

A Rhythmicity in the rate of Oxygen Consumption by the Manila clam, *Ruditapes philippinarum*

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Oxygen consumption by the Manila clam, *Ruditapes philippinarum* (Bivalvia: Veneridae) maintained in a culture tank for 7-10 weeks was measured with an automatic intermittent-flow-respirometer (AIFR). There was evidence for an endogenous tidal rhythm of 12.4 h in oxygen consumption, at irregular intervals of between about 7 and 18 hours during experiments of between 22 h 5 min and 70 h 32 min (duration) recording for fasting animals in constant darkness at constant temperatures in the absence of tidal rhythms.

INTRODUCTION

Many intertidal organisms show rhythms in their behaviour and physiology coinciding with the rise and fall of tides. The adaptative significance of such rhythms has been emphasized (Palmer, 1974; Enright, 1975). Recognition of tidal rhythms dates back to 1903, when such a rhythm was discovered in the green Turbellarian (*Convoluta roscoffensis*) (Bohn, 1903; Gamble and Keeble, 1903). Since then, similar rhythms have been described for many other intertidal organisms (Brown *et al.*, 1953; Sandeen *et al.*, 1953).

Rao (1954) reported that *Mytilus edulis* and *M. californianus* showed a tidal rhythm in their filtration rates. According to Bayne *et al.* (1976), however, neither Jørgensen (1960), Theede (1963) nor Davids (1964) could demonstrate the tidal rhythmicity in *Mytilus edulis* and Rao's technique for measuring filtration rate was criticized by Jørgensen (1960). Jørgensen (1960) found no correlations between the water transport in mussels and the tidal cycle or the level of intertidal zone at which the mussels are sampled. Circadian and circatidal rhythms of oxygen consumption in molluscs have been ob-

served by a number of researchers (Zann, 1973; Shirley and Findley, 1978; McCorkle *et al.*, 1979).

Previous investigations have examined pumping and respiration rates but have found no evidence of significant cycles in many bivalves species (Prof. Dr. J. Widdows, personal communication). Rhythmicity in shell gaping, and siphon extension, and also in adductor activity have been observed in bivalve under constant conditions (temperature, food) in the laboratory (Beentjes and Williams, 1986). Richardson (1988) investigated rhythms of band formation in the shell of *Tapes philippinarum* and suggested that these rhythms persist under non-tidal conditions. Although over the past 20 years some knowledge has been accumulated concerning the anatomy, neurophysiology, and pharmacology of the circadian system, it is still not known how circadian rhythms are really generated (Wollnik, 1992).

The Manila clam is one of the most important commercial bivalve species in the world (FAO, 1993) and is distributed in Korean, Chinese, Japanese and Philippine waters.

In this paper, we demonstrate an endogenous rhythm in oxygen consumption of an intertidal bivalve, the Manila clam, *Ruditapes philippinarum*,

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collected from the Korean tidal flats.

MATERIALS AND METHODS

The Manila clams (*Ruditapes philippinarum*) were collected on February 24, 1993 and June 23, 1993 from an intertidal zone in Garolim Bay (36° 58' N, 140° 20' E) on the west coast of Korea. The sampling area is aerially exposed during low-tide for 4-5 h and the mean tidal-range is 4.9 m. The collected clams were transported to Germany (it takes ca. 24 hours) and there cultured in sediments 15 cm deep with 15 liter of water. The Manila clams of 3-4 years old (shell length = 30.1-38.4 mm) were acclimatized to the experimental temperatures of 10 and 12°C over two weeks. As principle food, cultured marine algae (*Nannochloris spp.*) were provided. Approximately 100 ml algae 1.8×10^6 cells \cdot l⁻¹ per day was mixed with an artificial food concentrate (two drops-Liquifry Marine Interpet Ltd., England). The Manila clams were fed only once per day prior to the experiments. Feeding of the Manila clams, however, was stopped 48 h before their introduction into the respirometer, the animals were starved during the experiments in order to exclude a rise of oxygen consumption due to specific activity, i.e. feeding and digestion. In four experiments 11 animals were monitored with two to three animals being put in one chamber at a time. Oxygen consumption, therefore, represented an integration from two to three animals.

The oxygen consumption rate was measured by an automatic intermittent-flow-respirometer (AIFR) with one chamber system. The sea water (29-30‰) was filtered free of bacteria through a sterile membrane filter (with two Sartorius Capsule Filters, input 0.2 and output 0.07 μ m) to reduce background oxygen consumption and to prevent the buildup of microbial film in the apparatus (which was also thoroughly cleaned between experimental runs). During the experiments with durations of between 22 h 5 min and 70 h 32 min oxygen consumption and the water temperature (Farnell, Pt-100, Germany) were continuously measured. Oxygen saturation in the experimental chamber was always

maintained between 98.9 (highest) and 83.5% (lowest). After calibration of the oxygen probe (Esweiler, 15 μ PO₂, Germany), the measuring system was started and subsequently the experiment was controlled by a custom made computer program. The calculation of oxygen consumption occurred for intervals of 100, 200, or 300 seconds etc., depending on the expected consumption of the Manila clams. The oxygen consumption was calculated from the decrease of oxygen saturation during the experiment. When the oxygen saturation dropped below the predetermined limit, the magnetic drive gear pump (Ismatec Sa, MS-Z, Switzerland) and three way magnetic valve (Nortec, 332F, Germany) supplied the system with saturated seawater until the selected oxygen saturation level was reached. No measurements were made while flushing the chamber with oxygen saturated seawater from a storage tank to restore the upper oxygen saturation to 94.3-98.9%. After the end of each experiment, the chamber was rinsed with oxygen-saturated water and the probe voltage was examined to ascertain whether it had deviated from the gauge voltage at the beginning of the experiment. This voltage was also subsequently tested, between 6 and 24 hours and by measurements of the oxygen consumption without clams for reference. A detailed description for calculation methods and formulae of oxygen consumption are given by Kim (1994) and Kim *et al.* (in press).

RESULTS

Results of the experiments showed that Manila clams exhibited a rhythmicity in oxygen consumption (Table 1). The oxygen consumption in the clams from one experiment conducted between May and August (5.7-5.10, 1993 in Table 1) is shown as an example in the results section.

Oxygen consumption rate exhibited a rhythmicity during the experiments (Fig. 1a). The oxygen consumption rate varied from ca. 3 to near 0 ml O₂ g DW⁻¹ h⁻¹ as shown in Fig. 1a. The values of oxygen consumption in Fig. 1a were derived from the change in oxygen saturation (%) in Fig. 1b. Clusters

Table 1. Peaks and rates of oxygen consumption by *Ruditapes philippinarum* under different experimental conditions. (2-3 specimens of the Manila clams, *R. philippinarum*, were treated for 22 h 5 min-70 h 32 min. Statistical values were computed for each batch from these 174-2,215 measurements)

Date of measurement	5.7-5.10 (1993)	5.11-5.13 (1993)	8.13-8.14 (1993)	8.22-8.23 (1993)
Year-class	1990	1990	1989	1990
Individual number (n)	3	3	2	3
Mean shell length (cm)	3.1	3.3	3.8	3.0
Somatic-tissue weight (g DW)	0.51	0.71	0.83	0.48
Culture period (day) (after collecting)	72	76	51	60
Duration of the experiment (h)	70.5	42.1	22.5	22.1
Measurement interval (sec.)	102	76	304	304
Number of measured	2,215	1,910	174	233
Peak intervals (h)	7-18	7-10	8	8-10
Mean oxygen consumption (ml O ₂ g DW ⁻¹ h ⁻¹)	1.15 (SE=±0.001)	1.18 (SE=±0.005)	1.89 (SE=±0.003)	0.66 (SE=±0.01)
Temp.(°C)	10 (SE=±0.0006)	10 (SE=±0.001)	12 (SE=±0.006)	10 (SE=±0.005)
Oxygen saturation (%)	83.4-94.3 (SE=±0.06)	83.5-98.9 (SE=±0.07)	85.2-98.3 (SE=±0.18)	85.1-98.7 (SE=±0.22)
Salinity (‰)	29	29	30	29

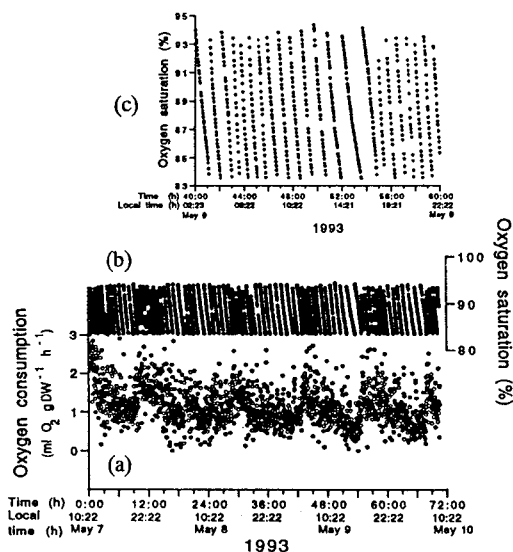


Fig. 1. Oxygen consumption at oxygen saturation levels of between 83.4 and 94.3% in three Manila clams ($n=3$), *Ruditapes philippinarum*, of shell length 30-33 mm (3 years old, dry weight 511.2 mg) at 10°C ($SE = \pm 0.0006$). Experiments were carried out on fasting animals under constant conditions (constant darkness, constant temperature), in the absence of tides (continuous immersion). (a) the rate of oxygen consumption showing periodicity, (b) changes in the level of oxygen saturation caused by animal activity over time, (c) enlargement of the variation in oxygen saturation (%) between 40 and 60 h after the start of the experiment.

of denser lines which depict values of oxygen consumption measured every 102 seconds indicate higher oxygen consumption (Fig. 1b). For example, there is a decrease in oxygen consumption between 51 and 55 h compared to the rate between 55 and 60 h (Fig. 1c). Such changes in rates of oxygen consumption over time were recognized by the steepness and intervals of lines as shown in Fig. 1b.

Fig. 1a is fitted to a weighted smooth curve of 10% and plotted in Fig. 2a. The mean oxygen consumption rate, averaged over the entire duration of the experiment and over the entire range of oxygen saturations (between 83.4 and 94.3%), was 1.15 ($SE = \pm 0.006$) ml O₂ g DW⁻¹ h⁻¹. The calculated oxygen consumption values at 90 or 85% of the saturation level over the duration of the experiment are shown in Figs. 2b and 2c. Again the endogenous rhythm was clear, there being a peak in oxygen consumption with a shorter than a day periodicity, although the amplitude was variable. The time taken for respiratory activity of the Manila clams to reduce oxygen saturation from maximal (i.e. 94.3%) to minimal saturation (i.e. 83.4%), namely, the rates of oxygen consumption during every set of measurements were compared. This gives an integrated indicator of oxygen consumption over all saturation values encountered by the animals. The rates in-

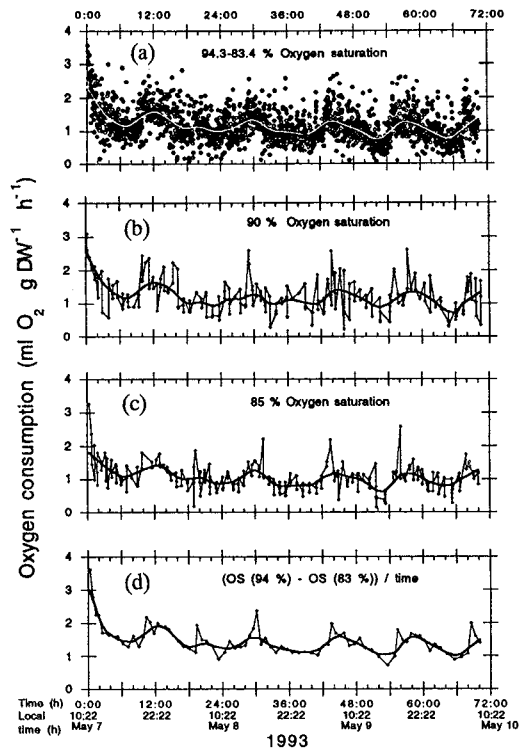


Fig. 2. Schematic time series indicating endogenous rhythms in oxygen consumption by three Manila clams, *Ruditapes philippinarum*, over time during constant conditions. These data were fitted by a weighted smooth curve of 10%. (a) oxygen consumption rates from the original data where saturation levels varied between 83.4 and 94.3%, (b) oxygen consumption rates using oxygen saturation values of 90%, (c) oxygen consumption rates using oxygen saturation values of 85%, and (d) the variation in the rate of oxygen consumption per hour [i.e. (oxygen saturation value at 94.3%-oxygen saturation value at 83.4%)/time].

volving times to reduce saturation to a minimum (i.e. 83.4%) are plotted against experiment duration in Fig. 2d.

The mean oxygen consumption at 85% oxygen saturation was lower (1.05 ± 0.03) than that at 90% saturation (1.20 ± 0.04) ml O₂ g DW⁻¹ h⁻¹. A difference of 5% in oxygen saturation related to a relatively wide difference (over 12%) of 0.15 ml O₂ g DW⁻¹ h⁻¹ in oxygen consumption rate. An extrapolation of oxygen consumption values to certain level would be possible, e.g. 1.05×1.12 , as if all values were measured at 90% saturation level.

DISCUSSION

During the initial three hours of the experiments, the oxygen consumption rates were higher than the remainder of the experiment. This initial rise may have been caused by stress experienced by clams during the set up of the experimental chamber. The gradual decrease in both magnitude and amplitude of the oxygen consumption was similar to that observed in the marsh periwinkle, *Littorina irrorata* (Shirely and Findley, 1978). The decrease in the rate of oxygen consumption over the whole experiment could be a response to starvation. Cederwall (1979) reported a decrease in oxygen consumption, which he similarly ascribed to starvation. Among the peaks, the last two coincided approximately with the tidal phase of 12.4 h (Figs. 1a and 2). We can see that the oxygen consumption rate of the Manila clams showed about two peaks per day (Fig. 1a), although an irregularity during the first 24 h of the experiment is somewhat puzzling. Either the clams have not reached a "steady state" (Jobling, 1981; Follum and Gray, 1987) under laboratory conditions yet or other unexpected factors are active. Widdows and Hawkins (1989) stated that the decline and variation in oxygen consumption during the initial 24 h is probably due to starvation and the switching off of metabolic costs associated with digestion and synthesis. However, an endogenous tidal rhythm of 12.4 h appeared to be predominant from the present results.

Evans (1972) observed that two bands a day were produced in the shells of cockles when they were emersed twice a day during neap tides. However, only one clear band was produced once a day when the animals were emersed at low spring tides. Since this observation, it has generally been accepted that band formation in the shell of molluscs can be linked to lunar or tidal rhythms. Richardson (1988) reported that Manila clams grown under simulated semi-diurnal conditions of emersion and immersion laid down clearly defined bands with an almost exact coincidence with the number of emersions. He observed, however, that *R. philippinarum* produces

weak microgrowth bands in the shell at approximate semi-diurnal periodicity under constant conditions in the laboratory.

The question arises as to whether or not the observed peaks are endogenous. From the beginning to the end of the experiments we never attempted to synchronize any of the external factors such as light, food and tide which might result in rhythmic activity. While studying rhythmicity in the shell deposition of the cockles, *Cerastoderma edule* Richardson *et al.* (1980) observed that there was no discernable influence of cycles of illumination on the banding patterns under any of the experimental conditions. It could be assumed that light might affect activity rhythms, although Manila clams live just under the sediment surface at depths of 2 to 6 cm (Kraeuter and Castagna, 1989) where dim light may penetrate through siphon channel. However, since the Manila clams showed a rhythm with a period shorter than a day, it may be assumed that illumination is of minor importance for the rhythmic activity of Manila clams.

Richardson (1988) observed that Manila clams grown under periodical supply of food, laid down defined bands whose number almost coincided with food supply. The rhythmicity with more than one peak per day observed in the present study was considered to be independent of feeding. The rates of oxygen consumption by mussels in the feeding increased exponentially with increase in absorbed ration (Bayne *et al.*, 1989), but such effects were reduced or eliminated by starving the clams during our experiments. To avoid direct effects of tides on the rate of oxygen consumption, rearing of the animals and experiments were conducted in the absence of tides. It appeared, therefore, that an endogenous process was not hindered for the the metabolic activity of Manila clam in the present study.

The observed endogenous rhythms of oxygen consumption in Manila clams, *R. philippinarum*, with a period shorter than a day under constant conditions may have some implications for the determination biological rhythms and of the oxygen consumption rate of other intertidal organisms.

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