

## ***Gracilaria vermiculophylla* (Ohmi) Papenfuss, 1967 (Rhodophyta, Gracilariaceae) in northern Europe, with emphasis on Danish conditions, and what to expect in the future**

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### **Abstract**

*Gracilaria vermiculophylla*, a red macroalga from the West Pacific, was discovered in western Germany (the Wadden Sea) in 2002 and has since also been observed in Sweden (from about 70 km south to about 80 km north of Göteborg), Denmark (Wadden Sea, Horsens Fjord, Limfjorden, Vejle Fjord, Holckenhavn Fjord, Øster Hurup Harbor) and eastern Germany (Kiel Bay). Today, less than 5 years following its first observation in the Wadden Sea the invader is common in many invaded regions, often being amongst the most abundant macroalgal species. *G. vermiculophylla* is successful in shallow protected soft-bottom estuaries and bays, typically in association with ubiquitous native invertebrates (lugworms, tube-building worms, mussels, cockles, snails). The invertebrates provide substratum for holdfast attachment and thalli incorporation, most likely increasing the stability of local *G. vermiculophylla* populations. We hypothesize that this substratum provision is highly important for its general invasion success. We also confirm that *G. vermiculophylla* can maintain growth at all salinities experienced along Danish coastlines (8.5-34 psu). In addition, laboratory experiments indicate that the ubiquitous grazer *Littorina littorea* has the potential to control *G. vermiculophylla* growth under specific environmental conditions, but also that *L. littorea* may facilitate small-scale dispersal within invaded locations, because grazing increases thalli fragmentation rates. Given its widespread distribution, rapid range expansion, and known ecological traits, *G. vermiculophylla* is clearly a permanent resident of northern European waters.

**Key words:** *Gracilaria vermiculophylla*, Grazing, Invasion, Denmark, Germany, Sweden

### **Introduction**

*Gracilaria vermiculophylla* (Ohmi) Papenfuss, 1967 is a red macroalga from the West Pacific that has been introduced to the East Pacific (Bellorin et al. 2004), the West Atlantic (Freshwater et al. 2006, Thomsen et al. 2006a), and the East Atlantic (Rueness 2005). In the East Pacific, West Atlantic and southern parts of the East Atlantic, introductions were difficult to

detect and follow, because native morphologically similar species were common prior to the arrival of *G. vermiculophylla*. In contrast, a more conspicuous invasion occurred in most of northern Europe as few *Gracilaria* species were common prior to arrival (north on the Swedish west coast in more saline waters, *G. vermiculophylla* could have been mistaken for the native *G. gracilis* and *Gracilariopsis longissima*, cf. Ahlgren 2005), and a detailed

invasion map is slowly emerging (e.g. from Sweden, see Nyberg 2006).

*G. vermiculophylla* has ecological traits that indicate it may become highly successful in protected shallow harbors, estuaries, and bays throughout the Baltic Sea. These traits include a high resistance to environmental stress, in particular low salinity (Wallentinus et al. 2004, Rueness 2005, Nyberg 2006, Thomsen and McGlathery in press), and an ability to recruit onto patchy hard substratum in great abundances (Thomsen and McGlathery 2006, Thomsen et al. 2007a, photo A in Annex 4) as well as sustain high growth of unattached small fragments (Rueness 2005, Nyberg 2006, Thomsen and McGlathery in press). In addition, *G. vermiculophylla* is known as a 'habitat-former' (Wallentinus and Nyberg in press) that interacts ecologically with native fauna (Thomsen and McGlathery 2005), creates habitats for a suite of associated epiphytes and invertebrates (Thomsen et al. 2006b), and potentially with large impacts on ecosystem metabolism (Tyler et al. 2005, Tyler and McGlathery 2006). Considering the ecological traits of *G. vermiculophylla*, its previous invasion history and the impacts associated with its habitat-forming characteristics, this invader has the potential to dramatically alter the ecology of shallow north European bays and estuaries, and it is therefore important to follow the invasion in detail. Our objective was to document *G. vermiculophylla*'s distribution and abundance in northern Europe. We especially focused on Horsens Fjord, the location in Denmark where this invader was first observed. We also describe the substratum *G. vermiculophylla* attaches to, report on its stress-tolerance and discuss potential future spread of *G. vermiculophylla*.

## Material and Methods

### *Distribution in north European waters*

Locations and year of introductions were mapped using literature and monitoring data, by email-queries to local researchers and by visiting invaded localities. Most invaded locations in Denmark and Sweden were visited in 2005 or 2006, including Mandø (western part), Vejle Fjord (inner part), Holckenhavn Fjord (inside of cause-way), Horsens Fjord (several sites, cf. next section), and the Göteborg area (6 sites, cf. Figure 4). At each site we surveyed the shallow subtidal zone (0-0.5 m) for a minimum of 30

minutes to (a) rank the abundance of *G. vermiculophylla* in relation to native macroalgae and (b) estimate abundance according to a simple rank-scale (rare, common, abundant, highly abundant). We did not visit invaded locations in Germany, but similar information was kindly provided by C. Buschbaum, G. Nehls, D. Schories and F. Weinberger.

### *Distribution in Horsens Fjord*

Horsens Fjord was the first known invaded Danish location, and four surveys were therefore conducted within this estuary to quantify how abundant *G. vermiculophylla* had become in a few years (the surveys all quantified percentage cover of *G. vermiculophylla*, but varied slightly with regards to sample extent and grain). Survey 1: Percent cover of the entire algal community was collected at permanent sampling locations each summer from 1989 to 2005 as part of the Danish National Marine Monitoring Program (13-16 locations until 1997, 8-9 locations thereafter, no sampling in 2000, 2002, or 2004). At each location percent cover of all hard substratum was estimated visually by divers at 1 to 5 depth intervals (depending on maximum depth range at the location). Each depth interval/sample point corresponded to the average percent cover based on searching  $3 \times 25$  m<sup>2</sup> areas (method described in detail by Stæhr et al. 2000 and Thomsen et al. 2007b). Three additional specific *G. vermiculophylla* surveys were conducted in July 2006. Survey 2: Twenty-four randomly selected locations were sampled using a similar method to survey 1, but only recording a single percent cover value per location (1 depth level). Survey 3: Diving transects were conducted at 9 randomly selected locations. At each location, a diver was dragged slowly after a boat while recording percent cover of *G. vermiculophylla*. 14-75 contiguous percent cover values (each of ca.  $5 \times 1$  m), and corresponding depth and GPS-coordinates were recorded per transect. Survey 4: Strong western winds created an opportunity for recording shallow video-transects on July 25 2006. Waters of 0-50 cm depth were exposed on the southern coastline and video-transects were recorded at 3 locations. At each location, 2-3 video-transect, each of ca.  $50 \times 1$  m, were recorded, and from these, percent cover was visually estimated from 30-40 random  $0.25 \times 0.25$  m still images per transect.

### Attachment types

The *G. vermiculophylla* attachment was quantified from randomly selected thalli at Mando, Holckenhavn Fjord, four locations in Horsens Fjord and six locations in the Göteborg area. Similar, but qualitatively-based, attachment information was obtained from locations in Germany (pers. com. D. Schories and F. Weinberger for Kiel Bay and G. Nehls and C. Buschmann for the German Wadden Sea). To quantify if attachment patterns in northern Europe were similar to *Gracilaria* attachment in other parts of the world, additional sampling was conducted at *Gracilaria*-dominated locations from each of three soft-bottom system: Hog Island Bay, Virginia, USA (*G. vermiculophylla*), Pauatahanui Inlet, New Zealand (*G. chilensis*), and Swan River, Australia (*G. comosa*) (two locations per estuary). At all locations, the attachment was classified into 10 categories: (1) unattached (= loose-lying, including partly buried in sediments), (2) partly buried under biogenic cast's (e.g. from lugworms), incorporated into (3) polychaete tubes, or (4) mussels byssal threads, or, attached to (5) live mussels, (6) live snails, (7) live cockles, (8) other live mollusk, (9) dead mollusk (shells), or (10) abiotic substratum (e.g. stones, boulders, consolidated rock surfaces, artificial substratum).

### Performance

To test the performance of *G. vermiculophylla* under multiple stressor and variable environmental conditions, a 4-factorial outdoor growth experiment was conducted that tested for interactive effects of salinity and light levels, grazing impact and number/size of initial thalli-fragments. The experimental design was as follows; 3 salinity levels (34, 17, 8.5 psu)  $\times$  2 light levels (top of 250 ml containers covered with transparent or black plastic)  $\times$  2 grazing levels ( $\pm$  addition of a single *Littorina littorea* snail)  $\times$  2 levels of added fragments ('1-large' vs. '3-small' fragments, same total biomass of ca. 1 gWW)  $\times$  4 replicates. Salinity treatments simulate ranges experienced in Danish waters, and were created by dilution of 34 psu seawater with tap water. Salinity was monitored regularly with a refractometer. Water was changed every 2<sup>nd</sup> day, and evaporation typically caused a 1-3% increase in salinity (as measured just before

water change). Light levels were manipulated to match the environmental extremes *Gracilaria* experience: High levels at shallow waters (clear plastic = 10% PAR reduction, LiCor PAR-Meter) and low levels at depth where unattached fragments often accumulate (black plastic cover = 90% PAR reduction). The snail, *L. littorea*, was chosen as model-grazer because (a) it has been shown to produce a strong grazing effect in 1-factorial tests (Gustafson 2005), and (b) it is commonly associated with *G. vermiculophylla* thalli (Nyberg, C., Thomsen, M.S., Wallentinus, I., unpubl. data, see also photo B in Annex 4). Effort was made to acclimatize snails to lower salinities over six days (day 1-2 = 25 psu, day 3-4 = 17 psu, day 5-6 = 8.5 psu). After the six days, the snails at 17 psu showed similar movement patterns compared to snails at 34 psu, but snails at 8.5 psu hardly moved, and were therefore not added to this salinity, causing the design to be unbalanced. Finally, thalli were added as either 1 or 3 fragments. This treatment is ecologically important because *G. vermiculophylla* frequently fragment due to physical and biological disturbances. Small fragments were created by breaking a random subset of large fragment into 3 pieces and allowing for a 24 h wound recovery. Wet weight and number of thalli-fragments was measured at the start (September 22, 2005) and end (October 1, 2005) of the experiment and 'Growth' and 'Fragmentation' was calculated as percent change of biomass and percent change in number of fragments over the experimental period.

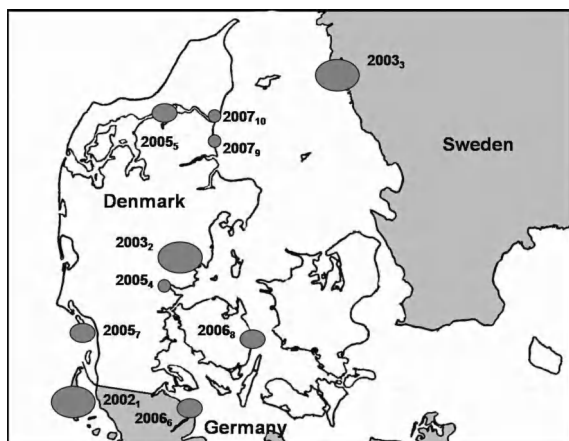
### Data analysis

Distribution pattern of *G. vermiculophylla* and its attachment status were analyzed graphically. In addition, *G. vermiculophylla* abundance in Horsens Fjord was correlated to depth (Spearman's  $r$ , using a single mean cover value for each of the 36 locations sampled in 2006). The growth experiment was analyzed with fixed-factorial ANOVA. Percent change in biomass had homogeneous variance (Levens test,  $P > 0.05$ ) and significant effects were evaluated at  $P < 0.05$ . Percent change in number of fragments had heterogeneous variance and could not be transformed to homogeneity (Levens test's,  $P < 0.05$ ). Significance was here evaluated at  $P < 0.01$  (Quinn and Keough 2002).

## Results

### Distribution in north European waters

*G. vermiculophylla* was observed in 2002 in the German part of the Wadden Sea (pers. com. G. Nehls), in 2003 around Göteborg, Sweden (Wallentinus et al. 2004) and Horsens Fjord (Survey 1, see below), in 2005 in Vejle Fjord (Survey 1, see below), Nibe and Gjol Bredninger (Danish Online News; [www.dr.dk/Regioner/Nord/Nyheder/Nordjylland/2007/04/19/](http://www.dr.dk/Regioner/Nord/Nyheder/Nordjylland/2007/04/19/)), and the Danish part of Wadden Sea (Mandø, Thomsen et al. 2005), in 2006, in Holckenhavn Fjord (pers. obs.), and Kiel Bay (pers. com. D. Schories), and in 2007 in Øster Hurup Harbor (pers. com. H. Knudsen) and Egense Harbor (pers. com. F. Andersen), cf. Figure 1, Annex 2.



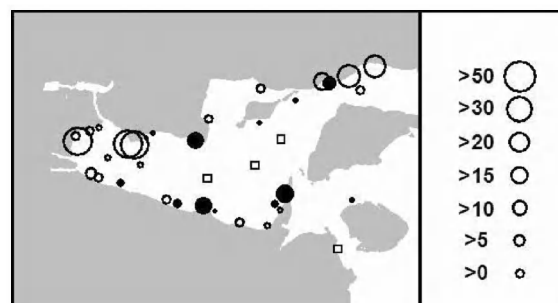
**Figure 1.** Distribution and relative abundance of *Gracilaria vermiculophylla* in Northern Europe. Invaded regions and the year of first observation was mapped using literature, monitoring data, queries and surveys. The circles sizes correspond roughly to the relative abundance of *G. vermiculophylla* at invaded regions. Subscripts indicate order of first observation, where 1 = Wadden Sea, Germany, 2 = Horsens Fjord, 3 = Swedish west coast, (circles size here based on 6 visited locations, cf. Nyberg 2006 for details), 4 = Vejle Fjord, 5 = Nibe and Gjol Bredninger in Limfjorden, 6 = Kiel Bay, 7 = Wadden Sea, Denmark (Mandø), 8 = Holckenhavn Fjord, 9 = Øster Hurup Harbor, and 10 = Egense Harbor (Annex 2)

Today, less than 5 years following the first observation in northern Europe, *G. vermiculophylla* has established large populations and is amongst the most abundant algae at many invaded locations (but with low abundance in Vejle Fjord and some of the wave-exposed locations in Sweden). For example, *G. vermiculophylla*, *Fucus vesiculosus* and green ephemerals (belonging to the genera of *Chaetomorpha* and *Ulva*, including former

*Enteromorpha*) were quantified to be the most abundant macroalgae at western Mandø in September 2005 and at Holckenhavn Fjord in August 2006 (Thomsen, M.S., unpubl. data).

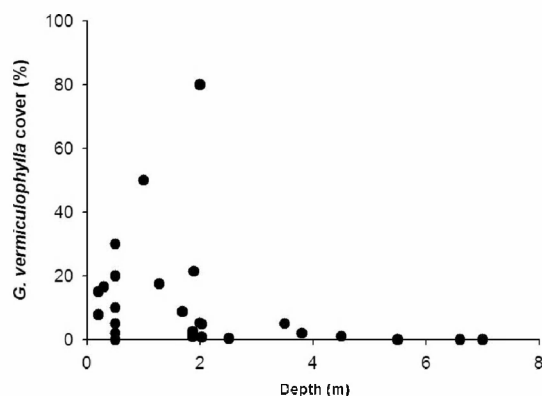
### Distribution in Horsens Fjord

In Survey 1, *G. vermiculophylla* was recorded for the first time in 2003 at shallow depth at 2 out of 9 stations with low cover ( $\leq 5\%$ ), and again in 2005 at 2 out of 9 stations, also with low cover ( $\leq 2\%$ ). It should be noted that 2005 data was collected immediately following a severe storm. *G. vermiculophylla* was also found with low cover in Vejle Fjord in 2005 at 1 out of 11 stations ( $< 5\%$ ). In Survey 2, *G. vermiculophylla* was found at 20 out of 24 stations, with cover ranging from 0 to 80% per location and a mean of 13.3% (SD = 20.5). In Survey 3, *G. vermiculophylla* was found on all nine locations, with mean transect covers ranging from 0.4 to 21.4% and an overall mean of 6.9% (SD = 7.7). Finally, in Survey 4, *G. vermiculophylla* was found at all 3 locations with a cover range of 2.5-24.1% per transect and an overall mean of 13.3% (SD = 21.8). In short, *G. vermiculophylla* was abundant throughout the fjord (Figure 2, Annex 3). There was a significant negative



**Figure 2.** Distribution and abundance of *Gracilaria vermiculophylla* in Horsens Fjord, July 2006. Circle sizes correlate with percentage cover. *G. vermiculophylla* was present at 20 out of 24 point-locations (black non-filled circles, black non-filled squares = 4 sampled locations without *G. vermiculophylla*), at 9 out of 9 'deep' dive transect-locations and at 3 out of 3 'shallow' video transect-locations (transect means shown, black filled circles). See Annex 3 for specific cover-values and corresponding geo-coordinates

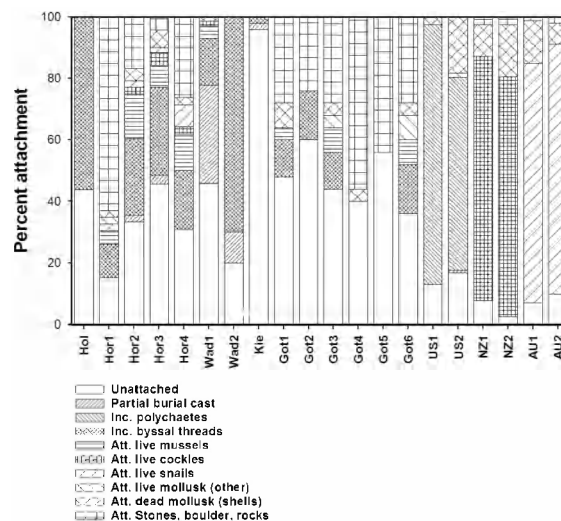
correlation between depth and percent cover ( $r = -0.42$ ,  $P = 0.011$ ,  $N = 36$ , Figure 3). Based on our surveys from 2006, we estimate that *G. vermiculophylla* and *Fucus vesiculosus* were the most abundant macroalgae in the inner and central parts of Horsens Fjord.



**Figure 3.** *Gracilaria vermiculophylla* abundance versus depth in Horsens Fjord. Percent cover and depth was quantified from 36 locations visited in July 2006 (including overall mean values from 12 transects)

#### Attachment type

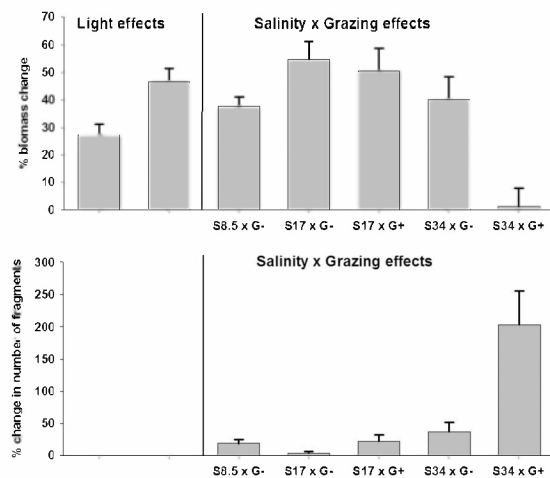
There was a general pattern showing high importance of biogenic associated attachment types at all sampled locations (Figure 4). Partial burial by biogenic casts, particularly by *Arenicola marina*, was common in the Wadden Sea. More strikingly, incorporation into mussel (*Mytilus edulis*) byssal threads was common in the Wadden Sea, Horsens Fjord, and Hockenhavn Fjord, and has been observed at all invaded regions in Northern Europe. An analog incorporation-type of attachment was dominating in Hog Island Bay (USA), although here facilitated by a polychaete (*Diopatra cuprea*). Biogenic associations between *Gracilaria* and native invertebrates were also common in Pauatahanui Inlet in New Zealand (*G. chilensis* attached to live cockles, mainly *Austrovenus stutchburyi*) and in Swan River in Australia (*G. comosa* attached to live snails, mainly *Batillaria australis*). At the Swedish locations (with high amounts of hard substratum) and in Kiel Bay (dominated by unattached thalli) biogenic associations were of relatively less importance. A reclassification of the percent attachment data (cf. Figure 4) into 'unattached thalli' (including thalli partly buried under casts), 'biotic attachment' (including incorporations) and 'abiotic attachment', show 36% were unattached, 42% were biogenically attached and 22% abiotically attached, indicating that biogenic structures are important in creating space for *G. vermiculophylla* in soft-bottom systems.



**Figure 4.** Attachment of *Gracilaria vermiculophylla* and other *Gracilaria* species. Attachment type was recorded from randomly selected thalli at Holckenhavn Fjord (Hol, N=32), 4 locations in Horsens Fjord (Hor 1-4, N=46, 48, 70, 42), Danish Wadden Sea (Wad1, N=72, western Mando), German Wadden Sea (Wad2, pers. com. C. Buschman and G. Nehls), Kiel Bay (Kie, pers. com. D. Schories and F. Weinberger), 6 locations on the Swedish west coast around Göteborg, including Särö (Got1, N=25), Sandö (Got2, N=25), Stora Amundön (Got3, N=25), Askim (Got4, N=25), Rörvik (Got5, N=25), and Saltholmen (Got6, N=25), 2 location in Hog Island Bay, Virginia, USA (US1, US2, N=77, 137), 2 locations in Pauatahanui Inlet, New Zealand (*G. chilensis*, NZ1, NZ2, N=39, 41) and 2 locations in Swan River, Australia (*G. comosa*, AU1, AU2, N=86, 103). Attachment was classified into groups as shown on figure legend. Att. = Attached, Inc. = incorporated

#### Performance

Biomass gain (= net growth) was detected at all treatment combinations. In particular, all complex interactions and all treatments associated with 'Fragmentation' were non-significant (Annex 1, but note near-significant 1-factor effect of fragmentation). In contrast, for the individual single factors, net growth was significantly higher (a) at high light over low light, (b) without grazers compared to with grazers, and (c) at 17 psu. In addition, the grazing  $\times$  salinity interaction was significant, clearly with highest grazing impact on algal biomass at 34 psu (little impact at 17 psu, no snails added to 8.5 psu, cf. figure 5 and discussion). Fragmentation rates were significantly highest in presence of grazers and at 34 psu.



**Figure 5.** Performance of *Gracilaria vermiculophylla*. A 4-factorial experiment tested for interactive effects of salinity (S34, S17, S8.5, units = psu), light (L+ = 10% PAR reduction, L- = 90% PAR reduction), grazing (G+ = 1 *Littorina littorea*, G- = no grazer) and 'number of fragments' on biomass change (top) and fragmentation rate (bottom) over the entire experimental period (+SE). *L. littorea* could not be acclimatized to 8.5 psu and was therefore not added to this salinity level. Only significant effects are shown, and significant interactions are given graphical preference over single factor significant results (i.e. significant salinity  $\times$  grazing, supersedes significant effects of salinity and grazing, cf. Annex 1)

Again, there was a significant grazing  $\times$  salinity interaction, with highest grazing impact on fragmentation at 34 psu (again little impact at 17 psu, and no snails were added to 8.5 psu, cf. figure 5). All more complex interactions as well as single factor effects of light and fragmentation were non-significant.

## Discussion

The large scale survey revealed that *G. vermiculophylla* is established with large populations in several regions in Denmark, Germany and Sweden, and being amongst the most abundant species in several of the invaded regions (although some of these regions are naturally species poor, due to low salinities, limited substratum, and large environmental fluctuations). This is 'impressive', considering the species was not present only few years earlier, and suggests similar invasion may occur in other bays and estuaries in Northern Europe. The extensive Danish macroalgal monitoring program, quantifying algal species-abundance patterns at >200 stations every second year

(Thomsen et al. 2007b) reassure that the time of Danish introductions are quite reliable (the Wadden Sea excluded, as it is not included in the monitoring program). Denmark has >70 distinct estuaries/coastal areas (Conley et al. 2000) and the invaded ones (Wadden Sea, Vejle Fjord, Horsens Fjord, Limfjorden, Holckenhavn Fjord and several smaller harbors) cover a range of sizes, drainage basins types, tidal effects, nutrient concentrations and salinity levels. The invaded regions differ with respect to tidal currents, salinity and wind exposure and the disjunct distribution pattern therefore do not reflect that these estuaries are particularly suitable regions, but rather indicate an invasion history of multiple independent introductions.

It is not known how *G. vermiculophylla* reached Europe, but potential vectors include oyster transplants, ballast waters, and ship hulls. Given that *G. vermiculophylla* is abundant on Virginian oyster reefs (and with high recruitment, Thomsen and McGlathery 2006, Thomsen et al. 2007a), has been found several times near oyster farms in France (Rueness 2005), and that *G. vermiculophylla*, to our knowledge, is not generally found in ballast water or on ship-hulls, transplantations of oyster seems a likely trans-Atlantic vector. Secondary dispersal between regions and estuaries is probably facilitated by entanglement to boat screws, fishing gear, trawling nets and various 'extensions' of smaller boats. For example, in Virginia we often observe *G. vermiculophylla* entangled to boat screws (cf. photos C-D in Annex 4) and many of these boats are regularly trailered between North Carolina and Virginia, particularly in the summer recreational fishing season. Interestingly, both states are today heavily invaded by *G. vermiculophylla* (Freshwater et al 2006, Thomsen et al 2006b), and these overland trips of a few hours can most likely be tolerated by the desiccation resistant *G. vermiculophylla* (Thomsen and McGlathery in press). Additional secondary dispersal routes include tumbling of the negatively buoyant fragments (i.e. mainly possible on small spatial scales) and transplantation of oysters and other mollusks (as for primary dispersal).

*G. vermiculophylla* and *Fucus vesiculosus* were the most abundant macroalgae in Horsens Fjord in July 2006, only 3 years after the discovery within this estuary (estimated with relatively high reliability, see above). It is noteworthy that *G. vermiculophylla* was uncommon in 2005 (Survey 1), suggesting a

dramatic increase from summer 2005 to summer 2006. However, the survey in 2005 followed a severe storm, and we speculate that *G. vermiculophylla* was relatively common also in 2005, but lost larger thalli due to wave forces, only leaving inconspicuous fragments attached to stones, mussels and incorporated to byssal threads. Given that *G. vermiculophylla* can sustain growth of 10% d<sup>-1</sup> under ideal conditions (Yokoya et al. 1999, Raikar et al. 2001, Nyberg 2006), spring and summer growth of fragmentary inocula that survived winter storms and cold conditions, could account for the high abundance observed in 2006. The data from Horsens Fjord also show that *G. vermiculophylla* was most abundant at shallow depth. Still, *G. vermiculophylla* is occasionally found in deeper waters (below 2 m), but these occurrences are probably related to advective transport of fragments, rather than in situ recruitment at depth. Such a strong depth effect on recruitment has previously been recorded in Virginia (USA) where *G. vermiculophylla* dominated at high elevations but *Codium fragile* ssp. *tomentosoides* and *Agardhiella subulata* were more abundant at lower levels (Thomsen et al. 2007a).

The importance of macroalgal attachment for the initial development of macroalgal blooms in soft-bottom systems, have recently been emphasized (Schories et al. 2000, Thomsen and McGlathery 2005, Thomsen et al. 2006b). Attachment provides a spatial fixation compared to unattached thalli that are more likely to drift with waves and currents (Thomsen 2004). Thus, spatial fixation is likely to increase population stability within a location. Spatial fixation can occur by recruitment onto abiotic (rocks and boulders) and biogenic surfaces (e.g. live or dead shells, cf. photo E, Annex 4) or by biogenic incorporation or partial burial (cf. photo F, Annex 4). The two latter processes are important because they do not require sexual fertilization and spore production, and it can therefore stabilize populations at locations where environmental conditions allow vegetative growth, but likely retard sexual reproduction (e.g. at low salinities, Zheng et al. 1987). Thus, the common incorporation into mussel byssal threads is likely to become a fixation process throughout estuaries in Northern Europe. The only other macroalga to be observed on similar incorporations was *Fucus vesiculosus* (Thomsen, M.S., pers. obs. and Albrecht 1998), one of the most abundant macroalgae in Denmark (Thomsen et al. 2007b). Although this particular invertebrate

facilitation was not observed in New Zealand, Australia or USA, analog *Gracilaria* facilitations by local ubiquitous invertebrates were dramatic; incorporation into polychaete tubes in Virginia, USA (Mangum et al. 1968) and recruitment onto live cockles (Healy 1980) and snail (Kirke et al. 1987) in New Zealand and Australia, respectively. The incorporation into polychaete tubes has been documented to increase small scale population stability of *G. vermiculophylla* (as *G. verrucosa*, Thomsen and McGlathery 2005), as has burial by biogenic cast and attachment to *Hydrobia* snails for *Ulva* (= former *Enteromorpha*) spp. (Reise 1983, Schories et al. 2000). It should be noted that incorporation facilitation provides a dynamic link between attachment types. For example thalli may frequently shift status of attachment from attached to stone to unattached (via storms) to partly buried (via sedimentation) to byssal incorporation (via mussel bed development) to unattached (via storms).

*G. vermiculophylla*'s general growth responses to variations in temperature, light and salinity are known from single-factorial laboratory experiments (Yokoya et al. 1999, Raikar et al. 2001, Rueness 2005, Nyberg 2006). These experiments have all shown that growth can be sustained at environmental extremes (e.g. 2-40 psu, 5-30°C, low and high light levels), traits of high importance for success in estuaries. Also, single-factorial laboratory experiments have indicated that native grazers may be able to control *G. vermiculophylla*, at least under ideal laboratory conditions (25 psu, 12:12 D:L, 10-14 °C, Gustafson 2005). However, none of these studies have quantified growth under more realistic (but still controlled) conditions with natural light and temperature fluctuations. We show that growth still is possible under all our investigated combinations of light, salinity, number-size of initial fragments, and grazer additions. For example, net growth was maintained at 90% reduction if surface light. Similar high dark-tolerance has been observed in populations from Virginia (Thomsen and McGlathery in press) and Sweden (Nyberg 2006). Indeed, *G. vermiculophylla* is capable of dark-uptake of both urea and amino acids (Tyler et al. 2005) and we have managed to keep this species alive in total darkness for >6 month in refrigerators (see also Nyberg 2006). These observations add to a growing list of stress-tolerant traits of this invader (Raikar et al. 2001, Aikins 2002, Rueness 2005, Nyberg 2006, Thomsen and

McGlathery in press). We observed a relatively strong effect of *L. littorea* grazing at 34 psu, similar to Gustafson (2005). This suggests that grazing may partially control biomass under high grazer densities. However, under field conditions, alternative food sources are generally available and high grazer densities are not necessarily kept for prolonged time periods, i.e. the reported grazing impacts likely reflects maximum impacts. The low grazing impact at 17 psu may be an experimental artifact, as grazers were collected from 34 psu sites (with 6 days of acclimatization). We did not observe differences in movement patterns of *L. littorea* between 17 and 34 psu, but metabolism and grazing rates are likely to differ, and we noted that more fecal pellets were produced in the 34 psu treatments. Thus, much longer acclimations may be necessary to observe full grazing effects at 17 psu, and we are therefore conducting supplementary grazing experiments using snails collected from sites of both high and low salinities to test specifically for grazer impact along the entire salinity gradient. An additional impact of *L. littorea* was a significant increase in fragmentation rates. Under in situ conditions, the key ecological grazer impact may indeed be that *G. vermiculophylla* is broken into smaller fragments that can disperse within and between regions (Freshwater et al. 2006). These small fragments are susceptible to incorporation into byssal threads, and *L. littorea* may thereby indirectly facilitate the establishment of more stable populations around mussel-beds, by provision of an abundant supply of small vegetative fragments.

In conclusion, *G. vermiculophylla* is less than 5 year following its discovery in northern Europe amongst the most abundant algae at several invaded locations in Denmark, Sweden and Germany. Grazing by native invertebrates may limit *G. vermiculophylla* biomass development under high salinity conditions, but facilitation processes, e.g. fragmentation caused by grazers and spatial fixation via incorporation processes and provision of hard substratum are likely more important, and will probably aid further establishment of *G. vermiculophylla* populations throughout Baltic bays and estuaries.

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**Annex 1**

Factorial ANOVA on *Gracilaria vermiculophylla* biomass changes. G = grazer ( $\pm$  *Littorina littorea*), L = light ( $\pm$  total shading), S = Salinity (34, 17, 8.5 psu), F = fragments added at T0 (1 vs. 3, total biomass of ca. 1 gWW in all treatments). Significant results ( $P < 0.05$  for biomass change and  $P < 0.01$  for fragmentation rate) are in bold and near-significant results in brackets ( $P < 0.10$  and  $P < 0.05$  respectively)

Source	DF	Biomass change (%)			Fragmentation rate (%)		
		SS	F	P	SS	F	P
G	1	7490.7	12.208	<b>0.0008</b>	137517.4	19.287	<b>0.0000</b>
L	1	7554.4	12.312	<b>0.0008</b>	11293.0	1.584	0.2121
S	2	17855.5	14.550	<b>0.0000</b>	184201.4	12.917	<b>0.0000</b>
F	1	2337.6	3.810	(0.0546)	32953.2	4.622	(0.0347)
G $\times$ L	1	52.7	0.086	0.7704	32100.7	4.502	(0.0371)
G $\times$ S	1	4758.6	7.755	<b>0.0068</b>	87517.4	12.275	<b>0.0008</b>
L $\times$ S	2	171.1	0.139	0.8701	13628.5	0.956	0.3891
G $\times$ L $\times$ S	1	1115.5	1.818	0.1816	32100.7	4.502	(0.0371)
G $\times$ F	1	1587.9	2.588	0.1118	6267.4	0.879	0.3514
L $\times$ F	1	192.3	0.313	0.5773	13684.3	1.919	0.1700
G $\times$ L $\times$ F	1	631.3	1.029	0.3136	14600.7	2.048	0.1565
S $\times$ F	2	2706.9	2.206	0.1172	49201.4	3.450	(0.0368)
G $\times$ S $\times$ F	1	25.5	0.042	0.8391	10850.7	1.522	0.2211
L $\times$ S $\times$ F	2	1838.0	1.498	0.2302	14253.5	1.000	0.3728
G $\times$ L $\times$ S $\times$ F	1	272.3	0.444	0.5073	29184.0	4.093	(0.0466)
Error	76	46633.3			541875.0		

**Annex 2**

Records of *Gracilaria vermiculophylla* in northern Europe in 2002-2007. Coordinates in decimal degrees latitude and longitude

Location	Latitude	Longitude	Record date	Collector	Abundance
1 Wadden Sea, Germany	54.90	8.32	Jul. 2002	G. Nehls <sup>1</sup>	Abundant
2 Horsens Fjord, Denmark	55.85	10.00	Aug. 2003	J. Nicholaisen <sup>2</sup>	Highly abundant
3 Goteborg archipelago, Sweden	57.80	11.81	Sep. 2003	I. Wallentinus <sup>3</sup>	Abundant
4 Vejle Fjord, Denmark	55.71	9.77	Jul. 2005	S. Schwærter <sup>2</sup>	Common
5 Limfjorden, Denmark	57.07	9.65	Jul. 2005	J. Deding <sup>4</sup>	Abundant
6 Kiel Bay, Germany	54.41	10.17	Aug. 2005	F. Weinberger, D. Schories <sup>5</sup>	Abundant
7 Wadden Sea, Denmark	55.33	8.54	Sep. 2005	M. Thomsen <sup>6</sup>	Abundant
8 Holckenhavn Fjord, Denmark	55.34	10.79	Aug. 2006	M. Thomsen <sup>7</sup>	Abundant
9 Øster Hurup Harbor, Denmark	56.83	10.30	Jan. 2007	H. Knudsen <sup>7</sup>	Rare
10 Egense Harbor, Denmark	57.03	10.32	May 2007	F. Andersen <sup>7</sup>	Rare

<sup>1</sup>Nehls G (2006). *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta) – a Pacific algae spreading on tidal flats of the Wadden Sea. <http://www.bioconsult-sh.de/en/referenzen/main.htm#poster>

<sup>2</sup>Collected as part of Danish National Monitoring Program, see also Thomsen et al. (2007b)

<sup>3</sup>Nyberg (2006)

<sup>4</sup>News story, Danish National Radio, see <http://www.dr.dk/Regioner/Nord/Nyheder/Nordjylland/2007/04/19/062325.htm>

<sup>5</sup>Schories D, Selig U (2006) Die Bedeutung eingeschleppter Arten (alien species) für die Euro-päische Wasserrahmenrichtlinie am Beispiel der Ostsee. Rostock. Meeresbiolog. Beitr. 15: 147-158

<sup>6</sup>Thomsen et al. (2005)

<sup>7</sup>Present paper

### Annex 3

Distribution of *Gracilaria vermiculophylla* in Horsens Fjord in July 2006. Coordinates in decimal degrees latitude and longitude. Survey 2 and 3 data collected by S. Schwärter, and Survey 4 data by M. Thomsen

Survey	Location	Latitude	Longitude	Depth (m)	Abundance (%)
2	1	55.8830	10.0814	0.5	30.0
2	2	55.8796	10.0813	0.5	30.0
2	3	55.8779	10.0455	0.5	20.0
2	4	55.8758	10.0040	0.5	5.0
2	5	55.8653	9.9685	0.5	5.0
2	6	55.8565	9.9183	1.0	50.0
2	7	55.8569	9.9138	1.0	50.0
2	8	55.8627	9.8941	0.5	2.0
2	9	55.8616	9.8881	0.5	5.0
2	10	55.8599	9.8782	0.5	5.0
2	11	55.8464	9.8884	0.5	10.0
2	12	55.8449	9.8936	0.5	5.0
2	13	55.8368	9.9392	0.5	5.0
2	14	55.8284	9.9885	0.5	5.0
2	15	55.8270	10.0073	0.5	2.0
2	16	55.8326	10.0159	0.5	2.0
2	17	55.8183	10.0547	0.5	0.0
2	18	55.8580	9.8797	2.0	80.0
2	19	55.8520	9.8999	3.8	2.0
2	20	55.8494	9.9219	4.5	1.0
2	21	55.8490	9.9672	6.6	0.0
2	22	55.8487	9.9993	7.0	0.0
2	23	55.8577	10.0173	5.5	0.0
2	24	55.8745	10.0714	3.5	5.0
3	1	55.8774	10.0504	1.3	17.5
3	2	55.8713	10.0274	1.9	1.0
3	3	55.8636	10.0026	1.9	2.4
3	4	55.8578	9.9593	1.9	21.5
3	5	55.8589	9.9259	2.0	4.9
3	6	55.8432	9.9083	2.0	5.2
3	7	55.8325	9.9719	2.0	0.7
3	8	55.8346	10.0125	1.7	8.8
3	9	55.8357	10.0645	2.5	0.4
4	1	55.8361	9.9313	0.2	15.0
4	2	55.8302	10.0038	0.3	16.5
4	3	55.8310	10.0304	0.2	7.8

#### Annex 4

Photos of *Gracilaria vermiculophylla*: A: Monospecific stand on an artificial recruitment panel in Horsens Fjord. B: Partial burial under lugworm casts and grazing by *Littorina littorea* in the Wadden Sea. C-D: Entangled to boat projections in Virginia and the Wadden Sea. E-F: Attached to *Littorina littorea* in Horsens Fjord. F: Incorporated into *Mytilus edulis* byssal threads in Holckenhavn Fjord. Photos by M. Thomsen

