Light Intensity Determines Temporal Niche Switching of Behavioral Activity in Deep-Water *Nephrops norvegicus* (Crustacea: Decapoda)

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Light Intensity Determines Temporal Niche Switching of Behavioral Activity in Deep-Water *Nephrops norvegicus* (Crustacea: Decapoda)

Juan José Chiesa,*[,1,2] Jacopo Aguzzi ,[†,2] José Antonio García,[†]
Francesc Sardà,[†] and Horacio O. de la Iglesia[‡]

*Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes-CONICET, Bernal, Argentina, [†]Instituto de Ciencias del Mar (ICM-CSIC), Barcelona, España, [‡]Department of Biology, University of Washington, Seattle, USA

Abstract The temporal distribution of behavioral programs throughout the 24-h day, known as temporal niche of a species, is determined by ecological factors that directly affect the adaptive value of the timing of specific behaviors. Temporal niche switching has been described in several species and is likely adaptive in habitats where the daily timing of those factors changes. Benthic species whose habitats span a wide range of water depths are exposed to considerable depth-dependent environmental changes. Temporally scheduled trawl surveys of the Norway lobster, *Nephrops norvegicus*, reveal that animals emerge from burrows at night on the shallow shelf (10-50 m deep), at crepuscular hours on the lower shelf (50-200 m), and at daytime on the slope (200-400 m). The mechanisms underlying nocturnality/diurnality switches are chiefly unknown, and *Nephrops* offers a unique model for their study. The depth-dependent decrease in luminance is a likely candidate determining the temporal distribution of behavior. The authors explored this possibility in the laboratory by exposing *Nephrops* to light:dark (LD) cycles of 470-nm monochromatic lighting that mimic conditions at the 100-m-deep shelf (10 lux) or the 300-m slope (0.1 lux). Two groups of animals were respectively exposed to each light intensity according to the following protocol: an initial 12:12 LD stage followed by constant darkness (DD), followed in turn by a second 12:12 LD stage. Activity at the burrow opening (*door-keeping* = *DK*), as well as full *emergence* (*E*), was continuously monitored. Under 10-lux LD cycles, most animals showed nocturnal *DK* activity—with some being crepuscular or diurnal—and all animals showed nocturnal *E* activity. In contrast, both behaviors were clearly diurnal in animals under 0.1-lux LD cycles. The phase of the nocturnal and diurnal *DK* rhythms detected respectively at 10 and 0.1 lux upon release into DD revealed that these rhythms are entrained circadian rhythms. The present data indicate that nocturnality/diurnality switches in *Nephrops* in its natural habitat, evidenced by captures at different depths, are likely determined by light intensity. This temporal niche switching involves different patterns of photic entrainment, leading to bona fide circadian diurnal or nocturnal phenotypes, as well as exogenous masking of behavioral outputs.

Key words *Nephrops norvegicus*, circadian, entrainment, invertebrates, masking, deep sea

1. To whom all correspondence should be addressed: Juan José Chiesa, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, B1876BXD Bernal, Buenos Aires, Argentina; e-mail: jjosechiesa@unq.edu.ar.
2. Both authors contributed equally to this work.

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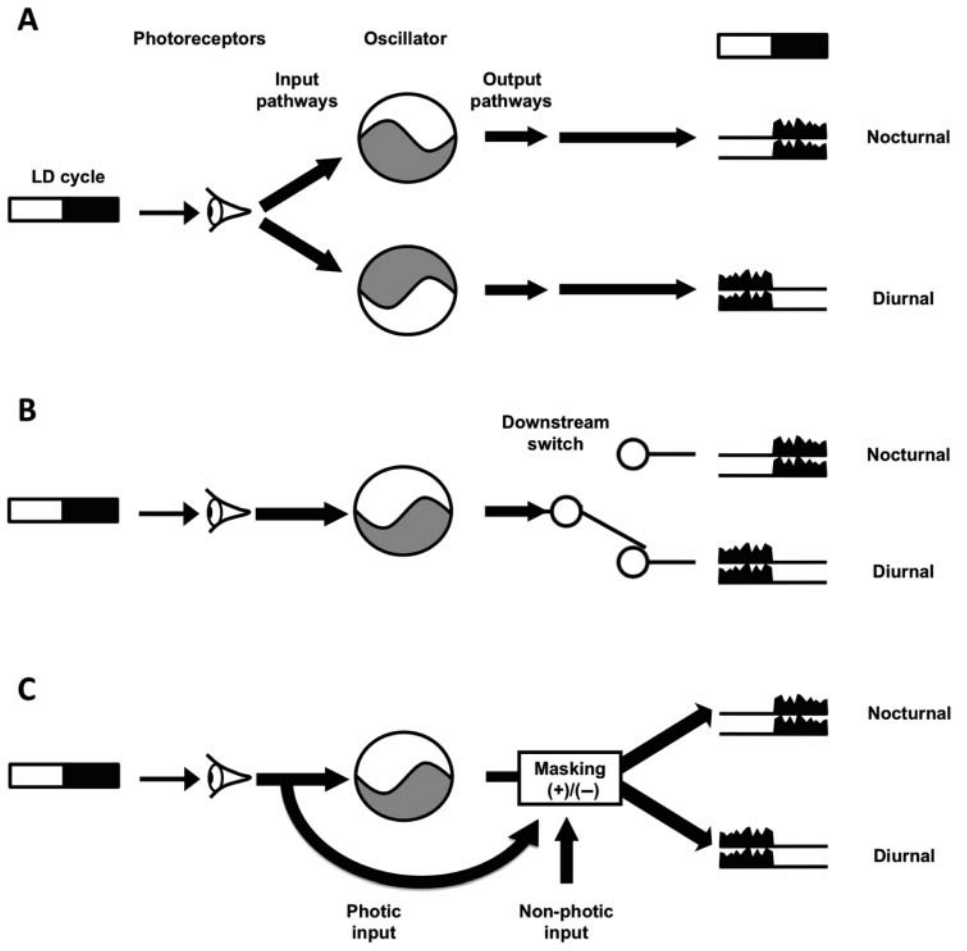

Figure 1. Schematic representation of mechanisms by which a circadian system could switch between nocturnal or diurnal temporal patterns. (A) The properties of the circadian oscillator could change so that its phase relationship to the LD cycle is different by almost 180°, leading to rhythms of activity that are oppositely phased. (B) The properties of the oscillator could be unaltered, but a switch in the output pathways regulating locomotor activity could alternate between nocturnal and diurnal patterns of locomotor activity. (C) The properties of the oscillator could be unaltered, but positive and/or negative masking could determine the rhythmic pattern of activity regardless of the oscillator phase relationship to the LD cycle.

The distribution of locomotor activity throughout the day is the result of an interplay between the output of circadian clock(s) entrained to 24-h cyclic environmental factors and the direct stimulatory (positive) or inhibitory (negative) effects of specific environmental factors on behavior, known as masking. For instance, a circadian clock that sustains locomotor activity during the day combined with positive masking by light on locomotion would typically result in a diurnal phenotype. Although under controlled laboratory conditions, nocturnality and diurnality may be easily distinguishable, these temporal niches are likely more plastic in the wild. Indeed, temporal niche switching appears to be a common feature in both vertebrates and invertebrates (see review in Mrosovsky and Hattar, 2005). Three basic mechanisms could account for temporal niche switches (Fig. 1). First (Fig. 1A), changes in oscillator properties could lead to changes in the phase angle of entrainment of the oscillator. For instance, relatively small changes in period may result in large changes in the phase angle of entrainment of the oscillator and therefore in changes of the time of day at which circadian locomotor activity will take place (Daan and Aschoff, 2001; Johnson et al., 2003). This is clearly the case in heterozygote *tau* mutant hamsters, which show a dramatically advanced onset of locomotor activity that renders them much more diurnal than their nocturnal wild-type siblings (Ralph and Menaker, 1988). Second (Fig. 1B), a circadian oscillator with a fixed phase of entrainment could govern distinctly phased locomotor activity rhythms if a switching mechanism downstream of the oscillator determines whether locomotor activity will take place during the day or night. This appears to be the case in some rodents, in which a similarly phased master circadian clock can sustain either nocturnal or diurnal phenotypes (Smale et al., 2003). Third (Fig. 1C), the predominance of locomotor activity in either the dark or light phase could be solely determined by positive and/or negative masking, regardless of the oscillator governance. This is the case in *Drosophila*, in which nocturnal moonlight leads to nocturnal activity (Kempinger et al., 2009), and it may be the case in nocturnal rodents (Cohen et al., 2010), in which

mutations that affect retinal sensitivity lead to unexpected diurnality (Mrosovsky and Hattar, 2005). The 3 mechanisms are not mutually exclusive, and indeed there is experimental evidence for all of them from studies in different species or different genotypes within the same species. However, the mechanism(s) underlying nocturnality/diurnality changes in species in which the temporal niche switches depending on habitat choice are unknown.

Benthic species distributed in the continental margin are exposed to LD cycles of varying light intensities depending on depth. They typically respond to LD cycles of "blue light" (470-480 nm) (Aguzzi et al., 2009), the sole source of radiation with sufficient energy to reach the deeper limits of the so-called twilight zone (Herring, 2002), and there is evidence that they may present temporal niche switching depending on the depth they inhabit and its associated light intensity. Populations of the Norway lobster, *Nephrops norvegicus*, can be found from less than 20 m depth at the *shelf* to up to 400 to 500 m depth at the *slope*. Temporally scheduled trawling shows a depth-dependent diel behavioral pattern (reviewed by Aguzzi and Sardà, 2008). On the upper-middle shelf (10-50-m depth), animals emerge at full-moon clear nights, probably due to a stimulatory effect of moonlight. On the lower shelf (50-200-m depth), animals show crepuscular emergence. Finally, animals exhibit diurnal emergence on the slope (400-430-m depth). Monochromatic blue light regulates burrow emergence, and this is in agreement with the absorbance spectrum of the species' visual pigments (Loew, 1974). However, it is still unknown whether the depth-dependent change in blue light intensity is sufficient to induce nocturnality/diurnality switches. If so, *Nephrops* would represent an ideal model to elucidate mechanisms of temporal niche switching in a species in which these switches likely confer high adaptive value.

Nephrops individuals held in aquaria with access to burrows can either be active at the burrow entrance ("door-keeping" (*DK*); Aguzzi and Sardà, 2008) or fully emerge from it ("emergence" (*E*); Chapman, 1980). These stereotypic behaviors have different ecological significance: while *DK* is related to territorial control, *E* is related to foraging and mating. The aim of the present study was to assess the depth-dependent photic regulation of *Nephrops* behavioral rhythms. We used monochromatic 470-nm wavelength LD cycles of 10 lux (mimicking sea shelf conditions) and 0.1 lux (mimicking sea slope conditions) and show that they respectively determine nocturnality and diurnality in both *DK* and *E*. Interestingly, our data indicate that this temporal niche switching involves different patterns of entrainment in *DK* behavior, which lead to bona fide circadian diurnal or nocturnal rhythms. Our results also show that masking by light, particularly of *E* behavior, contributes to the robustness of these nocturnality/diurnality switches.

MATERIALS AND METHODS

Animals, Housing, and Experimental Protocols

Adult males (average carapace length, 37.5 ± 4.4 mm) at intermoult stage were collected at night off the Ebro delta (northwestern Mediterranean; 40° 39′ N, 1° 13′ E; 40° 38′ N, 1° 11′ E) by a commercial trawler operating at 80 to 100 m depth. Nocturnal timing of sampling was chosen to avoid retinal damage due to sunlight exposure (Gaten, 1988; Gaten et al., 1990). Laboratory acclimation was carried out over 20 days in a lightproof isolated chamber under constant temperature (13 ± 0.1 °C, which corresponds to the constant temperature found in the Mediterranean below the 200-m deep thermocline (Salat, 1996). During this period, animals were exposed to a 12:12 h LD regime of 0.1 lux (Mavolux 5032B digital luxmeter, GMC Instruments, Nuremberg, Germany) using monochromatic light provided by blue LEDs/water clear lens (470 nm peak wavelength; model 67-1750, Digi-Key, Thief River Falls, MN). This simulates lighting conditions at 200 to 300 m depth (Aguzzi et al., 2009). Lights-on and lights-off were set at 0700 and 1900 clock time, respectively. Light intensity was gradually raised (lights-on) and extinguished (lights-off) throughout 30 min to acclimatize the eyes of animals and avoid optical damage (Gaten, 1988; Gaten et al., 1990). Animals were fed weekly with clams at random timings during acclimation but not during the experiments in order to avoid interference with the spontaneous patterns of locomotor activity (Fernández de Miguel and Aréchiga, 1994).

Throughout the experiments, animals were housed in individual polycarbonate aquaria re-creating selected environmental features of inhabited seabed areas (i.e., the presence of burrows) and of a substrate simulating the sediment texture. The movement of animals was tracked by means of vertical detection infrared beams in 2 different zones of the aquarium that allow discrimination between 2 different behaviors: *DK* (activity at the burrow entrance) and *E* (activity 20 cm away from it).

To detect activity rhythms under LD cycles and establish if they represented entrained circadian rhythms, we first exposed 2 groups of 11 animals each to 12:12 LD cycles (LD1) of 10 lux mimicking the shelf or 0.1 lux mimicking the slope. Second, we transferred the animals to constant darkness conditions (DD). Third, we reexposed the animals to LD cycles (LD2) of the same duration and intensity as LD1. Four individuals were discarded due to failure of the data acquisition system; thus, analysis was performed using an n of 10 for 10 lux and of 8 for 0.1 lux.

Behavioral Analysis

The tracking system reported the number of times per minute animals crossed each detection barrier. Prior to the analysis, these raw data sets were summed into 15-min bins and then smoothed by a moving average of 3 steps. Double-plotted actograms were obtained in 24-h format for visual inspection of rhythms.

The period value of rhythms was estimated separately for each stage by the chi-square periodogram analysis. Significant ($p < 0.05$) periodicities were identified, and the percentage of the total variance in the time series for each significant period was calculated from periodograms (Cambras et al., 2000), as a measure of the stability of rhythmic patterns (Chiesa et al., 2007). Only animals that showed significant periods in all the stages were included in the analysis below.

A 24-h waveform profile for *DK* and *E* activity was obtained for each animal in the 10- and 0.1-lux groups and for each LD stage (LD1 and LD2) separately. The percentage of activity occurring at the photophase, in relation to the total activity that each animal carried out throughout the 24-h period (i.e., "photophase activity"), was calculated from individual waveforms to assess either the diurnal or nocturnal distribution under LD. Behavioral patterns were identified as diurnal when photophase activity was above 60% and as nocturnal when it was below 40%, and this occurred at both LD stages. From individuals pooled into diurnal or nocturnal, the periodogram data, mean photophase activity, and the mean waveform were also obtained for comparisons between groups and LD stages. "Crepuscular" patterns were identified as those in which most of the activity appeared in coincidence with either L-D or D-L transitions.

In DD, the homogeneity of the LD cycles' effects on the circadian phase was studied using a Rayleigh z test (Batschelet, 1981). This test gives the significance in the clustering of phases distributed in circular coordinates (e.g., 24 h). The assumption for the analysis is that homogeneous entraining effects of the previous LD cycles on rhythms must cause a significant clustering of the individual circadian phases when animals are transferred to DD. The onset of the *DK* activity rhythm under 0.1 lux and 10 lux was estimated as follows. Data subsets of 24 h length were averaged during the first 5 days in DD. Then, the mean of this resulting waveform data was calculated and represented as a horizontal line onto the waveform plots. The activity onset was then defined as the time point at which activity values crossed and stayed above that line for at least 3 h. *E* activity data were excluded from this analysis due to the lack of significant free-running rhythms.

All chronobiological analysis was carried out using the software El Temps (Prof. Díez-Noguera, University of Barcelona, Spain). The variables obtained from periodogram and waveform analysis were represented as mean ± standard error of the mean (SEM). The 95% confidence interval (CI) for the mean period measured both under LD and DD was estimated to assess differences from the 24-h value. Two-way analysis of variance (ANOVA) considering 10-lux and 0.1-lux groups, as well as the stages as factors, compared the period and the percentage of variance for *DK* and photophase activity for *DK* and *E*. In all cases, significance was defined at the 95% level (i.e., $p < 0.05$).

RESULTS

The exposure of animals to 24-h LD cycles of different blue light intensity (10 lux or 0.1 lux) generated robust rhythms in the *DK* and *E* behaviors (Fig. 2). The periods of the DK rhythm under both photophase intensities were not statistically different from 24 h at either LD1 or LD2 stages (mean [CI] for 10 lux, LD1: 23.9 h [23.73-24.08 h], LD2: 24.04 h [23.96-24.13 h], $n = 7$; for 0.1 lux, LD1: 24.11 h [23.85-24.33 h], LD2: 23.96 h [23.73-24.18 h], $n = 5$). However, the activity distribution was strongly dependent on the photophase intensity. Animals exposed to 10-lux cycles showed a predominant nocturnal phenotype (Fig. 2A) during both LD stages. In contrast, all the animals exposed to the 0.1-lux photophase showed a clear diurnal pattern for both *DK* and *E* behaviors during the LD stages (Fig. 2B). After plotting each

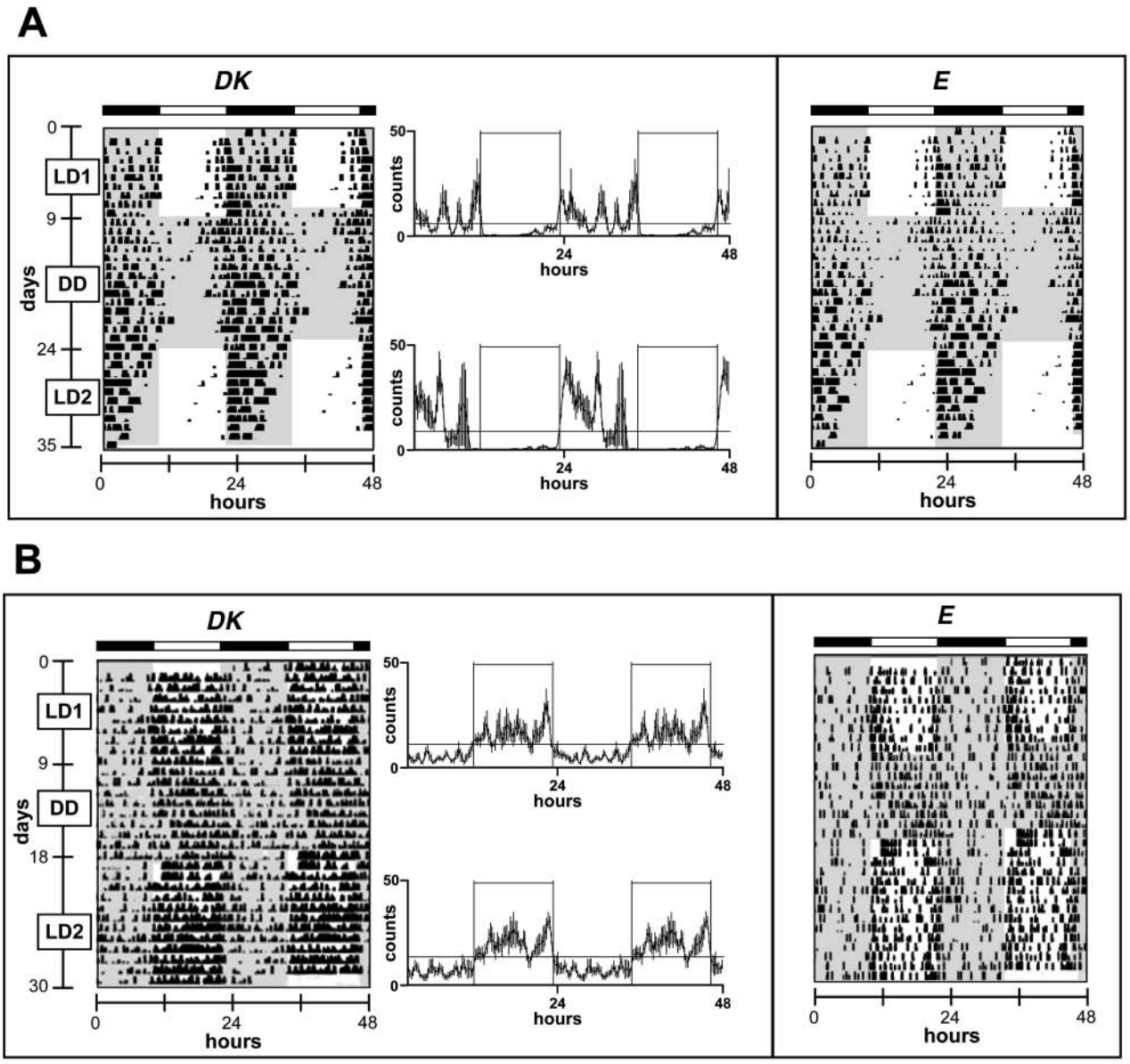

Figure 2. Representative *Nephrops* behavioral activity graphs double-plotted at modulo 24 h depicting rhythms in door-keeping (*DK*) and burrow emergence (*E*) activity under (A) 10 and (B) 0.1 lux during 3 experimental stages: a first 12:12 h LD cycle stage (LD1), followed by constant darkness (DD), in turn followed by a second LD cycle stage (LD2) equal to LD1. Panels on the left show *DK* activity in actogram and waveforms for data corresponding to LD1 and LD2 stages. *E* activity is shown for the same animal in the actogram on the right. White and black bars on top indicate photophase and scotophase hours of the LD cycle, respectively. Gray areas indicate darkness. The scale for activity bouts is normalized from 0% to 85%. In the waveform graphs, vertical bars represent the SEM, the horizontal line indicates the mean value of all waveform data, and the rectangles indicate the photophase.

animal's waveform, diurnal or nocturnal individuals were indentified as those respectively showing above 60% or below 40% *DK* activity within the photophase. For the 10-lux group, this criterion detected 4 nocturnal and 1 diurnal phenotypes. For 0.1 lux, it detected 5 diurnal phenotypes. Finally, 2 animals in the 10-lux group had obvious crepuscular activity (see below). The average waveforms calculated for both LD1 and LD2 stages in animals previously identified as nocturnal under 10 lux reveal this dominant phenotype, showing *DK* activity above the daily mean restricted to the scotophase (Fig. 3A). This procedure generated a clear diurnal distribution (Fig. 3B) for the 0.1-lux group, which showed activity above

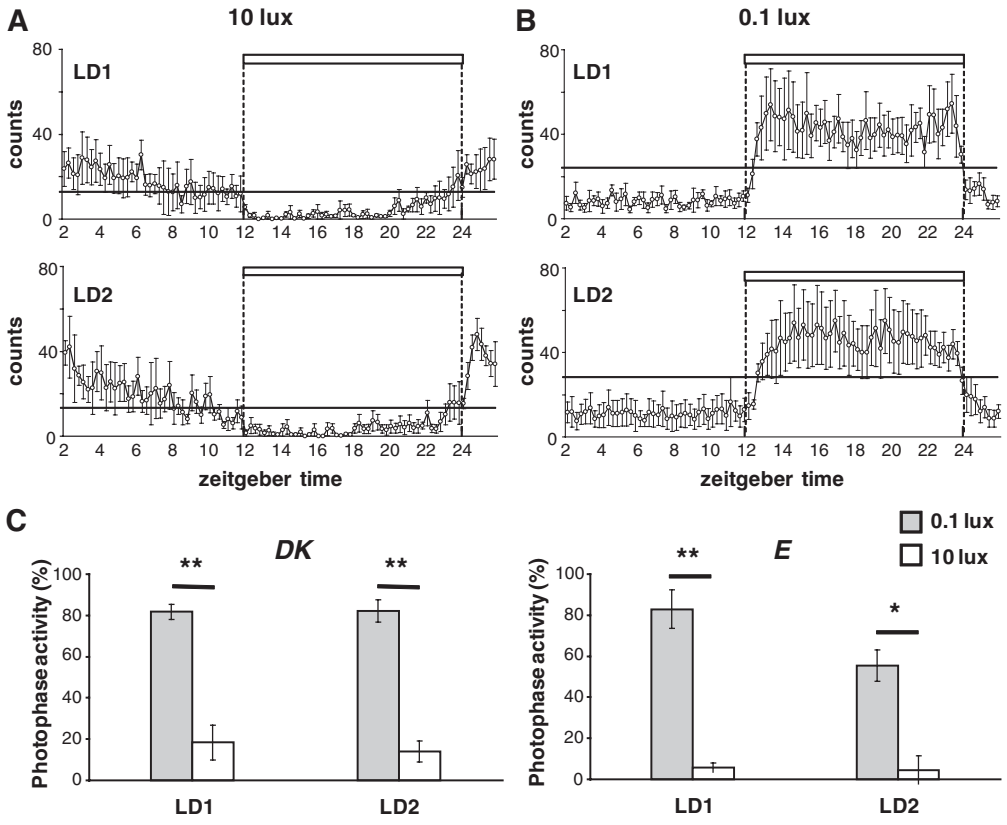

Figure 3. Average waveforms for nocturnal and diurnal individuals under (A) 10 and (B) 0.1 lux, respectively. Waveforms are plotted at modulo 24 h showing daily *DK* activity profile during both LD stages. White bar and dotted lines represent the photophase incidence (ZT12: lights-on, ZT24: lights-off). The horizontal line indicates the mean value of waveform data. (C) Mean (± SEM) percentage of door-keeping (*DK*) and emergence (*E*) activities exhibited during the photophase of both LD cycles stages, for both 0.1-lux and 10-lux groups. *$p < 0.05$, **$p < 0.005$, 2-way ANOVA and post hoc test.

the daily mean during the photophase. For both LD stages, the percentage of photophase *DK* activity (Fig. 3C) was significantly higher in diurnal animals under 0.1 lux than in nocturnal animals under 10 lux (2-way ANOVA, $F_{1,1,15} = 80.82$, $p < 0.0001$, Tukey test, LD1: $p < 0.0001$, LD2: $p < 0.005$). For each photophase intensity, the percentage of *DK* activity was similar comparing both LD stages (2-way ANOVA, $F_{1,1,15} = 0.08$, $p > 0.75$). Similar statistical results were found between intensity groups for *E* activity (Fig. 3C) (2-way ANOVA, $F_{1,1,15} = 35.14$, $p < 0.0001$) in both LD stages (Tukey test, LD1: $p < 0.001$, LD2: $p < 0.05$), with comparable percentage values for both groups between the stages (2-way ANOVA, $F_{1,1,15} = 1.75$, $p > 0.2$).

Significant free-running periods of *DK* activity were detected in the periodogram analysis in the majority of animals under DD conditions (7/10 for 10 lux, 5/8 for 0.1 lux). The mean values under 10 and 0.1 lux were calculated for these individuals, being statistically different from 24 h (mean [CI] for 10 lux: 24.43 h [24.09-24.78 h], $n = 7$; for 0.1 lux: 24.29 h [24.07-24.51 h], $n = 5$). The free-running period under DD was significantly higher than both periods measured at LD stages (2-way ANOVA, $F_{2,1,32} = 8.17$, $p < 0.005$, planned comparisons, LD1 vs. DD: $p < 0.001$, LD2 vs. DD: $p < 0.001$, LD1 vs. LD2, $p > 0.5$). The period values measured under DD were not affected by the previous photophase intensity (2-way ANOVA, $F_{2,1,32} = 1.84$, $p > 0.1$). Significant free-running periods of *E* activity were detected only in a few individuals of both groups (3/10 for 10 lux, 2/8 for 0.1 lux).

The periodogram percentage of variance of the *DK* activity rhythm was also compared between stages and light intensities. Significant differences were found for this variable when considering the stage (2-way ANOVA, $F_{2,1,32} = 5.85$, $p < 0.01$), being higher under LD1 (for 10 lux: 38.77 ± 6.19; 0.1 lux: 45.84 ± 8.20) and LD2 (for 10 lux: 39.27 ± 5.43; 0.1 lux: 29.43 ± 8.74) than DD (for 10 lux: 19.36 ± 4.29; 0.1 lux: 19.98 ± 2.72) (planned comparison, $p < 0.01$) but not different between LD1 and LD2 (planned comparison, $p > 0.1$). On the other hand, the percentage of variance was similar under both photophase intensities (2-way ANOVA, $F_{2,1,32} = 0.72$, $p > 0.4$).

Under both light intensities, the free-running rhythm of *DK* had in most cases a phase that was predicted by the phase of entrainment during LD1 (Fig. 2A for 10 lux, Fig. 2B for 0.1 lux). All 4 nocturnal animals exposed to 10 lux showed a free-running activity onset that coincided roughly with the extrapolated time of lights-off (ZT0). However, in

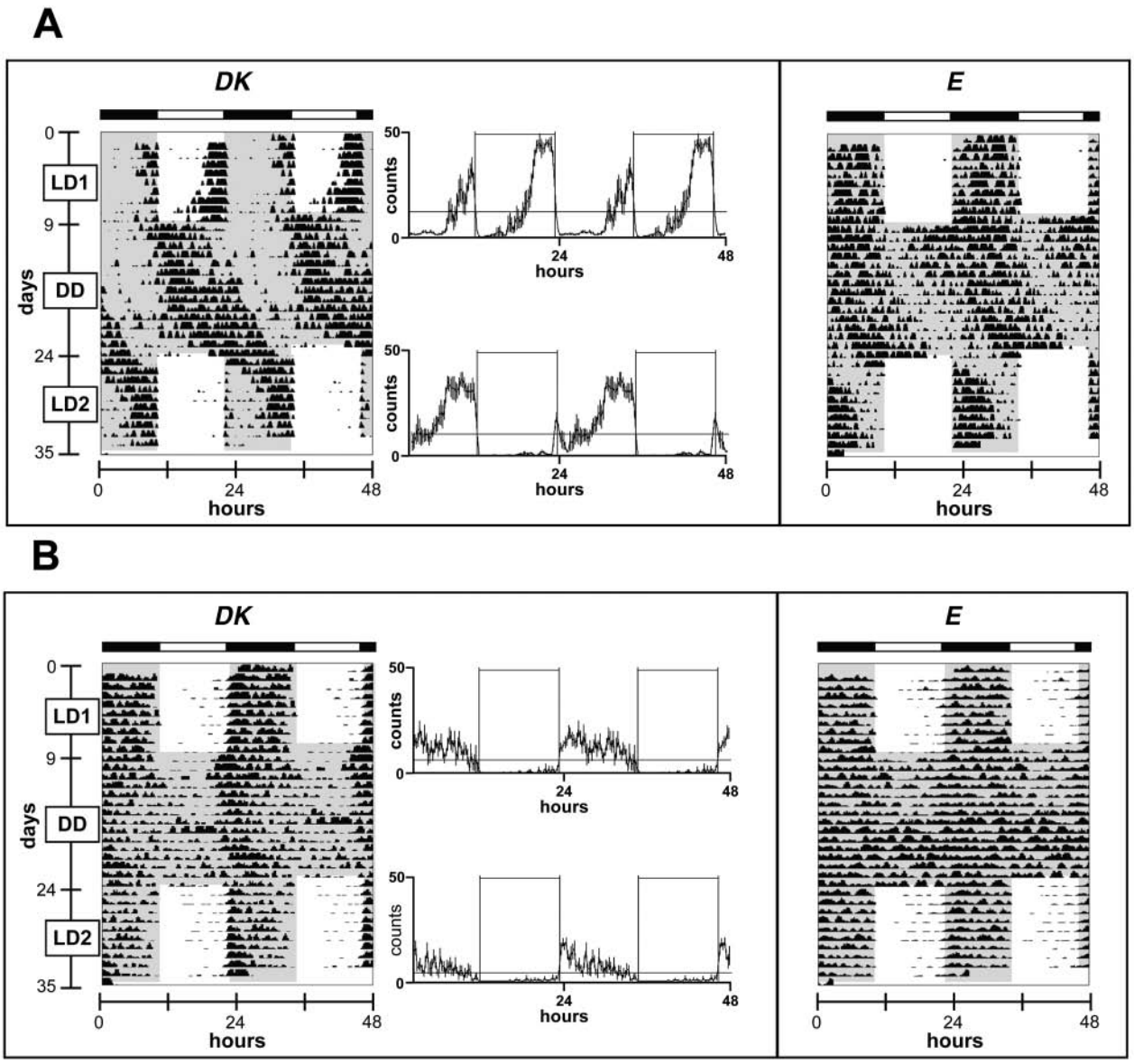

Figure 4. Door-keeping (*DK*) and burrow emergence (*E*) activity in individuals under 10-lux LD cycles that showed different phenotypes to that shown in Figure 2. (A) An individual with crepuscular phenotype for *DK* activity (two/seven 10-lux animals showed this crepuscular phenotype) and a strongly masked *E* activity rhythm. (B) An individual showing stable nocturnal *DK* circadian rhythm under LD and under DD but a fully masked *E* rhythm, which was arrhythmic under DD.

3/7 animals under 10 lux, the free-running phase was not predicted by lights-off as the outcome of a nocturnal pattern. Of these 3 animals, 1 was diurnal (not shown), 2 had crepuscular patterns (Fig. 4A), and they all showed free-running onsets that coincided approximately with the previous lights-on time (ZT12). Rayleigh *z* tests for 10-lux exposed animals confirmed a nonsignificant clustering of the free-running *DK* rhythm onsets (Fig. 5). In contrast to 10-lux-entrained animals, all 5 individuals under 0.1 lux showed diurnal rhythms whose free-running activity onset phases produced significant clustering in the Rayleigh *z* test in coincidence with the extrapolated time of lights-on (Fig. 5).

Figure 4 shows activity patterns of 2 animals exposed to 10 lux that were not as clearly nocturnal as most animals in this group. Figure 4A shows a 10-lux individual with crepuscular *DK* activity bouts coinciding with D-L and L-D transitions during LD1, possibly induced by lights-on and lights-off inhibition. Although in this animal the apparent phase of entrainment was crepuscular, its free-running rhythm

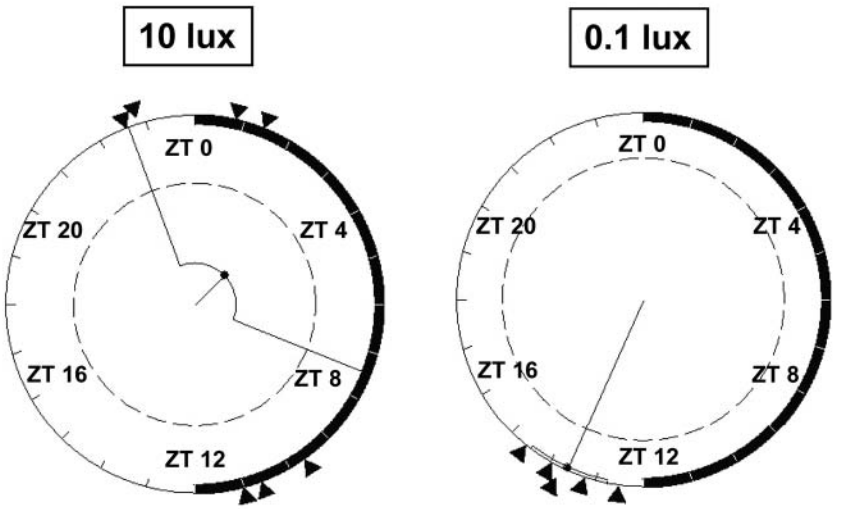

Figure 5. Outputs from Rayleigh *z* tests used to evaluate the clustering of door-keeping activity onsets (triangles) estimated the first 5 days under DD for the group of animals under 10 lux and those under 0.1 lux LD. Coordinates from 0 to 24 represent the previous zeitgeber times (lights-off at ZT0), and the black semicircle depicts the scotophase. The dotted circumference defines the threshold value for a significant *r* vector ($p < 0.05$). Clustering was only significant for animals under 0.1-lux LD cycles; $r = 0.98$, $r(0.05) = 0.75$.

upon release into DD clearly revealed a diurnal entrainment pattern. The activity pattern acquired a new phase relationship during LD2, and although still crepuscular, it became predominantly nocturnal. *E* activity in this animal, although circadian, was greatly inhibited by light during the photophase of both LD1 and LD2. The individual shown in Figure 4B had stable nocturnal entrainment of *DK* activity—confirmed by its free-running phase. In contrast, its nocturnal *E* activity was clearly the result of strong light inhibition, as it became predominantly arrhythmic during DD.

DISCUSSION

Although temporal niche switches in response to changes in the relative light/dark intensity in the LD cycle had been previously reported (Mrosovsky and Hattar, 2005; Kempinger et al., 2009), our study is the first one to show nocturnality/diurnality switches in a deep-water marine species that encounters dramatic light intensity changes depending on the habitat where each population lives. *Nephrops* showed predominantly diurnal activity patterns under dim (0.1 lux) blue LD cycles, but its activity was nocturnal under brighter (10 lux) blue LD cycles. This result strongly suggests that depth-dependent light intensity changes are a key determinant for the nocturnal and diurnal patterns of activity the species exhibits in nature, based on captures in the shelf and slope, respectively (Aguzzi and Sardà, 2008; Aguzzi et al., 2009).

Our results indicate that temporal niche switching in *Nephrops* is not merely the result of masking of locomotor activity by light but rather the result of a circadian system that can generate true nocturnally and diurnally entrained activity patterns depending on light intensity. This was evidenced by the fact that in both nocturnal and diurnal animals, the phase of the free-running rhythm was predicted by the phase of entrainment before releasing the animals into DD.

Two of the mechanisms outlined in Figure 1 could be responsible for the determination of nocturnality and diurnality in *Nephrops*. Variable light intensity could induce changes in the core properties of the oscillator(s) sustaining locomotor activity (Fig. 1A). Alternatively, the oscillator properties could be unaffected, and mechanisms downstream from the oscillator could switch between nocturnal and diurnal phenotypes depending on light intensity (Fig. 1B). Because there were no significant differences between the free-running period of animals released into DD from 0.1-lux LD cycles and that of animals released from 10-lux LD cycles, we favor the latter hypothesis—namely, that the temporal niche change results from a nocturnal/diurnal switch that can revert the output of the oscillator. Testing of this hypothesis would require identifying the location of the circadian oscillator governing locomotor activity and its molecular mechanisms, both of which are poorly understood in *Nephrops*, as well as in other decapods (de la Iglesia and Hsu, 2010).

While *DK* activity in both groups showed a true circadian modulation, *E* was not regulated as strongly by a circadian oscillator and showed a prominent exogenous light influence. This resulted in diurnal *E*

activity under 0.1-lux LD cycles and nocturnal *E* activity under 10-lux LD cycles. These patterns were likely due to respectively stimulatory (positive masking) and inhibitory (negative masking) effects of light on E activity (see Fig. 4). This dual modulation of locomotor activity by light has been previously described in nocturnal rodents, and mutations that deprive mice of rods and cones abolish positive masking but only moderately inhibit negative masking, suggesting that the two responses may rely on different photoreceptor systems (Mrosovsky et al., 1999). Masking was also evident for *DK* activity. Crepuscular *DK* activity emerged in some cases, and it appeared to be a consequence of inhibition by lights-on and lights-off, without a stable locking of the circadian phase (Fig. 4A). Furthermore, a significant increase in the periodogram percentage of variance under LD compared to DD reflects higher stability in waveforms due to masking of overt rhythms. Taken together, these results indicate that *DK* and *E* behaviors are coupled with different strengths to one or more circadian oscillators and are also under variable masking influence by light. *DK* activity likely represents a territorial behavior (Aguzzi and Sardà, 2008), and its predominantly endogenous control likely ensures coming out from burrows on a diel basis to regulate social behavior (Menesatti et al., 2009). In contrast, *E* behavior implies leaving the protection of the burrow and a potential exposure to predators. Masking is an adaptive behavioral trait in both vertebrates (Mrosovsky, 1999) and invertebrates (Page, 1989). It allows animals to "bypass," according to contingent ecological events (e.g., full moonlight [Kempinger et al., 2009] or the perception of a predator), the circadian modulation of a specific behavior. The stronger masking of *E* behavior by light found in the present study is consistent with this view.

Although all our experimental animals were sampled from the same depth (80-100 m), we detected behaviors typically encountered at the slope (diurnality) and shelf (nocturnality). This finding suggests that the different temporal niches encountered in *Nephrops* in the wild are the result of a plastic circadian system and not of a genetically determined behavioral phenotype linked to the population origin. This is consistent with studies on *Nephrops* population genetics that point to panmixia among populations from geographical zones that range from the North Atlantic to the East Mediterranean (Passamonti et al., 1997; Stamatis et al., 2004; Streiff et al., 2001). Nevertheless, it remains to be determined whether *Nephrops* individuals or their offspring migrate through different depths and whether the same genetic homogeneity found between different areas is present between different depths. The individuals in our study showed a wide range of rhythmic behaviors. These ranged from lack of true circadian rhythms—animals that showed no free-running rhythms despite their rhythmic behavior under LD conditions—to animals with robust circadian rhythms entrained to the different LD cycles (compare actograms in Figs. 2 and 4). This variability is not unexpected in animals sampled from natural populations; however, it remains to be determined whether the range of rhythmic behavioral patterns we encountered is truly representative of the species genetic variability.

The definition of species niche should include environmental changes across time and space (Kronfeld-Schor and Dayan, 2003). Temporal niche switches in marine species whose habitats span a wide range of water depths likely confer high adaptive value in response to depth-dependent changes in external variables. As in all decapods, *Nephrops* is at intermediate levels of marine food webs (Zaret and Suffern, 1976; Aksens and Giske, 1993; Onsrud et al., 2004), and its temporal niche is strongly conditioned by visual top predators (e.g., fish and cephalopods) as well as by its ability to catch prey. On the shelf, several decapod species, including *Nephrops*, cluster their activity phases at nighttime (Aguzzi et al., 2009), when prey is more easily available and predation by visual predators is low (Childress, 1995). In contrast, activity on the slope may be conditioned by other factors such as food availability, predator pressure, temperature, and light intensity itself. Those nonphotic factors that stably oscillate with a 24-h period could not only exert masking but also potentially serve as zeitgebers and "temporal-niche switchers." Of note, although the adaptive value of temporal niche switching may rely on the combined influence of multiple environmental factors in benthic species, our data strongly suggest that light intensity, as a reliable marker of depth, has been likely selected as a key determinant of these temporal switches.

In the present work, crepuscular activity was observed under 10-lux LD cycles but not under 0.1 lux. This difference suggests that the behavior of *Nephrops* might be controlled by a dual-circadian-oscillator system. Dual oscillators phase-locked to crepuscular light are also commonly observed in mammals

(Pittendrigh and Daan, 1976), insects (Helfrich-Förster, 2001; Stoleru et al., 2007), and crabs (Palmer, 2000; Thurman, 2001). The presence of crepuscular phenotypes in *Nephrops* suggests that switches between nocturnality and diurnality could be the result of a dual-oscillator system in which coupling between oscillators varies according to the relative light intensity of the light and dark phases.

In conclusion, our data indicate that the behavioral temporal niche in *Nephrops* is induced by differences in light intensity that mimic those that animals encounter between the sea slope and shelf. These nocturnality/diurnality switches are the result of a circadian system that can generate bona fide diurnal and nocturnal behavioral programs under different light intensity LD cycles, as well as of the masking effects of light.

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