

The Diurnal Modulation of the Circatidal Activity Rhythm by Feeding in the Isopod *Eurydice pulchra*

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The diurnal modulation of circatidal activity was studied in freshly caught *Eurydice pulchra* of different feeding states. Apparently fully fed specimens displayed definite circadian modulation, with greatest tidal activity at the time of expected night-time high tides, and apparently starved animals showed no circadian variation. Circatidal activity was also entrained in the laboratory using cycles of artificial agitation and the subsequent pattern of free-running rhythmicity was studied. Animals fed prior to entrainment displayed definite diurnal modulation of the pattern of circatidal swimming activity. Those fed after the entrainment regime, or not at all, displayed no apparent diurnal inequality of tidal activity peaks. No relationship between diurnal inequality and phototactic behaviour of freshly collected specimens was detected.

KEY WORDS: Rhythms, circadian, circatidal, feeding, *Eurydice*

INTRODUCTION

Eurydice pulchra (Leach) is a predacious cirrolanid isopod found commonly in the intertidal zones of high energy sandy beaches (Fish 1970). It swims and feeds actively only at high tide when the beach is flooded and remains buried in the sand throughout the low-tide period. In common with many intertidal species *E. pulchra* is

known to have an endogenous circatidal activity rhythm which cues emergence from the sand as the tide comes in and reburrowing after high tide (Jones and Naylor, 1970; Fish and Fish, 1972; Alheit and Naylor, 1976). This rhythm is known to be endogenous and is entrained by exposure to periods of wave turbulence in the surf at high tide (Hastings and Naylor, 1980).

Diurnal modulation of the circatidal rhythm in *E. pulchra* has been recorded both on the shore (Jones and Naylor, 1970; Fish and Fish, 1972; Alheit and Naylor, 1976) and in constant conditions in the laboratory (Hastings and Naylor, 1980). Diurnal modulation of tidally active behaviour on the shore can be easily explained in terms of the negatively phototactic behaviour of some parts of the population (Jones and Naylor, 1970; Macquart-Moulin, 1971, 1973). Constant negative phototaxis would not, however, explain the diurnal modulation in constant conditions. Recordings of *Eurydice pulchra* in constant conditions will sometimes show strong evidence of diurnal modulation and on other occasions no such modulation will be seen (Hastings and Naylor, 1980; Hastings, 1981; Reid and Naylor, 1985 and 1986).

Jones and Naylor (1970) showed that the response of *Eurydice* to light varied according to the animal's feeding state; starved animals were found to be positively phototactic while fed animals were negatively phototactic. As *Eurydice* appears to be more active on night-time high tides, it would appear that these two phenomena may be interrelated. The present investigation therefore set out to examine the relationship between feeding state, response to light (positive and negative phototaxis) and any diurnal modulation of the circatidal rhythm.

MATERIALS AND METHODS

Adult specimens of *E. pulchra* were collected from the exposed sandy beach at Llanddwyn, Anglesey, by towing a net in the surf on falling spring high tides when the animals are known to be most abundant (Fish and Fish, 1972; Alheit and Naylor, 1976). Juveniles of less than 4.0 mm body length were excluded from the study as it has been shown that they do not display diurnal modulation of their circatidal swimming pattern (Hastings and Naylor, 1980).

The fresh animals were divided according to either feeding state or phototactic response. To determine feeding state, animals were examined individually under a dissecting microscope with back lighting. For the purposes of this study, only those animals with obviously full or obviously empty guts were selected. The animals were then subdivided into groups of 20 and placed into the actographs as described below.

To determine phototactic response, animals were placed in a small tank with sea water and illuminated from one end. Samples were then drawn from those animals which were clustered around the light source and from those obviously repelled by it to the other end of the tank. Each sample was then placed in an identical tank and sampled again. Only those animals which were positively phototactic in both trials or negatively phototactic in both were subsequently used. Each sample was then placed in groups of 20 into the actographs as described below.

For the laboratory entrainment experiments, a population of *E. pulchra* was maintained in the laboratory without food for over a month. Any dead animals or exuvia were removed to prevent cannibalism. The animals were then divided into two groups. One group was allowed to feed to satiation on *Mytilus edulus*. The other group was checked to ensure empty hind guts and any animals which had apparently fed were discarded. The two groups were then subjected to an artificial agitation entrainment regime as described in Reid and Naylor (1985). The animals were placed in flasks provided with clean sand and sea water. Agitation, mimicking wave turbulence, was for 2 hours in every 12 hours for 4 days (8 cycles) and was applied around midday and midnight, as this is known to induce a strong circatidal activity pattern (Reid and Naylor, 1985). The light regime was 12L:12D with lights on at 0600 h and off at 1800 h. After the end of the last period of agitation, the fed animals were sorted and placed in groups of 20 into the actographs. The second, starved, group were subdivided into 2 groups. The first group was again checked for any evidence of having fed and then placed in groups of 20 into the actographs. The second group were allowed to feed to satiation on mussel flesh, checked and then placed into the actographs. In both cases feeding took less than 30 minutes, during which the other groups were held in similar conditions in neighboring containers. Examination

was by binocular microscope and as with the feeding period was under normal laboratory light conditions. Care was taken to ensure that, as far as possible, the only differences between the 3 groups were whether, and when fed.

The activity of all groups of animals was recorded in constant conditions (D:D, 15°C) in a controlled environment room. The actograph system used is as described in Reid and Naylor (1986). Results are expressed as percentages of the greatest number of activity events in any one 15 minute period (to permit direct comparisons between traces) against elapsed time. The data from each experiment are also plotted in the form of a 25 hour form estimate, allowing a comparison of the amplitude of activity on successive night (dark) and daytime (light) high tides (or agitation periods). A 25 hour form estimate, rather than 24 hours, is used as the induced rhythm is circatidal (c. 12.5 hours), the form estimate is thus of a bitidal, or lunidian period and not a solar (24 hour) day period. Results are again expressed as percentages for ease of comparison, with the night-time peak first in every case. To determine the significance of the differences between the two peaks of the form estimate, *t*-tests were performed on 6 hour segments of the data centred on the two peaks of activity. All data analysis, with the exception of Student's *t*-test, was carried out on a BBC "B" Microcomputer, using programs written by the author. *T*-tests were carried out using Statpack implemented on the DECsystem10 at U.C.N.W., Bangor.

RESULTS

The activity pattern in constant conditions of a group of 20 freshly collected *Eurydice pulchra* with obviously full guts is presented in Figure 1(a). The pattern is clearly circatidal but it can also be seen that activity on expected night-time high tides is greater than on expected daytime high tides. Analysis of the 25 hour form estimate of these data (Figure 1(b)) confirms that the amplitude of activity on night-time high tides is significantly greater than daytime high tides (Student's *t*-test $P < 0.001$). A group of 20 animals collected simultaneously and selected as having empty guts displayed a different pattern of circatidal swimming activity (Figure 2(a)) with

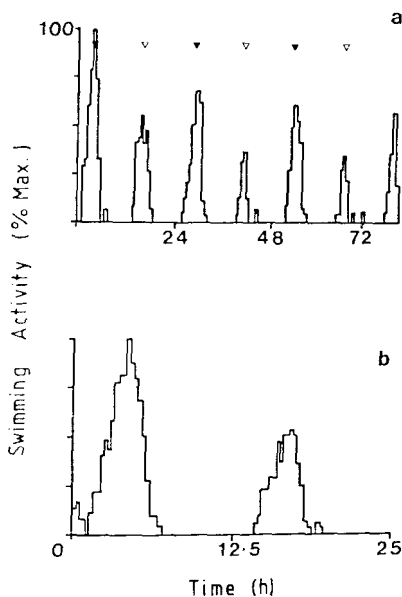


FIGURE 1 (a) 1/4 hourly swimming activity values recorded for 20 *E. pulchra* over 3 days in constant conditions after collection at spring high tide on the shore and selected as fed. Closed arrows indicate time of expected night-time high tides; open arrows indicate time of expected daytime high tides. (b) 25 hours form estimate of data in (a) above. The first peak occurs at night in this and in all subsequent form estimates.

little apparent difference between activity amplitude on night and daytime high tides. This is confirmed by the 25 hour form estimate of the same data (Figure 2(b)). No significant difference (Student's *t*-test $P = 0.2$) was detected between activity amplitude on night or daytime high tides.

To confirm the importance of feeding state in the modulation of expression of the circatidal rhythm, artificial entrainment experiments were carried out on a population maintained in the laboratory for over a month without food. Activity records for a group of 20 such animals which were then fed to satiation immediately before entrainment by simulated tidal turbulence are presented in Figure 3(a). The activity pattern is apparently circatidal with peaks coinciding with times of expected induced high tides. The amplitude of activity is, however, much greater at times

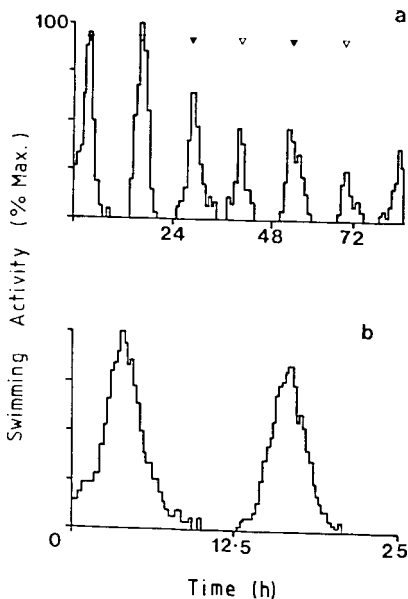


FIGURE 2 (a) 1/4 hourly swimming activity values recorded for 20 *E. pulchra* over 3 days in constant conditions after collection at spring high tide on the shore and selected as starved. Arrows as in Figure 1. (b) 25 hour form estimate of data in (a) above.

of expected agitation with the lights off. This is confirmed by the 25 hour form estimate of these data (Figure 3(b)), showing a significantly lower amplitude when agitation was expected with the lights on (Student's t -test $P < 0.0001$).

A different experimental design was used to determine whether feeding state acts directly to modify the expression of the circatidal rhythm or whether it does so by affecting the entrainment mechanism. A group of 20 previously starved animals was fed to satiation *after* entrainment and prior to recording its activity in constant conditions (Figure 4(a)). Unlike the animals fed *before* the entrainment regime was applied, this group displayed no significant difference (Student's t -test $P = 0.5$) between the amplitude of activity induced by expected activity with the lights off or on.

As a control to the two experiments above (cf. Figures 3 and 4), the activity pattern was recorded for a group of 20 starved animals entrained by agitation in a 12:12 LD regime without any feeding

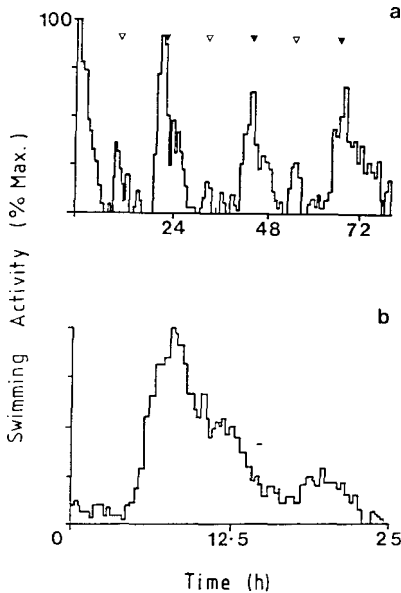


FIGURE 3 (a) 1/4 hourly swimming activity values recorded for 20 *E. pulchra* over 3 days in constant conditions after being starved for 1 month, fed to satiation and exposed to an agitation regime of 2 h every 12 h for 4 days (around noon and midnight). Arrows indicate time of expected agitation: closed-dark phase; open-light phase. (b) 25 hour form estimate of data in (a) above.

(Figure 5(a)). The pattern is clearly circatidal and there is no significant difference (Student's *t*-test $P=0.3$) between activity amplitude at times of expected agitation with lights on or lights off (Figure 5(b)). It would thus appear that the most important factor in inducing diurnal modulation of the circatidal rhythm in *E. pulchra* is the feeding state during entrainment of the circatidal rhythm.

Finally, since it has been reported that satiated *Eurydice* exhibit negative photoaxis (Jones and Naylor, 1970), it seemed worthwhile to determine whether animals which are negatively phototactic will also entrain a diurnally modulated circatidal rhythm. A group of freshly collected *eurydice* were therefore divided into negatively and positively phototactic animals. The subsequent activity patterns in constant conditions are presented in Figures 6(a) and 7(a)

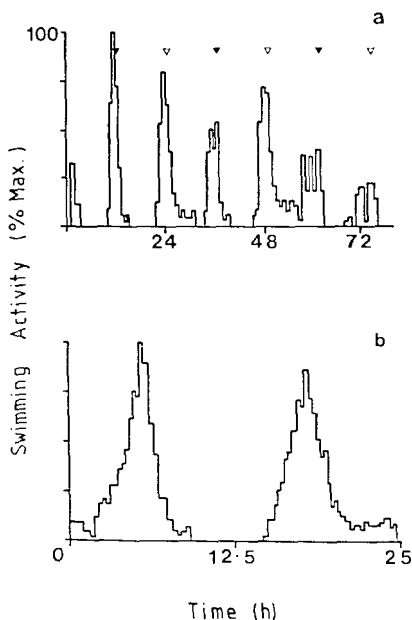


FIGURE 4 (a) 1/4 hourly swimming activity values recorded over 3 days in constant conditions for 20 *E. pulchra* treated as in Figure 3(a) but fed after agitation regime not before. Arrows as in Figure 3(a) above. (b) 25 hour form estimate of data in (a) above.

respectively. Both groups show clear circatidal patterns of swimming activity and there is no significant difference in amplitude between activity at times of expected night or daytime high tides (Figure 6(b), Student's *t*-test $P = 0.2$; Figure 7(b), Student's *t*-test $P = 0.5$). (The significance of these findings is discussed below.)

DISCUSSION

The present investigation unequivocally confirms the existence of diurnal modulation of the circatidal rhythm in *Eurydice pulchra* previously described by Hastings and Naylor (1980) and describes some conditions under which such modulation occurs. For example,

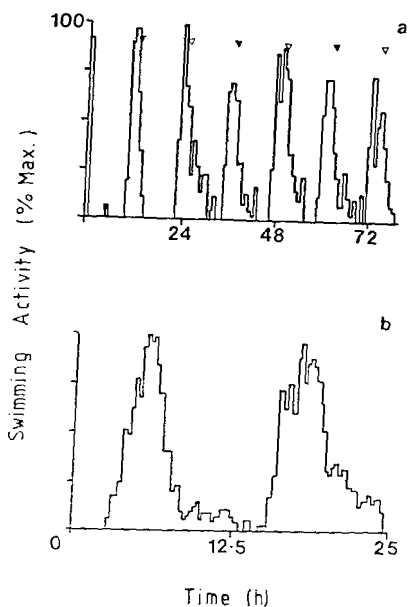


FIGURE 5 (a) 1/4 hourly swimming activity values recorded over 3 days in constant conditions for 20 *E. pulchra* treated as in Figure 3(a) but with no feeding at all. Arrows as in Figure 3(a) above. (b) 25 hour form estimate of data in (a) above.

animals freshly caught on the shore at spring high tides and selected as having full guts displayed a clear circadian modulation with a greater amplitude of swimming activity on night-time high tides. Furthermore, starved animals which were then fed to satiation in the laboratory prior to entrainment by an artificial agitation regime also showed a clear circadian difference in amplitude of total activity peaks. Moreover, those animals which were fed in the laboratory show a much more marked diurnal modulation when compared with freshly captured specimens, perhaps because, overall, they had more recently ingested food. It would appear, therefore, that feeding state is of great importance when considering the process of entrainment of circatidal rhythmicity in *Eurydice*.

Starved animals which were entrained by artificial tidal cycles of wave turbulence and then subsequently fed prior to being placed into the actographs, showed no apparent diurnal modulation

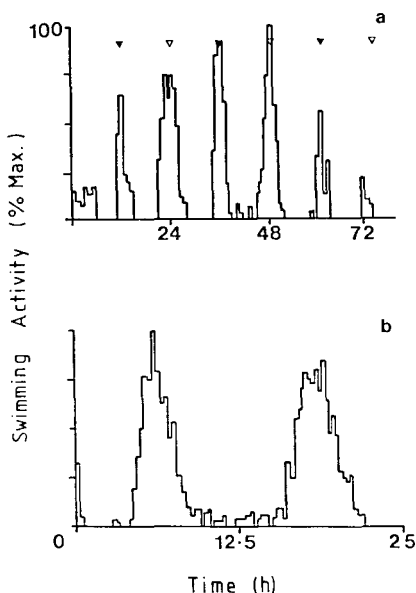


FIGURE 6 (a) 1/4 hourly swimming activity values recorded over 3 days in constant conditions for 20 *E. pulchra* after collection at spring high tides and selected as negatively phototactic (see Materials and Methods). Arrows as in Figure 1(a) above. (b) 25 hour form estimate of data in (a) above.

pattern. This suggests that the overt activity pattern is controlled by the endogenous rhythm and is not directly altered by a change in feeding state. Feeding state appears, therefore, to affect the rhythm by modifying the entrainment mechanism. This is analogous to the effect of low temperature on the circatidal rhythm, which was found to alter the amplitude of expression if applied during entrainment but not if applied after entrainment (Reid, 1986).

Previous work with *Eurydice* has suggested that feeding state is involved in determining the sign of the phototaxis response (Jones and Naylor, 1970). Animals which had fed recently were found to move away from light while those which had been starved move towards it. It might be expected, therefore, that as fed animals display a circatidal rhythm which is diurnally modulated, a similar pattern would be found in animals which display negative phototaxis. No such relationship was detected in the present

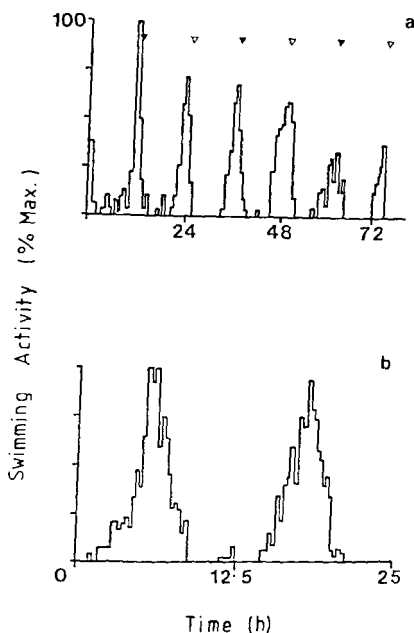


FIGURE 7 (a) 1/4 hourly swimming activity values recorded over 3 days in constant conditions for 20 *E. pulchra* after collection at spring high tides and selected as positively phototactic. Arrows as in Figure 1(a) above. (b) 25 hour form estimate of data in (a) above.

investigation. Furthermore, starved animals, previously shown to be positively phototactic (Jones and Naylor, 1970), would remain so even after a prolonged feeding bout (Reid 1988, in press). The control of phototactic sign in *E. pulchra* is clearly much more complex than the model postulated by Jones and Naylor (1972). Circatidally rhythmic variation in phototactic sign is now known to occur in this species (Reid, in press) and in others e.g. the sand beach amphipod *Synchelidium* (Forward 1980).

Circadian rhythms of emergence and swimming have been demonstrated in a number of crustaceans e.g. in the closely related species *Eurydice affinis* (Macquart-Moulin, 1973, 1977), and circadian modulation of circatidal swimming patterns have been described in *E. Pulchra* (Hastings and Naylor, 1980). However, the

demonstration in the present investigation of circadian modulation of the circatidal rhythm, dependent on feeding state, is new. The adaptive significance of such swimming predominantly on night-time high tides may be the avoidance of visual predators as suggested by Jones and Naylor (1970). A similar rationale has been suggested for the dusk spawning of some sciaenid fishes (Holt, Holt and Arnold, 1985) which may thus avoid high predation when the eggs are most concentrated. It is nevertheless important to consider why the animals should emerge and swim at night at all, particularly since they are known to take up to 3 weeks to digest fully the contents of the gut (Jones, 1968) and it would seem to be more sensible to avoid potentially risky excursions into the surf unless required. An explanation may be found in another important reason for swimming at high tide. Alheit and Naylor (1976) suggested that one of the functions of circatidal swimming on falling spring tides was to facilitate downshore migration and thus prevent stranding above the neap high water mark. While *Eurydice* may be able to tolerate stranding for extended periods above HWN (Jones, 1968; Ali, 1981), it would obviously be advantageous for the isopods to avoid it. Animals which had fed to near satiation would presumably not need to emerge and swim to forage but would still have to do so if swimming serves to alter their zonation pattern as suggested by Alheit and Naylor (1976). Further consideration of the adaptive value of rhythmic swimming in *Eurydice* is clearly required, not least to consider why feeding does not induce an immediate modulation of the circatidal rhythm. If circadian modulation has evolved to reduce the amplitude of daytime swimming activity in response to predation, it would be advantageous for it to do so without the delay implicit in the mechanism described here.

Two important points emerge from this study. First, circatidal swimming clearly functions as more than just a foraging exercise and is also involved in zonation maintenance. Second, the expression of circatidal activity rhythms is not a physiological imperative but can be modulated according to other parameters; in this case the recent foraging history of the individual animal.

References

- Alheit, J. and Naylor, E., (1976). Behavioural basis of intertidal zonation in *Eurydice pulchra* Leach. *J. exp. Mar. Biol. Ecol.*, **23**, 135-144.

- Ali, Y. O., (1981). Feeding biology of *Eurydice pulchra*. MSc thesis, University of Wales, 32 pp.
- Fish, S., (1970). The biology of *E. pulchra* (Crustacea: Isopoda). *J. Mar. Biol. Ass. U.K.*, **50**, 753-768.
- Fish, J. D. and Fish, S., (1972). The swimming rhythm of *Eurydice pulchra* Leach and a possible explanation of intertidal migration. *J. exp. Mar. Biol. Ecol.*, **8**, 195-200.
- Forward, R. B. (1980). Phototaxis of a sand beach amphipod: physiology and tidal rhythms. *J. Comp. Physiol.*, **135A**, 243-250.
- Hastings, M. H. and Naylor, E., (1980). The ontogeny of an endogenous rhythm in *Eurydice pulchra*. *J. exp. Mar. Biol. Ecol.*, **46**, 137-145.
- Hastings, M. H., (1981). The entraining effect of turbulence on the circatidal activity rhythm and its semilunar modulation in *Eurydice pulchra*. *Mar. Ecol. Prog. Ser.*, **4**, 85-90.
- Holt, G. J., Holt, S. A. and Arnold, C. R., (1985). Diel periodicity of spawning in Sciaenids. *Mar. Ecol. Prog. Ser.*, **27**, 1-7.
- Jones, D. A., (1968). The functional morphology of the digestive system in the carnivorous intertidal isopod *Eurydice*. *J. Zool. Lond.*, **156**, 363-376.
- Jones, D. A. and Naylor, E., (1970). The swimming rhythm of the sand beach isopod *Eurydice pulchra*. *J. exp. Mar. Biol. Ecol.*, **4**, 188-199.
- Macquart-Moulin, C. (1971). Modifications des reactions photocinetiques des peracarides de l'hyponeston nocturne en fonction de l'importance de l'enclairement. *Tethys*, **3**(4), 897-920.
- Macquart-Moulin, C. (1973). L'activite natatoire rythmique chez les peracarides benthoplanctoniques. Determinisme endogene des rythmes nycthemeraux. *Tethys*, **5**(1), 209-231.
- Macquart-Moulin, C. (1977). Le controle de l'emergence et des nages nocturnes chez les peracarides des plages Mediterranee. *Eurydice affinis* Hasen (Isopoda), *Gastrosaccus mediterraneus* Bacascu, *Gastrosaccus spinifer* Goes (Mysidacea). *J. exp. mar Biol. Ecol.*, **27**, 61-81.
- Reid, D. G. (1986). The rhythmic biology of a marine isopod crustacean. Ph.D. Thesis, University of Wales, 111 pp.
- Reid, D. G. and Naylor, E. (1985). Free-running, endogeneous semilunar rhythmicity in a marine isopod crustacean. *J. Mar. Biol. Ass. U.K.*, **65**, 85-91.
- Reid, D. G. and Naylor, E. (1986). An entrainment model for semilunar rhythmic swimming behaviour in the marine isopod *Eurydice pulchra* Leach. *J. Exp. Mar. Biol. Ecol.*, **100**, 25-35.