Influence of the Annual Change of Field Temperature on Resting Metabolism in Cultured Red Sea Bream

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To clarify the influence of the annual change of water temperature on resting metabolism in cultured red sea bream under field conditions, oxygen consumption was measured monthly throughout the year. The measurement was carried out at field temperature close to those occuring annually in the nearby fish farm. The annual change of resting metabolism showed a mountainous pattern as the level was higher in summer and lower in winter. The monthly change of the field temperature followed different loci between the season changes toward warmer weather and toward colder weather, and the annual change followed a markedly counter clockwise pattern. When field temperature was approximately the same between the two season changes, the level of metabolism was significantly lower in April and May near the warmer season than in November and December in the colder season. The regression lines were calculated between the field temperature and resting metabolism using natural logarithm value of resting metabolism as the season changes toward warmer weather and toward colder weather. These lines intersected at two points of 10.4℃ and 25.6℃ in field temperature.

Key word ; Cultured red sea bream, Resting metabolism, Annual field temperature

The adaptation mechanism in fish of a poikilotherms animal to the environmental water temperature has already been reported by many authors concerning enzymatic activity,[1-5] respiratory movement,[6] and resting metabolism[7-11] among fish species differing in habitat temperature and for the same fish acclimated to different temperatures.

Regarding the resting metabolism in these findings, Scholander *et al.*[2] compared the level of resting metabolism between fish species in the Arctic Zone and in the Tropical Zone by estimating the regression line of the resting metabolism to the water temperature which changed rapidly. It was found that the regression line distributed more highly in fish of the Arctic zone than in fish of the Tropical Zone and also that the level of resting metabolism at the habitat temperature was lower in fish of the Arctic zone than in fish of the Tropical Zone. This difference, however, was smaller than the difference estimated from the regression line. Furthermore, it was shown that fish in the Arctic Zone maintain a higher resting metabolism at very low temperatures.

Regarding on the resting metabolism in the same fish species to the annual change of field water temperature, Moore *et al.*[13] collected Atlantic midshipman, *Porichthys porosissimus* of sea-water fish in each of the four seasons by trawling and measured the resting metabolism after being acclimated to several water temperatures in the range of 10–30℃ for one week. They found that the resting metabolism of fish acclimated to low temperature was higher in the winter group than in the summer group at the same temperature, but for fish acclimated to temperatures above 20℃, the level in the winter group became lower than the levels in the spring and summer groups. Evans[14] measured the resting metabolism in pumpkinseeds, *Lepomis gibbosus,* of freshwater fish under the changes of water temperature in the range of 8–32℃ and the photoperiod reappeared in the field conditions in the laboratory. It was also reported that the annual change of resting metabolism was independent to the annual temperature cycle, and the change showed a likely mountainous pattern as the level was higher in summer than in winter and followed a markedly clockwise pattern.

Under the field situation conditions and in the same fish species, however, the annual change of resting metabolism has scarcely been found despite available data regarding the resting metabolism in red sea bream reviewed by Wu *et al.*[15] and Oikawa *et al.*,[16, 17] Therefore, the present study was undertaken to clarify the effect of the annual change of water temperature on the resting metabolism of cultured red sea bream in order to estimate the annual change of resting metabolism under field situation conditions.

Materials and Methods

Sample Fish

Cultured red sea bream, *Pagrus major* of commercial size that cultivated by a fish farmer in Tachibana Bay, Nagasaki prefecture were used in the present. Four or five specimens were purchased monthly during one year from April 1993 to April 1994 without August 1993, February and March 1994, and the total of sample fish were 45 specimens throughout the year. For recovering from the effect of transfer, the sample fish were held in a 500l FRP tank with water at the experimental temperature within 10 days before starting the experiment. They were fed daily moist pellets and starved for 24 h before starting the experiment.

Field and Experiment Temperatures

The temperature of sea water surrounding the fish farm in the bay was measured daily at 10 : 00 a.m. at 1 m depth and the mean value of water temperature each month was calculated as the field temperature. Concerning the temperature of sea water in the bay, it had already been determined that the difference of water temperature between the surface and the bottom was very small throughout the year, because the water depth in the bay is very shallow.

On the other hand, the experimental temperature was the value near the mean of water temperature for 14 days before the sampling date, and under this temperature the oxygen consumption in fish was measured.

Measurement of Resting Metabolism

The oxygen consumption of sample fish was measured with the equipment of our own original work as shown in Fig. 1, according to the procedure of Wu *et al.*[18]. The fish was gently fitted into a mobile fish holder in the espiration chamber and was held to take a resting condition. Oxygen consumption was measured at the experimental temperature by monitoring the depletion of dissolved oxygen concentrations in the respiration chamber with a D.O. meter (YSI, Model 58) and a recorder (Pantos Nihon Denshi Kagaku). The measurement was done continuously for at least 1 h. The resting metabolism was calculated by using a partially stable oxygen consumption more than 30 or 60 min and was expressed in a unit per kilogram body weight and 1 h (mg O_2/kg·h).

For avoiding measurement errors caused from transfer and handling stresses, we paid special attention to the following: the sample fish were held to recover from the effect of transfer and were used to the measurement after recovering to take some food. Then the sample fish were gently led into a water-filled bucket from a tank without handling and were settled for about 2–3 h in the respiration chamber covered with a black mat to prevent external disturbance.

Table 1. Collection date, field temperature, experimental temperature, specimen number, standard body length, and body weight on cultured red sea bream used in the present study

Collection date	Field temperature		Experimental temperature (ET) (℃)	Specimen number	Mean and Standard deviation.	
	Monthly mean (℃)	Mean for two-weeks before collecting (℃)			Standard body length (cm)	Body weight (g)
Apr. 20, 1993	15.5±1.3	14.9±0.9	15	4	26.58±0.66	629.23±35.70
May 27, 1993	18.2±0.9	18.6±0.4	18	4	27.22±1.20	571.69±54.53
Jun. 21, 1993	21.1±0.7	21.3±0.6	22	4	28.26±0.97	653.75±50.97
Jul. 31, 1993	22.7±1.2	23.4±0.3	25	4	29.88±1.19	636.54±28.71
Aug. 1993	25.0±1.0	–	–	–	–	–
Sep. 1993	23.9±0.6	–	–	–	–	–
Oct. 26, 1993	21.6±1.0	21.8±0.7	21	4	28.80±0.91	663.18±40.45
Nov. 30, 1993	19.5±1.3	18.1±0.8	18	4	28.70±0.35	695.78±42.77
Dec. 30, 1993	15.4±1.3	13.8±0.4	15	4	29.20±0.67	694.20±29.93
Jan. 31, 1994	13.0±0.9	12.2±0.6	13	4	28.73±0.96	708.90±59.93
Feb. 1994	12.1±0.4	–	–	–	–	–
Mar. 1994	12.5±0.5	–	–	–	–	–
Apr. 17, 1994	15.9±1.2	15.2±0.5	15	4	28.45±0.64	657.78±36.16
Total				36	28.36±1.37	634.99±101.97

These sample fish of market size were obtained monthly from a fish farmer at Tachibana Bay in Nagasaki Prefecture for a year from April 1993 to April 1994. The field temperarture at a depth of 1m in the fish culture area was measure daily at 10:00 a. m.

Results

Body Size of Sample Fish

The monthly mean values and standard deviations of standard body length and body weight on sample fish are shown in Table 1. The standard body length and body weight of fish specimens ranged from 25.8 cm to 30.2cm and from 554.8g to 773.5 g throughout the year, respectively. The variation of body weight and length was slightly different among fish specimens. The mean values showed a tendency which was smaller in April and July 1993 and April 1994 than the other months. The mean values were fairly good approximations, however, with the exception of April and July.

Annual Change of Field and Experiment Temperatures

The monthly value of field temperature increased gradually from April 1993 at 15.5±0.3℃ in the beginning of the experiment to August 1993 at 25.0±1.0℃, when it expressed the maximum level, and decreased gradually again toward February 1994 at 12.1±0.4℃, when it expressed the minimum level. It increased again toward April 1994 at 15.2±0.5℃.

On the other hand, the monthly change of experimental temperature approximated the above annual change of field temperature in spite of a slight difference (Table 1). The annual change of experimental temperature is illustrated in Fig. 2. The ranges of those annual changes were 12.9℃ at the field temperature and 12.0℃ at the experimental temperature, respectively.

Annual Change of Resting Metabolism

The resting metabolism is shown in Fig. 2 for the annual change and in Fig. 3 for the statistic comparison among

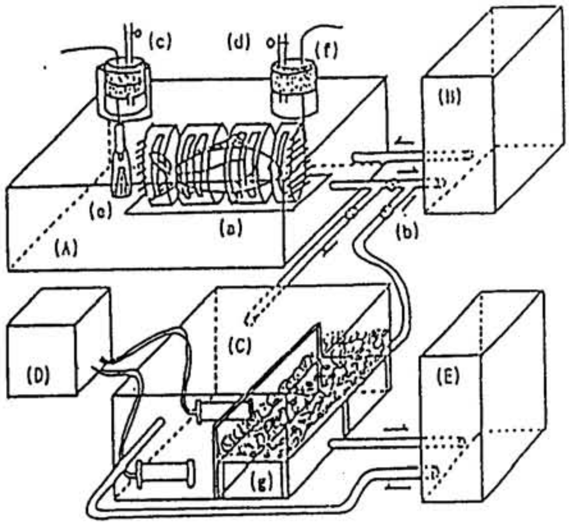


Fig. 1. The equipment for measuring the oxygen consumption of fish under resting condition. The fish is gently fitted into a mobile fish holder (a) in the main chamber (A) full of almost air-saturated sea water. Air bubbles in the sea water are revomed from the upper tube (c and d) and sea water is controlled at constant temperature by a thermoregulator (B). The oxygen consumption of fish under resting condition is measured by D.O. meter (YSI, model 58) (e), and pH value of sea water is checked by a pH sensor (f). In continous measurement for long time, sea water in the reservoir (C) is saturated by air pump (D) and is controlled at a constant temperature by another (E). When starting continous measurement, sea water in the main chanber is exchange through the synthetic wool filter (g) with sea water in the reservoir by openening the valves.

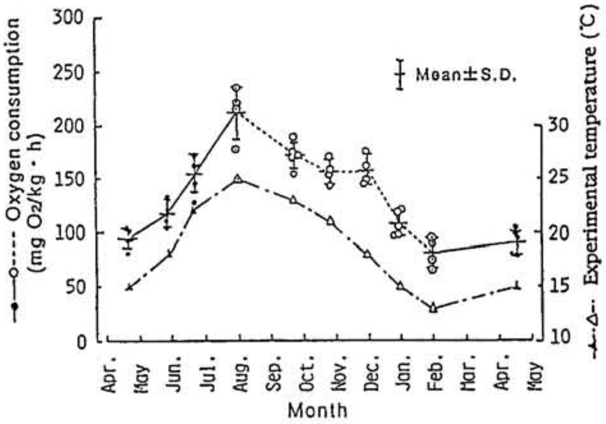
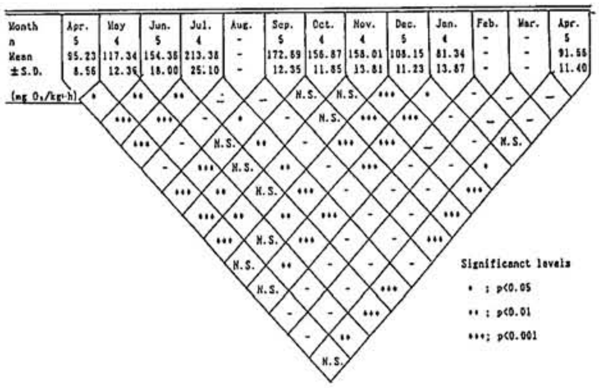


Fig. 2. Changes of the oxygen consumption and the experimental temperature with the lapse of month in cultured red sea bream. The circle and triangle symbols express oxygen consumption and experimental temperature, and the closed and open symbols express the two seasons changed from colder to warmer and from warmer to colder, respectively. The experiment temperature approximated to the field temperature in the fish farm is shown in Table 2-1.



Month	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
n	5	4	5	4	-	5	4	4	5	4	-	-	5
Mean	95.23	117.34	154.36	213.38	-	172.69	156.87	158.01	108.15	81.34	-	-	91.56
±S.D.	8.56	12.36	18.00	25.10	-	12.35	11.85	13.81	11.23	13.87	-	-	11.40
(mg O2/kg・h)													

Fig. 3. Comparison of mean value of oxygen consumption in cultured red sea bream among months, the significance level by F-test of difference in mean value of two months is given in the diagram. The one, two, and three asterisks show the significance levels of $p<0.050$, $p<0.010$, and $p<0.001$, respectively.

months. The monthly mean value of resting metabolism increased sharply and significantly from April 1993 at 95.23 ± 8.56 mg O2/kg·h to July 1993 at 213.38 ± 25.10 mg O_2/kg·h of the maximum level, and decreased somewhat slowly toward January 1994 at 81.34 ± 13.87 mg O_2/kg·h of the minimum level. However, the decline was smaller during the course from August to December and was significantly larger during the course from December to January. After reaching the lowest level the value increased slowly toward April at 91.56 ± 11.40 mg O_2/kg·h. The mean value of April 1994 at the end was approximately the same as that of April 1993 at the beginning. Namely, the annual change of resting metabolism showed a likely mountainous pattern as the level was higher in summer and lower in winter. The range of annual change was about ± 45% against the mean level all of the year, and the maximum level in July in summer was 2.6 times higher than the minimum level in January in winter. This annual change of resting metabolism showed a parallel pattern to that of experiment temperature.

Effect of Body Weight to Resting Metabolism

The relationship between body weight and resting metabolism is shown in Table 2 and Fig. 4. Regarding relationship between body weight and oxygen consumption per hour (mg O_2/h) for sample fish by each month, the distribution was divided into four quadrants by both mean values in the month and the frequency in each quadrant was counted. as in Table 2 (2-1). distribution of total frequency in each quadrant throughout the year showed a tendency of positive correlation that the oxygen consumption per hour (mg O_2/h) was higher in large fish than in small fish, although it was not biased significantly by χ2-test. However, the distribution of frequency in the relationship between body weight and oxygen consumption per kg and hour (mg O_2/kg·h) did not show a correlative tendency (Table 2-2)

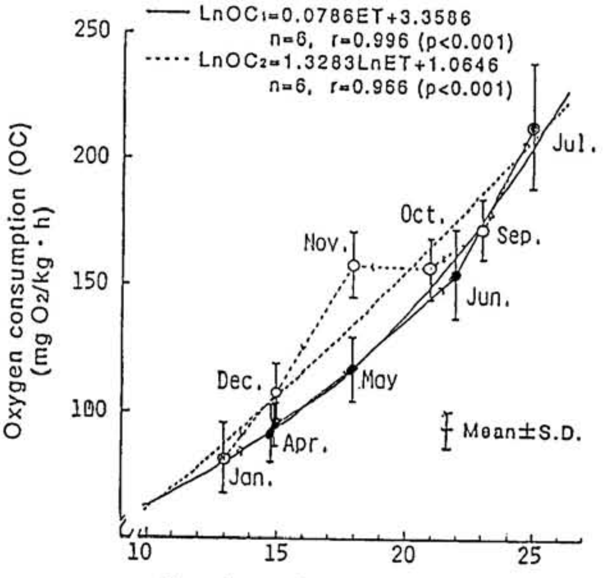


Fig. 4. The slope value of regression line between body weight and oxygen consumption in cultured red sea bream of each month from April to July plotted against their average body weight and experiment temperatures. The slope values of April, May, June, and July were calculated from fish number of ten, four, five, and four as shown in Table 2-1, respectively and their coefficients of correlation were significant by $p<0.050$.

Table 2 Relationships between relative body weight and relative oxygen consumption per hour(mg O_2/h) or relative oxyen consumption per body weight and hour(mg O_2/kg·h) under resting conditions in cultured red sea bream

2 − 1

		Relative body weight		Total
		Low	High	
Relative oxygen consumption (mg O_2/h)	High	9	15	24
	Low	13	8	21
	Total	22	23	45

$X^2=1.782$ (N. S.)

2 − 2

		Relative body weight		Total
		Low	High	
Relative oxygen consumption (mg O_2/kg·h)	High	11	11	22
	Low	12	11	23
	Total	23	22	45

$X^2=0.021$ (N. S.)

The distributions of oxygen consumption of mg O_2/h and mg O_2/kg · h in fish plotted against body weight by each month were divided relatively into four parts of high and low levels, and then total frequency in each of the four parts was calculated.

On the other hand, in regard to the relationship between body weight and oxygen consumption (mg O_2/h), the regression line and coefficient of correlation by each month were calculated. The slope value obtained from this regression line expressed the increasing rate of oxygen consumption with the increase of body weight, in other words, the metabolic activity per body mass. Unfortunately, in the present study, the coefficient of correlation was significant only in four consecutive months from April to July. If the slope values plotted against the body and experimental temperature, the slope values of these four months showed an insignificant correlation with the monthly mean values of body weight. These slope values, however, showed a significant positive correlation with the experiment temperatures (ET) as slope = $0.026\ \mathrm{ET} - 0.227$, $n=4$, and $r=0.978$ ($p<0.05$) (Fig. 4). From these results, it can be considered that the monthly change of resting metabolism in the present study was not caused by the difference condition of physique in sample fish but by the change of field temperature.

Effect of field temperature to resting metabolism

The monthly mean value of resting metabolism is plotted against the experiment temperature (field temperature) as illustrated in Fig. 5. The mean value in the season change toward warmer weather increased with the increase of experimental temperature, and then the mean value in the season change toward colder weather decreased with the decrease of experimental temperature. The change of resting metabolism with the experimental temperature showed logarithmic patterns, and followed different loci between two season changes, and then the annual change followed a marked counter clockwise rotation. Therefore, though the experiment temperature was approximately the same, the level of metabolism was significantly lower in April and May in the warmer season than in November and December in the colder season. These differences were significant; $p<0.05$ between April and December and $p<0.01$ between May and November.

The relationship between the experiment temperature (ET) and the resting metabolism (OC) was examined in several cases using both the measurement value and the natural logarithm (Ln) value by two seasonal changes. The coefficient of correlation was higher in the case using ET and Ln OC_1 than in the other cases of the season change toward warmer and of the season change to colder was higher in the case using Ln ET and Ln OC_2 than in the other cases. The regression line showing the highest correlation was $\mathrm{Ln}\ OC_1 = 0.0786\ \mathrm{ET} + 3.3586$, $n=6$, and $r=0.996$ ($p<0.001$) in the season change toward warmer weather and $\mathrm{Ln}\ OC_2 = 1.3283\ \mathrm{Ln}\ \mathrm{ET} + 1.0646$, $n=6$, and $r=0.966$ ($p<0.001$) in the season change to colder weather, respectively. These regression lines were in conformity with the measurement value.

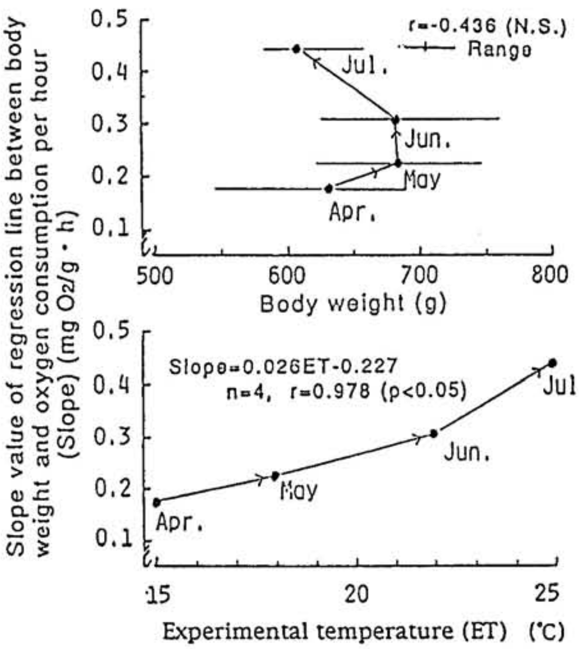


Fig. 5. Relationship between experiment temperature approximated to the field temperature nearby the fish farm and oxygen consumption in red sea bream. The symbols are the same as shown in Fig. 1. The solid and dotted stright lines attached an arrow show the change with the passage of month in the two seasons changed from colder to warmer and from warmer to colder, respectively. The solid and dotted lines express the regression line between experiment temperature and oxygen consumption in two seasons from colder to warmer and from warmer to colder, respectively.

The values of resting metabolism at optional field temperature were estimated with these two regression lines by two seasonal changes (Table 3). Two values in the season change toward warmer weather and in the season change to colder weather agreed at the field temperatures of 10.4℃ and 25.6℃. Therefore, these two regression lines intersected at two points of the field temperature.

Discussion

The annual change in resting metabolism of cultured red sea bream showed a mountainous pattern as the level was higher in summer and lower in winter (Fig. 2). This annual pattern agrees closely with annual pattern of resting metabolism in the pumpkinseeds that reported by Burns.[19] In regard to the influence of body weight on resting metabolism, for any given fish species, it has been reported that the increasing rate of metabolism decreases with body weight, although not in linear fashion. For a large number of

Table. 3 The oxygen consumption values of cultured red sea bream under resting conditions, in relation to the field temperature by two seasons from colder to warmer and from warmer to colder, their differences in two seasons, and their precentages of the difference

Field temperature	Oxygen consumption (mg O_2/kg · h)			$(OC_2 - OC_1) \times$
(℃)	OC_1	OC_2	$OC_2 - OC_1$	$100/OC_1$ (%)
10.00	63.11	61.75	−1.36	−2.15
10.42	65.23	65.22	−0.01	−0.01
11.00	68.27	70.09	1.81	2.66
12.00	73.85	78.67	4.82	6.52
13.00	79.89	87.50	7.60	9.52
14.00	86.43	96.55	10.12	11.71
15.00	93.49	105.82	12.32	13.18
16.00	101.14	115.29	14.15	13.99
17.00	109.41	124.96	15.55	14.21
18.00	118.36	134.81	16.46	13.90
19.00	128.03	144.85	16.82	13.13
20.00	138.50	155.06	16.56	11.96
21.00	149.83	165.45	15.62	10.42
22.00	162.08	175.99	13.91	8.58
23.00	175.34	186.70	11.36	6.48
24.00	189.67	197.55	7.88	4.15
25.00	205.18	208.56	3.38	1.65
25.63	215.60	215.57	−0.03	−0.01
26.00	221.96	219.71	−2.25	−1.01

OC_1 and OC_2 values of oxygen consumption in relation to the field temperature by two seasons from colder to warmer and of the contrary were estimated by substituting the field temperature for experimental temperature of two equation models between experimental temperature (ET) and oxygen consumption (OC_1) or (OC_2) as shown in Fig.5; $\text{Ln } OC_1 = 0.0786 \text{ ET} + 3.3586$ in season change from colder to warmer and $\text{Ln } OC_2 = 1.3283 \text{ Ln ET} + 1.0646$ in season change from warmer to colder, respectively.

fish ranging in body weight (W) from about 1mg to over 1000g, the rate of metabolism (M) has been found to obey the allometric relationship: $M/W = aWb-1$.[16,17] In cultured red sea bream, since the ranges of body weight either within the group or among the groups for sample fish used in the present study were narrow, the relationship between body weight and resting metabolism per unit kg body weight (mg O_2/kg·h) was found insignificant correlation, although there was one evidence of significant correlation ($p < 0.001$) obtained at 25℃ of sample fish with the body weight ranging from 84.0g to 684.5g differed from the data used for this annual resting metabolism (Fig. 6). Furthermore, in Fig. 4, the slope values of the relationship between the body weight and resting metabolism (mg O_2/h) for the sample fish used in the present study plotted against the body weight showed insignificant correlation, while the relationship between field temperature and these slope values showed positive correlation at significant level of $p < 0.05$. Therefore, it was possible further considered that the annual change of resting metabolism in cultured red sea bream was not caused by the difference physique condition of the sample fish, but caused of the body mass (Table 2 and Fig. 4).

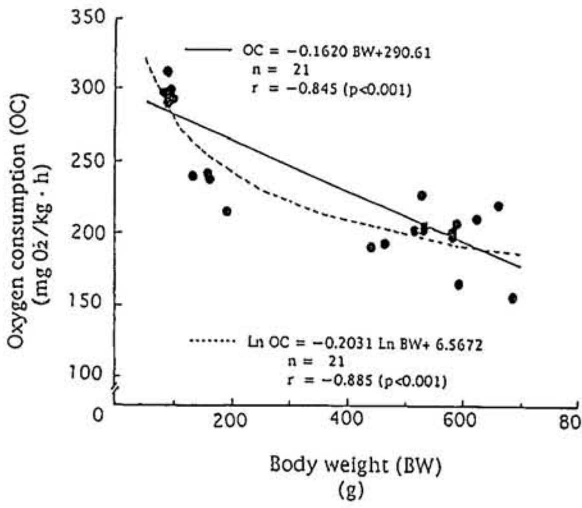


Fig. 6. Relationship between body weight (BW) and oxygen consumption at 25℃ of experimental temperature in cultured red sea bream differed from sample fish shown in Table 1. The solid and the dotted lines express the regression lines between body weight and oxygen consumption in two cases of these measurement values and natural logarithm values, respectively.

The relationship between field temperature and resting metabolism in cultured red sea bream showed a positive correlation. This finding was in general agreement with that have already been studied on many fish species from different climatic zones.[6, 12, 20]

The change of resting metabolism in cultured red sea bream to the annual change of field temperature exhibits different loci as the field temperature increase from spring to summer and decrease from summer to winter, and the annual change followed a markedly counter clockwise to the annual field temperature rotation (Fig. 5). Therefore, though the field temperature was approximately the same, the level of metabolism was lower in May near the warmer season than in November of the colder season (Fig. 5). This result suggests that the response of cultured red sea bream seem to be late in resting metabolism to the annual change of field temperature. Evans[21] considered that response of resting metabolism to the warm temperature changed from 8℃ to 18℃ and the reverse to the cold temperature changed from 18℃ to 8℃ required more time to be complete. However, the reverse temperature changed to the cold occurred more gradually. The same phenomenon has also been reviewed by Brett.[20]

The regression lines calculated using the natural logarithm value of resting metabolism as the season change toward warmer weather and as the season change to the colder weather intersected at two points of 10.4℃ and 25.6℃ in nfield temperature (Table 3). Furthermore, the difference of resting metabolism values estimated from two regression lines was largest at 17℃ and was 14.2%. From these results, it was possible further considered that these temperatures of 10.4℃ could be define as ultimate incipient lower limit and 25.6℃ as the ultimate incipient higher limit of field temperature that can be tolerated by cultured red sea bream.

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養殖マダイにおける安静時代謝の年間環境水温サイクルに伴う変化

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温帯の年間環境水温の変化が養殖魚の安静時代謝（RM）に及ぼす影響を明らかにするため，養殖マダイのRMを1年間経月的に測定した。RMは，1～7月の向暖期では水温の上昇に伴って漸次上昇し，7～1月の向寒期ではやや穏やかに下降した。従って，両期の同一水温下のRMは前者が後者より低かった。水温（t）とエネルギー消費量（RE）との間に$RE_1=e^{(0.0768t-2.3702)}$（向暖期）と$RE_2=e^{(1.3281Lnt-4.6639)}$（向寒期）の関係式が求められ，年間水温下の安静時エネルギー要求量の見積もりが可能となった。