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# Grazer specialisation and temperature effects on epiphytic fouling: conservation implications for a temperate African seagrass (*Zostera capensis*)

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**ABSTRACT:** Global change stressors can alter mutually beneficial interactions in structurally complex systems, leading to impaired functionality and service provision. Knowledge of how this is influenced by species identity and degree of specialisation is limited in seagrass systems, but these are key considerations for their conservation. Here, we used a mesocosm experiment to quantify effects of ocean warming on fouling in a temperate seagrass (*Zostera capensis*) system using sympatric specialist (Critically Endangered *Siphonaria compressa*) and generalist (*Fissurella mutabilis*) epiphytic limpet grazers. Results indicate an increase in fouling loads due to warming, by up to 15 times in the absence of grazers. Grazers differentially countered fouling, with the specialist providing a stronger cleaning function, especially at high temperatures. The specialist showed greater survival (80–90%) at higher temperatures, while survival for the generalist was reduced (10–20%). Our findings highlight the need for specialist, warm-tolerant grazers to be prioritised in seagrass conservation/restoration programs. This will likely establish positive feedback loops that benefit both seagrasses and grazers under warming conditions, with positive community- and ecosystem-wide ramifications.

**KEY WORDS:** Biodiversity · Ecosystem engineer · Warming · Seagrass · Grazing

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## 1. INTRODUCTION

Structure-forming, foundation species are key determinants of biodiversity and ecosystem multifunctionality (Jones et al. 1997, Bruno & Bertness 2001). By adding structure to otherwise homogeneous environments, foundation species create and regulate resource flows, typically increasing interaction web complexity and species/functional diversity (Jones et al. 1997, Borst et al. 2018). Mutually beneficial relationships are an important subset of interactions nested within foundational habitats (Bracken et al. 2007, Sheppard et al. 2017). This interaction generally results in an increase in the fitness or perform-

ance of 2 interacting species/groups, due to each providing critical ecological functions that ultimately benefit each other (Bracken et al. 2007, Sheppard et al. 2017). This interaction assumes great significance when a foundation species is involved, as any benefit accrued to the latter positively affects not only the species providing benefits to it, but also whole assemblages that depend on foundational structure. The persistence of hierarchically organised habitats and the unique biodiversity they support is thus integrally linked to the conservation of mutually beneficial interactions involving foundation species (Hughes et al. 2009). However, knowledge gaps, particularly around global change effects on these interactions

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across various foundational habitats, inhibit effective management and conservation of these ecosystems.

In coastal ecosystems, seagrasses rank amongst the most valuable of foundational habitats, providing essential socio-ecological services to the value of \$1.9 trillion annually (Waycott et al. 2009). However, these ecosystems are some of the most threatened on the planet, with rates of loss being on par with that of tropical rainforests and coral reefs (Waycott et al. 2009). Fouling, i.e. the proliferation of filamentous algae on leaves (principally through eutrophication coupled with consumer loss), is a leading cause of seagrass decline, primarily due to a reduction of light available for photosynthesis (Baden et al. 2003, van Montfrans et al. 2004, Östman et al. 2016). Fouling effects, however, can be limited and/or reversed by epiphytic grazers consuming algae, thereby improving physiological performance (van Montfrans et al. 2004, Baden et al. 2010, Svensson et al. 2012). Thus, substrate and/or resource (algae) provision by seagrasses positively impacts grazers, which in turn generates promotive effects on seagrasses, leading to mutually beneficial reinforcing feedbacks. However, the strength of such feedbacks is likely contingent upon environmental contexts in which these interactions occur. In this regard, stressors such as global warming have the potential to alter grazer–epiphyte interactions, but knowledge of how this manifests across different grazer regimes is limited. If epiphytic growth is favoured but grazer effectiveness weakened, then warming can shift seagrass systems to a degraded, algal-dominated state. However, if grazer effectiveness is strengthened, then warming may fortify the cleaning function provided by grazers. Understanding how grazer–epiphyte interactions are altered by warming is thus relevant to the management and conservation of seagrass ecosystems under warming conditions.

This study experimentally evaluates the effects of ocean warming on grazer–epiphyte interactions in a temperate seagrass (*Zostera capensis*) system in South Africa. *Z. capensis* is widely distributed from the South African temperate west coast to the tropical Kenyan coast in east Africa. This species is currently listed as Vulnerable by the IUCN Red List, due to declines in cover (Short et al. 2011). In South Africa, there are 2 systems that support large stands of *Z. capensis*: the Knysna Estuary and Langebaan Lagoon (the focal system in the present study) on the south and west coasts (Allanson & Herbert 2005, Pillay et al. 2010). In Langebaan, estimates indicate a 28% seagrass loss between 1960 and 2010, with a 98% loss reported in heavily disturbed areas, which

is thought to be linked to trampling and bait-collecting (Pillay et al. 2010). *Z. capensis* is predominantly intertidal in Langebaan, but has a fragmented distribution across a range of thermal regimes. In the warmer Geelbek site (Fig. 1), where temperatures can approach 30°C, *Z. capensis* is shorter but denser than at Oesterwal, which is cooler (maximum approximately 24°C). The Critically Endangered eelgrass false-limpet *Siphonaria compressa* (length = 4 mm) is the dominant seagrass-dwelling epiphytic grazer in Geelbek (maximum density ~ 400 m<sup>-2</sup>), while the generalist cape key-hole limpet *Fissurella mutabilis* (length = 20 mm), which is common on hard substrata, is the dominant seagrass-dwelling epiphytic grazer at Oesterwal (maximum density ~ 150 m<sup>-2</sup>). At the latter site, seagrass leaves are visibly fouled by filamentous algae, but at Geelbek, leaves are generally free of filamentous algae. Given the recent increasing trend in temperature in Langebaan Lagoon (Fig. 2), the central goal of this study was to evaluate warming effects on grazer–epiphyte interactions involving specialist (*S. compressa*) and generalist (*F. mutabilis*) seagrass grazers. Specifically, we hypothesised that *S. compressa* would exert stronger top-down impacts on seagrass epiphytes than *F. mutabilis*, given that the former is a specialist seagrass inhabitant. We also hypothesised that the grazing

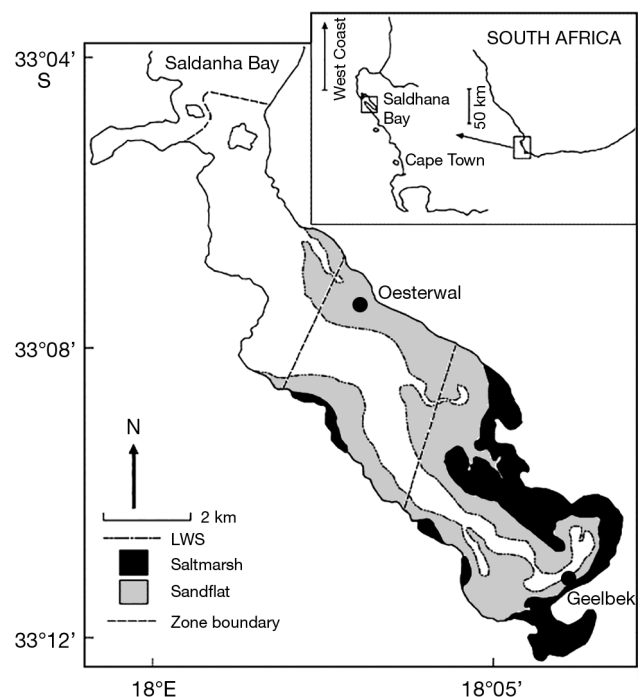


Fig. 1. Map of Langebaan, showing its geographical location within South Africa and approximate locations of sites from which limpets and seagrasses were collected for the mesocosm experiment. LWS: low water springs

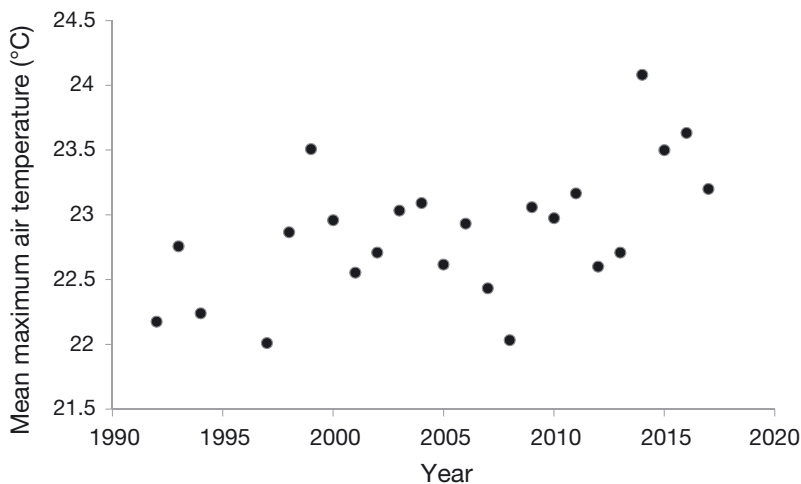


Fig. 2. Inter-annual variability in mean maximum air temperature recorded between 1990 and 2017 in Langebaan Lagoon. Data collected at Geelbek — courtesy of the South African Weather Service

impact of *S. compressa* would be stronger with increasing temperature, given that it occurs naturally in warmer conditions in the lagoon than *F. mutabilis*.

## 2. MATERIALS AND METHODS

Four header tanks (120 l) containing seawater (35‰) were individually aerated and heated (300 W Eheim Jager) to 18, 22, 26 and 30°C, corresponding to temperature ranges recorded in Langebaan Lagoon. Water was pumped (Atman PH-2500 submersible pump) from each header tank into 3 independent 80 l tanks (replicates for each temperature treatment) and back to the header tank at constant flow rates. Osram Lumilux Cool White (58 W) fluorescent tubes provided constant lighting on a 12-h day/night cycle. Three plant pots (diameter = 13 cm, depth = 10.8 cm) containing seagrass were added to each of the 80 l tanks following the removal of visible grazers and epiphytic algae on seagrass blades. The 3 seagrass pots were assigned to the following grazer treatments: no grazers (control), *Fissurella mutabilis* (2 per pot, 149 per m<sup>2</sup>) and *Siphonaria compressa* (5 per pot, 374 per m<sup>2</sup>; Fig. 3). Grazer densities in pots were determined from *in situ* densities measured during 4 prior seasonal surveys of seagrass beds in Langebaan Lagoon. In cases of deaths, limpets were added to maintain grazer densities as designated, but all mortalities were recorded. Salinity, nutrient levels, pH, oxygen and chlorophyll *a* (YSI 650 MDS 6600V2 multiprobe; Sera nutrient test kits) were monitored throughout the experiment to ensure consistency among replicates. Water in each system was changed

weekly to maintain water quality, which involved preheating water to designated temperatures. Following a 5-wk experimental period, the length and width (average of base, middle and top) of 10 leaves per pot were measured, from which surface area was determined (following Short & Duarte 2001). Fouling algae were simultaneously scraped off each of the 10 leaf samples and dried for 12 h at 60°C. Fouling measurements were pooled to generate a single mean value per pot per replicate, thus generating N = 36 (4 temperature treatments × 3 grazer treatments × 3 replicates = 36).

Data were analysed in the data analysis platform R. Two-way ANOVA was used to test the main and interactive effects of temperature and grazing on fouling, with *post hoc* Tukey's tests identifying within-treatment differences. Fouling data were transformed (Box-Cox) as they were not normally distributed or homogeneous (Shapiro-Wilk tests and QQ-plots). A significance level ( $\alpha$ ) of 0.05 was used for all analyses.

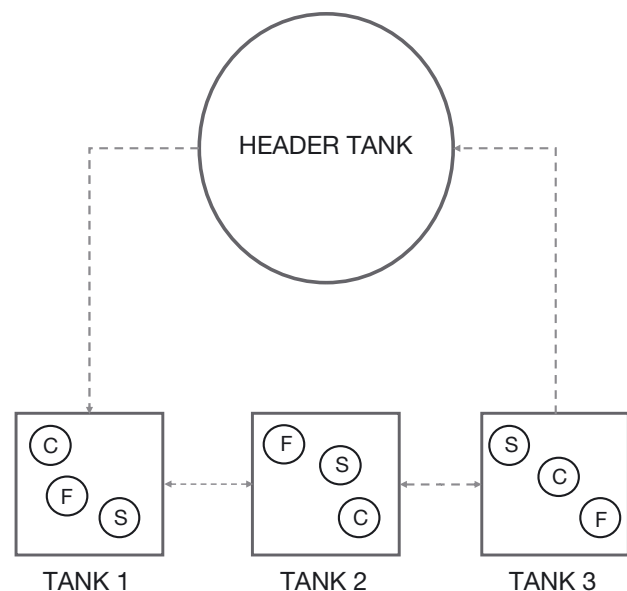


Fig. 3. Schematic showing the experimental layout used to test the effects of temperature and grazing on seagrass fouling. For each temperature treatment, header tanks containing seawater set to designated temperatures were circulated among 3 interconnected tanks, each of which contained seagrass with no grazers (C) and seagrass with *Fissurella mutabilis* (F) and *Siphonaria compressa* (S)

### 3. RESULTS

Temperature (ANOVA,  $F_{3,36} = 161.18$ ,  $p < 0.001$ ), grazing (ANOVA,  $F_{2,36} = 43.41$ ,  $p < 0.001$ ) and their interaction (ANOVA,  $F_{6,36} = 14.86$ ,  $p < 0.001$ ) significantly influenced microalgal fouling (Fig. 4A). Warming increased fouling in controls by up to 15 times at 30°C relative to the 18°C treatment. At the lowest temperature, grazing had no major effect on fouling, but grazer effects increased beyond the 22°C treatment, where *Fissurella mutabilis* and *Siphonaria compressa* presence decreased fouling by 10 and 20%, respectively, relative to controls. These effects were magnified at 26°C, with *F. mutabilis* and *S. compressa* removing 50 and 76% of microalgal fouling. Grazing by these limpets removed 15 and 37% of foul-

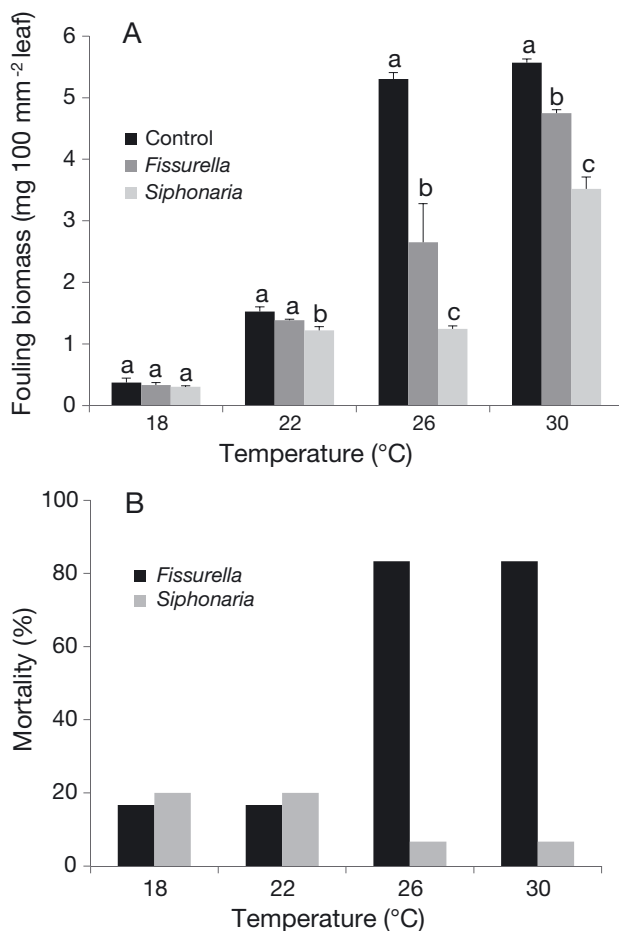


Fig. 4. (A) Variability in biomass (means  $\pm$  SE) of fouling epiphytes on *Zostera capensis* across temperature and grazing (*Fissurella mutabilis* and *Siphonaria compressa*) treatments. Different letters above bars indicate significant differences within temperature treatments. (B) Differences in limpet mortality across temperature treatments. NB: adult limpets were added to pots to offset mortality and maintain treatment densities

ing at 30°C. Warming had contrasting effects of mortality of *F. mutabilis* and *S. compressa* (Fig. 4B). The former was more vulnerable to warming, with mortality being roughly 80% at 26 and 30°C relative to a 16% mortality at 18 and 22°C. In contrast, *S. compressa* was more resilient to warming, with mortality ranging from 7 (26 and 30°C) to 20% (18 and 22°C).

### 4. DISCUSSION

Our findings shed light on the intricate ways in which ocean warming can influence grazer–epiphyte interactions in temperate seagrasses such as *Zostera capensis*. Our results show that in the absence of grazing, warming can elevate fouling of seagrasses, potentially shifting these systems towards a degraded, algal-dominated state in the long term. This is particularly concerning given that warming is likely to occur in parallel with coastal eutrophication, which is a key driver of catastrophic fouling in seagrasses (Baden et al. 2003, Campbell et al. 2017). In combination, eutrophication and warming may enhance seagrass stress levels by increasing epiphytic fouling loads. Such a shift to algal dominance can have major ecological and/or biological implications, mainly due to fouling depressing seagrass photosynthetic efficiency, growth rate and survival (Baden et al. 2003, Lee et al. 2007, Mvungi & Pillay 2019). At higher organisational levels, epiphytic fouling can reduce canopy cover through smothering, leading to the decimation of entire seagrass stands, and therefore losses in socio-ecological functions provided, including biodiversity support (Baden et al. 2003, Pillay et al. 2010).

However, our data indicate that warming-induced fouling can be attenuated by grazing, but the strength of this is species-specific, being likely dependent on grazer feeding specialisation and physiological tolerances to warming. In our study, the specialist eelgrass limpet *Siphonaria compressa* was clearly much more effective in controlling epiphytes than *Fissurella mutabilis*, especially under warming conditions. This is probably due to *S. compressa* being an obligate epiphytic grazer, with its co-evolution with *Z. capensis* (Allanson & Herbert 2005, Branch et al. 2010) leading to the development of specialist feeding traits adapted to grazing on epiphytes. In addition, its low mortality under high temperatures suggests that it has a high thermal tolerance, implying that its grazing impact is relatively robust against the warming conditions used in our study. Due to *F. mutabilis* not being a seagrass specialist but a hard substrate dweller (Branch

et al. 2010), it is probably less adapted for feeding on *Z. capensis* leaves, resulting in feeding constraints. This aspect, in conjunction with its low thermal tolerance (Fig. 2), likely explains the reduction in its effectiveness in controlling epiphytic growth at high temperatures. The contrasting thermal tolerances of limpets recorded in our experiment are in agreement with their *in situ* distributions, with *F. mutabilis* occurring predominantly in the cooler Oesterval site but with *S. compressa* occurring in Geelbek, which is warmer. Also apparent in Geelbek is the low levels of fouling of the seagrass blades, while levels at Oesterval are much higher. This provides circumstantial support for our experimental findings that *S. compressa* is more effective than *F. mutabilis* as an epiphytic grazer, especially at warmer temperatures. Lastly, it must be noted that the grazing effectiveness of *F. mutabilis* at high temperature is likely overestimated in this experiment due to the high number of dead individuals being replaced to maintain treatment densities.

It is worth recognising that the experimental design used in the present study is not ideal, particularly because it raises concerns regarding independence. This stems from the design of the recirculating system used in our study, in which water from each of 4 header tanks were circulated to 3 replicate tanks, from which biotic responses were assessed. Due to recirculation from the header tanks, there is theoretically the possibility that tanks were not independent. However, it is important to note that in experiments of this nature, header tanks are often used to recirculate water among connected mesocosms (Alsterberg et al. 2011, 2013, Eklöf et al. 2012), which is likely due to logistical constraints associated with fitting individual mesocosms with regulatory and monitoring equipment. It is also noteworthy that the increase in fouling that we recorded with increasing temperatures, has also been documented by Mvungi & Pillay (2019) in a later study on the performance of *Z. capensis* under warming conditions, which was based on the use of unconnected, independent mesocosms (i.e. without header tanks and re-circulating systems). Lastly, the significant interaction between temperature and grazing suggests that mesocosms were likely functionally independent as identical grazer treatments were used within each of the 9 tanks. In combination, these pieces of evidence suggest that the design used in our experiment may not invalidate our findings.

At a broader level, our results indicate that specialist grazers with high thermal tolerances could be important in mitigating warming effects in temper-

ate seagrass systems. As such, these grazers may need to be given conservation priority for warming-induced fouling to be managed. Given the warming trend observed in Langebaan Lagoon over the last 30 yr (Fig. 1), and the potential for warming to increase fouling levels, further protection of *S. compressa* and investment in restoration and/or expansion programs would be a critical component of the local seagrass management plan, given that *S. compressa* is a highly effective seagrass cleaner that can withstand warming conditions. In addition, *Z. capensis* transplantation programs aimed at restoring previously disturbed habitats may benefit from explicit inclusion of *S. compressa*. Transplantation programs that ignore the role of *S. compressa* as a grazer fail to harness the potential of reinforcing positive feedbacks that are robust against warming and that are likely to benefit both *Z. capensis* and *S. compressa*. This is particularly relevant locally given *S. compressa*'s Critically Endangered status. At a global scale, the identification of specialist, warm-adapted or -tolerant grazers and investment in their conservation and/or restoration may significantly improve survival of temperate seagrasses in the future, which in turn has positive implications for socio-ecological functions and service provision (Costanza et al. 1997, Orth et al. 2006). This may be an effective mitigation option because grazer conservation can be achieved at a local level through management intervention. Mitigating other warming stressors may not be as achievable at a local level given that these stressors are geographically pervasive and act over larger spatial scales.

In other hierarchically structured systems such as coral reefs, there has been significant interest in warm-tolerant species (e.g. mutualistic zooxanthellae) that benefit foundational ecosystems, based on the recognition that such species are central to the functioning and persistence of these systems under warming scenarios (Berkelmans & Van Oppen 2006). In contrast, this aspect has received little attention in seagrass ecosystems, where emphasis has generally been placed on maintenance of abiotic conditions required for seagrass persistence and proliferation, particularly by limiting anthropogenic disturbances locally. However, as indicated by our data, even in relatively pristine systems, warming may pose an important indirect threat to seagrasses by increasing microalgal proliferation. Under these conditions, conservation and management authorities may need to develop mitigatory strategies that extend beyond traditional approaches of limiting anthropogenic activities, including investing in grazer restoration programs.



Conservation research has, to a limited extent, focused on protecting sympatric species that are potentially linked to seagrass persistence (Hughes et al. 2009). In this regard, considerable attention has been afforded to charismatic vertebrates such as manatees and turtles, but scant attention has been paid to invertebrates, which are generally under-valued (Bracken et al. 2007). This has resulted in a poor appreciation of the ecological relevance of invertebrates in the persistence of seagrass ecosystems and hence, little support for their conservation. In this context, our findings provide important information on the ecological value of invertebrate grazers, particularly from a global change perspective, thus providing traction for their inclusion in seagrass conservation strategies. This is highly relevant at a local level, where the limpet *S. compressa* has received little conservation attention, despite its restricted distribution and it being South Africa's most endangered invertebrate (Allanson & Herbert 2005, Branch et al. 2010). In addition, knowledge of its general biology and causes of restriction is extremely limited, which in turn has major implications for seagrass conservation more broadly.

At its broadest level, this study emphasises the need to halt and reverse biotic homogenisation in seagrass ecosystems. In many marine systems, including seagrasses, chronic stressors from multiple human sectors has led to the rapid loss of biotic heterogeneity, leading to shifts from specialist to generalist assemblages (McKinney & Lockwood 1999). As shown by our data, past and/or future shifts to generalist grazer assemblages may be accompanied by losses of resilience against stressors such as fouling. Wilful management intervention is thus required to protect and restore specialist grazer assemblages, especially those that are robust against global change stressors. This notion is in line with calls for a halt to the shifting baseline syndrome (Pauly 1995), which occurs pervasively in marine ecosystems.

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#### LITERATURE CITED

- Allanson B, Herbert DG (2005) A newly discovered population of the critically endangered false limpet *Siphonaria compressa* Allanson, 1958 (Pulmonata: Siphonariidae), with observations on its reproductive biology. *S Afr J Sci* 101:95–97
- Alsterberg C, Hulth S, Sundbäck K (2011) Response of a shallow-water sediment system to warming. *Limnol Oceanogr* 56:2147–2160
- Alsterberg C, Eklöf JS, Gamfeldt L, Havenhand JN, Sundbäck K (2013) Consumers mediate the effects of experimental ocean acidification and warming on primary producers. *Proc Natl Acad Sci USA* 110:8603–8608
- Baden S, Gullström M, Lundén B, Pihl L, Rosenberg R (2003) Vanishing seagrass (*Zostera marina*, L.) in Swedish coastal waters. *Ambio* 32:374–377
- Baden S, Boström C, Tobiasson S, Arponen H, Moksnes PO (2010) Relative importance of trophic interactions and nutrient enrichment in seagrass ecosystems: a broad-scale field experiment in the Baltic–Skagerrak area. *Limnol Oceanogr* 55:1435–1448
- Berkelmans R, Van Oppen MJ (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proc R Soc Lond B Biol Sci* 273:2305–2312
- Borst AC, Verberk WC, Angelini C, Schotanus J and others (2018) Foundation species enhance food web complexity through non-trophic facilitation. *PLoS One* 13:e0199152
- Bracken MES, Gonzalez-Dorantes CA, Stachowicz JJ (2007) Whole-community mutualism: associated invertebrates facilitate a dominant habitat-forming seaweed. *Ecology* 88:2211–2219
- Branch GM, Griffiths CL, Branch ML, Beckley LE (2010) Two oceans. A guide to the marine life of southern Africa. Struik, Cape Town
- Bruno JF, Bertness MD (2001) Habitat modification and facilitation in benthic marine communities. In: MD Bertness, SD Gaines, ME Hay (eds) *Marine community ecology*. Sinauer Associates, Sunderland, MA, p 201–218
- Campbell JE, Altieri AH, Johnston LN, Kuempel CD, Paperno R, Paul VJ, Duffy JE (2017) Herbivore community determines the magnitude and mechanism of nutrient effects on subtropical and tropical seagrasses. *J Ecol* 106:401–412
- Costanza R, d'Arge R, De Groot R, Farber S and others (1997) The value of the world's ecosystem services and natural capital. *Nature* 387:253–260
- Eklöf JS, Alsterberg C, Havenhand JN, Sundbäck K, Wood HL, Gamfeldt L (2012) Experimental climate change weakens the insurance effect of biodiversity. *Ecol Lett* 15:864–872
- Hughes AR, Williams SL, Duarte CM, Heck KL, Waycott M (2009) Associations of concern: declining seagrasses and threatened dependent species. *Front Ecol Environ* 7: 242–246
- Jones CG, Lawton JH, Shachak M (1997) Positive and negative effects of organisms as physical ecosystem engineers. *Ecology* 78:1946–1957
- Lee K, Park SR, Kim YK (2007) Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *J Exp Mar Biol Ecol* 350:144–175
- McKinney ML, Lockwood JL (1999) Biotic homogenization: a few winners replacing many losers in the next mass extinction. *Trends Ecol Evol* 14:450–453
- Mvungi EF, Pillay D (2019) Eutrophication overrides warming as a stressor for a temperate African seagrass (*Zostera capensis*). *PLOS ONE* 14:e0215129
- Orth RJ, Carruthers TJ, Dennison WC, Duarte CM and others (2006) A global crisis for seagrass ecosystems. *BioScience* 56:987–996
- Östman Ö, Eklöf J, Eriksson BK, Olsson J, Moksnes PO, Bergström U (2016) Top-down control as important as

- nutrient enrichment for eutrophication effects in North Atlantic coastal ecosystems. *J Appl Ecol* 53:1138–1147
- ✦ Pauly D (1995) Anecdotes and the shifting baseline syndrome of fisheries. *Trends Ecol Evol* 10:430
- ✦ Pillay D, Branch GM, Griffiths CL, Williams C, Prinsloo A (2010) Ecosystem change in a South African marine reserve (1960–2009): role of seagrass loss and anthropogenic disturbance. *Mar Ecol Prog Ser* 415:35–48
- ✦ Sheppard C, Davy S, Pilling G, Graham N (2017) The biology of coral reefs. Oxford University Press, Oxford
- ✦ Short FT, Duarte CM (2001) Methods for the measurement of seagrass growth and production. In: FT Short, RG Coles (eds) *Global seagrass research methods*. Elsevier, Amsterdam, p 155–198
- ✦ Short FT, Polidoro B, Livingstone SR, Carpenter KE and others (2011) Extinction risk assessment of the world's seagrass species. *Biol Conserv* 144:1961–1971
- ✦ Svensson CJ, Baden S, Moksnes PO, Åberg P (2012) Temporal mismatches in predator–herbivore abundance control algal blooms in nutrient-enriched seagrass ecosystems. *Mar Ecol Prog Ser* 471:61–71
- ✦ van Montfrans J, Wetzel RL, Orth RJ (1984) Epiphyte–grazer relationships in seagrass meadows: consequences for seagrass growth and production. *Estuaries* 7:289–309
- ✦ Waycott M, Duarte CM, Carruthers TJ, Orth RJ and others (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proc Natl Acad Sci USA* 106:12377–12381

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