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Changes of Oxygen Consumption and Respiratory Quotient in the Tissues of Oysters during the Stages of Sexual Maturation and Spawning

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Summary

1. The seasonal changes of oxygen consumption and respiratory quotient (RQ) in the digestive diverticula (Dd), gill (G) and pallial margin (Pm) of the Japanese oyster, *Crassostrea gigas*, were followed manometrically to study the variations in utilization of glycogen and fat within these tissues in relation to their physiological activities. The oysters which had been cultured for two or three years in Onagawa Bay and Matsushima Bay separately were used as the materials.
2. The order of respiratory rate throughout the present study was as follows: Dd≧G>Pm.
3. All these tissues of the Matsushima Bay oysters in which the sexual maturation proceeded more rapidly and the spawning period was longer showed a marked decrease in respiratory rate at the spawning stage, while such a decrease was not observed in the Onagawa Bay oysters.
4. The RQ of G was about 1.0 in most cases, but that of Dd or Pm showed a great seasonal variation.
5. The RQ values of Dd and Pm were contrary to each other during the rapid stage of sexual maturation.
6. The seasonal change in RQ of Dd was more marked in the oysters from Matsushima Bay than in those from Onagawa Bay.
7. From the seasonal relationship between oxygen consumption and RQ, it was suggested that carbohydrates were more efficient as an energy source than fats not only in G but also in Dd and Pm.

Relatively little is known about the tissue respiration of bivalves, and only a few such studies have been made in recent years (1–7). There have been no reports on the comparative rate and seasonal change in oxygen consumption by the various tissues of oyster.

The numerical value of RQ is dependent on the chemical nature of the substance being oxidized within the organism (8–11). From many investigations of livestocks and birds, it has been well known that the herbivorous animals tend to have a higher RQ, while the carnivorous have a lower one. The value varies also during the conversion of food substances. Wierzuchowski and Ling reported quotients of 1.4 and higher in rapidly fattening hogs, and they cited a quotient of similar magnitude obtained by Benedict for the goose (10). The synthesis of oxygen-poor fats from the relatively oxygen-rich carbohydrates involves a liberation of carbon dioxide. On the other hand, the utilization of fats and proteins and their possible conversion to carbohydrates lowers the RQ values, and quotients below 0.7 have been observed in fasting, particularly in hibernating animals (10). According to Galtsoff (12), no definite trend in the changes of RQ could be detected in the American oyster, *Crassostrea virginica*. It varied throughout the year from 0.51 to 1.44. However, there are no available reports, as far as the author knows, on the seasonal changes in RQ of the various tissues of oyster.

Experimental Methods

The experiments were carried out from May to June in 1965 and from June to October in 1966. The Japanese oysters which had been cultured by the raft-culture method for two or three years in Onagawa Bay and Matsushima Bay separately were used as the materials. The comparative use of these two groups of oysters is considered to be favorable to the study of reproduction, because they differ in the rate of sexual maturation (13). After investigation of the mortality rate, observations on the gonadal condition of oysters were made macroscopically. The fresh tissues of Dd, G and Pm of four individuals were regularly sampled and minced with ophthalmic scissors or a razor for the manometric experiments.

The oxygen uptake and carbon dioxide evolution were measured at 25°C in a Warburg apparatus, with vessels of about 17 ml in capacity. Three reaction manometers and one thermobarometer were used in each measurement (14). The shaking rate was about 115 cycles per minute with an amplitude of 35 mm. Each vessel contained 70 to 100 mg (in wet weight) of digestive diverticula, 100 mg of gill or 100 to 150 mg of pallial margin in 1.8 ml of modified Herbst's artificial sea water buffered at pH 8.2 by the method reported in our preceding paper (13); 0.3 g of Tris (hydroxymethyl) aminomethane and 0.13 ml of 35% HCl were added to 100 ml of 90% artificial sea water. The final concentration of this solution was about 34‰ salinity. To trasfer carbon dioxide from the liquid phase to the gaseous phase, 0.2 ml of 3.5 N H_2SO_4 in the side arm was poured into the main chamber. To absorb carbon dioxide, 0.3 ml of 17% KOH and a filter paper were placed in the center-well (14). An example of the measurement in Dd is presented in Table 1. In this case, the volume of oxygen consumed for 20 minutes is 19.8 μl and the total volume of carbon dioxide evolved is 19.2 μl, therefore the RQ is 0.97.

TABLE 1. *Manometric Measurements of Oxygen Uptake and Carbon Dioxide Evolution in the Minced Digestive Diverticula of an Oyster*[*1]

Manometer		I ko_2[*2]$=1.373$ $kco_2=1.520$			II $kco_2=1.578$			III $ko_2=1.421$			Thermo-barometer	
Main chamber	Tissue	100.2 mg			100.5 mg			100.0 mg			–	
	Artifical sea water	1.8 ml			1.8 ml			1.8 ml			2.0 ml	
Side arm	3.5N H_2SO_4	0.2 ml			0.2 ml			–			–	
Center well	17% KOH	–			–			0.3 ml (with a filter paper)			–	
Time in minutes		R[*3]	D[*4]	C[*5]	R	D	C	R	D	C	R	D
0(pour H_2SO_4 into the main chamber of manometer II)		116.0	–	–	123.0	–	–	176.7	–	–	150.8	–
10		110.0	−6.0	−5.2	182.5	+59.5	+60.3	168.7	−8.0	−7.2	150.0	−0.8
20(pour H_2SO_4 into the main chamber of manometer I)		105.0	−11.0	−10.2	–	–	–	162.0	−14.7	−13.9	150.0	−0.8
30		177.0	+61.0	+60.8	–	–	–	–	–	–	151.0	+0.2

*1. sampled on September 3 in Onagawa Bay *2. k-flask constant (mm^2)
*3. reading (mm) *4. difference *5. correction

Results and Discussion

Gonadal Condition and Mortality Rate

In the Onagawa Bay oysters, sexes were not distinct in mid May. In mid June, however, they were distinct in a considerable number of oysters, though the sexual maturation was still at the early stage. Gonads developed rapidly from July to August and a small number of individuals showed a symptom of partial spawning early in September. As spawning was already finished late in September, its main period was presumed to be from the beginning to the middle of September. There was little mortality among oysters during culture.

In the Matsushima Bay oysters, sexes were distinct already in mid May. A partial spawning was observed in some individuals early in July. In the middle of August, the main spawning was completed in most oysters. However, the genital canals and gonoducts remained very clear in some, suggesting that spawning would occur till late in August at least. These observations indicated that the sexual maturation proceeded more rapidly and the spawning period was longer in the oysters of Matsushima Bay than in those of Onagawa Bay. In Matsushima Bay, the total loss due to the mortality during culture was estimated as about 12 per cent in mid August and about 20 per cent late in September.

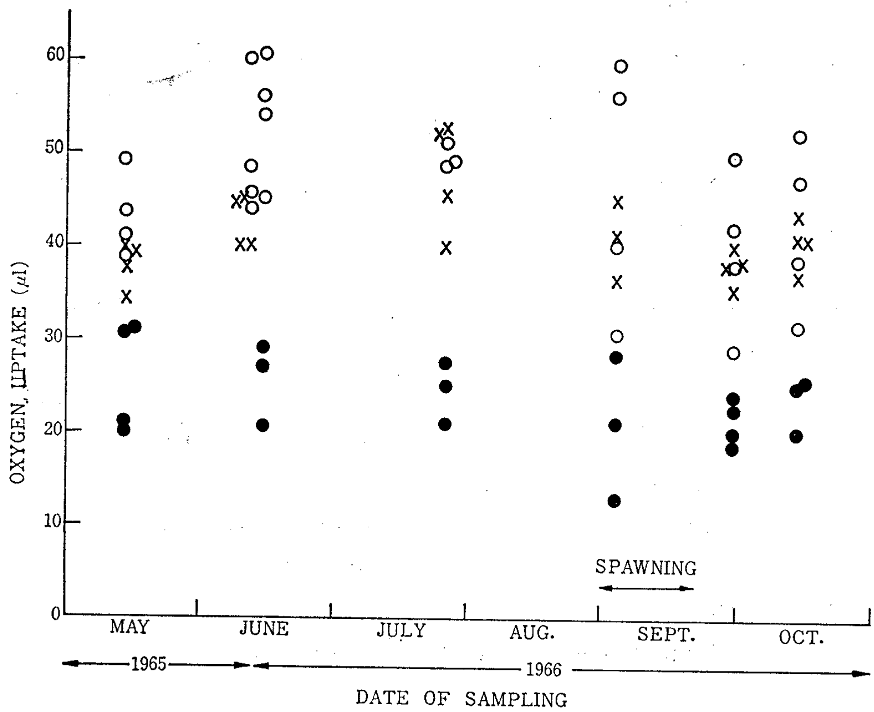

FIG. 1. Seasonal change in the oxygen uptake in tissues of oysters cultured in Onagawa Bay. Data represent values in μl per 100 mg of fresh tissue per hour. Temperature for measurement, 25°C. ○ Digestive diverticula, × Gill, ● Pallial margin.

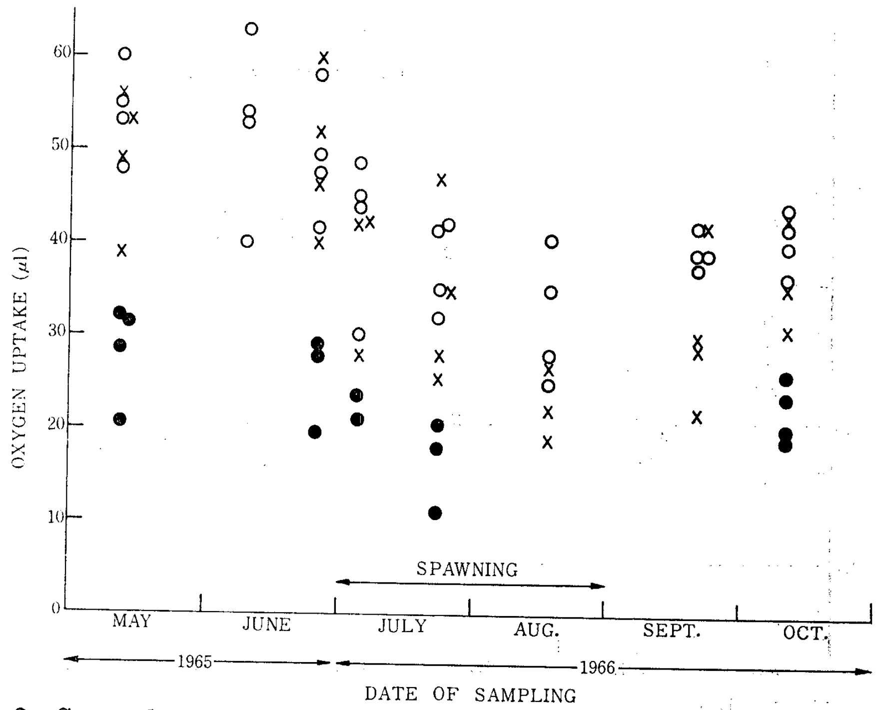

FIG. 2. Seasonal change in the oxygen uptake in tissues of oysters cultured in Matsushima Bay. Data represent values in μl per 100 mg of fresh tissue per hour. Temperature for measurement, 25°C. ○ Digestive diverticula, × Gill, ● Pallial margin.

Oxygen Consumption

The seasonal change of oxygen consumption in the tissues of Onagawa Bay oysters is presented in Fig. 1. The respiratory rate in Dd showed a slightly increasing tendency in June, or at the early stage of sexual maturation. It did not show a clear change after that until the spawning stage. Oysters indicating lower rates than those in June gained in number just after spawning. The rate in G showed a slightly upward trend from June to July, or from the early stage to the middle of sexual maturation, but it fell after spawning. No clear change was observed in Pm throughout the present study.

The seasonal change of respiratory rate in the tissues of Matsushima Bay oysters is presented in Fig. 2. All of the tissues indicated no definite variation in rate of oxygen consumption during the sexual maturation, but they showed a marked decrease at the spawning stage. This result suggests that the aerobic metabolism in the Matsushima Bay oysters declines at the stage when the mass mortality usually occurs (13, 15).

In oysters of both bays, the order of respiratory rate was as follows: Dd$\gtreqqless$G>

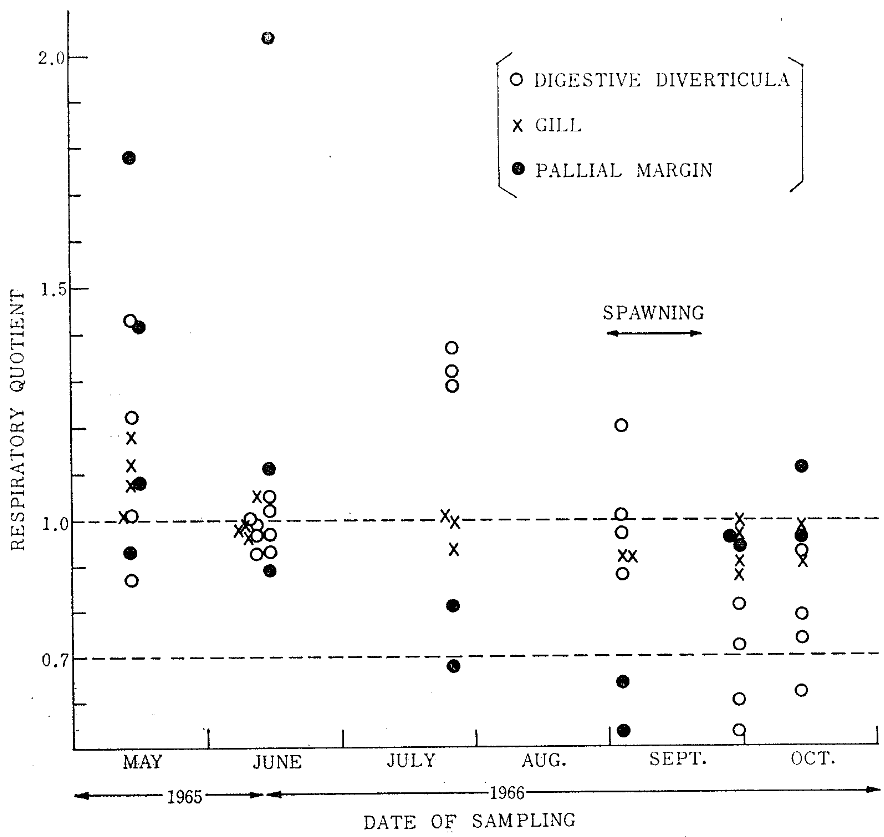

FIG. 3. Seasonal change in the respiratory quotient in tissues of oysters cultured in Onagawa Bay. Temperature for measurement, 25°C.

Pm. According to Kawai (2), that was G>Pm>Dd in the pearl oyster, *Pinctada martensii*, sampled in June. These results seem to indicate that these animals are widely different from each other in the order of oxidative metabolism among organs though equally marine bivalves.

Respiratory Quotient

As shown in Figs. 3 and 4, the seasonal change in RQ differed markedly with tissues. The RQ of G was about 1.0 in most cases, suggesting the utilization of carbohydrates as the main energy source in G. That of Dd or Pm, however, showed a great seasonal variation.

In Dd, high values of 1.43 or 1.22 were obtained when sexes were not distinct in most of the oysters. This result seems to suggest the conversion of carbohydrates to fats. When sexes became distinct, oysters showing the RQ of about 1.0 gained in number. During rapid sexual maturation the mean value of about 1.3 was observed in both bays, suggesting the active synthesis of fats. After that, the RQ in Dd showed a downward trend and it dropped to about 0.7 at the spawning stage (in Matsushima Bay) or just after this stage (in Onagawa Bay), suggesting the utilization of fats as the main energy source. Furthermore, values below 0.6

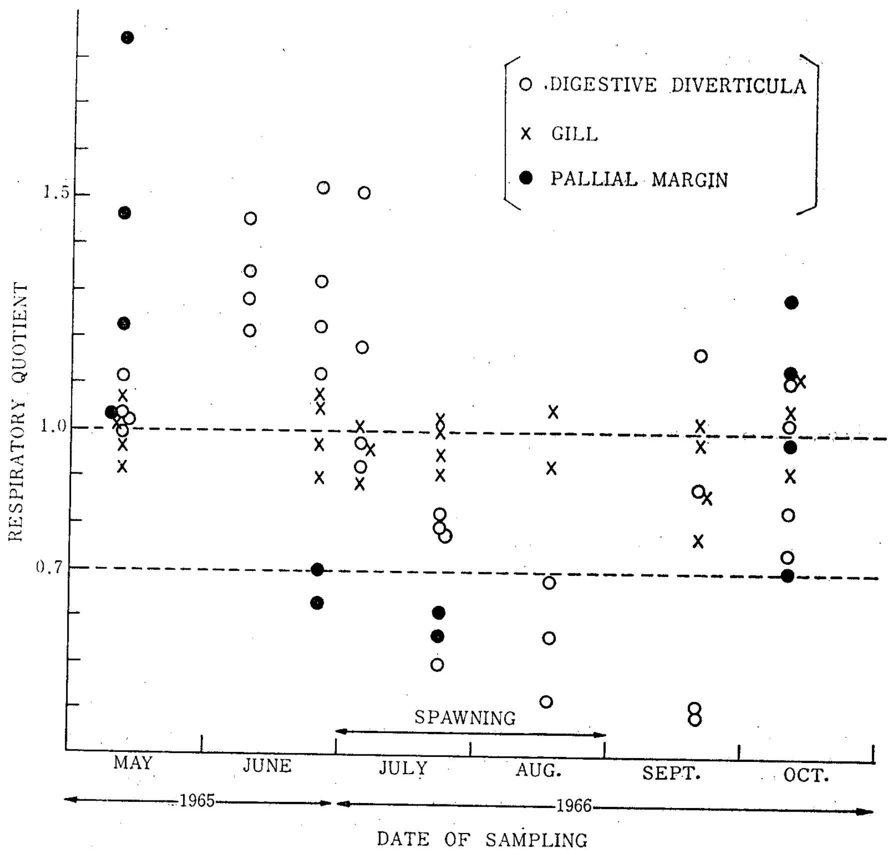

FIG. 4. Seasonal change in the respiratory quotient in tissues of oysters cultured in Matsushima Bay. Temperature for measurement, 25°C.

were obtained, suggesting the conversion of fats to carbohydrates. Extremely low values of about 0.4 were observed especially at the latter period of spawning and after that in Matsushima Bay. This result is interesting in relation to the abnormal mortality, because it usually occurs at these periods (13, 15). Although the RQ began to rise in both bays after these periods, the rise occurred more rapidly in Matsushima Bay. The above-mentioned results indicate that the seasonal change in metabolism of Dd is more marked in the oysters of Matsushima Bay than in those of Onagawa Bay.

In Pm, individuals having the RQ above 1.0 predominated in the oysters of both bays during the early stage of sexual maturation. Very high values of 2.04, 1.84 or 1.78 were found. These observations suggest the active conversion of carbohydrates to fats. The RQ, however, dropped to about 0.7 during the rapid sexual maturation when the mean value of about 1.3 was observed in Dd. This suggests the utilization of fats as the main energy source in Pm. Furthermore, values below 0.6 were found during spawning, suggesting the conversion of fats to carbohydrates. The RQ showed rapidly rising trend after spawning.

From the aforementioned results on the RQ in Dd and Pm it may be concluded that the respiratory substrate in these tissues varies with the gonad development of oyster.

Mori et al. (15) reported that total lipids or sudanophilic substances were found to deposit in the epithelia of the digestive diverticula and intestine of oysters during the stages of sexual maturation and spawning, and to show a drop in amount after spawning. In the previous paper of the author (16), the glucose-6-phosphate dehydrogenase system participating in production of reduced nicotinamide-adenine dinucleotide phosphate which is necessary for lipid biosynthesis was found to show activity in the epithelia of these digestive tracts during the stages of sexual maturation and partial spawning, and to show a drop in activity after spawning. It was also observed that the stored lipids in the connective tissue of oyster mantle decreased during the stages of sexual maturation and spawning (15). These results of histochemical studies seem to support the seasonal change in metabolism of Dd and Pm observed in the present study.

Seasonal Relationship between Oxygen Consumption and Respiratory Quotient

As previously stated, the RQ of G was about 1.0 in most cases, even when there was a marked variation in oxygen consumption as observed in the Matsushima Bay oysters. This suggests either that G cannot utilize fats as the respiratory substrate or that the metabolic rate of fats is too low to produce the large quantity of energy which is necessary for the physiological function of G. In the oysters of Matsushima Bay, the lowest rate of oxygen consumption in both Dd and Pm was found when their RQ was below 0.7, and in general high rates in Dd were obtained when this tissue showed an RQ of 1.0 or higher. These results suggest that carbohydrates were more efficient for the energy source than fats not only in G but also in Dd and Pm.

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