Coleoid cephalopods through time (Warnke K., Keupp H., Boletzky S. v., eds)

Berliner Paläobiol. Abh.	**03**	171-177	Berlin 2003

CIRCADIAN RHYTHMS IN *OCTOPUS VULGARIS*

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ABSTRACT

Biological rhythms enable organisms to measure time and to synchronize their endogenous behavior and physiology with the time constraints of their environment. Since the inhabitants of the littoral zone of marine environments are exposed to complex temporal and environmental changes, biological rhythms play an important role in their lives. Timekeeping is especially important for those with short lives like octopuses. Circadian rhythms, which are rhythms of about a day, are one of the most prominent of biological rhythms. They are ubiquitous through all phyla of the animal kingdom, light being one of the most important "Zeitgebers" for almost all of them. To examine the function of light as a synchronizer for their activity, four octopuses were held in a potentially entrained state with a 24-hour L:D (light/dark) cycle. After this phase the animals were placed in constant conditions to document free running activity with circadian components. The results of this study clarify why publications up to now show conflicting results about circadian aspects of *Octopus vulgaris* activity. While light was used inefficiently as a Zeitgeber for activity the results of our study proved the presence of an endogenous circadian rhythm in *O. vulgaris*. These generalistic and opportunistic animals may use several different time cues to synchronize their activity and behavior with the time constraints of their environment.

INTRODUCTION

Biological rhythms are ubiquitous phenomena in the animal kingdom. They enable organisms to anticipate cyclic changes in their environment and organize their lives correspondingly. Rhythms that persist in constant conditions are called freerunning. When a repeating external signal, called Zeitgeber or time cue, synchronizes the timing of these freerunning rhythms, they are entrained. The inhabitants of the littoral zone of marine environments are exposed to complex temporal and environmental changes. The magnitudes of the changes and their impact on physiological functions have forced many animals to develop circalunadian (rhythms of about the phases of the moon) or circatidal (rhythms of about the tides) clocks parallel to the circadian system (rhythms of about a day) (Naylor 1996). These rhythms enable organisms to measure time and to synchronize their endogenous behavior and physiology with the time constraints of their marine environment. The expression and endogenous basis of biological rhythms in marine phyla have been documented in many studies (Palmer 1996).

Octopuses are perhaps among the most spectacular inhabitants of the littoral zone. Besides their role as environmental barometer they have a high economic impact in many areas (Arreguin-Sanchez *et al.* 2000). In spite of their importance little information has been collected about the temporal patterns of their activity or their control. Studies on various *Octopus* species have usually concentrated on activity patterns without testing the balance of endogenous control and external Zeitgebers in their expression.

Reports on *Octopus vulgaris* of the Mediterranean proposed that the species was nocturnal. No nocturnal activity had been observed, and the basis of the conclusion was simply the observation that the dens were empty at night (Woods 1965, Altman 1967, Kayes 1974). Mather (1988) has reported daytime activity and nighttime den occupation of juvenile *O. vulgaris* in Bermuda. The limitations of these studies were that the animals were primarily observed during daytime. These conflicting reports about the timing of *O. vulgaris´* activity lead to the question of what they synchronize their activity with.

Evidence exists for the function of light as a circadian Zeitgeber in other cephalopods. Cobb and co-workers (1995) found that light affected the expression of activity in *Eledone cirrhosa*. *Sepia officinalis* increased buoyancy in the dark to facilitate swimming and decreased it in light to remain on the bottom (Denton & Gilpin-Brown 1961).

O. vulgaris lives in heterogeneous marine shelf habitats. It is a mobile predator, which feeds on fish, crustaceans, and molluscs. It inhabits the photic zone of the sea where sunlight is known to influence the activity of many organisms. Since these animals have both well-developed visual and camouflage systems, it may be expected that *O. vulgaris* activity may be strongly influenced by light. Our test for cyclic expression of activity was the behavior of individuals in an artificial light/dark (L:D) cycle where we were able to examine activity patterns more rigorously. Octopuses were held in a potentially entrained state with a 24-hour L:D cycle. After a phase of potential entrainment, the animals were placed in constant conditions (D:D) to document any free running activity with circadian components.

MATERIAL AND METHODS

Subjects

Four male *O. vulgaris* (3 - 9 cm mantle length) were collected in Naples, Italy (Stazione Zoologica di Napoli), and brought to Austria to our lab at the Konrad Lorenz Institute for Evolution and Cognition Research. Three of them were sub-adults and one was a mature animal. One animal became senescent and died in captivity after it had been in our lab for one and a half years. The other three were brought back to their place of capture and released**.**

Housing

Animals were held individually in 1,0 x 0,6 x 0,5 m glass tanks, which were part of two closed circulation systems of 1700 l and 2700 l artificial seawater with a turnover 24 times per day. Water was filtered with protein-skimmers, passive filters, UV-filters and denitrification tanks. Additional aeration in each tank produced a weak current. Water temperature was monitored and maintained at 21 degrees by a cooling system. Polysterol sheets isolated the back and the sides of the tanks. Animals were kept under a light/dark cycle with the light phase from 0800 to 2000. Between light and dark phases there were twilight phases, of one and a half hours in the morning and evening. They consisted of stepwise increases and decreases, respectively, of light intensity. Illumination during the day was provided by artificial light with a daylight emission spectrum. The light intensity on the water surface was 350 Lux. The animals were provided with a semi-natural habitat. Tanks had a sandy bottom with small rocks and a few large “living” stones with epigrowth, which provided building material for dens and shelters. An escape-proof plexiglass lid was used to cover the tanks. The animals were fed live and dead shrimp, mussels, crabs and fish.

Experiment

Condition 1 (L:D)

Animals were kept in a tank which was shaded from all visual influences by a plastic curtain and exposed to a 12:12 light-dark cycle (from 0800 to 2000 for animals 1 to 3 and for 0700 to 1900 (daylight saving) for animal 4. Light intensity was checked on the water surface during the light phase. At night the light intensity was below 1 Lux. A red light filter (Schott Glas filter) emitting light of a wavelength that could not be detected by the octopus was used to aid filming at night. In *Octopus vulgaris* a □max of 480 nm has been documented (Hamasaki 1968).

Condition 2 (D:D)

Animals were isolated from surrounding light by a photo resistant curtain. Within this curtain light levels could be held constant at about 15 Lux. This dim light level was used because permanent bright light has been

Table 1 Absolute and relative amounts of activity in the different light phases per LD cycle (24 hours)

	light	dark	twilight
Animal 1			
Absolute activity level	6.2 ± 0.9 h	2.5 ± 1.7 h	1.1 ± 0.6 h
Relative activity level	59.2 ± 8.2%	23.9 ± 15.9%	36.4 ± 19.3
Animal 2			
Absolute activity level	7.4 ± 2.0 h	3.0 ± 0.9 h	1.8 ± 0.7 h
Relative activity level	70.4 ± 19.5%	28.8 ± 8.2%	60.1 ± 23.0%
Animal 3			
Absolute activity level	5.5 ± 1.0 h	2.4 ± 1.3 h	1.3 ± 0.7 h
Relative activity level	52.9 ± 9.2 %	23.5 ± 12.3%	43.0 ± 25%
Animal 4			
Absolute activity level	8.2 ± 1.4 h	8.6 ± 0.5 h	2.4 ± 0.4 h
Relative activity level	77.8 ± 13.0%	82.3 ± 4.7%	80.1 ± 15.0%

reported to cause abnormal behavior and even death in fish.

Methodology

Animals were filmed with a digital video camera (Sony DVX 2000) for seven days each in condition 1 and 2, except for animal 1 which was only filmed for 6 days in condition 1 because of a power break. Recording was done for one second every 30 seconds using the interval recording program of the video camera. Time of feeding and maintenance of the tank was done at random times to avoid providing a circadian Zeitgeber for the animals.

Data Analysis

Analyses were done using Noldus Observer version 2.0. Data were later transferred into excel sheets and transformed into time series using the time intervals of 30 seconds. Categories of activity were defined: Resting (inactive): The octopus was sitting and not moving at all. Alertness or movement in place (active): The octopus moved without changing its location. Locomotion (active): The octopus changed its location.

One/zero measurements of these categories of activity were done every 30 seconds for each animal for the whole experimental period of two weeks (40320 measurements per animal). Rhythmicity in activity and behavior was quantified with autocorrelation analyses. The bases of the correlations are comparisons of the activity values for every 30 min with the previous case and all the preceding cases. Periodogram analyses were done to find the period of the rhythm. To characterize the rhythms quantitatively, the absolute (in hours) and relative (in percent) amounts of activity per 24 h and in different light phases were calculated. The absolute amounts of activity and behaviors were compared between L:D (condition 1) and D:D (condition 2) conditions. Averages are always presented as arithmetical means plus or minus the standard deviation (SD). Differences between two dependent data groups were analysed using the Wilcoxon-test**.**

RESULTS

Behavioral Responses to LD Cycles

Animals were active during both phases of the LD cycle but there was more diurnal activity. The differences in the relative amounts of activity during the three illumination-phases (light, dark and twilight) were significant for animal 1, 2 and 3 (Friedman-test: animal 1: $\chi^2 = 6.333$, $n = 6$, $p = 0.016$, animal 2: $\chi^2 = 8.857$, $n = 7$, $p = 0.012$, animal 3: $\chi^2 = 7.630$, $n = 7$, $p = 0.022$). They showed a predominance of activity in light conditions with an intermediate level during twilight phases (see Table 1). All animals showed activity during each LD cycle. Animal 1 had a daily activity mean of 40.9 ± 8.0% of the filmed 30 second intervals per LD cycle. For animal 2 the daily activity mean was 50.9 ± 9.1% per LD cycle. Animal 3 had a daily activity mean of 38.9 ± 5.1% of the LD cycle. Animal 4 was the senescent male. It differed from the other three individuals in two ways. It was about twice as active as the others. Animal 4 had a daily activity mean of 80.0 ± 7.4% in LD conditions. Octopus 4 did not show an activity preference for a particular light condition. The same frequencies of activity were found in all phases of the LD cycle.

Behavioral Responses to Constant Conditions DD

The amounts of activity were slightly lower in constant light conditions than in LD conditions (relative amounts of activity per 24 hours in DD: animal 1: 34.3 ± 6.0%; animal 2: 39.7 ± 11.3%; animal 3: 38.0 ± 6.4%; animal 4: 79.6 ± 5.9%). Although the difference in amount of activity was not significant for any animal there were individual differences in the total amount of activity per day, which were consistent over the seven cycles of constant conditions. Animal 4 was about twice as active as the other animals.

Autocorrelations and Periodograms

Autocorrelation statistics were used to quantify the rhythmic activity of the animals. Autocorrelations of animal 1 are shown as representative examples (Fig. 1, Fig. 2). All animals exhibited circadian rhythms in locomotor activity in DD conditions. Entrainment in LD was not perfect as the rhythm only approximated the period length of the LD cycle. The activity peaks were shifting more in LD conditions than in DD conditions. Animals therefore showed relative coordination with the time cue light in LD, which means that the rhythm sometimes drifts slightly away from the signal and then periodically regains it. Autocorrelation showed several peaks of activity and rest phases over the day in LD and DD conditions. In the periodogram analysis the dominant peak was around 24 (48 half hours see Figs 3, 4) hours in both LD and DD for each animal. Ultradian rhythms (rhythms that are shorter than about a day) were superimposed on the main circadian rhythm in each animal. The periodograms of animal 1 are shown as representative examples (Fig. 3, Fig. 4).

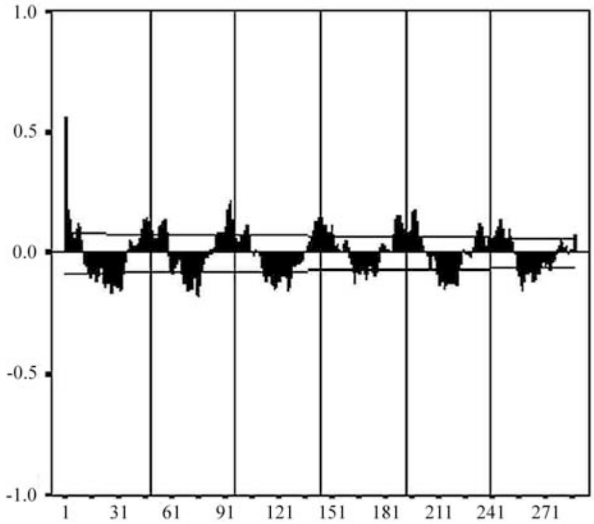

Fig. 1 Autocorrelation of half hour activity time intervals in LD are shown for animal 1. Black vertical lines represent 24 hours. Black horizontal lines represent significance limits

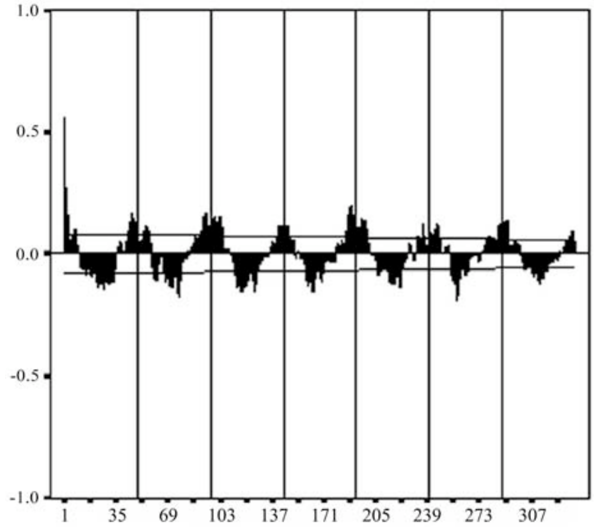

Fig. 2 Autocorrelation of half hour activity time intervals in DD are shown for animal 1. Black vertical lines represent 24 hours. Black horizontal lines represent significance limits

The results of the autocorrelation analyses showed that entrainment to the LD cycle were rather sloppy. The phase relationships between the Zeitgeber and the activity were not stable and there was only relative coordination. Still periodograms did show a mean period of 24 hours over the entire LD period. On the other hand, all animals showed a free running circadian rhythm in DD conditions. The period of the rhythm differed only slightly from 24 hours (autocorrelation), which fits the dominant peak of 24 hours periodicity in DD that was found in the periodograms. The ultradian cycles found in the periodograms were also present in the autocorrelation data as several peaks of activity and rest phases were found.

DISCUSSION

The results of this study clarify why publications up to now show conflicting results about circadian aspects of *O. vulgaris* activity. While light was obviously not efficient as a Zeitgeber for activity the results of our study proved the presence of an endogenous circadian rhythm in *O. vulgaris*. Octopuses only showed relative coordination between the rhythm of locomotor activity and the LD cycles. According to Aschoff's (1965) principles of Zeitgeber-rhythm interactions, relative coordination would be expected for weak time-giving cues. Either our light/dark cues were not strong enough for full entrainment or *O. vulgaris* use several different Zeitgebers to synchronize their activity and behavior with the environment.

The difference in light intensity used could have been considered to be sufficient. The range is higher than that found in coastal areas at a depth of 200 meters. These kinds of light conditions occur in the natural habitat of *Octopus vulgaris* (Kubodera & Tsuchiya 1993). Additionally there is no doubt that even low changes in luminous intensity have some influence on *O. vulgaris* activity. Moonlight caused unusually intense activity in *O. vulgaris* during the middle of the night (Kayes 1974) and full moonlight has approximately 0.25 Lux (Sitte *et al.* 1991). It is then possible that the levels of ambient light during the night in our study were below the level for a "dark response" in the animals. They did not use the light intensity changes as a strong Zeitgeber. The effect of the light conditions on the activity of the octopuses was then similar to a weak Zeitgeber, producing relative coordination.

Alternatively, light may only play a minor role in entrainment. Other environmental cues may be used to synchronize activity. This could be the reason for conflicting results on the time of activity that are common in octopod species. Mather (1988) reported that the selective pressure to temporally organize activity was not strong for *O. vulgaris* in Bermuda and thus their response was variable. Yarnall (1969) observed that the methods for prey catching in octopus included the use of chemotactile rather than visual search. Hence, they have not been selected for either diurnal or nocturnal activity. The availability of food may be a more important timing factor as starving animals have been reported to be mainly nocturnally active with a peak activity at about 2300. Wells and co-workers (1983) have reported that this pattern is preserved with evening feeding schedules but not ad libitum. Our octopuses were fed ad libitum, and they may have lacked this Zeitgeber to synchronize their activity.

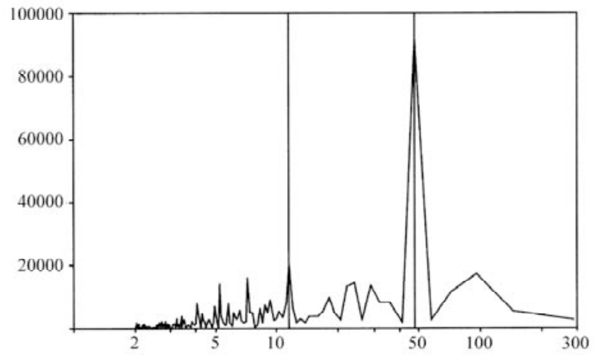

Fig. 3 The distribution of periodlength in LD is shown for animal 1. Periodlength is shown in half hour intervals. Dominant peaks are marked with black vertical lines

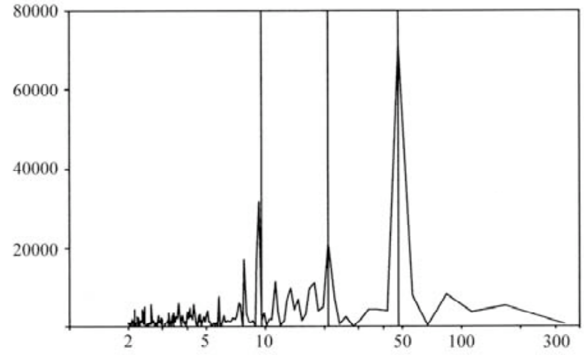

Fig. 4 The distribution of periodlength in DD is shown for animal 1. Periodlength is shown in half hour intervals. Dominant peaks are marked with black vertical lines

Avoidance of other octopod individuals and species could be another possibility for organizing *O. vulgaris* activity. Sympatric octopod species can share the same resources such as shelter or prey (Houck 1982).

Animals occupying similar niches in the same habitat should try to minimize competition and would do well to occupy differing parts of the day with their activity and rest. Temporal spacing and niche partitioning can be used to reduce interference with other octopods (Hanlon & Messenger 1996). Three Hawaiian shallow water octopod species maintained species-specific temporal spacing in locomotor activity in the laboratory as well as in the field (Houck 1982).

Predation pressure may also set activity cycles in *O.*

vulgaris. The evolution of coleoids is thought to have been strongly influenced by both competition and predation pressures from fish and marine reptiles from the Mesozoic onwards (Packard 1972). Predators of *O. vulgaris* are fish, marine mammals, birds and other cephalopod species (for a review see Hanlon & Messenger 1996), all of which have well developed eyes. Predation can be a critical factor in the distribution of octopods. Aronson (1991) found an inverse relationship between the number of predatory teleosts and the octopus population density.

In our experiment it was particularly animal 4, which differed from animals 1, 2 and 3 in these aspects. The amounts of activity found in animal 1, 2 and 3 agree with reports of Boyle (1980) that *O. vulgaris* were outside their homes between 40 and 60 percent of the time each day whereas animal 4 was nearly twice as active and hardly ever rested. Increased activity has been reported in senescent octopod males (Anderson pers. comm.) and animal 4 may have been a senescent male since it showed typical behavior for octopuses shortly before death like lack of feeding, retraction of skin around the eyes, uncoordinated movement, increased undirected activity, and white unhealing lesions on the body. Changes of time of activity with age have been reported for octopods (Mather 1984). The activity cycle of young *Octopus joubini* was altered from a slightly nocturnal to a strictly nocturnal one as they gradually changed from swimming to walking on the substrate in the first weeks of their lives.

Activity rhythms are especially important for the opportunistic but extremely vulnerable *O. vulgaris*. They can help minimize predation risk and maximize foraging efficiency. Still a larger sample size is necessary to answer open questions and to provide a better representation of the population of this particular species. Octopuses show divergent characters of activity in individual animals (Mather & Anderson 1993). Enlarging the sample size could clarify if results were more related to individual traits than to general species behavior. Biological rhythms play an important role in the life of many marine animals and timekeeping is especially important for those who have short lives like octopuses.

ACKNOLEDGEMENTS

We thank Flegra Bentivegna and Gianfranco Mazza from the Stazione Zoologica di Napoli for their help with collecting our octopuses. This study was supported by the Konrad Lorenz Institute for Evolution and Cognition Research and the science program of Niederösterreich.

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Received: 14 December 2002 / Accepted: 26 May 2003