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

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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.

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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 (A*risteus 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

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.

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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 $4*10^{-3} \, \mu E/m^2/s$ (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
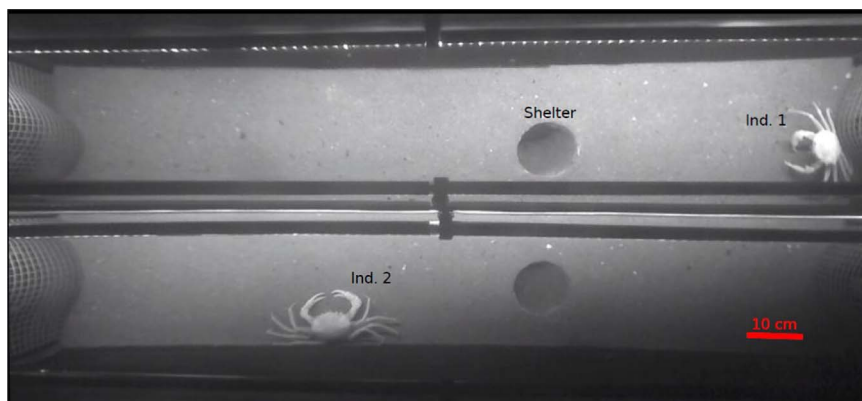

Fig. 1. Representative image of the experimental tank in which were settled (during all experimentation time) one individual at each runner with your respective shelter. For more details see Sbragaglia et al. (2013).

Table 1
Classification of crabs activities.

| Behavior	Definition
Feeding	The crab extracts putative food particles from the substrate using its chelipeds and then moving theme to the mouth (see video S4 in the supplementary material).
Exploring	The crab moves its walking limbs individually, touching the substrate or the walls of the tank (see video S5 in the Supplementary material).
Self-grooming	The crab cleans its body in two ways:
	– Cleaning its walking limbs by moving the chelipeds upon them (see video S6 in the supplementary material).
	– Cleaning its cheliped by passing it through the other cheliped (see video S7 in the supplementary material).
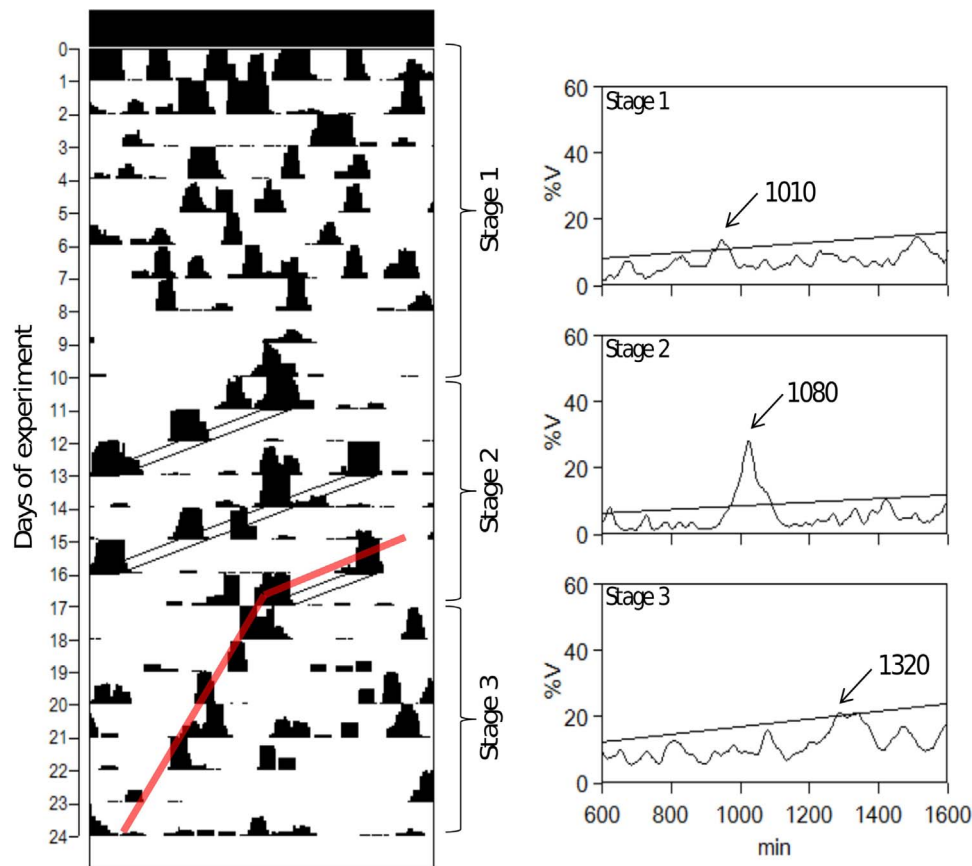
Fig. 2. Left: Actogram of one representative crab over 24 days during Experiment 1. The experiment is subdivided in three stages. Stage 1: ten days in constant darkness; stage 2: seven days in constant darkness with periodic water currents; stage 3: seven days in constant darkness. Locomotor activity is presented as centimeter traveled outside the burrow. Black bar at top indicate that the experiment was in total darkness. Periodic water current (10 cm s^{-1} of 2 h with a 18-h period) are represented is represented by open, oblique rectangles during stage 2 of the experiment. Red line indicates the phase of synchronization of locomotor activity. **Right:** The output of periodogram analysis of the locomotor activity is reported on the left for each stage of the experiment. The most significant peak is indicated by the arrow together with its value expressed in min. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

http://www.python.org). The algorithm works following different logic steps in order to automatically track the locomotor activity. Key steps of the process are described as follow. First of all, the differences among two consecutive frames are evaluated using background subtraction (OpenCV) method. The objects into one frame are identified focusing into a previously selected main Region Of Interest (ROI). Then, the area of each object is analyzed by dividing the main ROI into smaller areas (sROIs), adapted to the size of the object. Then, the image is binarized (two possible values each pixel), using Otsu algorithm to extract the profiles of all forms that are recognized in each sROIs. Finally, the centroid (position in a *x,y* plan) of the identified object is extracted and recorded. The centroid coordinates are used to calculate the distance covered by the object between two consecutive frames; in those cases where no consecutive detections of the same animal occurred (e.g. it is inside the burrow), the last detection is considered as the last position of the object. The final output is a time

series of movements as covered distances (cm) binned at 10 min intervals, for each crab.

Time series were represented with actograms (24-h-based) to discern the effects of both light and current cycles on the locomotor activity of individuals. A Chi-square periodogram (Sokolove and Bushell, 1978) was used to scan for significant ($P < 0.05$) periodicity in the $600 - 1600$ min range (equivalent to $10 - 27$ h) separately for each experimental stage. The percentage of variance (%V) was reported as a measure of the robustness of the rhythmic patterns (Refinetti, 2006). Moreover, the average locomotor activity during the second stage of both experiments was calculated for 2-hours periods during the following conditions: Experiment 1, within two hours before, during, and after the water current. Experiment 2, (a) at dusk (within one hour before to one hour after light-OFF), within two hours before and after dusk and (b) at dawn (within one hour before to one hour after light-ON), within two hours before and after dawn.
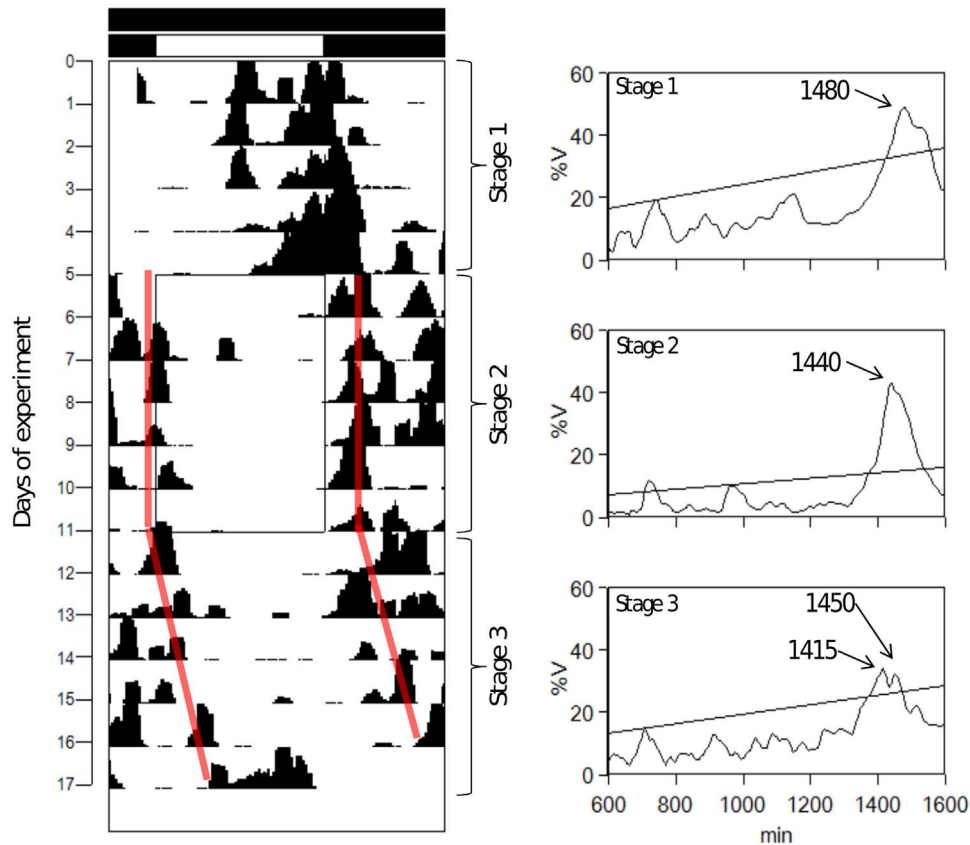
Fig. 3. Left: Actogram of one representative crab over 17 days during Experiment 2. The experiment is subdivided in three stages. Stage 1: five days in constant darkness; stage 2: six days with light-dark cycles; stage 3: six days in constant darkness. Locomotor activity is presented as centimeter traveled outside the burrow. Filled black bar at the top indicate constant darkness during stage 1 and 3. White-black bar and the square in the plot indicate light-darkness during stage 2. Red line indicates the phase of synchronization of locomotor activity. **Right:** The output of periodogram analysis of the locomotor activity is reported on the left for each stage of the experiment. The most significant peak is indicated by the arrow together with its value expressed in min. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
The two best generalized linear mixed-effect model for locomotor activity. Number of parameters (**N. par**), Akaike's information criterion (**AIC_i**), Akaike differences (**Δ_i**) and normalized weights of AIC (**w_i**). **F**: Factor (water current for experiment 1 and light for experiment 2). **D**: distance traveled. +/- indicate the incorporation/or not of the factor in the model. The models used are highlighted in bold.

Locomotor activity	Model	N. par	F	D	D:F	AIC_i	Δ_i	w_i
Experiment 1	**1**	**13**	**+**	**–**	**–**	**2163.4**	**0**	**0.976**
	2	18	+	+	–	2170.8	7.37	0.024
Experiment 2	**1**	**7**	**+**	**–**	**–**	**1457.3**	**0**	**0.751**
	2	13	+	+	–	1459.7	2.39	0.227

2.5. Behavioral analysis and data treatment

The analysis of the full-length videos was carried out by a trained operator through the focal animal sampling method (*sensu* Altmann, 1974). We quantified the occurrence of 3 distinct behaviors at stage 2 of both experiments: feeding, exploring, and self-grooming (Table 1). We counted the number of times a behavior was repeated by 10 s. That quantification was carried out during 2 h according to the different conditions: Experiment 1, before, during, and after water currents and Experiment 2, at the middle of darkness and light hours, at dusk, and at dawn (as previously defined; see above).

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We used linear and non-linear mixed effect models to analyse our behavioral data since these are particularly useful when there

is temporal pseudo-replication (repeated measurement). In fact, our experimental data were obtained from individuals that were measured repeatedly through time. We included "individuals" and "days" as random effect parameters into our mixed models (Littell et al., 2000). We analyzed the relationship between locomotor activity and independent variables (i.e. light and water currents) using a Generalized Linear Mixed Models (GLMM) with Gaussian error distributions. A model validation was applied to verify that underlying statistical assumptions were not violated. Normality of residuals was assessed by plotting theoretical quantiles versus standardized residuals (Q–Q plots). Homogeneity of variance was evaluated by plotting residuals *versus* fitted values, and influential data points were identified using Cook's distance method (Quinn and Keough, 2002). The validation procedure showed that there was no evidence of nonlinearity, but for all models, the diagnostic residual plots indicated heteroscedasticity, due to the inherent heterogeneity of variance within independent variables. Hereby, in order to obtain homogeneity of variance, we used Linear Mixed-Effects (LME) models with a variance–covariate structure (Zuur et al., 2007). Thus, a set of models with different variance structures were compared with the equivalent model without the LME ("nlme" package, Pinheiro et al., 2015) extension, using Akaike's Information Criterion (AIC; Akaike, 1998) and examination of plots of residuals versus fitted values. The model with the most-appropriate random structure was then used as a starting point for determining the most appropriate fixed structure.

A GLMM was also performed to determine if behaviors (dependent variable, see Table 1) vary according to the independent variables (i.e. light and water current cycles). Because of the nature
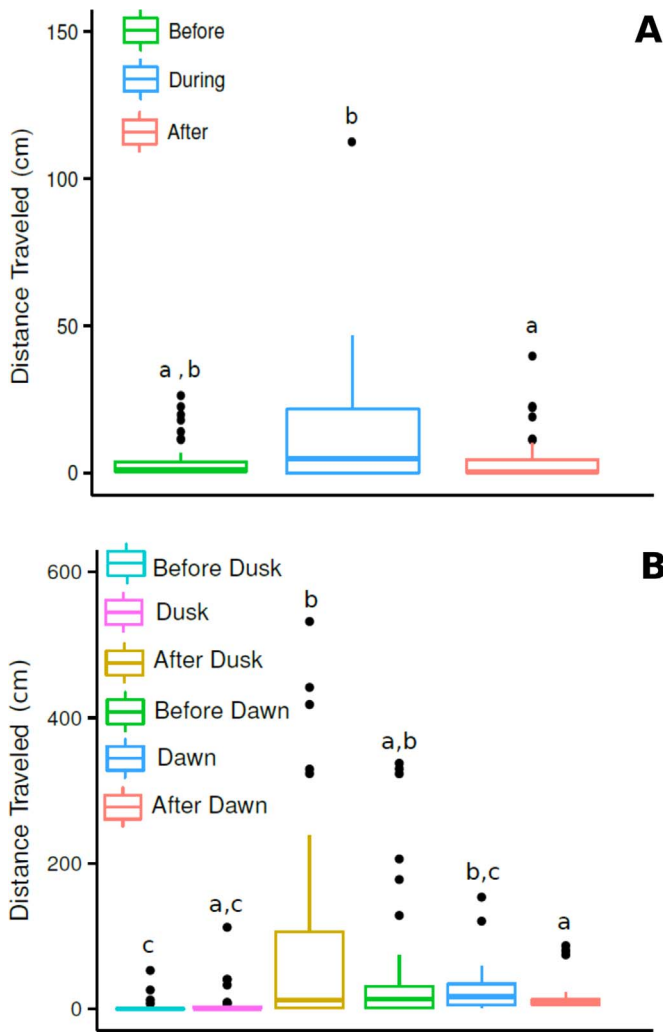
Fig. 4. Average Locomotor activity of crabs during two-hours periods in both experiments. A: (Experiment 1) observations were made within two hours before, during and after the water current. B: (Experiment 2) observations were made at dusk (within one hour before to one hour after light-OFF), within two hours before and after dusk; (b) at dawn (within one hour before to one hour after light-ON), within two hours before and after dawn. In each box-plot the median is indicated by the horizontal line, the top of the rectangle indicates the third quartile and the bottom of the rectangle indicates the first quartile. A vertical line extends from the top of the rectangle to indicate the maximum value, and another vertical line extends from the bottom of the rectangle to indicate the minimum value. The black dots indicate the outliers. Letters indicate significant differences based on Tukey post hoc tests).

Table 3

The two best generalized linear mixed-effect model for the 3 categories of behavior. Number of parameters (**N. par**), Akaike's information criterion (**AIC$_i$**), Akaike differences (Δ_i) and normalized weights of AIC (w_i). T: Time; F: Factor. +/- indicate the incorporation/or not of the factor in the model. NA indicates a model not estimated because all the parameters had wide confidence intervals overlapped with 0. The models used are highlighted in bold.

Behaviors	Model	N. par	T	F	T:F	AIC$_i$	Δ_i	w_i
Experiment 1								
Feeding	**1**	**9**	**−0.0085**	**+**	**+**	**1188.1**	**0**	**0.596**
	2	10	−0.009	+	+	1189.6	1.47	0.286
Exploring	**1**	**9**	**−0.0072**	**+**	**+**	**1746.8**	**0**	**0.539**
	2	10	−0.0072	+	+	1748.4	1.56	0.248
Self-grooming	1	NA	NA	NA	NA	NA	NA	NA
	2	NA	NA	NA	NA	NA	NA	NA
Experiment 2								
Feeding	**1**	**11**	**−0.021**	**+**	**+**	**1572.5**	**0**	**0.512**
	2	12	−0.021	+	+	1573.1	0.53	0.393
Exploring	**1**	**15**	**−3.8e−03**	**+**	**+**	**2157.4**	**0**	**0.97**
	2	11	−1.6e−02	+	−	2165.6	8.15	0.017
Self-grooming	**1**	NA	NA	NA	NA	NA	NA	NA
	2	NA	NA	NA	NA	NA	NA	NA

maximum likelihood with "*glmmADMB*" package (Skaug et al., 2014). We used the library "*MuMIn*" (Bartóm, 2015) to compute Akaike's weight (*wi*).

3. Results

3.1. Automated behavioral tracking

During experiment 1 (water currents cycle), two crabs escaped from the tank and were found dead on the floor at the end of the trial. In Fig. 2, we show a representative actogram of one crab together with periodogram analysis at the three stages of that experiment. During Stage 1, the crab had a free-running period (endogenous period in constant conditions) of 1010 min (16.83 h, V=13.55%). At stage 2, the crab exposed to periodic water currents, showed synchronized locomotor activity with a significant peak at 1080 min (18.00 h; V=28.01). At stage 3, when the crab was exposed to constant conditions, the free-running period was 1320 min (22.00 h; V=21.12%). It is important to notice that the phase of the locomotor activity (red line) at stage 3 was predicted by the phase during stage 2 (when the crab was synchronized to the water currents) indicating that periodic water currents entrained the crab locomotor activity (see Fig. 2).

During the experiment 2 (light-darkness cycle), again two crabs escaped from the tank and were found dead on the floor at the end of the trial. Fig. 3 presents the actogram and periodograms of a representative individual. At stage 1, the crab locomotor activity had a free-running period of 1480 min (24.66 h, V=48.73%). At stage 2, the locomotor activity was synchronized to the light-darkness cycle, with a period of 1440 min (24.00 h, V=42.86%). At stage 3, the crab was maintained in constant darkness and the locomotor activity free-run with two peaks: 1415 min (23.58 h, V=33.98%) and 1450 min (24.16 h, V=32.03%). The phase of locomotor activity at stage 3 was predicted by the phase of activity at stage 2 (Fig. 3, the red line), demonstrating entrainment of locomotor activity by light-darkness cycle.

The factors "water current" and "light cycle", respectively for experiments 1 and e 2, were incorporated in the selected mode (Table 2, see Akaike's weight), when considering crab locomotor activity. In experiment 1, locomotor activity was significantly higher "during" than "after" water currents, but no significant

of the data (counted data), these were analyzed using a set of models with either Poisson or negative binomial error distributions. Also, due to the high presence of zero-count values we used a zero-inflated distribution (Zuur et al., 2009).

In all cases, the AIC was used to assess models performance. In addition, we computed Akaike's weight (wi) for each candidate model (Franklin et al., 2001) through its computed AICc and the Δ values. The weights range between 0 and 1 are interpreted as the weights of evidence in favor of model i as the best model among the set of all candidate models examined (Burnham and Anderson, 2004). Finally, the models with the smallest AIC and the higher w_i values were chosen as the models that "best" represented the data. Then, where necessary, we conducted mean comparisons using a interaction means test in the "phia" package for mixed or multivariate linear models for repeated measures experiments (De Rosario Martines, 2015). All statistical analyses were conducted in R 3.3.1 (R Development Core Team, 2016). GLMM were fitted using
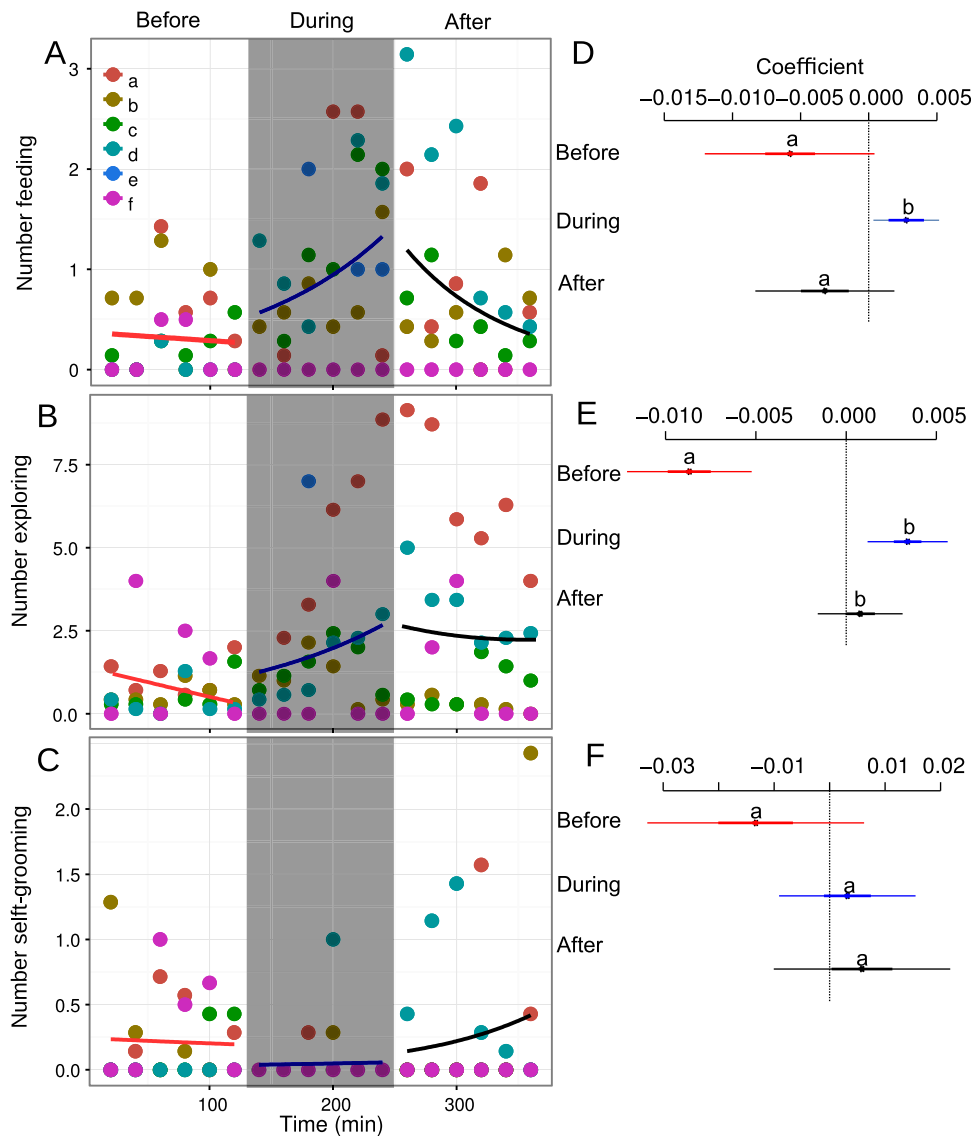
Fig. 5. Plot of the frequency of behavioral categories in experiment 1. Six-hours of daily observations were made in three different conditions: before (within two hours before the onset of water currents), during (during the water current, also indicated by the grey area), after (within two hours after the offset of water current). Dots indicate the mean of the frequency of behavior, while the different colors stay for different crabs. The lines in each graph indicate the estimated slopes that correspond to the plots on the right (D, E, F) that indicated the output of the *post hoc* test.

differences were found between "before" and "after" (Fig. 4A). In experiment 2, locomotor activity was significantly higher "after dusk" than at "dusk" or "before dusk"; then it was significantly higher at "dawn" than "after dawn", but without significant differences between "before dawn" and "dawn" (Fig. 4B).

3.2. Behavioral analysis

We found that the factors "water current", and "light cycle" for experiment 1 and 2, respectively, were incorporated in the selected model (Table 3; see Akaike's weight) indicating that both factors affect feeding and exploring behaviors, but not self-grooming. Thus, during experiment 1, the frequency of feeding behavior increased gradually after the onset of water currents and then decreased (Fig. 5A). The *post hoc* test showed that the estimated slope "during" water current is significantly different from slopes "before" and "after" (Fig. 5D). The frequency of exploratory behavior showed a decrease "before" the onset of water currents. It increased "during" currents and then it maintained a horizontal trajectory "after" (Fig. 5B). The *post hoc* test showed significant slope differences "before" when compared to "during" and "after"

(Fig. 5E). Finally, the frequency of self-grooming behavior did not show great fluctuations during the three different period of the experiment. Estimated slopes plotted in Fig. 5C are not representative in fact there were no significant differences with the *post hoc* test (Fig. 5F) and the parameters of the model had wide confidence intervals that overlapped with 0.

During experiment 2, the frequency of feeding and exploring behaviors followed similar patterns, but with some differences (Fig. 6A, B). The slopes of feeding behavior showed similar values (close to 0) at dawn, dusk, and night, being significantly different from the negative slope at day (Fig. 6D, see *post hoc* test). The slope of exploring behavior was close to 0 at night, it became negative and significantly different at dusk and dawn, in comparison to both day and night (Fig. 6E, see *post hoc* test). Finally, the slope reached the lowest negative value during the day with significantly differences with the rest of the periods (Fig. 6E). As previously observed in experiment 1, the frequency of self-grooming behavior did not show significant fluctuations (Fig. 6C) and the parameters of the model had wide confidence intervals and overlapped with 0 (Fig. 6F).
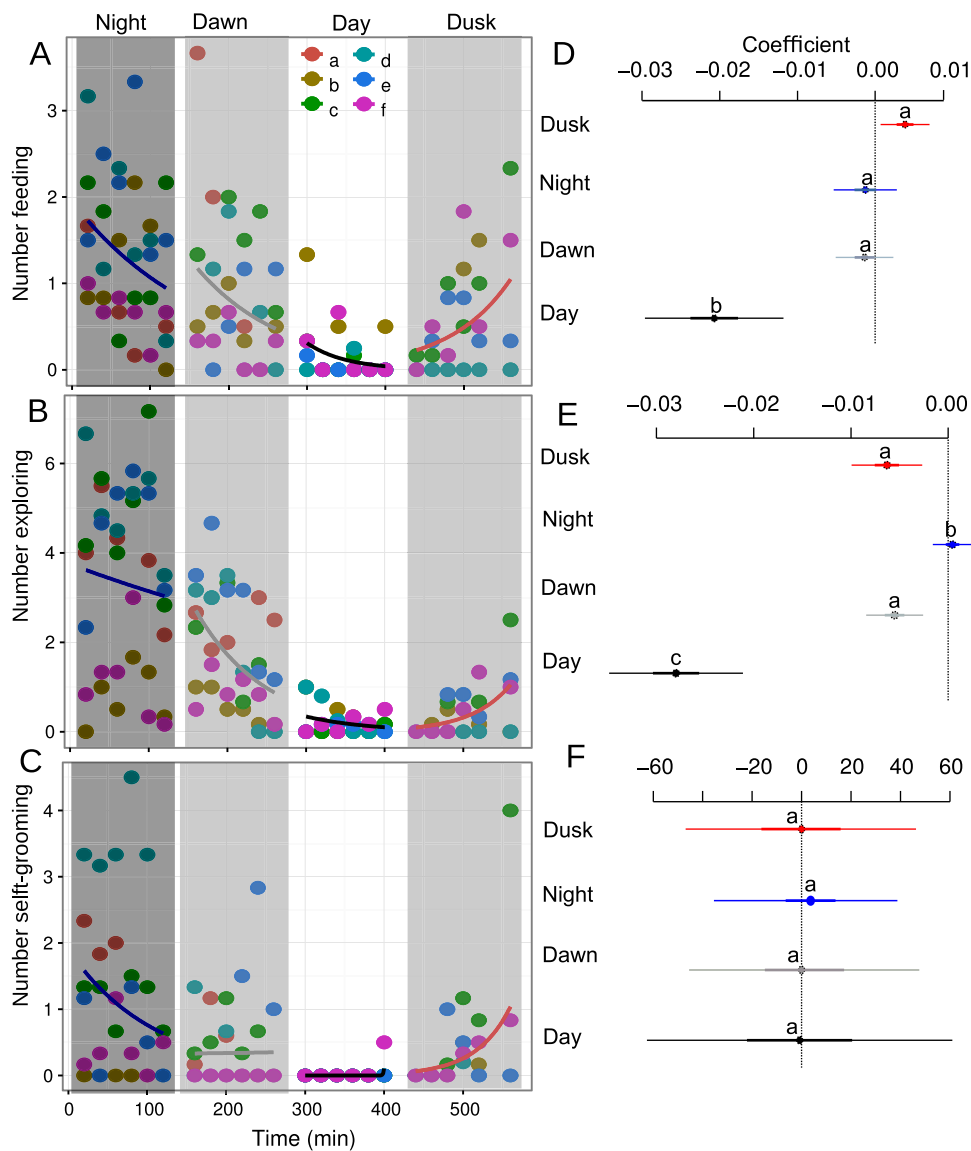
Fig. 6. Plot of the frequency of behavioral categories in experiment 2. Six-hours of daily observations were made in four different conditions: night (two hours in the middle of darkness hours, also indicated by dark grey area), Dawn (one hour before to one hour after light-ON, also represented by the light grey area), Dusk (one hour before to one hour after light-OFF), Day (two hours in the middle of light hours). Dots indicate the mean of the frequency of behavior, while the different colors stay for different crabs. The lines in each graph indicate the estimated slopes that correspond to the plots on the right (D, E, F) that indicated the output of the *post hoc* test.

4. Discussion

We provided the first insight on the behavior of the bathyal crab *Geryon longipes* using a laboratory to simulate two important environmental cues: current and light-darkness cycles. Locomotor activity results indicated that the endogenous clock may be entrained by both inertial (18 h) current and light-dark (24 h) cycles. Other analyzed items of the behavioral repertoire of the species (i.e. feeding and exploring) were also affected by currents and light-darkness cycles, highlighting the temporal cueing power of both cycles on the whole ethology of the species and not only on locomotion. Taken together, these results provide evidences on the role of inertial currents as putative regulator of rhythmic biology of deep-sea benthos.

4.1. Locomotor activity

Geryon longipes is able to synchronize its locomotor activity rhythms to both water currents and light-dark cycles, as previously suggested in another deep water crustacean, the Norway

lobster, *Nephrops norvegicus* (Sbragaglia et al., 2015). Here, we provide for the first time new insights on that synchronization of *G. longipes* to 18 h water currents cycles (see Fig. 2). Even if such phenomenon is not clearly observed in all the tested individuals, it is worth of attention because, to our best knowledge, it is the first laboratory documented evidence of such phenomenon in a deep sea species.

Intertidal organisms showed adaptive endogenous circatidal rhythms that free-run at about 12.4 h (Naylor, 2010) and allows organisms to anticipate tidal changes using environmental cues including turbulence/vibration, moonlight, salinity, and temperature fluctuations. Recently, it has been demonstrated that tidal currents may synchronize also the behavior and physiology of deep sea organisms (Wagner et al., 2007; Aguzzi et al., 2010; Sbragaglia et al., 2015). However, the existence of endogenous clocks in these organisms is far from clear. The Mediterranean deep water habitat is usually affected by currents with inertial periodicity instead of tidal one (Gasparini et al., 2004). The study we conducted here on *G. longipes* indicated that currents with inertial periodicity may have an important role in synchronizing

biological processes of deep-sea Mediterranean organisms.

Darkness triggered an increase in activity of *G. longipes* (Figs. 3, 4B). In experiment 2, the phase of locomotor activity at Stage 3 was predicted by the phase of activity at Stage 2 (see the red line in Fig. 3). This indicated that locomotor activity is entrainable by light-darkness cycles (as already observed for water currents cycle, see above). Even if such phenomenon is not clearly observed in all the tested individuals in this study, data indicated that crabs are able to synchronize their biological clocks to light-darkness cycle despite the species depth of distribution is within the twilight zone end (i.e. approx. 1000 m in the northwestern Mediterranean).

It should be noticed that at stage 1 of the experiment 2, G. longipes showed a free-running period of 24.66 h that differed from the 16.83 h recorded at stage 1 of experiment 1. This can be related to the fact that experiment 1 was run in October 2012, immediately after the sampling of crabs, while experiment 2 occurred almost one year after (June 2013). Fresh collected crab expressed a free-running period related to inertial currents that disappeared 8 months later. Such situation is common with intertidal animals that maintain free-running circatidal rhythmicity only for short period after sampling (Naylor, 2010).

4.2. Feeding, exploring, and grooming behaviors

Feeding behavior increased during water current stimuli, while the exploring behavior showed to increase both during and after water currents (see Fig. 5A and B). The increase of feeding behavior followed the same pattern of locomotor activity, on the contrary exploring behavior seemed to not be related to locomotion. We speculated that feeding and exploring behaviors may have different ecological correlation with water currents.

Feeding and exploring behaviors increased during darkness and decrease during light (see slopes in Fig. 6). Our laboratory data indicated a strong nocturnal phenotype (locomotor activity, feeding and exploring behaviors) of *G. longipes* that is also supported from previously documented evidences in the upper middle slope of the North-western Mediterranean Sea. Stomach fullness showed a diel pattern of variation with a clear peak at dawn (Maynou and Cartes, 1998). The diet of *G. longipes* is composed of several benthic invertebrates (Lagardère, 1977; Relini Orsi and Mori, 1977) and the crab is also described as scavenger. However, diet composition did not reveal significant differences between day and night (Cartes, 1993a). The absence of diel differences in diet composition could be explained considering the low contribution of mesopelagic (i.e. diel vertical migrators) preys in the diet (Cartes and Abello, 1992) even if the stomach fullness indicated a diel rhythms of feeding.

Laboratory data presented in this study on diel locomotor activity and feeding behavior of *G. longipes* matched with higher level of stomach fullness at dawn (Maynou and Cartes, 1998). However, results also indicated that periodic (18 h) water currents may play a role in the regulation of locomotion and feeding. *Geryon longipes* is also described as a carnivore (Cartes, 1993b; Fanelli et al., 2011, 2013b) and the behavior activity synchronized with water current could be related with the increment of activities of its preys. Thus, under this scenario, we could consider the periodic currents as a variable that could act in combination with light-darkness as synchronizers and modulators of bathyal benthic community in the Mediterranean Sea.

5. Conclusions

Present results point out the effects of blue light and currents on the regulation of behavior of bathyal organisms. A sampling schedule matching the local inertial periodicity of water currents on continental margins should be appropriate to detect both putative differences in the behavior of organisms and biodiversity changes due to behavioral components.

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