Effects of opportunistic algae on the early life history of a habitat-forming fucoid: influence of temperature, nutrient enrichment and grazing pressure

T. Alestra*, D. R. Schiel

Marine Ecology Research Group, School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

ABSTRACT: Fragmentation and loss of extensive populations of habitat-forming macroalgae have occurred worldwide as a result of human-driven coastal habitat degradation. Contributing to such declines may be the influence of opportunistic algae, which quickly respond to stresses such as nutrient loading and warmer sea temperatures. The early life stages of fucoids are particularly likely to be affected by the combined impacts of altered physical conditions and increased competition with stress-tolerant species. In this study, we examined the interactions between the early life stages of the intertidal fucoid *Hormosira banksii* and fast-growing, opportunistic green algae *Ulva* spp. Through a series of laboratory experiments, we tested (1) the impact of *Ulva* spp. on the settlement of *H. banksii* zygotes; (2) the impact of *Ulva* spp. on the rates of post-settlement survival and growth of *H. banksii* germlings under ambient and increased levels of temperature and nutrient concentration; (3) the interactions between the 2 taxa under grazing pressure of the abundant intertidal trochid *Lunella smaragdus*. *Ulva* spp. impaired the settlement of *H. banksii* zygotes, through both physical and chemical mechanisms, and the growth of the germlings; but *Ulva* spp. was also preferentially consumed by the grazers, thereby indirectly facilitating *H. banksii*. In addition, *Ulva* spp. responded positively to increased temperature and nutrients, while we found an indication that post-settlement survival of *H. banksii* may decline with warmer waters. These findings augment extensive field-based research addressing the loss of algal beds and shed light on biotic and physical mechanisms affecting the most critical phases of the life cycle of a key habitat-forming species.

KEY WORDS: Canopy · Competition · Ephemeral algae · Eutrophication · Germlings · Macroalgae · Temperature warming · *Ulva* spp.

INTRODUCTION

The structure and functioning of many marine ecosystems are shaped by the presence of either a single or a few habitat-forming species (Bruno & Bertness 2001). Well documented examples include canopy-forming macroalgae of the Order Fucales (fucoids), which support high levels of diversity and productivity on temperate intertidal rocky reefs worldwide, despite the extreme physical challenges of these habitats (Chapman 1995, Schiel 2006). It is not surprising, therefore, that the decline of large populations of fucoid algae reported worldwide, especially around urbanised areas, has caused great concern and stimulated extensive ecological research (Benedetti-Cecchi et al. 2001, Berger et al. 2004, Irving et al. 2009, Bellgrove et al. 2010).

In degraded environments, slow-growing perennial algae such as fucoids can be directly harmed by modified physical conditions (e.g. sedimentation,

*Corresponding author:
tommaso.alestra@pg.canterbury.ac.nz

eutrophication, chemical pollution) and by competition with stress-tolerant opportunistic species (Berger et al. 2004, Kraufvelin et al. 2006, Worm & Lotze 2006). Impacts on their early life stages are particularly likely to provide explanations for the regression of fucoid populations. These microscopic phases are known to suffer from high levels of mortality and represent a critical bottleneck in the development and persistence of adult stands (Vadas et al. 1992, Schiel & Foster 2006). Ephemeral algae can negatively affect the early demography of fucoids either by blocking the settlement of zygotes or by reducing the post-settlement survival rates of germlings (Worm et al. 1999, 2001, Berger et al. 2003, Isæus et al. 2004). Herbivory, however, may release fucoids from competition with opportunistic algae. In eutrophic environments, intense grazing pressure has been shown to facilitate weak competitors (i.e. perennial, slow-growing macroalgae) by preferentially consuming ephemeral species (Lubchenco 1983, Kim 1997, Worm et al. 1999, 2001).

Here, we examined the impacts of fast-growing, opportunistic algae, *Ulva* spp. (hereafter referred to as a single species, i.e. *Ulva*), on the early life history of the intertidal fucoid *Hormosira banksii*, which is a key structural component of intertidal algal assemblages in New Zealand and southeastern Australia. No other fucoid species in New Zealand is able to provide the same ecosystem services in mid-shore habitats (Schiel 2006), and there are long-lasting impacts on community diversity and productivity following *H. banksii* removal (Schiel & Lilley 2011, Tait & Schiel 2011). *Ulva* is a cosmopolitan genus comprising several species of fast-growing green algae commonly found in intertidal and estuarine habitats (Guiry & Guiry 2013). In eutrophic conditions, ulvoids can produce nuisance blooms, with detrimental ecological effects and serious economic consequences (Anderson et al. 1996, Valiela et al. 1997).

Building on earlier field-based work, we used a series of laboratory experiments to test the impact of *Ulva* on the early life history of *H. banksii*. These included tests of different levels of temperature, nutrients and grazing pressure on the interactions between the 2 species. *Ulva* is known to have allelopathic properties that inhibit zygote development (Nelson et al. 2003), so we began by testing whether such effects were evident on *H. banksii*.

We then tested the effects of the presence of *Ulva* on the rates of survival and growth of *H. banksii* in the first 6 wk of the post-settlement period. During this critical phase, the minute *H. banksii* germlings

may easily be overgrown and outcompeted by *Ulva*. Furthermore, increased nutrient concentration and rising seawater temperature may differentially affect the 2 species and their interactions. The abundance of fast-growing ephemeral species can be rapidly boosted by nutrient enrichment and warmer temperatures (Lotze et al. 1999, Lotze & Worm 2002, Steen 2004). The geographic range of numerous fucoids, in contrast, has shifted or retracted in response to water warming (Southward et al. 1995, Smale & Wernberg 2013), and there is experimental evidence linking these patterns to the thermal sensitivity of their early life stages (Andrews et al. 2014). In addition, the development of the early life stages of various fucoids, including *H. banksii*, can be impaired by elevated nutrient levels (Kevekordes 2001, Bergström et al. 2003). In combination with the presence or absence of *Ulva*, therefore, we also manipulated temperature and nutrient levels. We expected increased temperature and eutrophication to have a direct detrimental effect on the fitness of the germlings and to stimulate the growth of *Ulva*, thereby exacerbating its impact on *H. banksii*.

Finally, we assessed how the interactions between the 2 species can be modulated by the presence of the abundant intertidal trochid gastropod *Lunella smaragdus* (formerly *Turbo smaragdus*). This is one of the most abundant grazers in the intertidal zone of New Zealand, reaching densities of >100 individuals per m^2, and it is known to graze preferentially on ephemeral algal species (Walker 1998, Schiel 2006). In combination with presence or absence of *L. smaragdus*, we manipulated the density of *Ulva* to simulate the biotic consequences of eutrophication scenarios of increasing intensity. We expected to observe a negative impact of *Ulva*, proportional to its abundance, on the survival and growth of *H. banksii* germlings in the absence of grazing; a negative effect of *L. smaragdus* on both algal species, with a preference for *Ulva*; and, potentially, an indirect facilitative effect of *L. smaragdus* on *H. banksii* through the relaxation of competition with *Ulva*.

MATERIALS AND METHODS

The experiments were carried out in the facilities of the Edward Percival Field Station in Kaikoura, New Zealand. Settlers of the 2 agal species obtained from adult plants collected in the field were grown on 50 × 50 × 7 mm fibrolite plates. *Hormosira banksii* settlement density was not manipulated but was kept at ~300 cm^{-2} in all the experiments. These values are in

line with the settlement rates observed in the field in Kaikoura (Dunmore 2006). The plates were housed in plastic tanks (37 × 25 × 7 cm) filled with filtered (1 μm) and UV-sterilized seawater. Air pumps were used to aerate the tanks, and light racks provided illumination at an intensity of ~100 μmol m^{-2} s^{-1} under a 12 h light:12 h dark cycle. The plates were submerged throughout the duration of the experiments to exclude additional stress sources related to aerial exposure. A previous field-based study in Kaikoura showed a negative relationship between *H. banksii* germling survival and shore height, with 100% mortality in the high shore and increasing rates of survival at lower elevations (Dunmore 2006). By culturing *H. banksii* early life stages in benign and stable conditions, we were able to test the effects of the experimental factors more accurately.

Expt 1: Impact of *Ulva* on *H. banksii* zygote settlement

We compared the attachment of *H. banksii* zygotes experimentally settled on (1) unoccupied plates (control), (2) unoccupied plates housed in tanks previously occupied by *Ulva* (to test the impact of chemical alterations in the growth medium) and (3) plates 50% colonized by *Ulva* (to test the impact of substrate pre-emption). Each settlement condition was replicated in 2 tanks, each containing 4 plates.

Before the start of the experiment, 32 plates were distributed among 8 tanks (4 plates per tank), each filled with 4 l of seawater. The plates were soaked for 2 d, and successively, 4 of the tanks received 1 l of *Ulva* zooid solution, while 1 l of seawater was added to the remaining 4 to equalize the volume. All the plates were kept submerged at 17°C for 30 d, and the water was changed twice weekly. After 30 d, the plates seeded with *Ulva* were examined, and the percentage cover of *Ulva* was visually estimated using a 5 × 5 cm fine plastic grid divided into 25 subquadrats. Eight plates with 53.55 ± 5.55% (mean ± SE) cover of *Ulva* were selected to represent the treatment with 50% colonized substrates. These were reintroduced into 2 of the tanks originally seeded with *Ulva*. The remaining plates containing *Ulva* were removed from their tanks and replaced by 8 unoccupied plates. These constituted the treatment with unoccupied substrates housed in tanks previously occupied by *Ulva*. The remaining unoccupied plates (control) were not moved from their original tanks. After rearranging the substrates, 1 l of *H. banksii* zygote solution, obtained from ~20 adult plants, was added to each tank. After 24 h, all the plates were examined under a binocular microscope, and the number of settled zygotes was estimated in 3 haphazardly chosen 1 cm^2 areas.

Expt 2: Influence of temperature and nutrients on species interactions

We tested whether particular combinations of temperature and nutrient levels would favour *H. banksii* or *Ulva*. These factors were manipulated in crossed combination: (1) Temperature (17 vs. 20°C); (2) Nutrients (ambient vs. increased concentration) and (3) Culture type (*H. banksii* monocultures vs. mixed cultures).

We ran the experiment for 6 wk to assess the responses over a longer period of time compared to similar studies reported in the literature (Lotze & Worm 2002, Steen 2004). After 6 wk, however, it was no longer possible to culture *H. banksii* and *Ulva* in isolation, as other unwanted species (mainly microalgae) began to appear on the plates. The ambient sea water temperature, 17°C, was the maximum observed at Kaikoura during summer (Chiswell & Schiel 2001), the period of peak reproduction of *H. banksii*. The higher temperature, 20°C, was chosen in line with predictions by the IPCC (2007) for the end of the 21st century for New Zealand and Southern Australia. Increased nutrient concentrations were obtained through the addition of 20 μM N (nitrate and ammonia added at a ratio of 1:1) and 2 μM P (phosphate) per litre of seawater to match the values observed in eutrophic coastal areas around New Zealand (Barr 2007). Two temperature-controlled rooms equipped with independent thermostats were used to maintain the selected temperature levels throughout the experiment. It was not possible to allocate multiple rooms to the 2 temperature levels. The 2 rooms, however, were identical in all respects, and the experimental set up (i.e. the positions of tanks, lights and air pumps) did not differ between them. Within each room, 2 tanks were assigned to each possible combination of nutrient concentration and presence/absence of *Ulva*, with 4 plates in each tank.

Before the start of the experiment, each tank (16 in total) was filled with 4 l of seawater, and the plates were soaked for 2 d. One litre of *H. banksii* zygote solution, obtained from ~50 adult plants, was then gently poured over the plates in each tank. After 4 d, the water in each tank was replaced, and the initial abundance of *H. banksii* germlings was recorded.

The manipulation of temperature and nutrient levels was then initiated, and the tanks allocated to mixed cultures of *H. banksii* and *Ulva* received 1 l of *Ulva* zooid solution. The total volume of water was brought up to 5 l in each tank. The water was replaced twice weekly throughout the experiment, and nutrients were re-added to the +Nutrients tanks after each water change.

The plates were sampled after 2, 4 and 6 wk from the initial monitoring (4 d). At each sampling date, every plate was examined under a binocular microscope, and the number of *H. banksii* germlings was estimated as described for Expt 1. Percentage survival of *H. banksii* germlings was expressed as the proportional difference in the number of germlings in relation to the abundance recorded in the initial sampling. At the end of the experiment, the mean size of the germlings was calculated from 10 individuals randomly selected from each plate. These were detached from the plates and measured lengthwise using an eyepiece graticule. The growth of *Ulva* was also monitored throughout the experiment, with values of percentage cover recorded at each sampling date. In addition, the relative interaction index (RII; Armas et al. 2004) was calculated based on *H. banksii* germling percentage survival data recorded at each sampling date and used to describe the intensity and direction of the interactions between the 2 species under different combinations of temperature and nutrient levels. The RII is expressed as RII = $(Bw - Bo)/(Bw + Bo)$, where Bw and Bo represent the performance (percentage survival in this study) of the target species (*H. banksii*), with or without neighbours (*Ulva*), respectively. The RII ranges between 1 and −1, with negative values indicating competition and positive values showing facilitation. In this experiment, *H. banksii* survival rates in the presence of *Ulva* (Bw), recorded from the plates assigned to mixed cultures of the 2 species, were contrasted with mean values of Bo, calculated by averaging the survival rates of *H. banksii* germlings in the monocultures, separately for each combination of temperature and nutrient levels.

Expt 3: Influence of grazing on species interactions

We tested the impact of grazing by *Lunella smaragdus* on *H. banksii* germlings grown in isolation and in mixed cultures with various densities of *Ulva*. The experiment involved 2 factors: Grazing (present vs. absent) and Culture type (*H. banksii* monocul-

tures vs. mixed cultures with low and high density of *Ulva*). Each experimental condition was replicated in 2 tanks, each containing 4 plates.

Six medium-sized *L. smaragdus* (average shell length: 22.17 ± 0.41 mm) were collected and allowed to acclimatise to laboratory conditions for 2 wk before being introduced into the experimental tanks. Before the start of the experiment, the plates were soaked for 48 h in 4 l of seawater. One litre of *H. banksii* zygote solution, obtained from ~40 adult plants, was then added to each tank. The zygotes were allowed to settle and develop for 4 d before the first water change, which was followed by the inoculation of *Ulva*. Differing densities of *Ulva* were achieved by seeding the experimental tanks with different volumes of *Ulva* zooid solution. One litre of *Ulva* zooid solution was added to 4 tanks to obtain mixed cultures with a high density of *Ulva*, and 4 tanks were seeded with 0.5 l of the solution to create mixed cultures with a low density of *Ulva*. The total volume of water was then brought up to 5 l in each tank. The cultures were grown at 17°C for 30 d to allow the development of *Ulva*. The water was replaced twice weekly. After 30 d, the number of *H. banksii* germlings and percentage cover of *Ulva* were recorded. After this initial period, we started the experiment by adding 1 specimen of *L. smaragdus* to half of the experimental tanks assigned to each culture type.

The abundance of *H. banksii* germlings was monitored 2, 4 and 6 wk after the introduction of the grazers, and their percentage survival was estimated relative to the values recorded in the initial sampling. Ten *H. banksii* individuals from each plate were measured at the end of the experiment. Variations in the percentage cover of *Ulva* in relation to the initial values were also calculated at each sampling date. *H. banksii* germling percentage survival data were used to calculate the RII, describing species interactions relative to *Ulva* density and grazing pressure. Here, Bo represented the average percentage survival of *H. banksii* germlings in the absence of *Ulva*, calculated separately for the 2 levels of grazing pressure.

Statistical analyses

Zygote abundance data from Expt 1 were analysed using a 2-way ANOVA with the fixed factor Settlement Conditions (control vs. growth medium alteration vs. substrate pre-emption) and the random factor Tank (nested within Settlement Conditions).

Data of percentage survival and size of *H. banksii* germlings and the percentage cover of *Ulva* recorded at the conclusion of Expt 2 were analysed using a 4-way ANOVA with the fixed factors: (1) Temperature (17°C vs. 20°C); (2) Nutrients (ambient vs. increased concentration); (3) Culture type (*H. banksii* monocultures vs. mixed cultures) and (4) the random factor Tank (nested within Temperature, Nutrients and c Culture type). RII values relative to the final sampling date were analysed with a 3-way ANOVA including the same crossed combinations of the factors Temperature and Nutrients and the random factor Tank.

Data of percentage survival and size of *H. banksii* germlings and percentage cover variation of *Ulva* recorded at the conclusion of Expt 3 were analysed using a 3-way ANOVA with the fixed factors: (1) Grazing (present vs. absent); (2) Culture type (*H. banksii* monocultures vs. mixed cultures with low density of *Ulva* vs. mixed cultures with high density of *Ulva*) and (3) the random factor Tank (nested within Grazing and Culture type). RII values relative to the final sampling date were analysed with a 3-way ANOVA, including the fixed factors: (1) Grazing (present vs. absent); (2) Culture type (mixed cultures with low density of *Ulva* vs. mixed cultures with high density of *Ulva*) and (3) the random factor Tank.

When the variability among tanks was negligible (p > 0.25) pooling procedures were applied in accordance with to Underwood (1997). Cochran's *C*-tests were used to test for homogeneity of variances before each analysis. No transformation was required for any of the response variables. Student-Newman-Keuls (SNK) tests were performed for *a posteriori* comparisons of the means (Underwood 1997).

RESULTS

Expt 1: Impact of *Ulva* on *Hormosira banksii* zygote settlement

There were significant differences among treatments in the initial attachment of *H. banksii* zygotes ($F_{2,21} = 58.75$, p < 0.001, tested against the pooled term Tank + Residual; Fig. 1), resulting from allelopathy and space pre-emption. The average number of successfully settled *H. banksii* zygotes was ~300 cm^{-2} in the control tanks, whereas just over half that number attached where the medium had been previously occupied by *Ulva*, and about one-third that number attached when there was a 50% cover of *Ulva*.
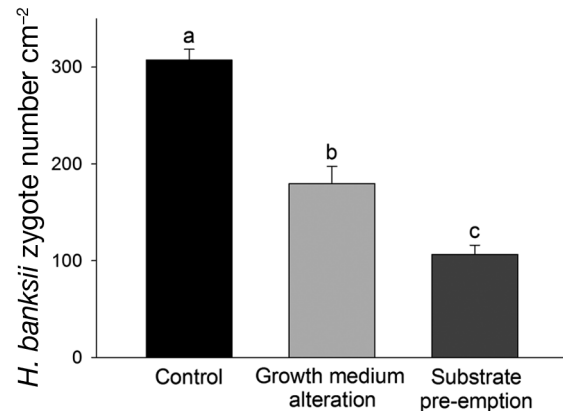

Fig. 1. Mean abundance (+1 SE) of attached *Hormosira banksii* zygotes recorded after 24 h on unoccupied plates (Control), unoccupied plates housed in tanks previously occupied by *Ulva* spp. (growth medium alteration) and plates 50% colonized by *Ulva* (substrate pre-emption); n = 8. The letters above the bars indicate the ranking of the means according to the SNK tests

Expt 2: Influence of temperature and nutrients on species interactions

At the start of the experiment, the mean ± SE number of germlings was 306.07 ± 12.35 cm^{-2} (n = 60). After 6 wk, the survival of *H. banksii* germlings was not significantly affected by temperature, nutrient levels or the presence of *Ulva* (Table 1A). Although 88.16 ± 3.05% of germlings survived at 17°C and 76.92 ± 3.04% at 20°C, these apparent differences were not significant ($F_{1,8} = 5.19$, p = 0.052). *H. banksii* germling sizes, however, were affected by *Ulva* (Table 1B, Fig. 2A). The germlings grown alone were significantly larger than those grown with *Ulva*, indicating an inhibitive interaction.

The growth of *Ulva* was stimulated by nutrient enrichment in the mixed cultures, with a clear separation of treatments becoming evident after 4 wk (Fig. 3B). Unexpectedly, *Ulva* also appeared in the *H. banksii* monocultures at ~4 wk and maintained percentage covers between 40 and 47% in the 17°C + Nutrients treatment but near zero in all others (Fig. 3A). This contamination was of unknown origin, but it may have occurred during the water changes. At the end of the experiment, however, *Ulva* was more abundant in the mixed cultures for all the combinations of temperature and nutrient levels. In the mixed cultures, its percentage cover was greater in the +Nutrients treatments and highest at 20°C, after 6 wk (Table 1C, Fig. 3A,B).

Throughout the experiment, the RII values were always close to zero, indicating the absence of either positive or negative impacts of *Ulva* on *H. banksii*

Table 1. ANOVA of percentage survival and size of *Hormosira banksii* germlings and percentage cover of *Ulva* spp. recorded at the final sampling date (6 wk) of Expt 2 in different culture types (*H. banksii* monocultures vs. mixed cultures) and under various temperature (17 vs. 20°C) and nutrient (ambient vs. increased concentration) levels. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

| Source of variation	df	A) *H. banksii* germling % survival		B) *H. banksii* germling size (µm)		C) *Ulva* % cover	
		MS	F	MS	F	MS	F
Temperature (T)	1	2013.77	5.19	156.25	0.01	284.77	1.20
Nutrients (N)	1	656.64	1.69	61256.25	4.56	8212.89	34.70***
Culture type (CT)	1	213.89	0.55	1035306.25	77.14***	17989.52	76.01***
T × N	1	172.27	0.44	56.25	0.00	37.52	0.16
T × CT	1	87.89	0.23	17117.80	1.28	2150.64	9.09*
N × CT	1	337.64	0.87	10850.35	0.81	1147.52	4.85
T × N × CT	1	337.64	0.87	42368.05	3.16	2413.27	10.20*
Tank (T × N × CT)	8	388.33	1.39	13421.22	4.41***	236.67	3.28**
Residual	48	279.48		3105.81		72.22	
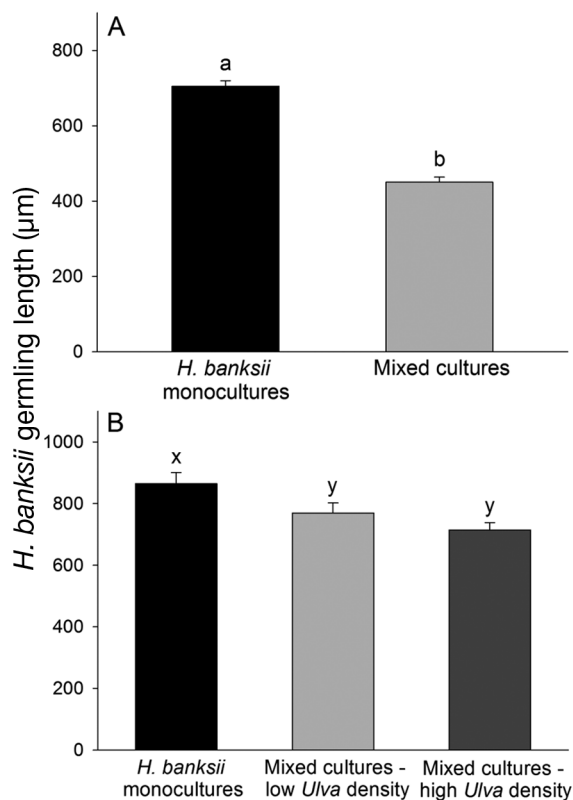
Fig. 2. Mean length (+1 SE) of *Hormosira banksii* germlings in (A) *H. banksii* monocultures and mixed cultures with *Ulva* spp. combined across all levels of temperature and nutrient concentration (n = 32), recorded at the end of Expt 2 (after 6 wk); (B) *H. banksii* monocultures and mixed cultures with low and high density of *Ulva* combined across all levels of grazing (n = 16), recorded at the end of Expt 3 (after 6 wk). The letters above the bars indicate the ranking of the means according to the SNK tests
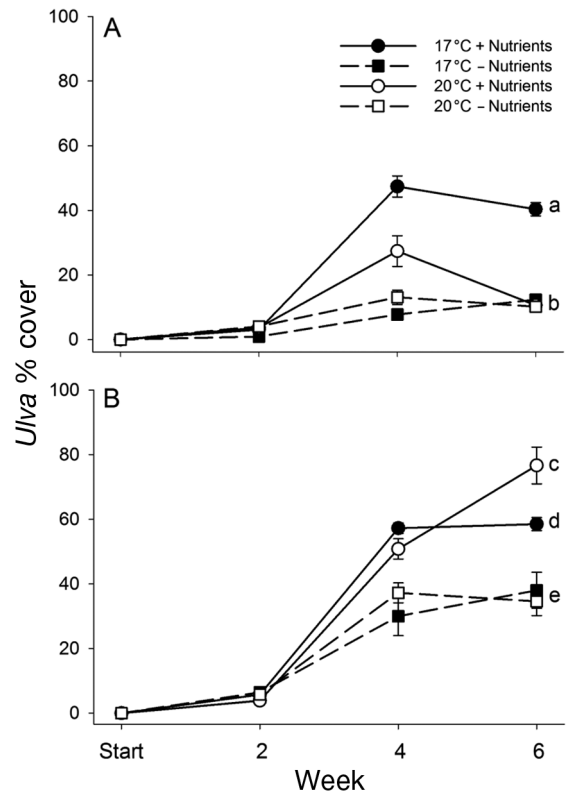

Fig. 3. Temporal variation in the mean percentage cover (±1 SE) of *Ulva* spp. in (A) *Hormosira banksii* monocultures and (B) mixed cultures under both ambient and increased levels of temperature and nutrient concentration (n = 8). The letters beside symbols show the ranking of the means according to the SNK tests for the final sampling date and allow comparisons of percentage cover values across graphs

Expt 3: Influence of grazing on species interactions

At the start of the experiment, the average number of germlings was 297.22 ± 12.58 cm^{-2} (n = 48). In the absence of *Ulva*, *Lunella smaragdus* rapidly compromised the survival of *H. banksii* to ~20% after 6 wk

germling survival. After 6 wk, the RII ranged from −0.06 ± 0.04 to 0.05 ± 0.06 and did not vary significantly with temperature ($F_{1,4} = 0.26$, p = 0.63) or nutrients ($F_{1,4} = 1.17$, p = 0.34).
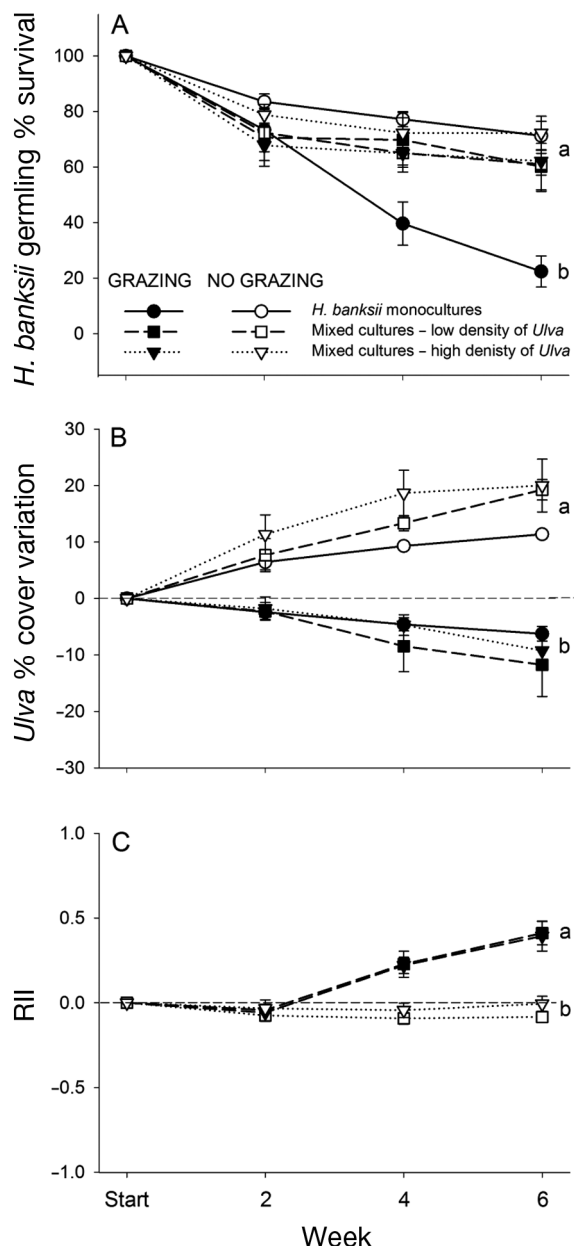
Fig. 4. Temporal variation, both in the presence and absence of *Lunella smaragdus*, in the mean values of (A) percentage survival (±1 SE) of *Hormosira banksii* germlings grown in mono- and mixed cultures with low and high density of *Ulva* spp. (n = 8); (B) percentage cover variation (±1 SE) of *Ulva* in the 3 different culture types (n = 8); (C) Relative interaction index (RII) (±1 SE) in mixed cultures with low and high density of *Ulva* (n = 8). The letters beside symbols show the ranking of the means according to the SNK tests for the final sampling date

(Grazing × Culture Type: $F_{1,42} = 6.52$, p < 0.01, tested against the pooled term Tank + Residual; Fig. 4A). All other treatments showed similar survival of *H. banksii* between 60 and 72%, indicating that *H. banksii* had protection from herbivory through the association

with *Ulva*. As in Expt 2, the size of the germlings was significantly larger when *H. banksii* was grown in isolation compared to the tanks with high and low densities of *Ulva*, across both levels of grazing pressure (Culture Type: $F_{2,42} = 5.77$, p < 0.01, tested against the pooled term Tank + Residual; Fig. 2B).

At the start of the experiment, the percentage cover of *Ulva* was 39.37 ± 3.80% and 65.84 ± 4.94% for the cultures with low and high density of *Ulva*, respectively. As happened in Expt 2, *Ulva* was also present in *H. banksii* monocultures, although with low coverage (9.24 ± 0.71%). Throughout the experiment, variations in the cover of *Ulva* were strongly influenced by grazers, regardless of the initial abundance of *Ulva*, with negative values recorded in the presence of *L. smaragdus* and positive values in its absence (Grazing: $F_{1,42} = 87.17$, p < 0.001, tested against the pooled term Tank + Residual; Fig. 4B). At the end of the experiment, the percentage cover of *Ulva* in the monocultures was reduced to very low values (2.92 ± 0.75%) when exposed to the action of the grazers, while it reached 20.52 ± 0.37% in the absence of *L. smaragdus*. Without grazing, *Ulva* cover increased to 65.73 ± 2.01% and 83.37 ± 3.90% in the mixed cultures with low and high density of *Ulva*, respectively. These same culture types had values of *Ulva* percentage cover equal to 20.54 ± 5.47% and 57.13 ± 10.61%, respectively, when *L. smaragdus* was present.

Throughout the experiment, RII values were always close to zero in the absence of the grazers (Fig. 4C). When *L. smaragdus* was present, however, the RII assumed positive values in both culture types (~0.45 after 6 wk; Grazing: $F_{1,28} = 51.80$, p < 0.001, tested against the pooled term Tank + Residual; Fig. 4C), which indicates a facilitative action of *Ulva* toward *H. banksii* germlings.

DISCUSSION

Our results provide a mechanistic understanding of initial conditions for attachment, settlement and growth which are likely to set the trajectory for the development of fucoid populations. We showed that common, opportunistic algae may benefit from high nutrients and temperature increase and have suppressive effects on the settlement and growth of a dominant fucoid. However, we also observed indirect facilitation of the perennial fucoid by the ephemeral algae moderated through preferential grazing.

Ulva may affect *Hormosira banksii* from the earliest stages of its life cycle, with chemical inhibition

compounding the impact of pre-emption of space. Zygote settlement was reduced by 42% compared to controls when there had been a prior presence of *Ulva*, while the lowest levels of settlement occurred when *Ulva* was present on the plates (−66% compared to the controls). This type of pre-emption of space by ephemeral algae interfering with the attachment of fucoid zygotes has been described in other studies (Worm et al. 1999, 2001, Berger et al. 2003, Isæus et al. 2004). In contrast, when unoccupied plates were translocated into tanks previously occupied by *Ulva*, the zygotes were probably affected by the legacy of chemical alteration left in the culturing medium by *Ulva* after its removal. The ability of ulvoids to produce and release allelochemicals is increasingly recognized (Nelson et al. 2003). These products often degrade rapidly (Jin & Dong 2003, Wang et al. 2007), but here we showed that they potentially affect *H. banksii* in the first 24 h of its life cycle even without a continuous secretion from fresh tissue. Similarly, Nelson et al. (2003) described the impact of the toxic properties of extracts from the green algae *Ulva fenestrata* and *Ulvaria obscura* on the development of *Fucus gardneri* zygotes.

In our 6 wk experiments, it was not survival but growth that was greatly compromised by the presence of *Ulva*. In one sense, this is surprising, given that there were allelopathic effects on attachment in Expt 1. If allelopathy were a major influence, it might be expected to also compromise the survival of settlers, but this was not evident in our study. Instead, there was suppressed growth of *H. banksii* in the presence of *Ulva*. This was probably due to nutrient depletion (Fong et al. 1993) and light attenuation (Brun et al. 2003) rather than chemical inhibition. The high tolerance shown by *H. banksii* germlings to interspecific competition with *Ulva* is remarkable since other studies of similar or shorter duration have described strong impacts of ephemeral species on post-settlement survival of the early life stages of various species of fucoids, both in field (Berger et al. 2003, Isæus et al. 2004) and laboratory contexts (Steen 2004). Reduced growth rates, however, may represent a serious handicap for a slow-growing species like *H. banksii*, with important implications for population persistence and replenishment. Living on the substratum, small germlings face challenging physical (e.g. sediment scouring and burial, canopy shading and sweeping) and biotic conditions (e.g. competition with low-lying algal mats and turfs, invertebrate grazing) that generally have only a minor influence on adults (Vadas et al. 1992, Schiel & Foster 2006). As a consequence, delayed develop-

ment and prolonged exposure to the rigours of benthic life may well result in increased mortality through time in natural conditions.

Our study also highlighted the importance of biotic and abiotic influences in regulating the outcome of the interaction between the 2 species. The herbivorous snail preferentially grazed on *Ulva* and, as a result, was diverted from *H. banksii*. Grazers are known to exert a strong top-down control on ephemeral species, which can indirectly facilitate the establishment of late successional, perennial macroalgae (Lubchenco 1983, Kim 1997, Worm et al. 1999, 2001). Our results are consistent with this large body of evidence and also indicate that the interactions between perennial and ephemeral algae may not necessarily be always negative. When competition affects growth but not mortality, species can simultaneously compete with and facilitate each other (Bruno et al. 2003). Even if the association with *Ulva* led to a competitive cost for *H. banksii*, attested by reduced growth rates, the reduction of consumption was an obvious advantage. Associational defence in marine and terrestrial habitats usually occurs when the focal species is surrounded by unpalatable neighbours (Hay 1986, Barbosa et al. 2009), but in this case, *Ulva* acted as a sink for the herbivores, and *H. banksii* benefitted from its elevated palatability. These results amplify the findings of field-based experiments in which *Lunella smaragdus* showed a marked preference for other ephemeral, disturbance-oriented species, like the brown algae *Adenocystis utricularis* and *Colpomenia sinuosa*, but had only minor effects on *H. banksii* (Walker 1998, Schiel 2006).

Increased temperature and nutrient levels synergistically enhanced the growth of *Ulva*. Similar results were found in other laboratory studies (Lotze et al. 1999, Lotze & Worm 2002, Steen 2004) and are consistent with the literature describing the association between coastal eutrophication and the rapid spread of opportunistic species leading to dramatic floristic changes (Valiela et al. 1997, Worm et al. 1999, Worm & Lotze 2006). In addition, we also observed a negative trend in *H. banksii* survival rates in response to temperature increase, although this was not statistically significant. Nonetheless, such findings are consistent with the results of further laboratory experiments in which we recorded reduced levels of survival for the germlings of *H. banksii* and 2 other fucoids (*Cystophora torulosa* and *Durvillaea antarctica*) when exposed to a temperature increase of 3°C during the initial 8 wk of their life cycle (T. Alestra & D. R. Schiel unpubl. data). Similarly, recent

field observations and laboratory experiments have shown that the juvenile stages of the fucoid *Scytothalia dorycarpa* have a narrow optimal temperature window and are severely affected by temperatures just 2°C above this range (Andrews et al. 2014).

Collectively, our experiments indicate that the strength and direction of fucoid-ephemeral interactions may vary temporally and spatially in response to environmental conditions and herbivore pressure. Altered physical conditions may potentially shift the balance of the interactions between the 2 species in favour of *Ulva*. Based on our results, an increased competitive impact of *Ulva* under high temperature and nutrient levels is likely to be associated with a reduced fitness of *H. banksii* germlings in warmer waters. Such a combination of long-term climatic changes and anthropogenic nutrient loading is particularly likely to affect sheltered coastal areas (Schiel 2013), which represent favourable habitats for many ephemeral species (Berger et al. 2003) as well as for *H. banksii* (Morton & Miller 1968) and for numerous other species of fucoids (Blanchette et al. 2000). Herbivory may release *H. banksii* from the competition with *Ulva*, and the latter species, in the presence of the grazers, may turn out to be an unexpected ally for the fucoid. As *L. smargdus* is by far the dominant grazer in southern New Zealand algal beds, the effects seen in the laboratory are likely to be relevant in natural contexts. In the field, however, the impacts of multiple anthropogenic influences on herbivore populations have the potential both to reduce and increase the importance of the ecological role of herbivores. For example, human exploitation of intertidal grazers has strong and well documented effects on the structure of algal communities (Castilla 1999, Aguilera & Navarrete 2007). Grazers′ metabolism and rates of consumption, on the other hand, are expected to increase under altered climate regimes (Hillebrand et al. 2009, O′Connor 2009).

It is clearly impossible to incorporate great natural complexity into short-term investigations carried out in artificial contexts. Laboratory experiments, however, allow manipulations difficult to perform in the field and provide the opportunity to identify the contribution of selected factors in isolation and to estimate their effects on target organisms. Given the ever increasing human pressure on coastal areas worldwide (Halpern et al. 2008), it is particularly important to have a mechanistic understanding of how organisms respond to altered physical and biotic conditions (Vinebrooke et al. 2004, Crain et al. 2008). In this regard, the limited knowledge of the factors affecting the early demography of numerous key

habitat-forming species represents a serious obstacle to the identification of the causes of their decline and the development of restoration strategies (Schiel & Foster 2006). Laboratory experiments are powerful tools to fill this knowledge gap (Irving et al. 2009), and the relevance of our findings may extend beyond the individual case of *H. banksii*, providing useful insights for other investigations.

LITERATURE CITED

Aguilera MA, Navarrete SA (2007) Effects of *Chiton granosus* (Frembly, 1827) and other molluscan grazers on algal succession in wave exposed mid-intertidal rocky shores of central Chile. J Exp Mar Biol Ecol 349:84−98

Anderson RJ, Monteiro PMS, Levitt GJ (1996) The effect of localised eutrophication on competition between *Ulva lactuca* (Ulvaceae, Chlorophyta) and a commercial resource of *Gracilaria verrucosa* (Gracilariaceae, Rhodophyta). Hydrobiologia 326-327:291−296

Andrews S, Bennett S, Wernberg T (2014) Reproductive seasonality and early life temperature sensitivity reflect vulnerability of a seaweed undergoing range reduction. Mar Ecol Prog Ser 495:119−129

Armas C, Ordiales R, Pugnaire FI (2004) Measuring plant interactions: a new comparative index. Ecology 85: 2682−2686

Barbosa P, Hines J, Kaplan I, Martinson H, Szczepaniec A, Szendrei Z (2009) Associational resistance and associational susceptibility: having right or wrong neighbours. Annu Rev Ecol Evol Syst 40:1−20

Barr NG (2007) Aspects of nitrogen metabolism in the green alga *Ulva*: developing an indicator of seawater nitrogen loading. PhD dissertation, University of Auckland

Bellgrove A, McKenzie P, McKenzie J, Sfiligoj B (2010) Restoration of the habitat-forming fucoid alga *Hormosira banksii* at effluent-affected sites: competitive exclusion by coralline turfs. Mar Ecol Prog Ser 419:47−56

Benedetti-Cecchi L, Pannacciulli F, Bulleri F, Moschella PS, Airoldi L, Relini G, Cinelli F (2001) Predicting the consequences of anthropogenic disturbance: large-scale effects of loss of canopy algae on rocky shores. Mar Ecol Prog Ser 214:137−150

Berger R, Henriksson E, Kautsky L, Malm T (2003) Effects of filamentous algae and deposited matter on the survival of *Fucus vesiculosus* L. germlings in the Baltic Sea. Aquat Ecol 37:1−11

Berger R, Bergström L, Granéli E, Kautsky L (2004) How does eutrophication affect different life stages of *Fucus vesiculosus* in the Baltic Sea?—a conceptual model. Hydrobiologia 514:243−248

Bergström L, Berger R, Kautsky L (2003) Negative direct effects of nutrient enrichment on the establishment of *Fucus vesiculosus* in the Baltic Sea. Eur J Phycol 38: 41–46

Blanchette CA, Thornber C, Gaines SD (2000) Effects of wave exposure on intertidal fucoid algae. Proc Fifth California Islands Symp 5:347–355

Brun FG, Vergara JJ, Navarro G, Hernández I, Pérez-Lloréns JL (2003) Effect of shading by *Ulva rigida* canopies on growth and carbon balance of the seagrass *Zostera noltii.* Mar Ecol Prog Ser 265:85–96

Bruno JF, Bertness MD (2001) Habitat modification and facilitation in benthic marine communities. In: Bertness MD, Hay ME, Gaines SD (eds) Marine community ecology. Sinauer Associates, Sunderland, MA, p 201–218

Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. Trends Ecol Evol 18: 119–125

Castilla JC (1999) Coastal marine communities: trends and perspectives from human-exclusion experiments. Trends Ecol Evol 14:280–283

Chapman ARO (1995) Functional ecology of fucoid algae: twenty-three years of progress. Phycologia 34:1–32

Chiswell SM, Schiel DR (2001) Influence of along-shore advection and upwelling on coastal temperature at Kaikoura Peninsula, New Zealand. NZ J Mar Freshw Res 35: 307–317

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. Ecol Lett 11:1304–1315

Dunmore RA (2006) Demography of early life stages of habitat-forming intertidal fucoid algae. PhD dissertation, University of Canterbury, Christchurch

Fong P, Donohoe RM, Zedler JB (1993) Competition with macroalgae and benthic cyanobacterial mats limits phytoplankton abundance in experimental microcosms. Mar Ecol Prog Ser 100:97–102

Guiry MD, Guiry GM (2013) AlgaeBase. National University of Ireland, Galway, www.algaebase.org (accessed 27 March 2013)

Halpern BS, Walbridge S, Selkoe KA, Kappel CV and others (2008) A global map of human impact on marine ecosystems. Science 319:948–952

Hay ME (1986) Associational plant defenses and the maintenance of species diversity: turning competitors into accomplices. Am Nat 128:617–641

Hillebrand H, Borer ET, Bracken MES, Cardinale BJ and others (2009) Herbivore metabolism and stoichiometry each constrain herbivory at different organizational scales across ecosystems. Ecol Lett 12:516–527

IPCC (Intergovernmental Panel on Climate Change) (2007) Climate change 2007: the physical science basis. In: Solomon S, Qin D, Manning M, Chen Z and others (eds) Contribution of Working Group I to the Fourth Assessment. Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Irving AD, Balata D, Colosio F, Ferrando GA, Airoldi L (2009) Light, sediment, temperature, and the early life-history of the habitat-forming alga *Cystoseira barbata.* Mar Biol 156:1223–1231

Isæus M, Malm T, Persson S, Svensson A (2004) Effects of filamentous algae and sediment on recruitment and survival of *Fucus serratus* (Phaeophyceae) juveniles in the eutrophic Baltic Sea. Eur J Phycol 39:301–307

Jin Q, Dong S (2003) Comparative studies on the allelopathic effects of two different strains of *Ulva pertusa* on *Heterosigma akashiwo* and *Alexandrium tamarense.* J Exp Mar Biol Ecol 293:41–55

Kevekordes K (2001) Toxicity tests using developmental stages of *Hormosira banksii* (Phaeophyta) identify ammonium as a damaging component of secondary treated sewage effluent discharged into Bass Strait, Victoria, Australia. Mar Ecol Prog Ser 219:139–148

Kim JH (1997) The role of herbivory, and direct and indirect interactions, in algal succession. J Exp Mar Biol Ecol 217: 119–135

Kraufvelin P, Moy FE, Christie H, Bokn TL (2006) Nutrient addition to experimental rocky shore communities revisited: delayed responses, rapid recovery. Ecosystems 9: 1076–1093

Lotze HK, Worm B (2002) Complex interactions of climatic and ecological controls on macroalgal recruitment. Limnol Oceanogr 47:1734–1741

Lotze HK, Schramm W, Schories D, Worm B (1999) Control of macroalgal blooms at early developmental stages: *Pilayella littoralis* versus *Enteromorpha* spp. Oecologia 119:46–54

Lubchenco J (1983) *Littorina* and *Fucus*: effects of herbivores, substratum heterogeneity, and plant escapes during succession. Ecology 64:1116–1123

Morton JE, Miller MC (1968) The New Zealand sea shore. Collins, London

Nelson TA, Lee DJ, Smith BC (2003) Are 'green tides' harmful algal blooms? Toxic properties of water-soluble extracts from two bloom-forming macroalgae, *Ulva fenestrata* and *Ulvaria obscura* (Ulvophyceae). J Phycol 39: 874–879

O'Connor MI (2009) Warming strengthens an herbivore–plant interaction. Ecology 90:388–398

Schiel DR (2006) Rivets or bolts? When single species count in the function of temperate rocky reef communities. J Exp Mar Biol Ecol 338:233–252

Schiel DR (2013) The other 93%: trophic cascades, stressors and managing coastlines in non-marine protected areas. N Z J Mar Freshw Res 47:374–391

Schiel DR, Foster MS (2006) The population biology of large brown seaweeds: ecological consequences of multiphase life histories in dynamic coastal environments. Annu Rev Ecol Evol Syst 37:343–372

Schiel DR, Lilley SA (2011) Impacts and negative feedbacks in community recovery over eight years following removal of habitat-forming macroalgae. J Exp Mar Biol Ecol 407:108–115

Smale DA, Wernberg T (2013) Extreme climatic event drives range contraction of a habitat-forming species. Proc R Soc Lond B 280:20122829

Southward AJ, Hawkins SJ, Burrows MT (1995) 70 years' observations of changes in distribution and abundance of zooplankton and intertidal organisms in the western English Channel in relation to rising sea temperature. J Therm Biol 20:127–155

Steen H (2004) Interspecific competition between *Enteromorpha* (Ulvales: Chlorophyceae) and *Fucus* (Fucales: Phaeophyceae) germlings: effects of nutrient concentration, temperature, and settlement density. Mar Ecol Prog Ser 278:89–101

Tait LW, Schiel DR (2011) Legacy effects of canopy disturbance on ecosystem functioning in macroalgal assemblages. PLoS ONE 6:e26986

Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge

Vadas RL, Johnson S, Norton TA (1992) Recruitment and mortality of early post-settlement stages of benthic algae. Br Phycol J 27:331–351

Valiela I, McClelland J, Hauxwell J, Behr PJ, Hersh D, Foreman K (1997) Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnol Oceanogr 42:1105–1118

Vinebrooke RD, Cottingham KL, Norberg J, Scheffer M, Dodson SI, Maberly SC, Sommer U (2004) Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos 104: 451–457

Walker W (1998) Grazing in the intertidal zone: effects of the herbivorous *Turbo smaragdus* on macroalgal assemblages. MSc dissertation, University of Canterbury, Christchurch

Wang Y, Yu Z, Song X, Tang X, Zhang S (2007) Effects of macroalgae *Ulva pertusa* (Chlorophyta) and *Gracilaria lemaneiformis* (Rhodophyta) on growth of four species of bloom-forming dinoflagellates. Aquat Bot 86:139–147

Worm B, Lotze HK (2006) Effects of eutrophication, grazing, and algal blooms on rocky shores. Limnol Oceanogr 51: 569–579

Worm B, Lotze HK, Boström C, Engkvist R, Labanauskas V, Sommer U (1999) Marine diversity shift linked to interactions among grazers, nutrients and propagule banks. Mar Ecol Prog Ser 185:309–314

Worm B, Lotze HK, Sommer U (2001) Algal propagule banks modify competition, consumer and resource control on Baltic rocky shores. Oecologia 128:281–293