Ann. Zool. Fennici 17: 221-231. 1980

Changes in the swimming activity of Pontoporeia affinis (Crustacea, Amphipoda) after exposure to sublethal concentrations of phenol, 4-chlorophenol and styrene

Magnus Lindström & Astrid Lindström

Lindström, M. & Lindström, A. 1980: Changes in the swimming activity of Pontoporeia affinis (Crustacea, Amphipoda) after exposure to sublethal concentrations of phenol, 4-chlorophenol and styrene. — Ann. Zool. Fennici 17: 221—231.

Amphipods of the species *Pontoporeia affinis* Lindström were subjected to sublethal concentrations of phenol (1—30 ppm), 4-chlorophenol (1—10 ppm), and styrene (2.3—46 ppm) in controlled constant-flow or static laboratory conditions at 4°C and a 12:12 h light-dark regime for 10 days in experiments lasting about 40 days. Exposure to toxicants led to changes in swimming behaviour (recorded with the aid of IR photocells). Phenols caused the activity to decrease, although the higher concentrations (17, 18 and 30 ppm phenol and 5 and 10 ppm of 4-chlorophenol) brought about temporary activation for a few days. The decrease in activity showed a clear correlation with increasing concentrations of toxicants. Styrene in static tests at initial concentrations 2.3—23 ppm had an immediately stimulating effect upon swimming activity. By contrast 35 and 46 ppm caused an immediate cessation lasting for several days, after which activity rose to many times the normal level. During the recovery period, the animals exposed to styrene regained ± the normal level of swimming activity, except those exposed to 69 ppm, which all died within a few hours. Volatilization of styrene from the aquaria was high in all experiments.

M. Lindström & A. Lindström, Tvärminne Zoological Station, 10850 Tvärminne, Finland.

1. Introduction

Recently, increasing attention has been paid to the effects of sublethal concentrations of pollutants on marine organisms. In conventional experiments, the toxicity of pollutants is investigated at LD 50 and LC 50 — the lethal dose or concentration causing 50 % mortality usually in 24, 48 or 96 h. However, the sublethal effects may well be of even greater importance than the acute lethal action, although they were not very spectacular at the individual level. For a survey of possible effects of sublethal concentrations of pollutants see Rosenthal & Alderdice. Sublethal exposure to toxicants can cause a decline in the population without any obvious reason, by affecting such processes as ingestion of food, reproduction, development, growth and behaviour (see e.g. Arthur & Leonard 1970, Carlson 1971, McKim & Benoit 1971, Winner & Farrell 1976 and Laughlin et al. 1978). Concerning behaviour, Kleerekoper (1976) suggests that in fish locomotion and especially orientation behaviour might serve as a suitable model for testing the toxicological effects of pollutants. Any major change in the behaviour of a species is of great importance for the continued existence of the species itself and for those other species that are ecologically dependent on it. For instance, in tests in which brook trout were exposed to sublethal concentrations of lead, Holcombe et al. (1976) showed that at a certain concentration the females did not succeed in spawning because of scoliosis which limited their mobility and agility.

---

[1] Report No. 629 from Tvärminne Zoological Station, University of Helsinki, SF-10850 Tvärminne

P. affinis Lindström is an ecologically important brackish and freshwater amphipod which is also of economic significance for fishery in the Baltic Sea. The importance of the species is largely due to its abundance. On the south coast of Finland in the waters around Tvärminne Zoological Station, where this investigation was made, *P. affinis* occurs on the soft bottoms in numbers of 7 000—12 000 ind/m^2 (e.g. Keynäs & Keynäs 1978). Segerstråle (1933) found that *P. affinis* and the mollusc *Macoma baltica* together dominate the biomass on soft bottoms in the northern Baltic.

P. affinis serves as food for many fish species such as Baltic herring, whitefish, smelt, cod, flounder, fourhorn sculpin and eel-pout (Segerstråle 1937, 1959, Aneer 1975), and also indirectly for eel, perch, cod and flounder, because *Pontoporeia* is eaten by *Mesidotea entomon* (Crustacea, Isopoda), a species which in turn serves as food for these fishes (Segerstråle 1959). For smelt and herring *Pontoporeia* is important as food during the autumn and particularly in November—December, when it lives more pelagically when approaching sexual maturity (Segerstråle 1937, Aneer 1975).

P. affinis is a decidedly nocturnal swimmer, which in the daytime burrows into the bottom mud and at night swims pelagically (Segerstråle 1950, Marzolf 1965). Its burrowing activity is of great importance for the mixing and oxidation of the organic matter in the bottom substrate. Previous investigations of the swimming activity under laboratory conditions with various light/dark regimes have been made by Donner & Lindström (1980) and Lindström & Lindström (1980). They concluded that because of its regularity the swimming activity of *P. affinis* could well be used as a measure of sublethal toxicity. *P. affinis* is also very easy to keep in aquaria.

This investigation considers the effects of phenol, 4-chlorophenol and styrene on the swimming activity of *P. affinis*. The acute toxicity of phenol to marine organisms has earlier been fairly thoroughly investigated in short-term toxicity tests (for literature see Buikema et al. 1979). Toxicity tests have shown that the chlorinated phenols are in general more toxic than pure phenol. For four fish species, for instance, the 48-h TL_m (median tolerance limit) was 11—21 ppm *o*-chlorophenol and 24—50 ppm for pure phenol (Pickering & Henderson 1966). For *Mesidotea entomon* 96-h LC 50 was 40—60 ppm for 4-chlorophenol and 177—186 ppm for pure phenol (Oksama & Kristoffersson 1979). Little is known about how styrene affects the marine environment, although discharges occur. Toxicity tests have been made with terrestrial mammals (see Härkönen 1978 for literature) and with fish (Pickering & Henderson 1966), sea urchins (Pagano et al. 1978) and brine shrimps (Price et al. 1974).

The present study constitutes an attempt to measure and quantify the effects of sublethal concentrations of styrene and phenol and to define the approximate concentrations at which these substances begin to affect the normal, nocturnal swimming activity of *P. affinis* in laboratory conditions.

2. Material and methods

The method used in this investigation was based upon the one developed by Donner & Lindström (1980). The present paper is a direct continuation of Lindström & Lindström's (1980) investigations on the circadian rhythm and swimming activity of *P. affinis*. The method is thoroughly described in the above papers.

2.1. Apparatus and maintenance of test animals

The test animals were collected from Tvärminne Storfjärd at 30—35 m depth with a van Veen grab. The samples, consisting of animals and bottom mud, were then transferred to a 300-l storage tank containing sea water at the same temperature as the samples. After this, the temperature was gradually lowered or raised to +4°C, usually within 12 h. The animals were acclimated for at least 1 week before the experiments.

The swimming activity of *P. affinis* was recorded in 5.5-l continuous-flow aquaria (33 cm long, 6 cm wide and 35 cm deep). From the storage tank ca 0.4 l bottom mud was put into each aquarium to a height of ca 2.5 cm. The mud served the test animals as both a food source and a burrowing substrate (Smith 1972). A bottom substrate was also essential for the development of an activity rhythm under a light/dark regime in the aquaria. In tests without a bottom substrate *P. affinis* swam continuously with high activity even in the daytime (Lindström & Lindström 1980).

The flow through the aquaria was regulated by a double overflow system. The water was unfiltered natural brackish water, salinity 5—6 ‰ (for further details see Kristoffersson et al. 1972). The flow of water was adjusted to 12 ml/min.

The temperature in all experiments was kept at +4°C. In these experimental conditions, even without flow, *P. affinis* can be kept alive for months — up to 165 days with only 15—27 % mortality (Donner & Lindström 1980). The oxygen content in the aquaria was over 8 mg/l at the end of the experiments. This was more than satisfactory for the needs of *P. affinis*, which under natural conditions, e.g. in Storfjärden, Tvärminne, lives in an environment with more than 7.8 mg oxygen per litre (Luotamo & Luotamo 1975) and has been found living at

oxygen concentrations of 1 ml/l in the Baltic Sea (Hessle & Vallin 1934).

Each test aquarium contained 35 or 40 animals with a length of 6—10 mm. The aquaria were placed in four light-tight chambers, two or three aquaria in each, the capacity of the system being at most 12 parallel tests.

The light-dark cycle in the experimental chambers was 12/12 h. The light period lasted from 7.00 to 19.00. The light intensity was $2—3 \times 10^{-2}$ lux. This value is about 4—5 log units above the absolute visual sensitivity threshold of *P. affinis* (Donner 1971). The light intensities were measured with an Airam UVM-8 LX luxmeter, calibrated in absolute units.

2.2. Measurement of swimming activity

The swimming activity of *P. affinis* was recorded with the aid of infrared light emitters and phototransistors connected to automatic counters giving outprints once an hour. The emitters and corresponding transistors were mounted opposite to each other along the long sides of each aquarium, four sets to each aquarium, about 2.5 cm below the water surface level.

2.3. Introduction of toxicants

In the continuous-flow toxicity tests the toxic substance was introduced into the tube carrying the brackish water supply. The water inlet discharged ca 20 mm above the bottom substrate, its diameter at this point being 1.0 mm, which ensured thorough mixing. A test with dyes showed that this was achieved. The aquaria reached 90 % of the desired concentration within 15—20 h, as calculated from formulae given by Sprague (1969) and ascertained by analyses.

2.4. Toxicity tests

Some 2 weeks before the toxicant was introduced the activity was recorded under clean, continuous-flow conditions. Pronounced rhythmic activity usually developed before the fourth day. The following ca 10 days are referred to here as the control period (C). This was followed by the exposure period (T) with the toxicant, usually for 10 days. Introduction of the toxicants began in the afternoon. The following day was taken as the first day of the exposure period. The exposure period was succeeded by a recovery period (R) of at least 10 days (Fig. 1). After this the animals were collected and counted.

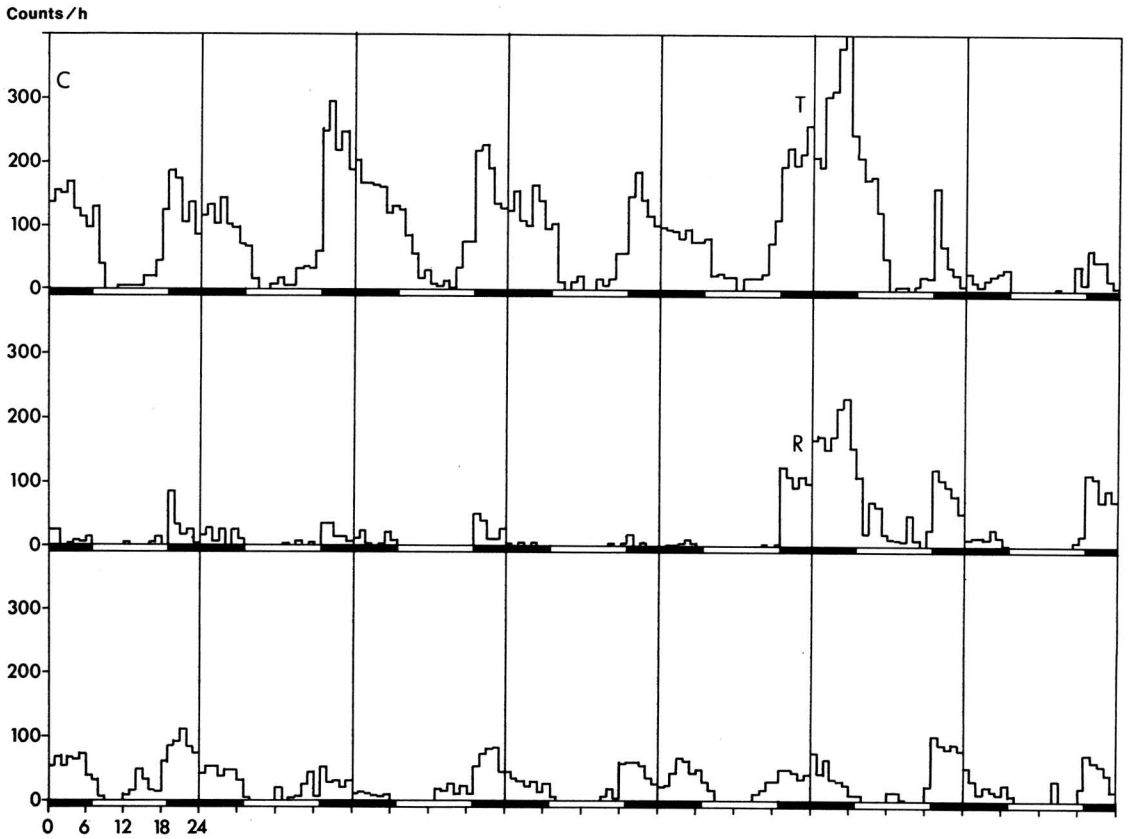

Fig. 1. Swimming activity of *P. affinis* during part of a test with 30 ppm phenol. C = control period, T = exposure period, R = recovery period. Activity is expressed as recordings/hour. Black bars = dark periods,

The swimming activity of *P. affinis* varies with the time of year (Lindström & Lindström 1980). Therefore the activity during T and R were always compared with the activity during the C period of the same experiment and expressed as a percentage of that activity. This made it possible to compare the results of experiments made at different times of year.

2.5. Tests with phenol and 4-chlorophenol

The stock solution was prepared from phenol (Merck p.a.) diluted with brackish water that had been filtered through double Whatman no. 4 filter papers. A peristaltic pump (Desaga) was used for the dosing apparatus. The phenol concentrations in the aquaria were analysed daily by the spectrophotometric method of Ochynski (1960).

2.6. Tests with styrene

During the C periods a continuous flow was maintained, but static conditions were used during the exposure period because of the low solubility of styrene. The styrene also volatilized from the aquaria; this was confirmed by analyses at different times of the test period. The stopping or starting of the flow had only a slight, temporary effect on activity.

The stock solution was prepared from monostyrene (Stymer Oy), and brackish water filtered in the same way as for phenol. The mixture, 1 ml styrene/1000 ml filtered brackish water, was shaken for 30 min in a shaking device (Desaga, 130 rev/min). After 48 h at +4°C the water phase was separated with a siphon and used as stock solution. Analyses showed that the stock solution contained 230 mg styrene/l. The gas-chromatographic styrene analyses were done at the Water Research Laboratory of the National Board of Waters, Helsinki.

Before the stock solutions were introduced a corresponding volume of water was drawn off from the aquaria. This water, 100 to 1550 ml, depending on the desired concentration, was then replaced with the solution of styrene at a flow rate of 2—5 ml/min in 0.5 to 5 h. The same peristaltic pump was used as in the tests with phenol.

3. Results

As a result of exposure to the toxicants the swimming activity of *P. affinis* either increased or decreased. The activity pattern with nocturnal swimming did not change in any experiment, even when the activity rose to many times the normal level.

3.1. Phenol

Test conditions

In the tests the animals were exposed to concentrations of phenol between 1 and 30 ppm. These concentrations were all sublethal. Although some animals died during the tests (see Table 1) mortality did not differ significantly from that of controls at the concentrations used.

In all aquaria with a phenol concentration of 10 mg/l or higher the concentration remained constant within narrow limits, but in the aquaria with 1—5 mg/l the concentration gradually fell during the test despite of the constant supply of test solution. In the tests with 1 ppm the phenol gradually began to decrease after ca 2 days until after 14 days it was ca 0.45 ppm. In the same manner 5 ppm decreased in 14 days to about 3.5 ppm. This decrease was probably due mainly to increased bacterial degradation (Kristoffersson et al. 1973, Buikema et al. 1979). When the exposure period ended and the flow was continued with clean brackish water any detectable amount of phenol was flushed away from all test aquaria within a few days.

Activity changes during the test period

The effect of exposure to phenol was either momentary stimulation of activity or inhibition from the very start (Fig. 1). At 1, 5, and 10 ppm the total activity per day diminished slowly throughout the exposure period. In the tests with 17 and 18 ppm activity increased considerably during the first few days of exposure, but then decreased far below the initial level.

At 30 ppm (Fig. 1) the activity, after half a day of slight activation, fell to almost zero and remained at this level for the rest of the exposure period of 7 days. This inactivity led us to suspect that 30 ppm had killed most of the animals, but the number of animals found alive after the experiment showed that this was not the case (Table 1).

Activity during the recovery period

During the recovery period the activity stabilized at different levels, though always lower

Table 1. Survival of *P. affinis* in phenol tests.

Phenol conc., ppm	0	1	5	10	17—18	30	30	30	30	30	30	30
Days of exposure		10	10	10	10	1	2	3	4	5	6	7
Surviving animals, %	94.7 ±7.3 (N=7)	100.0 97.5	82.9 100.0 100.0	100.0	87.5 95.0	91.4	85.7	85.7	91.4	97.1	94.3	94.3 97.1

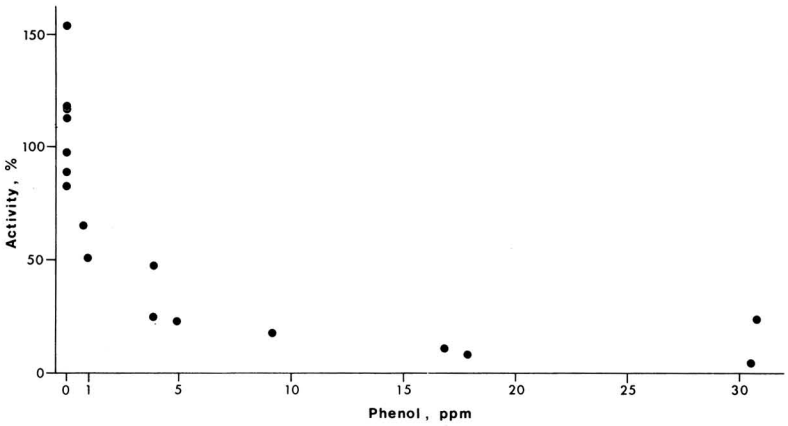

Fig. 2. Mean swimming activity of *P. affinis* during 10 days of recovery after exposure to various concentrations of phenol, expressed as a percentage of the mean diel activity during the control period. Preceding exposures lasted 10 days, except for 30 ppm, 7 days.

than that of the control period, depending on the concentration of phenol to which the animals had been exposed. A fairly good correlation was established between the decrease in activity and the phenol concentration. This is shown in Fig. 2, where for each concentration tested the respective activity during 10 days of R is expressed as a percentage of activity during 10 days of C.

Different exposure times

To investigate the effect of the exposure time on the activity of *P. affinis*, a series of tests was made in which the animals were exposed to 30 ppm phenol for 1 to 7 days. As the desired concentration was not attained until 15—20 h after the start of the test, the concentration in the 1-day exposure had only just reached 30 ppm when the recovery period began.

The immediate reaction of the animals was a slight stimulation on the first day of exposure, after which activity fell to almost zero and remained there for the whole exposure period, irrespective of its length. The difference between the treatments was evident during the recovery periods (Fig. 3). Even after exposure for 1 day the animals did not regain their former level of swimming activity when supplied with clean brackish water. They recovered to some extent, but the total activity level/day remained at about 50±30 % of the C level. The longer the period of exposure, the less complete was recovery. The decrease in activity in the control experiment (Fig. 3) depends on a natural fluctuation, also visible in Figs. 1 and 6. The survival percentages in the tests are given in Table 1.

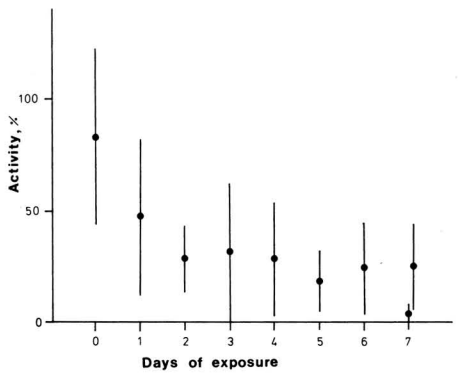

Fig. 3. Mean swimming activity of *P. affinis* during 12 days of recovery after exposure to 30 ppm phenol for various periods, expressed as a percentage of the mean diel activity during the control period. Black bar: *SD*.

3.2. 4-chlorophenol

In the tests with 4-chlorophenol four concentrations were used: 0.1, 1, 5 and 10 ppm. The exposure times were between 1 and 10 days. Two further tests with 5 ppm were made, in constant light and constant darkness, respectively, and a test with 10 ppm in constant light.

At the start of the exposure to 0.1 ppm no change in activity was observed. In three out of four tests with 1 ppm slight activation was noted for some days. The immediate reaction of *P. affinis* to 5 and 10 ppm was a strong increase in activity of short duration. An exception was 10 ppm in LL, in which activity immediately dropped to zero.

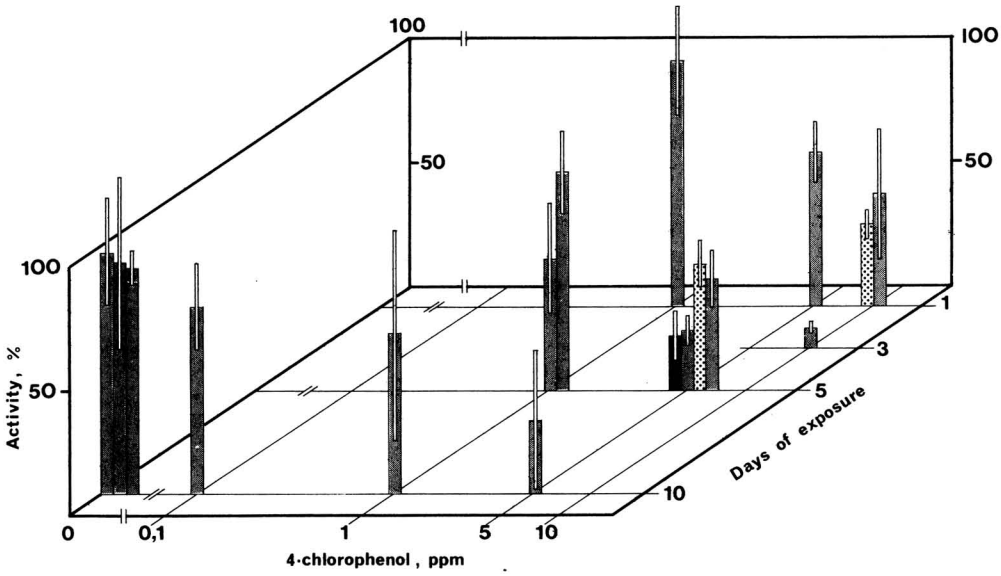

Fig. 4. Mean swimming activity of *P. affinis* during 10 days of recovery after different exposures to 4-chlorophenol, expressed as a percentage of the mean diel activity during the control period. Columns: stippled = LD, black = DD, dotted = LL. White bar : SD.

However, the increased activity in the different tests soon decreased to a level considerably below that of the control period, and in the test with 10 ppm fell to zero. Activity then persisted at a low level despite some degree of recovery. After the longer exposure times even the lower concentrations, 0.1 and 1.0 ppm, caused a more or less lasting reduction in activity. The activity during the recovery periods in relation to that of the control periods is shown in Fig. 4.

The "lowest" combination of concentration and exposure time that diminished activity was 0.1 ppm for 10 days, but not 1 ppm for 1 day.

Survival in the tests with 4-chlorophenol is shown in Table 2. At 10 ppm the toxicant was partly lethal in both 1- and 3-day exposures. These survival values can be compared to those in two tests with pure phenol, where the percentages of animals surviving exposure to 30 ppm for 7 days were 94.3 and 97.1, i.e. as high as in the control tests.

3.3. Styrene

Test conditions

The concentrations in the styrene experiments ranged from 2.3 to 69 ppm. These were theoretical initial concentrations calculated on the basis of the known concentration of styrene in the stock solution, 230 ppm, and on the percentage of aquarium water that was replaced with this stock solution.

Analyses in two tests showed that the styrene concentration had fallen to only 12 and 25 % of the initial values within 2 h after the aquaria were filled up at the start of the test. This fall was probably due to volatilization.

Table 2. Survival of *P. affinis* in 4-chlorophenol tests. N = 35 or 40.

4-chlorophenol conc., ppm	0	0.1	1	1	1	5	5	5	10	10
Days of exposure		10	1	5	10	1	5	10	1	3
Surviving animals, %	92.5 92.5 92.5[1]	95.0	92.5	82.5 96.0	95.0	82.5	95.0 87.5 82.5[2] 77.5[1]	90.0	82.5 70.0[2]	43.0

[1] = continuous dark, [2] = continuous light.

The styrene analyses (11 samples) were all made from the same batch of tests. However, the swimming activity of *P. affinis* followed the same patterns in all tests made with the same initial concentrations, indicating equal post-initial concentrations. The styrene concentrations referred to from here onwards are the calculated initial concentrations (C_i). Exact values are given only where samples were analysed.

The lower styrene concentrations, C_i less than 35 ppm, immediately stimulated the swimming of *P. affinis*, while a C_i of 35 ppm or higher caused immediate inactivity. During the recovery period the animals in all experiments regained about the same swimming activity as during the control period, except with C_i 69 ppm, in which all the animals died.

Stimulatory concentrations

The C_i 2.3 and 4.6 ppm caused only a minimal and temporary stimulation for the first 2 or 3 days of the test period. After this time the animals resumed their normal activity. The styrene may have volatilized almost completely by this time.

Two tests were made with C_i 11.5 ppm. In one there was temporary activation lasting only a few days, whereas in the other the activity doubled and remained at this level for almost the whole exposure period.

In the tests with C_i 23 ppm the activity increased to many times the normal level within a few hours after addition of styrene. The activity continued at a high level during the following days, then decreased, and had become normal by 11 days at the latest (Fig. 5).

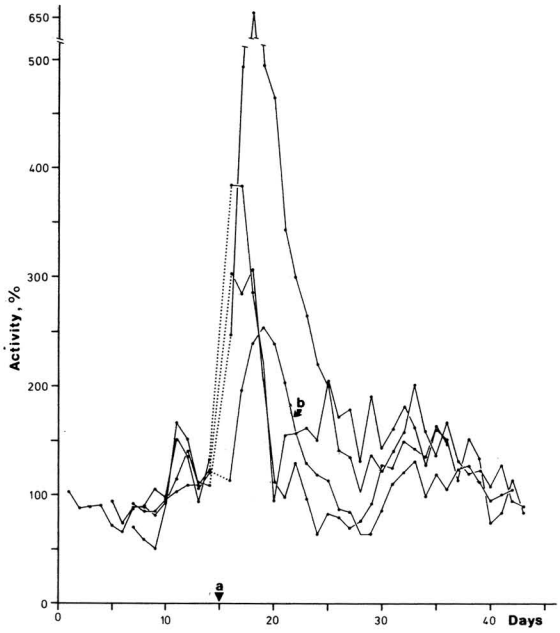

Fig. 5. Swimming activity of *P. affinis* during styrene experiments with an initial concentration of 23 ppm. Diel activity is expressed as a percentage of the mean diel activity during the 10 control days. Arrows indicate times of events; for explanations see text.

Inhibiting concentrations

At a C_i of 35 ppm styrene the activity of *P. affinis* immediately decreased to almost zero. During the following 4—5 days the animals swam very little. Then nocturnal activity began to increase again: after 3 days it had risen to two to three times the normal level, where it remained for some days, then returning to normal.

C_i 46 ppm affected swimming activity in much the same way as C_i 35 ppm. The decrease was more pronounced, however, and lasted 2 days longer. After the initial decrease the activity level rose to about the same level as for C_i 35 ppm for about 3 days, then returned to a more or less normal level (Figs. 6 and 7).

The highest concentration used, C_i 69 ppm, caused immediate total inhibition of activity, which fell to zero and remained there. At the end of the experiment it was noted that all the animals had died. Probably this had occurred in the early part of the exposure period, because all the animals were found on the surface of the bottom substrate and in advanced stages of decay.

Mortality in the styrene tests

The percentages of survivors are tabulated in Table 3. The duration of the exposure period is not mentioned because of the rapid volatilization of styrene. The last few days of "exposure" may in fact actually have been recovery days in terms of styrene content.

In the tests with C_i 35 and 46 ppm, in which mortality was high, it would have been better to correct the activity levels during the latter part of the exposure period and during the recovery period, so that they corresponded to a numerically complete test population. No correction was applied, however, because the experimental conditions did not permit counting of the animals during the experiment.

Concentration changes during the experiments

Analyses 2 h after the start of the tests with C_i 23 and 46 ppm showed that the con-

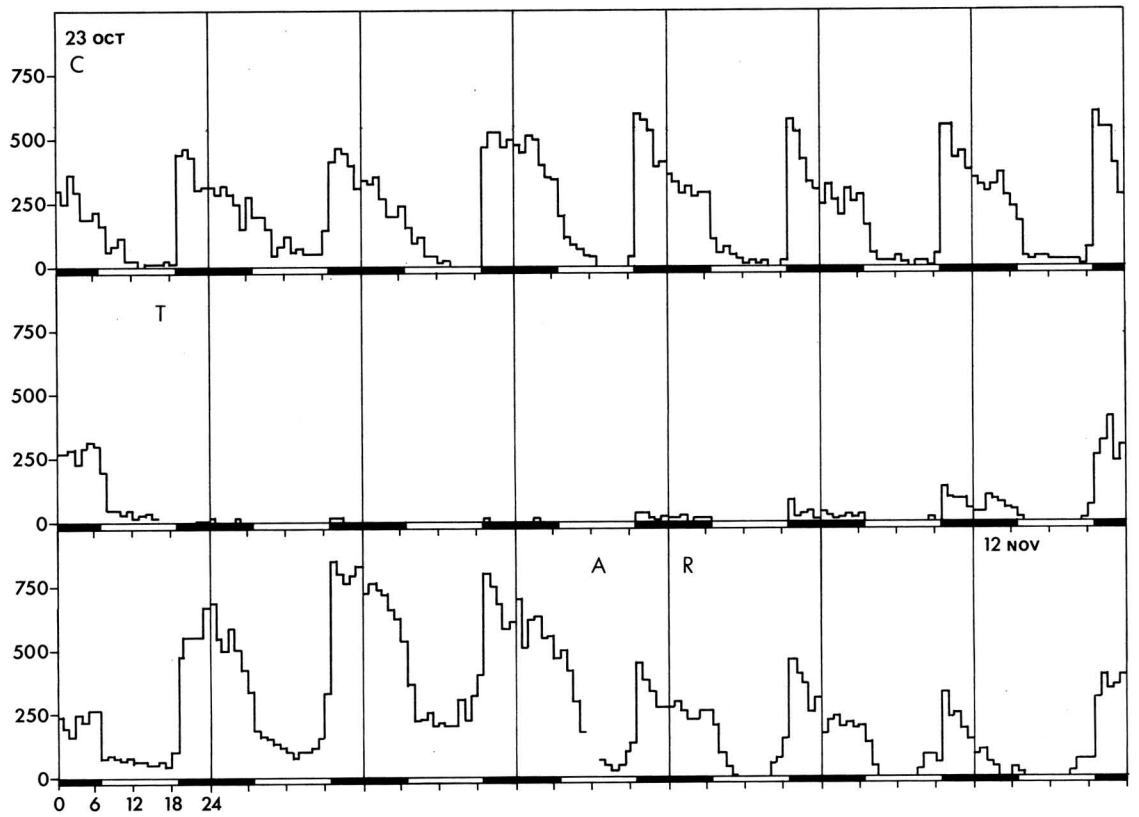

Fig. 6. Swimming activity of *P. affinis* during part of a 46 ppm (initial concentration) styrene experiment. C = control period, T = exposure period, R = recovery period. The activity is expressed as recordings/hour. Black bars = dark periods. At T and A the recording was interrupted during addition of test solution and sampling for analyses, respectively.

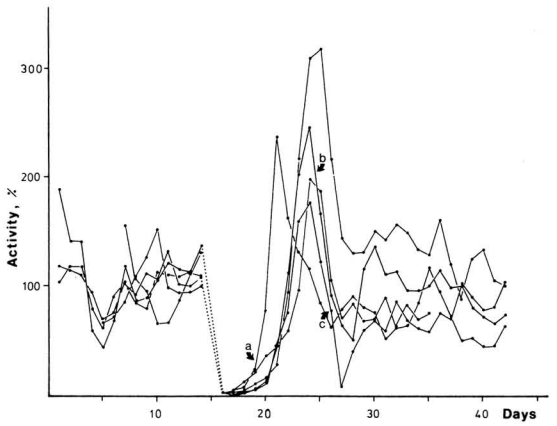

Fig. 7. Swimming activity of *P. affinis* during styrene experiments with an initial concentration of 46 ppm. Diel activity is expressed as a percentage of the mean diel activity during the 10 control days. Arrows indicate times of events; for explanations see text.

centrations had decreased to 3.3 (a in Fig. 5) and 12.5 ppm, respectively. Of these concentrations the former was immediately stimulatory whereas the latter immediately inactivated the animals for many days.

A concentration of 4.4 ppm was measured at the time when the activity began to rise in the test with the initially inactivating C_i 46 ppm (a in Fig. 7). The concentrations measured 1 day after the peaks of activity were 1.5 (A in Fig. 6, b in Fig. 7) and 0.1 ppm in the C_i 46 and 35 ppm tests. The animals in the latter test behaved as if the concentration had been lower than C_i 35 ppm — the activity after the period of inactivation began earlier than in the parallel tests with the same initial concentration.

Finally, in tests with C_i 23, 35 and 46 ppm the styrene concentrations were analysed at the time when the formerly increased activity returned to the normal level. The concentrations were then

Table 3. Survival of *P. affinis* in styrene tests. For the duration of exposure, see p. 227.

Styrene, initial conc., ppm	0	2.3	4.6	11.5	23.0	35.0	46.0	69.0
Number of tests	7	1	1	2	4	5	5	1
Surviving animals, %	94.7±7.3	100.0	97.5	96.3±5.3	98.0±1.3	84.7±14.8	68.6±19.1	0

0.4 (b in Fig. 5), 0.6 and 1.0 (c in Fig. 7) ppm, respectively.

4. Discussion

In this study only the swimming activity was recorded. The behaviour of the animals in the bottom substrate was not investigated.

Leppäkoski (1975) and Luotamo & Luotamo (1979), comparing the bottom fauna of clean and polluted waters, noted that *P. affinis* is one of the species that disappear even at low levels of pollution. The reason may be that the animals reacted to the pollutants with flight, having actively moved or been carried away from the area by water currents. On the other hand, if toxic substances were present in the area at concentrations too low to stimulate activity, the population may have diminished because of other sublethal effects of the toxicants.

The physiological state of a species and hence its susceptibility to toxicants may change with the seasons of the year. In tests made with fish, for instance, LC 50 values obtained at different times of year may differ by a factor of 2.5 (Ministry of Technology, London, 1968). No indications of such a difference could be seen in the results of our experiments with *P. affinis*.

Phenol and its derivatives are known to cause activation in animals of several groups. The sublethal effects of phenol on pike include increased sensitivity to light and vibrational stimuli (Kristoffersson et al. 1973). Oksama & Kristoffersson (1979) noted that exposure to low concentrations of phenol increased the swimming activity of the amphipod *Gammarus duebeni*. Similarly, Kaila & Saarikoski (1979) found that weakly ionized phenols (conc. 0.5—8.0 mM) caused motor excitation in intact crayfish.

In *P. affinis* exposure to phenol or 4-chlorophenol led to a change in swimming behaviour at all concentrations used. The immediate reaction to relatively high concentrations — 17, 18 and 30 ppm phenol, 5 and 10 ppm 4-chlorophenol — was an increase in activity for some days, but in the long run the change, even at low concentrations (1, 5 and 10 ppm phenol, 0.1 and 1 ppm 4-chlorophenol), was a clear decrease, which persisted throughout the recovery period of at least 10 days.

These effects can be compared with the results of phenol tests made on other crustaceans. Portmann (1970) gives the following LC 50 values for 48 h at 15° C: *Pandalus montagui* 17.5 ppm, *Crangon crangon* 23.5 ppm and *Carcinus maenas* 56 ppm. For *Crangon crangon* the following LC 50 values are also available: 30 and 25 ppm for 48 and 96 h at 15°C (Adema 1976, cited by Verschueren 1977). In the range 22—5°C, according to Portmann (1968), *C. crangon* does not tolerate phenol better at lower temperatures.

In our tests the toxicity of 4-chlorophenol was at least 3-fold that of pure phenol: 10 ppm 4-chlorophenol was partly lethal, whereas 30 ppm phenol caused no significant mortality. This is in good accord, for instance, with the observations of Oksama & Kristoffersson (1979) on *Mesidotea entomon*, in which 4-chlorophenol was about three times as toxic as pure phenol.

Even a brief exposure to a higher concentration of phenol had an irreversible activity-diminishing effect. After as little as 1 day in 30 ppm phenol *P. affinis* lost part of its natural swimming activity. The longer the exposure, the poorer was recovery. It should be kept in mind that mortality in these tests was very low even after the longest exposures.

These observations show that chronic exposure to phenol or 4-chlorophenol concentrations far below lethal values could have drastic effects on a *P. affinis* population. Among other things a change in swimming behaviour would affect the changes of males and females meeting for copulation, which apparently takes place during the nocturnal migrations (Segerstråle 1950).

The results of the tests with 30 ppm phenol and 5 ppm 4-chlorophenol (Figs. 3 and 4) might be interpreted to mean that after a minimum exposure of 2—3 days activity stabilizes at a certain level regardless of the exposure time. This would imply the existence of a concentration (less than 30 ppm for phenol, less than 5 ppm for 4-chlorophenol) that reduces activity to 50 % of the normal level, for instance, during chronic exposure. This would correspond to the LTC (lethal threshold concentration) values in conventional toxicity tests (Sprague 1969).

The most interesting feature of the styrene tests was that *P. affinis* became active at a certain concentration of styrene even after exposure to a higher inhibiting concentration. Because of the rapid diminution of the concentrations due to volatilization it was difficult to determine the activating concentration. In the tests which led to strongly stimulated activity immediately after the introduction of styrene the calculated initial concentration was 23 ppm, and the measured concentration 2 h later 3.3 ppm. Thus the activating concentration lies somewhere between 23 and 3 ppm, probably closer to the latter. In a test with an initially inhibiting concentration (46 ppm), analysis gave a value of 4.4 ppm at the time when the activity began to rise after a few days of inactivation (a in Fig. 7).

The availability of *P. affinis* as food for pelagic fishes depends largely on its swimming activity (Segerstråle 1937, Aneer 1975). Hence, the activation of *P. affinis* after a discharge of styrene might result in greatly increased feeding on the intoxicated animals by fish. Here it should be observed that even at the strongest concentration tested — C_i 46 ppm — the animals increased their activity, although the styrene concentration was partly lethal (30 % mortality).

Few other investigations have been made on the impact of styrene on marine animals. Price et al. (1974) have reported the value 68 ppm as 24-h TL_m for the arthropod *Artemia salina*. Pickering & Henderson (1966) gave the values 25.1—64.7 as 24-h TL_m for various fish species. Our tests with 46 and 69 ppm (Table 3) gave results in good accord with these values. Pagano et al. (1978) have tested the effects of styrene on the development of sea urchins. The concentrations tested, 5×10^{-3} M and 5×10^{-4} M (corresponding to ca 52 and 520 ppm), had various detrimental effects on fertilization and embryonic development.

The conclusion can be drawn that the method described here will be a useful tool for estimating sublethal effects of pollutants in the aquatic environment. This type of bioassay could easily be adapted for recording the activity of other aquatic animals, although its usefulness would depend on the specific behaviour of the species selected.

Acknowledgements. Our gratitude is expressed to Prof. Kai Otto Donner and Prof. Rolf Kristoffersson for valuable suggestions, discussions and availability for consultations and for constructive criticisms of the manuscript.

We are also grateful to the University of Helsinki for providing working facilities at Tvärminne Zoological Station, to the staff of the Station for practical assistance, to Mr. Veikko Matila (Sondi Oy) for constructing the recording devices, to Mrs. Kirsti Erkomaa, M.Sc., at the Water Research Institute of the National Board of Waters for the styrene determinations, to Mr. Ulf Eklund for photographic documentation of the material, and to Mrs. Jean Margaret Perttunen, B.Sc. (Hons.), who corrected our English language.

This investigation was supported by a grant from the Maj and Tor Nessling Foundation, for which we express our gratitude.

References

Aneer, G. 1975: Composition of food of the Baltic herring (Clupea harengus v. membras L.), fourhorn sculpin (Myoxocephalus quadricornis L.) and eel-pout (Zoarces viviparus L.) from deep soft bottom trawling in the Askö — Landsort area during two consecutive years. — Merentutkimuslaitoksen Julkaisuja/Havsforskningsinstitutets Skrifter 239: 146—154.

Arthur, J. W. & Leonard, E. N. 1970: Effects of copper on Gammarus pseudolimnaeus, Physa integra, and Campeloma decisum in soft water. — J. Fish. Res. Board Canada 27: 1277—1283.

Bernhard, M. & Zattera, A. 1970: The importance of avoiding chemical contamination for a successful cultivation of marine organisms. — Helgoländer Wiss. Meeresunters. 20: 655—675.

Buikema, A. L., Jr., McGinniss, M. J. & Cairns, J., Jr. 1979: Phenolics in aquatic ecosystems: a selected review of recent literature. — Mar. Environm. Res. 2 (2): 87—181.

Carlson, A. R. 1971: Effects of long-term exposure to carbaryl (Sevin) on survival, growth, and reproduction of the fathead minnow (Pimephales promelas). — J. Fish. Res. Board Canada 29: 583—587.

Donner, K. O. 1971: On vision in Pontoporeia affinis and P. femorata (Crustacea, Amphipoda). — Comment. Biol. 41: 1—17.

Donner, K. O. & Lindström, M. 1980: Sensitivity to light and circadian activity of Pontoporeia affinis (Crustacea, Amphipoda). — Ann. Zool. Fennici 17: 203—212.

Hessle, C. & Vallin, S. 1934: Undersökningar över plankton och dess växlingar i Östersjön under åren 1925—1927. (Summary: Investigations of plankton and its fluctuations in the Baltic during the years 1925—1927.) — Svenska Hydrografisk-Biol. Kommissionens Skrifter, N.S. (Biol.) 1(5): 1—94. Stockholm.

Holcombe, G. W., Benoit, D. A., Leonard, E. N. & McKim, J. M. 1976: Long-term effects of lead exposure on three generations of brook trout (Salvelinus fontinalis). — J. Fish. Res. Board Canada 33: 1731—1741.

Härkönen, H. 1978: Oireet, psyykkiset suoritukset ja neurofysiologiset löydökset styreenin altistumis-

vastesuhteen kuvaajina. (Summary: Symptoms, psychological functions and neurophysiological findings as indicators of the exposure-response relationship of styrene). — Ph.D. thesis, University of Helsinki.

Kaila, K. & Saarikoski, J. 1980: Inhibition of voltage-dependent potassium conductance by convulsant phenols in the medial giant axon of the crayfish. — Comp. Biochem. Physiol. 65 C: 17—21.

Keynäs, K. & Keynäs, L. 1978: Meiofauna in soft bottom sediments at Tvärminne, northern Baltic. Methods and preliminary results. — Memoranda Soc. Fauna Flora Fennica 54: 65—68.

Kleerekoper, H. 1976: Effects of sublethal concentrations of pollutants on the behavior of fish. — J. Fish. Res. Board Canada 33: 2036—2039.

Kristoffersson, R., Broberg, S. & Oikari, A. 1972: Effect of temperature change on some blood constituents and osmotic balance in the pike (Esox lucius L.) in brackish water. — Ann. Zool. Fennici 9: 212—218.

—»— 1973: Physiological effects of a sublethal concentration of phenol in the pike (Esox lucius L.) in pure brackish water. — Ann. Zool. Fennici 10: 392—397.

Laughlin, R. B., Jr., Young, L. G. L. & Neff, J. M. 1978: A long-term study of the effects of water-soluble fractions of No. 2 fuel oil on the survival, development rate, and growth of the mud crab Rhithropanopeus harrisii. — Mar. Biol. 47: 87—95.

Leppäkoski, E. 1975: Assessment of degree of pollution on the basis of macrozoobenthos in marine and brackish-water environments. — Acta Acad. Aboensis (B) 35 (2).

Lindström, M. & Lindström, A. 1980: Swimming activity of Pontoporeia affinis (Crustacea, Amphipoda) — Seasonal variations and usefulness for environmental studies. — Ann. Zool. Fennici 17: 213—220.

Luotamo, I. & Luotamo, M. 1975: Koverharin rauta- ja terästehtaan vesistövaikutuksista Tvärminne Storfjärdin vesialueeseen vuonna 1974. — Mimeographed. 22 pp. Tvärminne Zoological Station.

—»— 1979: Koverharin rauta- ja terästehtaan vesistövaikutuksista. Loppuraportti. — Mimeographed. Tvärminne Zoological Station.

Marzolf, G. R. 1965: Vertical migration of Pontoporeia affinis (Amphipoda) in Lake Michigan. — Publ. 13, Great Lakes Research Division, The University of Michigan.

McKim, J. M. & Benoit, D. A. 1971: Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (Salvelinus fontinalis). — J. Fish. Res. Board Canada 28: 655—662.

Ministry of Technology 1968: Water Pollution Research 1967. H.M.S.O. — 213 pp. London.

Oksama, M. & Kristoffersson, R. 1979: The toxicity of phenol to Phoxinus phoxinus, Gammarus duebeni, and Mesidotea entomon in brackish water. — Ann. Zool. Fennici 16: 209—216.

Ochynski, F. W. 1960: The absorptiometric determination of phenol. — The Analyst 85: 278—281.

Pagano, G., Esposito, A., Giordano, G. G. & Hagström, B. E. 1978: Embryotoxic and teratogenic effects of styrene derivatives on sea urchin development. — Scand. J. Work Environm. Health 4 (suppl 2): 136—141.

Pickering, Q. H. & Henderson, C. 1966: Acute toxicity of some important petrochemicals to fish. — J. Water Poll. Control Fed. 38 (9): 1419—1429.

Portmann, J. E. 1968: Progress report on a programme of insecticide analysis and toxicity-testing in relation to the marine environment. — Helgoländer Wiss. Meeresunters. 17: 247—256.

—»— 1970: The toxicity of 120 substances to marine organisms. — Shellfish Inform. Leaflet 19, Ministry of Agriculture, Fisheries and Food (UK).

Price, K. S., Waggy, G. T. & Conway, R. A. 1974: Brine shrimp bioassay and seawater BOD of petrochemicals. — J. Water Poll. Control Fed. 46 (1): 63—77.

Rosenthal, H. & Alderdice, D. F. 1976: Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. — J. Fish. Res. Board Canada 33: 2047—2065.

Segerstråle, S. G. 1933: Studien über die Bodentierwelt in südfinnländischen Küstengewässern. II. Übersicht über die Bodentierwelt, mit besonderer Berücksichtigung der Produktionsverhältnisse. — Comment. Biol. 4 (9): 1—80.

—»— 1937: Studien über die Bodentierwelt in südfinnländischen Küstengewässern. V. Das reife Pontoporeia affinis-Männchen ("P. weltneri") als Saisonnahrung für den Stint (Osmerus eperlanus L.). — Comment. Biol. 7 (3): 1—17.

—»— 1950: The amphipods on the coasts of Finland — some facts and problems. — Comment. Biol. 10 (14): 1—28.

—»— 1959: Synopsis of data on the Crustaceans Gammarus locusta, Gammarus oceanicus, Pontoporeia affinis, and Corophium volutator (Amphipoda Gammaridea). — Comment. Biol. 20 (5): 1—23.

Smith, W. E. 1972: Culture, reproduction, and temperature tolerance of Pontoporeia affinis in the laboratory. — Trans. Amer. Fish. Soc. 2: 253—256.

Sprague, J. B. 1969: Measurement of pollutant toxicity to fish I. Bioassay methods for acute toxicity. — Water Res. 3: 793—821.

Verschueren, K. 1977: Handbook of environmental data on organic chemicals. — 659 pp. New York.

Winner, R. W. & Farrell, M. P. 1976: Acute and chronic toxicity of copper to four species of Daphnia. — J. Fish. Res. Board Canada 33: 1685—1691.

Received 13. III. 1980
Printed 31. XII. 1980