Ann. Zool. Fennici 17: 213-220. 1980

Swimming activity of Pontoporeia affinis (Crustacea, Amphipoda) — Seasonal variations and usefulness for environmental studies[1]

Magnus Lindström & Astrid Lindström

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.

The swimming activity of the amphipod *Pontoporeia affinis* Lindström during different seasons of the year was recorded with the aid of infra-red photocells in laboratory conditions at 4°C and an LD 12:12 regime.

The animals exhibited nocturnal swimming activity which increased throughout the year, with a maximum at the time of sexual maturity in October — December. In the daytime the animals burrowed into the bottom mud and exhibited almost no swimming activity. The shape of the diel activity curve varied with the season. Reactions to light-on and light-off were more distinct in winter. In substrateless conditions swimming showed a different pattern, diurnal activity being high and even exceeding nocturnal activity.

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

1. Introduction

Pontoporeia affinis Lindström is an ecologically important species of soft bottoms in the Baltic Sea, in the brackish waters off the circumpolar coasts and in lakes in Northern Europe and North America (Segerstråle 1959). At night these amphipods rise from the bottom and become pelagic. This activity has been recorded and studied under laboratory conditions (Donner & Lindström 1980) and its occurrence in natural conditions has been demonstrated by catches from different depths at different times of day (Segerstråle 1937, Marzolf 1965b, McNaught & Hasler 1966). In the daytime *P. affinis* burrows into the bottom substrate, an activity that is of great importance for the mixing of the bottom (Segerstråle 1962).

The swimming activity of *Pontoporeia* is timed by light and by the animal's biological clock (Donner & Lindström 1980). Donner's (1971) electrophysiological measurements of the sensitivity of the *Pontoporeia* eye showed that the animal can be assumed not to have any perception of light at depths below 80—90 m in the Baltic. The local light conditions could perhaps explain why Wells (1968) obtained specimens of *Pontoporeia* in daytime samples above the bottom at all depths of 36.5 m and more in Lake Michigan.

The literature contains indirect evidence suggesting seasonal variations in the intensity of the swimming activity. Segerstråle (1937) showed that in November—December the food of the smelt (*Osmerus eperlanus*) consists largely of *P. affinis*. Aneer (1975) observed the same for Baltic herring (*Clupea harengus*). This indicates increased swimming activity of *P. affinis* during the time of sexual maturity, since there is reason to believe that neither the smelt nor the Baltic herring are bottom feeders to any appreciable extent. The attainment of sexual maturity in November— December is apparently controlled by light (Segerstråle 1970). Hence summer breeding is also observed in populations at depths exceeding 100 m in the Baltic Sea, where these animals cannot have any perception of light (Segerstråle 1967).

Donner & Lindström (1980), studying the circadian activity of *P. affinis*, found that the swimming activity may serve as a change-

[1] Report No. 628 from Tvärminne Zoological Station, University of Helsinki, SF-10850 Tvärminne.

sensitive parameter when the animals are exposed to environmental stress, e.g. to pollutants of various kinds.

The present paper reports the levels of swimming activity at different times of year. Knowledge of such variations in seasonal activity are of importance both from an ecological point of view and when the organism is tested for use in studies on the effects of various environmental factors. For this latter purpose the recording system used by Donner & Lindström (1980) was adapted to serve as a bioassay for sublethal toxicity tests.

2. Material and methods

The method used for recording the swimming activity of *P. affinis* was based on the same principle as that developed and described by Donner & Lindström (1980).

The test animals were caught in Tvärminne Storfjärd, SW Finland, with a van Veen grab at 30—35 m depth. The samples, including the mud, were transferred to a 300-l continuous flow storage tank at the temperature of the sample. The temperature was then gradually changed, usually within 12 h, until the storage and testing temperature, +4° C, was attained. The highest bottom sample temperature measured was +13°C; in winter it was even lower (+2°C) than the testing temperature. The animals were acclimated for at least 1 week in the 300-l storage tank before the experiments.

The system used for the experiments is shown in Fig. 1. The test aquaria were made of glass (33 cm long by 6 cm wide by 35 cm high). The water level was at 33 cm, which gave a water volume of 5.55 l. The equipment was made entirely of glass, silicone and polypropylene, which are relatively non-toxic materials (Bernhard & Zattera 1970).

In each aquarium 400 ml of natural mud (ca 2.5 cm deep) was placed as a bottom substrate. The mud provided the animals with both a burrowing and a food substrate (cf. Marzolf 1965a, Smith 1972). The mud was added and the water flow started 1 day before the introduction of the test animals. In two experiments made by Donner & Lindström (1980) with *Pontoporeia* individuals in the same conditions but without water flow and without additional

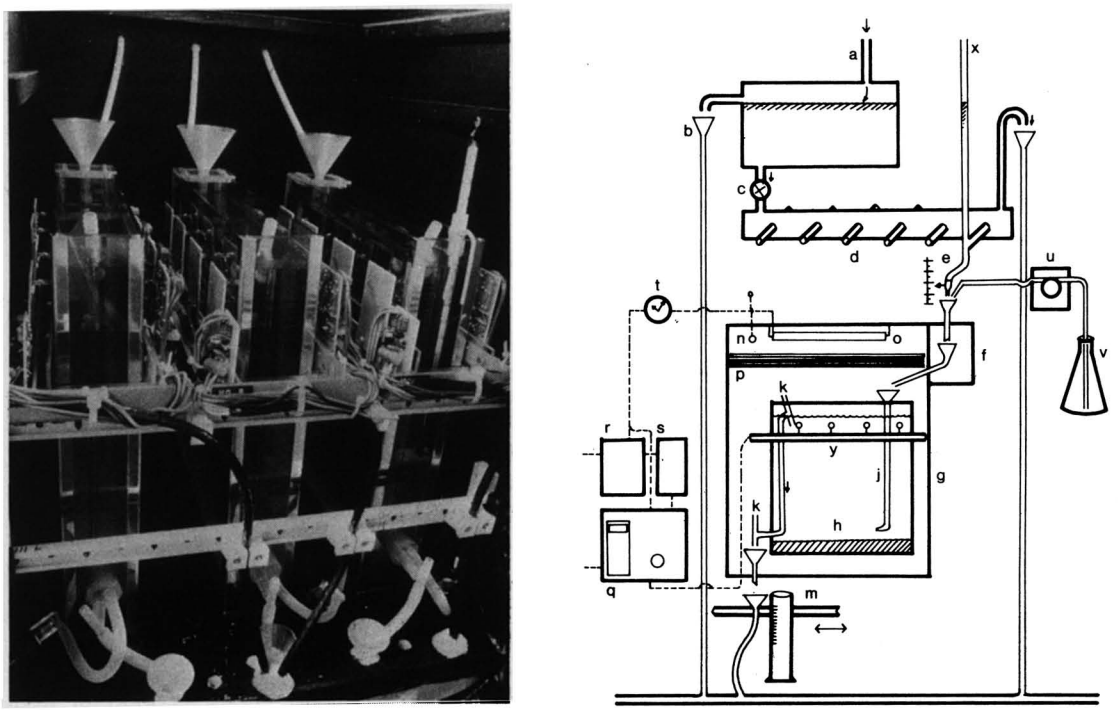

Fig. 1. Photograph and diagram of test equipment. — a. brackish water inlet, from the main water supply. — b. overflow to drainage. — c. valve. — d. water dispenser with second overflow. — e. syringe nozzles, adjustable in height. — f. light-tight box. — g. experimental chamber. — h. aquarium with bottom substrate. — j. water inlet, nozzle diameter 1.0 mm. — k. overflow and drainage from the aquarium. — m. measuring cylinders and drainage funnels mounted on a movable rod, allowing simultaneous sampling from all aquaria. — n. photoresistors for checking illumination. — o. fluorescent tubes, 24 V, 8 W. — p. opalescent light-absorbing acrylic filters. — q. event recorders and printers. — r. Ni-Cd battery, 24 V. — s. battery-loading device. — t. electronic timer for control of illumination. — u. peristaltic pump (used in toxicity tests). — v. (toxicant supply). — x. air-bubble outlet from the water dispenser. — y. counter frame with IR emitters and corresponding phototransistors.

nutrition except the bottom substrate, 73 and 85 % of the animals were still alive after 165 days.

The water used was natural unfiltered brackish water (salinity 5—6 $^{0}/_{00}$). The other properties of the water are described by Kristoffersson et al. (1972).

As a rule the experiments were conducted in continuous-flow conditions. For comparison, some experiments were made without flow. In these conditions the behaviour of the animals did not alter. The stopping or starting of the flow during an experiment caused a slight decrease in swimming activity, but in a day or two the activity had stabilized to its former level.

The flow of water to the aquaria was controlled by a double overflow system, and the flow to each aquarium respectively by regulation of the height of the inflow syringes. The flow was adjusted to 12 ml/min. Measuring cylinders (L, Fig. 1) attached to a movable bar in connection with the drainage system allowed simultaneous sampling from all aquaria.

Sprague (1969) recommends a 90 % replacement of water in 8—12 h for experiments with fish. The 90 % replacement time in our experiments was about 17 h. The oxygen content of the water was over 8 mg/l at the end of the experiments. During these a light oxidized layer developed at the surface of the bottom substrate, indicating good oxygen conditions. In the experiments without flow the oxygen content remained quite satisfactory, being over 8 mg/l.

For each aquarium 35 or 40 pre-adult or adult animals 6—10 mm long were selected. The animals were gently caught with a strainer from the storage tank and transferred to the aquaria 10 at a time. In some experiments it was impossible to avoid transferring some juveniles (under 4 mm long) with the bottom substrate.

Two or three aquaria were placed in each of four light-tight chambers; thus 8—12 parallel tests could be made simultaneously. The light in the chambers followed a 12/12 h light-dark cycle (LD 12:12), with the light period between 7.00 and 19.00 hours. Fluorescent tubes, 24 V, 8 W, above opalescent and light-absorbing acrylic filters in the lids of the chambers served as light sources. The light was thus dispersed diffusely over the test aquaria and reduced to $2—3 \times 10^{-2}$ lux measured with an Airam UVM-8 LX luxmeter. This light value is 4—5 log units above the absolute visual threshold of *Pontoporeia* (Donner 1971) and can be assumed to correspond approximately to the light conditions at about 30 m depth in this part of the Baltic Sea.

Swimming activity was recorded with the aid of IR-light-emitting diodes and IR-sensitive phototransistors (see Donner & Lindström 1980). When any of the IR beams was broken by a swimming animal a count was recorded.

The four emitters and corresponding phototransistors were mounted opposite to each other ca 2.5 cm below the water surface level along the long sides of the test aquaria (Fig. 1). The number of counts per hour was printed out numerically (Sodeco printers). In some experiments a recording position halfway down the aquarium was used simultaneously. The recordings from this level gave slightly lower values than those from higher up, but the shape of the activity curve was the same. In later experiments only the upper level was used.

The recordings from the different aquaria were not fully comparable, however, because the intercalibration of the recording units could not be made completely accurate.

Because a change in LD period or a recording failure would vitiate activity measurements of this kind, a standby power system was developed to be used during electric power breakdowns. The standby system (a battery of Ni-Cd cells on constant loading) was automatically coupled to the recorders and light tubes without a break in the event of a main power failure. During such power failures every recording unit had to depend on its own inbuilt quartz crystal timer.

3. Results

At latest 3 to 4 days after the animals had been transferred to the test aquaria a pronounced nocturnal rhythmic swimming activity developed (Fig. 2). For the present investigation we used the recordings from the first 10—14 stable days. Thereafter the experiments continued as toxicity tests (Lindström & Lindström 1980). In those experiments that were continued as controls — without addition of toxicants — the results from day 14 onwards are also used here. In these tests mortality did not exceed 10 % after a complete experiment lasting about 40 days. Most of the dead animals were then in an advanced stage of decay and could not be collected.

In interpreting the results we have considered both the total activity per day and the shape of the activity diagram as well as the ratio of the average activity during the L period to the average activity during the D period. The closer this ratio (the rhythmicity index, I_r) is to 1, the less pronounced is the rhythmicity (see Donner & Lindström 1980). For 49 experiments each lasting for 10 days the average I_r value was 0.23 ± 0.13 (*SD*).

3.1. The activity pattern

The light intensity of $2—3 \times 10^{-2}$ lux used in the experiments was strong enough to induce a marked rhythm with nocturnal activity in *Pontoporeia*. The swimming activity (Fig. 2) was high at night, 100 to 600 counts per hour, depending on the season of the year, and low, sometimes near zero, in the daytime (cf. Donner & Lindström 1980). It was fairly constant from day to day.

At light-off at 19.00 hours swimming activity increased abruptly. Activity values were usually highest within a few hours after light-off. The activity then gradually decreased towards light-on at 7.00 hours. In the beginning of the L period the animals sometimes maintained an activity

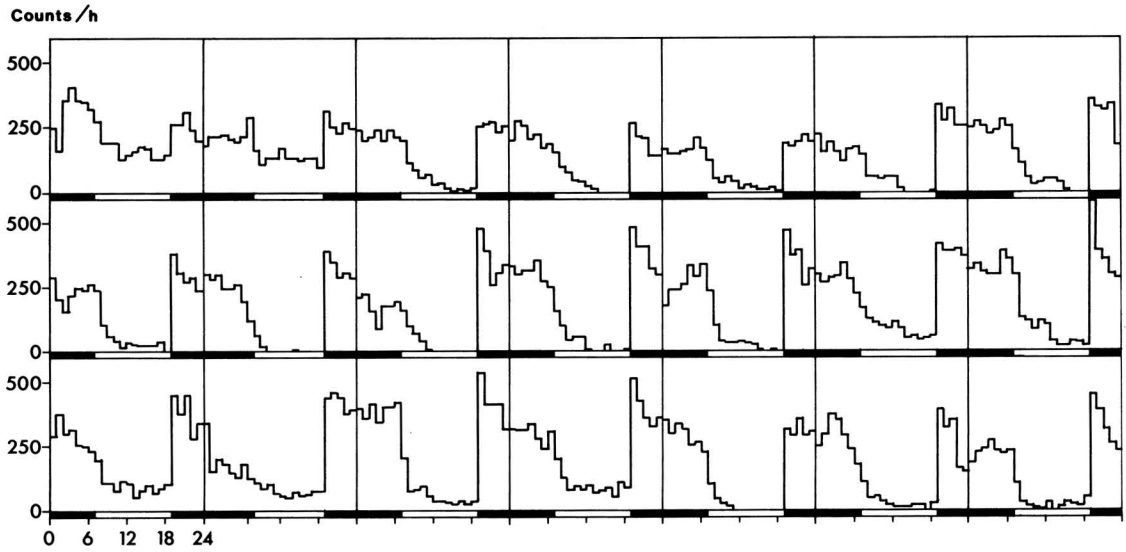

Fig. 2. Swimming activity of *P. affinis* during part of an experiment continued from October to November. Activity is expressed as recordings/hour. Black bars = dark periods.

higher than the average daytime level for a few hours. Towards the end of the L period the activity often started to rise about an hour before the light was switched off. If the light failed during the L period there was an immediate increase in activity. The behaviour of *P. affinis* in constant darkness is described by Donner & Lindström (1980).

3.2. Seasonal variations in the behaviour of *Pontoporeia affinis*

As shown by the series of experiments, the activity level increased during the course of the year from about 1000 counts per day in January to up to 7000 counts in October-November (Fig. 3). A sudden decrease then occurred from December to January. To confirm this phenomenon one test was started in mid-December and continued until mid-February. In this test, too, the activity decreased remarkably in early January. In February the animals were collected and counted. They were all alive; 33 were egg-bearing females, one was a sexually immature adult animal and the rest were smaller immature animals.

The shape of the daily activity curve also varied with the season. The prevailing 12-h L period in the experimental chambers differed from the natural conditions in the sea, being too long in winter and too short in summer. Despite this unnatural L period the animals tended to follow the outdoor conditions. This is seen in Fig. 4, which shows the averaged results of 10 tests in June 1979 and 9 tests in October 1978

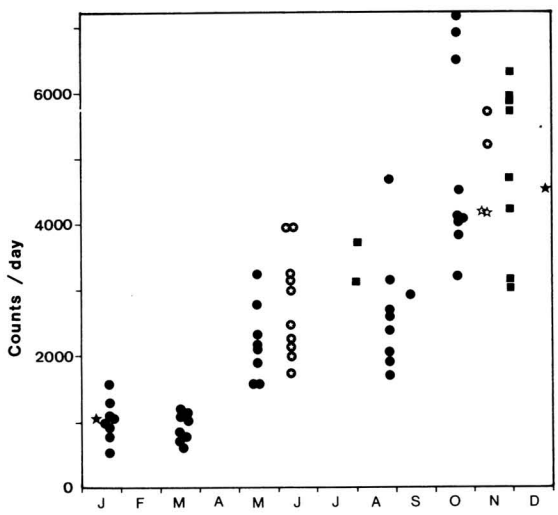

Fig. 3. Swimming activity level of *P. affinis* during the year. Each test is represented by one point (= average for 10 days). Empty star: 1976, square: 1977, circle: 1978, encircled star: 1979, filled star: one experiment continued from Dec. 1978 to Jan. 1979.

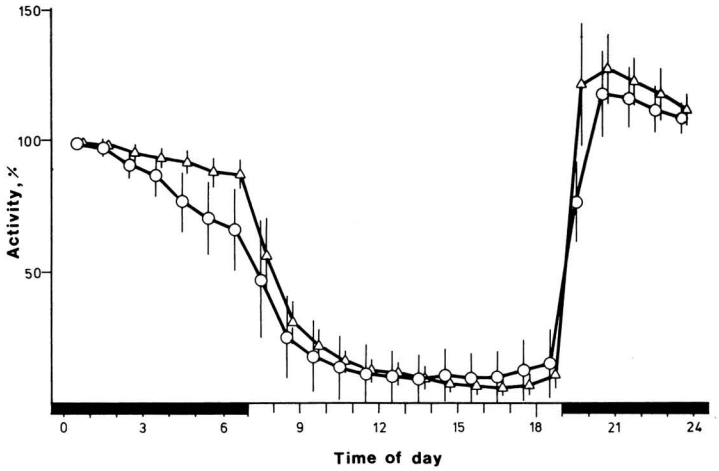

Fig. 4. Daily swimming activity of *P. affinis* during 10-day tests in June (circles) and October (triangles). Each point is a mean of the means of 10 (June) or 9 (October) tests, the bar being the *SD*. Daily values are calculated as percentages of the highest hourly value observed between 00 and 07 hours on the day in question.

(each test = mean of 10 days). As the total activity levels in June and October differed, the curves are drawn on a percentage basis, with the respective maximum activity values in the morning taken as 100 %. On October mornings the animals were still about as active at light-on at 7.00 hours as at midnight. In the summer their activity tended to decrease relatively steadily from midnight until morning. On October evenings the animals started to swim immediately the light was turned off. This is also seen in the October-November test in Fig. 2.

During the summer the animals reacted less promptly to light-off in the evenings, the increase in activity being slower. The time at which 20 % of the total evening activity was reached at different seasons is shown in Fig. 5.

3.3. Significance of the bottom substrate

The presence of a bottom substrate in the test aquaria tends to complicate the interpretation of the results of toxicity tests. The toxic substances may be absorbed on the mud in unknown quantities, or may react with it, in either case resulting in unpredictable test conditions. Moreover, they may be decomposed in different ways and in different amounts by other organisms present in the substrate. Consequently, it would be a great advantage if activity tests with toxic substances could be performed in aquaria without a bottom substrate. In order to examine the significance of the bottom substrate in the activity experiments one test was conducted in June without a bottom substrate and without continuous flow but otherwise in the same conditions as 10 parallel tests with substrate. Survival after 40 days of testing was 95 %. The activity pattern in the substrateless test differed markedly from the pattern normally shown, both in the shape of the diel activity curve and in the total activity level (Fig. 6). Earlier tests had confirmed that water flow has no effect on activity; tests with and without flow gave similar activity patterns. Hence, the results can be ascribed with confidence to the absence of a bottom substrate. The most striking feature of the behaviour in substrateless conditions was that in the beginning of the test the animals were more active in the daytime than at night, with a more or less diurnal I_r of 1.2 to 1.4. Not until day 14 did the activity in the daytime decrease, the index approaching

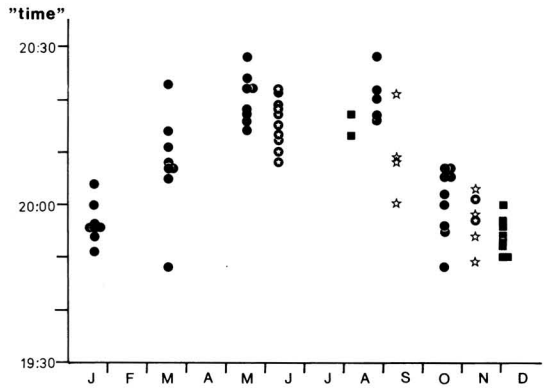

Fig. 5. Seasonal variation in reaction of *P. affinis* to light-off. Hour after light-off when activity has reached the 19.00—24.00 average value (= 20 % of the evening activity occurs before this hour). Every point (= test) is a mean value for 10 days. For symbols, see Fig. 3.

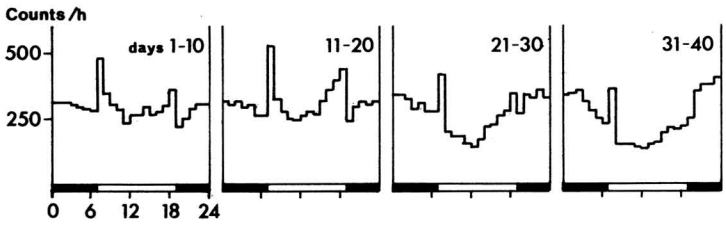

Fig. 6. Daily activity curve of *P. affinis* in substrateless conditions during 40 days. Columns are average values.

1.0. After day 20 the index remained under 1.0 and sank further to 0.3 on day 40, when the test was ended.

Fig. 6 shows another peculiarity of the behaviour of *Pontoporeia* in substrateless conditions: the animals reacted with activity peaks at light-on and at the hour just before light-off. This behaviour continued for the first 4 weeks of the test, whereafter the evening peak gradually disappeared.

The total diel activity was higher in substrateless conditions than in controls. This was due principally to the fact that diurnal activity was higher than nocturnal activity in the first part of the test and was about ten times the diurnal activity seen in normal conditions (Fig. 6 a,b). But the activity level at night was also elevated: ca 300 counts per hour against 200 in the controls.

4. Discussion

In laboratory conditions a rhythmicity in swimming activity developed in a few days and remained more or less constant throughout a period of 30 to 40 days. The presence of a bottom substrate proved necessary for the development of a clear rhythmicity. The diurnal swimming in substrateless conditions might be interpreted as an avoidance reaction to unsuitable substrates. This behaviour was accentuated at light-on when the animals simultaneously sought suitable burrowing sites. In the amphipod *Bathyporeia pelagica* a similar elevated activity in substrateless conditions was noted by Fincham (1970). The use of the activity pattern shown in substrateless conditions as a basis for behavioural studies is clearly not to be recommended. *P. affinis* lives on soft bottoms, and hence its activity on soft-bottom sites should be considered as its natural behaviour. In addition, the change in the activity pattern during the course of the experiment would complicate the interpretation of the results.

The differences in shape between the activity curves at different seasons may perhaps be due to the differences in total activity levels during the year. In summer the total activity level is relatively low, hence activity after light-off and before light-on is also limited. The increased tendency for vertical migration in autumn also results in higher activity immediately after light-off and before light-on.

An alternative explanation for the extended inactivation at the beginning and end of the D period in summer is that the animals swim according to the prevailing natural light conditions. The sun sets late in the summer evenings in Finland and if swimming activity is restricted to the dark periods the activity should be correspondingly delayed.

In autumn the artificial light in the aquaria kept the animals inactive at the beginning and end of the L periods, although in natural conditions they would have been active because darkness prevailed at these hours. When the light was finally switched off in the evenings the animals were ready to swim immediately and in the morning they kept swimming at as high a level of activity as in natural conditions until interrupted by the abrupt light-on. The experiments in January support the latter explanation, because although total activity was low the animals exhibited the activity pattern just described.

The reason for the seasonal differences in activity level is probably to be found in the reproductive cycle of the animal (Segerstråle 1950). In this part of the Baltic and in the light conditions and bottom depths from which they were caught, the animals reach sexual maturity in November-December (Segerstråle 1950).

At greater depths *P. affinis* breeds outside the cold season also (Segerstråle 1967). This must be taken into account when animals from deep waters are used for studies of rhytmicity or toxicity; the interpretation of the results may be complicated by the heterogeneity of the population with regard to the life stages.

Unfortunately, the individuals of the test populations could not be selected in advance with regard to sex or stage of development. Such a

procedure would have required a microscopical survey, which would have stressed the animals too much. Analyses of the test population after the tests showed that the fraction of sexually mature animals was usually too small to justify any conclusions.

If the elevated activity level noted in November-December is caused by sexual maturity, then what is the reason for the sudden decrease in activity between the December and the January tests? One explanation might be the heavier mortality among the males. According to Segerstråle (1950), males outnumber females in the pelagic part of the population and, when sexually mature, live for only 1 week after the final moult. However, in the test that continued from December to January and in which activity suddenly fell, the animals were all adult females or juveniles. No mortality had occurred, and no males had been present. Does this mean that females take part in the pelagic swimming to a greater extent than earlier believed? Of course, no conclusions can be drawn from this single test.

What fraction of the population swims actively at night? Marzolf (1965b) found that at the most 7.4 % of the adult or subadult population in Lake Michigan becomes pelagic at any one time. Here it should be pointed out that, according to Segerstråle (1977), the *Pontoporeia* of the North American lakes is another species, *P. hoyi*. Segerstråle (1950) mentioned that in the Baltic stock all individuals make excursions into the water. He concluded that as the males are more abundant in the pelagic part of the population they spend a longer time swimming pelagically than do the females. Direct nocturnal observations of our test aquaria with the aid of an IR viewer gave the impression that in most cases more than 50 % of the population was active at one time.

As the above discussion shows, the behaviour of *Pontoporeia affinis* in laboratory conditions can largely be predicted. This, together with the ease with which the animals can be maintained for long periods in aquaria, makes *Pontoporeia* a suitable test organism for experiments on sublethal toxicity. In optimal conditions and on a larger scale than was used in our experiments, it seems possible to keep *Pontoporeia* in aquaria for very long periods with successful breeding. Thus it might be possible to trace the impact of toxic substances for generations.

Acknowledgements. Our sincere thanks are due to Prof. Kai Otto Donner and Prof. Rolf Kristoffersson for valuable and inspiring discussions during the work and for their critical review of the manuscript.

We also wish to thank the University of Helsinki for providing working facilities at Tvärminne Zoological Station, the staff at the Zoological Station for practical assistance, Mr. Veikko Matila (Sondi Oy), who constructed the recording devices, Mr. Ulf Eklund for photographic documentation of the material, and Mrs. Jean Margaret Perttunen, B.Sc. (Hons.), who revised our English text.

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.

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

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.

Fincham, A. A. 1970: Rhythmic behaviour of the intertidal amphipod Bathyporeia pelagica. — J. Mar. Biol. Ass. U.K. 50: 1057—1068.

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.

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.

Lindström, M. & Lindström, A. 1980: Changes in the swimming activity of Pontoporeia affinis (Crustacea, Amphipoda) at exposures to sublethal concentrations of phenols and styrene. — Ann. Zool. Fennici 17: 221—231.

Marzolf, G. R. 1965a: Substrate relations of the burrowing amphipod Pontoporeia affinis in Lake Michigan. — Ecology 46 (5): 579—592.

—»— 1965b: Vertical migration of Pontoporeia affinis (Amphipoda) in Lake Michigan. — Publ. 13,

Great Lakes Research Division, The University of Michigan.

McNaught, D. C. & Hasler, A. D. 1966: Photoenvironments of planktonic Crustacea in Lake Michigan. — Verh. Int. Ver. Limnol. 16: 194—203.

Segerstråle, S. G. 1937a: Studien über die Bodentierwelt in südfinnländischen Küstengewässern. III. Zur Morphologie und Biologie des Amphipoden Pontoporeia affinis, nebst einer Revision der Pontoporeia-Systematik. — Comment. Biol. 7(1): 181.

—»— 1937b: 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 coast of Finnland — 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.

—»— 1962: Investigations on Baltic populations of the bivalve Macoma baltica (L.). Part II. What are the reasons for the periodic failure of recruitment and the scarcity of Macoma in deeper waters of the inner Baltic? — Comment. Biol. 24(7): 1—26.

—»— 1967: Observations of summer-breeding in populations of the glacial relict Pontoporeia affinis Lindstr. (Crustacea, Amphipoda), living at greater depths in the Baltic sea, with notes on the reproduction of P. femorata Kröyer. — J. Exper. Mar. Biol. Ecol. 1: 55—64.

—»— 1970: Light control of the reproductive cycle of Pontoporeia affinis Lindstr. (Crustacea, Amphipoda). — J. Exper. Mar. Biol. Ecol. 5: 272—275.

—»— 1977: The taxonomic status and prehistory of the glacial relict Pontoporeia (Crustacea Amphipoda) living in North American lakes. — Comment. Biol. 89: 1—18.

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.

Wells, L. 1968: Daytime distribution of Pontoporeia affinis off bottom in Lake Michigan. — Limnol. Oceanogr. 13 (4): 703—706.

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