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Inorganic skeleton of the demosponge *Halichondria panicea*. Seasonality in spicule production in the Baltic Sea

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Abstract Seasonality in spicule production of the demosponge *Halichondria panicea* (Pallas, 1766) was studied in the Western Baltic from July 1992 to July 1993. Spicule production increased in parallel with increasing temperatures in spring, being most pronounced during summer after release of larvae. Large proportions of up to 30% immature spicules occurred in the population during skeleton production peaks, and this resulted in reduction of mean spicule dimensions by ≃20%. Effects on spicule width were more obvious than on spicule length. In winter, when wave forces were largest, higher percentages of mineral skeleton were observed: 50% of dry weight compared to 40% in summer. Enhanced spicule densities in the tissues were not attained by marked spicule production but through a reduction in organic content by shrinking. In spring, growth of body tissue was faster than the build-up of skeleton, so that the relative amount of the latter was reduced. The indirect, growth-related developments of the skeleton were inversely proportional to temperature and directly to salinity, silicate concentrations and wave energy. Spicule size and the number of immature spicules were correlated with temperature and only slightly with salinity. No correlation with oxygen concentration or pH could be detected.

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Introduction

Skeletal characters of sponges are regarded as relatively stable compared to some of their other morphological features (e.g. Siribelli 1961). However, temperature, silicate concentration and wave force are said to influence inorganic skeleton content, spicule dimensions and rates of spicule growth in freshwater (e.g. Jørgensen 1944; Elvin 1971; Pé 1973) and marine demosponges (e.g. Stone 1970 a, b; Simpson 1978; Palumbi 1986).

Seasonality of spicule production has been studied in Western Baltic specimens of *Halichondria panicea*, the dominant demosponge in this area (Theede 1981). The biota of this region is subjected to substantial seasonal changes in water quality and weather conditions. Water temperature can vary by 15 to 20 C° over a year. In spring, algal blooms diminish soluble nutrients and silicate, from which *H. panicea* spicules are formed. In summer, oxygen saturation can be lowered by establishment of a thermocline. Winter storms occasionally replenish oxygen and salt content through the influx of North Sea water, and exert mechanical stress through breaking waves along the shorelines of the Western Baltic. Except for low oxygen concentrations, these stresses are more pronounced in the shallow waters of the sample area. Some conditions are more favourable than others and may provide a selective force for skeleton production and enhancement of body rigidity. A recurrent seasonal cycle of varying proportions in the inorganic and organic components of Western Baltic *H. panicea* has been observed (Barthel 1986). However, so far this has not been studied in the context of active spicule production.

Therefore, the present study examined indicators of spicule production in field samples throughout one year to determine variability in skeleton formation in this species. To trace seasonal effects, ambient water conditions were monitored in parallel. Two main approaches were used to detect possible changes in skeleton production. Firstly, changes in the relative amount of skeleton per unit tissue were recorded; secondly, increases in the numbers of smaller and slimmer "immature" spicules were used as indicators of ongoing skeleton production. We also attempted to observe whether mean spicule size changes seasonally, independent of any marked occurrence of immature spicules.

Materials and methods

Sample collection

Specimens of *Halichondria panicea* (Pallas, 1766) were collected at Bokniseck, Kiel Bight, Western Baltic, along predetermined transects on sandy boulder fields (lag sediment: Babenerd and Gerlach 1987) adjacent to the "Hausgarten", a restricted site of the Institut für Meereskunde (the sample site was a square area bounded by the following coordinates: 54°31′N, 10°01′E; 54°31′N, 10°00′E; 54°32′N, 10°01′E; 54°32′N, 10°02′E). Transects were aligned parallel to each other, at a 45° angle to the coast, at depths from 6 to 8 m. Transects were chosen at random during each trip and relocated by the Global Positioning System. From July 1992 to July 1993, monthly samples were taken by dredging red algae on which *H. panicea* grew as an epiphyte. In July 1992 and June 1993, additional samples were taken during the physiologically more active summer season (main food-energy demand is in the period from May to September: Barthel 1988).

Sample sizes were determined according to Bros and Cowell (1987) using a summer sample displaying large variation. From each dredge sample, 15 healthy, undamaged specimens of at least 4 cm^3 were chosen at random and kept alive in aerated fresh seawater for a maximum of 24 h.

The following ambient water characters at the sampling area were measured directly on each trip or obtained through the "Biologisches Monitoring Ostsee" project of the Institut für Meereskunde (H. Hansen personal communication): temperature (°C), salinity (‰), silicate content (μmol), oxygen content (%) and pH. Information on diatom blooms was also available through the same project (U. Horstmann personal communication).

The "Wetterbüro Schleswig" ("Schleswig Meteorological Office") of the German Weather Service provided wind speed and direction data measured at Olpenitz, roughly 12 km north of the sample site. No wind data were available in March 1993 because of equipment failure.

Sample preparation and data collection

All 15 sponges per sample were weighed after drip-drying on tissue paper to determine the fresh weight. After 48 h of freeze-drying and drying for 24 h at 100 °C, the dry weight (DW) was recorded. After 24 h in a muffle furnace at 550 °C, the ash weight (AW) was determined. Ash represents the "inorganic" fraction and is commonly used to describe the amount of siliceous skeleton in sponges (e.g. Stone 1970b; Palumbi 1986; Barthel 1991). Sponges are said to be isosmotic to changes in ambient salinities (Hartman 1958; Barnes 1980, pp 98–111). Salt constituted up to one-quarter of the ash. Thus, differences in water salinities probably explain the discrepancies previously noted for ash weights of samples from different sites (Palumbi 1986; Barthel 1991). As the Western Baltic is subject to relatively large fluctuations in ambient salinity (which varied between ~14 and 22 ‰ S during the study period), ash was corrected by subtracting the salt to obtain a more accurate estimate of the skeletal material (AWC). All absolute weight data were transformed to percentages for comparison between samples and to detect possible shifts in weight proportions, with the focus on the corrected ash weight.

For permanent spicule preparations, tissue samples from six randomly chosen specimens per sample were removed before weighing. Pieces of ~2 mm^3 were taken from the mesohyle of the youngest parts of the sponges to represent growth during the most recent conditions. Areas of fresh tissue growth were assumed to contain mainly new spicules. Tissue was dissolved at 80 °C in 500 μl of concentrated nitric acid until the coloured fumes ceased. After neutralisation and washing two to three times with absolute ethanol, spicules were resuspended by shaking thoroughly. A few drops (~250 μl) were placed haphazardly onto a microscope slide and burned to dry the spicules on the glass. Care was taken that the spicules did not sort for size across the slide (as would occur were the slide not absolutely level). Permanent mounts were made with corbit balsam (I. Hecht, Stadtrade 27, 24118 Kiel, Germany).

The spicules of *Halichondria panicea* are oxeas, small rods with two pointed ends. The lengths and widths of 70 spicules per slide were measured under a light microscope at × 250 (length) and × 400 (width). Length was the distance from point to point, width the distance across the widest part of the oxea. Spicules in the 1 mm periphery of the slide, together with broken, abnormally formed and unfocusable elements were ignored. Measurements were made along one of six transects chosen at random.

Criteria were established to define an "immature" spicule; i.e. one still in the process of being secreted at the time the sponge tissue was collected. During spicule formation in demosponges, width increases have been found to be more pronounced than length increases, with the most dramatic growth taking place during early secretion (Jørgensen 1944, 1947; Bergquist and Sinclair 1973). Thus, the ratio length:width was used to estimate which spicules were to be considered immature. Drawing criteria from bimodal frequency distributions of spicule size (Jones 1984), all spicules with a length:width ratio of ≥45 were considered immature. Splitting the two cohorts created an area of overlap between the two peaks of any bimodal distribution. Counts of immature spicules per sample were recorded as percentages and were used to pinpoint the level of skeletal production.

Data evaluation and assumptions

Seasonal differences in weight fractions, spicule dimensions and the relative amount of immature spicules were tested as Model II one-way ANOVAs, with sampling date as factor (= 15 levels). Spicule dimension data were analysed after taking means per sponge, thus reducing the nested design to a one-way ANOVA to account for hierarchy of data by subsampling (70 spicules per sponge, 6 sponges per month). Datasets of partial weights and relative amounts of immature spicules were transformed to make variation independent of the mean. All datasets used were homogeneous according to the Cochran test. Posthoc Tukey–Kramer tests were conducted after finding significant differences in the ANOVAs to see whether the difference was caused by just one outlier or whether several samples differed from each other.

Correlations of weight fractions, spicule sizes and number of immature spicules with ambient factors were tested by simple regressions. Except for fitting the regression for data versus oxygen polynomially at the power of 2, all other regressions were linear.

A number of assumptions were taken into account for regressions in order to estimate when environmental conditions were likely to affect the spicules found in the samples. It takes ~2 d at 21 °C for an oxea to form in *Ephydatia fluviatilis* which, with a mean length of 240 μm in free-living specimens (Weissenfels and Landschoff 1977), has slightly shorter spicules than *Halichondria panicea*. However, Weissenfels and Landschoff studied laboratory-reared sandwich cultures in which spicules never attained the mean size of spicules of free-living *E. fluviatilis*. With larger spicules in *H. panicea* and with usually much lower ambient temperatures at Bokniseck, we assumed a formation period of at least 7 d. Environmental factors can thus presumably affect each *H. panicea* spicule over a period of 7 d. After completion, spicules remain unchanged regardless of changing ambient conditions, but can move through the sponge tissues. For the transport of spicules into new body regions we assumed a period of 3 d (Kilian and Wintermann-Kilian 1979; Teragawa 1986). Therefore, mean values of temperature, salinity, silicate content, oxygen content and pH were calculated over a period of 7 d starting 10 d prior to sampling (the

10 d period included the 3 d when no spicule growth occurred and when, therefore, environmental measurements would have been irrelevant), and over the sampling depths of 6 to 8 m. In contrast, wave force is given as the maximum possible wave energy (wave height squared) which could have developed during this period according to wind and fetch (Darbyshire 1953: Formula 7). As epiphytic *H. panicea* at 6 to 8 m are comparatively sheltered from waves, only exceptionally large waves may affect them. Maximum wave energy was included into regressions in the same way as the means of the other factors (Gaines and Denny 1993). Estimates of wave energy are probably overestimates, but can be used to represent relative changes of mechanical stress acting on *H. panicea* at the sample site.

Results

Seasonality of inorganic skeleton and organic fractions in *Halichondria panicea*

The proportion of inorganic skeleton in Western Baltic *Halichondria panicea* varied considerably throughout the year (Fig. 1a; Table 1). This was not only due to a few outlying data points, but each monthly sample differed from at least one other monthly sample taken during that year. The same was true for the organic fraction. The seasonal trends of the salt-corrected mineral skeleton and the organic fraction varied inversely (Fig. 1a). The proportion of inorganic skeleton increased from 40% DW in summer 1992 to 50% DW in December 1992, whereas the organic fraction was roughly reciprocal to this, being 50% DW in summer 1992 and 30% DW in December 1992. Both components had about equal proportions of >40% DW in summer 1993. On an annual mean basis, the inorganic skeleton still constituted the larger part of the DW (46% DW compared to 41% DW for the organic fraction). Salt made up the remaining dry weight. Annual means for dry weight and water were 12 and 88% of the fresh weight, respectively.

The monthly proportions of inorganic skeleton and organic material were significantly correlated with four of the environmental factors (Figs. 1a; 2; Table 2): the proportion of skeleton decreased with increasing water temperature and increased in parallel with increasing salinity, water silicate content and wave force. The proportion of organic material increased with increasing temperature and with decreasing salinity, water silicate content and wave force. Both, temperature and salinity were closely correlated to seasonal changes in both inorganic and organic fractions ($P < 0.001$); silicate concentration and wave energy were less significant ($P > 0.006$). Salinity and silicate concentration showed stronger parallels with proportional changes in the organic fraction than in the mineral skeleton fraction. Neither oxygen content, nor pH of the water were related conclusively with proportions of either the inorganic skeleton fraction or the organic component.

Table 1 *Halichondria panicea*. Results of parametric analyses testing for seasonality in proportions of inorganic skeleton and organic fractions, spicule dimensions (*L* = length; *W* = width) and relative amount of immature spicules [*df* degrees of freedom for sample/for residual; *F* critical value of ANOVA; *P* probability of null hypothesis; ω^2 relative size of treatment effect; (*N*) overall sample size; *AWC* ash weight corrected for salt = inorganic skeleton; *AFDW* ash-free dry weight = organic substance]

Dependent factors	*df*	*F*	*P*	ω^2 (%)	(*N*)
AWC	14/205	24.099	0.0001	59.5	(220)
AFDW	14/205	59.898	0.0001	78.6	(220)
Spicule L	14/75	4.232	0.0001	33.5	(90)
Spicule W	14/75	5.065	0.0001	38.7	(90)
Immature spicules	14/75	3.401	0.0003	82.6	(90)

The exopinacoderm was visibly stronger in winter than in summer samples. The skeletal elements in the pinacoderm, which are arranged parallel to the surface, appeared to be more densely packed in winter. In some individuals in summer, the pinacoderm was so delicate that it was difficult to separate it intact from the rest of the sponge.

Seasonality of spicule dimensions and number of immature spicules in *Halichondria panicea*

Both spicule dimensions, length and width, differed significantly as a function of season (Fig. 1b; Table 1), with the largest seasonal difference in the sample of 22 July 1992.

Monthly mean spicule lengths and widths showed a similar seasonal trend (Fig. 1b): from a minimum in summer 1992 (227.7 and 6.0 μm, respectively) they increased to a small peak in September 1992 (277.7 and 8.5 μm), dropped slightly again in winter (11 December 1992: 273.7 and 7.4 μm), increased again, and were largest in April 1993 (309.1 and 9.2 μm). Thereafter, mean spicule size sank to another summer low (24 June 1993: 264.3 and 7.2 μm, respectively).

The decrease in mean spicule width was caused by the increasing numbers of immature spicules in the samples; spicule length was not noticeably affected (Table 2). Compared to spicule length, spicule width was thus the more sensitive indicator for periods of spicule production. Frequency distributions of spicule width displayed clearer size-class effects than those of spicule length.

A distinct bimodality of size-frequency distributions and a skewness towards the narrower spicules in summer implied intensive spicule production (Fig. 3). In autumn and winter, spicule width frequency distributions were normal in distribution, with spicule production slowing down and possibly ceasing altogether in individual sponges. In late winter and early spring, the distributions were slightly skewed towards narrower spicules, suggesting low-level skeleton formation. In late spring and summer 1993, pronounced bimodality of the frequency distributions indicated intensive spicule production.

Annual mean spicule dimensions of Western Baltic *Halichondria panicea* were 276.8 μm length, 8.0 μm width (absolute minima and maxima over sampling period = length 76.0 to 436.7 μm, width 1.5 to 14.9 μm).

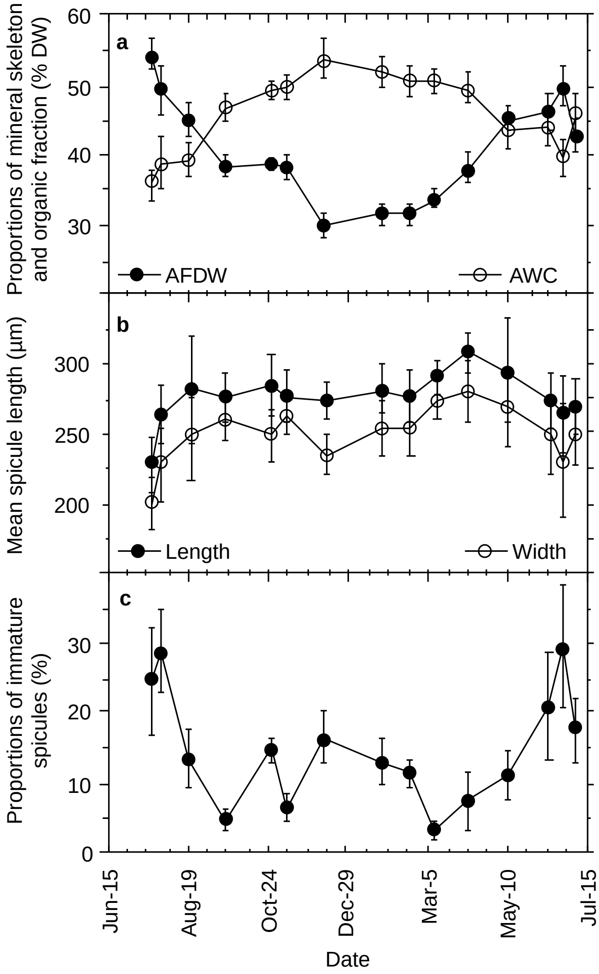

Fig. 1 *Halichondria panicea.* Skeletal characteristics of samples from Bokniseck, Kiel Bight, Western Baltic, during study period (22 July 1992 to 6 July 1993) (*error bars* are 95% confidence intervals). Seasonal trends in **a** mineral skeleton (*AWC* corrected ash wt) and organic fraction (*AFDW* ash-free dry wt), **b** mean spicule length and width, and **c** relative number of immature spicules per sample

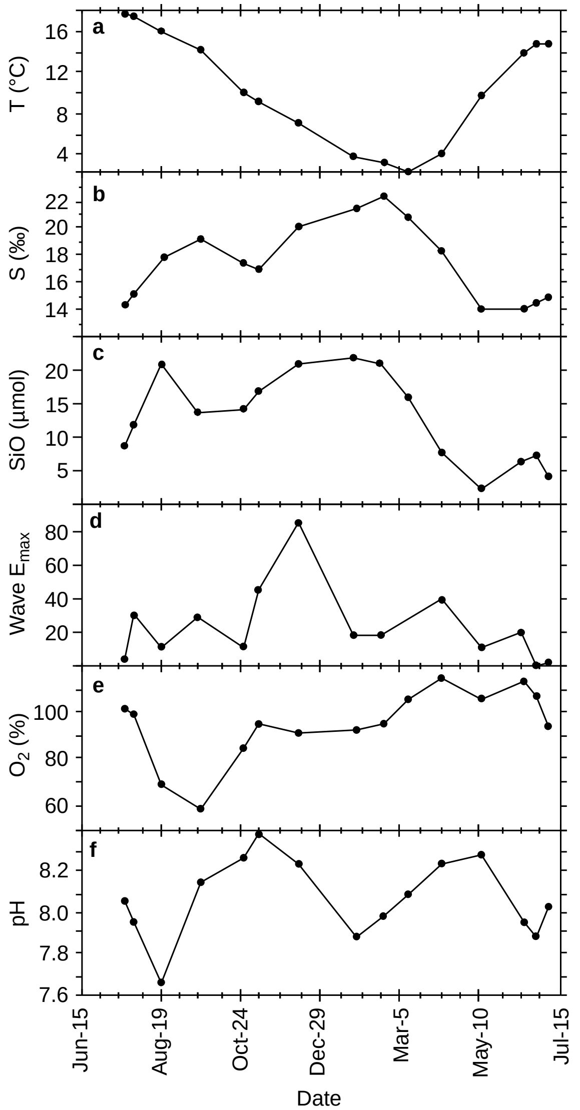

Fig. 2 Seasonal trends of environmental factors temperature, salinity, silicate concentration, maximum wave force, oxygen saturation and pH at Bokniseck, Kiel Bight, Western Baltic, during study period

Mean spicule length and width were inversely correlated with temperature (Figs. 1b, 2; Table 2). Salinity was not correlated with spicule size at the 95% probability level; however, there was a correlation at a lower probability of 85%. The parameters silicate content, water movement, oxygen saturation and pH were not related to spicule dimension in *Halichondria panicea*; regressions were highly insignificant.

The proportion of immature spicules differed significantly with season (Table 1). Higher percentages of immature spicules (almost 30%) were observed in the summers of 1992 and 1993 compared to low values of 3 to 4% in autumn and spring (Fig. 1c). There was a slight increase of the proportion of immature spicules in the winter 1992/1993; however, with a maximum of 17% immature spicules in December 1992, the increase was much lower than the summer peaks.

Only temperature showed any correlation with the number of immature spicules (Figs. 1c, 2; Table 2). The percentage of spicules in the process of formation increased in parallel with rising temperatures. There was some evidence that the number of immature spicules increased with decreasing salinity. However, as variability in salinity explained only 25% of the variance between monthly immature spicule counts, a larger sample size would be needed to resolve this aspect.

Discussion

The apparent increased content of inorganic skeleton in the winter samples of Western Baltic *Halichondria panicea* was not a direct result of increased spicule production. Despite evidence of low-level skeleton formation in winter, this was much less pronounced than in summer (when skeleton content was low), and thereby did not contribute to increasing rigidity. *H. panicea*

Table 2 *Halichondria panicea*. Results of regressions testing correlations between skeleton characteristics and environmental factors, and spicule mean size and relative number of immature spicules (*df* degrees of freedom for influencing factor/for residual; r^2 relative size of treatment effect; *T* temperature; *S* salinity; *silicate* silicate content; O_2 oxygen content; E_{max} maximal wave energy; *other abbreviations* as in Table 1)

Dependent factors	Source of regression	*df*	*F*	*P*	r^2 (%)	(*N*)
AWC	T	1/13	35.167	0.0001	73.0	(15)
	S	1/13	18.144	0.0009	58.3	(15)
	silicate	1/13	4.360	0.0570	25.1	(15)
	O_2	2/13	0.375	0.6949	5.9	(15)
	pH	1/13	3.287	0.0930	20.2	(15)
	E_{max}	1/12	7.266	0.0195	37.7	(14)
AFDW	T	1/13	32.773	0.0001	71.6	(15)
	S	1/13	53.678	0.0001	80.5	(15)
	silicate	1/13	10.589	0.0063	44.9	(15)
	O_2	2/13	0.649	0.5400	9.8	(15)
	pH	1/13	0.908	0.3581	6.5	(15)
	E_{max}	1/12	6.886	0.0222	36.5	(14)
Spicule L	T	1/13	8.941	0.0104	40.8	(15)
	S	1/13	2.322	0.1515	15.2	(15)
	silicate	1/13	0.074	0.7901	0.6	(15)
	O_2	2/13	0.582	0.5736	8.8	(15)
	pH	1/13	1.081	0.3175	6.0	(15)
	E_{max}	1/12	0.649	0.4363	5.1	(14)
Spicule W	T	1/13	9.248	0.0095	41.6	(15)
	S	1/13	2.563	0.1334	16.5	(15)
	silicate	1/13	0.015	0.9035	0.1	(15)
	O_2	2/13	0.817	0.4649	12.0	(15)
	pH	1/13	1.453	0.2495	10.1	(15)
	E_{max}	1/12	0.329	0.5769	2.7	(14)
Immature spicules	T	1/13	6.116	0.0280	32.0	(15)
	S	1/13	3.561	0.0817	25.0	(15)
	silicate	1/13	0.402	0.5371	3.0	(15)
	O_2	2/13	1.281	0.3132	17.6	(15)
	pH	1/13	2.188	0.1629	14.4	(15)
	E_{max}	1/12	0.774	0.3961	6.1	(14)
Spicule L	Immature spicules	1/13	7.926	0.0146	37.9	(15)
Spicule W	Immature spicules	1/13	24.827	0.0003	65.6	(15)

specimens increased in body mass to over twice their original size with rising temperatures from spring to late summer, then diminished in size after reproduction as the surrounding water became colder. This observation confirms results of previous studies on *H. panicea* from the same area (Barthel 1986, 1988, 1992). Sponges sampled in winter are smaller and appear shrunken and shrivelled compared to sponges sampled in summer (Schönberg personal observation). Thus, variation in the proportion of inorganic skeleton was indirectly controlled by variations in the amount of live tissue (Fig. 4), as proposed by Barthel (1986). We consider that this is a much faster process than direct regulation of the skeleton, i.e. faster than spicule production or loss of spicules.

By December 1992, the sponges in the Western Baltic were better adapted, being more resistant to wave forces, but were at the same time less physiologically active and produced fewer spicules. The proportion of organic matter was reduced, probably through the resorption of live tissue. This could fulfil a second function by providing an additional source of carbon in winter. Regrowth and tissue build-up with rising temperatures in spring and summer were rapid, involving the expenditure of much energy; as a result, production of scleroblasts appeared to be delayed in favour of other processes such as sexual reproduction. After release of larvae in early summer (Witte and Barthel 1994), new spicule production reached its highest levels. As a result, the absolute amount of skeleton increased, but the relative amount at this stage was markedly lower due to the growth of cellular tissue (Fig. 4). When *Halichondria panicea* began a new cycle of oogenesis in the autumn, spicule production dropped noticeably (Figs. 1c, 3), but the subsequent decrease in the organic fraction resulted in an increase in the relative amount of skeleton. This cycle may be repeated in individual specimens of *H. panicea* of the Western Baltic, which can grow for at least 2.5 yr (Witte and Barthel 1994). Cyclic variation in silicate content has also been recorded for *Chondrilla nucula* of the Ligurian Sea (Bavestrello et al. 1993): however, in this case the variability of the inorganic skeleton could not be related to environmental factors and was interpreted as a consequence of varying total number of spicules per specimen.

We found no evidence that the lower mineral skeleton content of *Halichondria panicea* in summer results from loss of spicules. There is an ongoing discussion as to whether sponges are able to dissolve siliceous structures (i.e. spicules) in order to reduce their skeleton fraction or to recycle silicate. *Chondrosia reniformis* selectively etches quartz particles incorporated into the ectosome

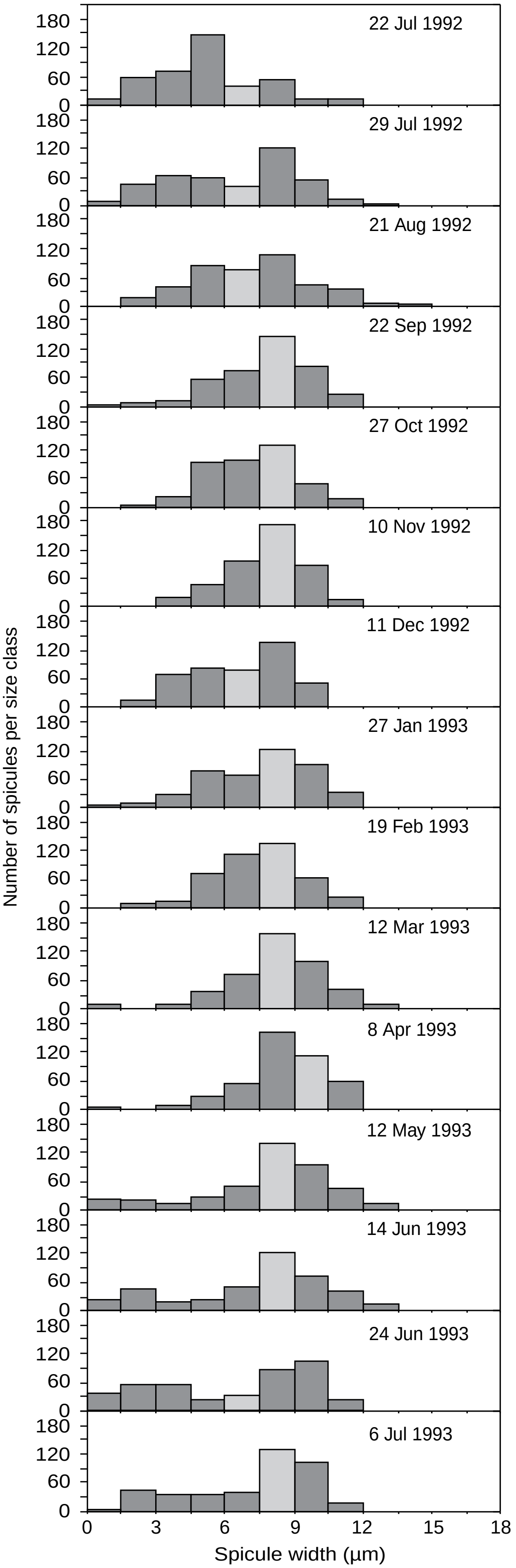

Fig. 3 *Halichondria panicea.* Spicule width-frequency distribution of spicules from mesohyle. Size class of monthly mean (N = 420) is indicated by lightest-shaded histogram for each date

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(Bavestrello et al. 1995). However, Bavestrello et al. found only intact spicules in their samples; thus, although they demonstrated that *C. reniformis* is able to dissolve siliceous material, there is no proof that this actually happens in the case of the spicules. One means of shedding spicules in *H. panicea* is by sloughing off the comparatively spicule-dense surface, the exopinacoderm. This has been observed in aquarium-reared specimens that cleaned their surface of detritus and fouling (Barthel and Wolfrath 1989). However, this would not be sufficient to explain the lower proportion of mineral skeleton in summer.

Temperature is probably the main factor regulating (indirectly) variation in the proportion of inorganic skeleton of *Halichondria panicea* in the Western Baltic; Barthel (1989) suggested that a certain temperature level triggers growth and other physiological activity. However, this still remains to be verified under controlled conditions in culture experiments. In the present study, the proportion of skeleton was also found to be positively related to salinity, silicate content of the water and wave force: even though salinities higher than those in the Western Baltic are believed to be physiologically beneficial for *H. panicea* (Barthel 1989), and silicate is an essential skeleton-building material, these environmental factors were strongly influenced by wind-driven currents and the resulting influx of North Sea water. Moreover, all three factors displayed an opposite trend to temperature. Thus, their individual effects can only satisfactorily be determined in transplant or culture experiments. Clone-transplant experiments in areas of weaker/stronger influence of wave forces in Torch Bay, Alaska, showed that epilithic *H. panicea* react directly to increased mechanical stress by enhancing tissue rigidity and spicule thickness (Palumbi 1984, 1986). Thus, it is possible that even in Western Baltic populations (which are exposed to only negligible tidal currents and which are protected against some wave force by the red algae on which they grow), strengthening of sponge tissues in

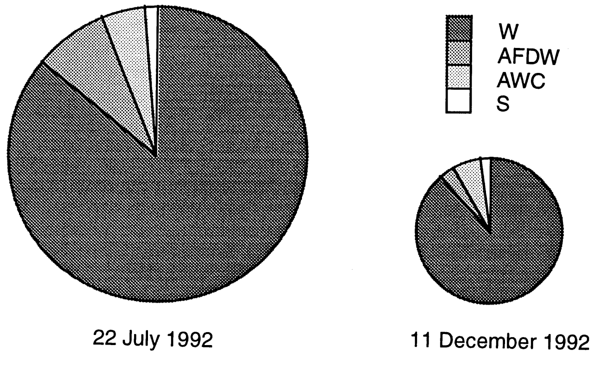

Fig. 4 *Halichondria panicea.* Body components (*W* water; *AWC* mineral skeleton; *AFDW* organic dry substance; *S* salt). Sponges are about twice as large in summer as in winter

response to increased wind-derived water movement would be observed were it not masked by temperature-related processes.

The observation of a stronger exopinacoderm in winter samples of *Halichondria panicea* could also be an adaptation to increased water movement during winter storms, since sand washes around in shallow depths, presenting a risk of abrasion and scouring. A structurally stronger surface layer would protect the sponge. This presumption is not supported by any data and must be confirmed by experiments.

The population studied underwent a comparatively brief period of annual spicule production. Peak production was in early summer, as evidenced by the bimodal frequency distributions of spicule width and a marked increase in the number of immature spicules (Figs. 1c, 3). The timing of spicule formation varies with location and probably with environmental factors. For example, in specimens at localities further north (Belt Sea, Kattegat and Skagerrak), intensive skeleton production seems to be prolonged into August to late September (Schönberg personal observations). Bimodality in frequency distributions of spicule width has been used before to demonstrate spicule production: *Haliclona rosea*, *H. elegans* and *Gellius angulatus* from the Irish Sea form spicules in spring to late summer (Jones 1984, 1987a, b, 1991). Earlier studies assessing growth rates of *Halichondria panicea* reported that specimens from the brackish Western Baltic are probably subjected to stress through low salinitiy, which may impair physiological functions (Barthel 1989). Hence, it seems that salinity stress may restrict spicule production at Bokniseck compared to localities further north, even though the correlation of the proportion of immature spicules and salinity at Bokniseck was not significant at the 95% probability level; the salinity gradient is the most pronounced environmental change along the north–south axis in the Western Baltic Sea.

Spicule dimensions of Western Baltic *Halichondria panicea* were highly variable within and between individuals and between seasons. Nevertheless, the range observed is considered to be normal for this species elsewhere (Hartman 1958, his Table 8; Vethaak et al. 1982, their Tables 1 and 5). Therefore, spicule size as such does not seem to be influenced as strongly by environmental variation as does the proportion of inorganic skeleton per unit tissue. The low salinity at Bokniseck, with an annual mean of about 17‰, did not appear to interfere with spicule formation. However, spicules decreased in size in summer, when temperatures increased (e.g. 22 July 1992: length 82% and width 75% of annual mean), an observation also noted for other marine demosponges (Jones 1987a, b, 1991; Bavestrello et al. 1993). In summer samples, decreasing mean spicule size is mainly due to increased numbers of immature spicules during skeleton production rather than to smaller sizes of completed spicules. Mean spicule size increased slightly in late winter to spring (8 April 1993: length 112% and width 116% of annual mean), even though there was evidence of some low-level spicule production in the studied population. This implies that spicules produced during the colder season attain a larger final size than those produced in summer. Jones (1987a) reported similar observations for *Haliclona elegans*. For *Microciona prolifera* in culture experiments, Simpson (1978) stated that particularly spicule width was inversely related to temperature. Hartman (1958) reported thicker spicules in populations of *Halichondria bowerbanki* and *Haliclona canaliculata* in winter than in summer, but did not relate this to temperature; he explained it as resulting from higher ambient silicate concentrations in winter. At Bokniseck, the difference in spicule width of *Halichondria panicea* was not correlated with mechanical stress due to waves during winter storms. The relative number of spicules was regarded to be more important to strengthening the tissues than the slight differences in individual spicule dimensions. Spicule size in *H. panicea* was not shown to be correlated with any ambient factors other than temperature; hence it would seem to be relatively independent of environmental variation. In contrast, Stone (1970a) and Jones (1991) found correlations between spicule dimensions in *Hymeniacidon perleve* and *Haliclona rosea* and ambient silicate concentrations. However, both, Stone's and Jones' conclusions were not supported by culture experiments.

Interactions between the environmental factors studied, additional environmental factors not considered, and events in the sponges' life cycle confuse the findings. For example, during spring and late summer, diatom blooms in the Western Baltic reduce the ambient concentration of dissolved silicate (von Bodungen 1975). *Halichondria panicea* seems able to compete for silicate for at least a short period of time, as a diatom bloom and peak spicule production occurred simultaneously in July 1992 (U. Horstmann personal communication). Other observations of diatom blooms coincided with lowered levels of skeleton formation in the sponges (August 1992, March 1993 and July/August 1993). It is possible that *H. panicea* can only compete with diatoms for dissolved silicate when most of its energy is allocated to spicule production, i.e. to silicate uptake. When temperatures start to rise in spring, most of its energy seems to be diverted to sexual reproduction. Despite otherwise seemingly ideal environmental conditions, spicule production may be low and the mean spicule size consequently relatively large.

This study demonstrates some aspects of spicule production in *Halichondria panicea*, but many questions remain to be solved, especially whether environmental factors found to correlate with skeleton characteristics are the true triggers of variations in its skeleton or whether they merely develop in parallel with other factors which were not considered. In order to verify these relationships with environmental factors, it will be necessary to examine hypotheses generated from field studies under controlled conditions in culture experiments, for which *H. panicea* has been shown to be suitable (Barthel and Theede 1986).

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