

Communication

Preliminary Results on the Daily and Seasonal Rhythms of Cuttlefish *Sepia officinalis* (Linnaeus, 1758) Locomotor Activity in Captivity

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Abstract: This research was aimed at characterizing the daily rhythms of locomotor activity of cuttlefish *Sepia officinalis* (Linnaeus, 1758), a promising aquaculture species, in a captive environment. Cuttlefish were distributed within two tanks equipped with infrared photocells, allowing the monitoring of locomotor activity continuously from August to February. Results showed a preference of cuttlefish for being active during the night in summer (24.2 ± 1.3 °C) and in autumn (18.4 ± 3.4 °C), with maximum activity peaks observed, respectively, at 01:09 h and 21:47 h, in close relation with sunset time. In winter (12.7 ± 1.4 °C), this preference changed, with higher levels of activity observed during the afternoon in December (15:20 h) and in January (18:13 h), thus suggesting this species as possessing a dualistic behaviour. Levels of activity progressively decreased during autumn and winter, likely related to low temperatures. The present study offers new insights on the behaviour of *Sepia officinalis*, presenting a variability of patterns across seasons, regulated by environmental synchronizers; photoperiod determining the activity periods, and temperature modulating the levels of activity. Such information may be a valuable tool for the improvement of cuttlefish culture protocols in aquaculture, and consequently its welfare.

Keywords: behaviour; cephalopod; cuttlefish; daily rhythms; environmental synchronizers; photoperiod

1. Introduction

Cephalopods are considered as an alternate group for aquaculture species diversification [1,2], not only because they are a good food source (highly appreciated in some worldwide markets, e.g., Portugal, Spain, Italy and Asia) and have the potential to quickly reach a market size, but also because they are considered animal models in several fields of research [3–5], and it is expected that such models are progressively obtained from bioteriums instead of being captured from nature.

Despite the recent advances in culture protocols of several cephalopod species [2], there is still little information available about the biological rhythms of cephalopods in captivity. In fact, to the best of our knowledge, there are only 4 published papers concerning this theme, and these are focused on octopus species [6–9]. This issue assumes increasing importance with the enforcement of EU welfare legislation (Directive 2010/63/EU), and this information is critical for the future update of Annex III (housing conditions) for cephalopods of that Directive, and for a future update of the Guidelines

for the Care and Welfare of Cephalopods in Research [10]. If it is considered that the European cuttlefish *Sepia officinalis* (Linnaeus, 1758) may have, at the least, two different sleep-like states [11], the importance of this study becomes even more evident.

Chronobiology is the field of science that examines periodic phenomena in living organisms and the mechanisms of synchronization with the natural cycles of the environment [12]. In anticipation of the cyclic changes in photoperiod and temperature [13,14], animals choose the most suitable moment to feed, reproduce or be active, thereby optimizing their biological processes [15,16]. Biological rhythms are classified as daily rhythms when the oscillation periodicity is around 24 h, and called circadian when the rhythm persists and freely runs under constant environmental conditions, proving their endogenous origin [17,18]. In teleost fish, each species shows daily patterns of locomotor activity that can go from diurnal and nocturnal, to crepuscular, or a combination of these. In some cases, within the same species, different individuals can show a great variability in their daily activity patterns [19]. In contrast to cephalopods, in the last two decades several studies have focused on the study of daily and circadian rhythms in teleosts, namely their behavioural and neuroendocrine rhythms [15,16,20]. A better knowledge of the circadian system is essential for the domestication of targeted species for aquaculture (cephalopods included) and for the optimization of existing production protocols.

In nature, cuttlefish are reported as being essentially crepuscular or nocturnal, although they may be active during daytime [21,22]. These authors revealed much about their reproductive biology, foraging behaviours, etc., but only in the natural habitat. Therefore, the aim of this research was to perform a first characterisation of the daily and seasonal behavioural rhythms of cuttlefish in captivity, by monitoring locomotor activity under natural conditions of temperature and photoperiod.

2. Results and Discussion

The mean cumulative mortality of both tanks is shown in Figure 1. Animals started dying after maturity, and due to normal senescence, from the beginning of December 2011 onwards.

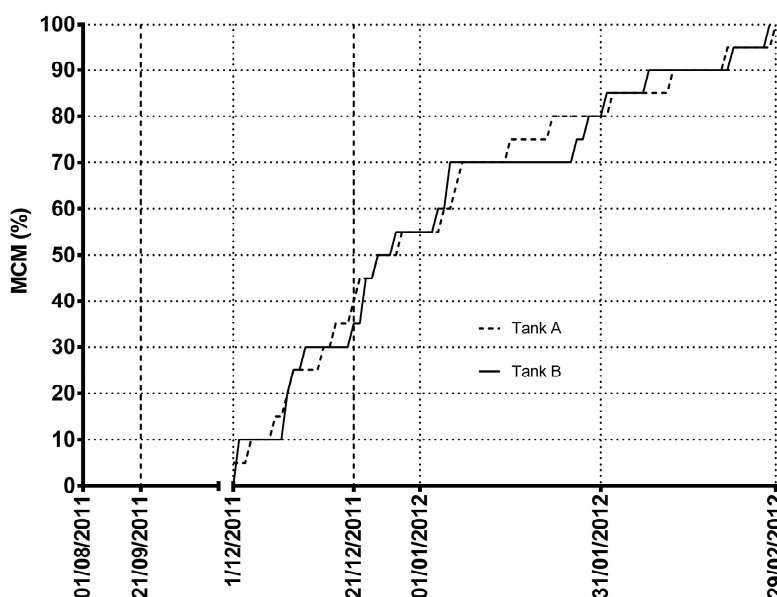


Figure 1. Mean cumulative mortality (MCM, %) of both cuttlefish stocks through the different seasons (bold vertical dash lines) and sets (vertical dash lines) used for studying the biological rhythms of the cuttlefish *S. officinalis*.

Cuttlefish displayed many behavioural variations during the seven-month duration of the study, with three different patterns identified, corresponding to the different seasons (summer, autumn and winter). In summer, when seawater temperature averaged around 24.2 ± 1.3 °C (Figure 2), the

activity was intense and markedly nocturnal (Figure 3a). The activity occurred predominantly after sunset (68.29%; $p < 0.0001$; Figure 3b), with some minor occurrences during daytime (mean values of 2.33 ± 1.26 vs. 5.92 ± 0.80 counts/10 min, respectively for day and night periods), namely at feeding times.

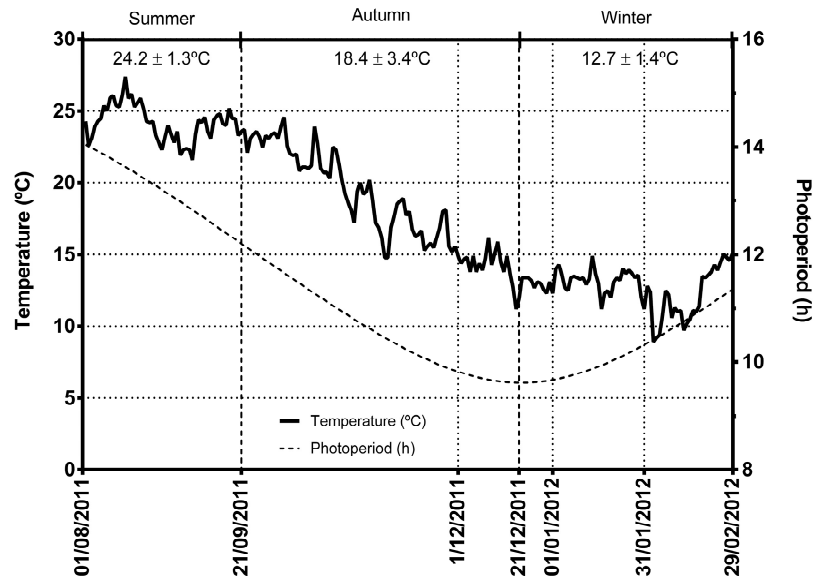


Figure 2. Temperature (°C) and photoperiod (hours of light) through the different seasons (bold vertical dash lines) and sets (vertical dash lines) used for studying the biological rhythms of the cuttlefish *Sepia officinalis*.

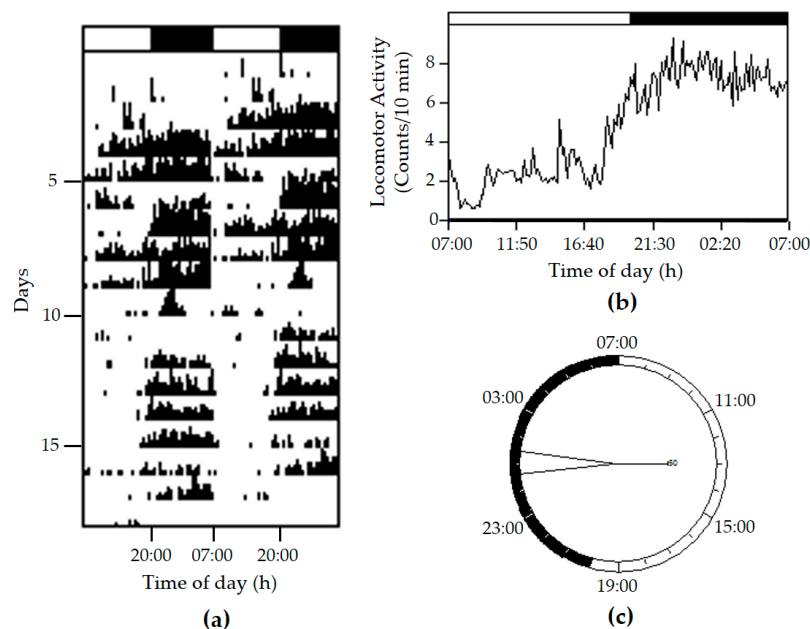


Figure 3. Representative actogram of locomotor activity of cuttlefish reared under natural conditions of photoperiod (13:11 Light:Dark), registered during summer (a) and respective mean waveform (b) and polar representation of the COSINOR analysis, depicting clockwise the daily cycle of activity (c). The white and black bars at the top of each graph represents the light (day) and dark (night) periods, respectively. The actogram is double-plotted for better visualization.

After 17:00h, in anticipation of the nocturnal phase, an increase of activity was observed, probably related to closure of the research station and the absence of anthropogenic disturbance. The application of the COSINOR analysis revealed a significant daily rhythm ($p < 0.0001$), with a mesor of 4.76 counts/10 min, amplitude of 2.76 counts/10 min and an acrophase located at 01:09 h, as depicted in the Polar representation of the COSINOR analysis (Figure 3c).

During autumn, despite the temperature drop (averaged at 18.4 ± 3.4 °C, Figure 2), such behaviour was maintained (Figure 4), but lower levels of activity were verified. Once again, there was a clear preference of cuttlefish for being active during night time (Figure 4a,b), with 33.88% and 66.12% of activity (0.95 ± 0.69 and 1.49 ± 0.66 counts/10 min), respectively, for day and night periods ($p < 0.001$). The COSINOR analysis confirmed the significance of the rhythm ($p < 0.01$), with a mesor of 1.28 counts/10 min, an amplitude of 0.27 counts/10 min and an acrophase located at 21:47 h (Figure 4c). Such acrophase change from summer to autumn (01:09 h to 21:47 h) is very likely to be related to sunset time. In fact, considering that in summer the sunset was around 20:00 h, and in autumn at 17:30 h, the acrophase occurred respectively 5 and 4 h after, reinforcing the idea of a strong synchronization of behaviour with the daily photoperiod cycle. Furthermore, the activity peaks seen, associated with feeding times, also reveal a synchronization role of this factor.

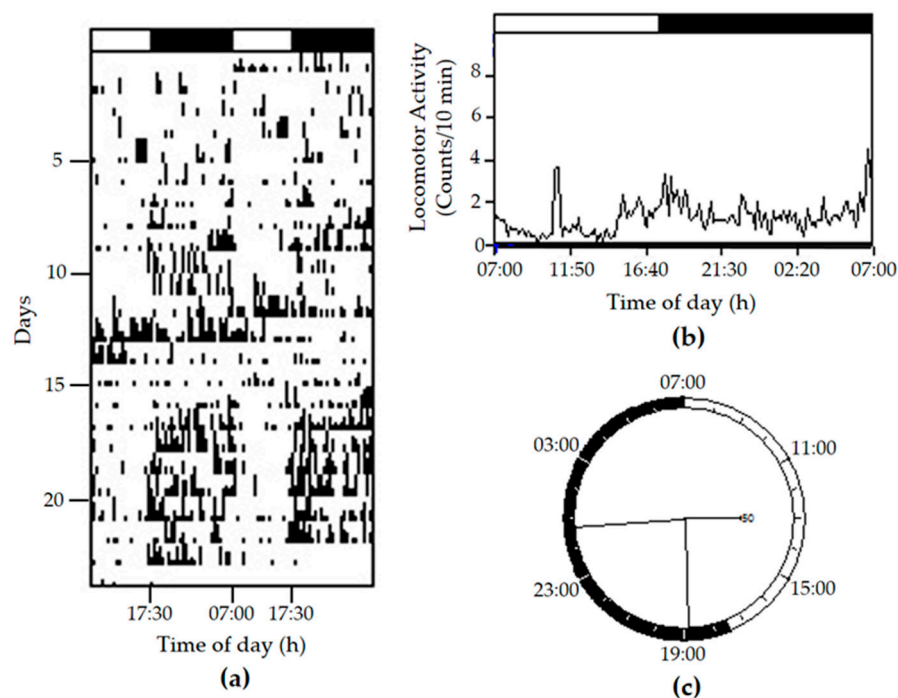


Figure 4. Representative actogram of locomotor activity of cuttlefish reared under natural conditions of photoperiod (11:13 LD), registered during autumn (a) and respective mean waveform (b) and polar representation of the COSINOR analysis, depicting clockwise the daily cycle of activity (c). The white and black bars at the top of each graph represents the light (day) and dark (night) periods, respectively. The actogram is double-plotted for better visualization.

Sepia officinalis has been reported to possess a diurnal feeding rhythm in the natural environment not regulated by lunar cycles, persisting in different moments of the annual cycle, with feeding activity occurring essentially in twilight periods [23], in accordance with the nocturnal behaviour in summer and autumn observed in the present study. Since these animals were hatched from wild eggs, it should not be surprising that they present a similar behaviour to that of wild animals. Denton and Gilpin-Brown [22] had previously identified daily rhythms of activity in this species, associated with changes in cuttlebone density regulated by light. In darkness, the density decreases and the cuttlefish becomes more buoyant,

acquiring a larger movement capacity and a higher rate of success in capturing prey. Moreover, cuttlefish are known to use polarization sensitivity during predation as well as intraspecific recognition and communication [24–26]. In line with such rhythms of activity, Jäckel et al. [27] observed daily oscillations in metabolic rates, with 30–40% rise in oxygen consumption after sunset. In agreement are the sleep-like states established by Frank et al. [11], which also follow a rhythmic pattern, with ca. 80% of resting time occurring during daytime, where their vision is affected by UV radiation [28]. All the above evidence, along with the present results, reinforce the rhythmic fashion of this species behaviour and physiology, with a preference of cuttlefish for being nocturnal in summer and in autumn. Other cephalopod species, such as *Eledone cirrhosa* [6] or *Octopus macropus* [9], also showed a nocturnal pattern, and have polarized vision [29,30], while for *O. vulgaris* results seemed to be inconsistent [8,9]. Such differences among cephalopod species might reflect their lifestyles in the natural environment, with the nocturnal behaviour being a clear advantage for predation. However, comparisons should be made with caution since not all studies mention the season when the tests were conducted, nor the temperatures associated. In winter, when temperature lowered to values of 12.7 ± 1.4 °C, the daily rhythm changed, with the highest levels of activity now being registered during the afternoon (Figure 5). For this data sub-set, two different patterns were identified (Figure 5a): one in December and another in January. In December (13.8 ± 1.1 °C, Figure 2), high levels of activity were registered during both day and night periods, related with reproductive behaviour and spawning events (data not shown). However, when data were plotted as a mean waveform (Figure 5b), a clear peak of activity could be observed in the middle of the day period (13:10 h). In this month, 47.06% of the total activity was registered during the day, while 52.94% occurred during the night. Although both percentages were very close to 50%, daytime activity was shown to be significantly higher than the one registered during the night (mean values 21.69 ± 6.14 and 16.94 ± 1.66 counts/10 min respectively, $p < 0.001$).

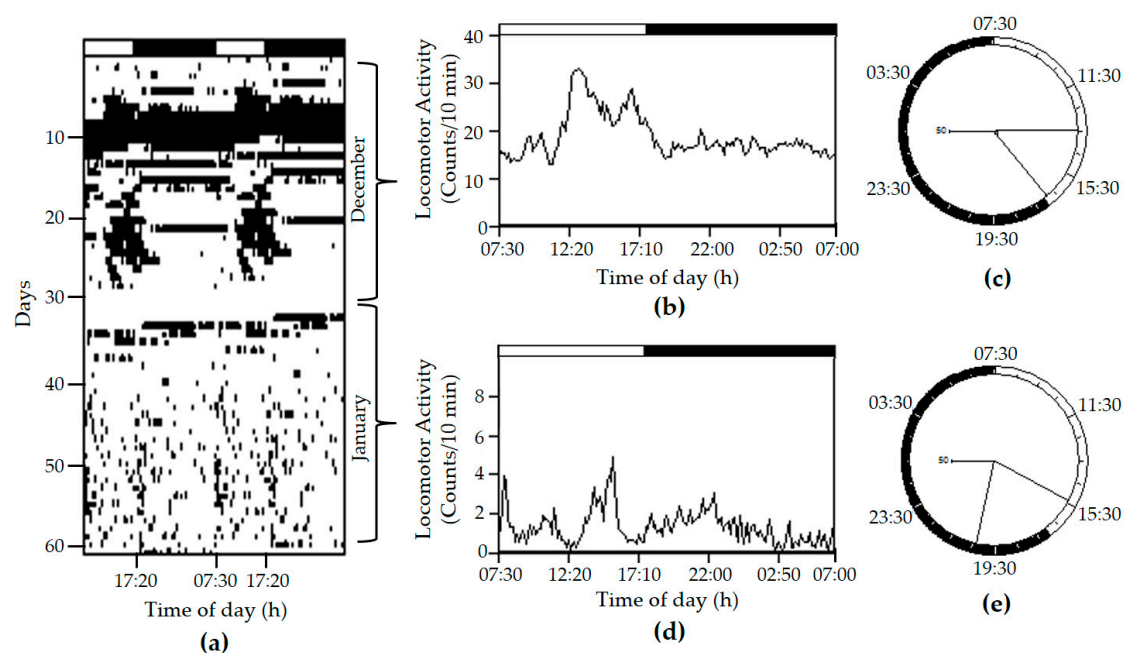


Figure 5. Representative actogram of locomotor activity of cuttlefish reared under natural conditions of photoperiod (10:14 LD) during winter (a) and corresponding analysis for the periods of December and January: mean waveform (b) and polar representation of the COSINOR analysis (c) for December and mean waveform (d) and COSINOR analysis (e) for January. The white and black bars at the top of each graph show the light (day) and dark (night) periods respectively. The actogram is double-plotted for better visualization.

During January (13.1 ± 0.8 °C, Figure 2), the levels of activity decreased greatly (Figure 5a), and the daily pattern was not so clear. The mean waveform (Figure 5d) showed a clear activity peak in late afternoon (15:40 h), however the locomotor activity did not show significant differences between day (1.45 ± 1.03 counts/10 min, 45.74%) and night (1.21 ± 0.65 counts/10 min, 54.26%) periods ($p > 0.05$), thus being considered arrhythmic for this period. Such accented decrease in activity could be either related with the low temperatures registered or with the decrease in number of animals associated with the mortality registered short after reproduction in these tanks [31]. The COSINOR analysis for the winter period was applied separately for each of these time intervals, and in both cases the rhythm was confirmed to be significant ($p < 0.001$): mesor of 18.90 and 1.31 counts/10 min, amplitude of 4.27 and 0.41 counts/10 min and acrophases at 15:20 h and 18:13 h, respectively for December and January (Figure 5c,e).

As with the photoperiod, temperature is also known to be a strong modulator of rhythms in aquatic animals, especially for ectothermic animals [20] like cuttlefish [32]. Indeed, the present results showed an accented decrease in activity along with the temperature drop (with the exception of the reproduction period in December), suggesting an important role of temperature in the synchronization of this species' behaviour rhythms, as verified for the photoperiod.

Cuttlefish showed a clear preference for being active during the night in summer and in autumn, while in winter this preference changed, with higher levels of activity observed during the afternoon, revealing a high plasticity of the circadian system of this species [19], and resembling a dualistic behaviour, which according to Eriksson [33], is “the ability to shift behavioural patterns and change from diurnal to nocturnal and vice versa at some stages of life history”. To the best of our knowledge, no similar evidence has been observed in cephalopods, however several fish species and the oyster *Crassostrea gigas* [34] have been described as possessing such capacity. One of the most common examples is the European sea bass *Dicentrarchus labrax*, which exhibited such behaviour in feeding patterns, under both natural [35] and controlled laboratorial conditions [36,37], being predominantly diurnal in summer, nocturnal in winter and then returning to diurnal behaviour in spring [36,38]. The cuttlefish seemed to present a similar behaviour in terms of locomotor activity, being very likely a dual species.

3. Materials and Methods

3.1. Ethical Statement

This experiment was performed under Projects SEPIABREED (FCT PTDC/MAR/120876/2010), SEPIATECH (PROMAR 31.03.05.FEP.002) and SEPIAMETA (FCT PTDC/MAR/102348/2008), which were approved before the entry into force of Directive 2010/63/EU [39] as national legislation in Portugal. Nonetheless, the present experiment did not cross the threshold set for regulation (i.e., procedures that may cause pain, suffering, distress or lasting harm equivalent to or higher than that caused by the insertion of a hypodermic needle in line with good veterinary practice). Also, ethics for biological rhythm research [40] were considered, and the cuttlefish involved in the present study were reared according to the best practice described by Sykes et al. [41] and Fiorito et al. [10].

3.2. Experimental Design

The present study was performed over 7 months (213 days) in 2011–2012 at the Centro de Ciências do Mar (CCMAR) Ramalhete Aquaculture Station (Ria Formosa, South Portugal— $37^{\circ}00'22.39''\text{N}$; $7^{\circ}58'02.69''\text{W}$). All cuttlefish hatched from eggs collected in the wild. The embryonic development of eggs, as well as hatchlings and juvenile's rearing, was performed according to the technology described by Sykes et al. [41]. Two groups of 20 cuttlefish juveniles (56.5 ± 17.8 g; with an age of 75 days after hatching) were reared until natural death (133.6 ± 59.4 g; and showing sex ratios of 1:1) in two 500 L rectangular outdoor tanks (with a bottom area of 1.0×1.0 m) provided with a constant seawater flow in an open system. These tanks were set up and covered with a green net, to diminish light

intensity. Parameters of water quality, such as temperature, dissolved oxygen saturation and salinity were monitored daily with an Oxiguard Handy probe and a VWR EC300 salinity meter. Environmental parameters oscillated naturally, on a daily basis and throughout the seven months of the study. Overall mean temperature was 17.9 ± 5.0 °C (range of 27.4–8.9 °C), salinity was 35.1 ± 1.7 g·L⁻¹ (range of 37.6–30.2 g·L⁻¹) and dissolved oxygen concentration was $100.3 \pm 4.6\%$ saturation (range of 88–110%). Animals were fed twice a day ad libitum with frozen grass shrimp *Palaemonetes varians* at 10 h and 16 h. Mean cumulative mortality (%) was determined daily.

In order to characterize this species chronotype, locomotor activity was monitored from August 2011 until February 2012 (across three different seasons). For the registration of locomotor activity, one photocell (E3S-AD62, OMRON, Kioto, Japan) was installed inside each tank (at mid water height—26 cm), connected to a motherboard (USB-1024HLS, Measurement Computing, Norton, MA, USA) which, in turn, was connected to a computer (Benq T2210HD). Every time a cuttlefish interrupted the infrared light beam (ca. 20 cm), an output signal was produced, which was recorded and stored in 10 min bins using specialized software (DIO98USB, University of Murcia, Murcia, Spain).

3.3. Data Analysis

Since data was expected to vary throughout the year, sub-sets were analysed separately (coinciding with seasons) [42], considering temperature and photoperiod natural oscillations (Figure 2). To avoid bias from a low number of individuals in the tanks due to natural mortality, this analysis ended when 80% of cuttlefish of each tank died (31/01/2012). In order to identify rhythm patterns for each data sub-set, activity records were plotted in the form of actograms and mean waveforms using the software *El Temps* (University of Barcelona, Barcelona, Spain; A. Diez-Noguera), representing, respectively, the locomotor activity along successive day cycles double-plotted on successive horizontal lines, and the mean activity along the 24 h cycle. Cuttlefish were either categorized as diurnal or nocturnal, depending on when (i.e., day or night, respectively) locomotor activity presented major values. All data was tested for normal distribution using the Shapiro-Wilk test [43]. Significant differences between mean diurnal and nocturnal activity counts were evaluated through a paired Student's *t*-test. Statistical difference was considered for $p < 0.01$. When no statistical differences between diurnal and nocturnal activity means were observed for a certain data sub-set, the animals were considered arrhythmic [44].

Statistical significance of activity daily rhythms was tested using a COSINOR analysis [45,46]. The 'amplitude' (one-half the peak-to-trough variation), 'mesor' (the time series mean), and 'acrophase' (peak time relative to the time scale) were determined by the least-squares approximation of cosine function.

4. Conclusions

In summary, *S. officinalis* has been observed as a nocturnal species during summer and autumn, turning to diurnal in December and becoming arrhythmic in January, in close relationship with the main environmental synchronizers: photoperiod determining the activity periods, and temperature modulating the levels of activity. Due to such observed capacity to change behaviour along the year, it has even been suggested to be a dual species. Such data is an added value for this species adaptation to aquaculture conditions, allowing the improvement of all kinds of culture husbandry protocols; e.g., knowing when this species is active during the 24 h of the day and at different seasons might help optimize handling, feeding and breeding protocols, and consequently this species' welfare under captive conditions.

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Author Contributions: C.C.V.O. and A.V.S. were responsible for the design and supervision of the study, carried out data analysis and respective interpretation of results and made major contributions to the manuscript. R.A.G. and P.A.F. carried out the setup of the experiment, were responsible for the maintenance of the animals, and for data collection. M.I.G.-M. drafted the manuscript, contributed to the figure analysis and was involved in revising the manuscript. All authors have read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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