Grazing by small crustaceans stimulates growth of *Ulva* spp. (Chlorophyta) through preferential consumption of epiphytes

Pauline Kamermans*, Erik-jan Malta, Jacobus M. Verschuure, Lonneke Schrijvers, L. Franca Lentz and Angela Tjin A Lien

Netherlands Institute of Ecology, Centre for Estuarine and Coastal Ecology,

P.O. Box 140, 4400 AC Yerseke, The Netherlands

*E-mail: kamermans@cemo.nioo.knaw.nl

Abstract

Eutrophication of shallow coastal waters often leads to blooms of macroalgae. Grazing by small crustaceans, such as amphipods and isopods, can reduce macroalgal biomass accumulation. The role of grazing by small crustaceans on *Ulva* spp. biomass development was investigated in the Veerse Meer, a brackish lagoon situated in the Southwest Netherlands. Exclusion of grazing in the field did not stimulate *Ulva* spp. growth. In fact, growth rates were higher in exclosures that allowed grazers to enter. Edibility tests identified the amphipod *Gammarus locusta* (L.), and the isopods *Idotea chelipes* (Pallas) and *Sphaeroma hookeri* Leach (Lejuez) as grazers on *Ulva* spp. However, when epiphytic diatoms were present on the *Ulva* spp. thalli, *Gammarus* and *Sphaeroma* did not graze on *Ulva* tissue. Only *Idotea* continued to graze on *Ulva* spp. A laboratory growth experiment revealed a positive effect of *Gammarus* presence on *Ulva* spp. growth, probably caused by preferential removal of epiphytic diatoms from the *Ulva* spp. thalli. The growth stimulation by epiphyte removing grazers such as *Gammarus* may explain the higher growth rates in the presence of grazers observed in the field. When determining the potential role of invertebrate grazers in controlling macroalgal biomass accumulation, it is important to include an assessment of the epiphyte abundance on the macroalgae, as preferential removal of epiphytes may stimulate growth and thus have the opposite effect.

Introduction

Eutrophication of shallow coastal waters often leads to blooms of macroalgae (Vollenweider, 1992). Bloom development implies that the loss factors are much smaller than the gain factors. Thus, loss rates due to grazing, decomposition, or export should be lower than growth rates. Waterfowl can consume substantial amounts of macrophytes (Kiørboe, 1980). However, in some areas birds do not play a role until the period after the biomass build-up phase (Lodge, 1991). Grazing by small crustaceans, such as amphipods and isopods, can also reduce macroalgal biomass accumulation (Valiela *et al.*, 1997). For example in the Venice lagoon in Italy, biomass increase of *Ulva rigida* was enhanced by 4-15 % when crustacean grazers were excluded (Sfriso and Pavoni, 1994). Geertz-Hansen *et al.* (1993) showed that, in the outer part of Roskilde Fjord in Denmark, invertebrate grazing pressure on *Ulva lactuca* matched the growth rate and prevented the development of a bloom. Grazing in the inner part, however, was negligible, which allowed biomass build-up.

Invertebrate grazing on macroalgae not only involves grazing on the algal thalli, but grazing can also be restricted to associated epiphytes. Effects of grazing on epiphytes have been studied more often in seagrasses than in macroalgae. In general, removal of epiphytes on seagrass leaves by invertebrate grazers stimulates growth of seagrasses (reviews by Orth and van Montfrans, 1984 and Jernakoff *et al.*, 1996). The mechanism of growth stimulation by epiphyte removal may include both improved light conditions for the host plant, and increased nutrient supply to the plant through herbivore mediated nutrient regeneration (Jernakoff *et al.*, 1996; Fong *et al.*, 1997). Some examples of preferential consumption of epiphytes growing on macroalgae over the macroalgal thalli themselves include grazing by amphipods on *Dictyota dichotoma* (Hudson) Lamouroux

(Hay et al., 1987), *Gracilaria asiatica* Zhang and Xia (Brawley and Fei, 1987) and *Sargassum filipendula* (Duffy, 1990). Experimental evidence of growth enhancement due to the epiphyte removal is also described for macroalgae. In a microcosm study, Brawley and Adey (1981) observed that removal of the amphipod *Ampithoe ramondi* Audouin by fish predators caused heavy epiphyte overgrowth of *Hypnea spinella* (C. Agardh) Kützing and a decrease in growth rates of the plants. Furthermore, Dudley (1992) showed that epiphyte grazing by stream insects increased the biomass of *Cladophora glomerata* considerably compared to grazer-free controls. However, Duffy (1990) observed that grazing by one of the three tested amphipod species reduced the biomass of *Sargassum filipendula*, because the grazer preferred the host plant to its epiphytes. The net effect of the grazing process on the macroalgal biomass accumulation in the field depends on the preference of the grazer species for macroalgae or epiphytes and the relative abundance of the different grazer species.

In the Veerse Meer, a brackish lagoon situated in the Southwest Netherlands, *Ulva* spp. blooms frequently occur and show a patchy distribution (Malta *et al.*, 2000). Most grazing by birds takes place after the biomass build up, but small crustaceans are present in the lagoon during the entire growing season of *Ulva* spp. (Coosen *et al.*, 1990). This group can potentially affect biomass accumulation. Here we present results of experiments, that were carried out both in the field and in the laboratory, to study the effect of grazing by small crustaceans on *Ulva* spp. biomass accumulation.

Material and methods

Edibility tests

Four *Ulva* species, *U. scandinavica* Bliding*, U. curvata* (Kütz) De Toni*, U. rigida* C. Agardh and *U. lactuca* L. (sensu Koeman and van den Hoek, 1981)*,* have been observed in the Veerse Meer lagoon. Identification of the different species present in the *Ulva* spp. bloom proved to be difficult, as morphological, molecular and ecophysiological characters show large overlaps (Malta *et al.*, 1999). Therefore, the species will be considered further as *Ulva* spp.. To determine which species graze on *Ulva* spp. a series of edibility tests was set up. The tests lasted 15 to 30 days. In each test, 20 individuals of one invertebrate species were placed in a beaker with an *Ulva* spp. disc (4.2 cm diameter) and one litre of lagoon water. A control beaker contained only an *Ulva* spp. disc and lagoon water. The beakers were placed in the dark at 15 ^{0}C and aerated continuously.

Three common invertebrate species from the Veerse Meer lagoon were tested: the isopods *Idotea chelipes* and *Sphaeroma hookeri*, and the amphipod *Gammarus locusta*. Separate treatments with old *Ulva* spp. discs and with young *Ulva* spp. discs were exposed to the different grazer species. In addition, clean young *Ulva* spp. discs were presented in Whatman GF/C filtered lagoon water. This treatment was included to test grazing on epiphyte free *Ulva* spp.. Prior to each test the crustaceans were starved in filtered lagoon water for 5 days. After each test the discs were visually inspected for the presence of holes in the thalli as well as the abundance of diatoms on the thalli. The latter was indicated by a brown layer. Microscopic observations confirmed that the brown layer on the *Ulva* thallus consisted of diatoms (P. Kamermans, pers. obs.). The occurrence of holes in *Ulva* spp. in the grazer treatment and the absence of holes in the control treatment indicated grazing on

Ulva spp., while clean green thalli in the grazer treatment compared to the presence of a brown layer on the disc in the control treatment indicated grazing on diatoms.

Exclosure experiment in the field

The field studies were conducted in the period from May to October at Middelplaten (51^0 32' 63'' N; 3^0 46'' 89'' E) and Kwistenburg (51^0 32' 61'' N; 3^0 50' 97'' E), two shallow sites (depth 80 cm) in the Veerse Meer lagoon. Growth rates of *Ulva* spp. were determined weekly at both sites using cages that were similar to the cages employed by Geertz-Hansen *et al.* (1993). The cages were made of 20 cm long Plexiglas tubes (20 cm diameter) cut lengthways in half. The ends and bottoms of these clear roofs were covered either with 1-cm plastic-coated steel mesh (large-mesh cages that allowed grazers to enter) or with 1-mm nylon netting (small-mesh cages that prevented grazers from entering). The Plexiglas roof construction ensured that light conditions were the same in large- and small-mesh cages. The cages were fixed to ropes that were tied between poles and suspended in the water column at 50 cm above the bottom to ensure average light intensities within the cages compared with those in the free-floating *Ulva* spp. mats.

At both sites, five cages with large mesh and five cages with small mesh were used. Each cage contained five *Ulva* spp. discs (4.2 cm diameter) that were punched from *Ulva* spp. thalli with a sharpened stainless steel tube. The cages were cleaned at intervals of 4 to 7 days. Every week, the growth rate was determined with new discs cut out of fresh *Ulva* spp. thalli. Before incubation, the total WW of the five discs in each cage was determined. Each week, the DW of fifteen extra discs was determined as described above to establish the WW to DW conversion factor. After one week, total WW per cage was

measured and growth was calculated as the increase in DW per cage with the following

formula of Geertz-Hansen *et al*. (1993):

$(\ln W_t - \ln W_0) / t$

where W_0 is the initial and W_t the final DW after t days of incubation.

Grazer abundance in the field

In July and August, the abundance of invertebrate grazers was determined at the

two study sites. Potential invertebrate grazers reach their greatest densities in these months

(Nienhuis and van Ierland, 1978; Nienhuis and Groenendijk, 1986). A 0.16 m^2 bottomless

cylinder (diameter 45 cm, height 45 cm) with a 1-mm net attached to the top was

randomly placed on the bottom. The net could be opened at the top and all material inside

the cylinder (macroalgae and invertebrates) was collected with a 1-mm dip net. Per site

three cylinder samples were taken. Potential invertebrate grazers, as based on Geertz-

Hansen *et al.* (1993), Horne *et al.* (1994) and our own edibility tests, were sorted from the

samples and their numbers counted. The samples were dried for 48 h at 60 ^{0}C and

weighed, after which they were combusted at 550 ^{0}C for 2 h and weighed again. From

this, the ash-free dry weight (AFDW) was calculated.

Flow experiment

It can be argued that experimental set-ups with cages will alter the environment

within the cages in such a way that factors other than grazing will also have an effect on

the growth rate of the *Ulva* discs. Flow conditions may be better in the large-mesh cages

compared to the small-mesh cages supplying more nutrients to the discs in the large- mesh

cages. To study this possibility, growth rate of *Ulva* spp. discs was determined in an

experiment without grazers. In each of two outside basins (2.5 x 1.2 x 0.5 m) six cages

were placed. The cages consisted of 17 cm long Plexiglas tubes with a diameter of 11 cm. The ends of the tubes were covered with 1-cm plastic-coated steel mesh (large-mesh treatment) or with 1-mm nylon netting (small-mesh treatment). Each basin contained three large-mesh cages and three small-mesh cages. The basins were filled with seawater from the Oosterschelde estuary, which was replaced every week. In one basin water was pumped around at a speed of 7 cm s^{-1} (flow treatment), while the water in the other basin was not moved (no-flow treatment). In an experiment, Parker (1981) showed that a current speed of 7.5 cm s^{-1} enhanced growth rates more than speeds of 15.0 or 22.5 cm s^{-1}. Per cage, two *Ulva* spp. discs were cut, introduced in the cages and processed as described above for the exclosure experiment. The flow experiment was conducted in September and October and lasted 5 weeks. To avoid specific effects caused by the basin, the pump was alternated between the basins at the beginning of each growth trial.

Laboratory growth experiment

The net effect of grazing by invertebrates on *Ulva* spp. growth was studied in an experiment carried out in May. About 1 gram wet weight of *Ulva* spp. was placed in each of six beakers with 400 ml aerated unfiltered lagoon water. In two beakers 15 *Idotea* were added, in two beakers 15 *Gammarus* were added and two beakers remained as control. The beakers were placed at a temperature of 15 ^{0}C and an irradiance level of 100 μE m^{-2} s^{-1} with a cycle of 12 h light and 12 h dark. *Ulva* spp. WW was determined on day 0, 3 and 8.

Statistical analyses

All data were tested for heteroscedacity with a Bartlett's test for homogeneity of variances (Sokal and Rohlf, 1995). Grazer abundance and biomass determined in the field,

as well as *Ulva* spp. growth in the exclosure and flow experiments scored as significant. The data continued to give significant results after several transformations. Therefore, these data were tested non-parametrically with Mann-Whitney U-tests. The data of the growth experiment in the laboratory did not score as significant in Bartlett's test. A two-way ANOVA was used to test the effect of time and treatment, as well as the interaction between time and treatment, on *Ulva* spp. weight (Sokal and Rohlf, 1995). The significance of differences between the separate treatments was determined with the Tukey-Kramer pairwise comparison test (Sokal and Rohlf, 1995). A significance level of 5% was used in all tests. The statistical analyses were conducted using the STATISTICA programme.

Results

Edibility tests

The edibility tests revealed that all 3 species investigated (*Idotea chelipes*, *Gammarus locusta* and *Sphaeroma hookeri*) consumed *Ulva* spp. (Table I). *Idotea* was the only species that consumed *Ulva* spp. under all circumstances (Table I). *Ulva* spp. in unfiltered seawater was not ingested by *Gammarus* and *Sphaeroma*, but diatoms present on the thalli were grazed by these species (Table I). Only when clean young *Ulva* spp. was presented in filtered seawater did *Gammarus* and *Sphaeroma* graze on the macroalga (Table I). These results indicate that *Gammarus* and *Sphaeroma* consumed *Ulva* spp. only when epiphytic diatoms were not present on the *Ulva* spp. thalli.

Exclosure experiment and grazer abundance in the field

Growth rates of *Ulva* spp., as established by the exclosure experiment, were high in May and June and declined thereafter (Fig. 1). At both sites, significantly higher growth rates were frequently observed in the large-mesh cages (Fig. 1). This indicates that exclusion of grazing did not stimulate growth. In contrast, growth was higher in the cages in which grazers were allowed to enter.

The occurrence of crustacean grazers showed large variability between species, samples and sites (Table II). *Gammarus locusta* was the dominant species in July, while *Idotea chelipes* and *Sphaeroma hookeri* were the most abundant species in August. In only three cases, significant differences between sites were observed. At Middelplaten, a significantly higher biomass of *Idotea* was found in July. This site also showed a significantly higher density and biomass of *Gammarus* in July. The density of *Sphaeroma*,

however, was significantly lower at Middelplaten in July. In general, potential invertebrate grazer density did not show large differences between sites.

Flow experiment

Maximal *Ulva* spp. growth rates observed in the flow experiment were similar to rates observed in the exclosure experiment in the field in September and October (compare Fig. 1 and Fig. 2). Occasionally, negative growth rates were found. This could be attributed to discs that were in a bad condition at the end of the growth trial, as they were slimy and showed decolorations. Higher *Ulva* spp. growth rates were found in the basin without current (Fig. 2). Contrary to the expectation, growth rates in the small-mesh cages were generally, and in some cases significantly, higher than in the large-mesh cages (Fig. 2). This indicates that the small mesh of the cages does not hamper growth.

Laboratory growth experiment

The growth experiment showed a slight increase in *Ulva* spp. biomass in the control treatment, indicating that the experimental conditions favoured growth of *Ulva* spp. (Fig. 3). A significant effect of treatment was found (Table IIIa). The presence of *Idotea* resulted in a significant reduction in *Ulva* spp. biomass (Fig. 3, Table IIIb). The presence of *Gammarus*, however, had the opposite effect, a significant increase in biomass compared to the control treatment was found (Fig. 3, Table IIIb). The clean green thalli in the *Gammarus* treatment appeared to be free of epiphytes. The differences between treatments increased as time progressed. This produced a significant interaction between duration of the experiment and treatment (Table IIIa). These results demonstrate a positive effect of *Gammarus* presence on *Ulva* spp. growth, probably caused by removal of epiphytic diatoms from the *Ulva* spp. thalli.

Discussion

Grazing by some species of small crustaceans can reduce macroalgal biomass (Geertz-Hansen et al., 1993; Sfriso and Pavoni, 1994; Duffy, 1990). Other invertebrate species feed preferentially on epiphytes occurring on the macroalgae and, as a result, may have a positive effect on macroalgal growth (Brawley and Adey, 1981; Dudley, 1992). The exclosure experiment in the Veerse Meer lagoon indicates that the negative effect of crustacean grazing on *Ulva* spp. was negligible. In fact, growth rates were higher in the cages that allowed grazers to enter. Exclusion of grazing by the small mesh produced lower *Ulva* spp. biomass increase. The results of the flow experiment do not provide evidence for reduced growth conditions for *Ulva* spp. in the small-mesh cages. In this flow experiment, growth was higher in the small-mesh cages than in the large-mesh cages. In some cases, weight loss instead of weight increase was found. In the flow experiment, the disc were constantly exposed to flow, while in the field periods without flow are not uncommon. The continuous flow may have been suboptimal for the *Ulva* spp. disc in the cages. This is supported by the bad condition of some of the disc at the end of the growth trials. In the basin without flow, the growth rates were generally similar between cages. Thus, the small-mesh cages did not reduce conditions for *Ulva* spp. growth.

Results from the exclosure experiment suggest that the presence of grazers had a positive effect on *Ulva* spp. growth. Data from the laboratory experiments offer a possible explanation for this observation. The edibility tests identified *Idotea chelipes* as the only invertebrate grazer that will consume *Ulva* spp. together with its epiphytes. *Gammarus* and *Sphaeroma* will only graze on *Ulva* tissue when epiphytes are not present. The grazing experiment in the laboratory indicates that cleaning of the *Ulva* spp. thalli by *Gammarus* had a positive effect on *Ulva* spp. growth. In the field, the amphipod

Gammarus and the isopod *Sphaeroma* will probably limit their consumption to epiphytic diatoms present on the *Ulva* spp. thalli. Consequently, the thalli can receive more light and grow faster.

Calculations with data from our laboratory grazing experiment show that one *Idotea* can consume an average of 0.036 g WW per day, which is 3 % of the initial *Ulva* spp. disc weight, while one *Gammarus* can stimulate *Ulva* spp. growth with an average of 0.002 g WW per day, which is 0.2 % of the initial *Ulva* spp. disc weight. Growth stimulation will most likely be larger in the field, because in the laboratory, light conditions were reduced compared to the field situation (respectively 100 μE m^{-2} s^{-1} in the lab and on average 250 μE m^{-2} s^{-1} in the field; Malta and Verschuure, 1997). In the field, part of the actual increase in biomass is probably consumed by *Idotea*. The ratio between *Idotea* density and the density of epiphyte removing grazers such as *Gammarus*, and the size of the individuals, determines whether the *Ulva* spp. biomass will increase or decrease. Furthermore, *Gammarus* will feed on epiphytes when they are present, but may switch to macroalgal biomass when epiphytes are scarce or absent. Thus, the abundance of epiphytes also affects the grazer impact by *Gammarus*.

Horne *et al*. (1994) found consumption of *Ulva* sp. by *Gammarus mucronatus* in an estuary with a low nutrient loading rate and not in an estuary with a high nutrient loading rate. When we relate these results to our data, this observation suggests that, under high nutrient conditions, *Gammarus* was not grazing on *Ulva*, but on its epiphytes. Epiphytes are generally more abundant at high than at low nutrient loading (Sand-Jensen and Borum, 1991).

In conclusion, *Ulva* spp. growth rate was enhanced by the presence of *Gammarus*. This probably resulted from preferential removal of epiphytic diatoms attached to *Ulva* spp. thalli. When determining the potential role of grazers in controlling macroalgal

biomass accumulation, it is important to include an assessment of the epiphyte abundance on the macroalgae, as preferential removal of epiphytes may stimulate growth and thus have the opposite effect.

Acknowledgements

References

Brawley, S.H. and W.H. Adey. 1981. The effects of micrograzers on algal community structure in a coral reef. Mar. Biol. 61: 167-177.

Brawley, S.H. and X.G. Fei. 1987. Studies of mesoherbivory in aquaria and in an unbarricaded mariculture farm on the Chinese coast. J. Phycol. 23: 614-623.

Coosen, J., Meire P., J.J. Stuart and J. Seys, 1990. Trophic relationships in brackish Lake Veere: the role of macrophytes. In: Trophic relationships in the marine environment. Proceedings of the 24th European Marine Biology Symposium. Eds. M. Barnes & R.N. Gibson, Aberdeen University Press, Aberdeen: 404-423.

Dudley, T.L. 1992. Beneficial effects of herbivores on stream macroalgae via epiphyte removal. Oikos 65: 121-127.

Duffy, J.E. 1990. Amphipods on seaweeds: partners or pests? Oecologia 83: 267-276.

Fong, P., J.S Desmond and J.B. Zedler. 1997. The effect of a horn snail on *Ulva expansa* (Chlorophyta): consumer or facilitator of growth? J. Phycol. 33: 353-359.

Geertz-Hansen, O., K. Sand-Jensen, D.F. Hansen and A. Chistiansen. 1993. Growth and grazing control of abundance of the marine macroalga, *Ulva lactuca* L. in a eutrophic Danish estuary. Aquat. Bot. 46: 101-109.

Hay, M.E., J.E. Duffy and C.A. Pfister. 1987. Chemical defences against different marine herbivores: are amphipods insect equivalents? Ecology 68: 1567-1580.

Horne, A., J. McClelland and I. Valiela. 1994. The growth and consumption of macroalgae in estuaries: the role of invertebrate grazers along a nutrient gradient in Waquoit Bay, Massachusetts. Biol. Bull. 187: 279-280.

Jernakoff, P., A. Brearley and J. Nielsen. 1996. Factors affecting grazer-epiphyte interactions in temperate seagrass meadows. Oceanography and Marine Biology: an Annual Review 34: 109-162.

Koeman, R.P.T. and C. van den Hoek. 1981. The taxonomy of *Ulva* (Chlorophyceae) in The Netherlands. Br. Phycol. J. 16: 9-53.

Kiørboe, T. 1980. Distribution and production of submerged macrophytes in Tipper Grund (Ringkøbing Fjord, Denmark), and the impact of waterfowl grazing. J. appl. Ecol. 17: 675-687.

Lodge, D.M. 1991. Herbivory on freshwater macrophytes. Aquat. Bot. 41: 195.224.

Malta, E.-j. and J.M. Verschuure. 1997. Effects of environmental variables on between-year variation of *Ulva* growth and biomass in a eutrophic brackish lake. J. Sea Res. 38: 71-84.

Malta, E-j., S.G.A. Draisma and P. Kamermans. 1999. Free-floating *Ulva* in the Southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison. Eur. J. Phycol. 34: 443-454.

Malta, E.-j., J.M. Verschuure and P.H. Nienhuis. 2000. Spatial variation of macroalgal biomass in a brackish eutrophic lake is caused by loss processes. Bot. Mar. accepted.

Nienhuis, P.H. and A.M. Groenendijk. 1986. Consumption of eelgrass (*Zostera marina*) by birds and invertebrates: an annual budget. Mar. Ecol. Prog. Ser. 29: 29-35.

Nienhuis, P.H. and E.T. van Ierland. 1978. Consumption of eelgrass, *Zostera marina*, by birds and invertebrates during the growing season in Lake Grevelingen (SW Netherlands). Neth. J. Sea Res. 12: 180-194.

Orth, R.J., and J. van Montfrans. 1984. Epiphyte-seagrass relationships with an emphasis on the role of micrograzing: a review. Aquat. Bot. 18: 43-69.

Parker, H.S. 1981. Influence of relative water motion on the growth, ammonium uptake and carbon and nitrogen composition of *Ulva lactuca* (Chlorophyta). Mar. Biol. 63: 309-318.

Sand-Jensen, K. and J. Borum. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquat. Bot. 41: 137-175.

Sfriso, A. and B. Pavoni. 1994. Macroalgae and phytoplankton competition in the central Venice lagoon. Environ. Technol. 15: 1-14.

Sokal, R.R. and F.J. Rohlf. 1995. Biometry. Third Edition. W.H. Freeman and company, New York, 887 pp.

Valiela, I., J. McClelland, J. Hauxwell, P.J. Behr, D. Hersh and K. Foreman. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. Limnol. Oceanogr. 42: 1105-1118.

Vollenweider, R.A., 1992. Coastal marine eutrophication: principles and control. In: Marine Coastal Eutrophication. Proceedings of an International Conference, Bologna, Italy, 21-24 March 1990, Elsevier, Amsterdam: 1-20.

Table I. Edibility of different food items for invertebrates from the Veerse Meer lagoon, + indicates that item was consumed - indicates that item was not consumed.

Grazer species	old Ulva in unfiltered lagoon water	young Ulva in unfiltered lagoon water	diatoms on old and young Ulva in unfiltered lagoon water	young Ulva in filtered lagoon water
Idotea chelipes	+	+	+	+
Sphaeroma hookeri	-	-	+	+
Gammarus locusta	-	-	+	+

Table II. Occurrence of invertebrate grazers at Middelplaten and Kwistenburg. Values are mean +SD (n = 3). Significantly higher values at sites, as determined by Mann-Whitney U-tests, are indicated: * = $P < 0.05$.

grazer species	date	Density ($\# \, m^{-3}$)	Density ($\# \, m^{-3}$)	Biomass ($g \, m^{-3}$)	Biomass ($g \, m^{-3}$)
	10 July	Middelplaten	Kwistenburg	Middelplaten	Kwistenburg
Idotea chelipes		91 (31)	57 (35)	0.25 (0.05) *	0.07 (0.02)
Sphaeroma hookeri		42 (33)	255 (64) *	0.10 (0.06)	0.22 (0.06)
Gammarus locusta		633 (362) *	289 (36)	0.64 (0.40) *	0.28 (0.01)
	29 Aug	Middelplaten	Kwistenburg	Middelplaten	Kwistenburg
Idotea chelipes		984 (367)	7555 (11555)	1.58 (0.71)	2.55 (2.97)
Sphaeroma hookeri		1453 (244)	810 (1021)	0.73 (0.31)	0.80 (0.76)
Gammarus locusta		68 (73)	34 (40)	0.10 (0.11)	0.05 (0.09)

Table IIIa. Statistical evaluation of the effect of treatment (no grazers present as control, or either *Idotea chelipes* or *Gammarus locusta* as grazers) on *Ulva* biomass in the laboratory growth experiment. Values are degrees of freedom (df), mean square (MS) and probability (P) of two-way ANOVA.

Source of variation	df	MS	P
Treatment	2	0.212	0.000
Time	2	0.005	0.166
Treatment x Time	4	0.091	0.000
Error	9	0.002	

Table IIIb. Matrix of pairwise comparison probabilities of Tukey-Kramer post-hoc test.

Treatment	*Idotea*	*Gammarus*	Control
Idotea	1.000		
Gammarus	0.000	1.000	
Control	0.000	0.002	1.000

Figure legends

Fig. 1. Growth rates (d^{-1}) of *Ulva* spp. discs at Middelplaten (a) in large-mesh cages (closed circles) and small-mesh cages (open circles) and at Kwistenburg (b) in large-mesh cages (closed squares) and small-mesh cages (open squares). Values are mean +SD (n = 5). Occasionally, the mesh became detached from the roofs and discs escaped from the cages. Growth rates were then based on means of less than 5 cages. This was the case for large-mesh cages at Middelplaten in week 1 n = 4, week 4,5 n = 3; small-mesh cages at Middelplaten in week 1,6,8 n = 4, week 4 n = 3, week 5 n = 2; large-mesh cages at Kwistenburg in week 2,22 n = 4, week 5 n = 3, week 4 n = 2; small-mesh cages at Kwistenburg in week 1,6,10,23,24 n = 4, week 4,5 n = 3. Significant differences in *Ulva* growth rates between large and small mesh-size cages as determined by Mann-Whitney U-tests are indicated with * = P < 0.05.

Fig. 2. Growth rates (d^{-1}) of *Ulva* spp. discs at Middelplaten (a) in large-mesh cages (closed circles) and small-mesh cages (open circles) in the flow basin and (b) in large-mesh cages (closed squares) and small-mesh cages (open squares) in the no-flow basin. Values are mean +SD (n = 3). Significant differences in *Ulva* growth rates between large and small mesh-size cages as determined by Mann-Whitney U-tests are indicated with * = P < 0.05.

Fig. 3. Biomass (g WW beaker^{-1}) of *Ulva* spp. in an experiment with different grazer treatments: no grazers as control (open diamonds), and the grazers *Gammarus locusta* (closed squares) and *Idotea chelipes* (closed circles). Values are mean +SD (n = 2).