



# A new data logger based on Raspberry-Pi for Arctic *Notostraca* locomotion investigations



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## ABSTRACT

A new data logger based on Raspberry-Pi to monitor the locomotion of Arctic invertebrates has been made, tested and deployed in field. The device uses infrared sensors to check in vivo the invertebrates picking up the locomotor activity, data are collected for the analysis. Thanks to the Raspberry Pi capabilities and features, the instrument proved to be suitable for extreme scenarios such as the polar environment, offering good performances obtained at a very low price. Preliminary test made in field have demonstrated its reliability. Some experimental considerations and the trend of the biological rhythms of the tadpole shrimp's locomotor activity are also discussed.

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## 1. Introduction

All living organisms have "biological clocks" that regulate physiological and behavioural functions by means of rhythms synchronous with the geophysical ones of the Earth. These rhythms have an evident adaptive value to allow the organisms to face predictable changes of their environment [1]. The presence of circadian clocks is independent from the environmental conditions and it has been demonstrated that those clocks can be adaptively modified to enable the time-keeping of species-specific in polar conditions [2–5]. For this reason, the polar animals that represent an excellent model for understanding the evolution of the adaptations to polar habitat, are particularly interesting regarding the biological rhythms and many studies use them to show the analysis of circadian rhythms. A useful parameter to study these rhythms is the spontaneous locomotor activity.

For small animals and invertebrates, the techniques for the detection of motor activity take advantage of sensors based on different technologies: ultrasound [6], infra-red beams [7], stabilimeters [8], running-wheels [9], capacity transducers [10], sound detectors [11], radars [12] and video-tracking [13]. Among them,

the techniques based on infra-red beam have numerous advantages: they allow the automatic monitoring of the general motor activity of the animals directly in its home cage without perturbing the pattern of its normal behaviour: it is not intrusive, it produces suitable output for direct computer analysis and it is adaptable to different conditions. Moreover, the circuit to manage the infra-red sensors is based on analog consumer electronic: inexpensive and usually easy to manage. Compared to the video-tracking system, infra-red does not need a separate illumination system for monitoring in conditions of low intensity or total absence of light.

Despite the choice of the sensor technology, previous set up were based on digital I/O board installed in full-size desktop computer [12,14]. Those systems were suitable for laboratory studies but not for field recording mostly because of their power needing and their size definitely not suitable for the shipment and deployment in remote locations.

Today, a new generation of devices, such as micro-controllers and CPU based microcomputers are available. Firstly introduced as single board computer for educational purposes they draw the attention of engineers, informatics and researchers because of their advantageous features: easy to program, portable, powerful, reliable and inexpensive; they are starting to be profitably used also in many field research.

These devices extend the possibilities of developing new automated systems easily adaptable to several experimental conditions [15–23]. Nevertheless, studies to verify the possibility to use them

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in extreme scenario such as polar or aerospace are not common in literature. For those scenarios, the key factors are reliability, resilience, low cost, low weight and small dimensions, especially in case of a network of sensors deployed on a wide area. As an example, the maintenance of a device placed in a remote area is complicated by environmental conditions and because of the intrinsic difficulty to receive new material in a base camp far away from the continent. So, the data logger must be simple, stable software and easy to deploy hardware setup.

The aim of this study is to develop, build and test a low-cost data-logger for monitoring and collect behavioural data on locomotor activity rhythms of high arctic invertebrate in natural extreme conditions. The proposed device uses infrared barrier as sensor, custom readout components and a Raspberry Pi (RPI) that controls the hardware and manage the data. For our purposes, the RPi was the optimal choice to drive the experimental setup since it meets all the requirement described above in terms of weight and dimensions. Obviously, the performances offered by the RPi are easily overcome by a Personal Computer (PC), although they are suitable for these kinds of applications and at the same time, the costs and the dimensions are also ten times smaller respect to a mid-range tower PC.

The species investigated is the *Lepidurus arcticus* (*Branchiopoda; Notostraca*), used for the first time in these kinds of research. The new system proposed wants to be the first step to provide bases for further genetic investigations on the function of the biological clock on arctic invertebrates.

## 2. Experimental set-up overview

### 2.1. General architecture of the data logger

The structure of the data logger is shown in Fig. 1. It is composed by an aquarium where the animal live, an electronic board for the conditioning of the signals that come from the aquarium and a RPi that acts as a controller of the measurements and data logger. To detect the locomotor activity of the animal, infrared barriers are mounted in the aquarium, usually three are sufficient to have a consistent detection. When the animal passes through the barrier, it signals its passage. The electric pulse generated then go through a conditioning circuit that adapt it to the input range of the I/O digital port of the RPi. Here, the signals are stored and processed according to the required pipeline.

Starting from the aquarium, the description of the single parts of the system follows. Fig. 2 shows an image of the aquarium. Each aquarium is made with a transparent plastic flask where the animals can be easily inserted (See Fig. 3).

Three different prototypes were made and tested with animals: a 200 ml flask with 4 IR sensors, a 200 ml flask with 6 IR sensors and a 40 ml flask with 9 IR sensors (not showed). We tested two different volumes and different area/sensors ratio flasks, since the movement's pattern of the *Lepidurus Arcticus* was not known *a priori*.

Locomotor activity is then detected and readout with the RPi.

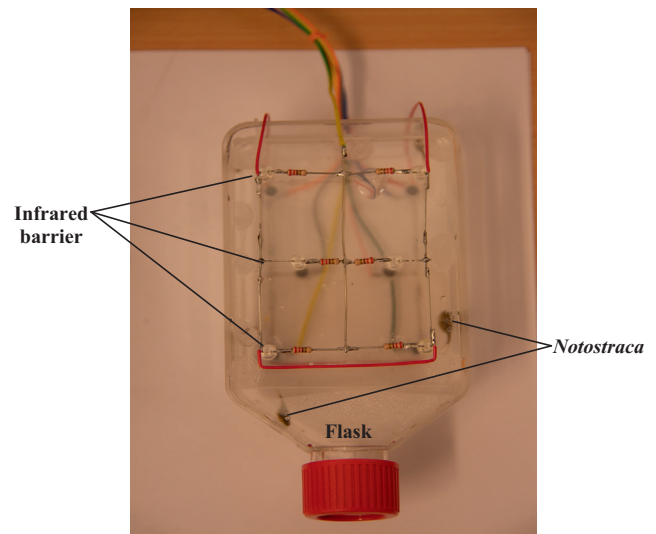


Fig. 2. The electronic aquaria with two *Notostraca* inside. It is possible to see the electronic components that realize the infrared barrier.

Infrared barriers are composed by an infrared emitting diodes with a narrow irradiance pattern, OP298B (OPTEK Inc., Carrollton USA), GaAlAs,  $\lambda$  890 nm,  $\phi$  25°,  $\varnothing$  3 mm, max output power 4.8 mW/cm<sup>2</sup> [24]; and wide receiving angle phototransistors, OP598B (OPTEK Inc., Carrollton USA) NPN,  $\lambda$  860 nm,  $\phi$  25°,  $\varnothing$  3 mm [25]. Compared to other diode-transistor couples, the couple OP298-OP598 offers a concentrated beam at different distances lens tip separation that is necessary for accurate measurements. The reliability of these products at low temperatures have been demonstrated for several applications [26]; they are also pretty easy to find on the market since they are used for enthusiast electronic projects. This aspect is fundamental in case of sudden necessity, because it is extremely difficult to manage a transport in a harsh scenario that implies a long and difficult trip.

To adapt the impedance and amplify the signal of each sensor the MC3303 operational amplifier [27] is used. A good gain-bandwidth product and a wide operational temperature range are offer from that device. An OR gate (74LS21 [28]) logically sums all the signals.

Then, a LM 555 [29] set in monostable mode provides the signal for the GPIO (General Purpose Input Output ports) of the RPi. This low cost single board credit card sized computer is the core of the instrument [30–34] with hardware and software features that makes it extremely interesting for this kind of application. The pins of the GPIO of the RPi are continuously readout by a script written in Python3 and the data are stored in text files on the mini SD card of the RPi. The software package we developed allows to easily modify the acquisition parameters, such as the sampling frequency, the collection time interval and the length of the measurement session.

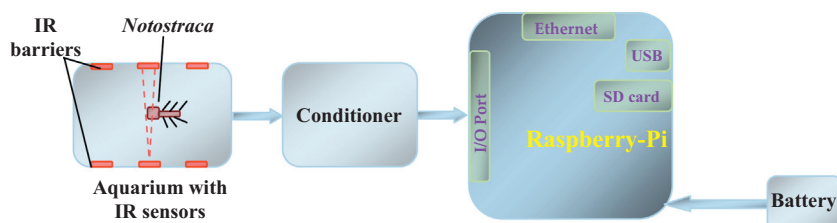


Fig. 1. Block scheme of the Data Logger.

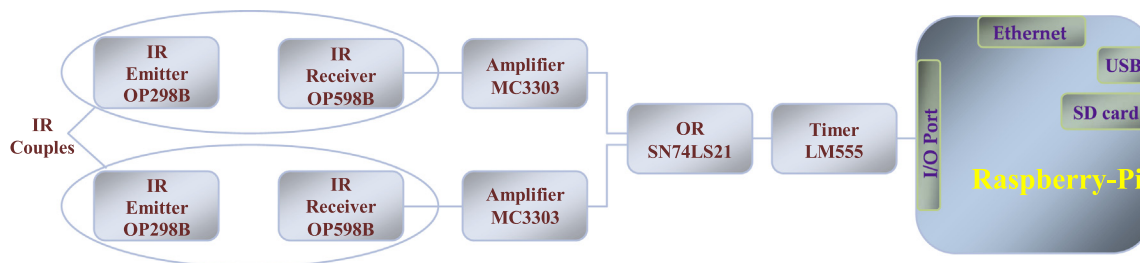


Fig. 3. Architecture of the signal conditioning circuit limited to only two IR sensors.

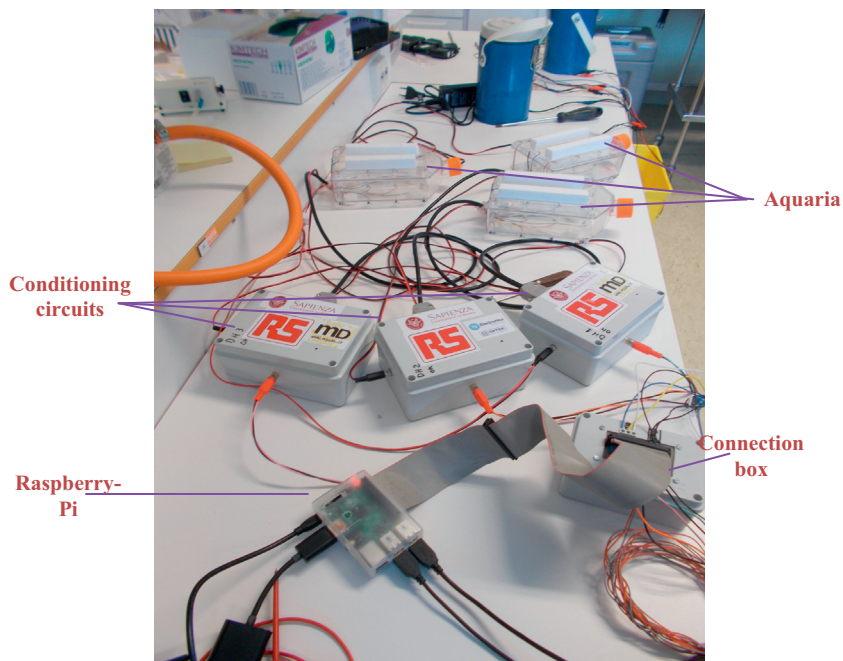


Fig. 4. The complete set-up.

Fig. 4 shows the instrument placed in laboratory. The RPi inside its transparent case; the conditioning circuits are mounted in the grey boxes, each module is connected to the RPi via a custom connection box, the data bus is made with a 40pin flat cable; on top of the picture the electronic aquaria are shown. The power is provided by a battery, properly sized for the Arctic field campaign.

## 2.2. Preliminary test

In order to be sure that the new data logger was able to correctly work at Arctic temperatures and considering the lacking information on the operative temperature on the RPi's datasheet [35], some functional preliminary test have been made. The aim of these tests was to certify the behaviour of the RPi in case of stressing test similar or worse of those that the card could meet in operative conditions. For this goal, we referred to the International Standard of IEC 60068 [36] series, which contains the fundamental information on environmental testing procedures and severities of tests. It consists of three parts:

- The first part, IEC 60068-1[37] – General and guidance, which deals with generalities;
- The second part, IEC 60068-2[38] – Tests – which publishes peculiar tests separately for several applications;
- The third part, IEC 60068-3 [39] – Supporting documentation and guidance, which deals with background information on a family of tests.

For our tests, we used the IEC 60068-2-1 [40], which deals with cold tests applicable to both non-heat-dissipating and heat-dissipating specimens and the IEC 60068-2-14 [41], which provides tests to determine the ability of components, equipment or other articles to withstand rapid changes of ambient temperature.

The aim of the cold test is to determine the ability of the board to be used, transported or stored at low temperature. The type of test made on the board is called “Ae” that indicates cold test for heat-dissipating prototypes with gradual change of temperature, specimen powered throughout. According to the standard, we considered that the specimen achieved the temperature stability during the test procedure. The sensors used to measure the chamber air temperature were located at such a distance from the board that the effect of the dissipation is negligible. The RPi was placed in the chamber, which was initially at lab temperature, about 24 °C. The temperature was then adjusted to –25 °C, appropriate for the severity of our test. Once the RPi under test reached a stable temperature, the specimen was powered on, temperature stabilized and then kept in the same conditions for 2 h (see Fig. 5). The RaspberryPi remained in the operating condition in accordance with the duty cycle and the loading condition. In our setup, we simulated the load connecting a resistor of 2000 Ω to the USB port to simulate the consumptions of the other sections of the electronics of the aquaria. The velocity of the air circulation was held very low to prevent any uncontrolled heat dissipation during the test. The RPi was then power cycled and

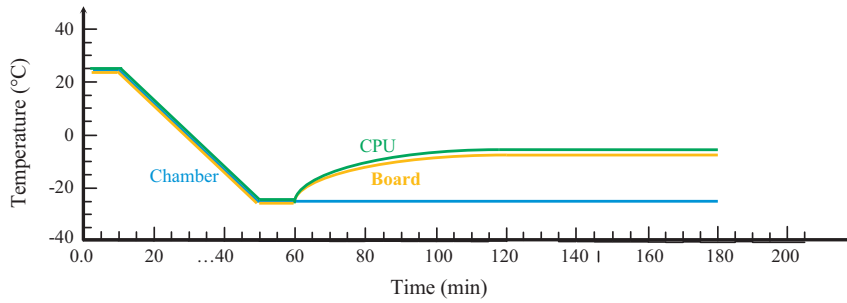


Fig. 5. Temperature of chamber, CPU and board in a test temperature realized according to the curve “Ae” of the IEC 60068-2-1: cold test for heat-dissipating specimens with gradual change of temperature.

checked fully functioning. The script loaded on board foreseen to switch on and switch off an on-board led and an external one connected to an I/O port with cycles of 1 s. As expected, the specimen remained in the operating condition in accordance with the duty cycle and at the loading condition. The change temperature rate within the chamber did not exceed 1 K per minute, averaged over a period of not more than 5 min. The temperature of incident air delivered to the sample was within  $\pm 2$  K from the test severity nominal temperature. The severity of the test, in terms of temperatures and duration of exposure, was chosen according to the standard and from the knowledge of the environment where the board would be used. To simulate the in field conditions we decided to set a lower temperature of  $-25$  °C and a duration of 96 h. As shown in Fig. 5, during the test, we measured the temperature of the chamber, of the RPi board and of the CPU. The first has been obtained using the embedded sensor, for the temperature of the RPi board we used a PT100 sensor directly mounted on it while, for the CPU we used its internal sensor. The graphs show how the three temperatures are similar from the test beginning to the switch on of the board; The thermal equilibrium between the board and the chamber is guaranteed by the slow decrease of the temperature and the low thermal inertia of the board with respect to the dimensions of the chamber. When the board is switched on, according to [42,43] the temperatures of the board and of the CPU gradually increase and settle to

15–20 °C above the chamber. Since the temperature of the CPU increases because of its activity.

At the end of the test, the board was kept in the chamber and the temperature was gradually increased to the environmental value. Even in this case, the rate of change of temperature within the chamber did not exceed 1 K per minute, averaged over a period of not more than 5 min. The RPi remained under the environmental conditions for a period of 1 h, adequate for the attainment of temperature stability. At the end, the board was visually inspected verifying that all was perfect, no oxidation or cracked soldering, and it was then tested again at environmental temperature for 12 h to conclude the test.

The second temperature test had the aim to check the behaviour of the RPi under sudden temperature changes. A change in temperature test is intended to determine the effect on the specimen of a change in temperature or a succession of changes in temperatures. The type of test made on the board is called “Na” that indicates rapid change of temperature with prescribed time of transfer and the test allows determining the ability of the RPi to withstand rapid changes of ambient temperature in air by alternate exposure to low temperature ( $-20$  °C) and to high temperature (80 °C) obtained with the use two separate chambers, one for the low temperature and one for the high temperature. Obviously, it was a concern for the operator to make the transfer between the two chambers, realized manually, in a very short time

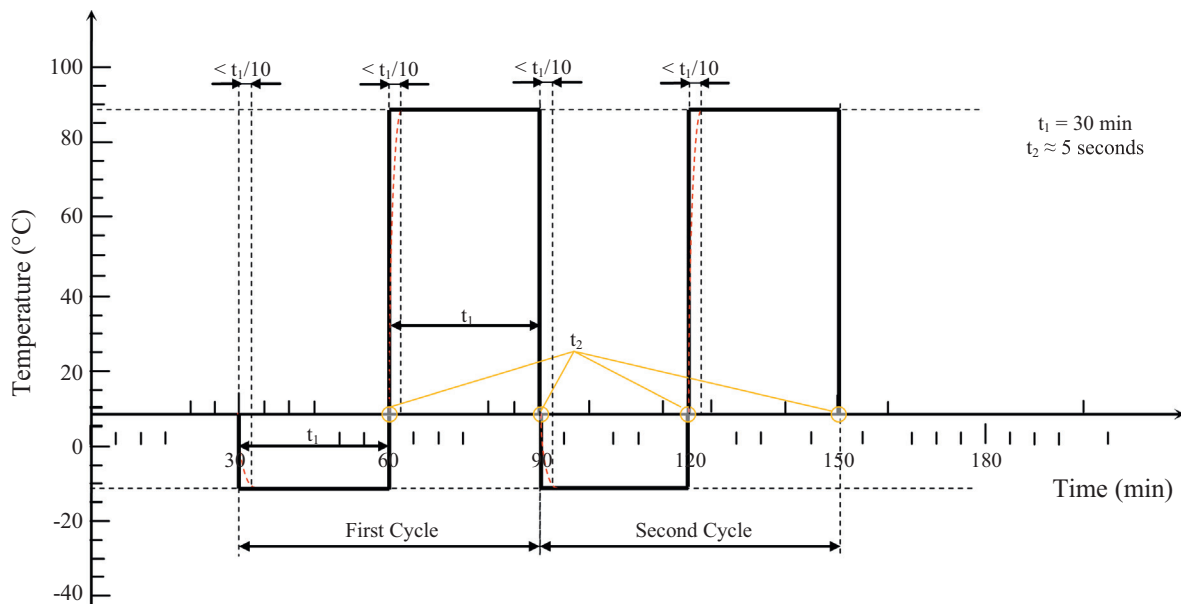


Fig. 6. Succession of changes of temperature realized according to the curve “Na” of the IEC 60068-2-14: rapid change of temperature with prescribed time of transfer.

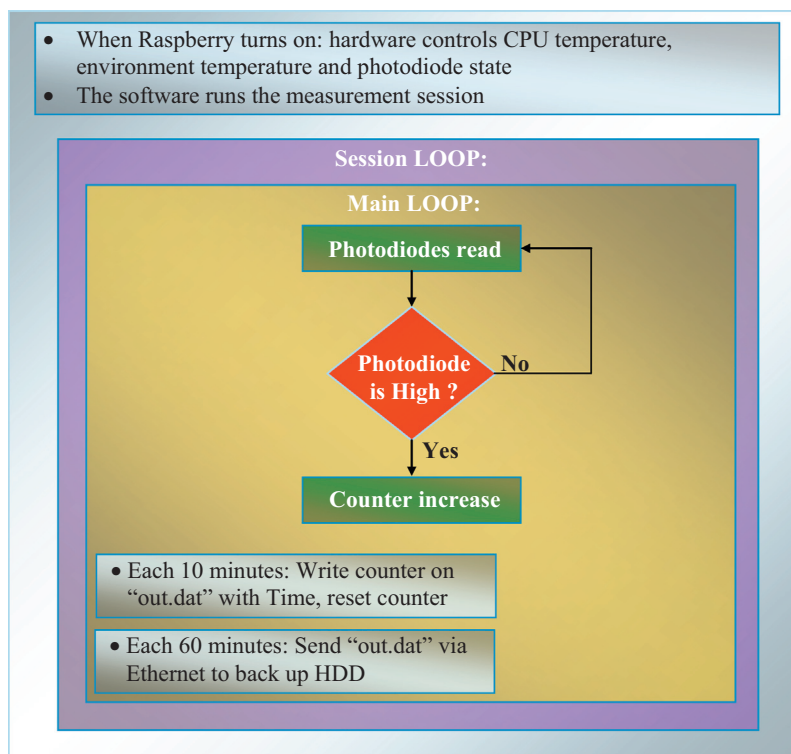


Fig. 7. Shows the block scheme of the software that runs inside the Raspberry-Pi.

( $t_2 \approx 5$  s) to avoid strong biases in the temperature measures. As suggested by the norms and considering the overall dimensions of the RPi, we decided an exposure time of 30 min ( $t_1$ ), therefore the exposure cycle time is of 1 h plus 5 s of transfer time. Fig. 6 shows the test made on the RPi.

The cycle has been repeated for five times as suggested by the norms. At the end of each test cycle, the RaspberryPi remained in standard atmospheric conditions for a period of 1 h for the attainment of temperature stability. The RaspberryPi has been visually examined and electrically tested before and after the test, controlling its good performances by means of the same script of the previous temperature test loaded on board and able to switch on and switch off a on board led and an external one connected to an I/O port with cycles of 1 s for 12 h.

### 2.3. The software

The software-hardware interface is made thanks to the *quick2-wire.gpio* library for python, freely distributed under GNU license [44]. Those methods allow the user to directly manage the Raspberry GPIO interface pins. In our script, in particular, we took advantage of the *pi\_header\_1.pin()* [44] function that allows to define the pins nature: input or output, so that the CPU is able to recognize them. The method is also able to open the pin communication with the function: *open()* [44].

The measurement technique is based on an integration cycle defined by the user under the format of a time in seconds inserted into a “while cycle” that defines the total measurement time.

Just as we are dealing with digital signals, we must read the pin state as a boolean value. This is obtained using the value function that returns the pin state in that format.

If the pin state is high it corresponds to a boolean ‘1’, otherwise is a ‘0’. We store and increase the integer variable *count\_n* within the time interval defined by the user. So, *count\_n* is the goal of

our measurement, is the variable that contains the scientific result. Data are stored in a file and eventually shown on screen for real-time spot checks. Fig. 7 shows the block scheme of the software that runs inside the Raspberry-Pi. This simple test script demonstrates how to readout a two channel setup and could be easily scaled to more complicated setups. We also point on the fact that we are taking advantage of the Python language coupled with the user-friendly RPi hardware interface to reach our goals.

## 3. Experiment

### 3.1. Animals

The tadpole shrimp *Lepidurus arcticus* (Branchiopoda, Notostraca) lives in freshwater lakes and temporary ponds in the Arctic region, where it has a circumpolar distribution [45]. *L. arcticus* is the only *notostracan* species found in permanent and temporary ponds in Svalbard. The lack of morphological changes since the past 250 million years has led to the description of *Notostraca* species as living fossils [46]. *L. arcticus* is also an important food item for birds. There is a lack of knowledge about the biology and ecology of this species, in literature there are few studies, unfortunately limited and incomplete.

In lakes and ponds, *Lepidurus* may be highly abundant and moves actively around on the sediment surface most of the time. *Lepidurus* is described as a scavenger, that eats sediments and small organisms living within. *L. arcticus* has a pelagic life style until it reaches the 5th instar when it becomes benthic and starts preying primarily on other invertebrates.

The arctic aquatic environment is nutrient-poor and a limited amount of food is available to the inhabitants. An optimal foraging strategy, therefore, would be to feed non-selectively on as many food items as possible. Although *Lepidurus* is primarily a benthic organism it is able to swim freely in the water column [45].

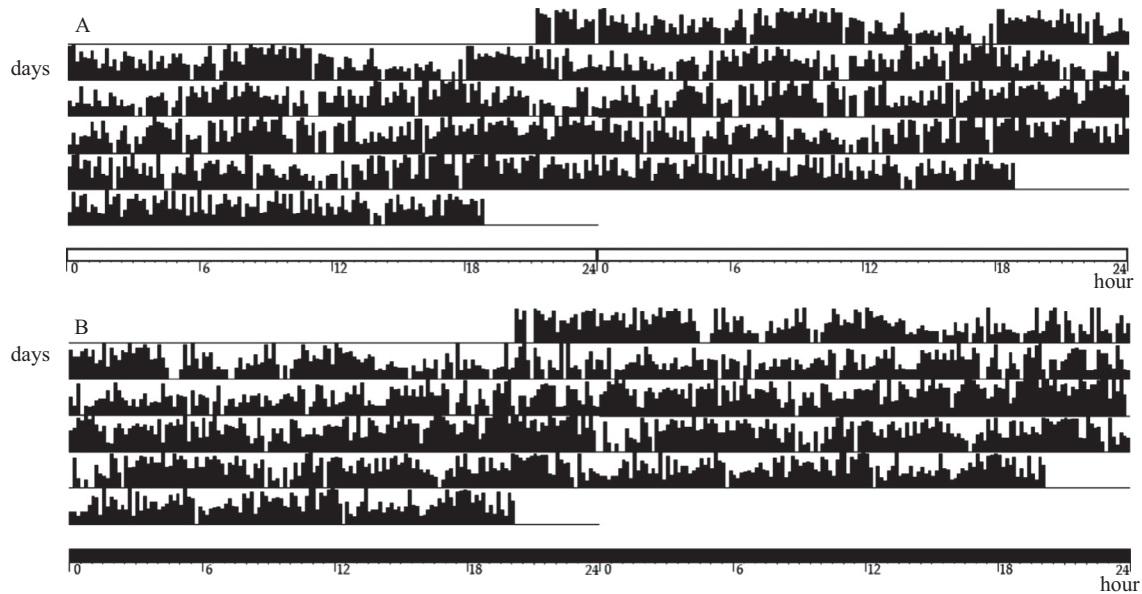


Fig. 8. Double-plot actogram of two representative animals: (A) sigle *L. arcticus* (L6) in natural condition (LL); (B) sigle *L. arcticus* (L9) in continuous darkness.

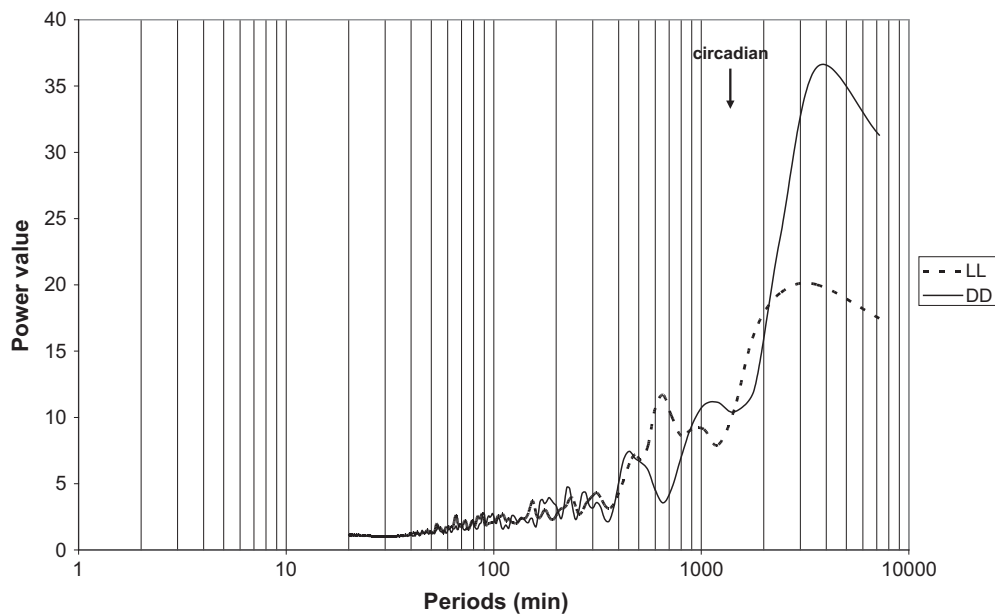


Fig. 9. Mean spectral analysis of all subjects. Power values are shown on the y-axis; periods (in minute) on a logarithmic scale are shown on the x-axis.

A quantitative analysis of the general locomotor activity is particularly interesting for the behavioural ecology of this species but also because they live in not so deep water and they are on the effect of the photoperiod.

*L. arcticus* may potentially act as an indicator species to environmental change in the Arctic freshwaters [47,48], but also as a model for study biological rhythms in high arctic animals [49].

Animals were caught in Solvatnet pond and Brandallaguna lake in August 2014 during CNR Arctic Summer Campaign 2014, using a small fishing net with handle. Solvatnet is situated in Ny-Ålesund (79° North), Brandallaguna is situated at 3.5 km from Ny-Ålesund on the island of Spitzbergen, Svalbard. The authorization to conduct this research were issued according to the Governor of Svalbard Ref. 2014/00729-2 a.512 (Fieldwork 2014, RIS-ID 10011).

### 3.2. The procedure

The tests were conducted in field and in laboratory. The field test has been done on the animals collected in Brandallaguna. Animals were placed in plastic flasks and transported in field inside a little hut, close to the lake, to protect the flasks from the direct light of the sun. Conditions of continuous light (LL) and food *ad libitum* was provided. The number of field test is limited because of safety: the presence of polar bears in the area and logistic, is literally difficult to reach the hut from the base camp.

For laboratory test, animals were transported in bottles of 5 litres and then placed in plastic flasks at  $10^{\circ} \pm 1^{\circ} \text{C}$  in a cold room (Kings Bay AS Marine Laboratory). Conditions of continuous light (LL) or continuous darkness (DD) and food *ad libitum* also in this case was provided.

The investigation lasted for a total of thirty days subdivided in slots of six days; in each of this slots five days were used to continuously monitor the animals while a day was used to pick up the data, check them and verify that the instruments was working properly.

### 3.3. Data analysis

Chronobiological parameters were computed with different methods. To estimate the circadian period and ultradian period, the Lomb-Scargle periodogram [50,51] algorithm have been used.

Spectral analysis was also performed. Time series were smoothed (3 point moving average) and the linear trend was removed before the final analysis. Power spectra were computed for each subject by Discrete Fourier Transform (DFT). Smoothed estimation of the spectra was obtained applying a Parzen's window. A one sample Kolmogorov-Smirnov test was used to check the random origin of the spectra (white noise). In power spectra, significantly different from white noise, peaks above 2.81 standard deviation in the time-stream ( $p < 0.001$ ) were considered significant [52]. A statistical test was done using non-parametric methods available in *Statistica* 6.0 (Stat-Soft, USA)[53]. The *t*-test for independent group was used to test significant difference among groups.

### 3.4. Results and discussion

During the arctic summer, the locomotor activity of nine single animals was recorded. Animals were monitored in different photo-periods: six single animals in continuous light (LL) and three animals in continuous darkness (DD), after 15 days of habituation in DD to avoid the after-effect.

The amount of activity per bin of 10 min was plotted in the actograms as a function of time, thus activity profiles were obtained (Fig. 8). The actogram is a graphical display of a time series along two time axes. The duration of a cycle (or predicted duration of a cycle) determines the length of each plot line. Successive cycles are plotted on successive lines.

The assessment of individual animals indicated that, under our experimental conditions, *L. arcticus* showed a continuous locomotor behaviour during the 24 h, but no rhythmicity was observed in actograms. For each single animal, the period (the time elapsed for one complete oscillation or cycle) in the range 18–30 h was computed. The magnitude of the oscillation (amplitude) is then calculated and gives us information on the parameters of the circadian rhythm. Under natural condition (LL) and continuous darkness *L. arcticus* did not show significant periods in circadian rhythmicity.

Spectral analysis (Fig. 9) did not show peaks around 24 h, but revealed significant peaks for ultradian rhythms in locomotor activity in each animal. Ultradian period computed in the range 1–18 h showed a mean value of 9.6 h in LL animals and 13.9 h for animals in DD.

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

## 4. Conclusions

In this study, we described an infrared device controlled by a RaspberryPi that allows to record the locomotor activity of a polar invertebrates in natural conditions. In this work we demonstrated the reliability offered by the RaspberryPi, certified with field tests

carried out for many days and in harsh environments, such as those in Arctic. This leads the important consequence that this technology offers new opportunities for researchers working in similarly difficult environments. The second important goal achieved is the reduction in dimensions and the weight with respect to a PC-based data logger. This is extremely important for researches in remote locations where weight and dimensions are a strong constrain for containing shipment and deployment costs. Our device is directly programmable, is based on open source codes and this increases the overall flexibility of the instrument. This feature is rarely present in commercial tools that provide only predetermined functions.

Compared to other similar devices, our system has surely low cost and portability quality essential for recording of the general locomotor activity in remote locations, natural conditions and extreme scenarios. Moreover, it supports useful features like the remote control and specific script for real time data analysis that will be implemented in the next improvements of the system. The research demonstrated the functionality of our data logger for the detection of the locomotor behaviour of tadpoles. We used this instrument to measure circadian and ultradian rhythmicity in locomotor activity of *Lepidurus arcticus*.

We argue that in the near future, the use of this device will allow to investigate on the ecology and evolution of activity patterns of the animals, but may also promote answers to fundamental functional and mechanistic questions in chronobiology.

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