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Influence of an anionic detergent (alkylbenzene sulphonate) on enzymes, moulting cycle and survival in the shrimp *Crangon crangon* L.

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Abstract

Shrimps *Crangon crangon* L. were exposed to 5, 7.5, 10, 25 and 50 ppm of the anionic detergent alkylbenzene sulphonate (ABS) added to brackish water (7 $^0/_{00}$ salinity). The animals were incubated in these solutions from one to nine days at 15° C or 20° C. After 24 h and 108 h of incubation the activities of arylsulphatase (E.C. 3.1.6.1), acid phosphatase (E.C. 3.1.3.2) and cathepsin D (E.C. 3.4.4.23) were assayed in homogenates of the hepatopancreas. The influence of the detergent ABS on the moulting cycle of the shrimps was also investigated. The activity of all acid hydrolases assayed descreased by 20 % to 50 % in the experimental shrimps, depending on concentration of the pollutant, as compared with the control group. The moulting cycle of the shrimps exposed to the action of the detergent was shortened from 31 % to 51 %, and the body weight during one moulting cycle was reduced by 24 % to 36 %. Survival times were reduced in the animals exposed to the action of the detergent. Total mortality of the shrimps occurred after 194 h, in 5 ppm of ABS at 15° C and after 108 h in 50 ppm. The 96 h LC_{50} for shrimp *Crangon crangon* L. under laboratory conditions was estimated as 27 ppm of alkylbenzene sulphonate.

Introduction

Detergents are increasingly finding wider application as washing and cleaning agents in household, industry, mining and shipping. In addition to heavy metals, pesticides, crude oil and its derivatives, detergents are the main source of marine coastal ecosystem pollution. In the waters of the Dead Vistula and the Bay of Gdańsk, the concentration of nonionic detergents was 1.7 to 2.6 ppm in 1973 (DREWA et al. 1975). At a time of increasing pollution of the seas with detergents, studies on survival and on pathophysiology of marine animals are gaining importance. The purpose of the present study was:

1. evaluation of the toxicity of alkylbenzene sulphonate to shrimp *Crangon crangon* under laboratory conditions,
2. elucidation, whether this detergent adversely affects the moulting cycle of the shrimp,
3. determination of the activity of three lysosomal acid hydrolases in the hepatopancreas of shrimps exposed to ABS detergent.

Material und methods

The experiments were carried out under laboratory conditions. They comprised toxicological, morphological and enzymatic studies with *Crangon crangon* after exposure to different concentrations of the anionic detergent alkylbenzene sulphonate (ABS).

Shrimps were collected during the summer months in coastal waters of Gdańsk Bay (7 $^0/_{00}$ salinity, 12–15° C). Groups of 30 shrimps were acclimated in plastic containers (26 x 20 x

30 cm; 10 l of brackish water, depth of water 20 cm). Mean body weight ranged from 300 to 400 mg, and body length varied from 25 to 40 mm. The animals were fed with chopped shrimps. A stock solution of 1 g of ABS/l was prepared from alkylbenzene sulphonate obtained from the "Pollena" plant in Gdańsk, which contained 80 % of pure ABS. To 10 l of brackish water ($7^0/_{00}$ salinity) in plastic containers, the proper amounts of stock solution were added to obtain final concentrations of 5, 7.5, 10, 25 and 50 ppm of ABS detergent (5, 7.5, 10, 25 and 50 ml, respectively).

Determinations of shrimp mortality at each detergent concentration, with and without aeration, at 15° C and 20° C were run in triplicate. Experiments were run indoors during summer months under natural light conditions. Dead animals were counted and removed from the tanks every 4 hours round the clock. The 96 h LC_{50} was read from the regression lines with the use of the graphic-logarithmic-probit method (BOJANOWSKA 1961, MILEWSKA 1964).

The solutions of the detergent, as well as the bottom sand in the tanks, were exchanged once daily.

For the enzymatic assay, the shrimps were caught at random and placed in Petri dishes on a layer of lignin to dry off the liquid. Freshly dissected hepatopancreas was homogenized with redistilled water in the proportion 1:9 (w/v) in a glass Potter homogenizer. Cell debris and nuclei were removed from the crude homogenate by centrifugation at 1000g for 10 min., and the supernatant was used for the enzyme activity determinations.

The effect of the detergent on the duration of the moulting cycle and on the increase in body weight per moulting cycle was extrapolated from phase A to phase D_{3-4}, i.e. from the phase of new carapace formation to the phase of discarding the old carapace (DREWA et al. 1983, KAMIGUCHI 1968).

Arylsulphatase activity was assayed by ROY's (1958) method, as modified by BŁESZYNSKI and DZIAŁOSZYŃSKI (1965). 0.1 ml of the fresh supernatant were added to 1.0 ml of 12 mM of 4-nitrocatechol sulfate K_2-salt (Serva) in phosphate buffer, pH 5.6. The mixture was incubated for 10 min at 37° C in a water bath. The extinction was read at 510 nm with a Specol spectrophotometer (Zeiss, Jena). The enzyme activity was expressed as the μM of 4-nitrocatechole liberated per 1 mg of protein.

The activity of acid phospatase was measured by the method of BESSEY et al. (1964), as modified by KRAWCZYŃSKI and OSIŃSKI (1967).

The activity of cathepsin D was determined according to the Anson method (COLLOWICK and KAPLAN 1955).

Protein content in the homogenates was assayed after LOWRY et al. (1951).

For the statistical evaluation of the differences between the experimental and control groups, Student's *t* test was used. Results at a probability level of 0.001 were accepted as highly significant, and those at the level of 0.01–0.025 as significant (DIEM and LENTNER 1977).

Results

Mortality

Since there were no differences in mortality and lysosomal enzyme activity between females and males, the mean results were calculated jointly for both sexes.

Shrimps exposed to 5 ppm and 50 ppm of the detergent at 15° C with aeration survived for 194 h and 108 h, respectively, at the most (Fig. 1). Temperature elevation by 5° C

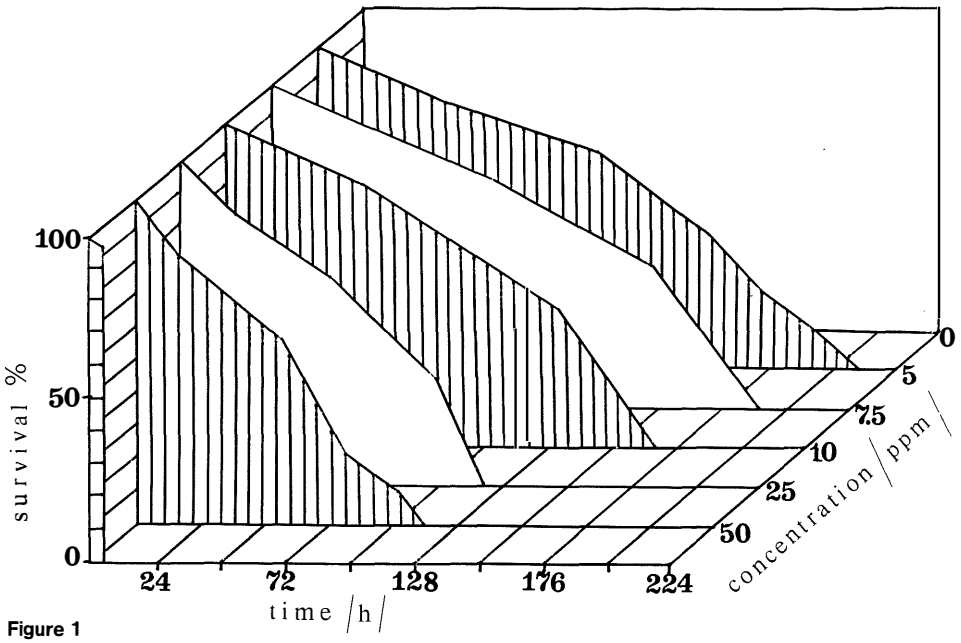

Figure 1

Mortality of *Crangon crangon* exposed to the detergent ABS at 15° C with aeration (60 l of air/10 l of solution/h). Total initial number of shrimps at day 0 was 90. Mortality curves have been calculated from three replicates of 30 shrimps each. Z-axis is not to scale. 0 = controls without detergent

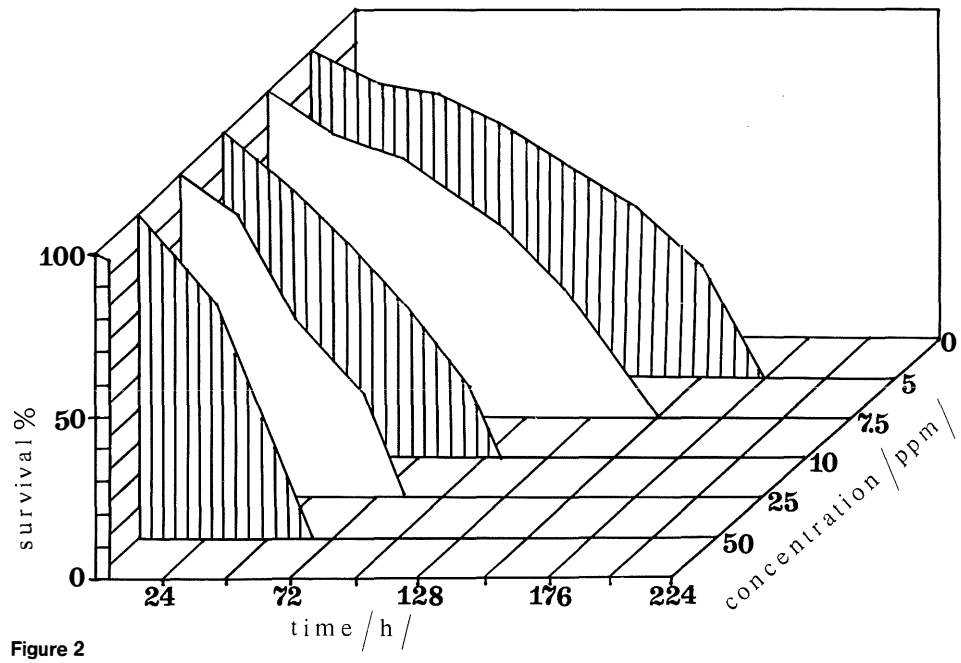

Figure 2

Mortality of *Crangon crangon* exposed to the detergent ABS at 20° C with aeration (60 L of air/10 L of solution/h).

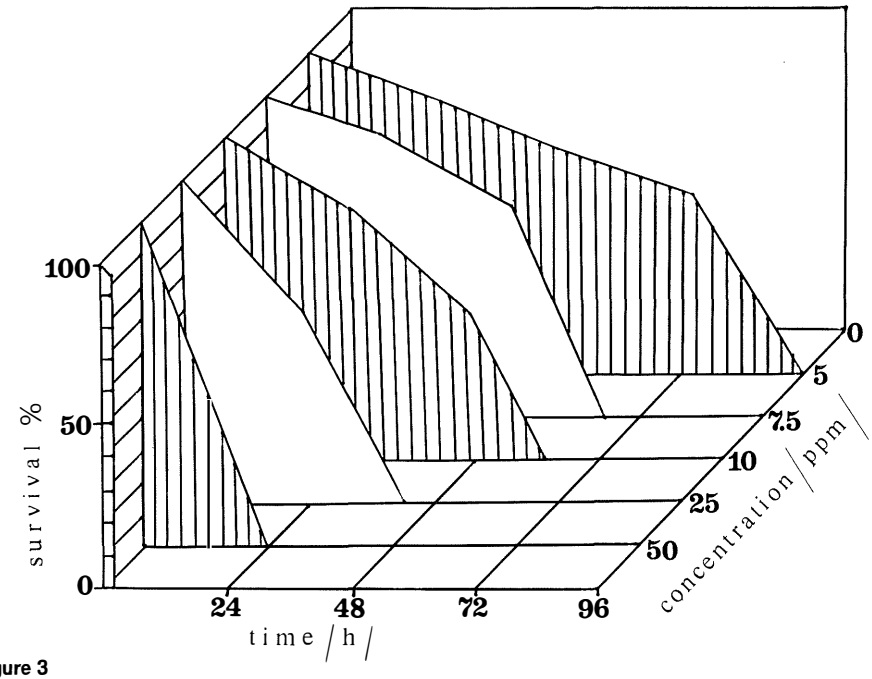

Figure 3

Mortality of *Crangon crangon* exposed to the detergent ABS at 15° C without aeration.

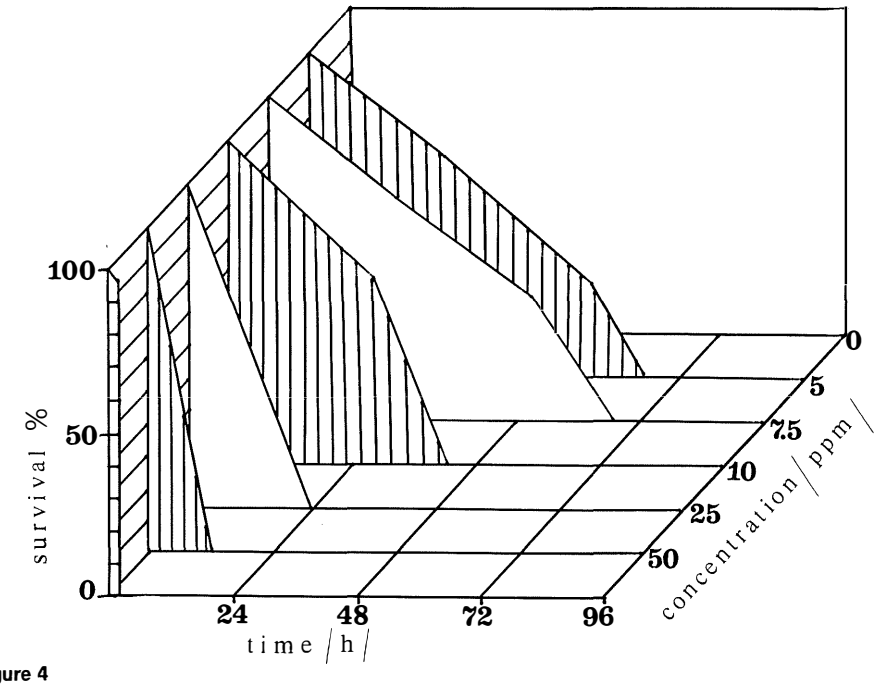

Figure 4

Mortality of *Crangon crangon* exposed to the detergent ABS at 20° C without aeration.

accelerated the death rate. At 20° C and concentrations of 5 ppm and 50 ppm with aeration, total mortality occurred after 168 and 64 h, respectively (Fig. 2).

All shrimps died after 12 h of exposure to 50 ppm and after 68 h at 5 ppm of the detergent at 20° C without aeration (Fig. 3, 4). All differences between the groups exposed to the detergent and the controls were highly significant ($p < 0.001$).

The 96 h LC_{50} of the ABS detergent for *Crangon crangon* at 15° C amounted to 27 ppm.

Effects on moulting cycle and growth

In controls at 15° C, the moulting cycle from phase A to phase D_{3-4} lasted 487 h, and the mean increase in body weight in one moulting cycle was 63 mg (Table 1).

Table 1

	Average moulting cycle and weight increase			
	Controls	ABS concentrations (ppm)		
		5	10	50
Duration of the moulting cycle (h) Mean ± S.D.	487 ± 45	475 ± 43	456 ± 40	434 ± 39
N	15	15	14	12
Increase of body weight during one cycle (mg) Mean ± S.D.	63 ± 9	59 ± 8	49 ± 9	40 ± 8
N	15	15	14	12

At a concentration of 5 ppm, the detergent exerted no significant effect on the duration of the moulting cycle and on the increase in body weight. At concentrations of 10 ppm and 50 ppm, the moulting cycle was shortened by 31 h and 53 h, respectively, and the increase in body weight was reduced by 24 % and 36 %, respectively ($p < 0.001$).

Effects on lysosomal enzymes activity

The activity of arylsuphatase in hepatopancreas after exposure of the shrimps to the detergent at 15° C with aeration is shown in Table 2.

Table 2

	Arylsulphatase activity (μM 4-nitrocatechole/mg protein)		
Period of exposure (hours)	Controls	Detergent ABS concentrations (ppm)	
		5	50
24	54.3 ± 6.0	49.7 ± 7.0	41.0 ± 5.0*)
108	56.2 ± 8.0	43.5 ± 4.0*)	38,2 ± 6,0*)

*) $p < 0.005$

After 24 h exposure to the concentrations of 5 ppm and 50 ppm of ABS detergent, the arylsulphatase activity decreased by 8.4 % and 24.5 %, respectively, and after 108 h exposure, enzyme activity was reduced by 23.0 % and 32.0 %, respectively.

Acid phosphatase activity in the homogenates of hepatopancreas of the shrimps exposed to the ABS detergent at 15° C with aeration is shown in Table 3.

Table 3

Acid phosphatase activity (μM p-nitrophenole/mg protein)

Period of exposure (hours)	Controls	Detergent ABS concentrations (ppm)	
		5	50
24	15.5 ± 1.7	14.0 ± 2.1	13.4 ± 1.9*)
108	16.4 ± 1.5	12.7 ± 1.6*)	11.1 ± 2.9*)

*) $0.01 < p < 0.025$

After 108 h exposure to the ABS concentration of 5 ppm and 50 ppm, the acid phosphatase activity dropped by 22.6 % and 32.3 %, and by 9.7 % and 13.5 %, respectively, after 24 h exposure. With the exception of the result obtained after 24 h exposure to the 5 ppm concentration, all remaining results differed significantly from those for the control group.

Cathepsin D activity in the homogenates of hepatopancreas of the shrimps exposed to the detergent ABS, at 15° C with aeration is shown in Table 4.

Table 4

Cathepsin D activity (μM tyrosine/mg protein)

Period of exposure (hours)	Controls	Detergent ABS concentrations (ppm)	
		5	50
24	26.4 ± 3.6	31.3 ± 3.5*)	34.0 ± 4.8**)
108	24.5 ± 4.1	20.9 ± 2.7	28.1 ± 3.5

**) $p < 0.001$ *) $0.01 < p < 0.025$

After 24 h exposure to ABS concentrations of 5 ppm and 50 ppm, acid phosphatase activity increased by 18.6 % and 28.8 %, respectively. After 108 h exposure, the activity at 5 ppm decreased by 12.2 %, and increased by 12.2 % at 50 ppm.

Discussion

Detergents penetrate into aquatic animals through the respiratory epithelium, alimentary system and integument. Although the integument exerts an isolating effect in crustaceans (GOLDRACE 1968, THANG et al. 1980), it is obvious that the thick carapace of a crab protects the body more effectively from detergents than the carapace of shrimps or krill (DREWA et al. 1977, DREWA 1981). Detergents bind to biological membranes, thus leading to impairment of their function and damage. These mechanisms have benn described by RAZIN (1972), ANASTASIU and JELESCU (1973), ZUBRZYCKA (1975), HELENIUS and

SIMMONS (1975), and ZASLAVSKY et al. (1978).The detergent-induced lability of biological membranes causes, among other things, changes in the ion composition of shrimp serum (ZBYTNIEWSKI et al. 1978).

Detergents induce changes in the permeability of cell membranes, as well as in membranes of mitochondria and lysosomes. According to the present results, lysosomes damaged by detergents, release acid hydrolases into the cytoplasm. Moreover, there is an evident relationship between the detergent concentration in the water and the activity of some lysosomal enzymes in the hepatopancreas of the shrimps. The higher the ABS concentration, the greater the difference in enzyme activity. The activities of arylsulphatase and acid phosphatase in hepatopancreas of the exposed shrimps are lowered, whereas the cathepsin D activity increased.

A similar phenomenon has been observed in krill *Euphausia superba* Dana (DREWA et al. 1981). After 1 h exposure to the detergent ABS, the activity of arylsulphatase increased, and then dropped considerably with longer exposure. The first phase of detergent action is characterized by mobilization of the lysosomal system and by increase in the activity of lysosomal enzymes, probably a defense mechanism leading to phagocytosis of destroyed cell structures. However, the increase in cell vacuolation and in the activity of lysosomal hydrolases ultimately leads to cell autolysis. The vacuoles observed were lysosomes. The high degree of hepatopancreas vacuolation after exposure to the detergent ABS confirms the increase in the number of lysosomes. The subsequent decrease in enzymatic activity is related to inactivation of enzymes by the detergent (DREWA et al. 1978). In the damaged cells, the detergent inhibits *de novo* synthesis of enzymes. At concentrations of 5 ppm and 50 ppm, ABS reduced the arylsulphatase activity by 12.7 and 18.0 μM, respectively (Table 2). The ABS detergent shortened the moulting cycle of the shrimps and reduced the increase in body weight (Table 1). At lowest concentration (5 ppm), the detergent shortened the moulting cycle by 12 h and reduced the increase in body weight by 6 %. The respective values for the 50 ppm concentration are 53 h and 36 %. The present results obtained under laboratory conditions are consistent with the observations of COWELL (1971) taken *in situ*. In polluted marine ecosystems, the increase in body weight of *Patella vulgata* is reduced by 30 %, as compared with pure waters. EDWARDS (1978) has reported a lowered increase in body weight, and moulting cycle disturbance in *Crangon crangon* larvae exposed to aromatic hydrocarbons. CZYŻEWSKA (1976) has observed higher mortality and slower development of the larvae of the crab *Rhithropanopeus harrisi* in a mixture of anionic and non-ionic detergents.

Cellular damage and inhibition of the mitotic cycle in the damaged tissues lead to the death of animals. The mortality of the shrimps exposed to the ABS detergent concentration of 50 ppm at 15° C with aeration is 100 % after 108 h of incubation.

COWELL (1971) observed high mortality of *Patella vulgata, Balanus balanoides* and *Littorina littorea* living in detergent-contaminated ecosystems. Also the mortality of *Arenicola marina* populations living near the coast of England has been found to be great after a single application of the detergent BP 1002.

Appropriate oxygenation and optimal water temperature are important factors lowering the mortality of *Crangon crangon* in water contaminated with the detergent ABS. According to the present results, the toxicity of the detergent increases with increasing temperature, and with decreasing oxygen content of the water. The detergent is less soluble at lower temperatures, the biochemical reactions are slower, animal motility is reduced, and therefore the survival of the shrimps exposed is longer. For krill *Euphausia superba*, 48 h LC_{50} of the detergent ABS is 200 ppm at 15° C (DREWA 1981), whereas 96 h LC_{50} for *Crangon crangon* at 15° C amounts to 27 ppm.

In summary: The detergent ABS induces an increase in the number of lysosomes, brings about changes in the activity of lysosomal enzymes and causes lability of cell and cell organelle membranes. ABS also disturbes the moulting cycle of *Crangon crangon* shortening it and reducing the increase in body weight of the shrimps in the cycle.

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Topics:
- Problems of aut- and synecology with special reference to the phenomenon of competition
- Food-web
- Experimental ecology

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Topics:
- Ecology of the Baltic coastal waters
- Morphological and physiological adaptations of organism to the brackish water environment

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Helsinki/Helsingfors, Finland, June 11–17, 1973, Institute of Marine Research, University of Helsinki

Topics:
- Production, food webs, and ecological models of the Baltic
- Indicator organisms/communities of the different environments in the Baltic

Merentutkimuslait. Julk./Havsforskningsinst. Skr. No. **239**, (1975), 355 pp. Editor: Å. Niemi.

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Topic:
- Structure, function and biological production of the Baltic ecosystem

Wydawnictwo Morskiego Inst. Rybackiego, Gdynia (1987), 327 pp. Editors: K. Siudzinski, L. Ludwig.

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Topics:
- Biological Features of the Baltic
- Structure, dynamics and biogeographical aspects of the Baltic ecosystems and the causes of their variability
- Physio-ecological aspects of conditions of life in the Baltic

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6th Symposium of the Baltic Marine Biologists

Århus, Denmark, August 20–26, 1979, Botanical Institute, Institute of Ecology and Genetics, University of Århus,
University of Copenhagen
Topic: – Relationship and exchange between the pelagic and the benthic biota

Ophelia, Suppl. **1** (1980), 304 pp. Editors: A. Møller Christensen, K. Muus.

7th Symposium of the Baltic Marine Biologists

Rostock, German Democratic Republic, August 31 – September 4, 1981, Sektion Biologie der Universität Rostock
Topics: – Proceedings in analysis of the ecosystem Baltic Sea
– Autecology and ecophysiology of brackish water organisms including pollution problems

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Lund, Sweden, August 10–14, 1983, Institute for Marine Ecology, University of Lund
Topic: – Recent changes in the Baltic ecosystem

Ophelia, Suppl. **3** (1984), 263 pp. Editors for the Org. Commitee of the Symposium: T. v. Wachenfeld, L. Edler, L.-E. Persson, Editor of Ophelia: K. Muus.

9th Symposium of the Baltic Marine Biologists

Turku/Åbo, Finland, June 11–15, 1985, Department of Biology, Åbo Akademi,
Department of Biology, University of Turku,
Archipelago Reseach Institute, University of Turku
Topics: – Ecology of coastal waters
– Interactions between and within species

Ophelia, Suppl. **4** (1986), 343 pp. Editor: K. Muus.

10th Symposium of the Baltic Marine Biologists

Kiel, Federal Republic of Germany, September 29 – October 3, 1987, Institut für Meereskunde an der Universität Kiel
Topics: – History of marine biological sciences in the Baltic Sea area
– Problems of eutrophication of the Baltic Sea
– Recruitment
– Ecotoxicology

Kieler Meeresforsch., Sonderh. **6** (1988), pp. Editors: H. Theede & W. Schramm.