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## Epibiota communities of the introduced and indigenous macroalgal relatives *Sargassum muticum* and *Halidrys siliquosa* in Limfjorden (Denmark)

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**Abstract** *Sargassum muticum* (Phaeophyceae, Fucales) has recently been introduced to Limfjorden (Denmark) where its closest relative is the indigenous *Halidrys siliquosa*. Previous studies have demonstrated large quantitative (canopy biomass) and qualitative (canopy persistence) differences in the habitat available to epibiota within the canopies of these two macroalgae. We therefore hypothesised that these algae would support different epibiota communities and tested this by sampling the epibiota of *S. muticum* and *H. siliquosa* on seven occasions throughout 1997 by enclosing entire thalli in mesh bags. We found 53 epibiota taxa and, with only one exception, they were all recorded on both host species. Species richness and abundance of epibiota exhibited clear seasonal variation on both host species, although epibiota biomass was seasonally constant on *H. siliquosa* but not on *S. muticum*. These patterns were consistent with the different life histories of the host species. There was a weakly negative correlation between thallus size

and epibiota biomass for both host species. When taking species-specific seasonal variation in thallus size into consideration, *S. muticum* and *H. siliquosa* were found to support significantly different epibiota biomasses. Multivariate analyses showed that epibiota community structure was different, although highly overlapping, between the two species, whereas there was an almost parallel temporal development in epibiota community structure. We conclude that it is unlikely that the introduction of *S. muticum* to Limfjorden has caused major changes in local epibiota community structure. However, the standing stock of epibiota is likely to have increased.

**Keywords** *Sargassum muticum* · *Halidrys siliquosa* · Epibiota · Canopy habitat · Community structure

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### Introduction

The introduction and subsequent expansion of *Sargassum muticum* (Yendo) Fensholt as a permanent member of the European algal flora has caused general concern about the effects on indigenous biota (Critchley et al. 1987; Ruessens 1989; Thelin 1989; Den Hartog 1997; Karlsson and Loo 1999). Several studies have indicated negative impacts by *S. muticum* on indigenous algal assemblages (Fletcher and Fletcher 1975; Ambrose and Nelson 1982; Viejo 1997; Cosson 1999; Staehr et al. 2000) but only a limited number of studies have addressed the possible consequences for macroalgal epibiota. Those that have, have suggested few adverse effects, apparently due to a low degree of habitat specificity among the epibiota (Withers et al. 1975; Jephson and Gray 1977; Viejo 1999). Nevertheless, following the introduction of *S. muticum* to Limfjorden, a shallow Danish sound, biologists noted conspicuously more epibiota associated with *S. muticum* relative to other algae (M. Lauersen, County of Ringkøbing, personal communication).

*S. muticum* was first recorded in Limfjorden in 1984 (Christensen 1984). Over a period of less than 15 years the population grew from a few isolated individuals to

become the dominant component of the macroalgal flora (Staehr et al. 2000). Such invasive behaviour is the normal pattern where *S. muticum* has been introduced (Knoepffler-Peguy et al. 1985; Núñez-López and Casas Valdez 1998; Cosson 1999; Fernandez 1999; Karlsson and Loo 1999).

*Halidrys siliquosa* (L.) Lyngb. is a close relative of *S. muticum* (Jensen 1974; Rousseau et al. 1997) and one of only a few large (>15 cm) erect indigenous macroalgae in Limfjorden. *S. muticum* and *H. siliquosa* both have coarsely branched thalli that create a relatively large canopy which may be a suitable habitat for a wide variety of fauna and flora (Mukai 1971; Withers et al. 1975; Haage 1976; Norton and Benson 1983; Aguilar-Rosas and Galindo 1990; Taylor and Cole 1994). During the course of a year, however, the habit of *S. muticum* is characterised by dramatic variation whereas the habit of *H. siliquosa* is much more constant (Wernberg et al. 2001): in spring, *S. muticum* grows from a small (10–15 cm) perennial basal structure into a large (100–120 cm) erect canopy before it senesces back in autumn, whereas *H. siliquosa* retains an erect canopy of intermediate size (~35 cm) throughout the year. We proposed that these quantitative (host size) and qualitative (host phenology) differences in resource (canopy habitat) availability were important to the epibiota community, and therefore tested the hypothesis that *S. muticum* and *H. siliquosa* support different epibiota biomass and species composition.

## Methods

### Study site

The study was conducted in Dråby Vig (56°49'92"N 08°50'70"E), western Limfjorden (Denmark). The study site was located on an eastern shore protected from the predominantly westerly winds. The surface temperature and salinity range annually from –1 to 25°C and 25 to 28‰, respectively (Groos et al. 1996). *S. muticum* has been present at the study site since the late 1980's (Wernberg-Møller et al. 1998a) and now forms dense beds from 2 to 4 m depth (Wernberg-Møller et al. 1998b). *H. siliquosa* is present as scattered individuals among the *S. muticum* (Wernberg-Møller et al. 1998b). All samples were taken from within the main *Sargassum* bed.

### Sampling

Epibiota were sampled once a month from May 1997 to January 1998, except October and December. Scuba divers haphazardly collected four to six individuals of each of *S. muticum* and *H. siliquosa* by carefully, but swiftly, placing a 1 mm mesh bag over the thallus of each alga (Norton and Benson 1983; Connolly 1995). The thallus was then detached from the substratum and brought ashore to be preserved in 70% ethanol.

In the laboratory, individually collected thalli were placed in white plastic trays and cleaned for epibiota by gentle flushing and brushing. Water from the trays was filtered through a 1 mm sieve to retain the epibiota. Fresh weight (FW) of the preserved *S. muticum* and *H. siliquosa* was measured after spinning the algal material in a mesh bag to remove excess water (Norton and Benson 1983). The epibiota was identified to lowest possible taxonomical level (usually species or genus). Faunal and algal taxonomy follow Hayward and Ryland (1996) and Nielsen et al. (1995), respectively. Individuals were counted for all taxa except colonial taxa (e.g. *Obelia*

spp.) and taxa otherwise without distinct individuals (e.g. filamentous algae). These taxa were registered as one individual. The wet weight (WW) of the preserved epibiota was determined after blotting with paper towels. For taxa with large individuals (e.g. *Styela clava*), WW was determined for each sample; whereas in taxa with small individuals (e.g. *Phytosia marina*), WW was estimated from mean values per individual obtained from measurements of large batches. Taxa with extremely small (often <1 mm) and inconspicuous individuals (e.g. Nematoda spp.) were registered as having a biomass of 0.001 g WW regardless of abundance. To alleviate the large differences in skeletal and shell components among taxa, epibiota WW was converted to ash free dry weight (AFDW) by applying conversion factors from Lappalainen and Kangas (1975), Ricciardi and Bourget (1998) and our own unpublished data.

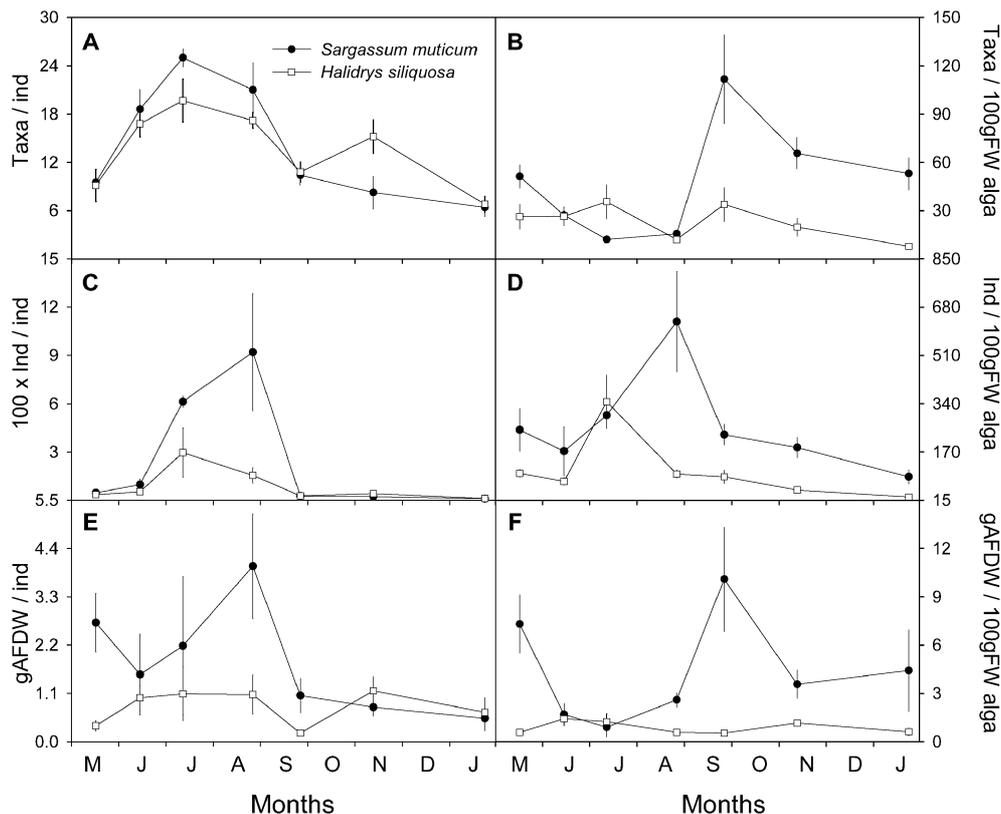
### Data analysis

A total of 70 samples, 35 from each species of macroalga, covering seven sampling times, were examined. Algal FW was used as a proxy for canopy habitat. This was justified by tight correlations between algal height and biomass (Wernberg et al. 2001) as well as height and canopy volume (sensu Hacker and Steneck 1990) for both macroalgal species (T. Wernberg, M.S. Thomsen and P.A. Staehr, unpublished data). Because of the relative importance of epibiota taxa with a few large or an obscure definition of individuals, biomass (mg AFDW/100 g FW alga) was the preferred unit for analysis and presentation, although primary data are also presented in other units commonly encountered in the literature (see Fig. 1).

First, using a mixed-model two-way ANOVA, we tested whether the canopy habitat offered by the collected specimens of *S. muticum* and *H. siliquosa* conformed to the general pattern hypothesised to influence epibiota ( $H_0$ : *S. muticum* and *H. siliquosa* offer equal amounts of canopy habitat, and there are no temporal differences in the amount of canopy habitat offered by either of these species). Then, using a mixed-model two-way ANCOVA, we tested whether *S. muticum* and *H. siliquosa* had different amounts of associated epibiota ( $H_0$ : *S. muticum* and *H. siliquosa* have equal amounts of associated epibiota at all sampling times when adjusting for differences in canopy habitat). In both analyses, algal species ('Species') was considered a fixed factor and sampling time ('Time') a random factor. Canopy habitat (thallus size, g FW) was the covariate. These analyses were performed on a slightly modified data set where, to balance the design, the mean of the four samples was added to each of the May data sets, and one sample was randomly removed from each of *S. muticum*/November and *H. siliquosa*/July data sets (see Table 2). Prior to analysis, data were log transformed to meet parametric assumptions of normality ( $\chi^2$  test,  $P < 0.05$ ) and homoscedasticity (Cochran's C-test,  $P < 0.05$ ).

To test if there were temporal differences and if epibiota communities were different between *S. muticum* and *H. siliquosa*, a two-way crossed ANOSIM was performed in PRIMER (Clarke and Gorley 2001) ( $H_0$ : there are no temporal differences and there are no differences in the epibiota communities of *S. muticum* and *H. siliquosa*). Bray-Curtis similarity matrices were constructed from both square root-transformed biomass data and presence/absence (p/a)-transformed data. The first transformation was chosen to reduce the influence of large variability among replicates, while retaining the quantitative information on the dominance of abundant taxa (Clarke and Warwick 1996), whereas the latter transformation was chosen to emphasise effects purely due to species distributions. The similarity data were plotted in nMDS plots. We used principal axis correlation (Belbin 1995) to correlate individual taxa to the pattern produced by the nMDS. This procedure yields a principal axis correlation coefficient (PCC) for each taxon, which can be interpreted as the weight of that taxon in driving the observed patterns (Belbin 1995).

**Fig. 1A–F** Seasonal development in epibiota loading on *Sargassum muticum* and *Halidrys siliquosa* given as species (taxa), abundance and biomass per individual (A, C, E) and per biomass of alga (B, D, F). Error bars are standard errors ( $n$  values can be found in Table 2)



## Results

Overall, there were no statistically significant differences in the biomass of the sampled *S. muticum* and *H. siliquosa* thalli (Table 1). The effect of sampling time and the interaction between host species and sampling time was, however, highly significant. There was also a highly significant difference in thallus biomass among species at different sampling times. These results agree closely with the observations of Wernberg et al. (2001), and suggest that the canopy habitat of the sampled specimens conformed to the quantitative and qualitative patterns hypothesised to influence the epibiota communities.

A total of 53 epibiota taxa, representing nine animal phyla (46 taxa) and three algal divisions (seven taxa), were found on the 70 individuals of *S. muticum* and *H. siliquosa* sampled (Table 2). With the exception of ‘Malacostraca (other)’, a quantitatively insignificant group that was found only on *S. muticum*, all taxa were found on both species of macroalgae. Arthropoda (Crustacea) was the most abundant phylum in terms of number of taxa, and Chordata (Urochordata) in terms of biomass.

The absolute species richness of epibiota per individual (Fig. 1A) followed the same temporal pattern on both macroalgae, with an increasing number of associated taxa from spring towards a summer peak in July. There was a tendency for *S. muticum* to have more species early in the year and *H. siliquosa* late in the year. The relative species richness per biomass (Fig. 1B) showed a markedly different pattern, where *S. muticum* had a distinct peak in

**Table 1** Test of differences in canopy habitat availability. ANOVA on LOG-transformed biomass (g FW) of collected *Sargassum muticum* and *Halidrys siliquosa* individuals

Effect	<i>df</i>	MS	<i>F</i>	<i>P</i>
Species	1	1.0515	1.75	0.23
Time	6	0.8903	15.4	<0.001
Species×Time	6	0.6013	10.4	<0.001
Residual	56	0.0580		
Cochran's <i>C</i>		<i>C</i> =0.22		

September and retained higher relative species numbers late in the year, while *H. siliquosa* did not appear to have any systematic temporal variation. The epibiota on both macroalgae showed a distinct summer peak in absolute (Fig. 1C) and relative (Fig. 1D) abundance. There was, however, a time lag between maximum abundance on *H. siliquosa* (July) and *S. muticum* (August). Biomass of epibiota (Fig. 1E, F) had a peak in early and late summer for *S. muticum* but was constant for *H. siliquosa*. *H. siliquosa* generally supported lower epibiota biomass than *S. muticum*.

The ANCOVA revealed homogeneity of slopes in regressions between algal size (g FW) and epibiota load (g WW/100 g FW alga) both between host species ( $P=0.66$ ) and among sampling times ( $P=0.99$ ), i.e. irrespective of host species and sampling time, the ratio of algal size to epibiota load remained the same. Correlation analysis on pooled data showed this common relationship between thallus size and epibiota load to be weakly negative but

**Table 2** Mean biomass of epibiota (mg AFDW) relative to biomass (100 g FW) of *S. muticum* and *H. siliquosa* ±S.E. × present without biomass (<5 mg AFDW/100 g FW alga)

Taxon	AFDW <sup>a</sup> (% of WW)	<i>Sargassum muticum</i>												<i>Haliodyx siliquosa</i>											
		May (n=4)	Jun (n=5)	Jul (n=5)	Aug (n=5)	Sep (n=5)	Nov (n=6)	Jan (n=5)	May (n=4)	Jun (n=5)	Jul (n=6)	Aug (n=5)	Sep (n=5)	Nov (n=5)	Jan (n=5)										
<b>Porifera (Calcarea &amp; Demospongiae)</b>		37.1±0.0	296±171	153±142	10±10	544±61	×	36±4	107±10	678±37	18±2	-	×	21±2	×										
<i>Leucosolenia</i> sp.	10.7	26±26	25±16	-	×	×	×	-	-	667±37	×	-	×	×	×										
<i>Haliichondria panicea</i> Pallas	10.7	507±226	248±170	65±55	9±9	44±6	×	-	24±3	×	16±2	-	×	21±2	×										
<i>Halielona oculata</i> Pallas	10.7	1,049±949	22±12	87±87	×	500±62	×	36±4	83±10	9±1	-	-	×	×	×										
<b>Cnidaria (Hydrozoa &amp; Anthozoa)</b>		9±4	53±30	×	91±45	×	336±15	67±4	67±4	76±4	8±1	×	107±6	38±3	9±1										
<i>Obelia</i> sp.	7.3 <sup>b</sup>	8±4	53±30	×	91±45	×	313±13	23±2	24±2	76±4	8±1	×	15±2	38±3	6±0										
<i>Sargatia</i> sp.	14.3	×	×	×	×	×	23±2	×	×	×	×	×	91±7	×	×										
<b>Nematoda</b>		×	×	×	×	×	×	×	×	×	×	×	×	×	×	×									
<b>Amelida (Polychaeta)</b>		81±28	32±4	11±2	241±51	109±4	179±4	62±4	81±3	30±3	10±0	×	113±8	10±1	6±1										
<i>Aprudridae</i> spp.	17.1	10±7	15±4	6±1	10±5	20±2	42±1	19±1	×	×	6±0	×	×	×	×										
<i>Nereidae</i> spp.	17.1	57±19	12±3	×	79±20	31±2	35±3	31±4	35±2	7±0	×	22±1	13±0	×	5±1										
<i>Phyllodoce</i> spp.	17.1	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×									
<i>Syllidae</i> spp.	17.1	×	×	×	151±52	57±3	99±4	12±2	39±4	21±2	×	14±1	98±8	8±1	×										
<i>Serpulidae</i> spp.	7.5 <sup>b</sup>	×	×	×	1,060±277	2,774±251	111±11	309±28	189±13	20±1	869±61	322±13	232±14	279±8	30±3										
<b>Polychaeta (other)</b>		81±31	639±420	460±419	1,060±277	2,774±251	111±11	309±28	189±13	20±1	869±61	322±13	232±14	279±8	30±3										
<b>Arthropoda (Crustacea)</b>		×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<i>Ostracoda</i> spp.	15.5	×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<i>Praunus inermis</i> Rathke	15.5	×	×	×	×	9±1	×	×	×	×	10±0	×	×	×	×										
<i>Praunus flexuosus</i> Müller	15.5	×	×	×	×	×	×	×	×	×	×	28±3	×	×	×										
<i>Latona baltica</i> Pallas	14.2	×	×	×	40±22	×	×	×	×	6±0	16±1	10±0	×	×	×										
<i>Iphimedia</i> spp.	16.0	×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<b>Gammaridae</b> spp.		×	×	×	×	16±2	×	×	×	×	×	×	×	×	×										
<b>Amphipoda</b> spp.		22±12	×	10±2	×	×	5±1	×	5±0	×	17±0	×	10±1	×	×										
<i>Jassa falcata</i> Montagu	16.0	×	×	×	×	×	×	×	×	×	6±0	×	×	×	×										
<i>Phitsica marina</i> Slabber	16.0	×	×	×	×	×	×	×	×	×	6±1	×	×	×	×										
<b>Amphipoda (other)</b>		52±21	16±16	×	35±35	10±1	37±4	235±29	23±2	10±1	48±2	86±2	24±3	23±2	28±3										
<i>Palaeomon adspersus</i> Rathke	16.0	×	×	×	336±59	401±22	68±8	68±8	23±2	×	×	197±14	213±7	39±2	×										
<i>Macropodia rostrata</i> L.	16.5	×	613±423	423±422	620±229	2,320±260	63±7	×	156±13	×	761±61	191±14	×	×	×										
<i>Corcyra maenas</i> L.	13.8	×	×	×	10±1	10±1	×	×	×	×	×	×	×	×	×										
<b>Malacostraca (other)</b>		1,401±701	80±79	48±26	14±9	1,605±201	316±21	135±12	31±3	241±16	212±23	75±3	25±3	40±4	56±7										
<b>Mollusca (Gastropoda &amp; Bivalvia)</b>		×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<i>Tectura</i> sp.	7.5	×	×	×	6±6	1,605±201	94±8	102±13	27±3	9±1	7±1	32±2	24±3	36±5	56±7										
<i>Gibbula</i> sp.	7.5	8±8	78±78	23±23	×	×	177±22	×	×	227±15	183±23	×	×	×	×										
<i>Littorina</i> sp.	7.5	1,378±707	×	6±1	×	×	×	×	×	×	×	×	×	×	×										
<i>Rissoa</i> sp.	7.5	×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<i>Bitium reticulatum</i> Da Costa	7.5	×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<b>Opisthobranchia</b> spp.		×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<b>Gastropoda (others)</b>		10±5	×	13±3	×	45±3	×	33±4	×	×	16±0	×	×	×	×										
<b>Mytilidae</b> spp.	5.8	×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<b>Cardiidae</b> spp.	5.8	×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<b>Bivalvia (others)</b>		×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<b>Bryozoa (Gymnolaemata)</b>		×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<i>Electra pilosa</i> L.	7.3	1,871±1860	117±56	45±23	193±51	1,570±118	467±49	120±13	×	64±7	59±4	28±2	5±1	10±1	14±1										
<b>Echinodermata (Asteroidea &amp; Echinoidea)</b>		1,719±1708	65±34	14±9	95±61	1,030±117	57±6	12±1	×	64±7	57±4	9±1	5±1	10±1	×										
<i>Asterias rubens</i> L.	11.2	1,52±152	52±48	31±19	98±25	540±67	410±50	108±12	×	×	×	20±2	×	×	×										
<i>Psammachinus militaris</i> Müller	3.5	1,951±172	434±204	165±60	913±232	3,485±153	2,140±69	3,680±282	16±2	312±23	38±2	108±3	48±4	740±27	484±32										
<b>Chordata (Urochordata)</b>		2.5	52±27	13±9	527±153	21±2	×	6±0	×	×	9±1	80±3	13±1	×	×										
<i>Clavelina lepadiformis</i> Müller	2.5	209±187	×	×	204±90	1,668±107	94±26	2,218±143	×	×	×	14±1	30±4	714±28	406±25										
<i>Ascidella aspersa</i> Müller	2.5	1,949±174	161±159	149±63	182±108	1,796±75	1,191±61	1,462±145	×	×	18±2	13±2	5±0	22±1	78±10										
<i>Stvela clava</i> Herdman	2.5	×	12±10	×	×	×	×	×	16±2	102±8	11±1	×	×	×	×										
<i>Borysthen salmoseri</i> Pallas	2.5	×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<i>Ascidacea (other)</i>	2.5	×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<b>Chordata (Euchordata, Osteichthyes)</b>		331±108	34±17	×	5±4	×	15±1	×	114±7	×	24±2	9±1	×	×	×										
<b>Chlorophyta, Rhodophyta, Phaeophyta</b>		×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<i>Chlorella</i> sp.	2.8 <sup>d</sup>	×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<i>Callithamnion</i> sp.	2.8 <sup>d</sup>	×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<i>Ceramium</i> sp.	2.8 <sup>d</sup>	×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<i>Polysiphonia</i> sp.	2.8 <sup>d</sup>	×	26±18	×	×	×	15±1	×	10±1	×	20±2	×	×	×	×										
<b>Rhodophyta (other)</b>		308±107	×	×	×	×	×	×	104±6	×	×	×	×	×	×										
<i>Ectocarpus</i> sp.	2.8 <sup>d</sup>	×	×	×	×	×	×	×	×	×	×	×	×	×	×										
<b>Phaeophyta (other)</b>		7,308±1810	1,685±685	887±606	2,594±442	10,092±400	3,566±110	4,409±314	564±21	1,425±52	1,238±61	580±13	530±21	1,154±21	601±28										
<b>Total</b>																									

<sup>a</sup> From Ricciari and Bourget (1998)  
<sup>b</sup> Value for shelled Gastropoda  
<sup>c</sup> Values for *Pungitius pungitius* L. derived from Lappalainen and Kangas (1975)  
<sup>d</sup> Own unpublished data for *Ceramium nodulosum* (Lightf.) Ducluz

**Table 3** Test of differences in epibiota biomass. ANCOVA for the effect of (host) species and (sampling) time on the relative epibiota load (biomass). Means adjusted for covariance with thallus size (g FW). Both epibiota biomass and thallus size were log transformed prior to analysis. *df* adjusted for the addition of two pseudo-samples in May

Effect	<i>df</i>	MS	<i>F</i>	<i>P</i>
Species	1	3.773591	5.993625	0.0499
Time	6	0.323532	2.008443	0.0808
Species × time	6	0.629601	3.908470	0.0026
Residual	53	0.161086		
Cochran's <i>C</i>		$C_{\text{epibiota load}}=0.31, C_{\text{thallus size}}=0.22$		

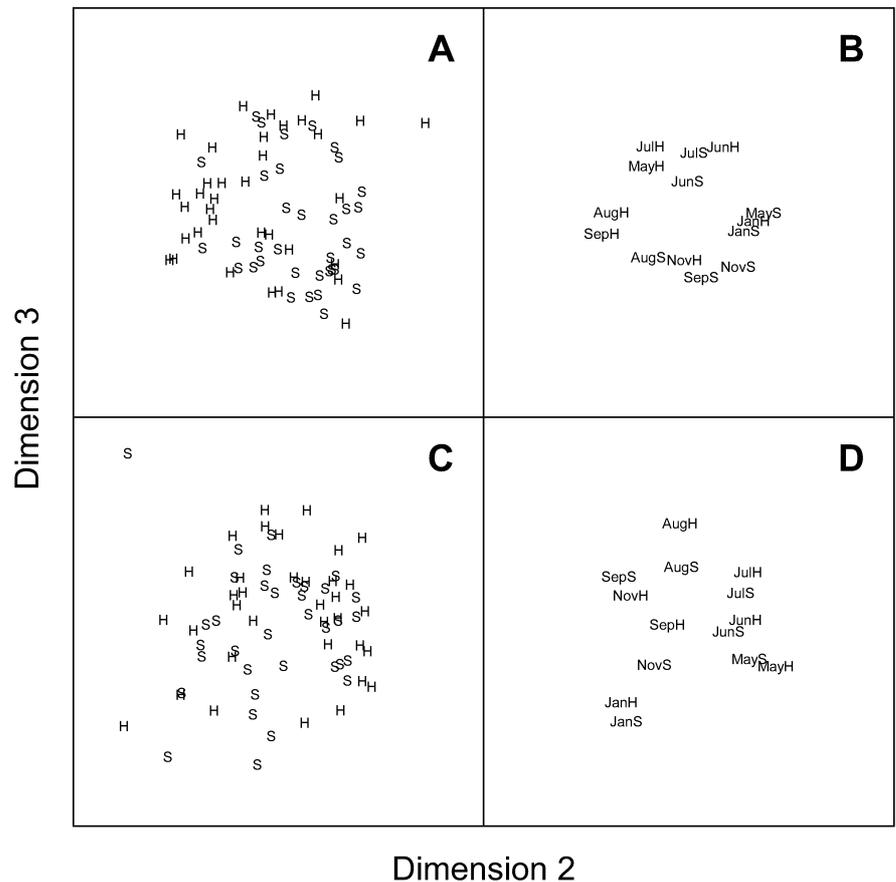
highly significant (Pearson Product Moment Correlation;  $r=-0.32, P=0.006, n=70$ ). Analyses on the means adjusted for differences in thallus size (Table 3) showed that the two macroalgae supported significantly different epibiota biomasses. There was no statistically significant effect of sampling time on epibiota biomass, suggesting a temporally constant epibiota load on both macroalgae when adjusted for temporal variation in thallus size. This result, however, must be interpreted cautiously as the *P* value is relatively low. The interaction between species and sampling time was highly significant, showing that time of year exaggerated differences in epibiota loading between the two host species.

A three-dimensional ordination of community data (Fig. 2A–D) was necessary to obtain satisfactory stress values. Only dimension 2 versus dimension 3 is presented

here, as this was the most informative plot in terms of apparent groupings. With both the p/a and the square root-transformed data, the ordination separated the samples into broad groups corresponding to samples from the two host species (Fig. 2A, C) and the different sampling times (Fig. 2B, D). These groupings were highly significant (Table 4). Global *R* values revealed that the species-wise groupings were generally weaker than the time-wise groupings, and that this pattern was most pronounced for the p/a-transformed data. This suggests that host species was important to epibiota biomass, whereas sampling time was important to epibiota community composition. The strong influence of sampling time on epibiota community structure can be seen in Fig. 2D as a clear unidirectional counter-clockwise succession of pair-wise monthly samples.

It was primarily the epibiota taxa with large, abundant individuals (Table 1), such as tunicates and crabs, that were responsible for the pattern (Table 5) seen in Fig. 2A, B whereas the patterns seen in Fig. 2C, D were mainly driven by the taxa with smaller, less abundant individuals. The differences in what taxa were driving these two ordinations suggest that differences between host species (more predominant with the square root-transformed data) are caused by differences in epibiota biomass of a constant subset of taxa rather than by differences in the kinds of associated taxa. The importance of small, infrequent taxa to the seasonal pattern (strongly dominant from the p/a-transformed data) suggests that the differences among

**Fig. 2A–D** nMDS plots of dimension 3 versus dimension 2 for all samples collected, and monthly averages for each algal species. **A, B** square root-transformed data (stress=0.20). **C, D** presence/absence-transformed data (stress=0.19). *S. S. muricatum*, *H. H. siliquosa*



**Table 4** Results of two-way crossed ANOSIM for the effect of (host) species and (sampling) time on epibiota loading.  $P < 0.001$  for all  $R$ .  $A$  square root-transformed data,  $B$  p/a-transformed data

		Global $R$
Species	A	0.338
	B	0.283
Time	A	0.492
	B	0.581

(sampling) times are caused by differences in community structure (i.e. species present) rather than by differences in epibiota biomass. In other words, the main difference between *S. muticum* and *H. siliquosa* is the abundance of epibiota, whereas the total epibiota community exhibits a clear parallel seasonal succession on both algae.

## Discussion

Our results show that the invasive *S. muticum* and the indigenous *H. siliquosa* support different amounts of epibiota, and that time of year exaggerates these differences. Further, our results suggest that the quantitative differences in epibiota load between *S. muticum* and *H. siliquosa* are matched only by relatively weak differences in epibiota community structure. Epibiota community structure on both host species showed a clear, almost parallel, temporal development.

Habitat architecture or complexity is important to epibiota, particularly to small crustaceans (Hacker and Steneck 1990; Duffy and Hay 1991; Hacker and Madin 1991; Taylor and Cole 1994). Jephson and Gray (1977) noted that *H. siliquosa* generally has few epibiota species compared to *S. muticum*. Although they have the same basic morphology (Jensen 1974), the thallus of *H. siliquosa* clearly has fewer and coarser branches than the annual thallus of *S. muticum*. Both Hacker and Steneck (1990) and Taylor and Cole (1994) found epifaunal densities to be significantly higher on algae with more complex thalli, suggesting that a similar mechanism could explain the quantitative differences between *S. muticum* and *H. siliquosa* in Limfjorden.

We found a negative correlation between thallus size and the relative amount of epibiota biomass supported.

This may seem counter-intuitive, but could be interpreted as an 'escape by growth' mechanism where the algae avoid detrimental grazing by satiating herbivores (Lobban and Harrison 1994). This is unlikely, however, as the ANCOVA showed no differences in slopes between the seasonally variable *S. muticum* and the seasonally constant (i.e. no 'escape growth') *H. siliquosa*. We consider it more likely that this negative correlation is an artefact of epibiota behaviour, rather than an algal growth strategy, as the primary site of attachment for some of the abundant (by biomass) sessile animals (e.g. *Styela clava*) was clearly around the main axis and holdfast on both host species, as it has also been observed on other macroalgae (Arrontes 1990). As the size of these parts of the thallus is relatively constant across a range of algal sizes and seasons (Wernberg-Møller et al. 1998b), the amount of epibiota which can be supported by them is independent of algal size. Smaller thalli will thus have a higher relative epibiota load than larger thalli. In *S. muticum*, this intra-individual epibiota distribution may be due to, or enhanced by, high levels of phenolic compounds produced by the rapidly growing thallus (Gorham and Lewey 1984). Jephson and Gray (1977) made similar speculations after observing extreme fouling of *S. muticum* around the time of senescence, when the concentration of these compounds drops (Gorham and Lewey 1984), something we also observed in Limfjorden around August/September. In contrast, Norton and Benson (1983) generally found more epifauna (mainly amphipods and gastropods) on the distal parts of the *S. muticum* thallus. *H. siliquosa* has been observed to slough off epidermal cells, a mechanism thought to control its epiphytes (Moss 1982).

Habitat quality (i.e. temporal stability) did not appear to be an important factor for epibiota community structure in Limfjorden, as *S. muticum* (highly variable habit) and *H. siliquosa* (constant habit) had almost identical species associated with them. Similar results were obtained by Viejo (1999) in northern Spain and Jephson and Gray (1977) in southern England. In New Zealand, Taylor and Cole (1994) studied ten different brown algae and found that most had distinct epifaunal assemblages, while individual epifaunal taxa showed little host specificity and were found on several host species. This general pattern of a lack of host specificity may be an epibiota adaptation to the fluctuating environment of temperate regions: host

**Table 5** Principal axis correlation coefficients (PCC) for the 10 taxa best correlated to (i.e. most determinant for) the three-dimensional nMDS ordinations.  $C$  Chordata (Urochordata),  $A$  Arthropoda (Crustacea),  $M$  Mollusca,  $An$  Annelida (Polychaeta),  $E$  Echinodermata (Echinoidae),  $Cn$  Cnidaria,  $Ch$  Chlorophyta

Square root-transformed data		P/a-transformed data	
Taxon	PCC	Taxon	PCC
<i>Ascidiella adspersa</i> (C)	0.80	<i>Rissoa</i> spp. (M)	0.74
<i>Styela clava</i> (C)	0.70	Polychaeta (other) (An)	0.74
<i>Macropodia rostrata</i> (A)	0.61	<i>Obelia</i> spp. (Cn)	0.73
<i>Carcinus maenas</i> (A)	0.61	<i>Clavelina lepadiformis</i> (C)	0.69
<i>Rissoa</i> spp. (M)	0.60	Mytilidae spp. (M)	0.69
<i>Praunus inermis</i> (A)	0.55	<i>Iphimedia</i> spp. (A)	0.68
<i>Phtisica marina</i> (A)	0.52	<i>Phtisica marina</i> (A)	0.66
<i>Littorina</i> spp. (M)	0.52	<i>Ceramium</i> sp. (Ch)	0.65
Serpulidae spp. (An)	0.51	<i>Praunus inermis</i> (A)	0.63
<i>Psammechinus miliaris</i> (E)	0.48	Nematoda spp.	0.63

phenology may not matter to many epibiota species because they follow the same seasonality as the algae. This is perhaps accentuated by the clear temporal change in epibiota observed in this and many other studies from temperate regions (Mukai 1971; Withers et al. 1975; Jephson and Gray 1977; Arrontes 1990; Kendrick and Burt 1997).

Crustacea comprised almost 30% of all epifaunal taxa found in this study. Norton and Benson (1983) and Pavia et al. (1999) found that this group of epifauna (mainly amphipods) were highly mobile and actively selected favourable host macroalgae. Gunnill (1982), however, found that no amphipods were able to colonize the intertidal furoid *Pelvetia fastigiata* (J. Agardh) De Toni over a 30 m distance, indicating that the range over which this selective mobility occurs is limited. Dispersal barriers may be a real problem in the establishment and development of an *H. siliquosa* epibiota community in Limfjorden because the individual plants are scattered several metres apart among the *S. muticum* individuals (Wernberg-Møller et al. 1998b).

Using a similar approach to us, Viejo (1999) estimated the impact of *S. muticum* invasion on mobile epifauna by comparing contemporary patterns of abundance and species distributions among *S. muticum* and indigenous equivalents (*Cystoseira nodicaulis* and *Fucus vesiculosus*). As she found no major differences in mobile epibiota associated with any of these algae, she concluded that local epibiota colonised *S. muticum* equally well as they colonised either of the indigenous host species, and thus that there was no effect of its invasion. Since no pre-invasion data were available, such an argument assumes that the total epibiota community had not already been changed, and this in turn probably depends on a combination of the relative abundance of the invader and the time it had been present or dominant in the system. This seriously reduces the power of any conclusion derived from a retained null hypothesis (i.e. no differences among invader and indigenous species). *S. muticum* has been a dominant component of the macroalgal flora at our study site for more than 10 years (Wernberg-Møller et al. 1998a), and because of large differences in abundance of the two macroalgae (Wernberg et al. 2001) it cannot be ruled out that the epibiota community structure radically changed with the invasion of *S. muticum* and that the contemporary epibiota community structure of *H. siliquosa* is, to a large degree, the result of a 'spill-over' effect. The observed quantitative differences in epibiota between *S. muticum* and *H. siliquosa* are, however, independent of this, and thus a real consequence of the introduction of *S. muticum*.

Based on the findings of this study, we find it unlikely that the introduction of *S. muticum* to Limfjorden has caused any major changes in local macroalgal epibiota community structure. It does, however, appear that the system now supports a much higher standing stock of epibiota than was previously the case. This appears to be a general pattern in the systems invaded by *S. muticum*, as similar conclusions were reached by Withers et al. (1975)

in southern England and by Viejo (1999) in northern Spain. The consequences of these quantitative changes are unknown at the system level, but they could include altered food webs because of increased availability of food items or increased grazing on algal propagules and recruits, perhaps reinforcing the position of *S. muticum* as a dominant due to the regenerative abilities of the genus (Kendrick and Walker 1994).

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