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Day–night and depth differences in haemolymph melatonin of the Norway lobster, *Nephrops norvegicus* (L.)

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ABSTRACT

Few studies have been conducted to quantify and understand the role of melatonin in invertebrates, and particularly in crustaceans and in deep-sea animals. In this study, we examined day–night differences in haemolymph melatonin of the burrowing decapod crustacean *Nephrops norvegicus* (L.) during exposure to cycles of monochromatic blue light (480 nm) and darkness cycles of 10 and 0.1 lx. These differential intensity conditions simulate illumination at the depth of the shelf (80–100 m) and of the slope (300–400 m), where these lobster populations are chiefly found in the Western Mediterranean Sea. Our objectives were: (a) to verify the presence of melatonin in the haemolymph of this species using liquid chromatography/tandem mass spectrometry (LC–MS/MS) and fluorescence HPLC (HPLC); and (b) to study the relationship between diel variations in melatonin concentration and locomotor rhythms, in order to examine whether the former influences behaviour. Melatonin was identified in LC–MS/MS by Q1 and Q3 mass peaks at an elution time of 3.7 min, and it was also detected by HPLC. Melatonin concentration was found to be two orders of magnitude higher at 10 lx (4.8 ± 5.3 ng ml^{-1}) than at 0.1 lx (0.06 ± 0.03 ng ml^{-1}). Also, the increase at daytime in 10 lx was absent in 0.1 lx. When the locomotor rhythm of animals exposed to both photoperiod regimes was compared, the diel periodicity was found to be preserved, but the timing of activity shifted from night to day. Extrapolating these data to the field, we interpret our results to mean that locomotor activity preserves its diel character, but not its phase and amplitude, in a bathymetric range where haemolymph melatonin reduces its concentration and rhythmic fluctuation.

1. Introduction

Melatonin is an evolutionarily conserved molecule involved in the transduction of photoperiodic information from the environment to the physiology of organisms (Arnoult et al., 1994; Hardeland, 1997). It has been detected in bacteria, eukaryotic unicellular organisms, macroalgae, plants, fungi, and various taxa of invertebrates and vertebrates (Hardeland et al., 1995; Van Tassel et al., 2001; Hardeland and Poeggeler, 2003; Pape and Lüning, 2006). The key enzyme regulating the biosynthetic pathway of melatonin, arylalkylamine N-acetyltransferase (aaNAT), is present not only in mammals but also in a broad range of invertebrates including decapods (Vivien-Roels et al., 1984; Smith, 1990; Vivien-Roels and Pévet, 1993; Itoh and Sumi, 1998; Withyachumnarnkul et al., 1992).

The presence of melatonin in invertebrates is well documented, but its role in regulating their physiology and circadian system is not yet fully understood (Vivien-Roels and Pévet, 1993). Rhythmic variations in the concentration of melatonin have been recorded in the

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eye and brain of insects over periods of 24 h (Abran et al., 1994; Hayes, 1989; Wetterberg et al., 1987; Itoh et al., 1995a, b) In crustacean decapods, melatonin rhythms have been measured in the optic lobes (Withyachumnarnkul et al., 1992, 1995), in the eyestalks, and in the haemolymph (Tilden et al., 2001a, b, 2003a, b). In crabs, eyestalks melatonin conveys information on environmental light intensity cycles to locomotor centres and coupled physiological processes (Tilden et al., 1997). For example, the increase in melatonin in the haemolymph occurs in association with locomotor activity peaks. Moreover, when exogenous melatonin is administered at mid-daytime phase it can induce a timing shift in glucose and lactate packs (Tilden et al., 2001a, b).

Nephrops norvegicus (L.) is a burrowing decapod that inhabits deep-water continental margin areas of the Atlantic Ocean and the Mediterranean Sea (Bell et al., 2006). Animals can be captured by trawl nets only when they emerge from their burrows. Daily fluctuations in the commercial catches of this species are usually considered a result of diel changes in burrow emergence behaviour in response to day–night cycles (reviewed in Aguzzi and Sardà, 2008). Field studies by temporally scheduled trawling reported population peaks in burrow emergence from night to day as the sampling depth increased. Emergence is nocturnal on upper continental shelves (10–50 m depth), but crepuscular on the lower shelves (100–150 m depth) (e.g. Farmer, 1975; Moller and Naylor, 1980). Fully diurnal emergence was recently reported on continental slopes (400–700 m depth) (Aguzzi et al., 2003a, b; Cristo and Castro, 2005). Blue light (480 nm) is the putative zeitgeber of emergence rhythms (Aguzzi and Sardà, 2008), and radiation of this wavelength is constantly present in the water column down to the lower border of the twilight zone (Jerlov, 1968; Herring, 2002) at a depth of approximately 1000 m in the Mediterranean (Margalef, 1986). It is unknown how the haemolymph melatonin concentration varies in *Nephrops* exposed to blue light cycles of different intensities, which simulate different depths and how any variations in concentration might be correlated with locomotor rhythmicity (Aguzzi and Sardà, 2008).

Melatonin in deep-water decapods should be studied to explain and predict the catchability and hence management of different species in terms of the behavioural and underlying physiological responses to environmental determinants such as the light cycle (Aguzzi and Sardà, 2008). In the present study, our objective was to verify the presence of melatonin in the haemolymph of *N. norvegicus* maintained under cycles of monochromatic blue light (480 nm) and darkness of 10 and 0.1 lx intensity, which served to simulate, respectively, conditions at depth on the shelf (80–100 m) and the slope (300–400 m) where most populations are found in the western Mediterranean (Aguzzi et al., 2003a, b). We aimed to measure the diel fluctuation in the concentration of haemolymph melatonin and to relate it to locomotor rhythms when animals are exposed to a light–darkness cycle of 0.1 lx (approx. $0.01\,\mu E_i\,m^{-2}\,s^{-1}$) as recorded here and of 10 lx (approx. $1\,\mu E_i\,m^{-2}\,s^{-1}$) as recorded in Aguzzi et al. (2008).

2. Materials and methods

2.1. Animals

Adult males (average carapace length, 40.5 ± 4.4 mm; see Table 1) at intermoult were collected off the Ebro delta (northwestern Mediterranean, within the following latitude and longitude ranges: 40° 39′N, 1° 13′E; 40° 38′N, 1° 11′E) by a commercial trawler fishing at a depth of 80–100 m. Animal collection was performed at night in order to avoid retinal damage due to sunlight exposure (Gaten et al., 1990). Laboratory acclimation was carried out over 15 days in a light-proof isolated chamber under conditions of constant temperature (13 ± 0.1 °C) and photoperiod regime, which simulated photic conditions at the depth at which the animals were sampled (80–100 m) (Aguzzi et al., 2003a, b). Specifically, the following photoperiod regime was used: monochromatic blue light (480 nm) and darkness cycle of 10 lx with lights-ON and -OFF timings at 7:00 and 19:00, respectively. Full-light intensity was acquired and extinguished within the following 30 min to acclimatize the animals' eyes and avoid optical damage. 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 (Fernandéz de Miguel and Aréchiga, 1994).

2.2. Experiments

The first experiment focused on searching for melatonin in the haemolymph of *Nephrops* and on determining whether its concentration showed day–night variations. Animals were exposed to the same photoperiod regime described above (10 lx); under this photoperiod condition, both adult males and females display clearly identifiable nocturnal locomotor rhythms (Aguzzi et al., 2008). After 15 days of acclimation in the animal facility, haemolymph was extracted at the central hour of the imposed night (00:00, 10 animals) and day (12:00, 9 animals) (Table 1).

The second experiment aimed to measure the day–-night variation in melatonin concentration in the haemo-

Table 1
Information on average ($\pm$s.d.) animals' size (carapace length; CL = mm) and melatonin haemolymph concentration ($ng\,ml^{-1}$), as well as, significant locomotor periodicity (*T*; hours) and waveform analysis outputs in average amplitude and mesor, for *Nephrops norvegicus* exposed to light–darkness cycles of intensity (*I*) equals to 10 and 0.1 lx.

I	*N*	CL	$ng\,ml^{-1}$	*T*	Mesor	Amplitude
10	19	41.8 ± 3.8	4.8 ± 5.3	–	–	–
0.1	20	39.2 ± 4.7	0.06 ± 0.03	23.99 ± 0.16	23.31 ± 18.36	95.28 ± 30.38