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ORIGINAL PAPER

Effect of simulated tidal currents on the burrow emergence rhythms of the Norway lobster (*Nephrops norvegicus*)

Valerio Sbragaglia[1] · **Jose Antonio García**[1] · **Juan Jose Chiesa**[2] · **Jacopo Aguzzi**[1]

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Abstract Light is the most important zeitgeber for the synchronization of biological rhythms in terrestrial organisms. In the sea, the light intensity progressively decreases, and tidal currents might control behavioural rhythms at disphotic depths. The Norway lobster, *Nephrops norvegicus*, is distributed from the upper shelf to middle slope areas. Its burrowing behaviour is under the control of a circadian system, and the effects of tidal currents have been inferred from catchability patterns. Male lobsters were collected from 100 m depth off the Ebro Delta, Tarragona, Spain (40° 39′N, 1° 13′E). Light intensity and water current cycles were simulated in the laboratory to investigate their combined effects on burrow emergence behavioural rhythms (June–July 2012). Periodic water currents (10 cm s^{-1}) inhibited *N. norvegicus* burrow emergence to a degree dependent on the relative phase between light and water current cycles. The lobsters preferred to remain inside the burrow in the presence of water currents. However, when they were outside the burrow, they spent more time orientated downstream during darkness hours. Moreover, four of the 15 lobsters showed that a current could act as a putative zeitgeber for the circadian oscillator, but further experiments are needed to confirm this finding. These results indicate that tidal current is an important parameter to consider when interpreting fishery-dependent data and data from video surveys, not only *N. norvegicus*, but for other deep-water epibenthic species.

Introduction

Light is the most important zeitgeber (i.e. environmental cue) that synchronizes the biological rhythms of terrestrial organisms (Dunlap et al. 2004). In contrast, in the sea, light intensity progressively decreases with depth, and other factors may be important for the synchronization of rhythmic biological processes (Aguzzi et al. 2011). In fact, the physical limit for penetration of sunlight (~1000 m in oligotrophic waters) defines a depth range known as the “twilight zone” (Hopkins 1985). Below this depth, tidal currents could replace sunlight as the synchronizer of animal behaviour and physiology (Wagner et al. 2007; Aguzzi et al. 2010). However, depth limits for light penetration depend on turbidity and other factors, and tidal regimes have strong geographical variability, depending on seafloor bathymetry, which results in numerous possible combinations of these cues.

The Norway lobster, *Nephrops norvegicus*, is distributed on muddy bottoms at 10–800 m depth from the northeast Atlantic Ocean to the western Mediterranean Sea (Farmer 1975; Bell et al. 2006; Johnson and Johnson 2013). *Nephrops norvegicus* has a strong burrowing habit, and its emergence behaviour can be subdivided into three different phases: door-keeping (lobsters at the burrow entrance with

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✉ Valerio Sbragaglia
sbragaglia@icm.csic.es

[1] Marine Science Institute (CSIC), Passeig Marítim de la Barceloneta, 37–49, Barcelona, Spain

[2] Laboratorio de Cronobiología, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes - CONICET, Roque Sáenz Peña 352, B1876BXD Bernal, Buenos Aires, Argentina

claws protruding out of the burrow, hereafter DK); emergence (totally out of the burrow, hereafter OUT); and concealment (completely in the burrow, hereafter IN) (Aguzzi and Sardà 2008). Such behaviour is under the control of the circadian system (24-h-based). A light-driven emergence behaviour rhythm has been characterized in the wild by fishing at different times: different diel (24-h) catchability patterns occur at different depths, nocturnal at 10–50 m and diurnal at 200–430 m (Farmer 1975; Bell et al. 2006; Aguzzi and Sardà 2008). Catchability in the wild has always been correlated with the emergence of individuals from their burrows, as observed by Main and Sangster (1985) and Newland and Chapman (1989). Recent laboratory experiments with artificial burrows have demonstrated that emergence behaviour is entrained by monochromatic blue light cycles of different intensities (as a proxy for depth) and that it shifts from the nocturnal phenotype to a diurnal one, as observed in the wild by trawling (Chiesa et al. 2010).

Rhythmic emergence behaviour in the laboratory has been studied in relation to the day–night modulation without considering the response of lobsters to water currents simulating tidal currents at the sea bottom. The sole laboratory evidence that *N. norvegicus* reacts to water currents (Newland et al. 1988) comes from a study on blind lobsters without burrow access, which showed that they assume a downstream orientation in flowing water. The first study on the effects of the neap and spring tides on *N. norvegicus* landings is now quite old (Storrow 1912), but the neap/spring phase of the tide sometimes exerts more influence than time of day, with spring tides depressing catches (Hillis 1971). Bell et al. (2008) also noticed an effect of neap and spring tides on catches of lobsters. In particular, the greater the current speed, the fewer lobsters were caught (Hillis 1996). That observation shifted attention from a cyclic event with neap/spring tides (correlated with the lunar month, 29.5 days) to a direct effect of water currents on lobster catchability. Thus, *N. norvegicus* is a good laboratory model for studying the combined effect of light and tidal cycles on the behaviour of deep-water epibenthic species.

Research on how periodic currents and day–night cycles influence behavioural rhythms of species is important for integrating individual behaviour into community and ecosystem dynamics (Schmitz et al. 2008; Aguzzi et al. 2015). However, laboratory experiments testing the putative effects of cyclic environmental variables on the behaviour of deep-water species are scarce due to the intrinsic difficulties in sampling and their laboratory maintenance. In this context, we investigated the effects of periodic water currents (12.4 h) and concomitant 24-h cycles of blue light–darkness on *N. norvegicus* burrowing behaviour.

Materials and methods

The actograph

An actograph was used to track *Nephrops norvegicus* behaviour (Sbragaglia et al. 2013a). Briefly, the actograph consisted of four tanks, each of which had two individual corridors (150 × 25 × 30 cm), in which the burrow emergence behaviour of lobsters was tracked by automated video image analysis. Each corridor was independent (no exchange of water between corridors), with sand glued on the bottom, an artificial burrow (because burrow size is correlated with lobster size, we built the artificial burrow to fit an average lobster with a cephalothorax length of 40 mm), 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. The burrow was inclined at ~30° in a direction opposite to the water current, and a diffuser 11 cm in diameter was used to reduce turbulence within the corridor. We used a constant inflow of water with an exchange rate of 4 L min^{-1} for each corridor; the water depth in the corridors was 28 cm. Blue light was used because marine crustacean decapods use this wavelength to synchronize their biological clocks (Aguzzi and Company 2010). Infrared illumination, which is invisible to *N. norvegicus*, was used to allow recording of their behaviour in darkness.

Four video cameras were used to track the behaviour of lobsters with a frame acquisition rate of 10 s^{-1}. The frames were automatically processed by a set of MATLAB functions, and time series of locomotor activity (cm) were obtained; for more information see Sbragaglia et al. (2013a). Moreover, all of the frames were assembled into a time-lapse video (hereafter referred to as the full-length video) for further characterization of lobster behaviour (see below).

Individual sampling and acclimation

Individuals were collected at night by a commercial trawler on the shelf (100 m depth) off the Ebro Delta (Tarragona, Spain; 40° 39′N, 1° 13′E). All sorting operations on deck and transportation to the laboratory followed methods of Aguzzi et al. (2008). In the laboratory, specimens were transferred to acclimation tanks 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); random feeding time, to prevent entrainment through food-entraining oscillators, as shown for crustaceans (Fernández De Miguel and Aréchiga 1994); light–dark cycles matching the summer photoperiod at the latitude of Barcelona (41° 23′ 0″N). Additionally, light-ON and light-OFF were progressively attained and extinguished within 30 min to acclimate the lobsters' eyes to light intensity changes. The acclimation facility contained individual cells (25 × 20 × 30 cm) made from plastic netting of different sizes, which allowed for full oxygenation and recirculation of the water without contact among individuals.

Experimental design

Fifteen adult males with a mean carapace length (CL; Mean ± SD) of 41.04 ± 4.85 mm were used in this study. Lobsters were never fed during the experiment, which is not harmful to the lobster. In fact, Stewart et al. (1972) showed that 3–8 months of starvation did not affect physiological parameters in *Homarus americanus*. Burrow emergence behaviour was studied over 29.5 days (1 lunar month) under a photoperiod matching the one at the latitude of Barcelona (~14 h light/10 h dark, June–July 2012). The ON/OFF switch of the blue LED was progressive (within 30 min). During light hours, the intensity was 4×10^{-3} µE m^{-2} s^{-1}, thus simulating a lower shelf condition (at ~100–150 m depth). The water temperature during the experiments was 13 ± 1 °C, and the dissolved oxygen content was always >9 mg L^{-1}.

We performed behavioural tests using Mediterranean individuals and exposing them to water currents with a periodicity typical of the Atlantic Ocean. Our approach is justified by the fact that *N. norvegicus* has a homogeneous population structure without signs of genetic divergence or isolation (Passamonti et al. 1997; Maltagliati et al. 1998; Stamatis et al. 2004). Every 12.4 h, lobsters were exposed to water currents of a 2-h duration with a speed of 10 cm s^{-1}. The semi-diurnal periodicity of 12.4 h simulates a periodic intensification of seabed current speed that has been proposed to entrain physiological rhythms in two species of deep-sea, North Atlantic fishes (Wagner et al. 2007).

Data treatment and behavioural analysis

Time series depicting locomotor activity out of the burrow were binned by 1-min intervals and represented with double-plotted actograms (24-h-based) to discern the effects of both light and current cycles on the behaviour 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) within the whole time series (29.5 days), and the percentage of variance (%V) was reported as a measure of the robustness of the rhythmic patterns (Refinetti 2006). Then, periodogram analysis was repeated for the lobsters that had shown a clear effect of the currents on their locomotor activity in their actograms. The analysis considered a selected number of days during which lobsters maintained clear synchronization with the water current cycle. At the same time, an average waveform (24.8-h-based) of the selected days was used to highlight the effect of water currents on locomotor activity.

The average locomotor activity during all of the days of the experiment was compared between light and darkness and among four different periods of the day: dusk (1 h before to 1 h after light-OFF); dawn (1 h before to 1 h after light-ON); day (1 h after light-ON to 1 h before light-OFF); and night (1 h after light-OFF to 1 h before light-ON). Then, we compared the sum of the locomotor activity 2 h before, during, and after the water current and plotted the values against the time of current onset. The overall temporal patterns (before, during, and after) were described using a gamma-family smoothing function.

In a second step, a trained operator visually analysed the full-length videos to characterize the lobsters' behaviour in flowing water. We quantified the amount of time that the lobsters spent in each of the three aspects of their burrowing behaviour (DK, IN, OUT) during the previously described periods of time (dawn, day, dusk, night). Finally, we also characterized the lobsters' behaviour outside the burrow in the presence of water currents using three categories: up- or down-stream body orientation without or with low displacement (<1 body length 10 s^{-1}), or active displacement (>1 body length 10 s^{-1}). This was usually performed with movements from one side to the other of the tank.

We square-root-transformed the data to satisfy the normality test and homoscedasticity of variance. A paired t test and one-way repeated-measures ANOVA (followed by the Tukey's multiple comparison procedure) were used to assess significant differences using SigmaPlot. In all analyses, we used a confidence interval of 95 %.

Results

The inspection of actograms evidenced robust diel burrow emergence activity in all individuals with peaks of locomotor activity at light-OFF (two representative actograms are presented in Fig. 1). Periodogram analysis of the whole time series (29.5 days) detected rhythmic activity in all tested individuals. Twelve lobsters showed diel (24 h) periodicity (mean ± SE = 24.06 ± 0.05 h; 23.37 ± 2.86 %V, $n = 12$), whereas three lobsters showed less robust tidal periodicity (mean ± SE; 24.86 ± 0.05 h; 9.50 ± 1.97 %V, $n = 3$).

Moreover, actograms indicated the presence of four lobsters with an evident synchronization to the water current cycle. We selected the days during which the

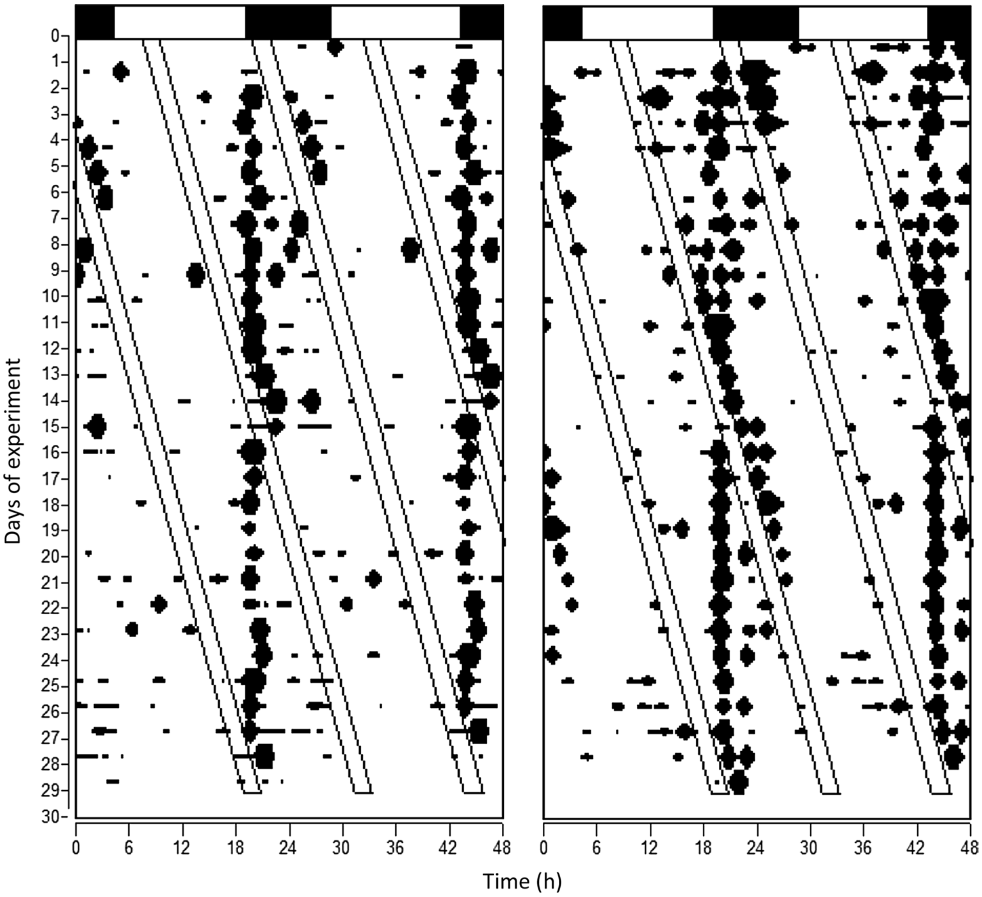

Fig. 1 Double-plotted (2 days in each *line*) actograms of two *N. norvegicus* over 29.5 days. Locomotor activity presented as centimeter travelled outside the burrow. *Open/filled bars* at *top* represent a 14-h light/10-h dark photoperiod. Flow cycle (10 cm s^{-1} of 2 h with a 12.4-h period) is represented by *open, oblique rectangles*

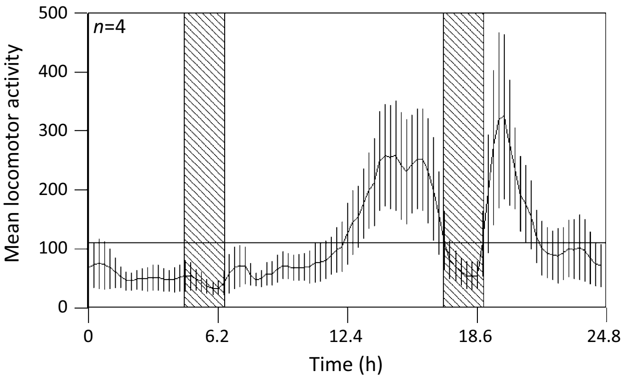

Fig. 2 Mean waveform (24.8-h-based) for locomotor activity of four lobsters with clear synchronization with the water current cycle (only selected days are reported as shown in Figure S1). *Vertical lines* represent the SE. The *horizontal line* represents the midline estimating statistic of rhythm (MESOR). Periodic water currents are identified by the *vertical shadowed* areas

four lobsters maintained this clear synchronization (see Fig. S1). Periodogram analysis indicated more robust tidal periodicity (mean ± SE = 24.85 ± 0.05 h; 55.92 ± 4.90 %V, $n = 4$) than previously observed. The average ($n = 4$) waveform (24.8-h-based) of the selected days was used to highlight the effect of water currents on locomotor activity (Fig. 2).

The locomotor activity of all lobsters was significantly (paired t test, $t14 = 5.432$, $P < 0.001$) higher during darkness (mean ± SE = 10 ± 2 cm, $n = 15$) than during light (mean ± SE = 6 ± 1 cm, $n = 15$). When looking in greater detail, lobsters were more active at dusk (mean ± SE = 13 ± 3 cm, $n = 15$) and night (mean ± SE = 10 ± 2 cm, $n = 15$) than at dawn (mean ± SE = 6 ± 2 cm, $n = 15$) and day (mean ± SE = 6 ± 1 cm, $n = 15$), with significant differences among the periods (ANOVA, $F(3,14) = 22.61$, $P < 0.001$).

The comparison of the sum of locomotor activity for all the lobsters 2 h before, during, and after the onset of water currents highlighted a behavioural locomotor response that was modulated by the time at which the currents were applied (Fig. 3). When the onset of currents occurred during the first hours of light (when lobsters were not active), there were no differences in the resulting smoothing curves. When the current onset was close to light-OFF (and lobsters began to be more active out of the burrow), the level of activity observed before and during the currents was the same, while the level of activity after the current was at its

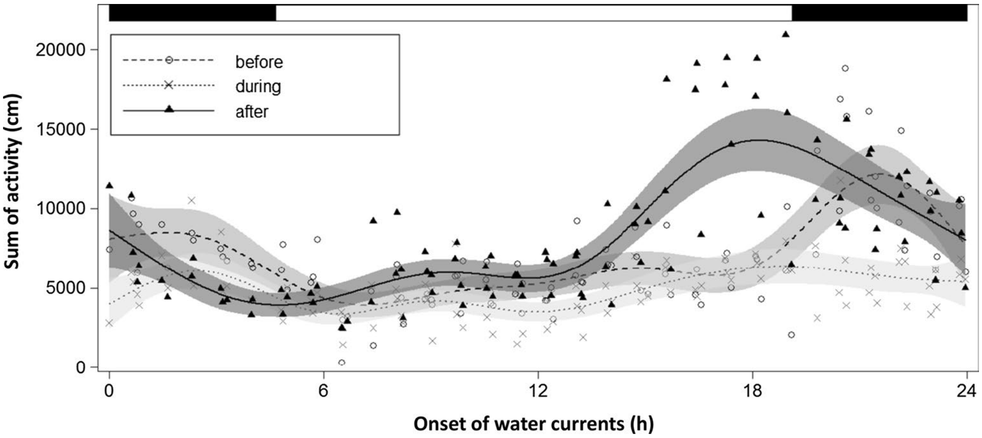

Fig. 3 Plot of locomotor activity ($n = 15$) 2 h before (*open circles*), during (*crosses*), and after (*filled triangles*) water currents plotted against time of water current onset. Data are presented along with a Gamma-family smoothing function (indicating the mean as a *dashed line* for *open circles*, a *dotted line* for crosses, and a *continuous line* for *filled triangles*), and the 95 % confidence interval is the *shaded grey-scale contours*. *Open/filled bars* at *top* represent a 14-h light/10-h dark photoperiod

maximum. The level of activity after the water currents started to decrease when the water currents onset occurred at the first hours of darkness, while the activity before the currents started to increase until reaching its peak. During the hours of darkness, there were two distinct peaks of activity, which occurred before and after the water current stimulus (Figs. 1, 2, 3). The activity following the water currents reached its minimum when the onset of currents was close to light-ON, while the activity before the water currents was still greater than the activity during the currents (indicating an inhibition of activity by water current).

The full-length videos indicated that the lobsters exposed to flowing water always spent a significantly higher amount of time inside the burrow than at door-keeping or outside the burrow (ANOVA, dawn: $F(2,14) = 63.21$, $P < 0.001$; day: $F(2,14) = 17.51$, $P < 0.001$; dusk: $F(2,14) = 9.63$, $P < 0.001$; night: $F(2,14) = 11.40$, $P < 0.001$; Table 1; Fig. 4). Activity outside the burrow during flowing water was higher at dusk and night than at dawn and day (ANOVA, $F(3,14) = 5.90$, $P = 0.002$; see Table 1; Fig. 4).

We also characterized the body orientation of the lobsters during flowing water by watching the full-length videos. There were no significant differences in the percentage of time that they spent moving or orientated up- and downstream at dawn, day, and dusk (though the power of the statistical test suggested caution when interpreting such results; see Table 1). However, when lobsters were outside the burrow at night, they spent a significantly greater amount of time orientated downstream than upstream or moving (mean ± SE = upstream: 12 ± 3 %, moving: 18 ± 6 %, downstream: 70 ± 4 %, $n = 9$; ANOVA, $F(2,8) = 66.33$, $P < 0.001$; see Table 1; Fig. 5).

Finally, the actograms of four of the 15 lobsters suggested that periodic water currents could have a putative role as a zeitgeber. In the actogram on the left (see Figs. 1 and S2), an individual showed two components of activity (i.e. peaks) during days 2–5, one in correspondence to the light-OFF and the other just after the current offset. When the current stimulus was too far from the light-OFF (>6:23 h, see Fig. 5), the lobster only showed the component of activity at light-OFF (day 6). Interestingly, during days 7–9, the component of activity that was previously synchronized to the current offset showed transients (i.e. it drifted to the left) that allowed it to resynchronize the phase with the major peak of activity at light-OFF. In fact, during days 10–11, the lobsters showed only one peak of activity. In the actogram on the right (see Figs. 1 and S2), during days 11–15, there was only one component of activity after the current offset (the diel peak of activity at light-OFF was inhibited). During days 16–21, two components of activity were visible: at light-OFF and after the current offset. During days 22–24, when the current stimulus was too far from light-OFF (>7:24 h, see Fig. S2), the lobster's activity showed only one major peak of activity at light-OFF, whereas the component of activity previously synchronized to the current offset showed transients (as observed for the other individual).

Discussion

We demonstrated that periodic water stimuli (as proxy of seabed tidal currents) influenced Norway lobster burrow emergence behaviour with a strength that is dependent on the phase relationship with the light–darkness cycle. Our results introduce new information regarding the response of *Nephrops norvegicus* to periodic hydrodynamic stimuli. First, lobsters preferred to remain inside the burrow in the

Table 1 Mean ± SE of the percentage of time spent by lobsters in different phases of burrow emergence during flowing water (behaviour during current) and in different orientation in the presence of the currents (body orientation during currents)

	% of time (mean ± SE)	*t*/*F*	*P*	*n*
Behaviour during current				
Dawn-DK (b)	11 ± 4			
Dawn-IN (a)	84 ± 6	63.208	<0.001	15
Dawn-OUT (b)	5 ± 3			
Day-DK (b)	14 ± 7			
Day-IN (a)	77 ± 9	17.505	<0.001	15
Day-OUT (b)	9 ± 6			
Dusk-DK (b)	13 ± 4			
Dusk-IN (a)	68 ± 8	9.628	<0.001	15
Dusk-OUT (b)	18 ± 8			
Night-DK (b)	15 ± 4			
Night-IN (a)	70 ± 9	11.400	<0.001	15
Night-OUT (b)	15 ± 8			
Body orientation during current				
Dawn-upstream	28 ± 19			
Dawn-downstream	58 ± 20	1.331	0.332[a]	4
Dawn-moving	14 ± 3			
Day-upstream	35 ± 12			
Day-downstream	53 ± 11	2.805	0.119[a]	5
Day-moving	12 ± 3			
Dusk-upstream	20 ± 9			
Dusk-downstream	63 ± 11	1.828	0.203[a]	7
Dusk-moving	29 ± 12			
Night-upstream (b)	12 ± 3			
Night-downstream (a)	70 ± 4	66.332	<0.001	9
Night-moving (b)	18 ± 6			

t/*F* represents the value of the statistics used to assess significant differences together with the *P* value and the *n*. Letters indicate the output of the multiple comparison post hoc test (a > b)

[a] The power of the test is below the desired value

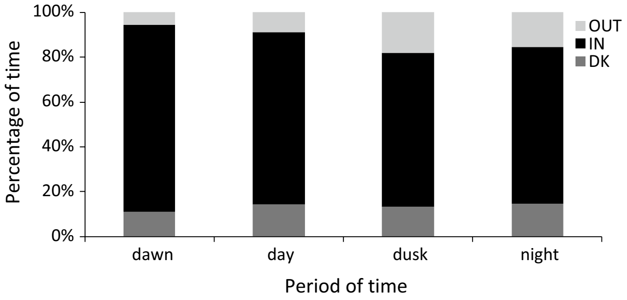

Fig. 4 Bars of percentage of average time spent by lobsters ($n = 15$) outside the burrow (OUT, *light grey*), inside the burrow (IN, *dark*), or at the burrow mouth (DK, *dark grey*) in the presence of flowing water at different periods of time: dawn (1 h before to 1 h after light-ON); day (1 h after light-ON to 1 h before light-OFF); dusk (1 h before to 1 h after light-OFF); night (1 h after light-OFF to 1 h before light-ON)

presence of water currents. Second, during water currents, some lobsters spent a reduced amount of time outside the burrow; this amount of time was greater at dusk and night, when lobsters are more active outside the burrow. Moreover, lobsters spent more time orientated downstream during darkness hours. Finally, our results suggest that water currents could entrain one of the components of the circadian oscillator, but further experiments are needed to clarify this aspect.

Lobsters showed the highest peak of diel activity around light-OFF as observed in previous studies (Atkinson and Naylor 1976; Hammond and Naylor 1977; Sbragaglia et al. 2013b). The response of lobsters to water currents was strictly dependent on the time at which the hydrodynamic stimulus was applied; however, lobsters' activity outside the burrow was still higher at dusk and night. This indicates that light-OFF is a crucial cue for the synchronization of the burrowing behaviour of *N. norvegicus* and that the light cycle is more important than the water current cycle.

It is important to notice that when the water currents coincided with the light-OFF, we observed a negative masking effect (i.e. suppression; sensu Mrosovsky 1999). In other words, the locomotor activity was inhibited and lobsters shifted their activity outside the burrow just after the offset of currents. Complex patterns of behaviour were previously observed when light–darkness and tidal cycles were studied simultaneously in marine organisms, and behavioural output usually depends on the relative phase between the cycles (Naylor 2010); examples are provided by a marine polychaete *Nereis virens* (Last et al. 2009) and by the horseshoe crab *Limulus polyphemus* (Watson and Chabot 2010).

Laroche et al. (1997) and Krumme et al. (2004) observed that recurring fish assemblages followed the combinations between tidal and light cycles in mangrove habitats. *Nephrops norvegicus* is a generalist predator and scavenger, and stable isotope studies indicate its role as a secondary consumer (Loc'h and Hily 2005; Johnson et al. 2013). Its behavioural pattern could affect the structure of the benthic community and, consequently, coupling of the benthopelagic compartment, thus periodically modifying biodiversity and trophic flow (Aguzzi et al. 2015). Krumme (2009) suggested considering the short-term variation caused by the interplay of tidal and light cycles during long-term monitoring programmes in intertidal zones. Our data indicated that the relative combination of tidal (12.4 and 24.8 h) and light cycles (24 h) could also be an important parameter for the deep-water benthic community and suggest the same attention to a design monitoring programme here.

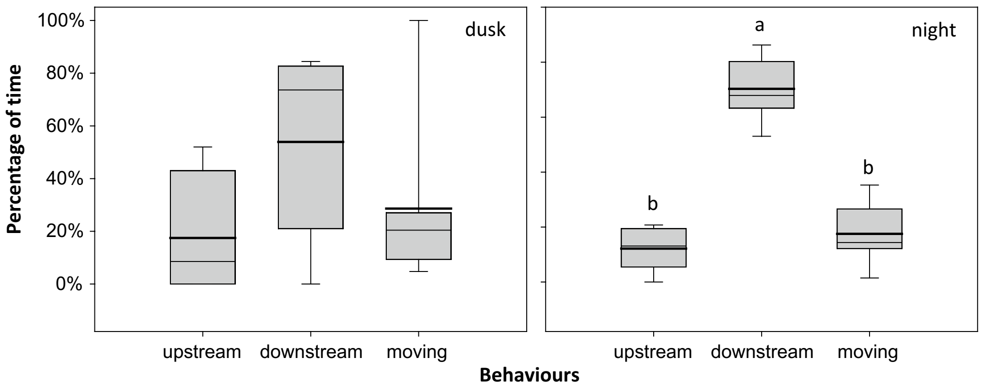

Fig. 5 Box plot of percentage of average time spent by lobsters orientated upstream, downstream, or moving during two periods of time: dusk (1 h before to 1 h after light-OFF; $n = 7$); night (1 h after light-OFF to 1 h before light-ON; $n = 9$). *Bold line* represents the mean. *Normal line* represents the median. The *grey box* represents the first quartile. *Lines* extending *vertically* from the *boxes* (*whiskers*) indicate variability outside the *upper* and *lower* quartiles. *Letters* indicate the output of multiple comparison post hoc test ($a > b$)

Water currents triggered concealment of lobsters. This behaviour may be of adaptive significance for minimizing the risk of predation. The predation risk experienced by *N. norvegicus* during peaks of current speed could be higher because predators' swimming activity may also be affected by water currents (Arnold 1981; Gibson 1992). For example, some deep-water continental margin fishes adjust their swimming behaviour in relation to the current speed (Lorance and Trenkel 2006). The most common predators of *N. norvegicus* in the Atlantic are the cod, *Gadus morhua*, haddock (*Melanogrammus aeglefinus*), dogfish (*Scyliorhinus canicula*), thornback ray (*Raja clavata*), and cephalopods (Thomas 1965; Chapman 1980; Johnson et al. 2013). Among *N. norvegicus'* predators, the cod seems to be the most efficient, and it has been demonstrated that the cod's horizontal and vertical displacements can be affected by tidal currents (Arnold et al. 1994; Michalsen et al. 1996; Pinnegar and Platts 2011). However, predation success by fish on *N. norvegicus* is usually low (Serrano et al. 2003), which suggests that a rapid retreat inside the burrow may be a successful anti-predator strategy.

Significant behavioural differences during flowing water were found only during the hours of darkness, when lobsters spent more time orientated downstream. There were no significant differences in behaviour during dawn, day, and dusk, but that result should be interpreted carefully because of the small number of individuals observed moving outside the burrow during those periods. Such a behavioural response from the lobsters could also be of ecological relevance in relation to its predators. Blind *N. norvegicus* in the laboratory orientated downstream in the presence of currents 7–20 cm s^{-1} (Newland et al. 1988). In the field, underwater video surveys documented a downstream orientation of lobsters when the current velocity showed a tidal periodicity with peaks at 10 cm s^{-1} (Newland and Chapman 1989). Newland et al. (1988) demonstrated that downstream orientation reduced the drag on lobster's body and might increase the probability of detecting fish predators that preferentially move upstream in water flow (Arnold 1981). Differences in body orientation could also be related to the efficiency of lobsters in detecting odour plumes in searching for food and assessing predation risk (Katoh et al. 2013). The way in which antennules are deployed in relation to water flow modifies the efficiency of aesthetascs (i.e. chemosensory hairs) in detecting water-borne odours (Koehl 2011). How body orientation in *N. norvegicus* influences chemical sensing is not known, but could be studied in the future.

We identified the presence of two distinct peaks during darkness hours, which suggest that the water currents could affect the phase of the circadian clock, rather than simply masking its output. However, the lack of experiments performed under constant conditions does not allow us to refer to it as entrainment. Further investigations could clarify whether the locomotor activity of *N. norvegicus* can be entrained by water current cycles. This question is relevant for benthic species on the shelf and upper slope, where light has been assumed to be the predominant zeitgeber synchronizing biological activity (Aguzzi et al. 2011). In a previous study at 2700 m depth in the northeast Atlantic, Wagner et al. (2007) showed that 12.4-h periodic peaks in current speed (similar to those in the present study) may represent a zeitgeber for demersal fish. *Nephrops norvegicus* possess mechanoreceptors that are distributed throughout the body (cuticular setae, first and second antennae, and statocysts) and are used for tactile exploration, perception of water movement, and detection of acoustic stimuli (Katoh et al. 2013). In decapod crustaceans, hydrodynamic stimuli and flow information are integrated by very sensitive mechanoreceptive neurons and interneurons connected to statocysts (Wiese 1976; Breithaupt and Tautz 1990; Katoh et al. 2013). Mechanoreceptors might also represent one of the input pathways to convey hydrodynamic information to the circadian system.

To the best of our knowledge, this is the first laboratory evidence that periodic water currents affect behavioural rhythm of a deep-water crustacean. The presence of an oscillator synchronized to light-OFF, which supports the splitting of locomotor activity into two components in the presence of periodic current stimuli, provides insight into the mechanism behind the spectral coordination (i.e. integration of various rhythms within an organism, sensu Refinetti 2012) of diel and tidal rhythms in this species. Aguzzi et al. (2011) presented a model of the *N. norvegicus* circadian pacemaker that assumed the presence of a population of oscillators with two basic properties: 6-h, phase-locked coupling and dumping. The data presented in Figs. 1 and S2 partially fit this model with the presence of two components, one that preserves its synchronization to the light-OFF and the other that follows the offset of water currents. Our data suggested that the phase coordination between the components may be >6 h and could reach 7:24 h (before loss of synchronization; see Fig. S2). Such a phenomenon was clearly observed in only a few individuals ($n = 4$). Interestingly, splitting (the presence of two separate phase components 180° apart) in mammalian model organisms is usually observed in a minority of tested individuals (Refinetti 2006). The optimization of physiological processes through the spectral coordination of diel and tidal rhythms has not received sufficient attention (Refinetti 2012), but it could be used as a determinant to assess the ecological significance of biological rhythms. Finally, we cannot exclude the possibility that a higher current speed and lower light intensity (simulating deeper water) could be more effective in eliciting such phenomena in a higher proportion of lobsters. Future experiments should also consider another important parameter such as water pressure, which can be an important environmental cue to synchronize activity to tidal cycles (Rice 1964; Fraser et al. 2003).

We have shown that periodic water currents simulated in the laboratory can modulate the behavioural rhythm of a deep-water species. Such results can guide future research on how the organization of circadian biology changes with depth. Moreover, these results suggest that the phases of diurnal or semi-diurnal tides are important when interpreting fishery-dependent and video survey data, not only for *N. norvegicus*, but also for other members of the deep-water benthic community.

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Compliance with ethical standards

Conflict of interest The authors declare the absence of conflict of interests for the data presented in this paper. Sampling and laboratory experiments followed the local legislation regarding animal welfare.

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