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Rhythms of activity and oxygen consumption in the common pond clam, *Ligumia subrostrata* (Say)

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Diurnal rhythms were found in the unionid clam *Ligumia subrostrata* for valve activity and oxygen consumption. Valve closures and valve gape were measured as indices of valve activity. Recordings were made after the animals were entrained to each of three photoperiods: 12 h light (L) : 12 h dark (D), 16 L : 8 D, and 24 L. Statistically significant ($P < 0.01$) rhythms of activity were found in 12 L : 12 D and 16 L : 8 D photoperiods with high activity occurring during the dark phase. The onset of darkness was indicated as the entraining factor. No rhythm of valve activity was found in 24 L. Flow-through respirometry of individual clams was used to determine if oxygen consumption paralleled activity rhythms. Rhythms of oxygen consumption were present in all three photoperiods with a change in light rather than the onset of darkness as the entraining factor. The activity rhythm was exogneous, whereas the oxygen consumption rhythm was an endogenous rhythm. Overall levels of activity and oxygen consumption were highest in the 16 L : 8 D regimen and depressed in constant light.

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Chez le lamellibranche unionidé *Ligumia subrostrata*, l'activité de la coquille et la consommation d'oxygène suivent des rythmes nycthéméraux. Au cours d'expériences, la fermeture et l'ouverture des valves ont servi d'indices d'activité de la coquille. L'activité a été enregistrée après adaptation des animaux à trois photopériodes différentes: 12 h clarité (C) : 12 h obscurité (O), 16 C : 8 O et 24 C. L'activité suit des rythmes significativement différents ($P < 0.01$) aux photopériodes 12 C : 12 O et 16 C : 8 O, cette activité étant à son maximum durant la phase d'obscurité. C'est le début de la période d'obscurité qui déclenche l'activité. La coquille n'a pas de rythme particulier d'activité lorsque la photopériode est de 24 C. La mesure de la consommation d'oxygène, évaluée au moyen d'un respiromètre, a permis d'établir si oui ou non l'activité respiratoire suit toujours l'activité de la coquille. L'activité respiratoire suit un rythme aux trois photopériodes, mais elle est déclenchée par les changements de lumière, plutôt que par le début de la période d'obscurité. Le rythme de l'activité de la coquille est exogène, alors que le rythme de l'activité respiratoire est endogène. L'activité de la coquille et la consommation d'oxygène sont en général plus importantes à la photopériode 16 C : 8 O et elles déclinent en présence de lumière continue.

[Traduit par le journal]

Introduction

Rhythmic changes in many physiologic activities have been noted to occur on a diurnal basis. Imlay (1968) has reported rhythms of valve activity in the freshwater clam *Elliptio complanatus catawbensis* (Lea) as a function of the light cycle. Diurnal as well as tidal activity rhythms have been reported for the marine molluscs (Palmer 1974; Bennett 1954). Rhythms of oxygen consumption have been observed in the marine gastropod molluscs *Littorina irrorata* (Shirley and Findley 1978), *L. littorea*, and *Urosalpinx cinereus* (Sandeen *et al.* 1954). Although oxygen consumption rates have been reported for both marine and freshwater bivalve molluscs (Bayne 1976; Booth and Mangum 1978; Dietz 1974; Waite and Neufeld 1977) the measurements have been confined to a few hours.

The object of this study was to investigate the presence of diurnal rhythms in oxygen consumption and valve activity of the common pond clam, *Ligumia subrostrata* (Say). These animals are not exposed to tidal effects as are the marine molluscs, but because they live in shallow water, they are exposed to photoperiod variations. Valve closures and valve gape were measured as indices of valve activity.

Materials and Methods

Male *Ligumia subrostrata* were collected from a pond near Baton Rouge, Louisiana. Animals used in the oxygen study were collected 11 March 1978; the animals used in the activity

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study were collected 14 June 1978. The clams were maintained at 20°C in an aerated aquarium containing artificial pond water (Dietz and Branton 1975) and were not fed. These animals have been maintained in the lab without feeding for more than a year without a substantial decrease in their total carbohydrates. Although recently collected animals have a higher rate of oxygen consumption than laboratory acclimated animals, the basis for this difference is not due to starvation (Dietz 1974). The clams were placed in a 12 h light (L): 12 h dark (D) regimen for a 7-day entrainment period before use. In both studies, the same animals were subsequently placed in an extended photophase photoperiod of 16 L : 8 D and then a 24-h constant light photoperiod for at least 7 days acclimation. The specimens used in the activity study had a mean length of 8.70 ± 0.2 (SD) cm and a mean dry tissue weight of 2.43 ± 0.4 g. The clams used for the oxygen study had a mean length of 6.35 ± 0.5 cm and a mean dry tissue weight of 0.92 ± 0.30 g.

Valve activity was measured using a modification of the arrangement described by Hiscock (1950). The clams were immobilized by cementing one valve to a Plexiglas substrate with epoxy while the free valve was attached to a Harvard Apparatus heart – smooth muscle transducer by a nylon suture. The animals were placed in 5 L of water in a 24 cm × 46 cm container and were situated so that the hinge and shell margins were in a horizontal plane. A Harvard Apparatus recorder was used to monitor valve activity. Valve activity was measured for 24-h periods for each animal in three photoperiods: 12 L : 12 D, 16 L : 8 D, and 24 L. Six numbered clams were used in each experiment. Valve gape was determined by assigning a value of 100% to the maximum degree of valve gape in each of the 24-h recordings and calculating the area under the activity curve for each hour. A computerized disc digitizing system was used to analyze the areas under the curves.

Respiration rates were measured using a flow-through respirometer and an International Biophysics Corporation differential oxygen analyzer (Findley *et al.* 1978). A single animal was placed in a 260-mL glass respiration chamber through which artificial pond water was pumped by a peristaltic pump at a rate of 10 mL/min. The chamber was enclosed in a box that could be made light-proof. An incandescent lamp with an intensity of 16 000 lx illuminated the chamber during photophase. During the scotophase the box was sealed. The entire apparatus was situated in a water table maintained at a constant 20°C. An Omniscribe strip chart recorder continuously recorded the oxygen consumption for 24-h periods in the 12 L : 12 D, 16 L : 8 D, and 24 L photoperiodic regimens. Respiration rates were measured for six clams; the same individuals were used for all three photoperiods. The data obtained from these measurements were converted to mean microlitres of O_2 consumed per animal per hour at standard pressure. The average animal weight was about 1 g.

An analysis of variance (ANOVA) model with animal and time as main effects was used to examine variance in valve activity and respiration rates with respect to time of day. This analysis requires the assumption of independence between animal and time; that is, the response over time is consistent from animal to animal. The use of the parametric ANOVA is appropriate since valve activity and oxygen consumption are essentially interval in nature, and the use of animal as a blocking-type main effect should compensate for the repeated measurements on each animal over 24 h. Independent orthogonal comparisons on blocks of time within a 24-h period were used for partitioning of the variance. An F statistic beyond the 95% region of acceptance was considered to be significant ($P < 0.05$), and an F statistic falling outside of the 99% region of acceptance was considered highly significant ($P < 0.01$). A Duncan's multiple range test was performed for valve closures, valve gape, and oxygen consumption to determine if the overall levels were significantly different ($P < 0.05$) in the three photoperiods.

Results

Ligumia subrostrata displayed rhythms of valve closure in the 12 L : 12 D and 16 L : 8 D photoperiods with the primary peak of activity occurring 1–2 h after the onset of darkness (Fig. 1). A decline in activity occurred at 0800 hours at the onset of the light phase in both 12 L : 12 D and 16 L : 8 D. No significant rhythm of valve closure existed in constant light conditions (Table 1). The number of valve closures was reduced in the 24 L photoperiod. The greatest number of valve closures per hour was 4 ± 4 in constant light as compared with 13 ± 7 in 12 L : 12 D and 11 ± 5 in 16 L : 8 D.

In addition to decreasing valve movement, *L. subrostrata* decreased the valve gape in 24 L. Measurements of percent valve gape per hour revealed highly significant rhythms similar to those of valve closure (Fig. 2). Maximum valve gape occurred during scotophase. The primary peak occurred 4 h after the onset of darkness in 12 L : 12 D (93 ± 7%

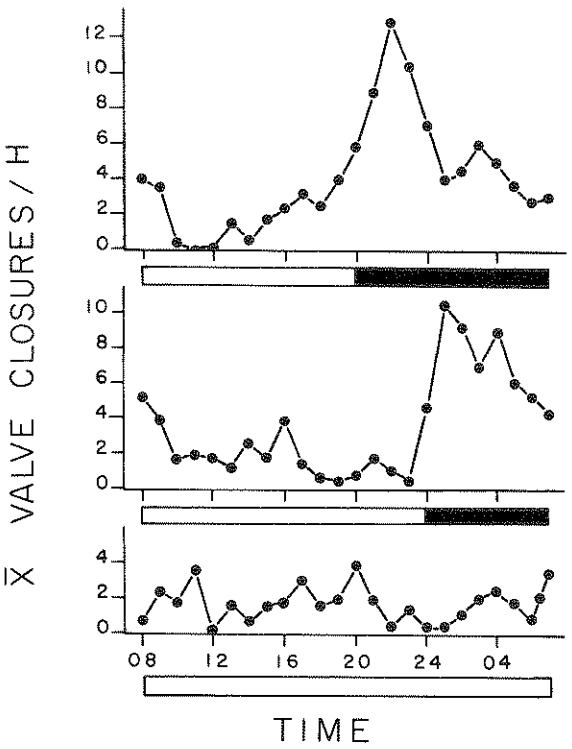

FIG. 1. Mean number of valve closures per hour for six clams on a 12 L : 12 D, 16 L : 8 D, and 24 L photoperiod. Bars below each graph depict the light–dark scheme. The open segment of the bar represents photophase, and the darkened segment represents scotophase.

TABLE 1. Analysis of variance (ANOVA) F statistics for valve closures, valve gape and oxygen consumption in 12 L : 12 D, 16 L : 8 D, and 24 L photoperiods

Photoperiod	Valve closures	Valve gape	Oxygen consumption	
			ANOVA	Orthogonal comparisons
12 L : 12 D	$F = 3.53^{**}$ (23, 115)	$F = 9.24^{**}$ (23, 115)	$F = 1.14$ NS (23, 106)	0800–1300 vs. 1400–1900 hours $F = 4.35^{*}$ (1, 106) 0800–1900 vs. 2000–0700 hours $F = 0.10$ NS 2000–0100 vs. 0200–0700 hours $F = 232.40^{**}$
16 L : 8 D	$F = 7.66^{**}$ (23, 115)	$F = 13.29^{**}$ (23, 115)	$F = 2.27^{**}$ (23, 107)	0800–1300 vs. 1400–1900 hours $F = 1.27$ NS (1, 107) 2000–0100 vs. 0200–0700 hours $F = 21.13^{**}$ 2300–0600 vs. 0700–2200 hours $F = 1477.59^{**}$
24 L	$F = 0.98$ NS (23, 115)	$F = 0.90$ NS (23, 110)	$F = 2.27^{**}$ (23, 84)	0800–1300 vs. 1400–1900 hours $F = 129.12^{**}$ (1, 84) 0800–1900 vs. 2000–0700 hours $F = 1.27$ NS 2000–0100 vs. 0200–0700 hours $F = 3.36$ NS

NOTE: $^{*}P < 0.05$; $^{**}P < 0.01$; NS indicates not significant at $\alpha = 0.05$. The numbers within parentheses represent degrees of freedom. Test statistics for orthogonal comparisons of blocks of time within a 24-h period are listed in addition to ANOVA F statistics for oxygen consumption.

valve gape/h) and 6 h after the onset of darkness in 16 L : 8 D (91 ± 6% valve gape/h). A decline in valve gape occurred at the onset of light in both the 12 L : 12 D (78 ± 15%) and 16 L : 8 D (62 ± 22%). As with valve closures, no significant rhythm of valve gape occurred under constant light conditions, and the overall level of percent valve gape was depressed throughout the 24-h period.

The rhythm of valve gape in these animals appears to be indirectly related to that of number of valve closures. After the peak of the valve closure rhythm, there is a substantial drop in the number of closures per hour for the duration of the dark phase, and the valves remain maximally open until the onset of the light phase.

The overall number of valve closures by *L. subrostrata* during the 12 L : 12 D photoperiod was significantly higher than during either the 16 L : 8 D or 24 L photoperiods (Table 2). The number of valve closures in 16 L : 8 D was not significantly different from the 24 L closures. The valve gape when the animals were on 16 L : 8 D was significantly higher than the 24 L valve gape.

No significant variance in rates of oxygen consumption was found over 24 h when the animals were on 12 L : 12 D (ANOVA). However, a highly significant difference in the respiration rates in time blocks 2000–0100 hours versus 0200–0700 hours and a significant difference in 0800–1300 hours versus 1400–1900 hours was found using orthogonal comparisons (Table 1). The 12 L : 12 D rhythm paralleled the 12 L : 12 D valve closure rhythm with the peak level of respiration (406 ± 253 μL O_2/animal h^{-1}) occurring 1 h after the onset of darkness, and a smaller peak (307 ± 70 μL O_2/animal h^{-1}) occurring at the onset of light (Fig. 3). Variance in oxygen consumption in animals on 16 L : 8 D was not significant using ANOVA, but a highly

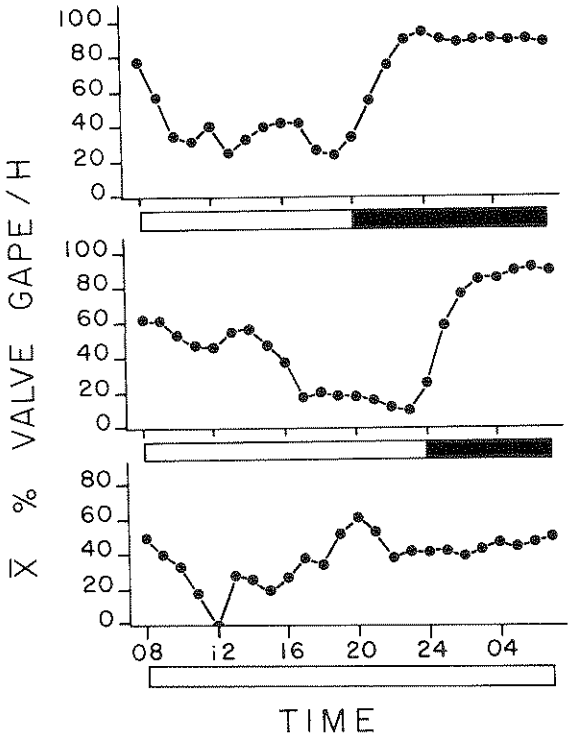

FIG. 2. Mean percent valve gape per hour for six clams on a 12 L : 12 D, 16 L : 8 D, and 24 L photoperiod. Bars below each graph depict the light–dark scheme. The open segment of the bar represents photophase, and the darkened segment represents scotophase.

significant difference was found between the dark hours, 2300–0600 hours, and the light hours, 0700–2200 hours, using orthogonal comparisons. The maximum level of oxygen consumption (481 ± 97 μL O_2/animal h^{-1}) occurred 5 h after the onset of light with a smaller increase (378 ± 94 μL O_2/animal h^{-1}) 2 h before the onset of light. The

TABLE 2. Duncan's multiple range test for means of oxygen consumption, valve closure, and valve gape data

Variable	Photoperiod		
Oxygen consumption, μL O_2/animal h^{-1}	16 L : 8 D	317.2 (18)	A
	12 L : 12 D	273.0 (22)	A, B
	24 L	226.8 (22)	B
Valve closures, no. closures/h	12 L : 12 D	6.3 (24)	A
	16 L : 8 D	2.8 (24)	B
	24 L	2.5 (24)	B
Valve gape, % valve gape/h	16 L : 8 D	50.3 (24)	A
	12 L : 12 D	48.5 (24)	A, B
	24 L	29.5 (24)	B

NOTE: Data with the same letters are not significantly different. The number within parentheses is the number of mean hourly observations for six clams.

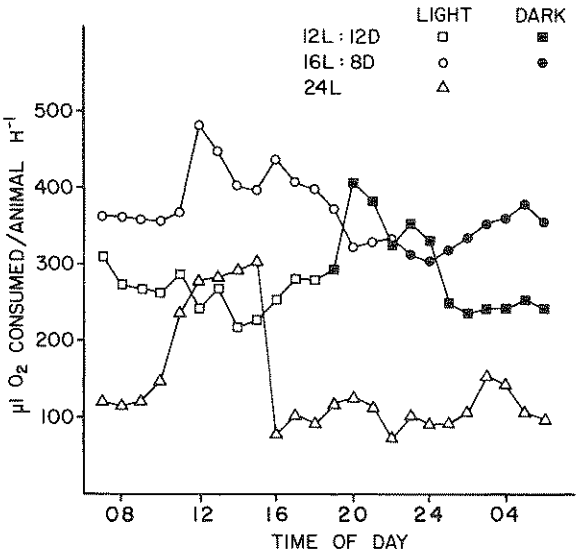

FIG. 3. Mean oxygen consumption rate in microlitres oxygen per animal per hour for six clams. Open symbols are rates during photophase. Closed symbols are rates during scotophase.

12 L : 12 D rhythm peaked approximately 12 h after the onset of darkness. The variance in oxygen consumption persisted in *L. subrostrata* in constant light conditions ($P < 0.05$; ANOVA) with the peak occurring at 0400 hours. In addition, highly significant differences in the respiration rates between the 0800–1900 hours and 2000–0700 hours blocks of time were found using orthogonal comparisons.

The minimum level of oxygen consumed by *L. subrostrata* in 12 L : 12 D was 217 μL O_2/animal h^{-1} as compared with the maximum level of 406 μL O_2/animal h^{-1}, an 87% increase above the minimum level. Animals in the 16 L : 8 D photoperiod showed a 58% increase from the minimum level of 304 μL O_2/animal h^{-1}, but the clams in constant light displayed an increase of more than 300% from the minimum rate of 72 μL O_2/animal h^{-1}. The average oxygen consumption for the animals in 16 L : 8 D was significantly higher than that in the 24 L photoperiod (Table 2). The animals in 12 L : 12 D had an intermediate rate of oxygen consumption, but were neither significantly higher than those in 24 L nor significantly lower than those in 16 L : 8 D.

Two short-term respiration patterns were detected. In the 12 L : 12 D photoperiod a sinusoidal rhythm with a period of approximately 5 min and an amplitude of 53 ± 12 μL O_2/ animal h^{-1} occurred in half of the animals tested and was sporadic with respect to time of day. The period and amplitude of the rhythm varied with each animal. This pattern did not appear in either the 16 L : 8 D or 24 L regimens. In the constant light regimen an unusual burst of oxygen consumption occurred every 3.5–4 h and lasted for approximately 30 min. Although the level of the bursts varied, the respiration rates were frequently more than doubled during the burst periods. There were no irregularities in valve activity that corresponded to these patterns of oxygen consumption.

Discussion

A diurnal rhythm of valve activity is evident in *Ligumia subrostrata* with the onset of darkness indicated as the phase-setting cue. The cue was demonstrable as the activity peak shifted correspondingly with a 4-h shift in the 16 L : 8 D scotophase. Valve activity rhythms have been reported for freshwater clams and for the Japanese pearl oyster (Kuwatani 1963; Salanki 1964; Imlay 1968). The oyster valves tended to be open more in response to the onset of darkness than to the onset of light. The valve closure and valve gape activity rhythms are exogenous rhythms that persist only while the activating stimulus is being received by the organism. Both activity rhythms disappeared after 7 days in constant light conditions. The response of the clam to light is believed to involve a dermal light sense associated with pigment cells since neither *Elliptio* nor other previously mentioned clams have differentiated visual organs (Imlay 1968).

The oxygen consumption rhythm was present in all three of the photoperiods. The cue appears not to be the onset of darkness as in the activity rhythm, but a change in light. The persistence of the respiration rhythm in constant light conditions indicates that the rhythm is endogenous. The last cue the animals received was termination of light in the 16 L : 8 D regimen. After the animals were used in the 16 L : 8 D study they were placed in constant

light for acclimation. The oxgen consumption rhythm persisted throughout the entrainment and study period of 14 days with the peak 24 L respiration level occurring at approximately the same time as the 16 L : 8 D peak level.

The oxygen consumption rates were significantly elevated in the 16 L : 8 D photoperiod. This phenomenon is comparable with the increased respiration levels of animals exposed to the longer daylight hours associated with summer months. However, the experimental temperature remained the same (20°C) in each of the photoperiods. The elevated respiration rates of laboratory-acclimated clams indicates that starvation is not a significant factor.

As activity, either locomotor or isometric, is an important factor in mediating the metabolic rate of an organism, a high activity period is often associated with increased oxygen consumption. Gartkiewicz (1922) found that respiration levels in *Anodonta* sp. during a resting period averaged only 0.018 mg O_2/h, but immediately upon opening, the level averaged 0.357 mg O_2/h then dropped to 0.254 mg O_2/h. The oxygen consumption data in *L. subrostrata* in the 12 L : 12 D photoperiod correlated with the 12 L : 12 D activity data. The 16 L : 8 D and 24 L oxygen and activity data were not as well correlated. Valve opening is primarily a passive process and conceivably, the closing of the valves is an energetically inexpensive process. Such valve movements would not greatly affect the respiration rate of the clam. Clearly, there are factors in addition to valve activity that are important in modifying the oxygen consumption rate in *Ligumia subrostrata*.

The observation of activity and oxygen consumption rhythms adds to the autecology of *Ligumia subrostrata*. The daily range of values recorded for *L. subrostrata* in both activity and oxygen consumption are more than ample to mask relationships associated with experimental manipulation. Maintaining animals in constant conditions in an attempt to dampen the endogenous rhythms may only compound the problem if they become free-running and less predictable.

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