Deep-Sea Research I 116 (2016) 165–173

Contents lists available at ScienceDirect

Deep-Sea Research I

journal homepage: www.elsevier.com/locate/dsri

First laboratory insight on the behavioral rhythms of the bathyal crab *Geryon longipes*

J.D. Nuñez [a,1], V. Sbragaglia [b,1], J.A. García [b], J.B. Company [b], J. Aguzzi [b,*]

[a] *IIMyC, Instituto de Investigaciones Marinas y Costeras, CONICET – FCEyN, Universidad Nacional de Mar del Plata, Funes 3250 (7600), Mar del Plata, Provincia de Buenos Aires, Argentina*

[b] *Institut de Ciències del Mar (ICM-CSIC), Pg. Marítim de la Barceloneta, 37-49, 08003 Barcelona, Spain*

ARTICLE INFO

Article history:
Received 27 April 2016
Received in revised form
20 August 2016
Accepted 23 August 2016
Available online 24 August 2016

Keywords:
Geryon longipes
Biological clock
Locomotor behavior
Inertial currents
Light-darkness
Blue light
Burrowing

ABSTRACT

The deep sea is the largest and at the same time least explored biome on Earth, but quantitative studies on the behavior of bathyal organisms are scarce because of the intrinsic difficulties related to *in situ* observations and maintaining animals in aquaria. In this study, we reported, for the first time, laboratory observations on locomotor rhythms and other behavioral observations (i.e. feeding, exploring and self-grooming) for the bathyal crab *Geryon longipes*. Crabs were collected on the middle-lower slope (720–1750 m) off the coast of Blanes (Spain). Inertial (18 h) water currents and monochromatic blue (i.e. 470 nm) light-darkness (24 h) cycles were simulated in two different experiments in flume tanks endowed with burrows. Both cycles were simulated in order to investigate activity rhythms regulation in Mediterranean deep-sea benthos. Crabs showed rhythmic locomotor activity synchronized to both water currents and light-darkness cycles. In general terms, feeding and exploring behaviors also followed the same pattern. Results presented here indicate the importance of local inertial (18 h) periodicity of water currents at the seabed as a temporal cue regulating the behavior of bathyal benthic fauna in all continental margin areas where the effects of tides is negligible.

1. Introduction

The deep sea is the largest and at the same time least explored biome on Earth (Ramirez-Llodra et al., 2010). Mesopelagic and bathypelagic depth-zones (> 1000 m) together, account for approximately the 75% of total seawater volume (Charette and Smith, 2010). Quantitative studies on the behavior of bathyal organisms are scarce because of the intrinsic difficulties related to in situ observations or laboratory maintenance. The studying of biological rhythms is of particular interest in a habitat where light intensity progressively decreases with depth, and other factors may synchronize rhythmic biological processes (e.g. Aguzzi et al., 2011). The physical limit for penetration of sunlight in oligotrophic waters is at approximately 1000 m (Hopkins, 1985) and monochromatic blue light (470 nm) is the only part of the spectrum reaching disphotic depths (Aguzzi and Company, 2010). Below this depth, periodic tidal currents could replace sunlight as synchronizers of animal behavior and physiology (Wagner et al., 2007: Aguzzi et al., 2010; Sbragaglia et al., 2015). In this scenario, the Mediterranean deep-sea habitat represents an interesting study area because tides are strongly reduced in intensity and there is evidence of cyclic water currents at the sea bottom driven by inertial motion (Gasparini et al., 2004). To date, a preliminary laboratory study on the locomotor activity of the Norway lobster (*Nephrops norvegicus*) as representative of deep-water megafauna has shown that biological rhythms can have a correlation with local inertial periodicity (18 h) measured in situ (Aguzzi et al., 2009).

Geryon longipes (Relini Orsi and Mori, 1977) is a brachyuran crab of bathyal muddy benthic communities of the Mediterranean Sea and northeast Atlantic Ocean (Abelló et al., 1988; Cartes and Sardà, 1992; Fanelli et al., 2013a). With the caridean shrimps (*Acanthephvra eximia* and *Pontophilus norvegicus*), the anomuran crab (*Munida tenuimana*) and the decapoda (*Aristeus antennatus*), *G. longipes* represents most of the biomass in those communities (Cartes and Maynou, 1998). Its bathymetric distribution spans from approximately 400 to 2000 m (Pérès, 1985: Cartes and Sardà, 1992) and is considered to possess burying as well as burrowing habits, as recently observed by remotely operated vehicles (see videos S1–3 in the supplementary material; courtesy of Aymà et al., 2016). *G. longipes* follows a seasonal pattern of reproduction with the highest percentage of ovigerous females occurring during winter-spring (Company et al., 2003). Its feeding activity (measured as daily-ration on ingested prey items), is low compared to

* Corresponding author.
E-mail address: jdnunez@mdp.edu.ar (J. Aguzzi).
[1] Authors have equally contributed to the paper.

http://dx.doi.org/10.1016/j.dsr.2016.08.007

other bathyal benthic decapod crustaceans. Stomach fullness shows a diel pattern of variation with a clear peak at dawn (Maynou and Cartes, 1998). Unfortunately, there are no quantitative measures of behavioral patterns of *G. longipes* under controlled laboratory conditions for a comparative coupling with field data. The characterization of behavioral rhythms in the laboratory is extremely important and would contribute to: (i) better understand the mechanisms governing biological rhythms of those animals dwelling at depths where the sun light is not present, and (ii) provide the first cross-comparison between field and laboratory data in a benthic species from bathyal depths. Here, we focused on the effect of simulated periodic water currents and monochromatic-blue light-dark cycles on the locomotor activity of *G. longipes*. Furthermore we studied the frequency of different behavioral patterns (feeding, exploring and self-grooming) under both simulated environmental conditions.

Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.dsr.2016.08.007.

2. Materials and methods

2.1. Sampling and acclimation

Individuals were collected in June 2014 off the coast of Blanes (Spain), on the middle-lower slope (i.e. between 720 and 1750 m), by trawl hauling, carried out with the *R/V* García del Cid. Once on board, crabs were immediately transferred to light-proof and refrigerated (13 ± 1 °C) containers. Then, individuals were transferred to the laboratory at the Marine Science Institute (Barcelona, Spain) in a light-proof chamber under the following conditions: constant temperature of 13 ± 1 °C, as reported for the western Mediterranean continental slope throughout the year (Hopkins, 1985). Under these conditions feeding time was randomized to prevent entrainment through food-entraining oscillators (see Fernández De Miguel and Aréchiga (1994)). Finally, light-dark cycles matching the local photoperiod. Additionally, light-ON and light-OFF were progressively attained and extinguished within 30 min, to avoid potential damages to crabs' eyes due to sudden light intensity changes. In fact *G. longipes*, as other deep sea species, is very sensitive to low level of light (Johnson et al., 2000, 2002).

2.2. Experimental tank

An actograph was used to track crabs behavior (Sbragaglia et al., 2013). Briefly, that actograph consisted of 4 tanks, each of which had 2 individual corridors ($150 \times 25 \times 30$ cm), where the locomotor activity of individual crabs was tracked by automated video-image analysis (Fig. 1). Each corridor was with sand glued on the bottom, an artificial burrow, a pump, and monochromatic blue (472 nm) and infrared (850 nm) LED illumination systems. The pump, together with a flume system, was used to create water currents independently in each corridor (a diffuser was used to reduce turbulence). Each corridor was endowed with a burrow that was inclined at $\sim$25° in a direction opposite to the flow. We used a constant inflow of water with an exchange rate of 4 L min^{-1} per corridor. The water depth of each corridor was of 28 cm. Blue light was chosen because at disphotic continental margin depths marine deep benthic decapods use this wavelength to synchronize their biological clocks (reviewed by Aguzzi and Company (2010)). Infrared illumination was used to allow recording of behavior in darkness. Four video HD cameras were used to record the behavior of crabs with a frame acquisition rate of 10 s. All frames were assembled into a time-lapse video (hereafter referred to as the full-length video) for further characterization of crab's behavior (see below).

2.3. Experimental design

We used a classic 3-stages experimental paradigm of chronobiology to assess the entrainment capability of crabs to light and water current cycles. We ran two different experiments using 16 adult males. Experiment 1 (16th of October – 8th of November 2012) was run in constant darkness (total darkness with only infrared LEDs on) and was subdivided in 3 stages: (i) 10 days in constant darkness; (ii) 7 days in constant darkness during which crabs were exposed, every 18 h, to water currents of 2-h duration with a speed of 10 cm s^{-1}; That setting simulated a periodic intensification of seabed current speed, as observed the latitude of sampling (Aguzzi et al., 2009); and finally, (iii) 7 days in constant darkness. Experiment 2 (19th June – 5th July 2013) also consisted of three stages: (i) 5 days in constant darkness; (ii) 6 days with blue light-dark cycles with a photoperiod matching the natural one at the latitude of Barcelona during the experimental trial (onset at 04:20 and offset at 19:20 UTC). The ON/OFF switching of the blue LED was progressive (within 30 min). During light hours, the intensity was $4*10^{-3}$ μE/m^2/s (simulating at about 200 m depth). Finally, (iii) we exposed animals to 6 days in constant darkness.

2.4. Automated Behavioral tracking and data treatment

The full-length videos were analyzed by an automated behavioral tracking routine, developed in Python, by means of OpenCV libraries (Python Language Reference, version 2.7; Available at

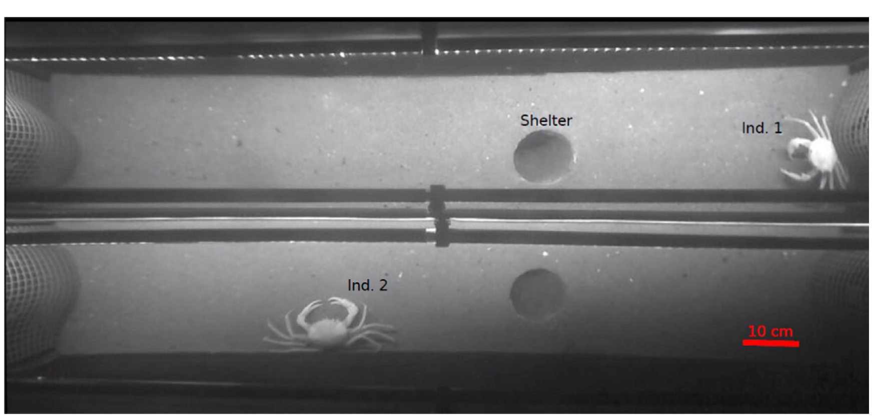

Fig. 1. Representative image of the experimental tank in which were settled (during all experimentation time) one individual at each runner with your respective shelter. For more details see Sbragaglia et al. (2013).