescens* captured during the fishing field trip.

Experimental procedures

Once in the laboratory, fish were transferred to a flow-through holding (quarantine) tank filled with fresh sea water to acclimate them to tank conditions. Concrete blocks / pavers were used to form reef-like habitats in each tank (Fig. 2.13).The volume of each tank was 1000 L. New fish were isolated from the fish caught previously, to prevent introducing disease to already acclimated fish. During the acclimation period, fish were regularly observed for skin conditions, possible parasites, and behavioural issues.
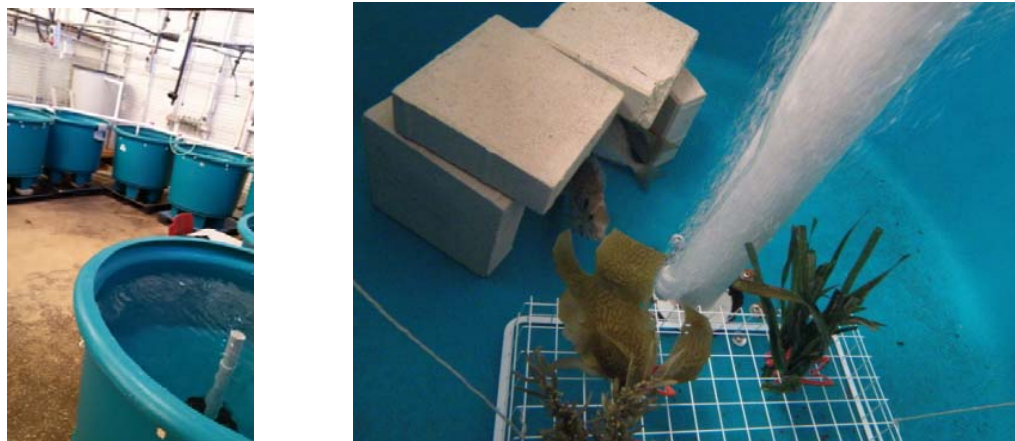
Fig. 2.13. Mesocosm facilities and holding tanks for feeding trials at Fremantle TAFE. Concrete blocks were used to create an artificial reef-like construction.

The choice experiment was conducted in 6 tanks simultaneously, with 3 adult *S. fuscescens* in each tank, and one control tank with control assays and no fish. For the feeding preference trials, 4 types of food sources – seagrass (*P. australis*, *Z. nigricaulis*) and macroalgae (*Sargassum sp.*, *E. radiata*) species – were introduced simultaneously in each tank and left there for 48 hrs for fish to consume (Fig. 2.13). Seaweed and seagrass were attached to the metal grid and placed into each treatment and control aquarium simultaneously (Paul et al., 1990; Ojeda & Muñoz, 1999) (Fig. 2.14). At the end of the experiment, seagrass and seaweed material was removed with a fine mesh net, re-blotted dry and re-weighed (g) to determine the amount consumed for each macrophyte (Targett & Targett, 1990). Differences between initial and final wet weights of different types of seagrasses and macroalgae were used to calculate percent of material consumed over the 24 h period. The control replicates in which the seagrass and algal biomasses were placed in aquaria with no fish, and collected after the same length of time that the herbivory experiments, provided the ability to determine whether the algal species and the seagrasses lose weight differentially, and if they do, to account for this in the analysis of the herbivory experiments. The biomass loss determined for the control assays was subtracted from the loss of seagrass and macroalgae biomass in the experimental tanks. Mean values were calculated for adjusted biomass loss for each seagrass and macroalgae species across 6 tanks. The temperature was consistent across the tanks (19-20^0).

Fig. 2.14. Seagrass shoots and macroalgae talli clustered together on metal grid to construct tethers and a set of food sources for the multiple-choice mesocosm experiment.

Pilot trials

Pilot trials were carried out at Fremantle TAFE facilities that have appropriate mesocosm and flow-through systems to undertake this study. The 'system-acclimation' period took from a few days to two weeks. Throughout this period, rabbitfish were supplied with small amounts of food every 1-2 days and monitored for feeding interest/behaviour and feeding-commencement. Commercial aquaculture marine pellets were used to feed fish, which were also supplemented with seagrass and macroalgae suspended in the tanks. Feeding commencement by the majority of fish was used to indicate that the fish were acclimated and ready for the trial. Only healthy fish were used in feeding experiments.

The fish were purged for two days before the beginning of the pilot trial (Bryan, 1975; Goecker et al., 2005). In total, 19 fish were involved in the pilot trial. Fish were tested in groups

of three, as solitary fish were noticed to not eat until other fish were introduced into the tank (Bryan, 1975; Paul et al., 1990; Pillans et al., 2004; Capper et al., 2006). Only the choice trial was conducted at this stage. Video observations were conducted directly and with the use of GoPRO cameras during the first half of the day, between 09:00 am and 01:00 pm. GoPRO cameras were placed in the tank filming fish behaviour for 2 h consecutively in each tank.

A no-choice grazing experiment, with four different food sources offered separately in different tanks, was not conducted due to the low effect of the multiple-choice trial and a lack of fish available for the experiment.

Statistical analyses

Statistical analyses were based on the seagrass and macroalgae biomass removed by fish from the tethered *P. australis*, *Z. nigricaulis, E. radiata* and *Sargassum sp.* replicates deployed in the testing tanks minus the biomass loss in the control replicates. Non-parametric Friedman's test was applied to determine if there is a preference for grazing on different food sources offered to *S. fuscescens* in mesocosm multi-choice feeding trials.

2.3   Permits and licences

Ethic approval was obtained from the ECU Animal Ethics Committee for the observational study in the marine reserves, including field research, underwater visual censuses, filming and tethering experiments *in situ* (ECU AEC15072 "Tropicalisation of temperate seagrass meadows"), as well as the pilot grazing rates and dietary preference study on the tropical *S.fuscescens* in mesocosm facilities (ECU AEC18157 "Feeding preferences and grazing rates of tropical consumers in temperate seagrass meadows").

The relevant licenses were obtained from the Department of Parks and Wildlife, WA and Rottnest Island Authority to conduct the research at Marmion Marine Park and Rottnest Island Marine Reserve. The permits were obtained from the DPaW to collect seagrass and macroalgae for tethers and feeding trials from Marmion Marine Park (SWO18442, SWO19191, CE005471). Rottnest Island Research Permit (2016/279740) was obtained for the collection of shoots from seagrass species *P. sinuosa* and *P. australis* within seagrass meadows in Rottnest Island Marine Reserve and underwater video observation of fish.

The relevant licence was obtained from the Department of Parks and Wildlife, WA (now Department of Biodiversity Conservation and Attractions) to take fauna for scientific purposes and, where authorised, keep it in captivity (Licence No. 08-001461-1). The permit from the Fisheries Department (now Department of Industries and Regional Development, Fisheries Division) allowed the collection of rabbitfish from the Marmion Marine Park (Exemption No. 3013). The Licence to collect flora for scientific purposes within CALM lands (CE005673) was obtained from the Department of Biodiversity Conservation and Attractions, WA.

3. Results

3.1 Level of grazing on temperate seagrass

3.1.1 Fish bite rates

Marmion Marine Park was the only region where fish bite marks were present on *P. sinuosa* shoots at all three sites and distances away from reef for both 2001 and 2016/17 (Fig. 3.1). A significant year effect was found for the percentage of *P. sinuosa* shoots with fish bite marks between 2001 and 2016/17 ($P < 0.0001$), but there were two- and three-way interactions between year, site and distance (Table 3.1), indicating that the patterns across years, sites and distances were not consistent.
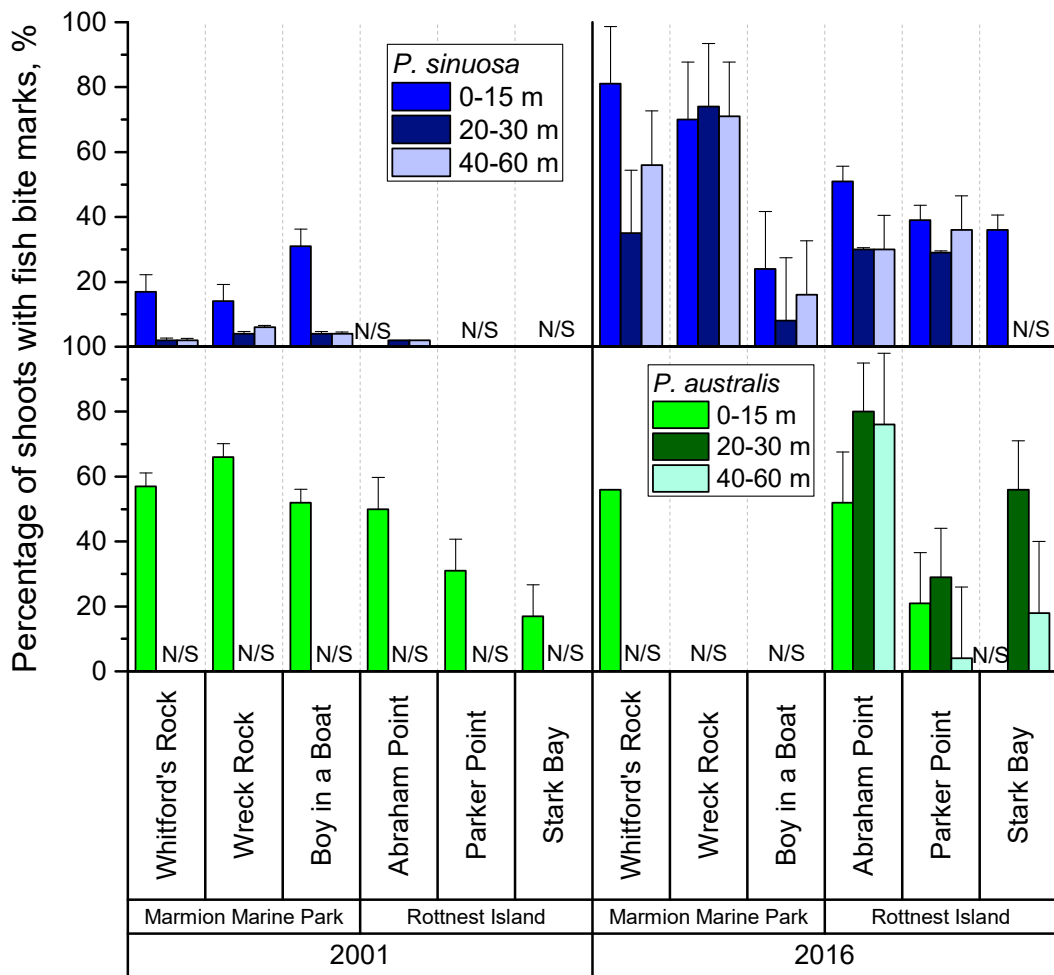
Fig. 3.1. Mean (±SE) percentage of shoots with fish bite marks for *P. sinuosa* and *P. australis* at different distance from the rocky reef (0-15 m, 20-30 m, 40-60 m) at MMP and RIMR in 2001 vs 2016/17. N/S means that no seagrass was present and sampled at those sites.

To further assess the differences between years and distances from reef, 2-way ANOVA was performed for each site separately. A significant year effect was found at Whitford's and Wreck Rock ($P < 0.01$ for both sites), but not at Boy in a Boat ($P = 0.356$) (Table 3.2). The percentage of shoots with bite marks differed significantly across distances from reef at Whitford's and Boy in a Boat sites ($P < 0.01$), which was dependent on year ($P = 0.0006$ and $P = 0.009$ respectively). This is likely due to the consistently higher percentage of *P. sinuosa* shoots with fish bite marks near the reef in 2001, compared to far less consistent trends in 2016/17. At Wreck Rock, no significant year difference was found for the percentage of *P. sinuosa* shoots with fish bite marks ($P = 0.427$), with no interaction between distance and year ($P = 0.077$).

Turkey's test showed that the percentage of *P. sinuosa* shoots with fish bite marks at Whitford's and Wreck Rock changed significantly in 15 years for each distance from reef ($P < 0.001$ for each combination). Turkey's test showed that the distance factor was not consistent across sites (Table 3.2). At Whitford's, all interactions between year-distance combinations were significantly different ($P < 0.01$), except for the 20-30 m vs 40-60 m distances from the reef in 2001 ($P = 1.0$). At Boy in a Boat, in 2001, the percentage of *P. sinuosa* shoots with fish bite marks in the nearest to the reef zone differed significantly from the further from the reef distances ($P < 0.01$), and also from 20-30 m distance in 2016/17 ($P = 0.0009$).

Table 3.1. Results of the three-way analyses of variance (ANOVA) assessing differences between three fixed factors: year, site within MMP, and three distances from reef for the percentage of *P. sinuosa* shoots with fish bite marks at MMP. Significant probabilities (highlighted in bold) were accepted at $P < 0.01$ as the data on the percentage of seagrass shoots with fish bite marks were not homogenous or normal after transformation.

	DF	Mean Square	F Value	P Value
Site	2	3044.740	93.806	**<0.0001**
Year	1	20533.500	632.625	**<0.0001**
Distance from reef	2	1655.407	51.002	**<0.0001**
Site x Year	2	4574	140.922	**<0.0001**
Site x Distance from reef	4	312.490	9.627	**<0.0001**
Year x Distance from reef	2	78	2.403	0.105
Site x Year x Distance	4	254.75	7.848	**0.0001**
Error	36	32.457	--	--
Corrected Total	53	--	--	--

Table 3.2. Results of the two-way analyses of variance (ANOVA) assessing differences in the percentage of *P. sinuosa* shoots with fish bite marks between two time periods and three distances from reef at each site, within MMP. Significant probabilities (at $P <$ 0.01) are highlighted in bold.

Site	Source	df	Mean Square	F Value	P Value
Whitford's Rock	Year	1	11453.915	432.725	**<0.0001**
	Distance	2	1468.502	55.479	**<0.0001**
	Year x Distance	2	385.278	14.555	**0.0006**
	Error	12	26.469	--	--
	Total	17	--	--	--
Wreck Rock	Year	1	18234.770	763.866	**<0.0001**
	Distance	2	21.765	0.911	0.427
	Year x Distance	2	75.935	3.181	0.077
	Error	12	23.871	--	--
	Total	17	--	--	--
Boy in a Boat	Year	1	22.646	0.921	0.356
	Distance	2	735.273	29.913	**0.00002**
	Year x Distance	2	171.404	6.973	**0.009**
	Error	12	24.579	--	--
	Total	17	--	--	--

The number of fish bite marks per *P. sinuosa* shoot near the reef differed significantly across the years and sites at MMP ($P < 0.0001$) (Fig. 3.2, Table 3.3), where bite marks were recorded only nearest to the reef zone (0-15 m) in both years. However, there was an interaction between Year and Site. Turkey's test revealed that bite marks per shoot increased between years at Whitford's and Wreck Rock, but not at Boy in a Boat. At Whitford's Rock, the average number of fish bite marks per shoot increased from 0.20 in 2001 to 1.51 in 2016/17, and at Wreck Rock from 0.15 to 1.08 (Fig. 3.2). The number of fish bite marks per *P. sinuosa* shoot was similar for Boy in a Boat across years (0.3 in 2001 vs 0.34 in 2016/17), with no significant difference observed (Turkey's test, $P = 0.679$).
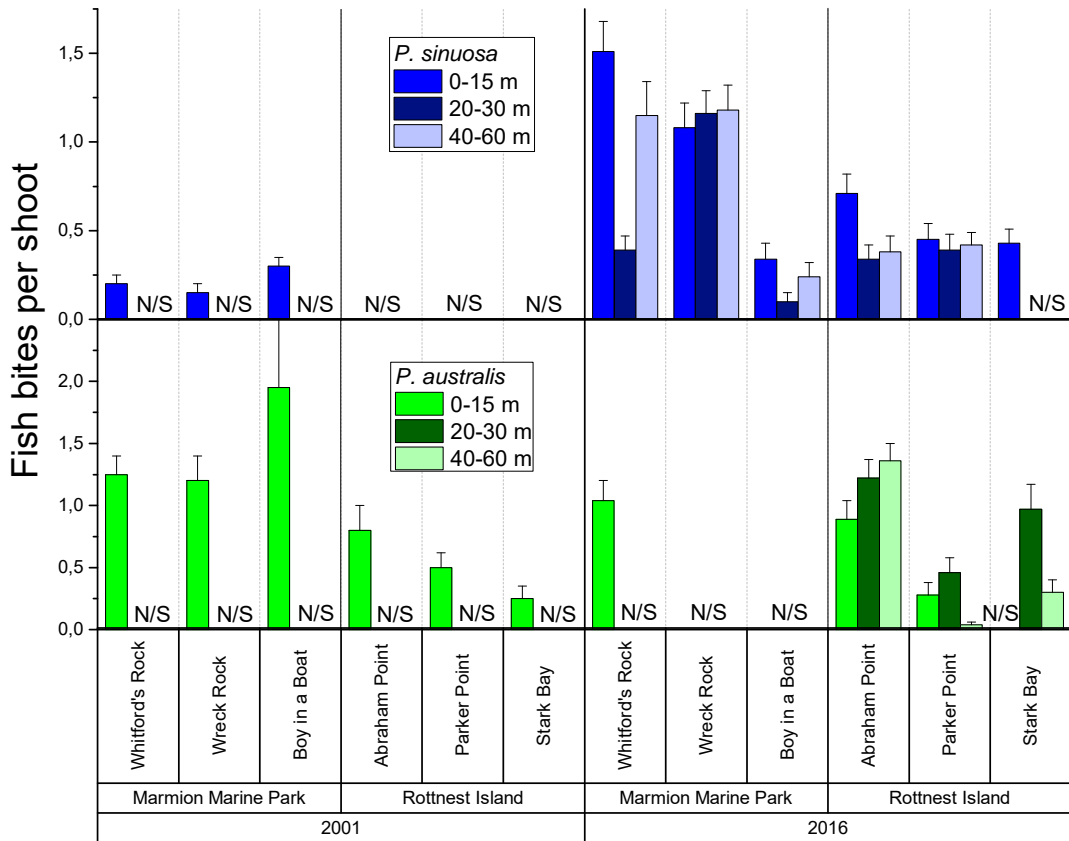
Fig. 3.2. Mean number (±SE) of fish bite marks per shoot on *P. sinuosa* and *P. australis* at different distances from reef (0-15, 20-30, 40-60 m) at MMP and RIMR in 2001 vs 2016/17. N/S = not sampled as, in 2016, seagrass species were not available close to reef, and, in 2001, samples were taken only for the close to the reef transect, since the incidents of some herbivory were not found further from reef.

Table 3.3. Two-factor analyses of variance (ANOVA) testing the differences in number of fish bite marks on *P. sinuosa* shoots at 0-15 m distance from reef at MMP in 2001 vs 2016/17. Significant probabilities were accepted at $P < 0.05$ as the data on the number of fish bite marks were homogenous and normal after transformation.

	DF	Mean Square	F Value	P Value
Year	1	2.637	1777.981	**<0.0001**
Site	2	0.435	293.644	**<0.0001**
Year x Site	2	0.640	431.734	**<0.0001**
Error	12	0.001	--	--
Total	17	--	--	--

Bite marks on *P. australis* shoots were only consistently sampled across more than one site in both years within region at RIMR, and this was restricted to seagrass next to the reef (0-15 m) (Fig. 3.1, 3.2). The proportion of *P. australis* shoots with bite marks next to the reef (0-15 m) did

not differ between years ($P = 0.211$), but was highly variable across sites ($P < 0.0001$, Table 3.4), ranging from 4 to 80% (Fig. 3.1). Similarly, number of fish bite marks on *P. australis* next to reef at RIMR did not differ between years ($P = 0.224$, Fig. 3.2, Table 3.5). However, the number of fish bite marks differed among sites ($P < 0.0001$), but this was dependent on years ($P = 0.001$). Turkey's test revealed that the number of fish bite marks on *P. australis* shoots decreased slightly between years at Parker Point (0.50 in 2001 vs 0.28 in 2016) ($P = 0.012$) but did not change at Abraham Point (0.80 in 2001 vs 0.89 in 2016) ($P = 0.159$) (Fig. 3.2).

Table 3.4. Results of the two-way ANOVA (Year x Site) assessing the differences in the variability of percentage of *P. australis* shoots with fish bite marks in the nearest to the reef distance at RIMR, in 2001 vs 2016/17. Values were significant with $P < 0.01$.

	DF	Mean Square	F Value	P Value
Year	1	48	1.846	0.211
Site	1	1875	72.115	**<0.0001**
Year x Site	1	108	4.153	0.075
Error	8	26	--	--
Total	11	--	--	--

Table 3.5. Two-way analyses of variance (ANOVA, Year x Site) testing the variability in number of fish bite marks on *P. australis* in the next to reef zone across RIMR sites and both time periods. Values in bold were significant with $P < 0.05$.

	DF	Mean Square	F Value	P Value
Year	1	0.007	1.734	0.224
Site	1	0.576	142.690	**<0.0001**
Year x Site	1	0.088	21.885	**0.001**
Error	8	0.004	--	--
Total	11	--	--	--

In 2001, when both *P. australis* and *P. sinuosa* could only be sampled at MMP sites, the number of bites per shoot differed significantly between species ($P = 0.024$) but not between sites ($P = 0.330$) (Table 3.6, Fig. 3.2). The number of bites per shoot was greater in *P. australis* (1.20 – 1.95 fish bites per seagrass shoot) compared to *P. sinuosa* (0.20 – 0.30) (Fig. 3.2). Similarly, the percentage of seagrass leaves with fish bite marks sampled at MMP in 2001 differed significantly between *Posidonia* species ($P < 0.0001$) but not between sites ($P = 0.611$) (Table 3.7, Fig, 3.1). In 2001, bite marks were generally higher on *P. sinuosa* shoots (14-31%) near reef (0-15 m) at MMP, compared to *P. australis* shoots (52-66 %) at the same location and distance from reef (Fig. 3.1).

In 2016/17, both species could only be sampled at RIMR sites, where the number of bites did not differ significantly between species ($P = 0.458$) or sites ($P = 0.381$) (Table 3.6, Fig. 3.2). As for the percentage of seagrass leaves with fish bite marks at RIMR in 2016/17, bite marks were found on 36-51% of *P. sinuosa* shoots adjacent to the reef (0-15 m), compared to 21-51 % of *P. australis* shoots at the same location in 2016/17 (Fig 3.1). Two-way ANOVA showed the

significant difference between sites ($P = 0.0006$) but not species ($P = 0.062$) (Table 3.7, Fig. 3.1).

Table 3.6. Two-way ANOVA assessing the differences in the variability of number of bites per shoot in *P. sinuosa vs P. australis* at near-reef zone at three sites at MMP in 2001 and at RIMR in 2016/17. Significant difference was accepted at $P < 0.05$.

Year and region		DF	Sum of Squares	Mean Square	F Value	P Value
2001, MMP	Seagrass species	1	2.343	2.343	39.062	**0.024**
	Sites	2	0.243	0.121	2.027	0.330
	Error	2	0.120	0.060	-	-
	Total	5	2.707	-	-	-
2016/17, RIMR	Seagrass species	1	0.052	0.052	0.829	0.458
	Sites	2	0.204	0.102	1.619	0.381
	Error	2	0.126	0.063	-	-
	Total	5	0.382	-	-	-

Table 3.7. Two-way ANOVA assessing the differences in the percentage of fish bite marks per shoot in *P. sinuosa vs P. australis* at near-reef zone at three sites at MMP in 2001 and at RIMR in 2016/17. Values in bold are significant with $P < 0.01$.

Year and region		DF	Sum of Squares	Mean Square	F Value	P Value
2001, MMP	Seagrass species	1	6346.888	6346.888	111.147	**<0.0001**
	Site	2	58.111	29.055	0.508	0.611
	Error	14	799.444	57.103	--	--
	Corrected Total	17	7204.444	--	--	--
2016/17, RIMR	Seagrass species	1	216.750	216.75	4.297	0.062
	Site	2	1548.750	774.375	15.354	**0.0006**
	Error	11	554.750	50.431	--	--
	Corrected Total	14	2212.400	--	--	--

3.1.2 Direct measures of herbivory and production

Direct estimates of *P. sinuosa* consumption based on tethering experiments in the near-reef zone (0-15 m) showed that grazing of seagrass by fish was highly patchy across sites and years, with grazing on this seagrass absent at RIMR in both years and very low in MMP in 2016/17 (Fig. 3.3). Because of a high incidence of zero values, statistical tests have not been performed to test for differences across years. In all sites at MMP, the mean consumption values ranged from $0.03 – 0.19$ cm^2 shoot^{-1} day^{-1} in 2001 compared to $0 – 0.008$ cm^2 shoot^{-1} day^{-1} in 2016/17 (Fig. 3.3). These grazing rates accounted for $0 – 41$ % of the productivity of *P. sinuosa* across the years and sites (Fig. 3.3). In 2016/17, when tethers were observed via video, no fish was shown biting *P. sinuosa* tethers, even when some consumption was observed at MMP. However, *Pelates octolineatus, Pelsartia humeralis, Odax cyanomelax, Siganus fuscescens* and *Kyphosus cornelii* were observed in *P. sinuosa* meadows.

In 2016/17, when *P. australis* was offered in tethers at sites in RIMR, the level of consumption on this seagrass was very low (0 – 0.12 cm^2 shoot^{-1} day^{-1}), accounting for 0 – 14 % of the productivity of the seagrass species (Fig. 3.3). Video footage showed that *S. fuscescens*, *Parma victoriae, K. cornelii, Rhabdosargus sarba* were present around the tethers, but no fish was observed biting on seagrass.
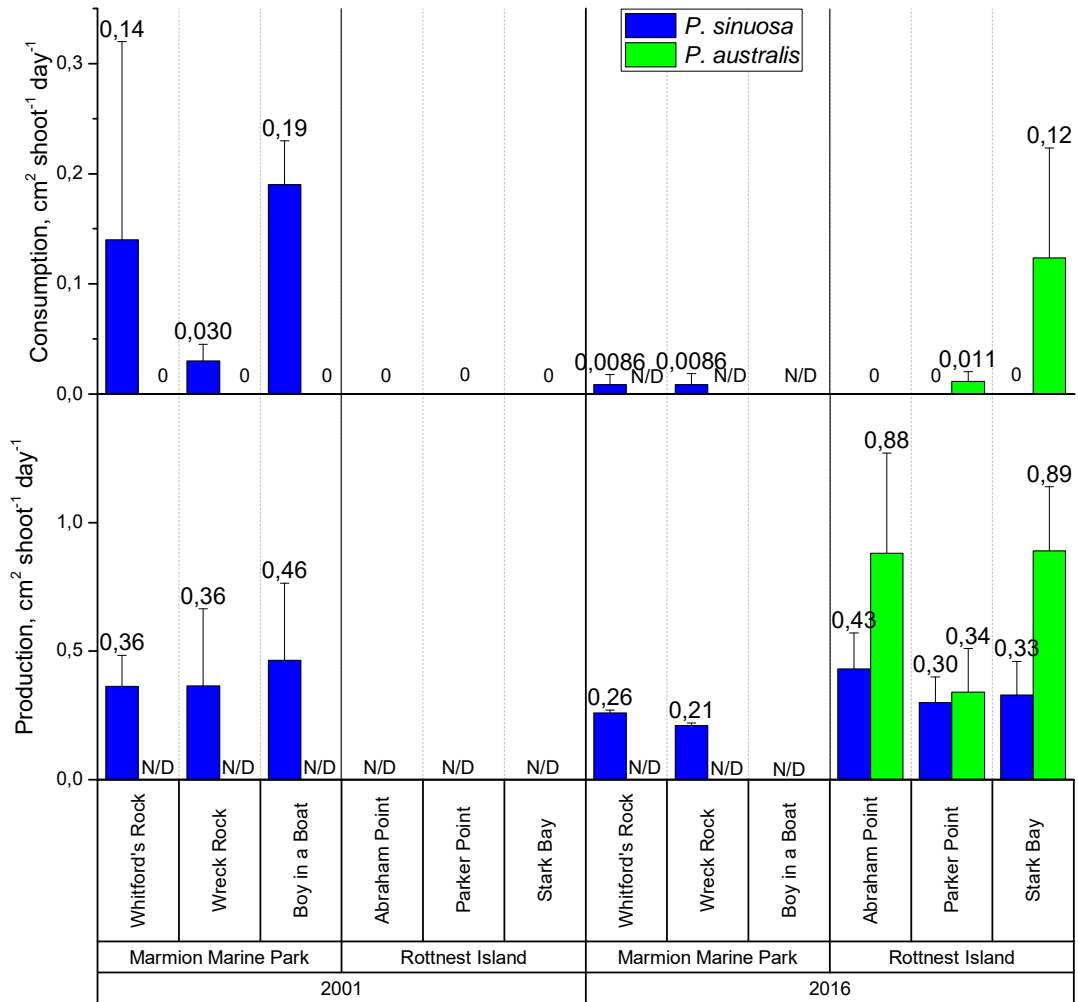
Fig. 3.3. Mean (± SE) consumption rates (cm^2 shoot^{-1} day^{-1}) and production (cm^2 shoot^{-1} day^{-1}) of the seagrasses *P. sinuosa* and *P. australis* in 2001 and 2016/17 in MMP and RIMR. N/D = no data available. Zero indicates no fresh fish bite marks found on tethers.

3.1.3 Herbivore abundance

The composition of herbivorous fish in seagrass meadows adjacent to reefs varied considerably between 2001 and 2016/17 (Table 3.8). Only two fish species encountered within the seagrass meadows at MMP and RIMR were common for both years: *S. fuscescens* and *K. cornelii*. Compared to 2001, the abundance of *S. fuscescens* is slightly higher at MMP in

2016/17 (Table 3.8). The abundances of *K. cornelii* were slightly lower in 2016/17 across both locations. *P. octolineatus* and *S. fuscescens* dominated the fish communities in seagrass meadows in 2016/17, and *Girella zebra* and *Kyphosus sp.* were the most abundant in 2001. *P. octolineatus, Pelsartia humeralis, Meuschenia sp.* and *Rhabdosargus sarba* were absent from UVC in 2001. In 2001, *Parma sp.* was common at MMP sites and absent from those sites in 2016/17. At RIMR, *Parma sp.* was encountered in low abundance in both years. *Odax acroptilus, Scorpis sp.* and *Girella zebra* were only recorded in 2001.

Out of the fish species being discussed in the thesis, only *Siganus fuscescens* was considered tropical.

Table 3.8. The abundance (mean ±SE) of herbivorous fish species surveyed using underwater visual censuses at MMP and RIMR (ind. per 400 m^2) in 2001 and 2016/17. In 2001, fish counts were not performed for Parker Point site. Zeros indicate no fish encountered.

Fish family	Fish species	2001					2016/17					
		MMP			RIMR		MMP			RIMR		
		Whitf	WR	Boy	AP	SB	Whitf	WR	Boy	PP	AP	SB
Terapontidae	*Pelates octolineatus*	0	0	0	0	0	4.6 (3.5)	160 (58.5)	0	20.6 (20.7)	0	0
	Pelsartia humeralis	0	0	0	0	0	0	41.1 (37.2)	0	0	0	0
Monacanthidae	*Meuschenia sp.*	0	0	0	0	0	0	1.5 (1.5)	0	0	0	0.7 (0.7)
Siganidae	*Siganus fuscescens*	0	0.83 (0.8)	0	0	0	0	0	38.1 (38.8)	2.3 (1.6)	0	0
Sparidae	*Rhabdosargus sarba*	0	0	0	0	0	0	0	0	1.1 (1.1)	0.7 (0.7)	0
Kyphosidae	*Kyphosus cornelii*	0	4.7 (2.9)	0	0.6 (0.4)	3.5 (3.0)	0	0	0	0	1.5 (0.7)	0
	Kyphosus sydneyanus	1.0 (0.4)	3.5 (3.0)	0	0	0	0	0	0	0	0	0
Labridae	*Odax acroptilus*	2.5 (0.8)	1.3 (0.4)	1.3 (0.2)	0	0	0	0	0	0	0	0
	Odax cyanomelax	1.08 (0.6)	0	0	0	0	0	0	0	0	0	0
Pomacentridae	*Parma sp.*	4.3 (1.4)	5.08 (1.1)	0.6 (0.4)	0.3 (0.2)	0.6 (0.4)	0	0	0	0	0	0
Scorpididae	*Scorpis sp.*	0	0.08 (1.1)	0	0	0	0	0	0	0	0	0
Girellidae	*Girella zebra*	0	0	0	1.3 (0.8)	8.6 (4.6)	0	0	0	0	0	0

Based on the MaxN for each herbivorous fish species from video footage of tethered seagrass shoots in 2016/17, *S. fuscescens* was observed at all sites (up to 9.3), except Abraham Point at RIMR (Table 3.9). Only one individual was recorded feeding within a *P. sinuosa* meadow, which was at Whitford's Rock, where it appeared to be targeting *Sargassum sp.* or *E.*

radiata present in the meadow, and occasionally seagrass. *P. octolineatus* had the highest MaxN (53.4), but only occurred at two sites and was rarely observed feeding on seagrass. A school of ca 40 individuals of *P. octolineatus* was seen only once biting seagrass within the meadow (but not the tethered shoots). *Odax cyanomelax* was observed in seagrass meadows at only two sites (MMP: 0.2 and 1.6) while both *Parma victoriae* and *P. occidentalis* were present only at two sites in RIMR (MaxN = 0.5 to 1.0). Both *Parma* species were observed around the deployed tethers, but were never observed biting them. *K. cornelii* (MaxN = 0.2-1.0) and *Rhabdosargus sarba* (MaxN = 0.2-3.8) were encountered in low abundance at four sites, but mainly at those in MMP. *Meuschenia sp.* was only encountered in seagrass meadows at Whitford's Rock (MaxN = 0.2).

Table 3.9. The MaxN abundance of herbivorous fish species (mean number of individuals ±SE) in 2016/17, taken from video footage of tethered seagrass. Zeros stand for no fish recorded.

Fish family	Fish species	2016/17					
		MMP			RIMR		
		Whitf	WR	Boy	AP	PP	SB
Siganidae	*Siganus fuscescens*	3.5 (0.8)	1.0 (0.4)	9.3 (8.1)	0	2.8 (1.5)	6.3 (2.9)
Monacanthidae	*Meuschenia sp.*	0.2 (0.2)	0	0	0	0	0
Terapontidae	*Pelates octolineatus*	3.3 (5.7)	53.4 (11.0)	0	0	0	0
	Pelsartia humeralis	0	1.4 (0.6)	0	0	0	0
Labridae	*Odax cyanomelax*	0.2 (0.2)	1.6 (0.3)	0	0	0	0
Kyphosidae	*Kyphosus cornelii*	0	0.2 (0.2)	0	0.3 (0.2)	1.0 (0.3)	0.2 (0.4)
Sparidae	*Rhabdosargus sarba*	0.2 (0.2)	0	0	0.3 (0.3)	3.8 (5.9)	0.2 (0.4)
Pomacentridae	*Parma victoriae*	0	0	0	0.5 (0.5)	1.0 (0)	0
	Parma occidentalis	0	0	0	0	0.6 (0.2)	0

3.2 Feeding preferences and grazing rates of tropical consumers in temperate seagrass meadows

3.2.1 Mesocosm trials

The level of loss of biomass was extremely low for all seagrass and macroalgae species. No significant difference in biomass loss was determined for *Z. nigricaulis*, *P. australis*, *E. radiata* and *Sargassum sp.* (Friedman: $\chi^2 = 1$, df = 3, $P = 0.801$), which is related to the almost absent grazing on all food choices. No fish was observed feeding using direct observations and underwater video filming. Most likely, the direct exposure of the tethers to some conditions of the mesocosm facilities (e.g. aeration system and water movements) facilitated deconstruction of tethers. Thus, in control tank, some minor losses (*P. australis* – up to 0.3 %, *Z. nigricaulis* – 1.2 %, *E. radiata* – 0 %, *Sargassum sp.* – 0.4 %), similar to those in the experiment tanks, occurred from decomposition of the tethers (this material was included biomass at the end of the trial). The losses in control tank were removed from the treatment.
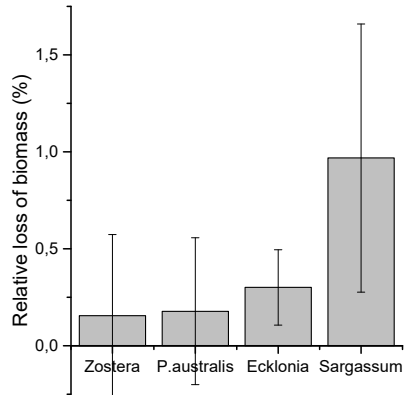
Fig. 3.4. The relative loss of biomass of *P. australis*, *Z. nigricaulis*, *E. radiata*, *Sargassum sp.* (mean, % ± SE) in aquarium-based feeding trials, to determine feeding preferences and grazing rates of *S. fuscescens*.

Based on video footage, no fish was observed feeding on or displaying any interest towards the tethered seagrass or macroalgae deployed. Fish were mostly observed hiding under the concrete blocks, demonstrating no active swimming or social behaviour (Fig. 3.5). Individual fish were observed to leave their shelters, but they demonstrated extremely cautious behaviour and no feeding interest towards the deployed tethers.
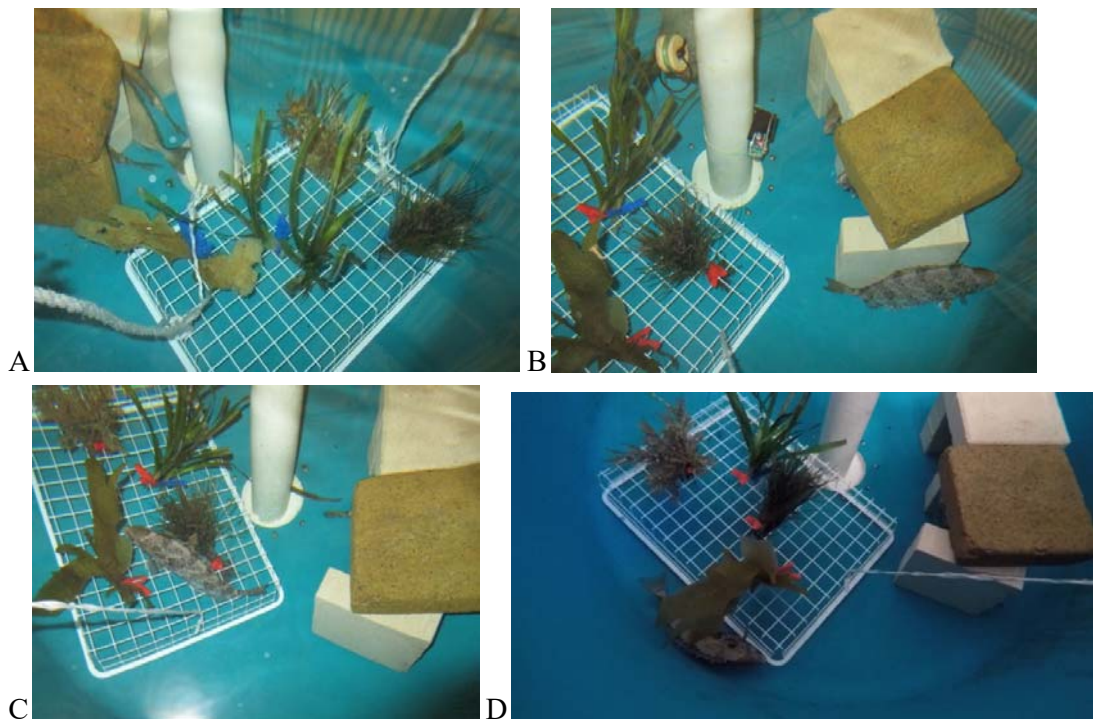
Fig. 3.5. *S. fuscescens* hiding in their shelters (A, B) for the whole duration of the direct observation or filming (most likely, for the whole duration of the feeding experiment), and individual fish (C, D) leaving their hides and staying motionless among deployed seagrass and macroalgae tethers.

3.2.2 Consumption rate experiments *in situ*

The comparison of the relative loss of biomass (%) per tethered seagrass (*P. australis*, *Z. nigricaulis*) and macroalgae (*Sargassum sp.*, *E. radiata*) species in three inshore locations at Marmion Marine Park in December 2017 revealed some patterns in grazing rates and dietary preferences of herbivorous fish species within temperate seagrass meadows (Fig. 3.6). The loss of biomass across tethered seagrass and macroalgae assays (accounting for autogenic changes) differed significantly at all locations: Wanneroo (Friedman: $\chi^2 = 20.16$, df = 3, $P = 0.0001$), Wreck Rock (Friedman: $\chi^2 = 13.08$, df =3, $P = 0.004$), and Whitford's Rock (Friedman: $\chi^2 = 14.52$, df = 3, $P = 0.002$). The Wilcoxon signed-rank test for pair comparison was applied then to identify the pairs of seagrass and macroalgae assays that differed significantly within each location. Compared to *Sargassum sp.* (Fig, 3.6), biomass loss at Wanneroo and Whitford's Rock was significantly higher for *E. radiata* (Wilcoxon: $P = 0.006$ for both locations), *Z. nigricaulis* ($P = 0.006$ for both locations) and *P. australis* ($P = 0.008$ and 0.014, relatively). The loss in biomass did not differ significantly among *E. radiata*, *Z. nigricaulis* and *P. australis* (Friedman: $\chi^2 = 5.2$, df = 2, $P = 0.074$). At Wreck Rock, biomass loss was significantly higher for *E. radiata* than *Sargassum sp.* ($P = 0.008$), *P. australis* ($P = 0.006$) and *Z. nigricaulis* ($P = 0.025$).
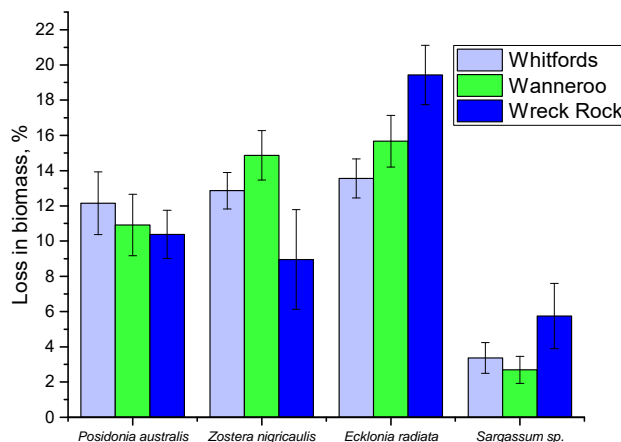
Fig. 3.6. Mean biomass loss (%) (±SE) per each seagrass (*P. australis*, *Z. nigricaulis*) and macroalgae (*E. radiata, Sargassum sp.*) species in inshore seagrass meadows at MMP (December 2017).

While biomass loss of *P. australis* did not differ from any other food source, the presence of separate fresh bite marks (a total of 2.08 cm^2 of the leaf area consumed) on *P. australis* leaves (Fig. 3.7) indicated direct grazing by herbivorous fishes. The counting of fish bite marks for *Z. nigricaulis* was hampered due to the quantity of thin unevenly long leaves (Fig. 3.8). For *E. radiata*, fresh fish bite marks were found on 20-60 % of deployed kelp thalli (0.16 – 7.16 cm^2 lost per thallus (Fig. 3.9). The irregular shape of *Sargassum sp.* thalli made it difficult to identify and count possible bite marks. However, some clearly identifiable fish bite marks were registered on macroalgae thalli retrieved after deployment (Fig. 3.10).

Fig. 3.7. *P. australis* leaf retrieved after deployment at MMP, Whitford's Rock location.

Fig. 3.8. Example of *Z. nigricaulis* shoots clustered together to construct tethers, showing the low possibility to identify and count possible fish bite marks.
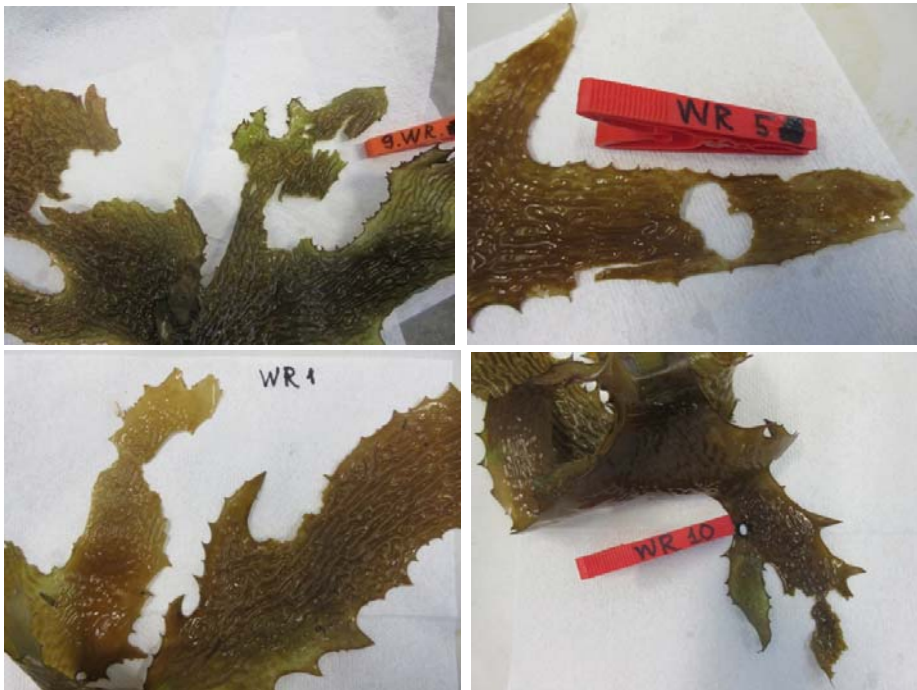
Fig. 3.9. Example of fish bite marks on *E. radiata* thalli retrieved after deployment at MMP.

Fig. 3.10. *Sargassum sp*. thallus retrieved after the deployment and separate leaves of *Sargassum sp.* with some identifiable fish bite marks.

Based on the video footage, no fish was observed feeding on the tethers in the feeding preference trials. Video revealed the presence of mostly carnivorous fish species (*Halichoeres brownfieldi, Upeneichthys vlamingii, Parupeneus spilurus, Notolabrus parilus, Sillaginodes punctatus, Coris auricularis, Enoplosus armatus*) that populate seagrass meadows and feed within seagrass targeting invertebrates or smaller fish (Fig. 3.11). Herbivorous-omnivorous fish species (*S. fuscescens, P. octolineatus, Pelsartia humeralis*) were less abundant in the area of research (Fig. 3.12). Fish rarely approached the tethers embedded or deployed at the edge of the seagrass meadows, expressing no obvious interest towards the tethered plants.
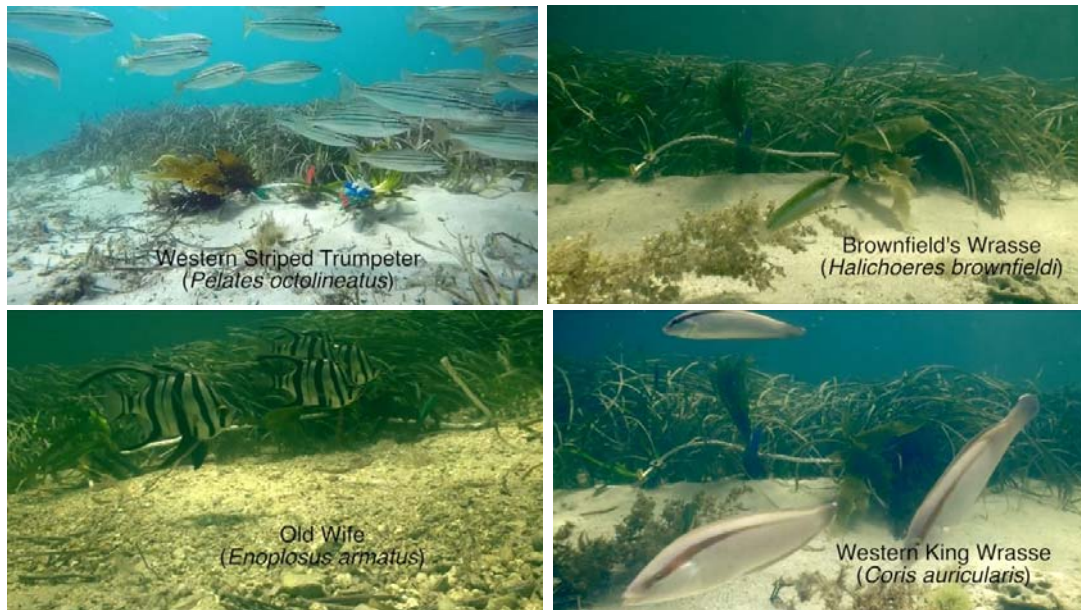
Fig. 3.11. Some fish species observed in seagrass meadows at MMP (photos retrieved from the video footage).

Fig. 3.12. *S. fuscescens* in seagrass meadows at MMP (photos retrieved from the video footage).

4. Discussion

4.1 Shifts in grazing on seagrass leaves in temperate seagrass meadows

Grazing patterns in 2001 and 2016/17 were inconsistent, varying between seagrass species and locations. Based on the number of bites on seagrass leaves, rates of consumption increased for *P. sinuosa* while no similar pattern was found for *P. australis*. This was in contrast to greater consumption of *P. australis* compared to *P. sinuosa* in 2001. The higher *P. sinuosa* consumption in 2016/17 is likely explained by the changed herbivorous fish species composition. A number of species known to feed on seagrass, including *P. octolineatus* and *S. fuscescens* (Paul et al., 1990; Fox & Bellwood, 2008; Al-Marzouqi et al., 2009; Fox et al., 2009; Lee et al., 2016) were more abundant during the sampling period in 2016/17 than 15 years earlier. Of those species, only the rabbitfish *S. fuscescens* is tropical. While this species was observed in low numbers in 2001, it appears that it has formed a self-sustaining population in the temperate region of Perth and even more southward after the 2011 heat wave event (Hyndes et al., 2016). In the study area, *S. fuscescens* was usually observed in small groups (with a maximum of 10 fish per school). A large school of *S. fuscescens* (ca 100 individuals) was filmed once, and this occurred in a no-take zone (Boy in a Boat) at Marmion Marine Park (Fig. 4.1). The fish were observed targeting brown (predominantly *Sargassum sp.*) and red macroalgae that are abundant in the area. Similar feeding behaviour of *Siganus* was never observed within the vast seagrass meadows where macroalgae was less abundant. The *in situ* video observation provided clear evidence of *S. fuscescens* consuming *Sargassum sp.* and red algae in the seagrass meadow next to rocky reef. Despite a total of 68 hours of video footage captured across the sampling periods, no footage showed *Siganus* biting seagrass. Thus, this tropical herbivore may not have contributed markedly to any increase in seagrass consumption, because they prefer macroalgae over seagrass (Paul et al., 1990; Pillans et al., 2004; Fox et al., 2009; Bennett et al., 2015), and this study was conducted near reefs where macroalgae were abundant.

The temperate limestone reefs in near-shore waters around Perth are characterised by productive and diverse macroalgal assemblages, including brown and red algae (Huisman & Walker, 1990; Wernberg et al., 2003; Kendrick et al., 2004; Smale et al., 2010). Research indicates that across the tropical to temperate gradient along the western coast of Australia, *S. fuscescens* consumes greater quantities of macroalgae, in particular red algae followed by brown algae (Avenant, 2018). This supports the findings of Pillans et al. (2004), who showed that red algae constitute the preferred food source for *S. fuscescens*, even though rabbitfish consume some seagrass and brown macroalgae. However, differences in the dietary composition of rabbitfish appear to correlate directly with the particular habitat and food availability in the habitat they occupy (Mantyka-Pringle & Bellwood, 2007; Fox & Bellwood, 2008; Hoey et al., 2013). Thus, larger proportions of macroalgae were consumed by *S. fuscescens* close to reefs, where the preferred algal food resources were available to them. Alternatively, the proportion of seagrass consumed increases in areas away from reef, apparently due to the reduced availability of macroalgae in seagrass meadows (Avenant, 2018). Similarly, the results of *in situ* experiments conducted in MMP in 2017 suggest that seagrass was fed on in equal amounts as kelp, although the herbivore associated with that consumption is unknown. The fact that *S. fuscescens* does

consume seagrass in temperate waters (Avenant, 2018) suggests that they will shift towards seagrass-based diet once macroalgae become less abundant or unavailable.

Fig. 4.1. A numerous school of *S. fuscescens* (ca. 100 individuals) filmed in a sanctuary site (Boy in a Boat, MMP) (photos retrieved from the video footage).

Feeding preferences of herbivorous fish are highly influenced by nutritional characteristics and caloric content of macrophytes, in particular the C:N ratio that may determine leaf palatability and the attractiveness of the plant as a food source (Heck et al., 2000; Cebrian et al., 2009; Prado & Heck, 2011; Jiménez-Ramos et al., 2017). This relationship between seagrass or macroalgal properties and feeding behaviour, however, can vary among fish species, as well as through space and time (Nowicki et al., 2018). As Pillans et al. (2004) demonstrated, the food preferences of *S. fuscescens* were not necessarily related to food quality. Instead, the selection of macrophytes by *S. fuscescens* was based on the rates of food assimilation, rather than on the absolute nutritional status of the algae. Thus, the values for both preference and assimilation efficiency of red algae in Pillans' et al. (2004) study were higher than *Zostera sp.* and brown algae. The results of the consumption rates for the *in situ* experiments, conducted in 2017 at MMP, align with Pillans' et al. (2004) results for *Zostera sp.* vs brown algae preference. In agreement with that study, consumption of *Z. nigricaulis* at MMP was generally higher than *Sargassum sp.*, suggesting a lower preference for these brown algae. Deeper insights into the effects of nutrient concentration and secondary metabolites could add some insight into explaining the feeding choices of *S. fuscescens* in temperate regions. In addition to the structural and chemical properties of macrophytes, the presence or absence of epiphytes on seagrass and macroalgal tissue could contribute to the explanation of the herbivory patterns I observed and clarify the results on consumption rates and feeding choices of fish (Marco-Mendez et al., 2017).

In relation to *Z. nigricaulis* consumption, an important aspect to be noted here concerns the specific environmental conditions (e.g. wave activity, current) and structural characteristics of *Z.*

nigricaulis (thin fragile leaves) that could contribute to deconstruction of the initially deployed material and its loss due to non-consumption related factors. No underwater video footage or direct observations was conducted to confirm that the biomass loss resulted exclusively from the herbivory on tethers, and not from an exposure to physical factors and pressure from the surrounding conditions of the local marine environment. However, clear grazing marks were observed on *P. australis* leaves, indicating that herbivorous fishes were actively feeding in on seagrass tethers.

In contrast to Vergés et al. (2014b) and Wernberg et al. (2013, 2016), who found the poleward expansion of tropical rabbitfishes to have a major impact on highly productive kelp forests in both Western Australia and the Mediterranean, our research suggests that tropicalisation of temperate seagrass meadows has not had much effect on seagrass consumption yet. Following the rate of migration, the associated herbivory from these tropical species on seagrass increases slowly. The fact that little seagrass was consumed by herbivorous species in the temperate region supports the idea that no detrimental effect will be caused to seagrass communities in the nearest future, as tropical species establish their populations in the temperate waters. However, once algal abundance is greatly reduced and/or rabbitfish abundance increases greatly, seagrass consumption rates in temperate waters along the western Australian coast will likely come to resemble that in tropical Australia.

High but variable abundances of *S. fuscescens* observed in 2016/17 suggest that this species has increased in abundance over the 15 years between studies, but these values are "snap shots" in two time periods. *Siganus* recruits were observed after the 2011 heat wave event and have since established self-sustainable populations (Lenanton et al., 2017), but there has not been ongoing monitoring of this or other tropical species in the region. It is possible that the population density has declined with the cooling of water temperatures during the 2016/17 sampling (Cahill et al., 2019), and therefore densities of these herbivores were not at levels as high as those observed after the heat wave event. Because sea temperatures and the frequency of heat wave events are expected to rise (Pearce & Feng, 2013; Hobday et al., 2016), this will likely increase the pressure that the communities of tropical herbivores, including *S. fuscescens*, exert on temperate seagrass ecosystems (Hyndes et al., 2016). Most likely, the risk for seagrass assemblages will increase once tropical herbivorous species increase their density and consumption of temperate seagrasses. This impact will depend on the variety of abiotic and biotic factors, including the existing fish assemblages in the area, the abundance of tropical species, the availability of food, and the feeding preferences that invading species develop in response to the changed environmental conditions.

4.2   Challenges encountered and suggestions for the future experiment design

Several issues arose during the study related to *in situ* observations of feeding and aquaria-based feeding preference experiments. The application of the *in situ* tethering can provide some valuable data when combined with underwater video observation, allowing the identification of fish communities and, hopefully, grazers targeting the tethered plant material. This approach has been used for numerous published studies on grazing on seagrasses and macroalgae (Mantyka & Bellwood, 2007; Bennett & Bellwood, 2011; Burkepile & Hay, 2011; Lefevre & Bellwood,

2011; Vergés et al., 2012; Michael et al., 2013). However, those studies have often occurred in the tropics where herbivorous fishes can be highly abundant (Meekan & Choat, 1997; Floeter et al., 2004; Bennett & Bellwood, 2011; Vergés et al., 2014b; Baskett & Schemske, 2018), or in regions like the Mediterranean Sea where the herbivore *Sarpa salpa* is highly abundant (Vergés et al., 2009; Prado et al., 2010; Steele et al., 2014). The lack of any video support for grazers biting tethers limits conclusions regarding tropicalisation when abundances of tropical species may not yet be high or may be very patchy in temperate regions. For future experiment designs, it is suggested to consider broad underwater video filming in the near-reef seagrass and macroalgal communities, where *S. fuscescens* are present. This video observation will help to determine the fish feeding behaviour and their preferences towards food sources naturally available to them in the temperate region.

Regarding *in situ* preference experiments, our research demonstrated low efficacy of the tethering technique to quantify the impact of seagrass herbivory *in situ*. The limitations could relate to the food choices offered to fish as well as local biotic and abiotic conditions. The positioning of seagrass and macroalgae tethers embedded in seagrass meadows or placed within the bare sand at the edge of the existing seagrass meadow could influence the experiment (Fig. 2.7, 2.11). The choice of food offered to the fish in form of tethers, did not differ from the food naturally available and usually abundant in the surrounding habitat, and therefore, the probability of fish targeting the tethers would be small. Herbivory pressure within seagrass meadows is known to vary depending on the exact location of the targeted shoots and their proximity to the edge of the meadow, with edge-positioned shoots experiencing the greater pressure compared to the centre-positioned specimens (Statton et al., 2015). Most likely, positioning the tethers outside of the meadow would make the food choices more "appealing" to grazers when examining herbivory in a particular seagrass meadow.

The largest issue encountered in this study related to using live fish for the feeding preference experiments in mesocosms, despite this *S. fuscescens* being used in a similar way in Queensland (Pillans et al., 2004). The large number of deaths and the limited grazing on natural food sources in the current study suggest that the population in the Perth region is susceptible to adverse handling and husbandry effects. A number of remedial measures should be applied, including the design for catching fish, the fish handling procedure, and their acclimation to the artificial tank conditions. To maximise the survival rate of the fish captured from the ocean for this particular research project, the fishing and handling procedure were altered on every collection to adapt to the ongoing observations in the response of fish to capture handling in the field or in the mesocosm facilities. Thus, the fish transporter was equipped by the oxygen tank to continuously oxygenate the seawater while capturing and transporting fish. The period of time fish are in the transport container from the time they are caught to when they are transferred to the flow-through mesocosm system should not exceed 6 hours (B. Roennfeldt, personal communication).

The rod and the line was found to be the best method of capture, provided the fish were being treated properly before and during transportation. The fishing gear and the experience of the angler determine where the hook embeds the fish (hopefully around the mouth, which causes less injuries for the captured fish). An enviro-net was used to remove the captured fish from the ocean water. When de-hooking the fish, they were left in the enviro-net and no wet cloth or

material touched the skin of fish as it removes the protective slime layer and increases the chance for fish to catch an infection. Additionally, the persistent use of barbless hooks could possibly increase the capture success of fish, reducing the possibility of tearing the mouth tissue when de-hooking the fish (see "Materials and Methods" section). In some cases, stomach bloat was observed in fish the day after capture. Two fish from this group did not survive, most probably resulting from the pollard that was used to attract fish in the ocean and bread used for bait, which is likely to have caused the stomach bloat. Capture methods and bait were modified by using kernels of corn, which were successfully used as bait for the remaining collection events. After being caught, fish were kept in the dark to reduce their stress.

Despite all these precautions, at the beginning of the acclimation process, some fish were noticed carrying what appeared to be a severe fungus (plus perhaps bacteria) (B. Roennfeldt, personal communication), which had been able to target the skin and fins of the fishes (Fig. 4.2). The fish affected by this transmittable disease (9 specimens in total) were isolated from the healthy fish and attempts to rescue these fish were undertaken. Most likely, the skin damage resulted from the capture regime that enabled the infection that was later transmitted between fish before or during transportation to the acclimation tank. The affected rabbitfish were not used for the feeding trials, and after few days in quarantine tank, they were euthanized using the prescribed overdose (170mg/L) of Aqui-S, following pre-sedation (Aqui-S 15mg/L) in their experimental tanks. No diagnostic analyses were performed to specify the disease.

Fig. 4.2. An individual *S. fuscescens* in the tank in mesocosm facilities. The fish is affected by some fungus or bacterial infection that targeted skin and fins of the fish.

As revealed after the feeding trials, one of the fish had an infestation of parasitic copepods which led to its demise (B. Roennfeldt, personal communication). These infestations appeared to be contagious and created risk to the other stock. Following the treatment and removal of the unhealthy fish from the area, the system was washed thoroughly in freshwater and then sterilised using oxalic acid and bleach to remove parasitic copepods from the culture system and facility. Informed by this experience, future attempts of using live *S. fuscescens* for aquarium-based experiments, should dip wild-caught / collected fish and dietary items to remove parasites, before bringing them into any culture system. If this rabbitfish population is carrying various diseases naturally, and they are at the limit of their natural thermal range, then it will be very easy to trigger disease development.

In addition to the described health issues, some aspects of social behaviour and biology of *S. fuscescens* should be further explored and considered when planning and setting the similar

feeding experiments in the future. *S. fuscescens* in the tanks showed a range of behaviour that is likely to have impacted the experiment. For example, evident bullying behaviour was noticed in a couple of tanks, when dominating males or females did not share hides (artificial reef constructions) with the other fish or kept chasing some other fish, leaving them with injuries and contributing to the risk of infection transmission. These aspects could influence the success of acclimation when keeping fish in tanks and the success of the feeding experiments when placing a certain number of fish together for one replicate.

The ability to capture a sufficient number of rabbitfish from local temperate waters proved to be a challenge (n = 225, as planned initially) in a short period of time. Rabbitfish were present around the temperate algal reef in the study region in small schools (7-10 fish). The use of the standard fishing gear with hook and line and the necessity to promptly transport the captured fish to the mesocosm facilities, excluded the possibility to catch more than 10 individual fish per one catching event.

In terms of the acclimation period, two weeks were proven to be enough for rabbitfish to acclimate for the mesocosm environment. The fish captured in November 2017 adapted and started feeding on aquaculture pellets after around 10-14 days. The fish caught later (January 2018), when the ocean water was warmer, started to feed almost on the day after they were captured. While these observations are limited to two times, it is plausible that this tropical species is less susceptible to handling at warmer temperatures, and therefore, water temperature seems to be a valid factor to consider when designing similar experiments in the future.

Surprisingly, fish in captivity demonstrated a clear interest towards the aquaculture plant-based pellets, but no interest to the natural food sources. Throughout the acclimation period, fish were fed using pellets supplemented with seagrass and macroalgae suspended in the tanks (see "Materials and methods" section), but they were purged for two days before the experiment started. No pellets were offered to fish to supplement their menu of seagrass and macroalgae during the feeding trial. However, when pellets were offered to fish after the tethers were removed from the testing tanks, they displayed fierce competitive behaviour for the pellets. Their lack of interest towards the deployed tethers contradicts an initial assumption about the attractivity of tethered seagrass and macroalgae species as a food source to herbivorous-omnivorous *S. fuscescens* within an artificial mesocosm environment. It is unlikely that this behaviour can be explained by the dependence on pellets that might have being developed in response to the feeding on pellets during the period of acclimation. For the majority of these fish, the acclimation period lasted for 6-10 days, which is unlikely to be sufficient for the development and consolidation of a sustainable feeding habit.

A variety of aspects regarding fish biology and behavioural ecology, as well as the catching and handling regime and mesocosm environment, should be thoroughly evaluated and considered when planning the procedure for the feeding preference experiments with a particular fish species. While used successfully in feeding trials elsewhere, there were numerous issues using this species in temperate Western Australia. The experiments conducted with live *S. fuscescens* here should be considered as a pilot study that provides valuable knowledge and draws attention to the important aspects to consider for the future research in this area.

5. Conclusions

Based on the current study, there is no clear evidence that the tropicalisation of *S. fuscescens* into temperate waters of Western Australia has altered temperate seagrass complexes yet. The results from the comparison between the current grazing rates on *Posidonia* sp. and those obtained in the same locations 15 years ago provided an important novel insight into the current pressure on temperate seagrass ecosystems via tropicalisation. Acknowledging the preference of the tropical *S. fuscescens* in regards of macroalgae in near-shore temperate waters, this research suggests their increasing contribution to the consumption of seagrass, but current consumption rates are relatively low. This observation supports the idea that the further expansion of the tropical herbivorous fish species and their establishment in the temperate reef-associated communities will result in their increased pressure on temperate seagrass meadows. Thus, our research demonstrated an increase in consumption of *P. sinuosa* in 2016/17 compared to 2001.

The increasing population size and associated herbivory from this tropical species on seagrass is likely to be gradual. While this tropical species was shown to have high levels of recruitment after the 2011 heat wave event (Lenanton et al., 2017), its ability to sustain high population size is likely to have been hindered by the colder conditions observed in the study region during the study period (Cahill et al., 2019). This is likely to have impacted the level of grazing by this species during the study period. However, predicted increasing sea temperatures over the coming decades are likely to enhance the species' population to grow and therefore increase consumption on seagrass. Hyndes et al. (2016) predicted that the increase in abundances of tropical species such as *S. fuscescens* will ultimately lead those temperate seagrass systems shifting from detrital-base to grazing-base systems.

The sampling program initiated in 2001 and repeated in 2016/17 provides base-line data and the opportunity to monitor and track the shift in abundances of tropical herbivores and resultant increases in grazing rates to test the above predictions. Furthermore, lesson learned from the capture, handling and husbandry of *S. fuscescens* in feeding trials in the current study will hopefully provide greater success for feeding preference experiments in the future.

References

Al-Marzouqi, A., Al-Nahdi, A., Jayabalan, N., & Al-Habsi, S. (2009). Stomach content analysis and length-weight relationship of the white-spotted rabbitfish Siganus canaliculatus (Park, 1797) from the Arabian Sea coast of Oman. *Journal of the Marine Biological Association of India, 51*(2), 211-216.

Alcoverro, T., & Mariani, S. (2002). Effects of sea urchin grazing on seagrass (*Thalassodendron ciliatum*) beds of a Kenyan lagoon. *Marine Ecology Progress Series, 226*, 255-263.

Aragones, L., & Marsh, H. (2000). Impact of Dugong grazing and turtle cropping on tropical seagrass communities. *Pacific conservation biology, 5*, 277-288.

Avenant, C. (2018). *Diet of the Tropicalised Herbivore Siganus fuscescens Across a Broad Latitudinal Gradient and Comparisons With Temperate Seagrass-associated Omnivorous Fishes.* (Master of Science (Biological Sciences)), Edith Cowan University, Perth. Retrieved from https://ro.ecu.edu.au/theses/2139

Ayvazian, S. G., & Hyndes, G. A. (1995). Surf-zone fish assemblages in south-western Australia: do adjacent nearshore habitats and the warm Leeuwin Current influence the characteristics of the fish fauna? *Marine Biology, 122*(4), 527-536. doi:10.1007/bf00350675

Azzurro, E., & Andaloro, F. (2004). A new settled population of the lessepsian migrant *Siganus luridus* (Pisces: Siganidae) in Linosa Island—Sicily Strait. *Journal of the Marine Biological Association of the United Kingdom, 84*(04), 819-821. doi:10.1017/S0025315404009993h

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., & Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs, Ecological Society of America, 81(2)*, 169-193.

Baskett, C. A., & Schemske, D. W. (2018). Latitudinal patterns of herbivore pressure in a temperate herb support the biotic interactions hypothesis. *Ecology Letters, 21*(4), 578-587. doi:10.1111/ele.12925

Bates, A. E., Pecl, G. T., Frusher, S., Hobday, A. J., Wernberg, T., Smale, D. A., Sunday, J. M., Hill, N. A., Dulvy, N. K., & Colwell, R. K. (2014). Defining and observing stages of climate-mediated range shifts in marine systems. *Global Environmental Change, 26*, 27-38.

Bell, S. Y., Fraser, M. W., Statton, J., & Kendrick, G. A. (2019). Salinity stress drives herbivory rates and selective grazing in subtidal seagrass communities. *PloS one, 14*(3), e0214308. doi:10.1371/journal.pone.0214308

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters, 15*(4), 365-377. doi:10.1111/j.1461-0248.2011.01736.x

Bellwood, D. R., & Choat, J. H. (1990). A functional analysis of grazing in parrotfishes (family Scaridae): the ecological implications. *Environmental Biology of Fishes, 28*(1), 189-214. doi:10.1007/bf00751035

Bennett, S., & Bellwood, D. R. (2011). Latitudinal variation in macroalgal consumption by fishes on the Great Barrier Reef. *Marine Ecology Progress Series, 426*, 241-252. doi:10.3354/meps09016

Bennett, S., Wernberg, T., Harvey, E. S., Santana-Garcon, J., Saunders, B. J., & Bellwood, D. (2015). Tropical herbivores provide resilience to a climate-mediated phase shift on temperate reefs. *Ecology Letters, 18*(7), 714-723. doi:10.1111/ele.12450

Booth, D. J., Figueira, W. F., Gregson, M. A., Brown, L., & Beretta, G. (2007). Occurrence of tropical fishes in temperate southeastern Australia: Role of the East Australian Current. *Estuarine, Coastal and Shelf Science, 72*(1–2), 102-114. doi:http://dx.doi.org/10.1016/j.ecss.2006.10.003

Bryan, P. G. (1975). Food habits, functional digestive morphology and assimilation efficiency of the Rabbitfish Siganus spinus (Pisces, Siganidae) on Guam. *Pacific Science, 29*(3), 269-277.

Burkepile, D. E., & Hay, M. E. (2011). Feeding complementarity versus redundancy among herbivorous fishes on a Caribbean reef. *Coral Reefs, 30*, 351−362. doi:10.1007/s00338-011-0726-6

Burkholder, D. A., Heithaus, M. R., Fourqurean, J. W., Wirsing, A., & Dill, L. M. (2013). Patterns of top-down control in a seagrass ecosystem: could a roving apex predator induce a behaviour-mediated trophic cascade? *Journal of Animal Ecology, 82*, 1192-1202. doi:doi: 10.1111/1365-2656.12097

Cahill, M., Pattiaratchi, C., & Feng, M. (2019). Persistent Cold Water off Western Australia. *Ocean Current.* Retrieved from http://oceancurrent.imos.org.au/news.php

Cambridge, M. L., & Hocking, P. J. (1997). Annual primary production and nutrient dynamics of the seagrasses Posidonia sinuosa and Posidonia australis in south-western Australia. *Aquatic Botany, 59*(3), 277-295. doi:http://dx.doi.org/10.1016/S0304-3770(97)00062-4

Cambridge, M. L., & McComb, A. J. (1984). The loss of seagrasses in Cockburn Sound, Western Australia. I. The time course and magnitude of seagrass decline in relation to industrial development. *Aquatic Botany, 20*, 229-243.

Capper, A., Tibbetts, I. R., O'Neil, J. M., & Shaw, G. R. (2006). Feeding preference and deterrence in rabbitfish Siganus fuscescens for the cyanobacterium Lyngbya majuscula in Moreton Bay, south-east Queensland, Australia. *Journal of Fish Biology, 2006*(68), 1589-1609.

Caputi, N., Jackson, G., & Pearce, A. F. (2014). *The marine heat wave off Western Australia during the summer of 2010/11 – 2 years on*. (250). Department of Fisheries, Western Australia.

Carruthers, T. J. B., Dennison, W. C., Kendrick, G. A., Waycott, M., Walker, D. I., & Cambridge, M. L. (2007). Seagrasses of south–west Australia: A conceptual synthesis of the world's most diverse and extensive seagrass meadows. *Journal of Experimental Marine Biology and Ecology, 350*(1–2), 21-45. doi:http://dx.doi.org/10.1016/j.jembe.2007.05.036

Cavanaugh, K. C., Kellner, J. R., Forde, A. J., Gruner, D. S., Parker, J. D., Rodriguez, W., & Feller, I. C. (2014). Poleward expansion of mangroves is a threshold response to decreased frequency of extreme cold events. *Proceedings of the National Academy of Sciences of the United States of America, 111*(2), 723-727. doi:10.1073/pnas.1315800111

Cebrian, J., Shurin, J. B., Borer, E. T., Cardinale, B. J., Ngai, J. T., Smith, M. D., & Fagan, W. F. (2009). Producer nutritional quality controls ecosystem trophic structure. *PloS one, 4*(3), e4929. doi:10.1371/journal.pone.0004929

Cheung, W. W. L., Meeuwig, J. J., Feng, M., Harvey, E., Lam, V. W. H., Langlois, T., Slawinski, D., Sun, C., & Pauly, D. (2012). Climate-change induced tropicalisation of marine communities in Western Australia. *Marine and Freshwater Research, 63*(5), 415-427. doi:10.1071/MF11205

Chilvers, B. L., Lawler, I. R., Macknight, F., Marsh, H., Noad, M., & Paterson, R. (2005). Moreton Bay, Queensland, Australia: an example of the co-existence of significant marine marlmal populations and large-scale coastal development. *Biological conservation, 122*, 559-571. doi:10.1016/j.biocon.2004.08.0

Chiu, S.-H., Huang, Y.-H., & Lin, H.-J. (2013). Carbon budget of leaves of the tropical intertidal seagrass Thalassia hemprichii. *Estuarine, Coastal and Shelf Science, 125*, 27-35. doi:https://doi.org/10.1016/j.ecss.2013.03.026

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., Raskin, R. G., Sutton, P., & van den Belt, M. (1997). The value of the world's ecosystem services and natural capital. *Nature, 387*(6630), 253-260.

Cresswell, G. R., & Domingues, C. M. (2009). The Leeuwin Current south of Western Australia. *Journal of the Royal Society of Western Australia, 92*, 83-100.

Dawson, T. P., Jackson, S. T., House, J. I., Prentice, I. C., & Mace, G. M. (2011). Beyond predictions: biodiversity conservation in a changing climate. *Science, 332*(6025), 53-58. doi:10.1126/science.1200303

Dickens, L. C., Goatley, C. H. R., Tanner, J. K., & Bellwood, D. R. (2011). Quantifying Relative Diver Effects in Underwater Visual Censuses. *PLoS One, 6*(4), e18965. doi:10.1371/journal.pone.0018965

Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, J. P., Chan, F., English, C. A., Galindo, H. M., Grebmeier, J. M., Hollowed, A. B., Knowlton, N., Polovina, J., Rabalais, N. N., Sydeman, W. J., & Talley, L. D. (2012). Climate change impacts on marine ecosystems. *Ann Rev Mar Sci, 4*, 11-37. doi:10.1146/annurev-marine-041911-111611

Dos Santos, V. M., Matheson, F. E., Pilditch, C. A., & Elger, A. (2012). Is black swan grazing a threat to seagrass? Indications from an observational study in New Zealand. *Aquatic Botany, 100*, 41-50.

Duarte, C. M. (2002). The future of seagrass meadows. *Environmental Conservation, 29(02)*, 192-206. doi:10.1017/S0376892902000127

Duarte, C. M., & Cebrian, J. (1996). The fate of marine autotrophic production. *Limnological Oceanography, 41*(8), 1758-1766.

Duarte, C. M., Middelburg, J. J., & Caraco, N. (2005). Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences, 2*(1), 1-8. doi:10.5194/bg-2-1-2005

Feng, M., Caputi, N., & Pearce, A. (2012). Leeuwin Current. *Marine Climate Change in Australia. Impacts and Adaptation Responses 2012. Report Card*, 61-80.

Floeter, S. R., Behrens, M. D., Ferreira, C. E. L., Paddack, M. J., & Horn, M. H. (2005). Geographical gradients of marine herbivorous fishes: patterns and processes. *Marine Biology, 147*, 1435-1447. doi:10.1007/s00227-005-0027-0

Floeter, S. R., Ferreira, C. E. L., Dominici-Arosemena, A., & Zalmon, I. R. (2004). Latitudinal gradients in Atlantic reef fish communities: trophic structure and spatial use patterns. *Journal of Fish Biology, 64*(6), 1680-1699. doi:10.1111/j.l095-8649.2004.00428

Fodrie, J. F., Heck, K. L., Powers, S. P., Graham, W. M., & Robinson, K. (2010). Climate-related, decadal-scale assemblage changes of seagrass-associated fishes in the northern Gulf of Mexico: Tropical fish in the Nothern Gulf of Mexico. *Global Change Biology, 16*(1), 48-59. doi:10.1111/j.1365-2486.2009.01889.x

Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marba, N., Holmer, M., Mateo, M. A., Apostolaki, E. T., Kendrick, G. A., Krause-Jensen, D., McGlathery, K. J., & Serrano, O. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature Geosci, 5*(7), 505-509. doi:http://www.nature.com/ngeo/journal/v5/n7/abs/ngeo1477.html#supplementary-information

Fourqurean, J. W., Manuel, S., Coates, K. A., Kenworthy, W. J., & Smith, S. R. (2010). Effects of excluding sea turtle herbivores from a seagrass bed: Overgrazing may have led to loss of seagrass meadows in Bermuda. *Marine Ecology Progress Series, 419*, 223–232. doi:10.3354/meps08853

Fox, R. J., & Bellwood, D. R. (2008). Remote video bioassays reveal the potential feeding impact of the rabbitfish *Siganus canaliculatus* (f. *Siganidae*) on an inner-shelf reef on the Great Barrier Reef. *Coral Reefs, 27*, 605-615. doi:10.1007/s00338-008-0359-6

Fox, R. J., Sunderland, T. L., Hoey, A. S., & Bellwood, D. R. (2009). Estimating ecosystem function: contrasting roles of closely related herbivorous rabbitfishes (Siganidae) on coral reefs. *Marine Ecology Progress Series, 385*, 261-269.

Fraser, M. W., Kendrick, G. A., Statton, J., Hovey, R. K., Zavala-Perez, A., & Walker, D. I. (2014). Extreme climate events lower resilience of foundation seagrass at edge of biogeographical range. *Journal of Ecology, 2014*(102), 1528-1536. doi:10.1111/1365-2745.12300

Fujita, D. (2010). Current status and problems of isoyake in Japan. *Bull. Fish. Res. Agen., 32*, 33-42.

Gericke, R. L., Heck, K. L., & Fodrie, F. J. (2014). Interactions between Northern-Shifting Tropical Species and Native Species in the Northern Gulf of Mexico. *Estuaries and Coasts, 37*(4), 952-961. doi:10.1007/s12237-013-9733-x

Gillanders, B. M. (2007). Seagrass *Marine Ecology* (pp. 457-484). South Melbourne, Vic: Oxford University Press.

Goecker, M. E., Heck, J. K. L., & Valentine, J. F. (2005). Effects of nitrogen concentrations in turtlegrass Thalassia testudinum on consumption by the bucktooth parrotfish Sparisoma radians. *Marine Ecology Progress Series, 286*, 239-248.

Heck, K. L., Fodrie, F. J., Madsen, S., Baillie, C. J., & Byron, D. A. (2015). Seagrass consumption by native and a tropically associated fish species: potential impacts of the tropicalization of the northern Gulf of Mexico. *Marine Ecology Progress Series, 520*, 165-173.

Heck, K. L., Hays, G., & Orth, R. J. (2003). Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology Progress Series, 253*, 123–136.

Heck, K. L., Pennock, J. R., Valentine, J., Coen, L., & Sklenar, S. A. (2000). Effects of nutrient enrichment and small predator density on seagrass ecosystems: An experimental assessment. *Limnology and Oceanography, 45*(5), 1041-1057. doi:10.4319/lo.2000.45.5.1041

Heck, K. L., & Valentine, J. F. (1995). Sea urchin herbivory: evidence for long-lasting effects in subtropical seagrass meadows. *Journal of Experimental Marine Biology and Ecology, 189*, 205-217.

Heck, K. L., & Valentine, J. F. (2006). Plant–herbivore interactions in seagrass meadows. *Journal of Experimental Marine Biology and Ecology, 330*(1), 420-436. doi:0.1016/j.jembe.2005.12.044

Heithaus, M. R., Alcoverro, T., Arthur, R., Burkholder, D. A., Coates, K. A., Christianen, M. J. A., Kelkar, N., Manuel, S. A., Wirsing, A. J., Kenworthy, W. J., & Fourqurean, J. W. (2014). Seagrasses in the Age of Sea Turtle Conservation and Shark Overfishing. *Frontiers in Marine Science, 1*. doi:10.3389/fmars.2014.00028

Helmuth, B., Mieszkowska, N., Moore, P., & Hawkins, S. J. (2006). Living on the Edge of Two Changing Worlds: Forecasting the Responses of Rocky Intertidal Ecosystems to Climate Change. *Annual Review of Ecology, Evolution, and Systematics, 37*(1), 373-404. doi:10.1146/annurev.ecolsys.37.091305.110149

Hobday, A. J., Alexander, L. V., Perkins, S. E., Smale, D. A., Benthuysen, J., Burrows, M. T., Donat, M. G., Feng, M., Straub, S. C., Oliver, E. C. J., Scannell, H. A., Sen Gupta, A., Wernberg, T., Holbrook, N. J., & Moore, P. J. (2016). A hierarchical approach to defining marine heatwaves. *Progress in Oceanography, 141*, 227-238.

Hobday, A. J., & Pecl, G. T. (2014). Identification of global marine hotspots: sentinels for change and vanguards for adaptation action. *Reviews in Fish Biology and Fisheries, 24*(2), 415-425.

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The Impact of Climate Change on the World's Marine Ecosystems. *Science, 328*(5985), 1523-1528. doi:10.1126/science.1189930

Hoey, A. S., & Bellwood, D. R. (2009). Limited Functional Redundancy in a High Diversity System: Single Species Dominates Key Ecological Process on Coral Reefs. *Ecosystems, 12*(8), 1316-1328. doi:10.1007/s10021-009-9291-z

Hoey, A. S., Brandl, S. J., & Bellwood, D. R. (2013). Diet and cross-shelf distribution of rabbitfishes (f. Siganidae) on the northern Great Barrier Reef: Implications for ecosystem function. *Coral Reefs, 32*, 973-984. doi:10.1007/s00338-013-1043-z

Hopper, S. (2009). OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. *Plant and soil, 322*, 49-86.

Horn, M. H. (1989). Biology of marine herbivorous fishes. *Oceanogr Mar Biol Annu Rev, 27*, 167-272.

Huisman, J. M., & Walker, D. I. (1990). A catalogue of the marine plants of Rottnest Island, Western Australia, with notes on their distribution and biogeography. *Kingia, 1*, 349-459.

Hutchins, J. B. (1991). Dispersal of tropical fishes to temperate seas in the southern hemisphere. *Journal of the Royal Society of Western Australia, 74*, 79-84.

Hutchins, J. B. (1994). A survey of the nearshore reef fish fauna of Western Australia's west and south coasts - the Leeuwin Province. *Records of the Western Australian Museum, Supplement 46*, 1-66.

Hutchins, J. B., & Pearce, A. F. (1994). Influence of the Leeuwin Current on Recruitment of Tropical Reef Fishes at Rottnest Island, Western Australia. *Bulletin of marine science, 54*(1), 245-255.

Hyndes, G. A., Francour, P., Guidetti, P., Heck, K. L., & Jenkins, G. (2018). The Roles of Seagrasses in Structuring Associated Fish Assemblages and Fisheries. In A. W. D. Larkum, G. Kendrick, & P. J. Ralph (Eds.), *Seagrasses of Australia: Structure, Ecology and Conservation* (pp. 589-627). Netherlands: Springer International Publishing.

Hyndes, G. A., Heck, K. L., Vergés, A., Harvey, E. S., Kendrick, G. A., Lavery, P. S., McMahon, K., Orth, R. J., Pearce, A., Vanderklift, M., Wernberg, T., Whiting, S., & Wilson, S. (2016). Accelerating Tropicalization and the Transformation of Temperate Seagrass Meadows. *BioScience, 66*(11), 938-948. doi:10.1093/biosci/biw111

Hyndes, G. A., Nagelkerken, I., McLeod, R. J., Connolly, R. M., Lavery, P. S., & Vanderklift, M. A. (2014). Mechanisms and ecological role of carbon transfer within coastal seascapes. *Biological Reviews, 89*(1), 232-254. doi:10.1111/brv.12055

Jackson, E. L., Rowden, A. A., Attrill, M. J., Bossey, S. J., & Jones, M. B. (2001). The importance of seagrass beds as a habitat for fishery species. *Oceanography and Marine Biology: An Annual Review, 39*, 269-303.

Jernakoff, P., & Nielsen, J. (1997). The relative importance of amphipod and gastropod grazers in Posidonia sinuosa meadows. *Aquatic Botany, 56*(3–4), 183-202. doi:http://dx.doi.org/10.1016/S0304-3770(96)01112-6

Jiménez-Ramos, R., Egea, L. G., Ortega, M. J., Hernández, I., Vergara, J. J., & Brun, F. G. (2017). Global and local disturbances interact to modify seagrass palatability. *PloS one, 12*(8), e0183256. doi:10.1371/journal.pone.0183256

Kaiser, M. J., Attrill, M. J., Jennings, S., Thomas, D. N., Barnes, D. K. A., Brierley, A. S., Hiddink, J. G., Kaartokallio, H., Polunin, N. V. C., & Raffaelli, D. G. (2011). *Marine Ecology: Processes, Systems, and Impacts* (O. U. Press Ed. Second ed.).

Kendrick, G., Harvey, E., Wernberg, T., Harman, N., & Goldberg, N. (2004). The role of disturbance in maintaining diversity of benthic macroalgal assemblages in southwestern Australia. *The Japanese Bulletin of Phycology, 52*, 5-9.

Kirkman, H. (1997). *Seagrasses of Australia*. Department of the Environment, Canberra.

Kirkman, H., & Walker, D. I. (1989). Regional studies—Western Australian seagrasses. In A. W. D. Larkum, A. J. McComb, & S. A. Shepherd (Eds.), *Biology od seagrasses: A treatise on the biology of seagrasses with special reference to the Australian region* (pp. 157-181). Amsterdam: Elsevier.

Kirsch, K. D., Valentine, J. F., & Heck, K. L. (2002). Parrotfish grazing on turtlegrass Thalassia testudinum: evidence for the importance of seagrass consumption in food web dynamics of the Florida Keys National Marine Sanctuary. *Marine Ecology Progress Series, 227*, 71-85.

Lal, A., Arthur, R., Marbā, N., Lill, A. W., & Alcoverro, T. (2010). Implications of conserving an ecosystem modifier: increasing green turtle (Chelonia mydas) densities substantially alters seagrass meadows. *Biological conservation, 143*(11), 2730-2738.

Last, P. R., White, W. T., Gledhill, D. C., Hobday, A. J., Brown, R., Edgar, G. J., & Pecl, G. (2011). Long-term shifts in abundance and distribution of a temperate fish fauna: a response to climate change and fishing practices. *Global Ecology and Biogeography, 20*(1), 58-72. doi:10.1111/j.1466-8238.2010.00575.x

Lavery, P. S., Mateo, M. A., Serrano, O., & Rozaimi, M. (2013). Variability in the carbon storage of seagrass habitats and its implications for global estimates of blue carbon ecosystem service. *PLoS One, 8*(9), e73748. doi:10.1371/journal.pone.0073748

Lee, C.-L., Huang, Y.-H., Chien-Hsun, C., & Hsing-Juh, L. (2016). Remote underwater video reveals grazing preferences and drift export in multispecies seagrass beds. *Journal of Experimental Marine Biology and Ecology, 476*, 1-7.

Lefevre, C. D., & Bellwood, D. R. (2011). Temporal variation in coral reef ecosystem processes: herbivory of macroalgae by fishes. *Marine Ecology Progress Series, 422*, 239−251. doi:10.3354/meps08916

Lenanton, R. C. J., Dowling, C. E., Smith, K. A., Fairclough, D. V., & Jackson, G. (2017). Potential influence of a marine heatwave on range extensions of tropical fishes in the eastern Indian Ocean—Invaluable contributions from amateur observers. *Regional Studies in Marine Science, 13*, 19-31. doi:https://doi.org/10.1016/j.rsma.2017.03.005

Ling, S. D., Johnson, C. R., Frusher, S. D., & Ridgway, K. R. (2009). Overfishing reduces resilience of kelp beds to climate-driven catastrophic phase shift. *Proceedings of the National Academy of Sciences, 106*(52), 22341-22345. doi:10.1073/pnas.0907529106

Longo, G. O., & Floeter, S. R. (2012). Comparison of remote video and diver's direct observations to quantify reef fishes feeding on benthos in coral and rocky reefs. *Journal of Fish Biology, 81*(5), 1773-1780. doi:10.1111/j.1095-8649.2012.03441.x

Longo, G. O., Hay, E. E., Ferreirra, C. E. L., & Floeter, S. R. (2018). Trophic interactions across 61 degrees of latitude in the Western Atlantic. *Global Ecology and Biogeography*. doi:https://onlinelibrary.wiley.com/doi/pdf/10.1111/geb.12806

Lough, J. M. (2008). Shifting climate zones for Australia's tropical marine ecosystems. *Geophysical Research Letters, 35*(14). doi:10.1029/2008GL034634

MacArthur, L. D., & Hyndes, G. A. (2007). Varying foraging strategies of Labridae in seagrass habitats: Herbivory in temperate seagrass meadows? *Journal of Experimental Marine Biology and Ecology, 340*, 247-258. doi:10.1016/j.jembe.2006.09.017

Mallet, D., Wantiez, L., Lemouellic, S., Vigliola, L., & Pelletier, D. (2014). Complementarity of Rotating Video and Underwater Visual Census for Assessing Species Richness, Frequency and Density of Reef Fish on Coral Reef Slopes. *PLoS One, 9*(1), e84344. doi:10.1371/journal.pone.0084344

Mantyka-Pringle, C., & Bellwood, D. (2007). Direct evaluation of macroalgal removal by herbivorous coral reef fishes. *Coral Reefs, 26*, 435-442. doi:10.1007/s00338-007-0214-1

Mantyka, C. S., & Bellwood, D. R. (2007). Macroalgal grazing selectivity among herbivorous coral reef fishes. *Marine Ecology Progress Series, 352*, 177-185. doi:10.3354/meps07055

Marco-Mendez, C., Ferrero-Vicente, L. M., Prado, P., & Sanchez-Lizaso, J. L. (2017). Epiphytes and nutrient contents influence Sarpa salpa herbivory on Caulerpa spp vs. seagrass species in Mediterranean meadows. *Estuarine, Coastal and Shelf Science, 184*, 54-66. doi:http://dx.doi.org/10.1016/j.ecss.2016.11.005

Mariani, S., & Alcoverro, T. (1999). A multiple-choice feeding-preference experiment utilising seagrasses with a natural population of herbivorous fishes. *Marine Ecology Progress Series, 189*, 295-299.

Marsh, H., O'Shea, T. J., & Reynolds, J. E. (2011). *Ecology and Conservation of the Sirenia: Dugongs and Manatees*. Cambridge: Cambridge University Press.

Maxwell, J. G. H., & Cresswell, G. R. (1981). Dispersal of tropical marine fauna to the Great Australian Bight by the Leeuwin Current. *Australian Journal of Marine and Freshwater Research, 32*, 493-500. doi:10.1071/MF9810493

Meekan, M., & Choat, J. (1997). Latitudinal variation in abundance of herbivorous fishes: a comparison of temperate and tropical reefs. *Marine Biology, 128*(3), 373-383. doi:https://doi.org/10.1007/s002270050103

Michael, P. J., Hyndes, G. A., Vanderklift, M. A., & Vergés, A. (2013). Identity and behaviour of herbivorous fish influence large-scale spatial patterns of macroalgal herbivory in a coral reef. *Marine Ecology Progress Series, 482*, 227-240.

Nagelkerken, I. (2007). Are non-estuarine mangroves connected to coral reefs through fish migration? *Bulletin of marine science, 80*(3), 595–607.

Nowicki, R., Fourqurean, J., & Heithaus, M. (2018). The Role of Consumers in Structuring Seagrass Communities: Direct and Indirect Mechanisms. In A. W. D. Larkum, G. A. Kendrick, & P. J. Ralph (Eds.), *Seagrasses of Australia* (pp. 491-540): Springer International Publishing.

Nykjaer, L. (2009). Mediterranean Sea surface warming 1985–2006. *Climate Research, 39*(1), 11-17.

Ojeda, F. P., & Muñoz, A. A. (1999). Feeding selectivity of the herbivorous fish *Scartichthys viridis*: effects on macroalgal community structure in a temperate rocky intertidal coastal zone. *Marine Ecology Progress Series, 184*, 219-229.

Ozvarol, Y., Ertan, O. O., & Turna, I. I. (2011). The grazing effect of *Siganus luridus* Ruppell, 1828 on *Posidonia oceanica* (L.) Delile, 1813 meadows in Turkish Mediterranean coast (Gazipasa/Antalya). *Journal of Food, Agriculture and Environment, 9*(1), 531-533.

Parker, J. D., & Hay, M. E. (2005). Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters, 8*(9), 959-967. doi:10.1111/j.1461-0248.2005.00799.x

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics, 37*, 637-669. doi:10.2307/annurev.ecolsys.37.091305.3000

Paul, V. J., Nelson, S. G., & Sanger, H. R. (1990). Feeding preferences of adult and juvenile rabbitfish Siganus argenteus in relation to chemical defenses of tropical seaweeds. *Marine Ecology Progress Series, 60*, 23-34. doi:0171-8630/90/0060/0023

Pearce, A. F., & Feng, M. (2007). Observations of warming on the Western Australian continental shelf. *Marine and Freshwater Research, 58*, 914-920.

Pearce, A. F., & Feng, M. (2013). The rise and fall of the "marine heat wave" off Western Australia during the summer of 2010/2011. *Journaal of Marine Systems, 111-112*, 139-156.

Pearce, A. F., & Hutchins, J. B. (2009). Oceanic processes and the recruitment of tropical fish at Rottnest Island (Western Australia). *Journal of the Royal Society of Western Australia, 92*, 179-195.

Pennings, S. C., Ho, C. K., Salgado, C. S., Wieski, K., Dave, N., Kunza, A. E., & Wason, E. L. (2009). Latitudinal variation in herbivore pressure in Atlantic Coast salt marshes. *Ecology, 90*, 183-195.

Perry, A. L., Low, P. J., Ellis, J. R., & Reynolds, J. D. (2005). Climate change and distribution shifts in marine fishes. *Science, 308*(5730), 1912-1915. doi:10.1126/science.1111322

Peterson, C. H., & Renaud, P. E. (1989). Analysis of feeding preference experiments. *Oecologia, 80*, 82-86.

Pillans, R. D., Franklin, C. E., & Tibbetts, I. R. (2004). Food choice in *Siganus fuscescens*: influence of macrophyte nutrient content and availability. *Journal of Fish Biology, 64*(2), 297-309. doi:10.1111/j.0022-1112.2004.00261.x

Poloczanska, E. S., Brown, C. J., Sydeman, W. J., Kiessling, W., Schoeman, D. S., Moore, P. J., Brander, K., Bruno, J. F., Buckley, L. B., Burrows, M. T., Duarte, C. M., Halpern, B. S., Holding, J., Kappel, C. V., O'Connor, M. I., Pandolfi, J. M., Parmesan, C., Schwing, F., Thompson, S. A., & Richardson, A. J. (2013). Global imprint of climate change on marine life. *Nature Climate Change*(3), 919-925. doi:10.1038/NCLIMATE1958

Poore, A. G. B., Campbell, A. H., Coleman, R. A., Edgar, G. J., Jormalainen, V., Reynolds, P. A., Sotka, E. E., Stachovicz, J. J., Taylor, R. B., Vanderklift, M. A., & Duffy, J. E. (2012). Global patterns in the impact of marine herbivores on benthic primary producers. *Ecology Letters, 15*, 912-922. doi:10.1111/j.1461-0248.2012.01804.x

Prado, P., & Heck, K. (2011). Seagrass selection by omnivorous and herbivorous consumers: Determining factors. *Marine Ecology Progress Series, 429*, 45-55. doi:10.3354/meps09076

Prado, P., Romero, J., & Alcoverro, T. (2010). Nutrient status, plant availability and seasonal forcing mediate fish herbivory in temperate seagrass beds. *Marine Ecology Progress Series, 409*, 229-239. doi:10.3354/meps08585

Prado, P., Tomas, F., Alcoverro, T., & Romero, J. (2007). Extensive direct measurements of Posidonia oceanica defoliation confirm the importance of herbivory in temperate seagrass meadows. *Marine Ecology Progress Series, 340*, 63-71. doi:doi: 10.3354/meps340063

Rasher, D. B., Hoey, A. S., & Hay, M. E. (2013). Consumer diversity interacts with prey defenses to drive ecosystem function. *Ecology, 94*(6), 1347-1358.

Roa, R. (1992). Design and analysis of multiple-choice feeding-preference experiments. *Oecologia, 89*, 509-515.

Sala, E., Kizilkaya, Z., Yildirim, D., & Ballesteros, E. (2011). Alien Marine Fishes Deplete Algal Biomass in the Eastern Mediterranean. *PLoS One, 6*(2), e17356. doi:10.1371/journal.pone.0017356

Sen Gupta, A., Brown, J. N., Jourdain, N. C., van Sebille, E., Ganachaud, A., & Vergés, A. (2015). Episodic and non-uniform shifts of thermal habitats in a warming ocean. *Deep Sea Research Part II: Topical Studies in Oceanography, 113*, 59-72. doi:http://dx.doi.org/10.1016/j.dsr2.2013.12.002

Serisawa, Y., Imoto, Z., Ishikawa, T., & Ohno, M. (2004). Decline of the Ecklonia cava population associated with increased seawater temperatures in Tosa Bay, southern Japan. *Fisheries Science, 70*(1), 189-191. doi:10.1111/j.0919-9268.2004.00788.x

Short, F., & Duarte, C. (2001). Methods for the measurement of seagrass growth and production. In F. T. Short & R. G. Coles (Eds.), *Global Seagrass Research Methods* (pp. 155-182). Amsterdam: Elsevier Science B.V.

Smale, D. A., Kendrick, G. A., Waddington, K. I., Van Niel, K. P., Meeuwig, J. J., & Harvey, E. S. (2010). Benthic assemblage composition on subtidal reefs along a latitudinal gradient in Western Australia. *Estuarine, Coastal and Shelf Science, 86*(1), 83-92. doi:https://doi.org/10.1016/j.ecss.2009.10.016

Smale, D. A., & Wernberg, T. (2013). Extreme climatic event drives range contraction of a habitat-forming species. *Proceedings of the Royal Society of London B: Biological Sciences, 280*(1754). doi:10.1098/rspb.2012.2829

Sorte, C. J. B., Williams, S. L., & Carlton, J. T. (2010). Marine range shifts and species introductions: comparative spread rates and community impacts. *Global Ecology and Biogeography, 19*(3), 303-316. doi:10.1111/j.1466-8238.2009.00519.x

Statton, J., Gustin-Craig, S., Dixon, K. W., & Kendrick, G. A. (2015). Edge Effects along a Seagrass Margin Result in an Increased Grazing Risk on Posidonia australis Transplants. *PloS one, 10*(10), e0137778. doi:10.1371/journal.pone.0137778

Steele, L., Darnell, K. M., Cebrián, J., & Sanchez–Lizaso, J. L. (2014). Sarpa salpa herbivory on shallow reaches of Posidonia oceanica beds. *Animal Biodiversity and Conservation, 37*(1), 49-57.

Suarez-Jimenez, R., Hepburn, C. D., Hyndes, G., McLeod, R. J., Taylor, R. B., & Hurd, C. L. (2017). Importance of the invasive macroalga *Undaria pinnatifida* as trophic subsidy for a beach consumer. *Marine Biology, 164*(113), 1-9. doi:10.1007/s00227-017-3140-y

Targett, T. E., & Targett, N. M. (1990). Energetics of food selection by the herbivorous parrotfish Sparisoma radians: roles of assimilation efficiency, gut evacuation rate, and algal secondary metabolites. *Marine Ecology Progress Series, 66*, 13-21. doi:0171-8630/90/0066/0013

Thomson, J. A., Burkholder, D. A., Heithaus, M. R., Fourqurean, J. W., Fraser, M. W., Statton, J., & Kendrick, G. A. (2015). Extreme temperatures, foundation species, and abrupt ecosystem change: an example from an iconic seagrass ecosystem. *Global Change Biology, 21*(4), 1463-1474. doi:10.1111/gcb.12694

Tomas, F., Box, A., & Terrados, J. (2011). Effects of invasive seaweeds on feeding preference and performance of a keystone Mediterranean herbivore. *Biological Invasions, 13*(7), 1559-1570. doi:10.1007/s10530-010-9913-6

Tomas, F., Turon, X., & Romero, J. (2005). Effects of herbivores on a *Posidonia oceanica* seagrass meadow: importance of epiphytes. *Marine Ecology Progress Series, 287*, 115-125.

Underwood, A. J. (1981). Techniques of analysis of variance in experimental marine biology and ecology. *Oceanography and Marine Biology. Annual Review, 19*, 513-605.

Unsworth, R. K. F., Taylor, J. D., Powell, A., Bell, J. J., & Smith, D. J. (2007). The contribution of scarid herbivory to seagrass ecosystem dynamics in the Indo-Pacific. *Estuarine, Coastal and Shelf Science, 74*(1–2), 53-62. doi:http://dx.doi.org/10.1016/j.ecss.2007.04.001

Valentine, J. F., & Duffy, J. E. (2006). The Central Role of Grazing in Seagrass Ecology. In A. W. D. Larkum, R. J. Orth, & C. M. Duarte (Eds.), *Seagrasses: Biology, Ecology and Conservation* (pp. 463-501). Dordrecht: Springer.

Valentine, J. F., & Heck, K. L. (1999). Seagrass herbivory: evidence for the continued grazing of marine grasses. *Marine Ecology Progress Series, 176*, 291-302.

Vergés, A., Alcoverro, T., & Ballesteros, E. (2009). Role of fish herbivory in structuring the vertical distribution of canopy algae *Cystoseira spp.* in the Mediterranean Sea. *Marine Ecology Progress Series, 375*, 1-11. doi:10.3354/meps07778

Vergés, A., Bennett, S., & Bellwood, D. R. (2012). Diversity among Macroalgae-Consuming Fishes on Coral Reefs: A Transcontinental Comparison. *PLoS One, 7*(9), e45543. doi:10.1371/journal.pone.0045543

Vergés, A., Steinberg, P. D., Hay, M. E., Poore, A. G. B., Campbell, A. H., Ballesteros, E., Heck, K. L., Booth, D. J., Coleman, M. A., Feary, D. A., Figueira, W., Langlois, T., Marzinelli, E. M., Mizerek, T., Mumby, P. J., Nakamura, Y., Roughan, M., van Sebille, E., Gupta, A. S., Smale, D. A., Tomas, F., Wernberg, T., & Wilson, S. K. (2014a). The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. *Proceedings of the Royal Society of London B: Biological Sciences, 281*(1789). doi:10.1098/rspb.2014.0846

Vergés, A., Tomas, F., Cebrian, E., Ballesteros, E., Kizilkaya, Z., Dendrinos, P., Karamanlidis, A. A., Spiegel, D., & Sala, E. (2014b). Tropical rabbitfish and the deforestation of a warming temperate sea. *Journal of Ecology, 102*(6), 1518-1527. doi:10.1111/1365-2745.12324

Walter, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., T.J.C., B., Fromentin, J. M., Hoegh-Guldberg, O., & Bairlein, F. (2002). Ecological responses to recent climate change. *Nature, 416*, 389-395.

Watson, D. L., & Harvey, E. S. (2009). Influence of the Leeuwin Current on the distribution of fishes and the composition of fish assemblages. *Journal of the Royal Society of Western Australia, 92*, 147-154.

Watson, D. L., Harvey, E. S., Anderson, M. J., & Kendrick, G. A. (2005). A comparison of temperate reef fish assemblages recorded by three underwater stereo-video techniques. *Marine Biology, 148*(2), 415-425. doi:10.1007/s00227-005-0090-6

Watson, D. L., Harvey, E. S., Fitzpatrick, B. M., Langlois, T. J., & Shedrawi, G. (2010). Assessing reef fish assemblage structure: how do different stereo-video techniques compare? *Marine Biology, 157*(6), 1237-1250. doi:10.1007/s00227-010-1404-x

Waycott, M., Duarte, C., Carruthers, T. J. B., Orth, R. J., Dennison, W. C., Olyarnik, S., Calladine, A., Fourqurean, J. W., Heck, K. L., Hughes, A. R., Kendrick, G., Kenworthy, W. J., Short, F. T., & Williams, S. L. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences of the United States of America, 106*(30), 12377-12381. doi:10.1073/pnas.0905620106

Wernberg, T., Bennett, S., Babcock, R. C., de Bettignies, T., Cure, K., Depczynski, M., Dufois, F., Fromont, J., Fulton, C. J., Hovey, R. K., Harvey, E. S., Holmes, T. H., Kendrick, G. A., Radford, B., Santana-Garcon, J., Saunders, B. J., Smale, D. A., Thomsen, M. S., Tuckett, C. A., Tuya, F., Vanderklift, M. A., & Wilson, S. (2016). Climate-driven regime shift of a temperate marine ecosystem. *Science, 353*(6295), 169-172. doi:10.1126/science.aad8745

Wernberg, T., Kendrick, G., & Phillips, J. (2003). Regional differences in kelp-associated algal assemblages on temperate limestone reefs in south-western Australia. *Diversity and Distributions, 9*, 427-441. doi:10.1046/j.1472-4642.2003.00048.x

Wernberg, T., Russell, B., Thomsen, M., Gurgel, C. F., Bradshaw, C., Poloczanska, E., & Connell, S. (2011). Seaweed communities in retreat from ocean warming. *Current Biology, 21*, 1828-1832. doi:10.1016/j.cub.2011.09.028

Wernberg, T., Smale, D. A., Tuya, F., Thomsen, M. S., Langlois, T. J., de Bettignies, T., Bennett, S., & Rousseaux, C. S. (2013). An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. *Nature Clim. Change, 3*(1), 78-82. doi:http://www.nature.com/nclimate/journal/v3/n1/abs/nclimate1627.html#supplementary-information

Westera, M. B., & Lavery, P. S. (2006). A comparison of hole punch and needle punch methods for the measurement of seagrass productivity. *Aquatic Botany, 85*, 267-269. doi:10.1016/j.aquabot.2006.05.007

White, K. S., Westera, M. B., & Kendrick, G. A. (2011). Spatial patterns in fish herbivory in a temperate Australian seagrass meadow. *Estuarine Coastal and Shelf Science, 93*(4), 366-374. doi:10.1016/j.ecss.2011.05.006

Wilson, S. K., Depczynski, M., Fisher, R., Holmes, T. H., O'Leary, R. A., & Tinkler, P. (2010). Habitat Associations of Juvenile Fish at Ningaloo Reef, Western Australia: The Importance of Coral and Algae. *PLoS One, 5*(12), e15185. doi:10.1371/journal.pone.0015185

Yamaguchi, A. (2010). Biological aspects of herbivorous fishes in the coastal areas of western Japan. *Bull. Fish. Res. Agen., 32*, 89-94.

York, P. H., Hyndes, G., Bishop, M. J., & Barnes, R. S. K. (2018). Faunal Assemblages of Seagrass Ecosystems. In A. W. D. Larkum, A. Kendrick, & P. J. Ralph (Eds.), *Seagrasses of Australia: Structure, Ecology, Conservation* (pp. 541-588). Netherlands: Springer International Publishing.

Zarco-Perello, S., Wernberg, T., Langlois, T. J., & Vanderklift, M. A. (2017). Tropicalization strengthens consumer pressure on habitat-forming seaweeds. *Scientific Reports, 7*(1), 820. doi:10.1038/s41598-017-00991-2

Zemke-White, L. W., Choat, J., & Clements, K. (2002). A re-evaluation of the diel feeding hypothesis for marine herbivorous fishes. *Marine Biology, 141*(3), 571-579. doi:10.1007/s00227-002-0849-y

Zieman, J. C. (1974). Methods for the study of the growth and production of turtle grass, Thalassia testudinum. *Aquaculture, 4*, 139-143.