

## Factors affecting the burrowing activity of *Macoma balthica* (L.)<sup>1</sup>

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The dependence of the burrowing activity of *Macoma balthica* (L.) on size, water temperature, bottom quality, salinity, "starvation" and exposure to low cadmium concentrations were tested under laboratory conditions. In response to adverse stimuli, small specimens burrowed more rapidly than large ones. Burrowing was more rapid at 16°C than at 4°C. Keeping *Macoma* on a hard bottom significantly retarded subsequent burrowing activity on a soft sand bottom. Storage without food at 14°C for 3 months did not affect the rate of burrowing of large specimens but slightly reduced that of small ones. Preliminary tests with cadmium showed that its effects were dependent on the concentration used and the length of exposure. In individuals exposed for 24 h, cadmium at 2 and 5 ppm completely inhibited all burrowing activity, and at 1 ppm diminished it clearly. Exposure to 0.5 ppm Cd caused first an increase in burrowing activity, and then a decrease.

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### 1. Introduction

The biology of *Macoma balthica* (L.), including its abundance, size, age, spatial distribution, growth, reproduction, feeding, respiration, and general chemical composition, has been described by several authors (see e.g. SEGERSTRÅLE 1960, 1962, 1965; DE WILDE 1973, 1975; BEUKEMA 1973, BEUKEMA *et al.* 1977). Some work has also been done on the behaviour of *Macoma*, especially on crawling and feeding (BRAFIELD & NEWELL 1961, BRAFIELD 1963, SWENNEN 1969, HULSCHER 1973). Little attention, however, has been paid to the factors that influence burrowing in this species (BREUM 1970).

Our aim in studying the burrowing behaviour of *Macoma* was to find out whether this behavioural reaction could conveniently be used as a biotest in controlled experimental conditions. The short-term ("slug dose") effects of low concentrations of cadmium are presented here as examples. Comparative experiments, using the routine described here, are now in progress with other heavy metals.

### 2. Material and methods

The specimens used were collected in June—August 1977 from fixed sampling stations, one just outside Tvärminne Zoological Station and two from Pojo Bay (Station IV and Station Bå). The animals were collected from depths of 4—7 m (no tidal effect) and from soft mud bottoms, usually with a van Veen sampler. The salinity was measured at each sampling site, and the specimens were stored and tested at this salinity (3—7 ‰). They were kept in the laboratory in high-quality plastic aquaria (25×20×12 cm), with a ca. 4-cm layer of sieved (1 mm sieve) and thoroughly washed dune sand on the bottom, and a ca. 5-cm layer of natural, aerated, brackish water upon it. Aquaria temperatures were 8° C and 14° C. Specimens were tested in identical aquaria. To avoid any effect of crowding, not more than 20 animals were tested in each aquarium. Only animals which had burrowed into the sand in the storage aquaria were used in the tests. Tests were performed in the morning, as preliminary tests suggested a diurnal rhythm in burrowing activity (cf. DE WILDE 1975). The test was started by placing the animals on the sand and counting the number that had burrowed down at successive time intervals. The effect of size was tested with two size groups, small (shell length 6—12 mm) and large (15—22 mm) specimens. The effect of temperature was studied by testing both size groups at temperatures ranging from 4° C to 16° C. All animals were acclimatized to temperature changes of 5° C for 4—5 days before the tests. The effect of bottom quality was tested by letting specimens stay on a bare (plastic) bottom for 2—3 days, and then testing the animals on sand. When these animals had been in the test aquaria for 2—3 days, they were

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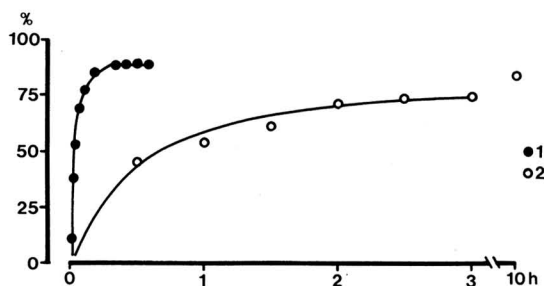


Fig. 1. The burrowing activity of (1) small ( $n = 100$ ), and (2) large ( $n = 100$ ) *Macoma* from Tvärminne ( $8^{\circ}\text{C}$ ,  $6\text{‰}$  sal.). Percentage of animals burrowed plotted against time.

dug up from the sand and tested again. The effects of maintenance without food were tested in a group of animals kept for several months in the laboratory at  $14^{\circ}\text{C}$  and  $6\text{‰}$  salinity. Tests were performed monthly. The variation burrowing activity within a group of animals of the same size class taken from the same sampling station at the same time was also determined. Cadmium was chosen as a model for studying the extent to which burrowing behaviour was affected by a heavy metal pollutant. Small *Macoma* were exposed to 0.5, 1.0, 2.0 and 5.0 ppm concentrations of Cd ( $3\text{ CdSO}_4 \times 8\text{ H}_2\text{O}$  pa) for 24 h at  $14^{\circ}\text{C}$  and  $6\text{‰}$  salinity. The tests were made in aquaria without sand to minimize the absorption of Cd from the medium (cf. SAWARD *et al.* 1975). After 24-h exposure the animals were tested and then left in the clean test aquaria and their survival noted for 15 days. In addition to the 24-h exposure tests, small animals were exposed to 0.5 ppm Cd for 72 h and tested for 30 min every 24 h. Animals sampled at the same time from the same sampling sites as the exposed animals served as controls.

### 3. Results

Small specimens were more active than large ones. Fig. 1 shows the results of tests on small and large specimens from the same sampling site and tested in identical experimental conditions. In the first 30 min 90 % of the small specimens burrowed down but only 45 % of the large ones. In small animals burrowing activity was highest during the first 15 min, and in large animals during the first 150 min (Fig. 1). Repeated control experiments (with small specimens) showed that animals collected at the same sampling station at the same time and kept and tested in the laboratory under the same conditions gave activity curves that were reproducible within limits of  $\pm 5\%$ .

Both large and small specimens sampled just outside the Tvärminne Zoological Station

(salinity about  $6\text{‰}$ ) burrowed down into the sand quicker than animals collected from the two other sampling sites (sal. about  $3\text{‰}$ ), see Fig. 2. This difference was apparent at all temperatures.

Burrowing activity was in general higher at  $16^{\circ}\text{C}$  than at  $4^{\circ}\text{C}$ . For example, 73 % of the small specimens (from Station IV) tested at  $16^{\circ}\text{C}$  burrowed down during the first 15 min. When they were tested at  $4^{\circ}\text{C}$  the figure was only 40 %. For the large specimens the respective values were 68 % and 35 % in 6.5 h.

Keeping *Macoma* on a bare (hard) bottom for 2–3 days retarded burrowing activity. This effect was clear at all temperatures tested (Fig. 3).

A group of small and large specimens collected in June were kept in the laboratory

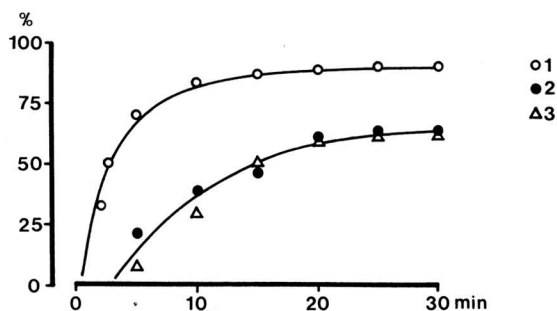


Fig. 2. The burrowing activity of small *Macoma* from (1) Tvärminne ( $n = 100$ ,  $8^{\circ}\text{C}$ ,  $6\text{‰}$  sal.), (2) Station IV ( $n = 100$ ,  $8^{\circ}\text{C}$ ,  $3\text{‰}$  sal.) and (6) Station Bå ( $n = 100$ ,  $8^{\circ}\text{C}$ ,  $3\text{‰}$  sal.). Percentage of animals burrowed plotted against time.

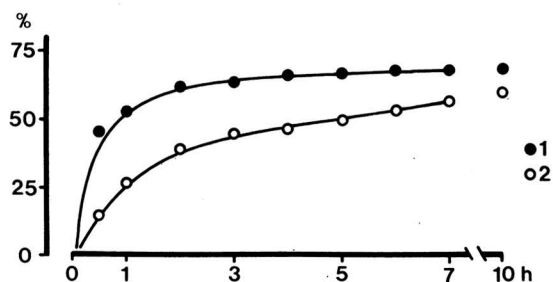


Fig. 3. The burrowing activity of large *Macoma* after 2–3 days on (1) a soft bottom and on (2) a hard bottom ( $n = 400$ ,  $4\text{--}16^{\circ}\text{C}$ ,  $3\text{--}7\text{‰}$  sal.). Percentage of animals burrowed plotted against time.

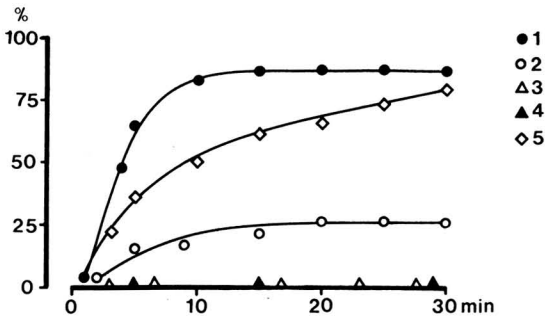


Fig. 4. The burrowing activity of small *Macoma* after 24-h exposure to (1) 0.5, (2) 1.0, (3) 2.0, and (4) 5.0 ppm Cd (5 = control, 14° C, 6 ‰ sal.). Percentage of animals burrowed plotted against time.

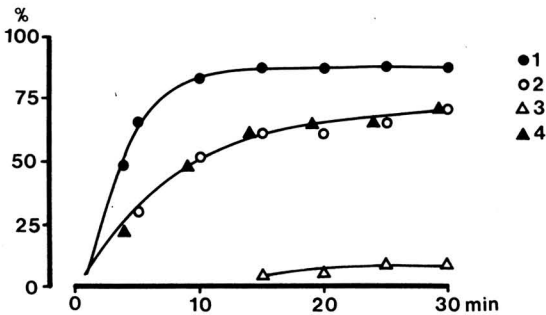


Fig. 5. The burrowing activity of small *Macoma* after (1) 24, (2) 48, and (3) 72 h exposure to 0.5 ppm Cd (4 = control, 14° C, 6 ‰ sal.). Percentage of animals burrowed plotted against time.

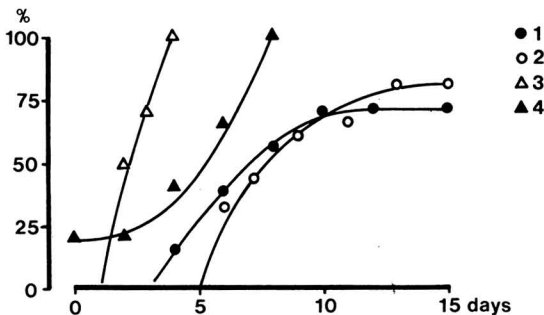


Fig. 6. Cumulative death rate of small *Macoma* in natural sea water after exposure to Cd. 1 = 0.5 ppm for 72 h, 2 = 1.0 ppm for 21 h, 3 = 2 ppm for 24 h, 4 = 5.0 ppm for 24 h (14° C, 9 ‰ sal.). Percentage dead plotted against time.

without food at 14° C for several months. They were tested monthly. Maintenance under laboratory conditions without food ("starvation") did not appear to affect the burrowing behaviour of large specimens. When tested in

September they showed the same activity curve as 3 months earlier; 85 % had burrowed down in 3 h. The mortality was 3 % per month. "Starving" had some effect on small *Macoma*. In June and July 50 % of the animals had dug down in 2 min. In August 50 % had dug down in 5 min and in September in 7 min. In June and July 95 % and 100 % had dug down in 30 min, but the figures fell in August and September to only 75 % and 80 %, respectively. The mortality was 5 % per month. Exposure to cadmium had a clear effect on the burrowing behaviour of *Macoma*. After 24-h exposure to 2 and 5 ppm Cd, not a single animal burrowed into the sand, although all were alive (Fig. 4). Of the animals exposed to these concentrations 50 % were dead after 2 and 5 days, respectively (Fig. 6). Only 25 % of the animals exposed to 1 ppm for 24 h burrowed down in 30 min; 50 % of the animals were dead after 8 days (Fig. 6). 24-h exposure to 0.5 ppm Cd stimulated burrowing activity. After 48-h exposure the animals had about the same activity curve as controls, and after 72-h exposure only 10 % burrowed down (Fig. 5). When exposed to 0.5 ppm Cd for 72 h and then transferred to clean test aquaria, 50 % of the animals were dead after 7 days, but when exposed only for 24 h before transfer all animals were alive after 15 days.

#### 4. Discussion

The difference in burrowing activity between large and small animals is quite evident. The reasons for this difference are not clear, however. DE WILDE (1975) found that medium-sized *Macoma* (10–40 mg) consumed more oxygen than large ones (>40 mg). HULSCHER (1973) found a negative correlation between shell length and the depth to which *Macoma* burrowed into the substrate. He stated that large *Macoma* tend to come up on to the surface to obtain better oxygen conditions. BRAFIELD (1963) had observed the same thing in his research with *Macoma*. Slower burrowing activity may be associated with the lower oxygen consumption and probably lower metabolic rate. The fact that large animals have to perform more mechanical work when burrowing down might also come into the picture. The difference in burrowing activity between animals from Tvärminne and from

Pojo Bay may have been partly due to the difference in salinity. However, REMANE & SCHLIEPER (1971) report that *Mytilus edulis* has similar respiration rates in salinities ranging from brackish to fully marine. Thus other factors are evidently involved.

Temperature had a clear effect on burrowing activity. The animals were more active at 16° C than at 4° C. DE WILDE (1975) found that food uptake decreased at temperatures above 10° C and at high temperatures food uptake became very low. Those apparently contradictory observations fit KINNE's (1963) statement that: "Various functions of an organism, such as locomotion, growth and reproduction, may have somewhat different temperature ranges".

Diminished burrowing activity was noted when specimens had been kept on a bare (hard) bottom for 2—3 days. Unsuccessful efforts to burrow on a hard bottom clearly affected the animals, for when they were transferred to a soft bottom their burrowing was retarded. Whether this was a result of adaptation or due to fatigue remains to be shown.

Keeping *Macoma* for 3 months under laboratory conditions without food seemed to affect only the small animals, which presumably have a higher metabolic rate than large ones, so that "starving" affects them sooner. HULSCHER (1973) reports that even when infected with trematodes *Macoma* could be kept alive in the laboratory for over a year; whether the animals were fed or not was not mentioned. In this respect, *Macoma* meets one of the criteria for

a useful "world" species for laboratory research.

Although at higher concentrations or on longer exposure cadmium retards the activity of organisms, at low concentrations or on short-term exposure it seems to stimulate activity. This "stimulating effect" of Cd has been demonstrated even at cellular level by BERLAND *et al.* (1977), using the diatom *Skeletonema costatum* as a test organism. The death rate after exposure to Cd seems to be positively correlated with concentration and time of exposure. However, the time taken for 50 % of the animals to die after 24-h exposure to 5 ppm Cd was 5 days, but after 24-h exposure to 2 ppm Cd only 2 days. On exposing the brine shrimp *Artemia salina* to mercuric chloride, BROWN & ASHANULLAH (1971) similarly found an increase in toxicity with decreasing concentrations. This phenomenon remains unexplained.

In conclusion, the burrowing behaviour of *Macoma balthica* seems to be very sensitive to stress and to stimulating factors. The burrowing behaviour can be used as a biotest for sublethal concentrations of heavy metals and presumably for other stress factors. It is important, however, that the animals used in a biotest should all be taken from the same locality, sampled at the same time and kept under identical laboratory conditions.

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