

A STUDY OF DIURNAL PERIODICITY IN OXYGEN
CONSUMPTION OF THE SMALL ABALONE
(*HALIOTIS DIVERSICOLOR*
SUPERTEXTA LISCHKE)¹

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Rong-Quen Jan, Kwang-Tsao Shao and Kun-Hsiung Chang (1981) A Study of diurnal periodicity in oxygen consumption of the small abalone (*Haliotis diversicolor supertexta* Lischke). *Bull. Inst. Zool., Academia Sinica* 20(1): 1-8. This respiratory study of the small abalone (*Haliotis diversicolor supertexta* Lischke) is based on measurements of dissolved oxygen levels in a closed flow-through system for 24-hour periods. Abalone which had been starved and exposed to a natural light regime (daylight/dark 12:12) showed a clear diurnal change of oxygen consumption, viz., 20% more at night than during the day. On the other hand, abalone which had been fed and exposed to natural light, or starved and kept in constant light, do not exhibit this diurnal change.

The small abalone *Haliotis diversicolor supertexta* is a common prosobranch mollusc (family Haliotidae) found in subtidal rocky shores of eastern and northern Taiwan. In recent years, because the natural populations of abalone have been overexploited, fishermen have been propagating the abalone artificially and raising them in intertidal ponds, as well as in the open sea. High mortalities, of which the causes are not clearly understood, have been experienced during raising and transporting abalone. Studies of the physiology and ecology of this animal may be essential in revealing the causes of mortality and establishing a scientific basis for better operation.

Numerous studies have shown that in many molluscs respiratory regulation is not entirely constant but varies with changes in temperature (3,4,9,26), weight (22-25), and oxygen tension (1,2,7). However, these investigators have neglected the possible effect of a diurnal periodicity in

respiratory responses. Although clear circadian rhythms in oxygen consumption of marine prosobranchs have been reported⁽⁶⁾, Uki and Kikuchi⁽²¹⁾ pointed out that oxygen consumption of *Haliotis discus hannai* increased from dusk to midnight and decreased from midnight to midday. Since factors governing this phenomenon remain obscure, more detailed studies are warranted.

The present investigation is based on the premise that an understanding of oxygen consumption of animals which show a diurnal periodicity requires consideration of both endogenous factors as well as the exogenous environmental factors. Thus, the present study concentrates on the photoperiodicity and the food consumption of abalone previously acclimated to different light regimes.

MATERIALS AND METHODS

Specimens of *Haliotis diversicolor supertexta* were collected by SCUBA diving at depths of

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8–10 m off Gichi coast on the mid-eastern Taiwan during the summer of 1979. The animals were then transferred to the laboratory of the Institute in Taipei and held in aquaria containing small reefs and biofilters. Abalones were reared in the laboratory for more than 50 days before experiments started.

All the experiments were conducted in a closed flow-through system (Fig. 1), which was composed of four components: (1), an 80 l glass container for storing water; (2), a 0.88 l acrylic 'mixing chamber' for mixing the water, this chamber was connected to five identical transparent acrylic 0.88 or 0.39 l 'respiration chambers' by distributing tubes; (3), five overflow chambers with BOD bottles; (4), a bubbling tank for reoxygenating.

Four experiments (Table 1) were conducted with abalone of various body sizes. The abalone in the experiments were: (1), held without feeding and under a natural light regime (daylight/dark 12:12) (Series No. 1); (2), fed with alga and kept under natural light regime (Series No. 2); (3), acclimated to natural light followed by deprivation of food under conditions of no light (0 lux, D) or illumination at 500 lux (L) (Series No. 3D or 3L); (4), acclimated to dark, followed by deprivation of food under conditions of no light (0 lux, D) or illumination at 500 lux

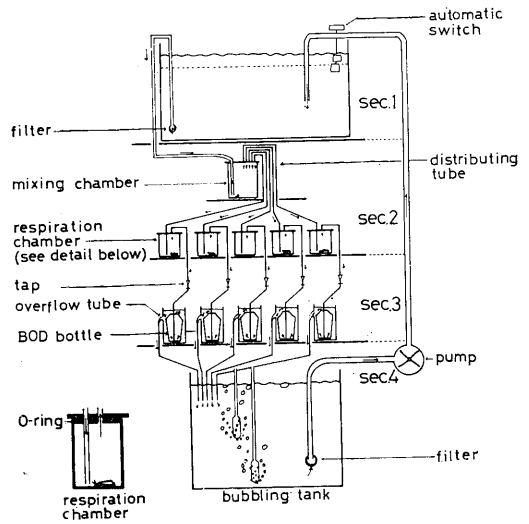


Fig. 1. Flow-through experimental set-up. (Not to scale)

(Series No. 4D or 4L).

During the experiment, water was drawn through the chambers by siphonal action. The flow rate was regulated according to the size of the animals, by adjusting the tap in section 3 (Fig. 1). The rate varied less than 4% within 24 hours. It was attempted to regulate the rate to a level which would give, at most, 25% reduction in oxygen tension, the oxygen level in the different experiments actually ranged from 70.8% to

TABLE I.
Conditions for the 24-hour period experiments

| Series No. | No. of size groups | Acclimated light | Experimental light | Experimental food supply | Temp. (°C) |
|------------|--------------------|----------------------------------|------------------------|---|------------|
| 1 | 8 | A natural light regime; 12:12 LD | Natural light (4 days) | A food supply stopped from four days before DO measurement | 24±0.3 |
| 2 | 8 | Same as above | Same as above | A food supply stopped from one hour before DO measurement | 24±0.2 |
| 3D | 4 | Same as above | Dark; 0 lux (24 hours) | A food supply stopped from three days before DO measurement | 23±0.2 |
| 3L | 4 | Same as above | 500 lux (24 hours) | A food supply stopped from five days before DO measurement | 23±0.2 |
| 4D | 4 | A dark condition for 53 days | Dark; 0 lux (24 hours) | A food supply stopped from three days before DO measurement | 23±0.2 |
| 4L | 4 | Same as above | 500 lux (24 hours) | A food supply stopped from five days before DO measurement | 23±0.2 |

(): Time elapsing before the 24-hour measurements started.

83.2% saturation.

The water flowing from the respiration chamber was sampled by using BOD bottles at two hour intervals, from 10:00 AM to the same time next day. Dissolved oxygen (DO) in the sea water was measured and the oxygen consumption rate was calculated in mg O₂/individual/hour. These diurnal oxygen consumption rates of abalone of each size group were then converted to percentages of mean oxygen consumption rate for the 24-hour period. These serial percentages are defined as diurnal relative oxygen consumption rates.

For each experiment, the diurnal relative consumption rates were grouped into day or night samples. The statistical difference between the two samples was evaluated using both Bartlett's *B* parameter and Student's *t* test⁽¹⁹⁾.

For each experiment, the relationship between wet body weight and oxygen consumption rate was determined according to the power equation, $R = aW^b$, where *R* is the oxygen consumption in mg O₂/individual/hour, *W* is the wet body weight in g, *a* is the intercept, and *b* is the slope of the regression.

The DO was measured by a Rexnord Model 650 Multianalyzer with a Weston and Stock Model 33 dissolved oxygen probe installed with a membrane of 0.5 mil. The probe was calibrated every 24 hours by Winkler's method⁽²⁰⁾.

The wet weight of abalone with shell, after removal of epizoon, was measured to 0.01 g on a double beam balance (Kyoto Ishida). Light intensity was measured by a Tokyo Photo-Electrics Model ANA-500 lux meter (accuracy ± 15%).

RESULTS

1. For abalone held without feeding and under a natural light regime:

In the eight size groups of abalone, during a 24 hour period, the mean relative oxygen consumption rate is 91.10% in the day and 109.43% at night (Fig. 2). The oxygen consumption at night is significantly higher than that in the day, ($p < 0.01$).

A linear relationship is found between the logarithm of both oxygen consumption rate and wet body weight (Fig. 3). The regression equation is:

$$R = 0.1036W^{0.8124}, r = 0.9365 \quad (1)$$

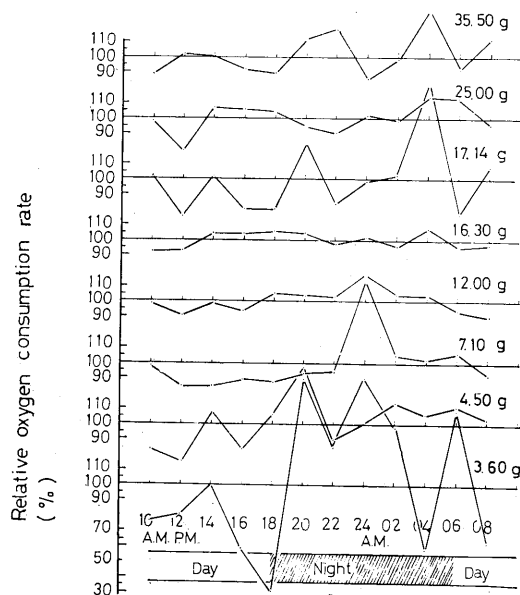


Fig. 2. Diurnal changes of relative oxygen consumption rates in different size groups of starved abalone.

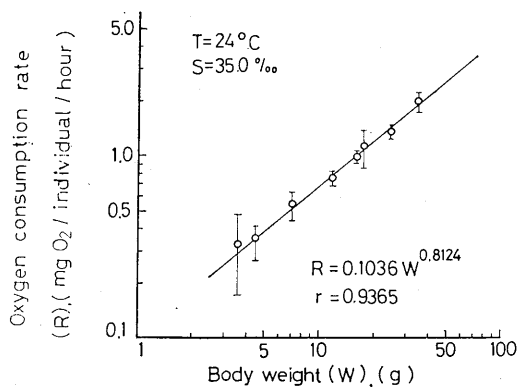


Fig. 3. Relationship of oxygen consumption to wet body weight of starved abalone under natural light during a 24-hour period, each circle and bar represents mean ± SE of twelve measurements.

2. For abalone fed with alga and under a natural light regime:

The mean relative oxygen consumption rate is 98.94% in the day and 100.98% at night, (Fig. 4). The abalone fed with alga does not show a significant diurnal change in oxygen consumption ($p > 0.05$).

The relationship between oxygen consumption and wet body weight of this group is shown in Fig. 5. The regression equation is

$$R = 0.0970W^{0.8028}, r = 0.9908 \quad (2)$$

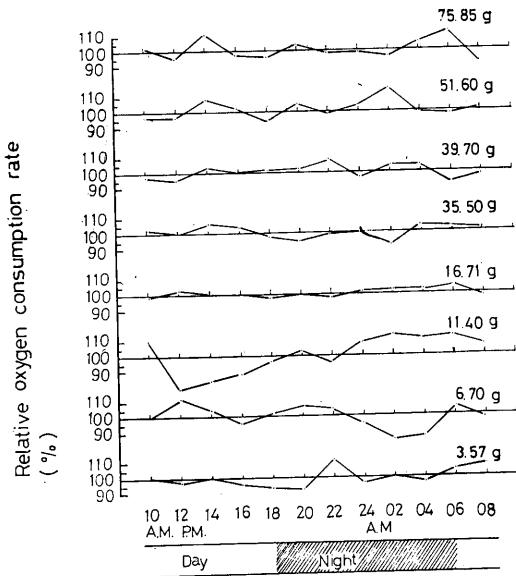


Fig. 4. Diurnal changes of relative oxygen consumption rates in different size groups, of abalone fed with alga.

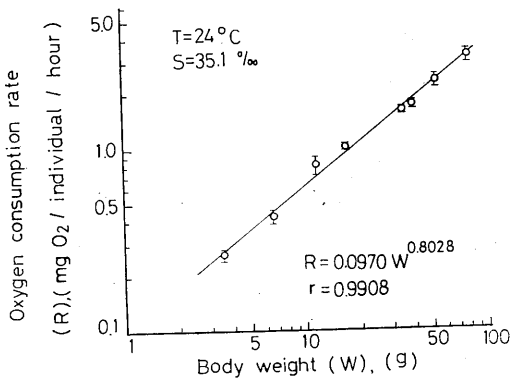


Fig. 5. Relationship of oxygen consumption to wet body weight of abalone, fed with alga, during a 24-hour period.

3. Abalone acclimated to natural light previously, then, deprived of food supply, and kept in the dark (D) or under 500 lux (L) illumination for a 24 hour period.

There is a marked difference in oxygen-consumption rate between the abalone held in the dark and light, as illustrated in Fig. 6, which presents the oxygen consumption for four size groups of abalone.

The relationship between oxygen consumption and wet body weight of abalone held in dark of light are shown as follows

$$0 \text{ lux } R = 0.0972W^{0.8107}, r = 0.9931 \quad (3)$$

$$500 \text{ lux } R = 0.0724W^{0.7771}, r = 0.9913 \quad (4)$$

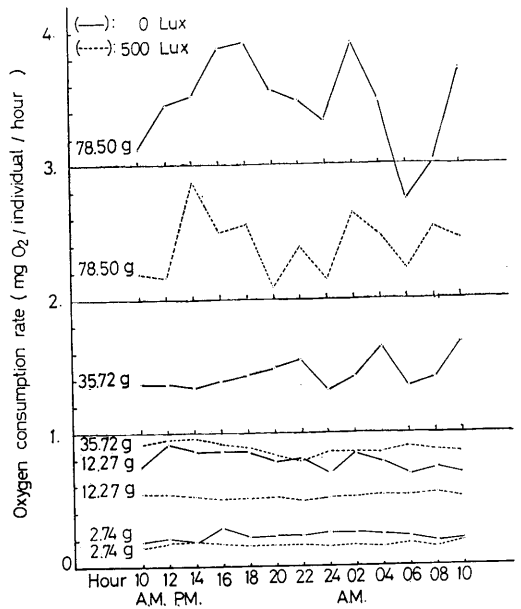


Fig. 6. Oxygen consumption, of four size groups, of natural light acclimated abalone under two light intensity regimes.

4. Abalone acclimated to a dark environment previously, then, deprived of food supply, and kept in the dark (D) or under 500 lux illumination (L) for a 24-hour period.

When the light intensity was maintained at 500 lux. the oxygen consumption rates of dark-acclimated abalone differed markedly from

those of abalone continuously in the dark (Fig. 7). The regressions equation are:

$$0 \text{ lux } R=0.1228W^{0.7426}, r=0.9844 \quad (5)$$

$$500 \text{ lux } R=0.0866W^{0.7750}, r=0.9864 \quad (6)$$

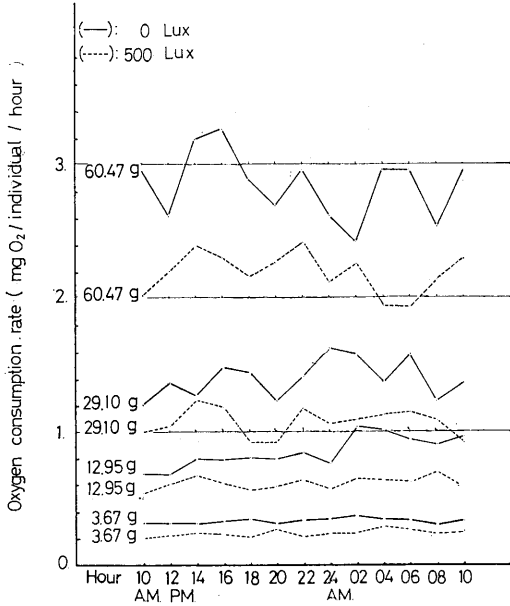


Fig. 7. Oxygen consumption of darkness acclimated abalone.

Diurnal relative oxygen consumption rates, under constant illumination, were calculated from the sum of the oxygen consumption rates determined at the same time in both (D) and (L) experiments, for each size group, of abalone (Fig. 8). The relative oxygen consumption rate is 99.19% in the day and 100.19% at night. The result also show that, under constant illumination, there is no diurnal change of oxygen consumption in starved abalone ($p > 0.05$).

It does not appear to be a persient circadian rhythm, either: When the relative oxygen consumption rates of the eight groups shown in fig. 8 were divided into twelve subgroups according to time of measurement, the result of analysis of variance was non-significant ($p > 0.05$).

Diurnal changes of relative oxygen consumption rates of abalone maintained without

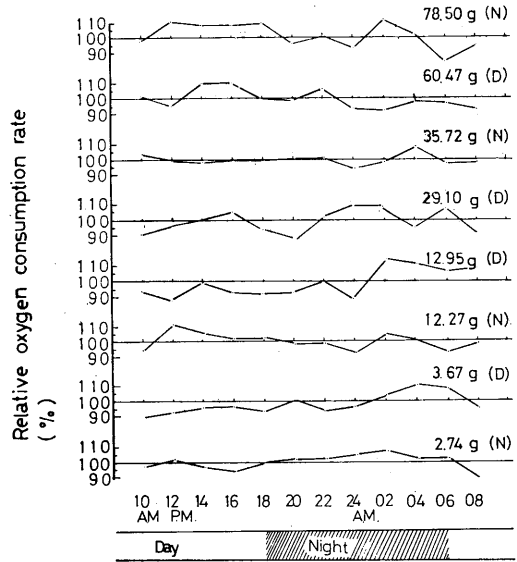


Fig. 8. Diurnal changes of the relative oxygen consumption rates of both natural light acclimated (N) and darkness acclimated (D) abalone, under constant light regimes.

or with feeding under a natural light regime, and without feeding but under constant illumination, are summarized in Fig. 9. The marked fluctuation, occurring among animals kept without feeding and under natural light, exhibits a clear diurnal periodicity as mentioned above.

Regression from six 24-hour period experiments were treated by covariance analysis.

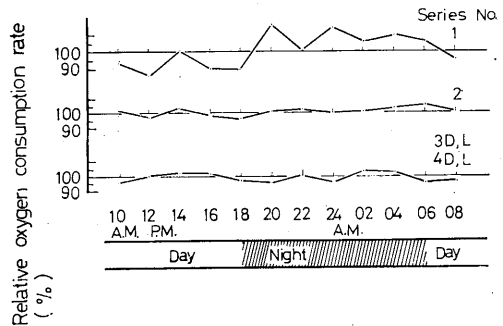


Fig. 9. Diurnal changes of mean relative oxygen consumption rates of starved (1), fed (2), and constant light illuminated (3D, L; 4D, L) abalone.

The result indicates that there is no significant difference among regressions in the value of b ($p > 0.05$), and a common slope, $b = 0.7857$, is estimated. It is, therefore, possible to replace the six regressions by new equations with a common b and adjusted means. These new equations corresponding to the original equation series are as follows

$$R = 0.1423W^{0.7857} \quad (1')$$

$$R = 0.0860W^{0.7857} \quad (2')$$

$$R = 0.1002W^{0.7857} \quad (3')$$

$$R = 0.0679W^{0.7857} \quad (4')$$

$$R = 0.1044W^{0.7857} \quad (5')$$

$$R = 0.0807W^{0.7857} \quad (6')$$

Both experiments 3D and 3L were completed with the same specimens, as were experiments 4D and 4L. The oxygen consumption of abalone, previously acclimated to a normal diel light cycle, was 1.47 times in darkness (0 lux) when comparing with that in the light (500 lux), as shown by comparing the regression constants of equations (3') and (4'); it was 1.29 times that of abalone acclimated to darkness by comparing the constants of equations (5') and (6').

DISCUSSION

There are only a few published values for oxygen consumption rates of *Haliotis*. Olsen⁽¹⁴⁾ found that $b = 0.72$ for *H. fulgens* at 20.5°C, $b = 0.84$ for *H. corrugata* at 25°C, $b = 0.44$ for *H. refescens* at 12.5°C and 0.46 at 16.5°C. Uki and Kikuchi⁽²¹⁾ found that $b = 0.8025$ for *H. discus hannai*. In present study, b varies from 0.7426 to 0.8124 for *H. diversicolor supertexta*. These values were obtained under different experimental conditions, and with a wet weight ranging from 3.57 to 78.50 g. However, covariance analysis indicated that $b = 0.7857$ was a reasonable estimated value to apply in our experiments. In the absence of non-empirical criteria on which to base a value, $b = 0.7847$ was chosen and proved useful in the quantitative analysis and comparison among the experimental results.

It has been reported that light intensity affects the level of respiration of abalone. Sagara and Araki⁽¹⁵⁾ found that the oxygen consumption of veligers of both *H. gigantea* and *H. discus* in darkness corresponded to 1/2 that in the light (about 1500 lux). But on the other hand, the experimental results on *H. discus hannai*⁽¹⁶⁾ and *H. diversicolor supertexta* (the present experiments) showed that the oxygen consumption of adult abalone were larger in the dark than in the light. This difference may, at least in part, imply a different phototaxis occurring at these two stages of life cycle.

Shepherd⁽¹⁷⁾ demonstrated that crevices, caves and cavities under boulders provide a refuge for *H. roei*, *H. ruber* and *H. scalaris* and juveniles of other species. He also said that, except for nocturnal feeding excursions, these species seem to be confined to such places by the activity of their predators. Many predators have been recorded e.g., fishes, crabs, lobsters, octopuses and sea stars^(5,14,17,18). However, the authors found that *H. diversicolor supertexta*, whether collected from the natural environment or rearing ponds, would move toward shaded areas under the reefs even when no predator was present (pers. obser.) Meadows and Campbell⁽¹⁰⁾ have discussed those factors affecting habitat selection of aquatic invertebrates. They found that chemical or physical factors had a greater influence on habitat selection. The authors suggest that light intensity is an important factor leading to a quantitative difference in oxygen consumption as well as to habitat selection. However, the relationship among oxygen consumption, light conditions and negative phototaxis in abalone remains to be studied.

Diurnal periodicity exhibited by animals in nature may be due to light and temperature changes occurring during the 24-hour day-night period, or may be regulated by the biological clock for the circadian rhythm^(6,13). Nimura and Oshima⁽¹²⁾ reported that there are apparently diurnal changes in oxygen consumption of *H. sieboldii*, *H. gigantea* as well as *H. discus hannai*. Uki and Kikuchi⁽²¹⁾ studied *H. discus hannai*

under constant temperature for a 24-hour period and found that the abalone consumed 20% more oxygen from dusk to midnight than from midnight to midday. In the present study, it was found that *H. diversicolor supertexta* consumes 20% more oxygen at night only when both starved and exposed to a natural diel cycle of light. However, when the light intensity was kept constant during the experiment or food (*Ulva* sp.) was supplied in the system, there was no evidence of a diurnal change in the oxygen consumption.

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九孔日週性耗氧量之探討

詹榮桂 邵廣昭 張崑雄

本報告主旨在探討九孔 *Haliotis diversicolor supertexta* Lischke 耗氧量的日週性變化情形。

實驗結果顯示，饑餓的九孔在自然的日夜光照變化下，耗氧量會有日週性的改變；亦即，在夜間的耗氧量比在日間的多 20%。但是，餵過的九孔在自然光照變化下，或饑餓的九孔在固定光照下，耗氧量都不會呈現上述的日週性變化。