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Interspecific Variations in the Circadian Rhythm of Bimodal Oxygen Uptake in Four Species of Murrels

Jyoti S. D. Munshi, Ajoy K. Patra, Niva Biswas and Jagdish Ojha

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Abstract Studies of bimodal oxygen uptake in four species of murrels, genus *Channa*, at different periods of the 24 h day regime showed distinct circadian rhythm in their metabolism. The metabolism of the four species remained higher in the period extending from dusk (16:00～18:00) to dawn (04:00～06:00). *C. marulius* showed the highest O_2 uptake (66.4 ± 0.5 ml $O_2 \cdot kg^{-1} \cdot h^{-1}$) during midnight (24:00～02:00), *C. striatus* (78.7 ± 18.6) and *C. gachua* (95.6 ± 2.6) during early parts of the night (20:00～22:00) and *C. punctatus* (57.5 ± 1.1) during dusk (16:00～18:00). Of the total oxygen uptake, *C. marulius*, *C. striatus*, *C. gachua* and *C. punctatus* extracted about 84.5%, 67.7%, 53.4% and 86.8% of oxygen through aerial routes respectively. In all the species the lowest or the second lowest rate of oxygen uptake was recorded at noon, and during this period gill breathing dominated over aerial breathing in *C. striatus* and *C. gachua*. The circadian rhythm of their oxygen uptake has been correlated with the diurnal fluctuation of metabolism of the swamp ecosystem. A general metabolic wheel hypothesis has been postulated.

Organismic physiology and behaviour is often rhythmic and these rhythms will persist in the laboratory in the absence of photoperiod and the various physico-chemical factors of the environment in which organisms live under natural conditions. Because they do persevere, it is concluded that they are under the control of a so-called biological clock. Often in the artificial constancy of the laboratory, the periods of these rhythms deviate slightly from the ones displayed in nature and are referred to as circadian (Palmer, 1976).

In India there are many swampy areas infested with floating water-hyacinth, *Eichhornia crassipes*, and/or rooted *Cyperus* communities. In summer the water lodged in these areas become hypoxic and hypercarbic. The physico-chemical factors of this adverse ecological environment show rhythmic fluctuations at different hours of the day. These rhythms seem to govern the physiology and behaviour of an interesting group of air-breathing fishes which thrive well in such swampy areas. These fishes exhibit various degrees of bimodal gas exchange. The relative dependence of the fish on gill- and air-breathing have been studied in a few dual-breather species (Hughes and Singh, 1970, 1971; Singh and Hughes, 1971, 1973; Singh, 1976; Lomholt and Johansen, 1976; Ojha et al., 1978). However, little is known about the interspecific variations in the circadian rhythm of bimodal oxygen uptake in fishes (Patra et al., 1978).

This paper reports observations and detailed measurements of the interspecific variations in the circadian rhythm of bimodal oxygen uptake in four closely related species of murrels of the genus *Channa* and correlates them with the fluctuations in some of the physico-chemical factors of their natural habitat—the swamps. Murrels belong to the family Channidae of the order Channiformes. They are widely distributed in the fresh-water swamps and ponds of temperate and tropical Asia and tropical Africa. Of about 21 species, reported four, i.e. *Channa marulius* (Hamilton), *C. striatus* (Bloch), *C. gachua* (Hamilton) and *C. punctatus* (Bloch), are commonly found in the swampy areas of northern India. All are air-breathing with a pair of suprabranchial chambers which assist the gills in gaseous exchange (Munshi, 1962).

Materials and methods

Murrels were collected from the swamps of North Bihar, India, in July, 1977 and maintained in glass aquaria in the laboratory. The fishes were fed goat liver, small prawns and earthworms on alternate days during a minimum acclimatization period of 10 days in the laboratory. The fishes were kept fasting for 24 hours before experiments. No feeding was done during experiments.

Bimodal oxygen uptake from air and still water was measured in a closed glass respirometer containing 3 *l* water (initial O_2 content $4.75\ ml\,O_2 \cdot l^{-1}$; pH 7.2~7.3) and 1 *l* of air. The fish had free access to air through a semicircular hole of about 8 cm in diameter in a disc float of a thermocol material that separated the water/air interface of the respirometer. KOH in a petri dish placed on the float absorbed CO_2. The air phase of the respirometer was connected to a manometer. Imbalance in the levels of the manometer fluid reflected uptake of oxygen when the CO_2 is absorbed by KOH. The fishes were acclimatized in the respirometer at least 12 hours before the readings were taken. The respirometer was placed in a constant temperature bath.

Experiments were carried out at $30 \pm 1°C$ in summer in an air-conditioned room. A diffused light was available to the fish through the semicircular hole in the disc float. Observations showed that the fishes could locate the breathing hole more readily, because it was the only source of light.

The concentration of dissolved O_2 in the water was estimated by Winkler's volumetric method (Welch, 1948). Aquatic O_2 uptake was calculated from the difference between the O_2 levels of the ambient water in the respirometer before and after the experiment and the volume of water in the respirometer. Uptake of O_2 from air was calculated from the range of imbalance of the levels of the manometric fluid in the manometer and by the use of combined gas law equations and vapour pressure (Dejours, 1975). Mean values of oxygen uptake of a series of observations of adult fishes at standard temperature pressure dry (STPD) and standard deviations were calculated. pH of ambient water was measured by an electronic pH meter (Systronics). Equivalent energy utilization was calculated from the caloric equivalent of O_2 ($4.8\ mgO_2 \cdot l^{-1}$; Winberg, 1956). Paired *t*-tests were employed to test the level of significance of the differences between the sample means of the bimodal oxygen uptake during various hours of the day.

Results

$\dot{V}o_2$ ($ml\,O_2 \cdot kg^{-1} \cdot h^{-1}$) from air as well as water in four species of genus *Channa* and their total energy cost round the clock were investigated and the data have been summarized in Table 1, and diagrammatically presented in Fig. 1.

1. *Channa marulius*

This species showed its maximum rate of $\dot{V}o_2$, $56.1\ ml\,O_2 \cdot kg^{-1} \cdot h^{-1}$ from air at midnight (24:00~02:00) with a moderate high, $42.5\ ml\,O_2 \cdot kg^{-1} \cdot h^{-1}$ in early hours of the evening (16:00~18:00). The minimum rate of oxygen uptake was recorded at noon ($19.05\ ml\,O_2 \cdot kg^{-1} \cdot h^{-1}$) (Fig. 1). The oxygen uptake from air at early hours of the night (20:00~22:00) was significantly different ($P < 0.05$) from that obtained during noon (12:00~14:00).

Oxygen uptake from water remained more or less constant at all periods of the day regime except at midnight (24:00~02:00) when this value was found to be the lowest (Fig. 1).

The highest value of total $\dot{V}o_2$ ($mlO_2 \cdot kg^{-1} \cdot h^{-1}$) was observed at midnight (66.4) followed by 52.6 and 51.0 at 20:00~22:00 and 04:00~06:00 respectively. The lowest value (30.6) was recorded at noon (12:00~14:00). The differences in the total oxygen uptake between 08:00~10:00 and 16:00~18:00, 12:00~14:00 and 24:00~02:00 were significant ($P < 0.05$).

2. *Channa striatus*

The period of highest O_2 uptake (53.3 $ml\,O_2 \cdot kg^{-1} \cdot h^{-1}$) from air was in the night (20:00~22:00) with a secondary peak (41.1) in the midnight (24:00~02:00) and the lowest uptake (14.0) was recorded at noon (12:00~14:00) (Fig. 1).

The highest rate of $\dot{V}o_2$ ($31.3\ ml\,O_2 \cdot kg^{-1} \cdot h^{-1}$)

from water was recorded in the morning hours (04:00~06:00), then $\dot{V}O_2$ dropped down to 13.7 in the next few hours (08:00~10:00). In the rest of the periods of the day only very slight variations were observed (Fig. 1).

The highest value for total O_2 consumption (78.7 ml $O_2 \cdot kg^{-1} \cdot h^{-1}$) was recorded in the night (20:00~22:00) followed by 71.7 ml $O_2 \cdot kg^{-1} \cdot h^{-1}$ in the dawn (04:00~06:00). The lowest value for total $\dot{V}O_2$ (32.9) was obtained in the morning hours (08:00~10:00), and then in the noon (12:00~14:00) (Fig. 1). There was a significant difference ($P<0.05$) in the oxygen uptake between noon (12:00~14:00) and midnight (24:00~02:00).

3. *Channa gachua*

The maximum $\dot{V}O_2$ (63.4 ml $O_2 \cdot kg^{-1} \cdot h^{-1}$) from air occurred in the midnight (24:00~02:00) with a second peak (62.4) in the morning (08:00~10:00). The minimum (9.9) oxygen uptake was recorded at noon (12:00~14:00) (Fig. 1).

The oxygen consumption through the gills from water was at its peak (44.6 ml $O_2 \cdot kg^{-1} \cdot h^{-1}$) in the night (20:00~22:00) followed by 38.4 in the early hours of the day (04:00~06:00) and lowest (10.5) during the 08:00~10:00 period.

Total oxygen uptake rate was highest (95.6 ml $O_2 \cdot kg^{-1} \cdot h^{-1}$) in the night (20:00~22:00) and minimum (41.3) at noon (12:00~14:00) (Fig. 1). The difference in the rate of $\dot{V}O_2$ between the midnight (24:00~02:00) and noon (12:00~14:00) periods was found to be statistically highly significant ($P<0.01$).

4. *Channa punctatus*

The highest rate of oxygen uptake through aerial route was found to be 49.6 ml $O_2 \cdot kg^{-1} \cdot h^{-1}$ in the (dusk) evening (16:00~18:00) and the

Table 1. Interspecific variations of circadian rhythm in oxygen uptake of some air-breathing murrels at 30±1°C. Measurement of oxygen uptake was made three times for each species and period in all sections except for *C. marulius* at 24:00~02:00, for which the measurement was made two times. * TEU, total equivalent energy utilization ($Kcal \cdot kg^{-1} \cdot h^{-1}$).

Species (Mean body weight in g)	Hours of day	Oxygen uptake (ml $O_2 \cdot kg^{-1} \cdot h^{-1}$) Aerial	(%)	Aquatic	(%)	Total	TEU*
Channa marulius (93.0)	04:00~06:00	39.5± 0.0	(77.5)	11.5± 0.0	(22.5)	51.0± 0.0	0.245
	08:00~10:00	35.3± 5.1	(75.5)	11.5± 1.4	(24.5)	46.8± 8.6	0.225
	12:00~14:00	19.0± 3.7	(62.3)	11.5± 0.1	(37.7)	30.8± 3.8	0.147
	16:00~18:00	42.5± 0.0	(78.6)	11.6± 0.0	(21.4)	54.1± 0.0	0.260
	20:00~22:00	41.3± 0.0	(78.6)	11.3± 0.0	(21.4)	52.6± 0.0	0.252
	24:00~02:00	56.1± 1.8	(84.5)	10.3± 1.3	(15.5)	66.4± 0.5	0.319
Channa striatus (82.0)	04:00~06:00	40.4±14.5	(56.4)	31.3± 0.6	(43.6)	71.7±15.2	0.344
	08:00~10:00	19.2± 1.5	(58.4)	13.7± 0.3	(41.6)	32.9± 1.2	0.158
	12:00~14:00	14.0± 0.5	(40.7)	20.4± 7.7	(59.3)	34.4± 7.2	0.165
	16:00~18:00	23.4± 0.9	(52.9)	20.8± 9.8	(47.1)	44.3± 9.2	0.213
	20:00~22:00	53.3±20.2	(67.7)	25.4±11.6	(32.3)	78.7± 8.6	0.378
	24:00~02:00	41.1± 1.2	(61.6)	25.5±14.0	(38.4)	66.6±13.4	0.320
Channa gachua (30.0)	04:00~06:00	39.7± 5.7	(50.8)	38.4± 3.8	(49.2)	78.1± 9.3	0.375
	08:00~10:00	62.4± 0.0	(85.6)	10.5± 0.0	(14.4)	72.9± 0.0	0.350
	12:00~14:00	9.9± 4.1	(24.0)	31.4± 7.0	(76.0)	41.3± 3.7	0.198
	16:00~18:00	40.7± 3.6	(72.9)	15.1± 1.2	(27.1)	55.8± 2.5	0.268
	20:00~22:00	51.0± 0.0	(53.4)	44.6± 2.6	(46.6)	95.6± 2.6	0.459
	24:00~02:00	63.4± 5.8	(76.2)	19.8± 5.4	(23.8)	83.1± 9.4	0.399
Channa punctatus (93.0)	04:00~06:00	40.9± 0.4	(79.2)	10.7± 1.7	(20.8)	51.6± 1.1	0.248
	08:00~10:00	30.7± 8.9	(67.4)	15.4± 2.3	(32.6)	46.0± 9.7	0.221
	12:00~14:00	32.8± 1.5	(75.6)	11.3± 1.0	(24.4)	44.0± 1.1	0.211
	16:00~18:00	49.6± 2.1	(86.8)	7.9± 1.8	(13.2)	57.5± 1.1	0.276
	20:00~22:00	43.2± 2.1	(83.4)	9.0± 1.1	(16.6)	52.2± 1.2	0.250
	24:00~02:00	35.4± 0.6	(76.7)	10.7± 0.7	(23.3)	46.1± 1.0	0.221

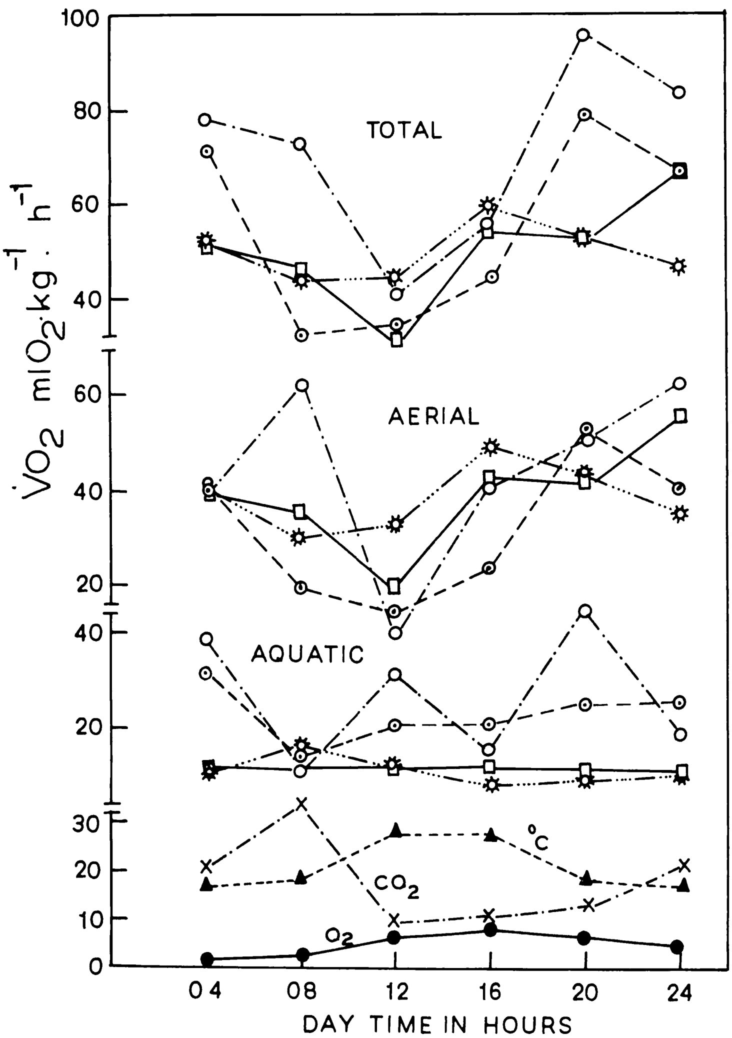

Fig. 1. Circadian rhythm of bimodal oxygen uptake in four species of murrels and its relation with some of the physicochemical fluctuations of their natural habitat. □——□ *C. marulius*; ⊙----⊙ *C. striatus*; ○·-·-·○ *C. gachua*; ☼···—☼ *C. punctatus*. ●——● Dissolved O_2 $ml \cdot l^{-1}$; ▲------▲ Temperature °C; ×-·-·-× Free CO_2 ppm; in the natural habitat.

lowest (30.7) in the morning (0.8:00～10:00). In other periods of the circadian cycle there were slight variations in aerial oxygen uptake (Table 1, Fig. 1). The peak value of aquatic oxygen uptake (15.4 $mlO_2 \cdot kg^{-1} \cdot h^{-1}$) occurred in the morning (08:00～10:00), followed by 11.3 at noon (12:00～14:00) and the lowest (7.9) at dusk (16:00～18:00). Aquatic $\dot{V}o_2$ at other periods of the circadian cycle showed slight fluctuations (Fig. 1).

The highest total O_2 uptake (57.5 $mlO_2 \cdot kg^{-1} \cdot h^{-1}$) was recorded in the evening (16:00～18:00) and the lowest (44.0) at noon (12:00～14:00) like all other previous species studied. The difference in the oxygen uptake values between 24:00～02:00 and 12:00～14:00 were statistically significant ($P<0.05$).

Discussion

The discovery of biological rhythm in oxygen consumption in air-breathing fishes is interesting. This is a physiological adaptation of air-breathing fishes in relation to the fluctuations of oxygen and carbon dioxide contents of their natural habitat.

The abilty of a murrel to obtain oxygen from the water will vary with the oxygen tension of the water in swamps and the capacity of the gills to extract it from the water. In general the gills are not so well developed in the murrels (Hakim et al., 1978). All the four species of *Channa* show distinct circadian rhythm in their metabolism. This rhythm seems to be associated precisely with the diel fluctuations of oxygen and carbon dioxide tensions of the water in swamps (Fig. 1).

This study on murrels clearly indicates that there are large interspecific variations in the circadian rhythm of their total metabolic rate. Differences in the percentage of aerial oxygen uptake in the four species of murrels may be due to interspecific variations in the oxygen uptake efficiency of the bimodal gas exchange machinery (Hakim et al., 1978; Ojha et al., 1978). While two species (*C. striatus* and *C. marulius*) are obligate air-breathers, the other two are facultative ones. However, certain common features in their behaviour in the respirometer have been noted, viz., (i) all are more active at night as they become very restive and frequently take air-breaths; (ii) during the day the fishes breathe quietly exchanging gases mostly with gills, and behave like oxygen conformers; (iii) and interestingly enough all the species show very low metabolic rates at mid-day.

Behavioural studies of the four species indicate that they avoid bright light and hide themselves under the coverage of macrovegetation. They come out in the open waters after dusk in search of prey and are more active at night.

Two distinct microenvironments in terms of dissolved oxygen and free CO_2 were found in the open and vegetation covered water areas. Generally, dissolved oxygen was lower, free CO_2 higher, pH lower and temperature lower under the water hyacinths than in the "open" water (Ultsch, 1973; Rai and Datta-Munshi, 1979). A sort of diel fluctuation of O_2 and free CO_2 in the two microhabitats have also been recorded (Rai and Munshi, 1979).

The availability of these data should enable some interesting ecological conclusions to be drawn about animals inhabiting swamps. The dissolved oxygen under macrovegetation depletes rapidly to almost zero ($1.26\,ml\,O_2 \cdot l^{-1}$) by 04:00, especially in the summer, when the O_2 demand of most aquatic organisms is greatest. As such, most of the vertebrates found in swamps are either entirely dependent upon aerial breathing (snakes, tortoises) or supplemental air-breathers like murrels. Further, the dependence upon aerial breathing of an organism utilizing bimodal (air and water) gas exchange can be evaluated as a function of time of day.

Thus the two metabolic systems, one of habitat (swamp) and the other of fishes, are closely interlocked with each other, one influencing the other. The general metabolism of the swamp may be contemplated as a big metabolic wheel which drives all the small metabolic wheels of different biotic communities. There is some sort of feedback mechanism also in which the metabolic activities of different biotic communities influence the whole metabolism of the ecosystem. Assemblage of air-breathing fishes form an integral part of the swamp ecosystem since their origin several million years ago. The circadian rhythm has now become an inherent property of their system which they transmit even under the artificial conditions of the laboratory.

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Literature cited

Dejours, P. 1975. Principles of comparative respiratory physiology. North-Holland Publishing Co., Amsterdam, 253 pp.

Hakim, A., J. S. D. Munshi and G. M. Hughes. 1978. Morphometrics of the respiratory organs of *Channa punctatus*. J. Zool., Lond., 184: 519~543, figs. 1~6.

Hughes, G. M. and B. N. Singh. 1970. Respiration in an air-breathing fish, the climbing perch, *Anabas testudineus*. I. O_2 uptake and CO_2 release into air and water. J. Exp. Biol., 53: 265~280, figs. 1~7.

Hughes, G. M. and B. N. Singh. 1971. Gas exchange with air and water in an air-breathing catfish, *Saccobranchus fossilis*. J. Exp. Biol., 55: 667～682, figs. 1～7.

Lomholt, J. P. and K. Johansen. 1976. Gas exchange in the amphibious fish, *Amphipnous cuchia*. J. Comp. Physiol., 107: 141～157, figs. 1～9.

Munshi, J. S. D. 1962. On the accessory respiratory organs of *Ophiocephalus punctatus* (Bloch) and *Ophiocephalus striatus* (Bloch). J. Linn. Soc. Lond. (Zool.), 44: 616～626, figs. 1～32.

Ojha, J., O. P. Dandotia, A. K. Patra and J. S. D. Munshi. 1978. Bimodal oxygen uptake in relation to body weight in a fresh-water murrel, *Channa* (=*Ophiocephalus*) *gachua*. Z. Tier. Physiol. Tierernährg. u. Futtermittelkde, 40: 57～66, figs. 1～2.

Palmer, D. J. 1976. Introduction to biological rhythms, their property, and clock control. In J. D. Palmer, ed.: An Introduction to biological rhythms. Academic Press, New York, 1～30, figs. 1～20.

Patra, A. K., Niva Biswas, J. Ojha and J. S. D. Munshi. 1978. Circadian rhythm in the bimodal oxygen uptake in a fresh-water swamp-eel, *Amphipnous* (=*Monopterus*) *cuchia*. Ind. J. Exp. Biol., 16(7): 808～809, fig. 1.

Rai, D. N. and Jayashree Datta Munshi. 1979. The influence of thick floating vegetation (water hyacinth; *Eichhornia crassipes*) on the physico-chemical environment of a fresh-water wetland. Hydrobiologia (Hague), 62(1): 65～69, figs. 1～5.

Rai, D. N. and J. S. D. Munshi. 1979. Observations on diurnal changes of some physico-chemical factors of three tropical swamps of Darbhanga, North Bihar. Com. Physiol. Ecol., 4(2): 52～55.

Singh, B. N. 1976. Balance between aquatic and aerial respiration. In G. M. Hughes, ed.: Respiration of the amphibious vertebrates, Academic Press, London, 125～165, figs. 1～19.

Singh, B. N. and G. M. Hughes. 1971. Respiration of an air-breathing catfish, *Clarias batrachus* (Linn.). J. Exp. Biol., 55: 421～434, figs. 1～6.

Singh, B. N. and G. M. Hughes. 1973. Cardiac and respiratory responses in the climbing perch, *Anabas testudineus*. J. Comp. Physiol., 84: 205～226, figs. 1～11.

Ultsch, G. R. 1973. The effects of water hyacinths (*Eichhornia crassipes*) on the microenvironment of aquatic communities. Arch. Hydrobiol., 72(4): 460～473.

Welch, P. S. 1948. Limnological methods. McGrow Hill, New York.

Winberg, G. G. 1956. Rate of metabolism and food requirements of fishes. (Original in Russian). Trans. series (194), Fish. Res. Bd. Canada.

(P. G. Department of Zoology, Bhagalpur University, Bhagalpur-812007, India).

タイワンドジョウ属４種の酸素消費量の内日変動

Jyoti S. D. Munshi・Ajoy K. Patra・Niva Biswas・Jagdish Ojha

タイワンドジョウ属 *Channa* の４種の空気と水からの酸素摂取量には顕著な概日リズムが見られ，薄暮（16～18時）から薄明（4～6時）までの夜間に高い値を保つ．酸素摂取量が最高値を示す時間とその値は，*C. marulius* では0～2時，66.4±0.5 ml $O_2 \cdot kg^{-1} \cdot h^{-1}$，*C. striatus* では20～22時，78.7±8.6 ml $O_2 \cdot kg^{-1} \cdot h^{-1}$，*C. gachua* では20～22時，95.6±2.6 ml $O_2 \cdot kg^{-1} \cdot h^{-1}$，*C. punctatus* では16～18時，57.5±1.1 ml $O_2 \cdot kg^{-1} \cdot h^{-1}$ であった．これに対し酸素摂取量が最低値を示す時間は概ね各種とも正午からの２時間に見られ，その時間には *C. striatus* と *C. gachua* では鰓呼吸の比率が空気呼吸のそれより大きかった．最高酸素摂取量のうち空気呼吸の占める率は，*C. marulius* では 84.5%，*C. striatus* では 67.7%，*C. gachua* では 53.4%，*C. punctatus* では 86.8%であった．酸素摂取の概日リズムは，沼地生態系の物質代謝の日周変動に相関していた．