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Diel Rhythms of Oxygen Consumption and Activity Level of Juvenile Flounder *Paralichthys olivaceus*

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The diel rhythms of oxygen consumption and activity level were measured over a 24-hr period in three size groups of starved juvenile flounder *Paralichthys olivaceus* by continuous-flow respiratory and video-observation analysis, respectively. In the small group (mean body weight ± SD, MBW: 3.3 ± 0.9 g), two peaks of oxygen consumption were observed at 03:00 and 17:00 hours, and the hourly metabolic rates were not significantly different between dark and light periods. In the medium group (MBW: 20.3 ± 3.7 g) and the large group (MBW: 58.5 ± 7.1 g), only a single peak was observed at 04:00 or 06:00 hours, and the hourly consumption rates were significantly higher in the dark than in the light. For all groups, the highest oxygen consumption rates occurred under conditions of weak light or darkness. The routine metabolic rate is described by the equation $\log M = 0.643 \log W - 0.387$, where M is the oxygen consumption rate (mgO_2/kg/hr) and W is body weight (kg) at 20°C. The diel pattern in activity rate (duration of swimming per hour in minutes) was similar to the pattern of oxygen consumption; fishes were more active during the night (mean activity rate = 5.0 min/hr) than during the day (mean activity rate = 2.2 min/hr). The relation between rate of oxygen consumption and activity rate is discussed.

Key words: oxygen consumption, juvenile flounder, diel rhythms, activity

Diel and seasonal changes in the environment affect many biological functions. Fish metabolism is partly regulated by endogenous diel rhythms and exogenous diurnal cycles.[1] Thus knowledge of daily rhythms of oxygen consumption and the influence of environmental conditions is of great importance for the scientific aquaculture of fishes.

Measurement of oxygen consumption by monitoring a respiratory chamber is complicated by diel variations of oxygen concentration. In a previous study by Beamish,[2] the average routine oxygen consumption of *Salvelinus fontinalis* (Mitchill) decreased during the first 2 or 3 days of a starvation period, after which it remained approximately constant, along with the standard oxygen consumption rate. More recently investigations of the metabolic rate of fish have focused on the presence of diel rhythms in feeding activity, but there is little information on the diel patterns of oxygen consumption.[3-5]

The Japanese flounder *Paralichthys olivaceus* is a commercially important fish in the coastal waters of Japan. There are few studies of the parameters of oxygen consumption rate by the flounder, although the specific metabolic rates of larvae and adults have been reported.[6,7] Kurokura *et al.* found that specific oxygen consumption of 11 to 31-day-old flounder is significantly lower in the morning than during the rest of the day.[8] Honda found a positive group effect in flounder maintained in tanks without bottom sand.[9] Most previous studies have measured oxygen consumption rates over periods of 12 hours or less. This study was carried out to determine the diel pattern of oxygen consumption and discuss the relationship between oxygen consumption and activity rate of juvenile flounder during a starvation period. In addition, the effect of body size on oxygen consumption is discussed for juvenile flounder.

Materials and Methods

Experimental Fish

The juvenile flounder used in these experiments hatched at the Miyako Station, Japan Sea-Farming Association, and were reared at the Hirame Aquatic Center, Suttsu, Hokkaido. Fish weighing 2–3 g were transported to the Usujiri Fisheries Laboratory, Faculty of Fisheries, Hokkaido University for experiments. Fish were acclimated to the experimental temperature of 20.0 ± 0.5°C for five days in two net containers positioned at the surface of a 1000-liter tank. The rate of temperature change was approximately 1–2°C per day. The light regime at the culture room was approximately 12h D:12h L (100 lux at the surface of the tank), but during video recording of activity at night, the light at the surface of the tank was 15 lux. Fish were fed commercial pellets (Kyowa Ltd.) once a day. Daily feed rations were about 2% of body weight. In each trial, two fish were placed in a single chamber to avoid the group effect.[9] After a starvation period of 36 hours, the fish were placed in a respirometer for 12–15 hours (overnight) to acclimate. Throughout the trial the flounder could move freely within the chamber.

Experiment I

Oxygen consumption was measured using a continuous flow respirometer. The experimental design was similar to that described by Jobling and Davies,[10] consisting of a test chamber and water bath, through which water flowed at a controlled rate to a 100-liter catchment reservoir (Fig. 1). The test chambers were constructed from milky white plastic buckets and were covered with a transparent hermetical cover. Water leaving the respirometer was re-oxygenated in the catchment reservoir by strong aeration with a bubbler. The dissolved oxygen concentration of seawater in the respirometer never fell below 70% of the air saturation level. Water temperature was maintained with an aquacooler unit and a heater. The seawater outflow from the respirometer chamber was directed past an oxygen electrode. The oxygen electrode and oxygen meter were coupled to a chart recorder. Oxygen levels were measured and recorded with a YSI model 58 oxygen meter (Yellow Springs Instrument Co., Yellow Springs, U.S.) and a Charter recorder (Rika Denki Ltd., Japan), respectively. The oxygen concentration levels of seawater in the test chamber were monitored continuously. The oxygen concentration levels of the surrounding bath were measured every 6 hours over a 24-hour period. The actual oxygen consumption of the chamber was continuously recorded. The oxygen concentration rate of the fish was calculated as the difference between the value recorded for the chamber containing fish and that of the surrounding bath at that time.

Diel rhythms in the oxygen consumption of flounder weighing from 2.4–67.5 g were examined for a small size group (mean body weight±SD, MBW=3.3±0.9 g), a medium group (MBW=20.3±3.7 g) and a large group (MBW=58.5±7.1 g) in July, September, and November, 1995, respectively. The small, medium and large groups were examined in 5, 3 and 5 trials, respectively.

Experiment II

Diel rhythms in the activity level of 12 fish weighing from 5.3–8.3 g were measured in August 1995. While the oxygen consumption rates were measured, the activity of the fish in the respirometer was recorded with a video camera (Panasonic NV-M35) to compare the relationship between oxygen consumption and activity. The camera continuously recorded over a 48-hr period in order not to disturb the fish. The activity rate (duration of swimming per hour in minutes) of the juvenile flounder was measured using the same methods described in Sims *et al.*[5] Using the clock on the video screen and frame-by-frame analysis, the number of minutes the fish were active each hour and the frequency of motion were determined for 5 experimental trials.

All stated means include ± the standard deviation of the mean. All statistical tests in this study are *t*-test.

Results

Experiment I

Hourly oxygen consumption rates of juvenile flounder over a 24-hour period were highly variable (Fig. 2). A single peak was observed at 03:00–06:00 hours for the medium and large size groups. In the small size group, fluctuation of oxygen consumption rate was observed, but a distinct peak was not found. In all groups, the highest oxygen consumption rates occurred under conditions of weak light or darkness.

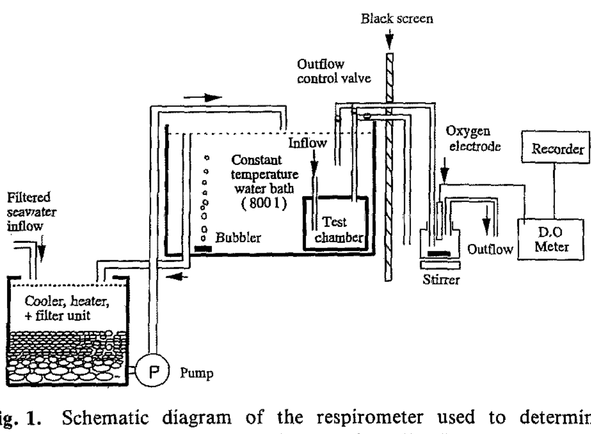

Fig. 1. Schematic diagram of the respirometer used to determine oxygen consumption rate in the juvenile Japanese flounder *Paralichthys olivaceus*.

D.O. meter: dissolved oxygen meter.

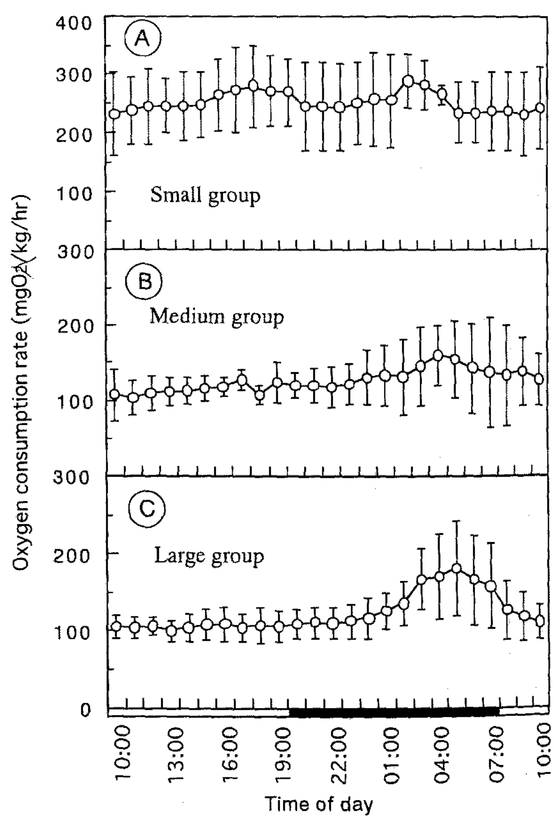

Fig. 2. The diel rhythms of oxygen consumption in three size groups of starved juvenile flounder over a 24-hr period at 20°C.

The dark horizontal bar indicates the hours of darkness. Circles and vertical bars indicate mean and standard deviation, respectively. The groups included 10 fish in 5 trials (A), 6 fish in 3 trials (B) and 10 fish in 5 trials (C).

The hourly oxygen consumption rates of the medium and large groups were significantly higher during the dark period than during the light period ($P<0.05$ and $P<0.01$, respectively) (Table 1). The mean hourly oxygen consumption rate of the large group increased by 28.7% from 108.2 mg/kg/hr in the light to 139.5 mg/kg/hr in the dark. In contrast to the above two groups, the oxygen consumption rate of the small group was not significantly different between the dark and light periods ($P=0.79$). The hourly oxygen consumption rates of the small and medium groups were not significantly different during the first half of the night (19:00-01:00 hours) and the second half (01:00-07:00 hours) ($P>0.50$ and $P>0.10$, respectively). However, the oxygen consumption rate of the large group was significantly lower during the first half of the night than during the second half ($P<0.01$).

The relationship between body weight and oxygen consumption is usually described by an equation of the form:[3,11,12]

$$M=aW^b$$

where M is oxygen consumption rate (mg/ind./hr), W is body weight (g), and a and b are constants representing the oxygen consumption of a fish of unit weight. Logarithmic transformation of this equation yields a straight line relationship:

$$\log M=b\log W+\log a.$$

In this study, the relationship (Fig. 3) between the rate of oxygen consumption over a 24-hour period and body weight is expressed as follows:

$$\log M=0.643\log W-0.384\ (n=15,\ r^2=0.966).$$

The rate of oxygen consumption increased with increasing body weight of juvenile flounder, but the rate of consumption per unit body weight decreased with increasing body weight.

Experiment II

The diel pattern in activity levels was similar to the pattern of oxygen consumption, but the relative changes were different (Fig. 4). The mean activity level of 5.0 min/hr during the night was 2.3 times longer than the mean level of 2.2 min/hr during the day ($P<0.05$). The fluctuation of oxygen consumption increased with increasing activity.

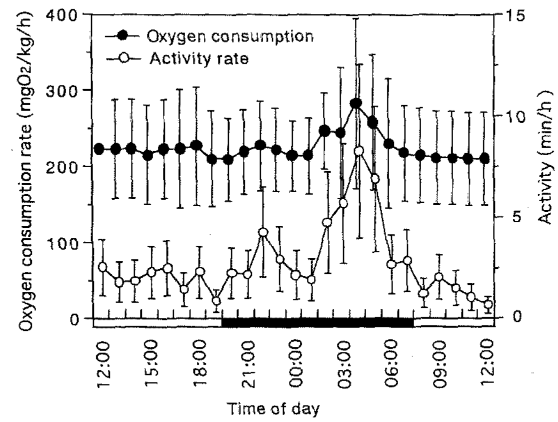

Fig. 3. Diel trends of oxygen consumption and activity rate in the juvenile flounder (body weight: 5.3-8.3 g) starved during four trials at 25°C.
The dark horizontal bar indicates the hours of darkness.

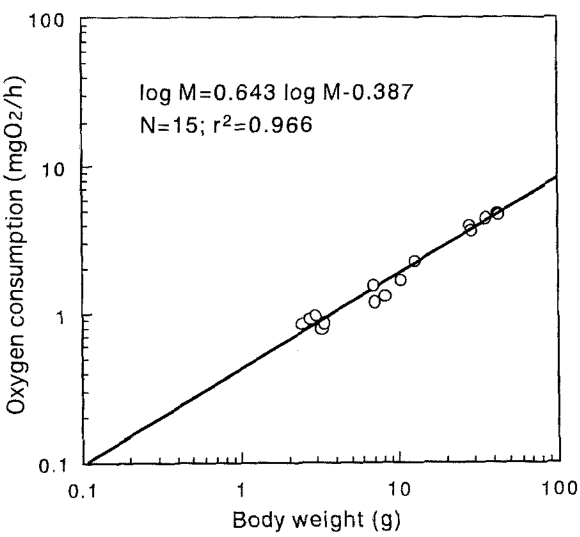

Fig. 4. Relationship between the rate of oxygen consumption (mgO_2 ind./h) and body weight (g) for juvenile flounder *Paralichthys olivaceus* at 20°.

Discussion

The rate of oxygen consumption by the medium and large size groups of juvenile flounder increased significantly at night. Similar patterns in oxygen consumption have been recorded for other fish species, including the mullet (*Mugil cephalus*),[13] the spotted grunter (*Pomadasys commersonni*)[11] and the lesser spotted dogfish (*Scyliorhinus*

Table 1. The mean rate of oxygen consumption (mgO_2/kg/hr) of different size groups of the juvenile flounder *Paralichths olivaceus* during light and period over a 24-hour period

Size	Light (07:00-19:00)	Dark (19:00-07:00)	Probability	Dark: First-half (19:00-01:00)	Dark: Second-half (01:00-07:00)	Probability
Small group	250±49	259±39	$P>0.75$	254±51	264±29	$P>0.50$
Medium group	117±18	133±25	$P<0.05$	122±15	145±11	$P>0.10$
Large group	108±21	139±30	$P<0.01$	117±15	161±25	$P<0.01$

canicula).[5] An opposite pattern, however, of increased oxygen consumption during the day has been reported for sockeye salmon (*Oncorhynchus nerka*).[14] As the flounder grew, oxygen consumption showed a diel rhythm with a peak occurring in the early morning.

Several studies have measured the diurnal variation of activity for flounder. Adult fish in the field leave the sea bottom at dusk and return to the bottom at daylight.[15,16] Miyazaki *et al.* noted that juveniles cultured in a hatchery usually bury into the bottom sand at daylight and emerge at night.[17] In our study, the level of activity during the night (4.5 min/hr) was higher than during the day (2.0 min/hr), with the peak of activity occurring in the second half of the dark period.

The activity level of many fish species has been shown to be directly related to oxygen consumption. Kausch measured the daily rhythms of activity and oxygen consumption of young carp (*Cyprinus carpio*) and found a significant correlation between the two, with two peaks in both occurring in starved fish over a 24-hour period.[18] Sims *et al.* found the level of oxygen consumption and activity of the dogfish (*Scyliorhinus canicula*) were relatively high but varied at night. In our study, the rate of oxygen consumption and activity levels were measured every hour over a 24-hour period as the fish fasted. Although the relative change of activity was larger than the relative change in the fluctuation of oxygen consumption rate, the two were clearly related. Our data suggest that the fluctuation of oxygen consumption for starved fish is mainly a result of its activity. The higher activity level under conditions of weak light or darkness may be the consequence of the adjustment of an internal clock (indigenous cycle) controlled by external cues.

The components of the equation $M=aW^b$ for oxygen consumption vary greatly for different fish species.[12,13,18] In the present study, a value of 0.64 was determined for the weight exponent b for juvenile flounder. Winberg suggested a weight exponent of 0.81 to be appropriate for goldfish (*Carassius auratus*).[12] There is interspecific variation in the value of b, but a mean value of 0.86 has been suggested for fish.[19] However, a vast amount of information published shows that the values for the weight exponent fall between 0.40 and 0.90.[11,13] The value of b is usually temperature independent and has no apparent relationship to morphological change.[8,14,15] Jobling (1982) suggested that metabolic rates of plaice (*Pleuronectes platessa*) are low compared to those for round-bodied fish under similar conditions.[20] The results of the present study suggest that the value of b for juvenile flounder is lower than that for round-bodied fish.

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