WADI Project

Protocol for locomotor activity rhythm study on amphipods in the Maremma Regional Park (Italy) and in Lake Maryuit (Egypt)

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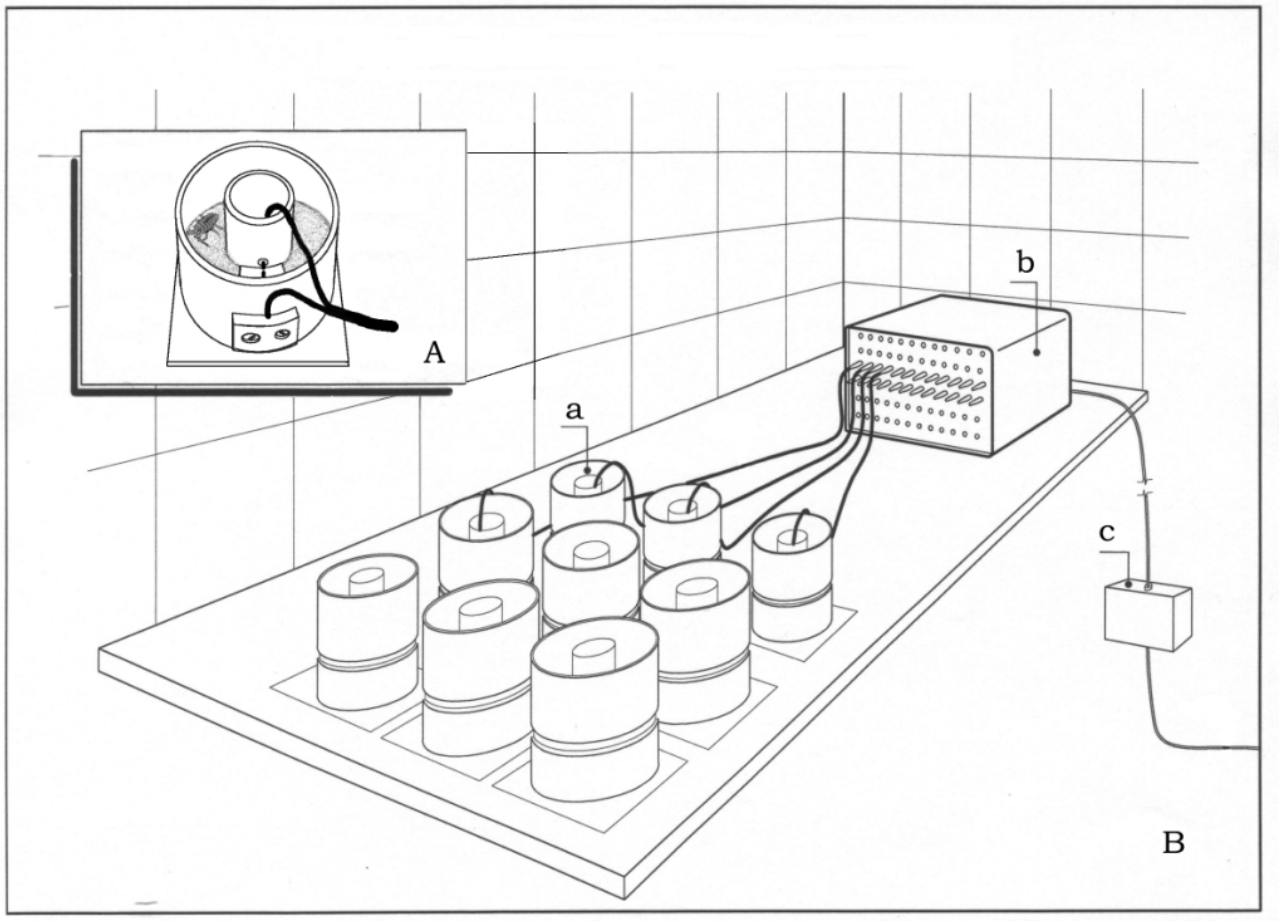
Adult amphipods (longer than 0.5cm) were collected from the site by hand or by an entomological putter, placed in a covered bucket with some moist sand and aerated during the travel to the laboratory.
The recording of the activity of single specimens started within 24h from the collection.

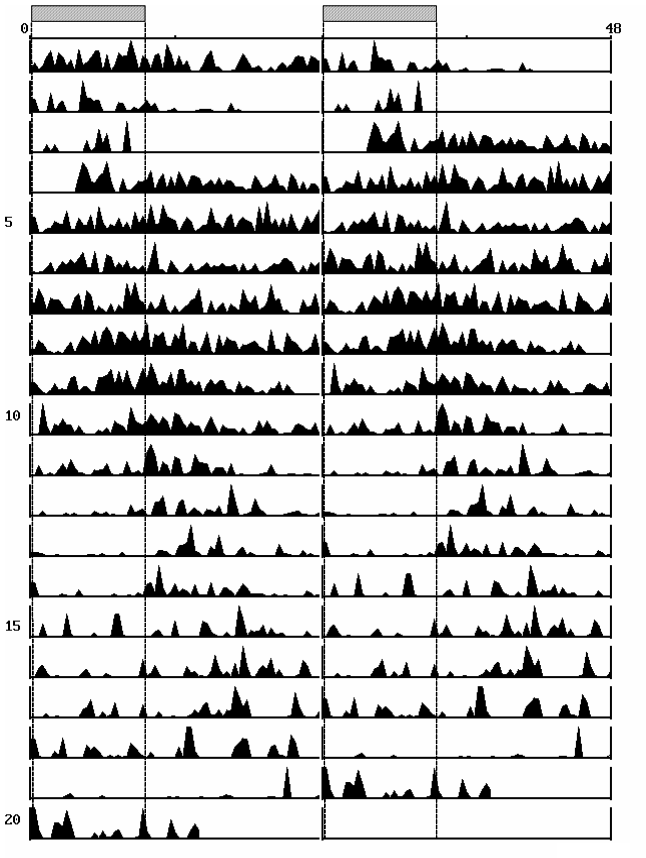
Recording system:
The apparatus for rhythm recording consisted of 40 opaque, annular PVC chambers (12 cm in diameter, 16cm high) with a concentric cylinder (∅ 3.5cm) as support and protection for the infrared emitter (Pk 950nm, 40mW, 100mA). The infrared receiver (Pk 950nm) was positioned at a corresponding height on the external side of the recording chamber. A Plexiglas basement 3cm high was the basis on which the infrared ray crossed the annulus of the recording chamber. The chamber was filled with about 3 cm of moist substratum (sand or soil depending on the amphipod species) and covered by a black polythene sheet to prevent water loss. Dry fish food (TetraMin) was provided *ad libitum* on a piece of filter paper positioned at the opposite side of the chamber with respect to the infrared ray. The individuals (one in each recording chamber) disrupted the infrared ray, and the number of interruptions were transferred to a computer (output: 5V for 5mS lasting) every 20 min, via a data logger, using the *Sand24* software program (D.D. Green, Biosciences Electronics Workshop, University of Birmingham, Birmingham, U.K.). The recording lasted 21 days in constant dark conditions at a constant temperature of 18°±1°C.

Processing Software:
The *Roto24* (D.D. Green, Biosciences Electronics Workshop, University of Birmingham, Birmingham, U.K.) software was used to separate the final file in 40 separate files (one for each channel) and the *Chart35* (D.D. Green, Biosciences Electronics Workshop, University of Birmingham, Birmingham, U.K.) to obtain an actogram for each animal. Period analysis was done with the *Time series* software (Gerard Harris Computing, Rockwell Park, Bristol, U.K.) producing periodograms (length and definition of the period).

Data analysis:
On each sample we calculated the survival, number of active animals (animals being active for more than 2/3 of the whole length of the recording), number of periodic animals (those chosen among the active animals and having a significant periodogram), period length (in hours) and definition (Signal to Noise Ratio, i.e. ratio between the value of the Periodogram Correlation Ratio and the 95% probability line in the Periodogram graph).



A: recording chamber with two cables for infrared emitter and receiver
B: whole apparatus with a. recording chambers, b. logger, c. interface to the personal computer.



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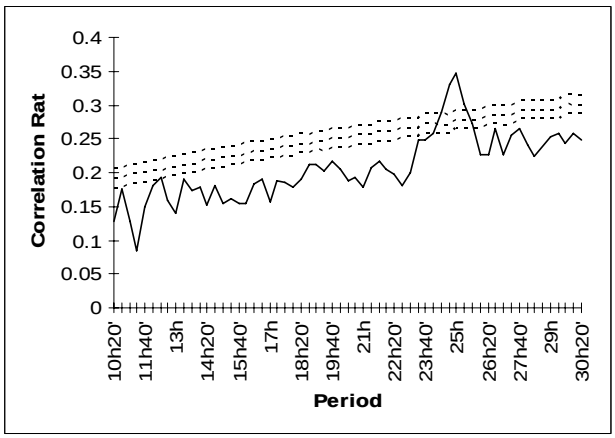

D

C: Actogram of a single individual, bars at the top of the figure indicate times of natural darkness. Actograms show the activity of each individual expressed as a percentage of the maximum recorded each day. Activity recorded in each 24 h is double plotted on the right of and below first 24 h.
D: Significant periodogram of the same individual, on the y axis the period definition and on x axis the period length.