

# Effects of *Pilayella littoralis* on *Fucus vesiculosus* recruitment: implications for community composition

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**ABSTRACT:** With increasing eutrophication in coastal areas, mass developments of annual filamentous algae, such as *Pilayella littoralis* and *Enteromorpha* spp., have increased. Simultaneously, the perennial macroalga *Fucus vesiculosus* has declined in many areas in the Baltic Sea. To test the effects of *P. littoralis* on *F. vesiculosus* we performed a number of laboratory and field studies. The most severe negative effect observed was the reduced number of settled germlings (95 %) when *P. littoralis* was present on the substrate prior to seeding of *F. vesiculosus* eggs. A low concentration (0.1 %) of exudates from *P. littoralis* reduced the attachment rate of *F. vesiculosus* during the initial 12 h, while higher concentrations of exudates (5 to 10 %) negatively affected germination and rhizoid development. These results could explain for the negative correlations that have been observed between *F. vesiculosus* and annual filamentous algae in the Baltic Sea. Our field data showed that the biomass of filamentous algae was higher in the summer than in the autumn, suggesting that recruits from the summer-reproducing *F. vesiculosus* may encounter more competition from *P. littoralis* and other filamentous algae than do recruits from the autumn-reproducing populations. The summer- and autumn-reproducing *F. vesiculosus* host similar abundance and biomass of their associated flora and fauna; hence, a potential shift from summer- to autumn-reproducing *F. vesiculosus* would not change the overall community composition in this ecosystem.

**KEY WORDS:** Summer-reproducing *Fucus vesiculosus* · Autumn-reproducing *Fucus vesiculosus* · *Pilayella littoralis* · Post-settlement survival · Community composition · Filamentous algae · Attachment · Interference competition · Allelopathy

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## INTRODUCTION

Over the past few decades, mass developments of annual filamentous algae, such as *Enteromorpha* spp., *Pilayella littoralis* and *Cladophora* spp., have increased in coastal areas around the world (Rosenberg et al. 1996, Schramm & Nienhuis 1996, Johansson et al. 1998, Eriksson et al. 2002), including the Baltic Sea (Hällfors et al. 1984, Cederwall & Elmgren 1990, Eriksson et al. 1998, Rönnberg & Mathiesen 1998, Lotze et al. 1999, Vahteri et al. 2000). At the same time, the distribution of the perennial macroalga *Fucus vesiculosus* has become more restricted (Kangas et al. 1982, Kautsky et al. 1986, Eriksson et al. 1998, Schramm 1999, Middelboe &

Sand-Jensen 2000), except for some local recovery in recent years (Kautsky 2000, Nilsson et al. 2004).

*Fucus vesiculosus* (hereafter *Fucus*) is the only large brown alga in the northern Baltic proper, where it provides one of the most species-rich habitats, offering protection, food supply and spawning area to many different groups of fauna (Haage 1975, Wallentinus 1979). The diversity and abundance of associated fauna in these communities is high, as shown in a study by Haage (1975). Due to the high biodiversity of the *Fucus* community and its ecological significance in the Baltic Sea (Kautsky et al. 1992), it is important to understand the various factors that structure this community and its biodiversity.

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Laboratory and field-based studies have shown that a great number of complex factors and interactions may underlie the overall decline of *Fucus* populations in the Baltic Sea (Kangas et al. 1982, Vogt & Schramm 1991, Berger et al. 2004). In addition, processes involving egg settlement and survival of recruits may represent a bottleneck for fucoid algae (Vadas et al. 1992, Serrão 1996). Further, negative correlations between percentage cover of filamentous or foliose species and large perennial fucoid species have been observed (Worm & Chapman 1996, Worm et al. 1999), suggesting interspecific interactions between these 2 different forms of algae. The mechanism underlying the negative correlations is not always clear; however, space occupation by filamentous algae has been shown to have a strong negative effect on the settling success of *Fucus* (Worm et al. 2001, Berger et al. 2003).

*Pilayella littoralis* (hereafter *Pilayella*) is a common fast-growing epilithic and epiphytic brown alga that occurs in the upper littoral zone in the Baltic Sea, co-occurring with *Fucus*. It dominates during spring and early summer (Wallentinus 1979, Kiirikki & Lehvo 1997, Lotze et al. 1999) and starts to detach and decay in mid-summer (Aneer 1987). In a laboratory study, Aneer (1987) found that some filamentous algae, including *Pilayella*, had negative effects on fish egg survival, and suggested that the high natural mortality of Baltic herring *Clupea harengus* may be locally correlated to the toxic exudates of brown filamentous algae. Decomposition of filamentous algae locally generates high levels of nutrients (Vahteri et al. 2000) such as nitrate, phosphate and ammonium, which can all have damaging effects on the early life stages of macroalgae (Kevekordes 2001, Bergström et al. 2003). Thus, high abundance of detaching *Pilayella* during *Fucus* reproduction may have negative consequences for the recruitment of *Fucus*. Besides nutrients, exudates from *Pilayella* may also contain chemicals or secondary metabolites that can be harmful to *Fucus* recruits (McInnes et al. 1984).

*Fucus* reproduction peaks in 2 different periods in the Baltic Sea: May–June and September–October. These different patterns of reproduction are believed to be expressions of different genotypes of *Fucus* (Berger et al. 2001). Genetic analysis of *Fucus* aiming to cast light on these 2 different reproduction patterns is still in progress, and it is as yet unclear whether there are 2 distinct genotypes. We will therefore refrain from using the word ‘genotype’ in this article. Since the biomass of *Pilayella* peaks in spring, it is possible that fucoid populations that reproduce in autumn encounter less competition from this filamentous alga, and hence have an advantage over fucoid populations that reproduce during the *Pilayella* outbreak.

This study primarily aimed to test whether *Pilayella* has negative effects on *Fucus* settlement and recruitment (i.e. attachment, germination, rhizoid development and survival). To evaluate potential effects *in situ*, the biomass of *Pilayella* and total filamentous algae, as well as the biomass of *Fucus* juveniles, was measured in the field during the 2 periods of *Fucus* reproduction. Possible ecological consequences of a shift from summer- to autumn-reproducing *Fucus* were also investigated by sampling associated flora and fauna from individuals representing the 2 different reproductive patterns. Seven hypotheses (H-1 to H-7) were addressed, and to answer these hypotheses we conducted both laboratory and field studies.

## MATERIALS AND METHODS

**Preparation of *Pilayella* exudates.** Fresh *Pilayella* was collected at the Askö Laboratory, Sweden (58° 49' N, 19° 39' E), in May 2000, and sub-samples were placed in aquaria filled with seawater of the same salinity and temperature as the water in the sampling area (6.5‰ salinity, 13 ± 1°C). To test the hypothesis that exudates from decaying *Pilayella* have stronger negative effects on *Fucus* recruitment than do fresh *Pilayella* (H-1), samples were placed either in the light and aerated (FP, fresh *Pilayella*) or in the dark and not aerated (DP, decaying *Pilayella*) (approximately 0.5 g dry weight l<sup>-1</sup> water). The latter treatment was conducted to prompt *Pilayella* decay, a phenomenon which naturally occurs in summer after *Pilayella* detachment. Material was left under these conditions for 3 d before the exudate was filtered off. Half of the *Pilayella* exudate was directly used in the germination, survival and rhizoid-development experiment, while the other half was frozen for later use in the attachment experiment. Since nutrients are known to have negative effects on the attachment and recruitment of macroalgae (Kevekordes 2001, Bergström et al. 2003), we analysed the nitrate, ammonium and phosphate content of the exudates, according to methods described by Valderrama (1995).

**Effects of exudates on *Fucus* attachment.** To test whether initial attachment of fertilized *Fucus* eggs is prevented by exudates of *Pilayella* (H-2), a laboratory experiment was carried out in October 2002. *Pilayella* exudates (FP and DP) were diluted to 0.1, 1, 5, 10 and 25 % of the original solution, and 8 ml of each concentration (and of the seawater control) was placed in acid-washed petri dishes (10 ml total volume) (total n = 60 FP, 60 DP and 12 control). Ripe *Fucus* (>50 individuals) was collected 2 d before the experiment at 2 locations 100 km apart. Egg release and fertilization were induced according to methods described in Bergström

et al. (2003); 2 ml of egg suspension (ca. 150 to 200 eggs) was added to the petri dishes, which were then immediately transferred to a room held at a constant temperature of  $13 \pm 1^\circ\text{C}$  with a 12:12 h, light:dark cycle which mimicked natural conditions where the samples had been collected. Three replicates were removed from each treatment at 6, 12, 24 and 48 h after fertilization to estimate the attachment success. Each removed dish was carefully agitated and the treatment medium containing non-attached zygotes was poured into an empty glass tube. Both the attached and non-attached zygotes were preserved with Lugol's solution. The number of zygotes was counted under a stereo microscope and the percentage of attached zygotes was calculated.

#### **Germination, survival and rhizoid development.**

The hypothesis that *Pilayella* exudates negatively affect germination, survival and rhizoid development (H-3) was tested in May 2000. Ripe receptacles of *Fucus* were collected in the Askö area and brought to the laboratory, where they were stored damp and in the dark in plastic bags at  $4^\circ\text{C}$ , after sex was determined and the males and females separated. When gamete release was expected, female and male receptacles were placed separately in the same concentrations of *Pilayella* exudates as were used in the attachment experiment (control, 0.1, 1, 5, 10 and 25%) at  $13 \pm 1^\circ\text{C}$ . Eggs and sperm from treatments with the same concentration of exudates were then mixed and left for fertilization for 1 h. Fertilization success was determined using a solution of 0.0001% Calcofluor White (see Serrão et al. 1996). After fertilization, 5 ml of the suspension of fertilized eggs was transferred to petri dishes pre-filled with 5 ml of the various treatment media ( $n = 5$  dishes per treatment). The number of zygotes in each petri dish was counted after 24 h, the germination frequency was checked after 5 d and the survival and number of germlings producing rhizoids was checked after 12 d. The percentage of surviving germlings was calculated as the difference between Days 12 and 5.

**Substrate competition.** To test whether *Pilayella* physically prevents establishment of *Fucus* germlings (H-4), small stones (5 to  $10\text{ cm}^2$ ) with attached *Pilayella* ( $n = 10$ ) were collected in the Askö area in June 2001. To standardize the coverage of *Pilayella*, the turfs were cut to uniform height (ca. 3 cm), representing a biomass of approximately 0.2 to  $0.3\text{ g dry wt cm}^{-2}$ . The stones were also washed to exclude fine sediments trapped in the turfs. Stones from the same area without *Pilayella* were used as controls ( $n = 10$ ). Fertilized eggs were seeded onto the stones, which were then stored in an outdoor tank for 3 mo before the surviving germlings were counted. On each stone, the germlings present on a randomly chosen area of  $3.1\text{ cm}^2$  were counted under

a stereomicroscope. The germlings were recalculated to number of germlings  $\text{dm}^{-2}$  substrate.

**Occurrence of filamentous algae, *Fucus* juveniles and macrofauna in the field.** To investigate the hypothesis that zygotes from the autumn-reproducing *Fucus* encounter less competition with filamentous algae than do zygotes from the summer-reproducing populations (H-5), the occurrence of *Pilayella* and other filamentous algae was quantified during the 2 different periods of *Fucus* reproduction (May and September) in 2001. The biomass of 1 to 2 yr old *Fucus* juveniles under the canopy of summer- and autumn-reproducing *Fucus* was also sampled to investigate the hypothesis that the autumn-reproducing *Fucus* achieve greater recruitment success than do the summer-reproducing *Fucus* (H-6). Samples of both summer- and autumn-reproducing *Fucus* were collected to examine further the null hypothesis that *Fucus* is inhabited by similar abundance and biomass of associated macrofauna and epiphytic filamentous algal, irrespective of reproduction pattern (H-7). Individuals from 5 summer- and autumn-reproducing *Fucus* stands were collected randomly with net bags (1 mm mesh size) at different sites in the Askö area ( $n = 3$  for both summer- and autumn-reproducing *Fucus* collected in May, and  $n = 3$  for autumn- and  $n = 4$  for summer-reproducing *Fucus* collected in September) at approximately 0.3 to 0.5 m depth. Razor blades were used to clear the *Fucus* of epiphytic filamentous algae from which macrofauna were separated and counted. The area under the collected *Fucus* stand was sampled using a square frame ( $0.25 \times 0.25\text{ m}$ ), with an attached net bag (1 mm mesh size); a scraper was used to clear the vegetation. The epilithic filamentous algae and 1 to 2 yr old *Fucus* juveniles sampled inside the frame were separated out for weighing. The macrofauna, filamentous algae, *Fucus* individuals and juveniles were dried to constant dry weight at  $50^\circ\text{C}$ . The biomass of epiphytic filamentous algae and the abundance and biomass of associated fauna were recalculated as 100 g dry wt *Fucus*, and the epilithic algae and *Fucus* juveniles were transformed to  $\text{g dry wt m}^{-2}$ .

**Statistical analyses.** Statistical analyses were performed using SPSS 9.0.1 for Windows and Statistica 6.1. Homogeneity of variance was tested with Cochran's test, and normality of residuals with the Kolmogorov-Smirnov ( $N > 50$ ) and Shapiro-Wilk ( $N < 50$ ) tests. A general linear model (GLM) was used in the attachment experiment to evaluate the effects of treatment (FP and DP), concentration and time on attachment, and Tukey post-hoc testing was used for a *posteriori* comparisons. Due to the heterogeneity of the variances, a Mann-Whitney *U*-test was used to determine whether the percentages of germination, survival and rhizoid development differed between treatment

Table 1. Nutrient concentrations ( $\mu\text{mol l}^{-1}$ ) in the various solutions of *Pilayella littoralis* exudates. Values in **bold** numbers indicate very high concentrations, according to the Swedish EPA assessment of water quality (Naturvårdsverket 1999)

Exudate conc. (%)	Fresh			Decaying		
	NO <sub>3</sub> -N	NH <sub>4</sub> -N	PO <sub>4</sub> -P	NO <sub>3</sub> -N	NH <sub>4</sub> -N	PO <sub>4</sub> -P
0.1	<0.001	<0.001	<0.001	<0.001	0.02	0.05
1	<0.001	<0.001	<0.001	0.02	0.2	0.5
5	0.02	0.03	<0.001	0.09	0.9	<b>2.5</b>
10	0.03	0.06	<0.001	0.2	1.9	<b>5.0</b>
25	0.08	0.15	0.03	0.4	<b>4.7</b>	<b>12.6</b>

media (FP and DP). A 1-way ANOVA with a post-hoc test that does not assume equal variances (Dunnnett T3 test) was thereafter used to test for differences between concentrations. Data pertaining to the abundance of established germlings on stones were transformed ( $\log(x + 100)$ ) to meet the assumptions of homogeneity of variance, and a 1-way ANOVA was used to test for differences between stones with and without *Pilayella*.

Data from the field study (epiphytic and epilithic *Pilayella* and total filamentous algae, *Fucus* juveniles under the canopy and biomass and abundance of associated fauna) were analysed using a 2-way ANOVA with 'season' and '*Fucus* period of reproduction' as fixed factors. The data pertaining to epiphytic and epilithic *Pilayella* were transformed ( $\text{Arctan}(x + 100)$ ), as were the data pertaining to total epilithic filamentous algae, *Fucus* juveniles and abundance of *Radix balthica* (formerly *Lymnaea peregra*) ( $\log_{10}(x + 1)$ ), so as to meet the assumptions of homogeneity of variance.

## RESULTS

### Nutrient analysis of *Pilayella* exudates

Nutrient concentrations (in  $\mu\text{mol l}^{-1}$ ) in the original exudates from fresh and decaying *Pilayella* were 0.3 and 1.7 NO<sub>3</sub>-N, 0.6 and 18.9 NH<sub>4</sub>-N and 0.1 and 50.5 PO<sub>4</sub>-P, respectively. The proportions of nutrients in the different concentrations of exudates are shown in Table 1.

### Effects of exudates on *Fucus* attachment

Attachment of *Fucus* was initially inhibited (6 to 12 h) by exudates from both fresh and decaying *Pilayella* (Fig. 1). Approximately 20% of the fertilized eggs attached in the 5 different concentrations (0.1, 1.0, 5, 10 and 25%) of both exudates (FP and DP) at 6 and 12 h after fertilization, while about 80% managed

to attach in the controls (Tukey post-hoc,  $p < 0.05$  in all cases). At 24 and 48 h after fertilization neither exudate ( $p > 0.05$  in both cases) produced negative effects, and at 48 h, 80 to 98% of all zygotes had attached irrespective of treatment.

### Germination, survival and rhizoid development

The effect of exudates on germination, rhizoid development and survival of *Fucus* germlings did not differ between the 2 treatment media (FP and DP) (Mann-Whitney  $p > 0.05$ , in all cases). The lowest tested concentration (0.1%) of fresh *Pilayella* exudate had a positive effect on germination ( $p < 0.05$ ), while a high concentration (25%) of both FP and DP exudates affected germination negatively (Dunnnett T3 post-hoc  $p < 0.01$  and  $p < 0.05$ , respectively) (Fig. 2A). The rhizoid development process was more sensitive to the treatments, and was

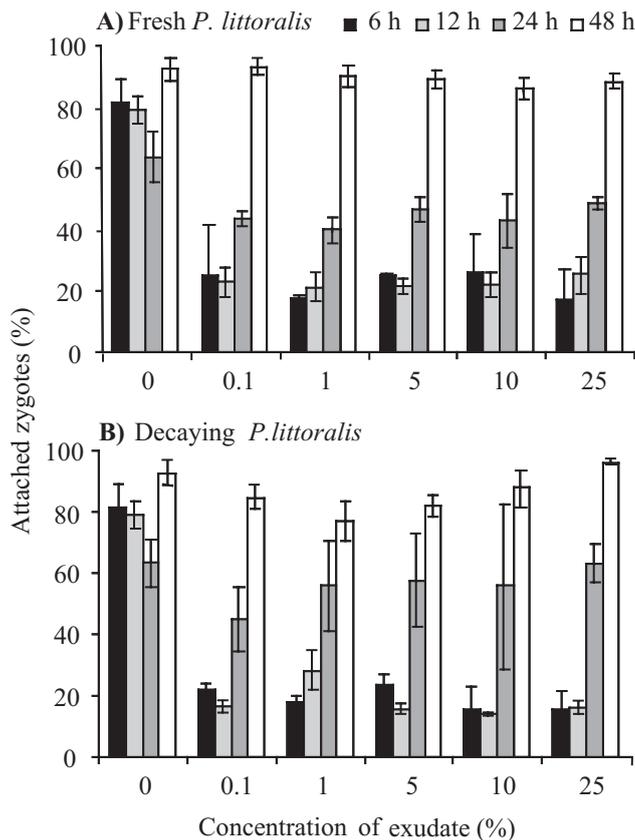


Fig. 1. *Fucus vesiculosus*. Attachment (%) of germlings at 6, 12, 24 and 48 h in exudates from (A) fresh and (B) decaying *Pilayella littoralis*. Bars show mean value  $\pm$  SE

already reduced in 1% of DP ( $p < 0.01$ ) and 5% of FP ( $p < 0.05$ ) exudates (Fig. 2B). Similar concentrations had a positive effect on the survival of the germlings (12 d), where FP exudates enhanced survival at 1 and 10% ( $p < 0.01$  and  $< 0.05$ , respectively) while DP exudates had a positive significant effect at concentrations of 10 and 25% ( $p < 0.001$  and  $< 0.01$ , respectively) (Fig. 2C). The survival rate in the controls was, however, rather low at approximately 25% (Fig. 2C).

### Substrate competition

Fewer *Fucus* germlings became established on substrates on which *Pilayella* was present prior to settling. Fewer than 5 germlings  $\text{dm}^{-2}$  became established on stones with turfs, while about 100 germlings  $\text{dm}^{-2}$  became established on stones free from epilithic *Pilayella* ( $p < 0.001$ ), representing a 95% reduction in settled germlings.

### Occurrence of *Pilayella* and total filamentous algae

The biomass of *Pilayella* as epiphytes on *Fucus* and the total biomass of epiphytic filamentous algae differed significantly ( $p < 0.001$  and  $< 0.05$ , respectively) between the 2 collection periods (May and September), the highest biomass being found in May, while no difference was detected between summer- and autumn-reproducing *Fucus* (Table 2, Fig. 3A). The dominant epiphytic filamentous alga in May was *Pilayella*, while the brown alga *Dictyosiphon foeniculaceus* contributed the highest epiphytic biomass in September.

The same relationship in biomass variation between May and September was found for the epilithic filamentous algae, with a significantly higher biomass of *Pilayella* and total filamentous algae occurring in May ( $p < 0.01$  in both cases) than in September, with no difference between summer- or autumn-reproducing *Fucus* (Table 2). The biomass of epilithic *Pilayella* was approximately 34.5 and 3.3 g dry wt  $\text{m}^{-2}$  in May and September, respectively, while the biomass of total epilithic filamentous algae was slightly higher, at 35.4 and 4.9 g dry wt  $\text{m}^{-2}$  for the same periods.

### Juveniles under the canopy of *Fucus*

There were no significant ( $p > 0.05$ ) differences between season (i.e. May and September) or between summer- and autumn-reproducing *Fucus* in terms of biomass of 1 to 2 yr old juveniles collected under the *Fucus* canopy (Table 2, Fig. 3B).

### Associated macrofauna in *Fucus*

The 5 most common macrofaunal species living in *Fucus* were the crustaceans *Gammarus* spp. and *Idotea baltica*, the gastropods *Theodoxus fluviatilis* and *Radix balthica* and the bivalve *Mytilus edulis*. The abundance of these species and the total number of collected fauna did not differ between summer- and autumn-reproducing *Fucus* stands ( $p > 0.05$ ) (Fig. 3C). The only species that differed significantly in abundance ( $p < 0.01$ ) between sampling times was *Gammarus* spp., with the highest numbers occurring in May (no data shown).

The biomass of associated fauna followed the same pattern as did the abundance data, and did not differ significantly between summer- and autumn-reproduc-

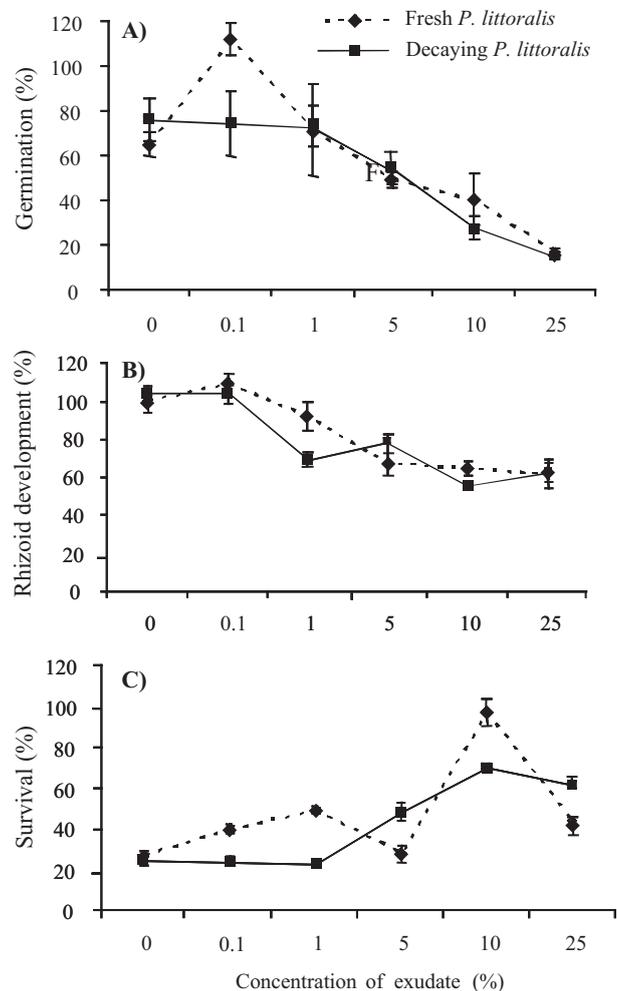


Fig. 2. *Fucus vesiculosus*. (A) Germination frequency at 5 d after fertilization, (B) rhizoid development at 12 d after fertilization and (C) survival frequency at 12 d after fertilization of germlings in treatment media with fresh and decaying *Pilayella littoralis* (mean  $\pm$  SE)

Table 2. Results of the 2-way ANOVAs testing for differences in the biomass of epiphytic and epilithic *Pilayella littoralis* and total filamentous algae as well as *Fucus vesiculosus* juveniles between different collection times, i.e. season (May and September), and different periods of *F. vesiculosus* reproduction (i.e. summer- and autumn-reproducing *F. vesiculosus*). ns =  $p > 0.05$

Source of variation	df	MS	F	p
<b>Biomass of epiphytic <i>P. littoralis</i> 100 g<sup>-1</sup> dry wt <i>F. vesiculosus</i></b>				
Season	1	0.000	30.19	<0.001
Period of reproduction	1	0.000	0.22	ns
Season × period of reproduction	1	0.000	0.52	ns
Residual	8	0.000		
<b>Biomass of total epiphytic filamentous algae 100 g<sup>-1</sup> dry wt <i>F. vesiculosus</i></b>				
Season	1	1205.3	11.00	<0.05
Period of reproduction	1	2.7	0.02	ns
Season × period of reproduction	1	74.1	0.68	ns
Residual	8	109.6		
<b>Biomass of epilithic <i>P. littoralis</i> m<sup>-2</sup></b>				
Season	1	0.000	21.24	<0.01
Period of reproduction	1	0.000	0.15	ns
Season × period of reproduction	1	0.000	0.27	ns
Residual	9	0.000		
<b>Biomass of total epilithic filamentous algae m<sup>-2</sup></b>				
Season	1	2.496	18.22	<0.01
Period of reproduction	1	0.000	0.00	ns
Season × period of reproduction	1	0.013	0.09	ns
Residual	9	0.137		
<b>Biomass of <i>Fucus</i> juveniles m<sup>-2</sup></b>				
Season	1	0.348	3.39	ns
Period of reproduction	1	0.321	3.13	ns
Season × period of reproduction	1	0.345	3.36	ns
Residual	9	0.103		

ing *Fucus* ( $p > 0.05$ ) (no data shown). The only faunal species of which the biomass differed significantly between the collection seasons was again *Gammarus* spp., with the highest biomass occurring in May ( $p < 0.05$ ) (no data shown).

## DISCUSSION

### Effects of *Pilayella* exudates

This report demonstrates that the negative impacts of *Pilayella* exudates on *Fucus* may inhibit *Fucus* recruitment in areas with great abundance of *Pilayella*. The results further show that the toxic products in *Pilayella* may not, as hypothesised, be released mainly by decomposing *Pilayella* (H-1), but also from viable populations.

The hypothesis that the initial attachment process of *Fucus* zygotes would be sensitive to exudates of *Pilayella* (H-2) was, however, supported. Zygotes were most sensitive to the exudates at 6 and 12 h after fertilization, and initial attachment was reduced even at the lowest concentration tested. The later life stages, i.e. germination

(5 d) and rhizoid development (12 d), were also negatively affected by *Pilayella* exudates, but only at higher concentrations. The survival of zygotes (12 d) was in fact positively affected by approximately the same high concentrations; thus our hypothesis (H-3) could only partly be supported. These results suggest that *Fucus* sensitivity to potentially toxic substances was related to its life stage.

After fertilization, *Fucus* embryos must undergo a series of critical developmental steps, including axis fixation, cell wall deposition, secretion of adhesive products, germination of rhizoid and cell division (Kropf 1997, Brownlee & Bouget 1998). Our results indicate that *Pilayella* exudates may contain substances that retard or inhibit these processes. Inhibited attachment in the littoral zone may involve an increased risk that *Fucus* may be disturbed by waves or currents during the settling process, resulting in unsuccessful settlement. Recently, Bergström et al. (2003) showed that the attachment and early post-fertilization processes in *Fucus* are sensitive to high levels of nutrients. As concentrations of 5 to 25% of exudates of decaying *Pilayella*

contained very high levels of nutrients (2.5 to 12.6  $\mu\text{mol l}^{-1}$  phosphate, 0.9 to 4.7  $\mu\text{mol l}^{-1}$  ammonia), observed negative impacts at these concentrations may be an effect of nutrient toxicity. However, the decline in germination and the reduction of rhizoid development at exudate concentrations of 5 to 25% were similar with exudates from both fresh and decaying *Pilayella*, despite nutrient levels being much less in the exudates from fresh *Pilayella*. The attachment at 6 and 12 h was also reduced in both treatment media at the lowest tested concentration (0.1%, <0.1  $\mu\text{mol l}^{-1}$  phosphate, nitrate and ammonia), so this reduction was unrelated to nutrient levels. Therefore, nutrient level is not the factor causing the retarded attachment, and may only partly explain the adverse effects on germination and rhizoid development. The results imply that *Pilayella* contains substances other than nutrients that are toxic to or interfere with early life stages in *Fucus*.

A recent study has shown that 2 common green macroalgae (*Ulva fenestrata* and *Ulvaria obscura*) from the northeastern Pacific had similar toxic properties in reducing development of *Fucus gardneri* Silva zygotes and oyster larvae (Nelson et al. 2003). It was suggested

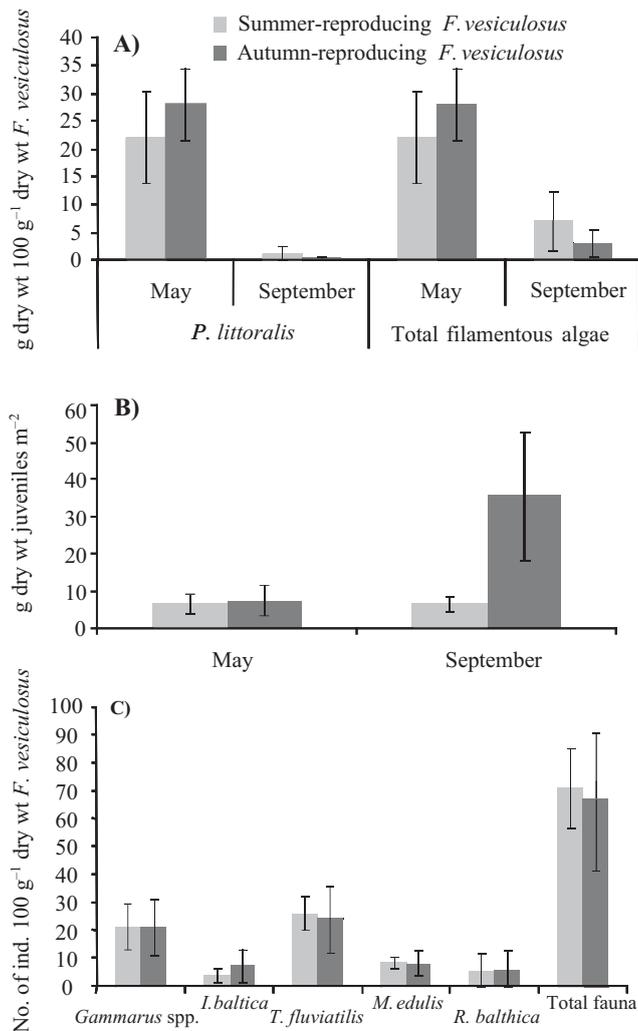


Fig. 3. (A) Biomass of epiphytic *Pilayella littoralis* and total filamentous algae ( $n = 3$ ). (B) 1 to 2 yr old juveniles of *Fucus vesiculosus* collected in 2 seasons (i.e. May and September) under the canopy of summer- respectively autumn-reproducing *F. vesiculosus* ( $n = 3$  for all variables except juveniles collected in September under the canopy of summer-reproducing *F. vesiculosus* which has  $n = 4$ ). (C) Abundance of the 5 most common macrofaunal species and the total number of macrofauna in summer- and autumn-reproducing *F. vesiculosus* ( $n = 7$  for summer- and  $n = 6$  for autumn-reproducing *F. vesiculosus*). The species are *Gammarus* spp., *Idotea baltica*, *Theodoxus fluviatilis*, *Mytilus edulis* and *Radix balthica* (formerly *Lymnaea peregra*). Bars show mean value  $\pm$  SE

that the toxic impacts of blooms of these green algae might be more important than are generally recognized (Nelson et al. 2003). As *Pilayella* is one of the dominant algae in the Baltic Sea in summer (Wallentinus 1979, Kiirikki & Lehvo 1997, Lotze et al. 1999), any toxic substance released by this species may have significant consequences in coastal zones. Toxic substances released by *Pilayella* may, for instance, contain

polyphenolic substances known as phlorotannins—known to be produced by brown algae, including *Pilayella* (Ragan & Glombitza 1986). The negative effects we found of *Pilayella* exudates on the early life stages of *Fucus*, could be a consequence of these substances, which have been suggested as having allelopathic effects on unicellular (McLachlan & Craige 1964, 1966) and crustose red algae (Fletcher 1975). *Pilayella* exudates may also contain organobromine compounds that are produced naturally by brown algae (Gschwend et al. 1985) for chemical defence and as hormones (Gribble 2000). These substances are easily released into the surrounding water (Gschwend et al. 1985). Since low concentrations of bromine inhibit germination and apical hair formation in *Fucus* (Andersson et al. 1992), it is also possible that organobromine compounds from *Pilayella* exudates are responsible for the negative effects shown in this study. The structure of phenols and the occurrence of brominated compounds in *Pilayella* are so far unknown, and research aimed at identifying these substances is needed.

The positive effect of high concentrations of exudates on *Fucus* survival may indicate a process of germling 'hardening'—i.e. the surviving germlings have become more resistant during exposure to adverse conditions (Chapman & Fletcher 2002). It may also be reasonable to believe that the higher concentrations of exudates contained substances that stimulated surviving germlings. The survival rate in the control was low (around 25%), and limiting nutrient levels in this treatment may explain this result. The higher nutrient levels in the *Pilayella* exudate treatments than in the control may, for example, have benefited growing germlings which survived the critical attachment and germination processes. The results also suggest and confirm that survival during the early life stages is a non-linear function of age (Brawley & Johnson 1991), which emphasises the importance of including various life stages in toxicity tests and other ecologically relevant experiments.

### Physical impact

Besides the chemical impact, *Pilayella* also, as hypothesised (H-4), substantially reduced *Fucus* recruitment by physical means if it was present on the substrate prior to settling of *Fucus* embryos. The inhibition of *Fucus* recruitment is likely to be a result of space pre-emption. This may explain the observed negative correlation between cover of filamentous algae and cover of *Fucus* in the Baltic Sea (Worm et al. 1999). Similar interference competition has been found to occur between *Fucus* and both the common green

algae *Cladophora glomerata* (Berger et al. 2003) and *Enteromorpha* spp. (Worm et al. 2001).

### Field relevance and ecological consequences

As hypothesised, the field studies revealed great differences in the biomass of epiphytic and epilithic *Pilayella* and total filamentous algae between the 2 different periods of *Fucus* reproduction, the highest biomasses occurring in May (H-5). The smaller amount of filamentous algae (especially *Pilayella*) in autumn may give zygotes from autumn-reproducing *Fucus* an advantage over zygotes from summer-reproducing *Fucus*. However, the biomass of 1 to 2 yr old juveniles did not differ between the summer- and autumn-reproducing *Fucus*. Hence, the result did not support the hypothesis that the autumn-reproducing *Fucus* would have higher juvenile biomass than would the summer-reproducing *Fucus* (H-6). This result may be due to very high variation in juvenile biomass under the autumn-reproducing *Fucus*. Thus, a larger field study is required to determine the true differences in recruitment success between the summer- and autumn-reproducing *Fucus*.

The field studies further revealed that the biomass of epilithic *Pilayella* growing under the *Fucus* canopy in May (35 g dry wt m<sup>-2</sup>) was lower than implied by measurements made in similar areas without macroalgal canopy, where *Pilayella* may reach over 200 g dry wt m<sup>-2</sup> (Wallentinus 1979, S. Råberg unpubl. data). Since our attachment experiment showed that the settling rate of *Fucus* zygotes was retarded in the presence of *Pilayella* exudates, zygotes in the field may easily be transported away from the area of *Fucus* canopy and hence be forced to compete with even higher abundances of epilithic *Pilayella* and filamentous algae in general. Besides physical competition and allelopathic interactions, filamentous turfs also tend to trap fine sediment and particles (Eriksson et al. 1998, Airoidi 2003 and references therein), further decreasing the possibility of *Fucus* recruits establishing themselves on suitable substrate. Based on the present results and other observed negative correlations between filamentous algae and *Fucus* (Worm et al. 2001, Berger et al. 2003), we predict that the likelihood of *Fucus* re-invading or colonizing habitats already dominated by a flora of annual filamentous algae are small. Such interference competition would be of minor importance in exposed areas, however, where the whiplash effect of adult *Fucus* prevents large-scale establishment of filamentous algae (Kiirikki 1996).

The higher biomass of *Pilayella* in summer indicates that the autumn-reproducing *Fucus* may encounter less competition with *Pilayella* for substrates and less nega-

tive interactions with exudates, and therefore have a competitive advantage over summer-reproducing *Fucus*. According to the null hypothesis (H-7), there were no differences in the amount of epiphytic and epilithic *Pilayella* and total filamentous algae or abundance and biomass of associated macrofauna between summer- and autumn-reproducing *Fucus*. Therefore, a potential shift from a community dominated by summer-reproducing *Fucus* to a community dominated by autumn-reproducing *Fucus* would not change the overall species composition in the ecosystem.

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