Temporal modification in cardiac rhythmicity of *Nephrops norvegicus* (Crustacea: Decapoda) in relation to trawl capture stress*

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SUMMARY: The effects of trawling on cardiac rhythmicity of *Nephrops norvegicus* (L.) are still mostly unknown. Ultradian rhythms reported in previous studies may result from trawling capture stress, thus disappearing following acclimatisation to laboratory conditions. To test this hypothesis, 34 time series of cardiac activity data recorded in constant darkness were studied by Fourier analysis. Spectral decomposition of time series was obtained by defining the fundamental or circadian harmonic (CH) in 24-h together with 9 submultiples of this period. The power content (PC) of each harmonic was estimated in data segments of 24-h duration (days), giving graphic matrices of PC values over consecutive days. Values of PC for 9 submultiples were summed and studied in a block named ultradian band (UB). The modification in the PC of the CH and of the UB was evaluated during laboratory acclimatisation. A significant increase in the PC of the circadian harmonic component (CH) over consecutive days of testing was observed. These findings suggest that, rather than being a product of dim light environmental fluctuations experienced by the animals from the deep waters of the continental slope, ultradian periodicity could well be caused by the stress of capture.

Keywords: *Nephrops norvegicus*, stress, cardiac rhythms, constant darkness, Fourier analysis, circadian and ultradian rhythms.

RESUMEN: MODIFICACIÓN TEMPORAL DE LA RITMICIDAD CARDÍACA DE *NEPHROPS NORVEGICUS* EN RELACIÓN AL ESTRÉS DE CAPTURA POR ARRASTRE. – Los efectos de la pesca de arrastre sobre la ritmicidad cardíaca de *Nephrops norvegicus* (L.) son aún desconocidos y los ritmos ultradianos descritos en estudios previos podrían ser consecuencia del estrés de captura por el arte de pesca de arrastre, desapareciendo éstos al continuar la aclimatación en el laboratorio. Para probar esta hipótesis, 34 series temporales de datos de actividad cardíaca registrados en oscuridad constante fueron estudiadas mediante análisis de Fourier. Se obtuvo la descomposición espectral de las series definiendo el armónico fundamental o armónico circadiano (CH) en 24 h junto con 9 submúltiplos de este período. Se estimó el contenido de potencia (PC) de cada armónico en segmentos de datos de 24 h de duración (días) obteniéndose matrices gráficas de valores del PC en días consecutivos. Los valores del PC de los 9 submúltiplos fueron sumados y estudiados en un bloque llamado banda ultradiana (UB). Fue evaluada la modificación diaria del PC del CH y de la UB durante la aclimatación en el laboratorio. Se observó un incremento significativo en el PC del componente armónico circadiario (CH) durante los días consecutivos. Estos hallazgos sugieren que la periodicidad ultradiana no sería producida por las fluctuaciones ambientales de luz ténue experimentadas por el animal en aguas profundas del talud continental, sino que estarían provocadas probablemente por el estrés de captura.

Palabras clave: *Nephrops norvegicus*, estrés, ritmos cardíacos, oscuridad constante, análisis de Fourier, ritmos circadianos y ultradianos.

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Introduction

Metabolic rates in decapod crustaceans provide a reliable measure of their response to stress conditions (Reddy and Bhagyalakshmi, 1994; Aagaard, 1996). Several types of stress affect their physiology during trawl fishing (Bergmann *et al.*, 2001). For example, capture of *Nephrops norvegicus* (L.) by trawling, its handling on board, and its consequent aerial exposure, even for a short time, results in marked hyperglycaemia (Aguzzi *et al.*, 2004c) along with consistent variations in haemolymph ammonia and lactate (Schmitt and Uglow, 1997; 1998). When animals are transferred to the laboratory for physiological tests, a decreasing trend in behavioural and metabolic parameters (i.e., locomotor activity, oxygen consumption and heart rate) is often recorded as a probable consequence of acclimatisation to laboratory conditions (Aguzzi *et al.*, 2003a).

During the decade 1975-85, British authors studied *N. norvegicus* behavioural and underlying physiological rhythms in relation to burrow emergence behaviour (Atkinson and Naylor, 1976; Oakley, 1979; Moller and Naylor, 1980), which deeply affects the populations' catchability on a diel base (Chapman and Howard, 1979; Aguzzi *et al.*, 2003b). Specimens captured by creeling on British shelves at shallow depths (20-150 m) were used in laboratory tests on locomotor and coupled neurohormonal rhythms, showing a strong nocturnal regulation (Atkinson and Naylor, 1976; Aréchiga *et al.*, 1980). In the last few years, the growing fishery pressure on western Mediterranean stocks in deeper-water areas of the continental slope (300-500 m) (Abelló *et al.*, 2002) have renewed the interest in studies on behavioural and physiological rhythmicity associated with burrow emergence and consequent catchability. Laboratory tests on locomotor and cardiac rhythmicity were conducted on trawl-captured specimens, showing high percentages of circadian animals, along with a minority of ultradian ones (Aguzzi *et al.*, 2004a,b). Ultradian rhythms were reported in these studies for the first time, as a unique feature of continental slope animals, apparently due to dim daily light intensity fluctuations experienced by slope animals, impairing the correct functioning of their biological clock (Aguzzi *et al.*, 2003b). Nevertheless, doubts on the origin of ultradian rhythms as a product of trawl capture stress still persisted (Aguzzi, 2002).

For western Mediterranean continental slope *N. norvegicus*, studies on behavioural and physiological rhythmicity in relation to fishery require the capture of specimens by relatively long-lasting trawling, given the deep water distribution of exploited populations in the area (Abelló *et al.*, 2002). The effects of trawling stress on the animals' biological rhythms are still mostly unknown. Ultradian periodicities may result from stress of capture, hence disappearing with acclimation to laboratory conditions, rather than being the product of local dim light intensity fluctuations impairing biological clock functioning (Aguzzi *et al.*, 2004b). To test this hypothesis, modifications of a deterministic physiological variable, such as the heart rate, were screened during the first week of acclimation of animals to laboratory conditions in constant darkness. Time series of ultradian and circadian periodicity obtained in an earlier study (Aguzzi *et al.*, 2004a) were used for this purpose.

MATERIAL AND METHODS

Material

A total of 34 time series of nearly one-week duration, showing significant circadian (n = 25) and ultradian (n = 9) periodicities in a previous study (Aguzzi *et al.,* 2004a) were analysed. Time-series, as number of heart beats per 10 minutes, were recorded from intermoult adult specimens of *N. norvegicus* captured by trawling by a commercial fishing vessel on the western Mediterranean continental slope (400-450 m). Once on board, the animals were immediately transferred to individual plastic tanks and transported to the laboratory within 2 h after capture. In the laboratory, the tanks were endowed with an external individual system of circulation and filtration of the seawater. The isolation of the specimens prevented possible synchronisation of animal physiology through dissolved metabolites. The water temperature during the tests was 13 ºC (± 0.1°C), corresponding to that found on the western Mediterranean continental slope throughout the year (Salat, 1996). Animals were not fed during the experiments to avoid entrainment of cardiac rhythm upon timing of feeding (Fernández de Miguel and Aréchiga, 1994). Cardiac activity was recorded in constant darkness using the CAPMON monitoring equipment (Depledge and Andersen, 1990), by connecting each specimen to a lightweight infrared emitter/detector electrode placed on the anterior dorsal region of their carapace, above the heart. Data were continuously stored on a computer. Time-series analysis was performed using the integrated package for chronobiological studies “El Temps” (Díez-Noguera, 1999, University of Barcelona).

Methods

The temporal modification in the periodicity of the cardiac rhythm was studied with Fourier analysis. A fundamental function of circadian periodicity (Circadian Harmonic, CH) was modelled since the majority of time series (73.5%) recorded by Aguzzi *et al.* (2004a), showed a 24-h periodicity. Nine submultiple periods (constituting an Ultradian Band, UB) were also obtained by spectral decomposition of the circadian harmonic, in order to cover a wide array of ultradian periods (from the 2^{nd} harmonic of 12-h period to the 10^{th} harmonic of 2.4-h period). Time-series segments of 24-h duration were adjusted by least squares to all modelled harmonics (CH and its UB submultiples), giving corresponding Power Content (PC) values as a measure of their fit (i.e. the percentage of data variance explained by a harmonic). For each time-series, a matrix of PC values for each harmonic was therefore obtained over consecutive days. Each matrix was graphically represented by using a scale of grey: from black, representing the highest PC value (i.e. maximum fitting), to white as the lowest (i.e. minimum fitting). This representation allowed the easy visualization of the temporal modification in the periodicity of time series in terms of changes in their fit (PC) to the harmonics of the modelled set.

The temporal modification of cardiac activity rhythms was globally evaluated by considering PC values for the CH component and for the UB harmonics as two different blocks. A mean PC for the CH component was obtained per day by averaging in matrices all corresponding values. PC values obtained from the 2^{nd} to the 10^{th} UB harmonic were summed together for each day in each matrix. The resulting sums were then averaged in all matrices for corresponding days. Computed PC averages for CH and UB were plotted along with corresponding standard deviations *versus* the days of the experiment. A linear regression analysis (ANOVA, $p < 0.05$) assessed the presence of any significant trend in mean PC estimates for the CH and UB harmonics.

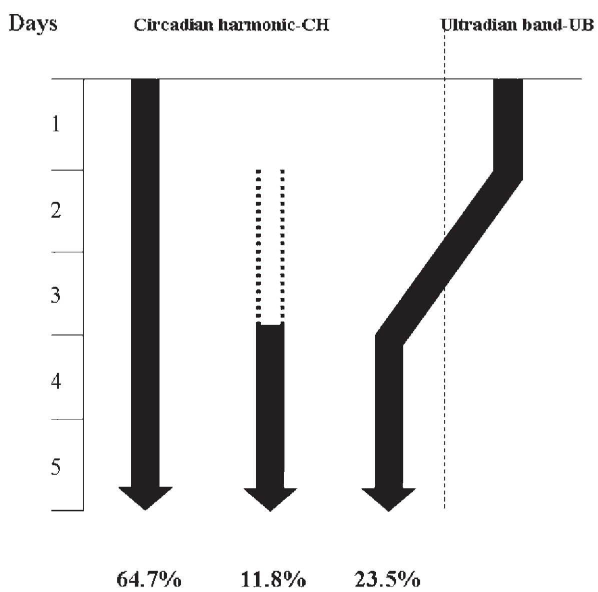

FIG. 1. – *Nephrops norvegicus*. Percentages of animals showing higher power content (PC, the black arrow) corresponding to the modelled circadian harmonic (CH) or its ultradian submultiples (Ultradian Band - UB) over consecutive days of acclimatization to laboratory constant darkness (n = 34). Three different groups of animals can be distinguished according to the immediate or delayed presence of high PC values corresponding to CH. In the group of those showing delay, some show the transformation of ultradian periodicity (high PC at UB) into circadian periodicity (high PC at CH).

RESULTS

Fourier analysis identified composite scenery in the temporal modification of the PC of the CH and UB harmonics over consecutive days of cardiac testing in all 34 time series analysed. The datasets were divided into different groups (Fig. 1): time series in which the circadian harmonic (CH) was constantly present from the beginning of the test (n = 22), and time series in which the CH was absent at the beginning of tests (n = 12), but gradually appeared over time. In this latter category, a group (n = 8) showed defined ultradian harmonics (UB) at the beginning of tests, while in the remaining time series (n = 4) no defined UB harmonics appeared.

Graphical PC matrices of different time series are reported as an example for each category quoted above. In Figure 2a, the animal showed a persistent circadian periodicity in its cardiac activity, as indicated by the presence of highest PC values corresponding to the modelled CH (circadian harmonic) throughout the whole testing period. In Figure 2b, the circadian periodicity appeared two days after the start of cardiac monitoring: a gradual increase in the PC of the CH harmonic occurred in the first couple of days, being maximum from the third day, but no defined UB harmonic was present. The data set of Figure 2c showed high PC values for both the CH and the UB harmonic of 12-h periodicity at the

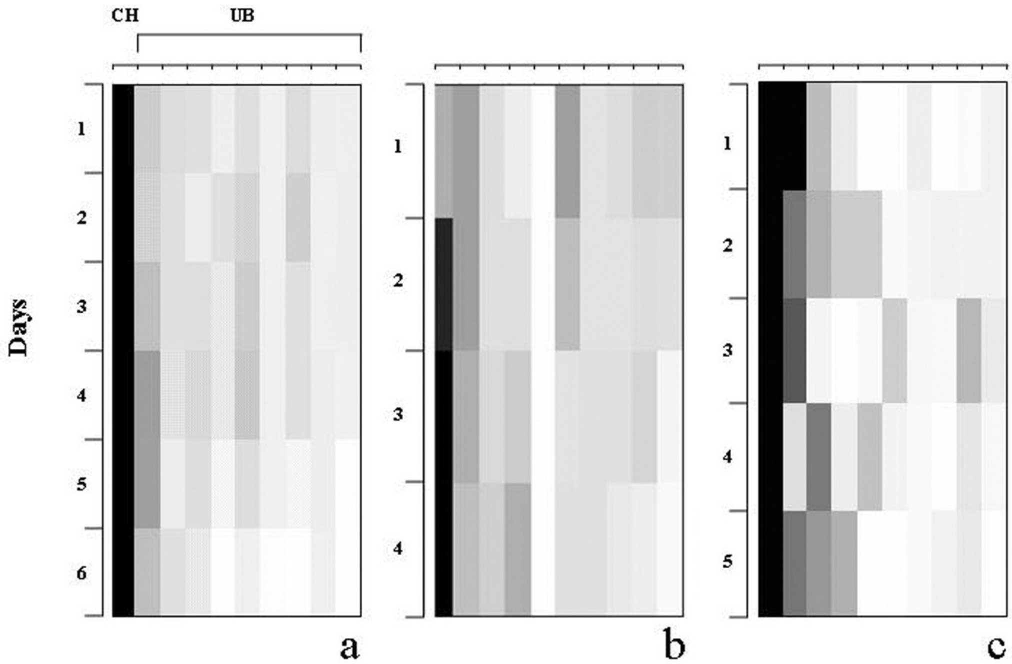

FIG. 2. – Graphical Power Content (PC) matrices as computed by Fourier analysis on time series of cardiac activity data of *N. norvegicus* kept in constant darkness. Changes in PC value from black (the highest value) to white (the lowest value) of the circadian (CH) and ultradian (UB) harmonics (columns) are represented over consecutive days (rows). A) a strong circadian periodicity is preserved (i.e. the black band is present in the CH column during the 6 days), B) the circadian periodicity appear (i.e. the black band gradually appear in the CH column from the 2nd day), C) the circadian component persist during all the experiment while the PC of the UB component corresponding to 12-h gradually disappear from the first day.

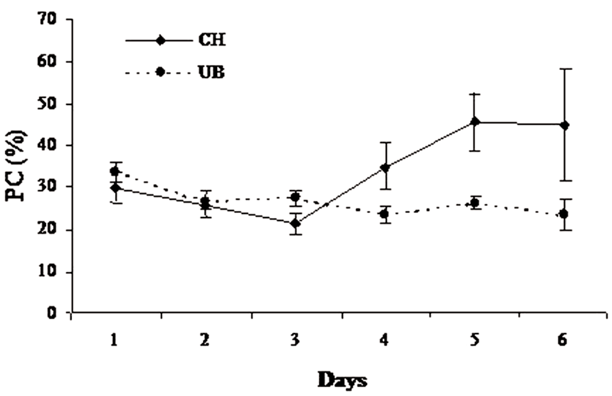

FIG. 3. – Modification of mean PC values of the CH and UB band components over consecutive days of cardiac testing in constant darkness. For a significant increase in the PC of the CH component, the UB band shows a concomitant decrease. Vertical bars, standard deviations.

beginning of the test; from the second day on, the PC of the UB harmonic showed fluctuations without defining a clear pattern of variation, the circadian harmonic (CH) being the strongest.

Figure 3 reports the temporal modification in the mean PC of the CH and UB harmonics over consecutive days of test in constant darkness in all 34 time series considered. The circadian component showed a general reinforcement over consecutive days, while ultradian components gradually diminished their PC. In fact, the PC of CH presented a slight but significant temporal increase ($p = 0.019$; $b = 0.20$). Reciprocally, the PC of the UB band showed a small but significant temporal decrease over time ($p = 0.006$; $b = -0.23$). A general stabilisation in the PC of the CH harmonic appeared after the fourth day.

DISCUSSION

This study showed that *Nephrops norvegicus* from the western Mediterranean continental slope may present a temporal modification in their cardiac rhythmicity over consecutive days of testing in constant darkness in 35.3% of the tested animals. A progressive and significant reinforcement of the circadian harmonic component was observed with a certain stabilisation after the fourth day. Contemporarily in this temporal window, ultradian components, present at the beginning, gradually disappeared from 23.5% of the data sets of tested specimens. These results suggest that a disruption of the animals' cardiac rhythmicity, as caused by stress of capture, gradually takes place in a consistent percentage of animals immediately after capture. Circadian periodicity is restored after acclimatisation to laboratory conditions.

The present findings agree with those obtained from studies on other metabolic parameters that are typically altered by this fishing method. Glucose metabolism was studied in *N. norvegicus* in relation to creel and trawling fishing stresses (Spicer *et al*., 1990; Schmitt and Uglow, 1997; Aguzzi *et al*., 2004c). These studies showed a marked hyperglycaemia as a physiological response to trawl capture, handling and aerial exposure of animals on board during their sorting from the nets. Initial high oxygen consumption measures were also obtained as a result of trawling stress in animals of the western Mediterranean slope (Aguzzi *et al*., 2003a). A consequent decrease in glucose haemolymph concentration and oxygen consumption were thus recorded only after the animals' acclimatisation to laboratory conditions (Aguzzi *et al*., 2004b,c).

Generally, studies focusing on invertebrate cardiac rhythms consider for analysis datasets collected immediately after the animals' transfer to laboratory conditions. Datasets of the first days of test are not eliminated and when a decreasing trend is present it is removed, assuming that high initial behavioural and metabolic measures may mask the underlying rhythmicity (Aagaard *et al*., 1995; Aagaard, 1996; Rovero *et al*., 1999). However, the present data suggest that stress of capture may generate not only a decreasing trend in measures, but also ultradian components in time series immediately after capture that gradually disappear during the process of acclimatisation to laboratory conditions. Thus, impaired results on cardiac rhythmicity may be obtained when time series of data recorded before acclimatisation are analysed. This consideration should be carefully taken into account when marine species are considered for physiological rhythms testing, since the stress generated by the methods of capture may impair or confound the results. As suggested by the present study, a four-day acclimatisation period to laboratory conditions should be planned before cardiac testing in *N. norvegicus*, when the objective of the study is to determine circadian rhythms in relation to animals' behaviour and underlying physiology in their environment. Conversely, cardiac testing during the acclimatisation period may be important in revealing the pattern of functioning of the cardiac pacemaker since stress may generate ultradian rhythmicity.

In decapods, light is suggested to trigger the coupling of different circadian oscillators into a functional circadian clock (e.g. Reid and Naylor, 1993; Warman and Naylor, 1995; Palmer, 2000). Cardiac beating is produced by a small group of pacemaker cells generating bursts of impulses (Taylor, 1982). Hence, the presence of ultradian periodicity in cardiac activity of *N. norvegicus* inhabiting the western Mediterranean continental slope was suggested to be provoked by too dim environmental light intensity fluctuations at this depth [e.g. in June midday peak in light intensity of 2.5 $m\mathrm{E_i}\ \mathrm{m^{-2}}\ \mathrm{s^{-1}}$ at 100-110 m and 2.10^{-7} $m\mathrm{E_i}\ \mathrm{m^{-2}}\ \mathrm{s^{-1}}$ at 400-430 m (Aguzzi *et al*., 2003b)]. Conversely, as reported in this study, circadian cardiac rhythmicity reinforces itself in constant darkness after a few days of acclimatisation. Also, marked light intensity-driven catch patterns made by a single peak over a 24-h cycle, phased at midday, were recently found in *N. norvegicus* populations of the continental slope (Aguzzi *et al*., 2003b). This result suggested that light at this depth is still capable of exerting its action on the rhythmic emergence behaviour of *N. norvegicus*, and possibly also on the underlying cardiac pacemaker. Nevertheless, emergence and its underlying physiology on the slope still represent a challenging issue nowadays, since its regulation by other factors, such as the daily presence-absence of the epibenthic-pelagic prey in the proximity of the bottom (Al-Adhub and Naylor, 1977), also needs to be taken into account.

In conclusion, the progressive reinforcement of circadian periodicity in constant darkness suggests that, rather than being a product of environmental dim light intensity fluctuations experienced by the animals on the slope, ultradian periodicity could well be caused by the stress of capture. Additional studies on *N. norvegicus* cardiac rhythms should be performed for durations longer than one week to further elucidate to what extent pacemaker uncoupling may effectively produce ultradian rhythms in constant darkness, as could currently happen in the dim-light intensity environment of the slope, or to examine whether it is effectively caused by trawling stress.

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