Seasonal changes in condition and biochemical constituents in the soft part of Macoma balthica (Lamellibranchiata) in the Tvärminne brackish water area (Baltic Sea)

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The condition of *Macoma balthica* (L.), taken from a soft bottom at a depth of 7–8 m, in 6 ‰ S, was highest in the summer months of June–August and lowest in spring during March–April. The condition rose rapidly soon after spawning in May. The tissue water content (%) and "shell component index" were lowest at the time of the best condition. Shell growth began in May and slowed down in August when the winter ring began to form.

In September, medium-sized *Macoma*, of 12–16 mm shell length, showed the best condition but the histogram was quite level from 6 to 20 mm. Smaller animals (6–12 mm), kept in the laboratory at 6–12°C from July to September, had a better condition and greater dry weight than freshly collected animals of similar size. The water content of the soft part (%) increased from the categories of smaller shell length to larger animals.

About 100 % of *Macoma* used in the seasonal studies from this sampling station, which can be assumed free from pollutants, were infected by trematode matacercariae in the extrapallial space. 11–55 % of animals analysed monthly carried ≥20 metacercariae per shell half. This heavy infection did not obviously affect the condition of the host bivalve.

The lipid level of the soft part (14–24 % of dry weight) was much higher than in *Macoma* in the Wadden Sea, Netherlands, and conversely the protein level (40–58 %) was lower. The glycogen level was the same (9–32 % of dry weight). The seasonal variation in these levels was very similar to that found in the Netherlands, but the onset of the most prominent changes in spring, increase in glycogen and lipid levels and decrease of proteins, took place in the Tvärminne brackish water area one month later than in the Wadden Sea. Also, the maximum value of lipids was later here than in the Netherlands.

In May *Macoma* lost about 25 % of the weight at their soft part during spawning, but in February–March, after being kept for 2 weeks at 10–12°C in the laboratory, the loss due to spawning was smaller. Keeping *M. balthica* in the laboratory on a minimal diet for 6 months from late winter to early autumn (4–6°) decreased their condition to one third of that found in the wild state. Their water content was greater, while the glycogen level remained very much lower than in the wild. The lipid level did not differ so markedly. The effects caused by keeping *M. balthica* in the laboratory depend on the initial condition of the molluscs, the season and stage of their reproductive cycle, the temperature in comparison to their natural ambient temperature, and the nutritional conditions in the aquarium.

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1. Introduction

Macoma balthica (L.) is a widely distributed deposit-freeder inhabiting different kinds of soft bottoms, often forming dense *Macoma* communities. There have been numerous investigations on the growth and/or biological production of *Macoma balthica* from different geographical locations and different kinds of bottom sediments (e.g. Segerstråle 1960, Lammens 1967, Gilbert 1973, Green 1973, Bergh 1974, Chambers & Milne 1975, Warwick & Price 1975, McLusky &

Allan 1976, Beukema et al. 1977, Ankar 1980, Bachelet 1982, and Nichols & Thompson 1982).

Recently, when water pollution has more commonly been taken into consideration, the active treatment of water and sediment by bivalves has been evaluated afresh (e.g. Bergh 1974, Black 1980). *Macoma balthica* is used as an indicator in long-term observations on pollutants in the Baltic Sea (ICES 1974). Besides bioaccumulation of pollutants from its ambient (e.g. Langston 1978, Kaitala 1981), *M. balthica* indicates low concentrations of toxicants through

increased mortality (Shaw et al. 1976), changes in burrowing activity (Lindén 1977, Eldon & Kristoffersson 1978, McGreer 1979, Eldon et al. 1980, Møhlenberg & Kiørboe 1983), and through deterioration of certain tissues (Eldon et al. 1980). The sensitivity of behavioural changes is dependent on the season (Eldon et al. 1980). Further, the relationship between the body size of *M. balthica* and concentration of heavy metals in its tissues varies due to different growth and feeding rhythms in different populations and even within the same population (Strong & Luoma 1981). Thus, when one interprets the effect and fate of toxicants one must first be conversant with the physiological state of the test animal.

Very few descriptions of variations in the basic physiology and biochemistry of this species have been published. Changes in metabolism (e.g. oxygen consumption) due to different temperatures have been described by Spärck (1936) and de Wilde (1975). The reproductive cycle of *M. balthica* has been studied intensively by Battle (1933) in Ontario, Canada, by Caddy (1967) in England and by Lammens (1967) and de Wilde & Berghuis (1978) in the Netherlands, and Gilbert (1978) in Massachusetts, USA. One study concerned the seasonal changes in dry weight and chemical composition of *M. balthica* in the Dutch Wadden Sea (Beukema & de Bruin 1977). These results will not necessarily apply to *M. balthica* here in its brackish water and more boreal location.

In this article I shall describe the condition and biochemical composition (water content and glycogen, lipid and protein contents of the soft part) of *M. balthica* in the brackish water area of Tvärminne Zoological Station near the mouth of the Gulf of Finland, where the salinity is about 6 ‰. Seasonal changes and spawning, together with the effect of laboratory conditions on these biochemical components and on general condition are described, and a note is appended concerning trematode infection of the extrapallial space.

2. Material and methods

Macoma balthica were collected with a van Veen bottom sampler just outside Tvärminne Zoological Station (59° 50′N, 23° 15′E) from a depth of 7–8 m. This collection site (Halsholmen–Sundholmen) can be assumed to be free from pollutants. The salinity is about 6 ‰ and tidal action is negligible. The location is usually covered by ice for about four months, from late December to late April, but the duration of the ice cover varies greatly from year to year. The water temperature at that depth is coldest (about 0°C) in February–March and it begins to rise steeply in May, being usually at its highest in August (about 16°C in 1981). In July 1982 the rise in temperature was slower than in 1981 and it began to decrease earlier than in 1981. However, November 1982 was exceptionally mild.

Sampling was begun in 1981. In 1982 samples were taken nine times. *Macoma* were sieved using their natural water and a 7 mm mesh sieve. Bivalves were collected in a bucket filled with Baltic water at its natural temperature. In the laboratory the animals were put in a plastic container with aerated running brackish water at 6–8°C. The animals were left to purge themselves for at least two days.

Medium-sized animals were chosen for the analyses, and both sexes were included. The overall weight of each individual was first measured after gentle blotting with cellstoff. When disturbed, the mollusc contracts its adductors and a certain amount of water is left in the mantle cavity. The soft part was then freed with a preparation knife and gently blotted with a filter paper, before being weighed. Parasites on the inner surface of the shells were stained with Trypan Rot in brackish water and assessed. The shells were wiped off and their wet weights and lengths were measured.

For glycogen analysis, the weighed soft parts from about five bivalves were pooled and homogenized twice in 5 % TCA in an ice bath. Glycogen was precipitated from the supernatant liquid twice with 94 % ethanol and washed with ethanol and diethylether. Two parallel pooled analyses were usually made at a time. The purities of the glycogen yields were determined with anthron analyses (modified from Scott & Melvin 1953), and on the basis of this a correction was made to the analyses. The percentage yield was determined with an artificial bivalve body made from weighed amounts of glycogen (Merck), albumin, olive oil, NaCl and water, and on the other hand with weighed amounts of glycogen. The yield was found to be about 90 % and the proper correction was made to the analyses. The glycogen content was calculated as a percentage of unit wet weight.

The total lipid content of the soft part was extracted and purified by the method developed by Folch et al. (1957). The lipid content was determined as a percentage of unit wet weight. About ten individuals were analysed at each sampling.

Proteins in the soft part (% of unit wet weight) were determined using the method established by Lowry et al. (1951). Weighed soft parts were frozen with liquid nitrogen and stored in a deep-frozen state until being analysed.

The dry weights of soft parts and their water contents were determined from other individuals than the ones used for determination of biochemical components after drying at 100°C for 48 h. Glycogen, lipid and protein levels as a percentage of dry unit weight were calculated using mean dry material percentages.

The indices describing condition were calculated from the measured data of the bivalves. The "shell component index" was calculated as follows: shell wet weight × 100/(shell wet weight + soft part wet weight). "Fatness" was calculated as: soft part wet weight × 100/(weight of whole animal–shell weight). The denominator is thus the sum of the weights of the soft part and water in the mantle cavity. The condition factor was calculated using the dry weight: soft part dry weight, mg/(shell length, cm)^3.

Effects of keeping *M. balthica* in the laboratory for different times, and condition parametres characteristic to different size categories of *M. balthica* in September:

I) Condition characteristics of different size categories of *M. balthica* in September. *M. balthica* collected at the beginning of July 1982 were kept in running brackish water with aeration at 10–12°C for 4 weeks and thereafter with sand on the bottom with aerated running water at 6–8°C for about another 4 weeks. The photoperiod was 12 h L and 12 h D. They were sampled in early September. Water contents and dry weights of the soft parts, shell wet weights and condition indices were measured for classes of different shell lengths. These characteristics were compared with the respective figures in freshly collected *M. balthica*.

II) Effects of different temperatures in February–March. Bivalves were collected at the end of January 1982. They were kept in aerated running brackish water at 6–8°C for 2 weeks (12 h L, 12 h D). One group was then kept at 10–12°C for

another 2 weeks, the control group being maintained at 6–8°C. At the beginning of March the spawning degree of the animals was checked under a binocular microscope. They were roughly classified as unspawned, partly spawned or spawned. Condition indices and water contents were compared with the natural values in early February and early March.

III) Six-month and three-month experiments. Bivalves collected at the end of January 1981 were kept in the laboratory in stagnant water sith aeration at 4–6°C (12h L, 12 h D) until August. They had washed and sieved (1 mm mesh) sand on the bottom to burrow in. The water was changed about twice a month. Another group was kept in similar conditions from March to September 1981. One group of *Macoma* were kept on a bare bottom from the beginning of July to the end of September. After the experiments, water content, glycogen and lipids, together with condition indices, were measured. The stage in the reproductive cycle was checked by means of gonadal smears.

3. Results

The condition factor and "fatness" of *M. balthica* were highest in the summer months of June–August, being 14.0–14.5 and c. 61–62 %, respectively (Fig. 1). The lowest condition appeared in spring, March–April, the condition factor being 7.5–8.5 and "fatness" 51–52 %. "Fatness" was especially low (47.5 %) in recently spawned individuals during May. Compared to the "fatness" of unspawned individuals (60.5 %), spawned and partly spawned animals had lost 22 % of their fatness index. The respective decrease in the condition factor amounted to 26 % of the condition factor of unspawned individuals. In May 1981 the mean condition of all the individuals analysed was low (condition factor 7 and "fatness" 47.5 %) but they were not checked to see whether they had spawned or not. The condition rose rapidly in May and June and fell again drastically in August and September. The decrease then ceased in autumn and continued again in winter. "Fatness" even rose temporarily in October–November 1982. In November 1981 the condition factor was significantly lower than in November 1982. The "shell component index" was clearly a mirror-image of the fatness index, being 43–45 % in summer and about 47–49 % at other times.

Almost all individuals, examined at various times of the year, were infected by trematode metacercariae (Table 1). These parasites live between the mantle and shell. The degree of infection had a slight tendency to be greater in summer and early autumn than in winter and spring. About 21–55 % of *M. balthica* examined were infected by ≥20 parasites per shell half from June to September. No clear correlation between the condition of the host individual and the degree of infection could be seen.

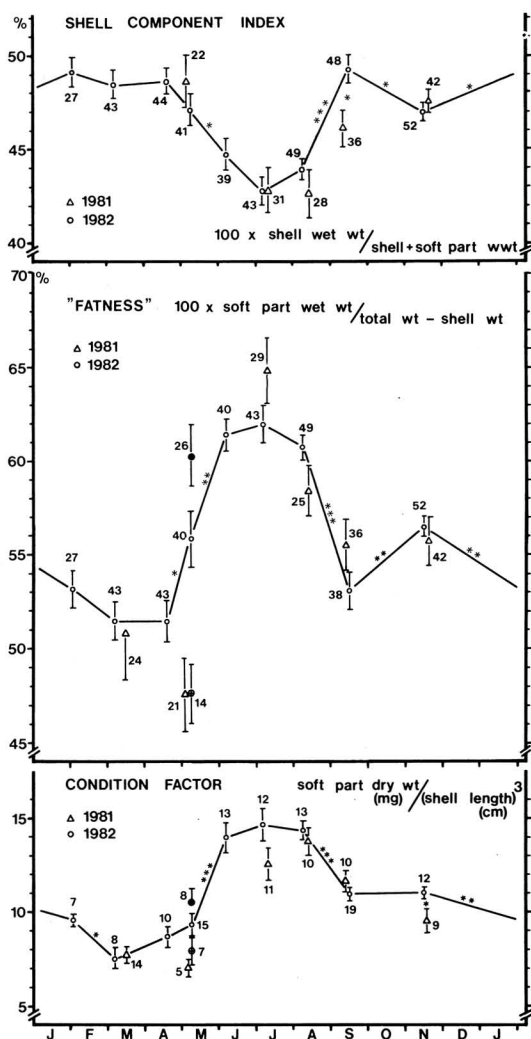The blooming of green algae commenced in April and a green colour was observable in the

Fig. 1. Graphs showing seasonal changes in the condition of *M. balthica* expressed as shell component index, "fatness" and condition factor. Means ± SEs and numbers of specimens analysed are given. The triangles represent results from 1981 and circles from 1982. In May the upper solid circles represent means for the unspawned animals and the lower solid circles means for the spawned animals. The cirle between them is the total mean. Probability levels have been obtained using a two-sided Student's *t* test: * = *p*<0.05, ** = *p*<0.01, *** = *p*<0.001.

digestive glands of *M. balthica* as early as this. In May the digestive glands were even very dark green in colour. In June the colour was masked by the yellow brown colour of stored lipids. A green band in the centrifuged TCA precipitate left after the glycogen extraction procedure was seen throughout the summer until the autumn.

Shell growth had begun in most individuals in early May (Fig. 2). The formation of more

Table 1. Frequency (%) and extent (% infected by ≥20 parasites/shell half) of infection by trematode metacercariae between the mantle and shell in *Macoma balthica* at different seasons during 1981 and 1982. (*N*) = number of specimens analysed.

	Frequency		Extent	
	1981(*N*)	1982(*N*)	1981	1982
February		100(27)		11
March		100(38)		21
April		100(40)		15
May	100(16)	100(41)	19	15
June		97(33)		24
July	100(31)	100(43)	23	21
August	100(26)	96(49)	27	24
September	96(26)	100(20)	23	55
November	100(37)	100(52)	19	19

translucent shell material and retardation of growth began as early as August in most individuals. In November 1982 the translucent "winter ring" was usually obvious, but in some specimens this either seemed to be lacking or more calcified material had been deposited outside an existing ring.

In early March only 1 out of 43 individuals (2 %) had partly spawned. In mid-April 19 % had partly spawned and on May 7th, 1982, 40 % had spawned at least partly, to judge by their appearance under the binocular microscope. In early June sex could be determined only in 12 % of the individuals, i.e. they were unspawned or only partly spawned. Sex could be recognized in half of the animals in August and in most individuals in September.

The water content of the soft part of *Macoma* (Fig. 3) was smallest (about 77–78 %) in summer, or June–August. After August it increased gradually through the autumn and winter until it reached about 85 % in March, after which it began to decrease. The most appreciable decrease occurred between May and June. The water contents of spawned and unspawned animals did not differ significantly in May. The glycogen content, percentage of soft part wet weight (Fig. 3), was lowest, about 1.5–2.2 %, from February to May, and between May and June it increased drastically up to about 7.2 %. After that it
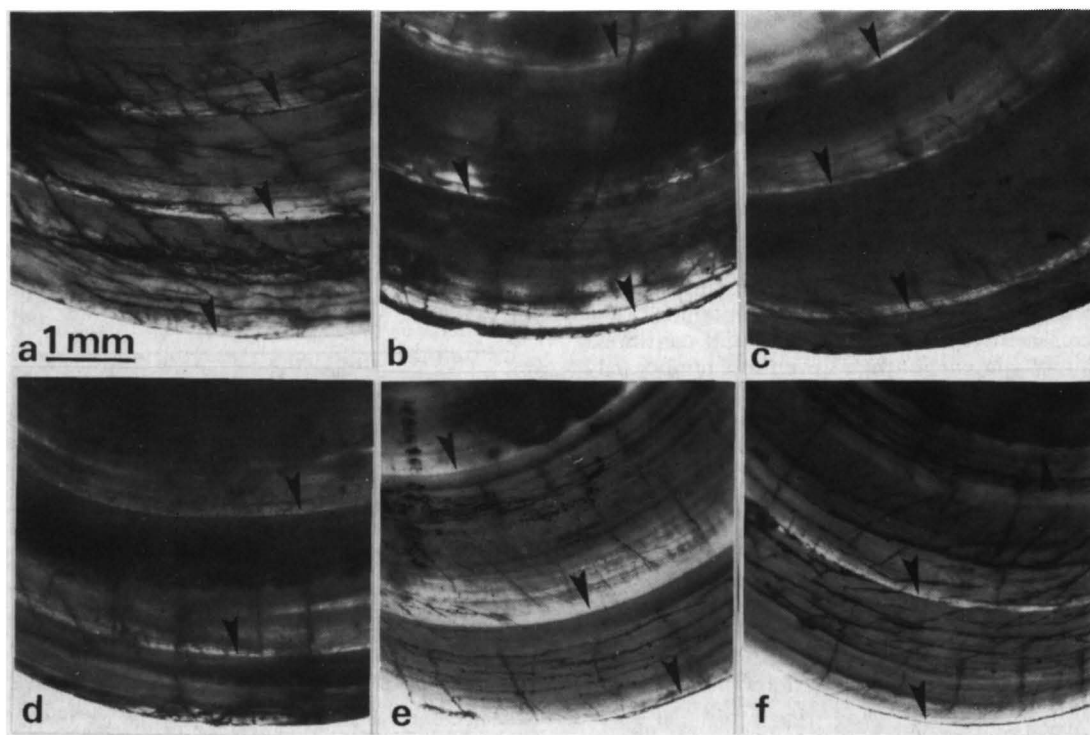

Fig. 2. Shell margins of *M. balthica* from spring to autumn photographed from the anterior edge with transparent light. The scale is the same for all pictures. The arrows indicate "annual rings" ("winter-rings"). The lengths of the shells were c. 11–13 mm. a) Shell which has not commenced growth in early May. b) Second shell in May, in which growth has obviously begun. c) to e) Shell growth progress from beginning of June (c) and beginning of July (d) to early August (e) when the "annual ring" begins to form. f) Shell in November. A "winter ring" is clearly visible. The narrow darker band at the margin is the periostracum stained with Trypan Rot (see methods).
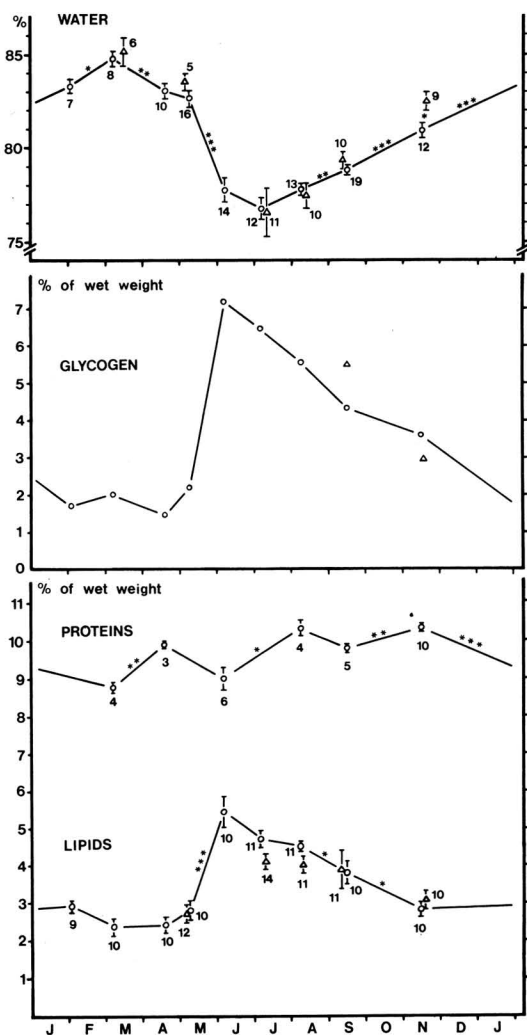
Fig. 3. Seasonal changes in water, glycogen, protein and lipid contents of the soft part of *M. balthica* (% of unit wet weight). For explanations see Fig. 1.

excluding the tissue water, which varies quite considerably throughout the year, is most prominent in the protein graph (c.f. Figs 3 and 4). It then more closely resembles the course of the water content graph. The protein level was highest in March–April, about 58 % of dry weight, and lowest in June, about 40 % of dry weight. The graphs for glycogen and lipids were only slightly levelled out by this calculation. The glycogen level varied between 9 and 13 % in February–May and increased up to 32 % up to June, decreasing thereafter. The lipid level was about 14–17 % from
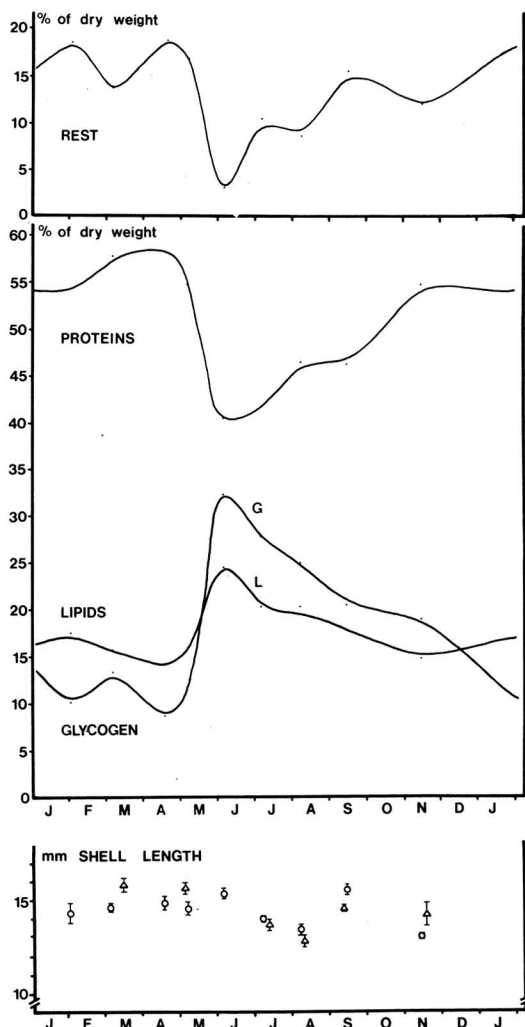

Fig. 4. Seasonal changes in the glycogen (G), lipid (L) and protein levels in the soft part of *M. balthica* (% of unit dry weight). The "rest" graph is obtained by subtracting the sum of glycogen, lipids and proteins from 100 %. At the bottom mean shell lengths ($\pm SE$) of the bivalves used in the entire seasonal study are given.

decreased steadily until February. The protein content (Fig. 3) varied between 8.8 and 10.4 % of wet weight.

The lipid content graph greatly resembled that of the glycogen content. However, it reached the spring level, about 2.4–2.9 % as early as November. The highest mean lipid content was 5.4 % in June. In late summer, 1981 the lipid content appeared to be lower than at an equivalent time in 1982, but the difference was not significant. In June 1981 it was not measured.

The levels of glycogen, lipids and proteins as percentages of dry weight allow generalized graphs like Fig. 4 to be drawn. The effect of

November to May. It then rose to 24 % in June and decreased until November. The "rest" graph is composed of salts and undetermined organic compounds.

The mean shell lengths of the animals used in the seasonal analyses above are given at the bottom of Fig. 4. The means varied between 12.7–15.8 mm in 1981 and 13.0–15.5 mm in 1982. In order to show a possible size dependence of the condition indices used above, these were measured in September 1982 for different size categories of *M. balthica*.

I) *Condition characteristics of different size categories of M. balthica in September.* The weight of the soft part of *Macoma* increases sigmoidally as the shell length increases from 6.0 mm (class 1, dry weight 2.6 mg) to 21.9 mm (class 8, dry weight 59 mg) (Fig. 5). The increase in the dry weight is retarded in animals over 18 mm (beyond the class 6). *Macoma* of smaller size categories kept in the laboratory (L) had higher mean dry weights than *Macoma* of equivalent sizes taken from the sea (S) in September. The wet weight of the shell at first increases slowly in smaller *Macoma*, then the increase accelerated with a further increase in shell length (Fig. 5). Keeping *Macoma* in the laboratory from early July to early September did not lead to differences in the weights of their shells as compared with freshly collected *Macoma*.

"Fatness" was greatest in the medium-sized *Macoma*, 12.0–15.9 mm, (classes 4 and 5), so that

about 52 % of their shell interior was "flesh" (Fig. 6). Animals kept in the laboratory, especially smaller ones, were fatter than those taken from the sea.

The histogram for the shell component index was a close mirror-image of the fatness index. The shell represented over 50 % of the weight of shell + soft part in smaller and larger animals and less than 50 % in the medium-sized animals (Fig. 6).

The water content of the soft part rose gradually in conjunction with increasing size from about 76 % in the size category 6.0–7.9 mm to about 81.5 % in the 20.0–21.9 mm group (Fig. 6). The condition factor was highest (11.3) in medium-sized bivalves (12.0–15.9 mm). In large animals (20.0–21.9 mm) it was low (6.3), but otherwise the histogram was quite level. Of those *Macoma* kept in the laboratory, the three smallest categories up to 11.9 mm showed a better condition factor (12.2–13.3) than those taken from the sea (9.8–10.4).

II) *Effects of different temperatures in February–March.* When 25 *M. balthica* had been kept in an aquarium at 10–12°C for two weeks in February and analysed in early March, 12 of them were classified as spawned, 5 as partly spawned and 8 unspawned. Of 12 control animals at 6–8°C, one was partly spawned. From natural *Macoma* in early March one out of 43 was partly spawned.

During February–March, in the natural habitat at about 0°C, the condition factor of
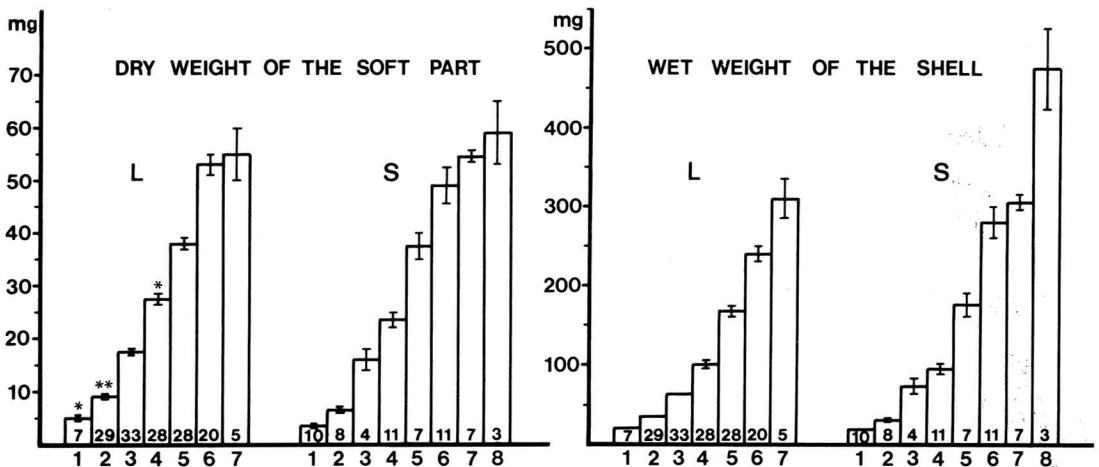

Fig. 5. Mean dry weights of the soft part and mean wet weights of the shell (±*SE*) in different size classes (shell length) of *M. balthica* kept in the laboratory (L) or taken from the sea (S), analysed in September 1982 (experiment I).

Division into size classes is as follows: 1 = 6.0–7.9 mm, 2 = 8.0–9.9 mm, 3 = 10.0–11.9 mm, 4 = 12.0–13.9 mm, 5 = 14.0–15.9 mm, 6 = 16.0–17.9 mm, 7 = 18.0–19.9 mm, 8 = 20.0–21.9 mm. Numbers inside the columns represent the numbers of individuals analysed. Asterisks indicate statistically significant differences between animals kept in the laboratory and animals taken from the sea. Student's *t* test, two-sided probabilities: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.
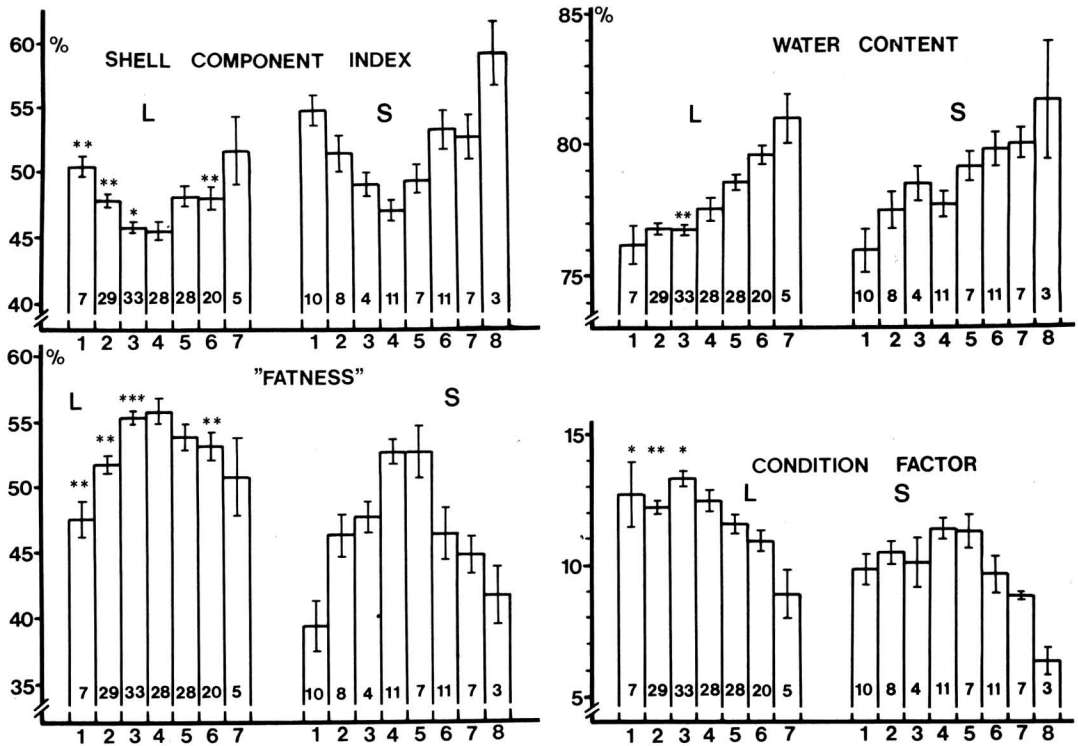
Fig. 6. Condition characteristics of different size categories of *M. balthica* in September 1982. Animals freshly caught from the sea (S) are compared with animals kept in the laboratory (L) for about two months. For explanation see Fig. 5.

Macoma decreases. Part of the material is replaced by increasing tissue water content so that the fatness index does not decrease significantly (Figs 1, 3 and 7). When *Macoma* were kept in the laboratory at 6–8°C or 10–12°C their water contents did not increase beyond the value in February (Fig. 7). The condition factor and "fatness" decreased only in partly spawned and spawned animals (b and c) as compared to those in February. The decrease of "fatness" (18 %) in spawned animals (c) as compared to the "fatness" of unspawned animals (a) was due to small decreases in both water content and dry weight, neither of which alone decreased significantly. The respective decrease of the condition factor was only about 14 %. The unspawned animals at 6–8°C and 10–12°C (a) had very similar water contents and condition.

III) *Six-month and three-month experiments.* In *M. balthica* kept in the laboratory for six months (analysed in August or September) the water content of the soft part was noticeably greater than in natural animals at the same time of the year (Table 2). "Fatness" was about half of the
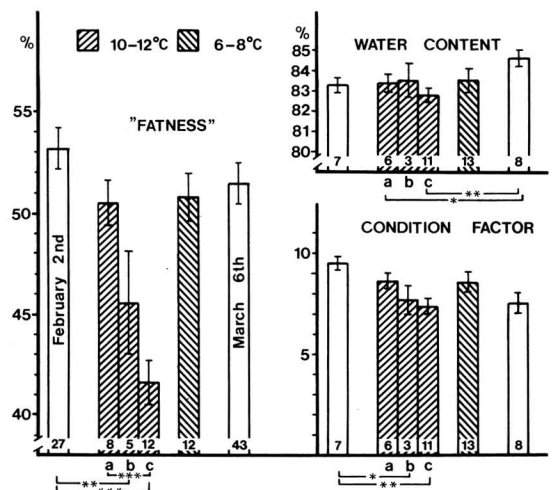

Fig. 7. Comparison of condition and water content of *M. balthica* kept in the laboratory at different temperatures from February to March with values in the wild during February and March 1982 (experiment II). For the experimental arrangement see under methods. a = unspawned animals, b = partially-spawned animals, c = spawned animals.

Table 2. Effects of keeping *M. balthica* in the laboratory at 4-6°C for 3 or 6 months on the mollusc's condition, and water, glycogen and lipid contents of the soft part (experiment III). P = Significance on basis of Student's *t* test (* = $p<0.05$, *** = $p<0.001$, ns = not significant) or percentage of test value from natural value.

	6 months	(N)	P	Wild	(N)	P	3 months	(N)
Analysed in August 1981								
water content (%)	83.97±1.08	(7)	***	77.43±0.66	(10)			
"fatness" (%)	29.86±1.25	(17)	51 %	58.42±1.37	(25)			
condition factor	4.04±0.47	(7)	29 %	13.74±0.73	(10)			
lipids (% of wet weight)	3.38±0.21	(8)	ns	4.01±0.23	(11)			
lipids (% of dry weight)	21.09		119 %	17.77				
shell length (mm)	10.88±0.58	(18)		13.77±0.30	(26)			
Analysed in September 1981								
water content (%)	82.94± 1.67	(8)	*	79.28±0.44	(10)	ns	80.86±1.79	(8)
"fatness" (%)	27.63±1.21	(24)	49 %	55.48±1.37	(36)	*	50.50±2.14	(29)
condition factor	4.08±0.63	(8)	35 %	11.65±0.53	(10)	ns	8.82±1.48	(8)
glycogen (% of wet weight)	0.67		12 %	5.51		73 %	4.03	
glycogen (% of dry weight)	3.93		15 %	26.59		79 %	21.05	
lipids (% of wet weight)	2.48±0.25	(8)	*	3.87±1.37	(11)	ns	3.29±0.27	(11)
lipids (% of dry weight)	14.54		78 %	18.68		92 %	17.19	
shell length (mm)	12.48±0.31	(27)		14.48±0.18	(36)		13.00±0.63	(30)

normal value and the condition factor had decreased to as low as one third of normal. The glycogen level was only 15 % of its normal value but lipid levels did not differ so markedly from those in the wild. In most individuals the gonadal cycle was not in phase with the normal rhythm as though many of them had failed to spawn. In *Macoma* kept in the laboratory for three months (analysed in September) trends were similar to those in the six-month experiment though they were not quite so clear.

4. Discussion

The annual cycle of the condition of *M. balthica* is here in the main similar to that described for the Dutch Wadden Sea (Beukema & de Bruin 1977), with the exception that the sharp increase begins about one month later here, in early May. Here the condition remains high from June to August, whereas in the Wadden Sea it shows a downward trend after June. Near Aberdeen in Scotland the most marked increase in the dry weight of *M. balthica* (14 mm) is still later, in June-July (Chambers & Milne 1975). The condition there shows a similar decline in late summer (July-August) as was experienced near Tvärminne (August-September).

The condition factor is usually calculated using ash-free dry weight. If the condition factor is recalculated assuming that the "rest" graph in Fig. 4 represents ash content, it changes the figures only slightly. Condition factors for *Macoma* calculated in both these ways correspond closely to those described by Beukema & de Bruin (1977).

The spawning of *M. balthica* either coincides with the beginning of growth or it precedes it. Most spawning takes place here in May. Feeding on green algae has sometimes commenced as early as April but very rapid growth does not begin before May. The storing of material is so rapid that the loss due to spawning is soon restored. The spawning time in Scotland (Chambers & Milne 1975) is from the energy standpoint very different from that in Finland. *M. balthica* spawns there when the condition of the animal is decreasing to its minimum. Here spawning coincides with the active feeding period, and at the same time the animal's condition is beginning to improve. The amount of material lost at spawning was found here to be about the same as in the Wadden Sea (25 %, de Wilde & Berghuis 1978). This is dependent on the season because in March only 14-18 % was lost at spawning induced by a temperature rise (experiment II). Possibly the gonads were not fully developed or spawning was not perfect, or the animals had consumed their sexual products.

The spawning times of *Macoma* differ to some degree in different geographical regions. In the Thames Estuary in England *Macoma balthica* spawns in April-June and to a lesser extent in autumn (Caddy 1967). This second spawning was not evident in the Lyncher estuary (Warwick & Price 1975). In Aberdeen in Scotland *M. balthica* spawns quite early, from February to April (Chambers & Milne 1975). In the Dutch Wadden Sea *M. balthica* spawns in April-May (Lammens 1967, de Wilde & Berghuis 1978). In Ontario's Passamaquoddy bay, Canada it spawns intermittently at least from July to September Battle 1933. In Massachusetts according to Gilbert (1973, 1978) it spawns in May. In San Francisco Bay, California, near the southern limit of its range, it has again two spawning seasons: January-March and July-August (Nichols & Thompson 1982). Here, near Tvärminne, some

individuals partly spawned in April and most spawning occurred in May. Partial and intermittent spawning of *M. balthica* has been observed earlier, at least in tidal areas, (Battle 1933, Lammens 1967, Caddy 1967, de Wilde & Berghuis 1978 and Nichols & Thompson 1982).

Apart from by hormones, the spawning of *M. balthica* is controlled by temperature, temperature fluctuations, and lunar and tidal cycles (de Wilde & Berghuis 1978, Battle 1933). The critical temperature at which spawning of *Macoma* usually takes place is around 10°C (Caddy 1967, Lammens 1967, de Wilde 1975, de Wilde & Berghuis 1978). The result of experiment II is in agreement with this. But in May when most *Macoma* spawn here the ambient water temperature is still rather far from 10° (about 6°C) and also daily fluctuations are small. The mud temperature may rise more slowly than the water tempearture in May and there is a temperature difference between the ambient mud and the water pumped in by a bivalve. Possibly the good nutritional condition allows spawning to be accomplished. The spawning of *M. balthica* can be delayed in the laboratory by low temperatures (Lammens 1967). This was also seen in experiment III where many animals failed to spawn at a temperature of 4–6°C.

Keeping *M. balthica* in the laboratory at 6–8° or 10–12°C (experiment II) during February–March stopped the rise in their tissue water content which would have continued in Nature at about 0°C at that time. Also the decrease in their condition factor slowed down. High tissue water content may be correlated with maturing gonad (c.f. Pekkarinen 1984, in press) and/or with poor condition in late winter. Perhaps *Macoma* do not feed actively at that time. This period may represent an "extremely low temperature", at which, according to Lammens (1967), food intake in *M. balthica* is prevented, due to a decrease in metabolic processes, resulting in abatement of ciliary activity and other body processes. However, according to de Wilde (1975) there is remarkably high feeding activity even at temperatures below 0°C. In experiment II *M. balthica* may even have taken some food from the water. If the decrease in water content in spring is due to spawning, why was there no difference in the water contents of the spawned and unspawned *Macoma* in May? The water content may not decrease precisely at spawning, but a little later. After spawning, the gonad appears to be filled with water.

Shell growth usually takes place between spawning and new proliferation of gonads (Lammens 1967). The so-called "winter ring" or "annual ring" begins to form in late summer

when growth is retarded. In most investigations the beginning of gonadal development has been recorded as August (Lammens 1967, Gilbert 1973, de Wilde & Berghuis 1978). Here in Finland sex could be determined with a binocular microscope in half of the individuals in August and in most individuals in September. In the Wadden Sea, according to de Wilde & Berghuis (1978), gonadal development was slow — males and females were not recognisable before December, although gonad development began in August. There is again a different situation in Scotland, where the gonad started to develop by the end of May and mature animals were present by mid-July, when the dry weight of the soft part was at its maximum (Chambers & Milne 1975).

When we use condition indices such as "fatness" and condition factor which are calculated in relation to shell size, it must be appreciated that the shell grows from about May to August and the volume inside the shell also grows. Shell growth is, however, masked (also in the shell component index) by major changes in other factors. The growth of the soft part (storing of material) is so rapid in May–August that in spite of increasing shell volume "fatness" of the soft part ("in relation to the shell volume") increases in May and remains high until August. At the same time the "flesh" contains the least tissue water. The fatness index includes also the tissue water so that it reflects the annual cycle of the animal's condition (or reproductive cycle) in a different way than the condition factor. Lawrence & Scott (1982) used a nearly similar index for oysters, with the exception that they used the dry weight of the soft part instead of the wet weight.

Shell growth of *Macoma* usually almost completely ceases for winter, but in Massachusetts it continues throughout the year (Gilbert 1973). The condition of *Macoma* at Tvärminne was significantly better in November 1982 than November 1981. In some individuals even the shell seemed to have continued its growth. It should be mentioned that November 1982 was the second most mild November of the century. This mild and sunny weather has, of course, influenced primary production and this has been reflected in the food of *Macoma*.

There was no clear indication that the heavily infected (≥20 metacercariae / shell half) *Macoma* had a lower condition. The practically 100 percent infection was a "normal" situation in this population. The slight increase in the degree of infection in summer may result from new infections. *Macoma* are known to bury deepest in winter in an intertidal mud habitat in England (Reading & McGrorty 1978). If infected animals do not bury deeply (Hulscher 1973), they may be

preyed upon by predators more often than healthy animals in winter when food is scarce. This parasite, owing to its unusual infection site, may be different from that which is said to cause *Macoma* crawling tracks on the sediment (Swennen 1969, Swennen & Ching 1974).

Regarding the condition and water content of different size groups of *Macoma* (experiment I), the tendency towards a lower condition factor in larger specimens results from their greater water content, which replaces some dry material (Fig. 6). The increase of dry weight with increasing shell length is retarded in larger specimens of over 18 mm, although shell length growth is also retarded. The condition factor of *Macoma* in the Wadden Sea is nearly independent of shell length in the range 12–20 mm (Beukema & de Bruin 1977). Near Tvärminne the condition factor can roughly be assumed as constant from 6 to 20 mm. On the other hand de Wilde (1975) found the condition factor of *Macoma* in the Wadden Sea to be greatest (over 11) in large animals (over 20 mm) in a certain year, in late summer. In another year the greatest condition factor (10) was in medium-sized animals. The smallest condition factor (6.6–7.6) was in the smallest animals. The discrepancy between this and my results may be due to different growth curves and different nutritional and temperature conditions in the Wadden Sea compared to Tvärminne.

One reason for the "fatness" being smaller in larger animals may be that shell volume in proportion to shell length increases in larger animals, owing to the shell material being deposited partly under the former shell margin. The "fatness" of *Macoma* from different localities cannot be compared, because the form of the shell and its concavity may vary from place to place due to different growth rates (Segerstråle 1960). The lower "fatness" of smaller bivalves partly results from their smaller water content. If the water content of the soft part correlates with the gonad development, the younger *Macoma* should have greater water content than the older ones in late summer, because they spawn earlier (Lammens 1967, Kalliola, personal information) and therefore they are expected to be at a more advanced stage in the reproductive cycle than the older ones in late summer. When the animals in experiment I were taken into the laboratory much of the period of shell growth had already passed. If most of the growth had not already occurred the animals would have been assigned to categories of smaller shell length than those to which they would have properly belonged. However, the increment of the soft body is probably syncronized with shell growth.

The condition factor of *Macoma* decreases very significantly in the wild in August. In smaller animals which had been kept in the laboratory for July–August the condition factor (and dry weight) was still quite high in early September (experiment I). The reason for this must be the temperature. The water temperature was lower in the laboratory, at least during the latter half of the experimet (6–8°C). In their natural habitat the temperature at that time was about 13–15.5°C. Feeding activity in *Macoma* declines above 10°C (de Wilde 1975), while oxygen consumption increases at higher temperatures (Spärck 1936, de Wilde 1975). Since smaller *Macoma* have a greater oxygen consumption per unit weight and thus a higher metabolic rate than larger *Macoma* (de Wilde 1975), the decrease of energy reserves is more marked in them. The condition histogram of different-sized *Macoma* could be different in mid-summer at the time of best condition. The high condition factor of smaller *Macoma* in the laboratory may partly owe to favourable sizes of nutritional particles flourished in the aquarium. Differences existing between wild individuals and those kept in the laboratory may also result from siphon cropping by predators in the sea. Regeneration calls for excess energy, at least where larger amounts of the siphon are removed or siphons are repeatedly preyed on and food is limited (c.f. Trevallion 1971, Hodgson 1982). It is not known why small *Macoma* should be more susceptible to this.

Comparing experiment I and the three-month experiment III, which were made in different years, it can be seen that the decreases in condition indices were smaller in experiment I (1982). In the three-month experiment III (1981) the bivalves were living on bare bottom in stagnant water, and the time the experiment ran was a little longer than in experiment I. Bivalves which are not allowed to burrow may become "stressed" (Wikander 1981).

The effects on the condition of *M. balthica* arising from keeping the animals in the laboratory thus depend on the initial condition of the organisms, the season and the stage of their sexual cycle, the temperature compared to the temperature in the wild, and the nutritional conditions in the laboratory (c.f. experiments I, II and III and the seasonal analyses, and Pekkarinen 1984, in press).

The most striking feature concerning the biochemical composition of the soft part of *Macoma* is the higher level of lipids and contrastingly lower level of proteins compared to those in the *Macoma* of the Dutch Wadden Sea (Beukema & de Bruin 1977). Here the smallest mean lipid level (about 14 % of the dry weight, the correction for ash-free dry weight causing little change) is about the same as the highest mean in the Wadden Sea (nearly 15 % of ash-free dry

weight). The glycogen level is very similar to that of Dutch *Macoma*. The lipid and glycogen contents in *Macoma* in the Kiel Bight (southern Baltic Sea) (8–21 % and 11–22 % of ash-free dry weight, respectively, Graf et al. 1982) were somewhat smaller than at Tvärminne.

The major changes in the levels of glycogen, lipids and proteins here begin about one month later than in the Wadden Sea, the initial point here coinciding with early May. The maximal values for glycogen and lipids and protein minimums occur in June, as is the case with protein and glycogen culminations in Dutch *Macoma* (Beukema & de Bruin 1977). However, the lipid maximum in the Dutch *Macoma* is much earlier, occurring in May, then decreasing rapidly through May, June and July. The decrease here is much slower. In the Kiel Bight the lipid and glycogen levels show two peaks, one in early summer and the other in autumn, September–October (Graf et al. 1982). The increase there begins in March, just after the settling of the spring bloom. The coinciding of rapid storing of lipids and glycogen with settlement of green algae was also evident in Finnish *Macoma*.

The protein level of the gonad of the Pismo clam, *Tivela stultorum* is low during early gametogenesis (a little over 20 %, Giese et al. 1967). In gravid gonad the protein level increases. The course of the level of carbohydrates is opposite to this, they amount to 40–50 % during early gametogenesis and 25–35 % in mature gonad. Glycogen accumulation in May–June does not take place in the gonad tissue of *Macoma* because the gonad nearly disappears at that time. In *Tivela* the gonad is large even when gametes are absent and it perhaps contains much accessory tissue rich in nutrients (Giese et al. 1967). The decrease in the protein level in *Macoma* in May may be partly due to losses at spawning and partly due to special accumulation of glycogen and lipids causing the protein level to remain lower.

When looking over the results of the six-month experiment (experiment III) it must be taken into account that the bivalves missed the rapid accumulation period of glycogen and lipids which occurred in the wild state in early summer. Under extreme stress glycogen is almost completely utilised in the tissues of *Macoma* but lipid levels do not fall so markedly. The even higher lipid level in the clams of the first six-month experiment than in wild *Macoma* may be partly explained by their smaller body sizes. In late summer 1983 smaller *Macoma* (9–11 mm) showed higher lipid levels than medium-sized (13–15 mm) and larger (17–19 mm) *Macoma* (unpublished results by the author). Although the "fatness" and condition of *Macoma* are low in spring, the protein levels and even lipid levels of the molluscs are high. Thus, despite their decreasing soft body size they are nutritionally valuable for predators.

According to preliminary histochemical examinations, *Macoma balthica* accumulates lipids in vast amounts in its digestive gland. Part of this reserve fat may be channelled into the growing oocytes. The digestive gland of *Tivela stultorum* contains about 10–20 % (of dry weight) lipids while other tissues, apart from the gills and mature ovarium (10 %) are poor in lipids (Giese et al. 1967). In *Mytilus edulis*, too, the digestive gland serves as a storage organ and it also regulates the distribution of nutrients to the body tissues. (R. J. Thompson, according to Gabbott & Bayne 1973). Seasonal histochemical studies on glycogen and lipids in the tissues of *M. balthica* are in progress.

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