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SHORT COMMUNICATION

Locomotor activity rhythms in high arctic freshwater crustacean: *Lepidurus arcticus* (Branchiopoda; Notostraca)

Vittorio Pasquali*

Neuroscience Section, Department of Psychology, SAPIENZA University of Rome, Rome, Italy

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Introduction

All living organisms have “biological clocks” that regulate physiological and behavioural functions by means of rhythms similar to the geophysical rhythms of the earth; these rhythms have an evident adaptive value to enable organisms to anticipate and, hence, to prepare for predictable changes in their environment (King & Takahashi 2000). This occurs as interaction between the clocks and exogenous time cues (Zeitgebers), the most prominent of which is the 24-h light–dark (LD) cycle. However, in polar environments, the strength of this Zeitgebers is greatly reduced around the summer and winter solstices, when the sun never sets or never rises.

The presence of circadian clocks is independent of the environmental conditions. There are recent evidences that when daily transitions of dusk and dawn are not present, reindeer and ptarmigan that live in the Arctic do not exhibit circadian rhythms in their behaviour and physiology. Free-ranging reindeer do not exhibit 24-h locomotor activity rhythms in summer and winter (continuous light and continuous darkness). Differently, during the equinoxes, animals express a circadian regulation of locomotor activity (van Oort et al. 2005, 2007). Seasonal absence of circadian rhythmicity has been recorded also in the daily activity of the Svalbard ptarmigan (Stokkan et al. 1986; Reierth & Stokkan 1998). New molecular and hormonal evidence suggest that reindeer living at high latitudes in the Arctic lack the underlying biological clock necessary for generating circadian rhythmicity (Stokkan et al. 2007; Lu et al. 2010).

The diversity of behavioural responses, even within the limited number of species tested, is surprising and suggests that several factors may be involved in regulating circadian variability. It has been proposed that circadian clocks can be adaptively modified to enable species-specific time-keeping under polar conditions (Daan & Aschoff 1975; van Oort et al. 2007; Lu et al. 2010; Bloch et al. 2013). Thereby, clocks would be “fitted” to specific aspects of the ecology and behaviour of an organism.

Animals everywhere are confronted by environments that demand specialized behavioural and metabolic responses; for those of us intent on understanding the adaptive significance of clocks and rhythms, arctic organisms represent an excellent model in chronobiological studies.

*Email: vittorio.pasquali@uniroma1.it

The aim of the study was to collect behavioural data on locomotor activity rhythms of high arctic invertebrate *Lepidurus arcticus* to provide bases for further genetic investigations on the function of the biological clock on arctic invertebrate.

Materials and methods

Animals

The *L. arcticus* (Branchiopoda, Notostraca) lives in freshwater lakes and temporary ponds in the Arctic region, where it has a circumpolar distribution (Arnold 1966). *L. arcticus* is the only notostracan species found in permanent and temporary ponds in Svalbard. The sampled pond is situated in Ny-Ålesund (79° North) on the island of Spitzbergen, Svalbard. Animals were caught in Solvatnet pond in August 2014 during CNR Arctic Summer Campaign 2014, using a small fishing net with handle. Permits to conduct this research were issued according to the Governor of Svalbard Ref. 2014/00729-2 a.512 (Fieldwork 2014, RIS-ID 10011).

Procedure

Animals were transported to the laboratory in 5 l bottle and there they were placed in plastic flasks (250 ml) at 10 ± 1 °C in a cold room (Kings Bay AS Marine Laboratory). Conditions of continuous light (LL) or darkness (DD) were applied to different subgroups; food was provided *ad libitum*. Locomotor activity was recorded automatically by infrared beams mounted on a cell culture flask linked to a computer. Animals were monitored continuously for 5 days.

Data analysis

Chronobiological parameters were computed by different analyses. To estimate the circadian period and ultradian period, I analysed the data by the Lomb–Scargle periodogram (Lomb 1976; Scargle 1982).

Spectral analyses were also computed. Time series were smoothed (three-point moving average) and the linear trend was removed before the analysis. Power spectra were computed for each subject by discrete Fourier transform. Smoothed estimation of the spectra was obtained by a Parzen window. A one-sample Kolmogorov–Smirnov test was used to check the random origin of the spectra (white noise). In spectra significantly different from white noise, peaks above 2.81 standard deviation ($p < 0.001$) were considered significant (Conte et al. 1995).

Statistical testing was done using nonparametric methods implemented in Statistica 6.0 (Stat-Soft, USA). The Kruskal–Wallis nonparametric analysis of variance was used to test significant difference among groups.

Results and discussion

During the arctic summer, the locomotor activity of nine single animals (L1–9) and three groups of four animals (G1–3) each, was recorded. Animals were monitored in different photoperiod: six single animals (L2–3–4–5–6–8) and three groups (G1–2–3) of animals in natural condition (LL), three single animals (L1–7–9) in continuous darkness (DD) after 15 days of habituation in DD.

The amount of activity per bin of 10 min was plotted as a function of time, thus obtaining activity profiles (Figure 1). The assessment of individual animals and groups

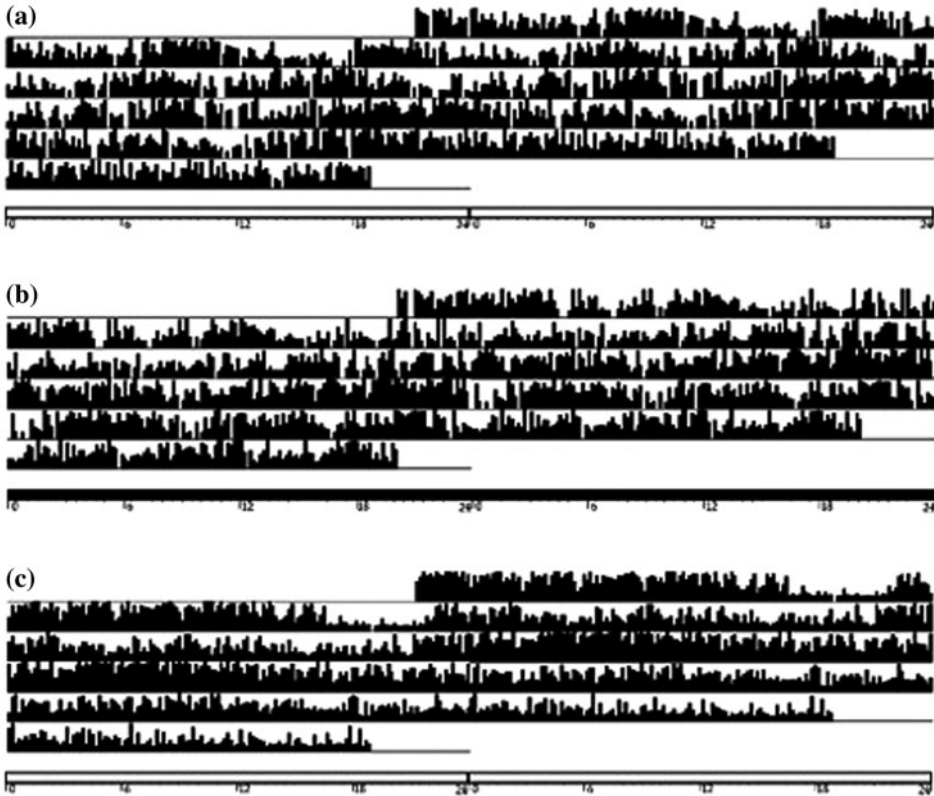


Figure 1. Double-plot actogram of three representative animals: (a) single *L. arcticus* (L6) in continuous light; (b) single *L. arcticus* (L9) in continuous darkness, and (c) a group of four *L. arcticus* (G3) in continuous light.

indicated that under our experimental conditions, *L. arcticus* showed an around the clock locomotor behaviour, but no rhythmicity was observed in actograms.

For each single animal and group, the tau in the range 18–30 h and its acrophase were computed. Under natural condition (LL), *L. arcticus* ($n = 6$) did not show circadian rhythm and only four animals showed a significant rhythmicity with a mean tau of 26.1 – 5.6 (mean – SD, range 18 – 30 h). For the acrophase, only one animal was significant $L2 = 16.1$ h. Single *L. arcticus* ($n = 3$) were also recorded in DD after 15 days of habituation to avoid the after-effect. Only two animals showed a significant rhythmicity in the range 18–30 h, $L1 = 23.1$ and $L7 = 18.1$; acrophase was only in $L7 = 16.3$. Three groups were also recorded in LL condition, they showed a period of 26.7–1.5 (mean SD, range 25–27.9) and a wide acrophase 12.2 – 6.4 h (mean – SD, range 5.9–18.7 h).

No statistical difference was observed between LL single, DD single and LL group ($H2 = 2.50$, $p > 0.05$, $n = 9$) for the tau and the acrophase ($H2 = 0.53$, $p > 0.05$, $n = 5$).

Spectral analysis (Figure 2) showed no peaks around 24 h, but revealed significant peaks for ultradian rhythms in locomotor activity in each animal. Period computed in the range 1–18 h showed a mean value in LL animals at 9.6 h 4.4 h (mean SD, range 4.2–17.1 h) and 13.9 h – 5.7 h (mean – SD, range 7.4–17.9 h) for animals in DD. In the “group” condition, the value was 9.8 h – 2.1 h (mean – SD, range 8–12.5 h).

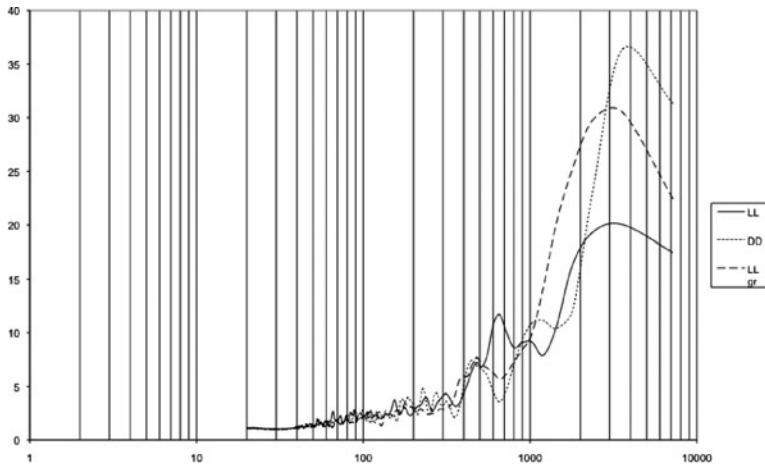


Figure 2. Mean spectral analysis of all subjects. Power values are shown on the y -axis; periods (in minute) on a logarithmic scale are shown on the x -axis.

No statistical differences were observed for the ultradian tau between the three conditions tested ($H_2 = 1.24$, $p > 0.05$, $n = 12$).

This first study on the behavioural analysis of the locomotor activity rhythms in freshwater tadpole shrimp *L. arcticus* certainly deserves further investigation. The data presented here emphasizes that *L. arcticus*, recorded individually and in groups in natural condition during the arctic summer, do not show circadian rhythm.

One could argue that the recordings were short to detect the circadian rhythm, it should be noted that the spectral analysis is a very sensitive methodology and certainly the presence of a low-amplitude rhythmicity would be identified. Indeed, studies conducted on troglitic groundwater invertebrate, *Spelaeomysis bottazzii*, with short recording of 3–5 days showed the presence of the circadian rhythm with a very low amplitude (Pasquali et al. 2007). Therefore, I can hypothesize that *L. arcticus* did not exhibit any circadian rhythm, not for an artifact, but for real non-expression. Coherently, periodogram and spectral analysis showed presence of ultradian rhythms with several peaks in the range 1–10 h. This rhythmicity was observed by the author on a wide variety of organisms (Pasquali et al. 2010; Pasquali & Sbordoni 2014 mice).

Irrespective of whether the mechanism is a stopped or disconnected arctic animals' clock, the result is an absence of daily time-keeping during constant polar days and nights. Is there a general selective pressure to lose circadian control at the poles?

The ... circadian organization to manage daily energy challenges, is a useful generalization rather than a rigid rule. The 24 h light–dark cycle may impose a strong environmental rhythmicity on an organism, but it does not necessarily follow that feeding and metabolic rhythms must follow a circadian rhythmicity ... (Hazlerigg 2014).

The cited sentence well describes an opinion to be taken into consideration when working with animals adapted to very specific habitats (e.g. polar regions, caves). Such animals may have evolved a mechanism to sustain prolonged activity around the clock without damage (Cavallari et al. 2011; Pasquali & Sbordoni 2014).

The mechanisms underlying around-the-clock activity are not well understood, but the available data suggest that several different mechanisms may lead to this extreme form of chronobiological plasticity. The prevalence of around-the-clock activity in phylogenetically distant species and the diversity of underlying mechanisms are consistent with convergent evolution. When circadian organization may indeed not be critical, ultradian organization of behaviour and physiology could also allow temporal coordination (Bloch et al. 2013).

It is too early to describe what kind of temporal organization these animals have or if their biological clock works. What we see at the moment is that *L. arcticus* in continuous light or dark do not show circadian rhythmicity, as observed in reindeer of the high arctic.

Further comparative studies are needed to determine the species' characteristics that lead to differential dependence on circadian control in arctic animals and in *L. arcticus*. Among the other approaches, genetic studies will contribute to the comprehension of the mechanisms related to around-the-clock activity.

Approaching these questions may not only shed light on the ecology and evolution of activity patterns of animals, but may also promote answers to fundamental functional and mechanistic questions in chronobiology.

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