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INFLUENCES OF TEMPERATURE AND SALINITY ON OXYGEN CONSUMPTION OF TISSUES IN THE AMERICAN OYSTER (*CRASSOSTREA VIRGINICA*)

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Abstract—1. QO_2 of three oyster tissues was used to assess responses to alterations in temperature and salinity.

2. Cold-acclimated gill tissue showed good acclimatory ability, while mantle tissue showed little and muscle none.

3. Among warm-acclimated tissues gill and mantle showed partial acclimation and muscle none.

4. Upon dilution of the sea water medium gill and mantle tissues showed increased QO_2, while muscle tissue exhibited no alteration.

5. No significant alterations in tissue QO_2 took place in response to increased salinity of the medium.

INTRODUCTION

The ability of poikilothermic organisms to adjust various metabolic rate functions upward or downward in response to changing environmental conditions is a well documented phenomenon (Vernberg & Vernberg, 1972; Vernberg, 1975; Prosser, 1967, 1973; Read, 1964; Kinne, 1963, 1964; Segal, 1961). Often, however, the exact mechanisms of such compensatory reactions remain unknown. The inability of an organism to cope with varying situations may limit the distribution and even the survival of the species.

Temperature and salinity fluctuations are pervasive aspects of the estuarine environment, affecting many organisms in the Chesapeake Bay. The American oyster, *Crassostrea virginica* (Gmelin) is subjected to wide fluctuations in temperature and salinity regimes on a circannual basis, and even more rapid changes associated with large influxes of fresh water due to prolonged summer storms. Adaptive responses in the respiratory system of intact animals to changes in temperature and salinity have been the subject of several studies (Galtsoff, 1964; Loosanoff, 1950; Korringa, 1952; Nicol, 1967). However, except for the study of Percy *et al.* (1971) there exists little information regarding the responses of individual tissues of *Crassostrea virginica* to thermal and osmotic stress.

Evidence cited by Galtsoff (1964) seems to indicate lack of acclimation (type 4 of Precht, 1958) in intact animals. Preliminary experiments conducted in our laboratory have yielded data supporting this conclusion. However, it has been suggested that the apparent maintained respiratory depression in cold environments may actually be due to a decrease in the ventilation rate, since several studies have shown that it requires the transport of 15–16 l. of water for each milliliter of oxygen consumed (Gordon, 1972; Nicol, 1967).

MATERIALS AND METHODS

Collection and housing of animals

Adult oysters (*Crassostrea virginica*) approx 10 cm long and 4–5 cm wide (3–4 yr old) were collected from several locations in the Tangier Sound region of Chesapeake Bay. They were held in fiberglass aquaria containing filtered, aerated sea water (Instant Ocean) 20‰ salinity. Each tank contained individual temperature and photoperiod controls. The temperature of the holding tank was adjusted to the temperature at which the animals had been living in the wild. While in the holding tanks animals were exposed to a 10L:14D photoperiod regimen. The pH was monitored daily and remained at 8.1.

Temperature studies

Just prior to the beginning of acclimation the temperature of the holding tank was raised or lowered over a 24 hr period to the acclimation temperature for the particular experiment. Animals were then acclimated to 5, 15 or 25°C (±0.5°C) for not less than 21 days at 20‰ salinity and a 10L:14D photoperiod. 15–18 hr before tissue samples were removed, several experimental animals were taken from the acclimation tank and each was placed in a chamber of 1 l. capacity containing aerated, filtered, circulating sea water 20‰ salinity. The temperature of the water flowing through these chambers was then raised or lowered over the indicated number of hours to the experimental temperature. Oysters were opened by carefully cutting both the adductor muscle and the hinge ligament. Samples of gill, mantle and adductor muscle tissue were prepared by the method outlined by Percy *et al.* (1971). A gill sample consisted of four pieces of tissue 0.5–1 cm on each side cut from all of the demibranchs of each animal. Samples of mantle tissue were cut only from the thin, central portion of the organ and measured approx. 0.5–1 cm on each side. Adductor muscle samples were cut only from that portion of the muscle containing the striated fibers. Slices were made parallel to the fibers using a Stadie–Riggs microtome (A. H. Thomas Co.).

Oxygen consumption was measured using a Gilson GRP 14 respirometer. Samples were placed in 16 ml flasks containing 3 ml of filtered sea water 20‰ salinity, pH 8.1 and air as the gas phase. The center well of the flasks was filled with 0.2 ml of 10% KOH. The shaking rate was 110/min. For each group of samples oxygen consumption was measured at one temperature over the following ranges: gill 5–29°C, mantle 5–27°C, muscle 13–30°C.

After a 45 min equilibration period manometer readings were made at 30 min intervals over a 2 hr period. Tissue samples were then removed from the flasks and taken to dryness in an oven at 80°C for 48 hr (Quick, 1971).

Respiratory rates (QO_2) are expressed as microliters of O_2/hr per g dry wt.

Salinity studies

Animals were acclimated for 21 days to 15°C and 20‰ salinity in tanks containing recirculating, filtered artificial sea water. A 10L:14D photoperiod regimen was imposed. At the end of this period, designated day 0, tissue respiration was measured in gill, mantle and muscle samples from animals in each of the three tanks at 15°C as previously described.

The salinity in one tank was then lowered over a 24 hr period to 10‰, a second tank was allowed to remain at 20‰ and a third tank had the salinity adjusted to 30‰. QO_2 of tissue samples from each salinity was then measured at 15°C each day for the next 21 days in the manner previously described.

Statistical treatment of data

Acclimation can be measured by comparing rate functions. If complete acclimation to temperature has occurred there should be no significant difference in the rate functions of two groups when measured at their respective acclimation temperatures. When measured at an intermediate temperature if acclimation has occurred there should be an overshoot or undershoot of the rate function, i.e. translation of the rate–temperature curve.

Acclimatory response to salinity change was monitored by measuring oxygen consumption and comparing the QO_2 of tissue samples removed from organisms from each of the three acclimation tanks during the 21 day experimental period. All measurements in this part were made at 15°C.

The Student's *t*-test was used to determine whether or not the differences found were statistically significant at the 5% level ($P = 0.05$). Each point in the figures represents the mean of samples from at least 14 specimens.

RESULTS

Temperature studies

Respiratory rates of gill, mantle and adductor muscle tissue differed from one another in several respects. Figures 1, 2 and 3 show that at any given temperature the QO_2 of gill tissue was higher than that of either mantle or adductor muscle. The latter tissue always exhibited a QO_2 lower than that of the others at any given temperature. Figure 1 shows the mean oxygen consumption of gill samples from the three acclimation groups over the respective temperature ranges. Pertinent statistical analysis and Q_{10} data are presented in Tables 1–4.

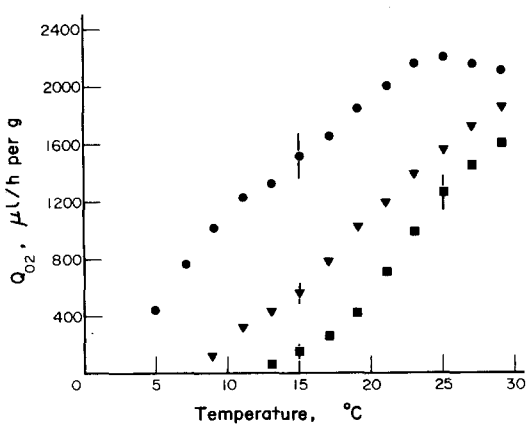

Fig. 1. Mean QO_2 of gill tissues acclimated to 5°C (circles), 15°C (triangles) and 25°C (squares) over the entire experimental range. Vertical bars represent ±2 S.E. of the mean.

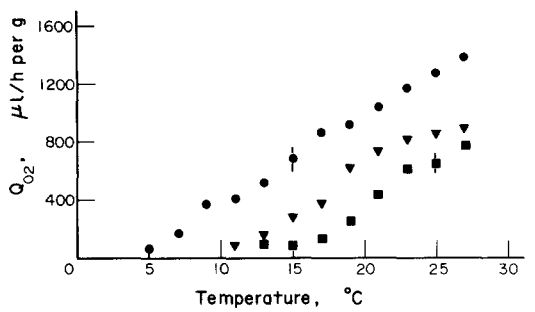

Fig. 2. Mean QO_2 of mantle tissues acclimated to 5°C (circles), 15°C (triangles) and 25°C (squares) over the entire experimental range. Vertical bars represent ±2 S.E. of the mean.

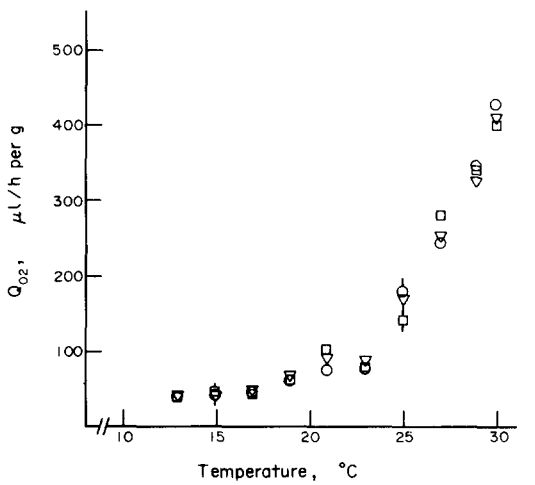

Fig. 3. Mean QO_2 of muscle tissues acclimated to 5°C (circles), 15°C (triangles) and 25°C (squares) over the entire experimental range. Vertical bars represent ±2 S.E. of the mean.

When animals were transferred from 15 to 5°C the QO_2 fell to a level which could not be measured (Q_{10} of 4.39). After a period of 21 days or more at 5°C the QO_2 rose to a mean value of 450 µl/hr per g dry

Table 1. Comparison of mean QO_2 of gill samples measured at the acclimation temperatures

Acclimation temperature (°C)	Mean QO_2 ±2 S.E.	Value of *P* vs. 5°C	vs. 25°C
5	450 ± 16		0.001
15	560 ± 29	0.01	0.001
25	1272 ± 63	0.001	

Table 2. Comparison of mean QO_2 of gill samples measured at 15°C

Acclimation temperature (°C)	Mean QO_2 ±2 S. E.	Value of *P* vs. 5°C	vs. 25°C
5	1523 ± 70		0.001
15	560 ± 29	0.001	0.001
25	150 ± 9	0.001	

Table 3. Q_{10} values for QO_2 of gill samples within acclimation groups

Acclimation temperature (°C)	Temperature range (°C)	Q_{10}
5	5–25	2.22
15	11–29	2.71
25	13–29	1.60

Table 4. Acclimated Q_{10} values for QO_2 of gill samples

Groups compared (°C)	Q_{10}
5 vs. 15	1.24
15 vs. 25	2.27

Table 7. Acclimated Q_{10} values for QO_2 of mantle samples

Groups compared (°C)	Q_{10}
5 vs. 15	4.16
15 vs. 25	2.70

Table 8. Q_{10} values for QO_2 of mantle samples within acclimation groups

Acclimation temperature (°C)	Temperature range (°C)	Q_{10}
5	5–27	3.93
15	11–27	3.95
25	13–27	4.22

wt. This value is significantly different from that obtained for 15°C acclimated tissues at 15°C (Table 1). The acclimated Q_{10} value between 5 and 15°C acclimated tissues is 1.24. The QO_2 of 5°C acclimated tissues when measured at 15°C is significantly different from that of 15°C controls (Tables 2 and 4). These results indicate at least partial acclimation. The entire curve for QO_2 of gill tissues from 5°C acclimated animals is displaced upward of the curve for 15°C controls. This translation of the rate–temperature curve probably indicates a change in the levels of enzyme activity (Prosser, 1973).

When oysters from 15°C were warmed the QO_2 of gill tissue increased with a Q_{10} of 2.79. After a period of 21 days or more at 25°C the QO_2 fell to a mean value of 1272 which is significantly different from that of 15°C acclimated tissues at 15°C. The acclimated Q_{10} value is 2.27 (Tables 1 and 4). Both of these results suggest that little acclimation has occurred.

Responses of mantle tissue to altered temperature are shown in Fig. 2. Upon transfer to cold water (5°C) the QO_2 dropped to an immeasurable level. Following 21 days or more of acclimation the QO_2 rose to 68 μl/hr per g dry wt. This value is significantly different from that obtained in 15°C acclimated mantle tissue when measured at 15°C (Table 5). In addition, QO_2 values measured at the control temperature (Table 6) showed significant differences between 5 and 15°C acclimated groups. These results, along with the acclimated Q_{10} values presented in Table 7, and the upward displacement of the rate–temperature curve lead to the conclusion that some compensation took place. Similar results obtained in tissue samples from warm-acclimated animals (Tables 5–7) indicate that partial acclimation possibly occurred but, as in the previous group, was far from complete.

Similar Q_{10} values over the respective recording ranges within acclimation groups (Table 8) indicates lack of rotational change and, therefore, little reliance on alternate enzyme pathways (Prosser, 1973).

In none of the three muscle acclimation groups could reliable data be obtained below 13°C. Table 9 shows statistically similar post-acclimation QO_2 values at 15°C for all three muscle tissue groups. These results, along with the similar Q_{10} values (Table 10) and lack of translational change imply lack of acclimation. Of interest is the fact that the QO_2 for

Table 5. Comparison of mean QO_2 of mantle samples measured at the acclimation temperatures

Acclimation temperature (°C)	Mean QO_2 ±2 S.E.	Value of P vs. 5°C	Value of P vs. 25°C
5	68 ± 9		0.001
15	283 ± 17	0.001	0.001
25	651 ± 32	0.001	

Table 6. Comparison of mean QO_2 of mantle samples measured at 15°C

Acclimation temperature (°C)	Mean QO_2 ±2 S.E.	Value of P vs. 5°C	Value of P vs. 25°C
5	683 ± 40		0.001
15	283 ± 17	0.001	0.001
25	99 ± 13	0.001	

Table 9. Comparison of mean QO_2 of muscle samples measured at 15°C

Acclimation temperature (°C)	Mean QO_2 ±2 S.E.	Value of P vs. 5°C	Value of P vs. 25°C
5	44 ± 6		0.9
15	43 ± 10	0.9	0.9
25	45 ± 8	0.9	

Table 10. Q_{10} values for QO_2 of muscle samples within acclimation groups

Acclimation temperature (°C)	Temperature range (°C)	Q_{10}
5	13–30	3.78
15	13–30	3.87
25	13–30	3.78

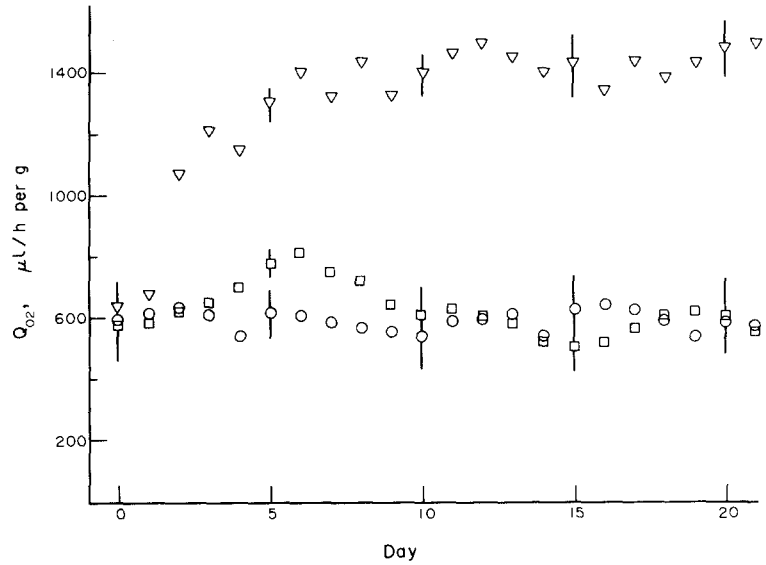

Fig. 4. Mean QO_2 of gill tissues acclimated to 10‰ (triangles), 20‰ (circles) and 30‰ (squares) salinity during the 21 day experimental period. Vertical bars represent ±2 S.E. of the mean.

all three muscle tissue groups remained quite low between 13 and 23°C (2.20 for 15°C acclimated animals) but increased markedly between 23 and 30°C (8.73 for 15°C acclimated animals).

Salinity studies

Figure 4 illustrates the responses of gill tissue to increased and decreased salinities. One day prior to the salinity change the QO_2 of tissue samples from the three tanks showed no significant differences. By the second day following alteration of salinity the QO_2 of gill tissues from animals in dilute sea water exhibited an increase. The differences in QO_2 of tissues from dilute (10‰) sea water and "control" (20‰) tanks became larger on subsequent days and remained significant (Table 11) throughout the duration of the experiment. Gill tissues from animals transferred to concentrated (30‰) sea water showed only a transient difference in QO_2 from that of "controls" which disappeared by day 10.

Table 11. Comparison of mean QO_2 of gill samples from different salinities

Day	Salinity groups compared (‰)	Value of *P*
0	10 vs. 20	NS*
	10 vs. 30	NS
	20 vs. 30	NS
5	10 vs. 20	0.001
	10 vs. 30	0.001
	20 vs. 30	0.001
10	10 vs. 20	0.001
	10 vs. 30	0.001
	20 vs. 30	NS
15	10 vs. 20	0.001
	10 vs. 30	0.001
	20 vs. 30	NS
20	10 vs. 20	0.001
	20 vs. 30	NS

*NS = not significant.

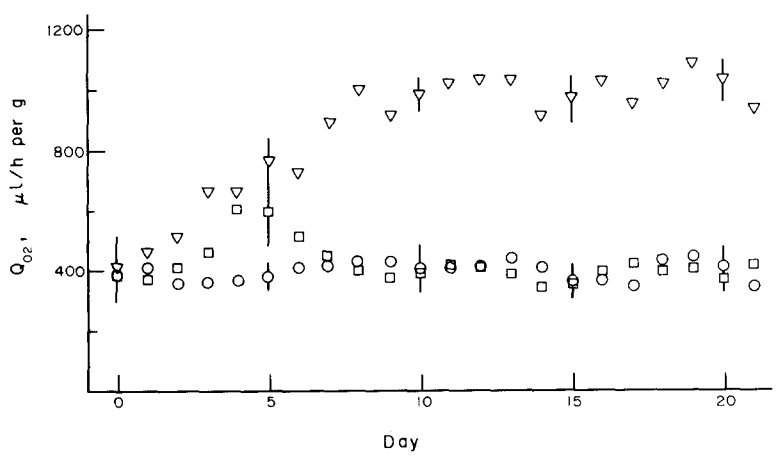

Fig. 5. Mean QO_2 of mantle tissues acclimated to 10‰ (triangles), 20‰ (circles) and 30‰ (squares) salinity during the 21 day experimental period. Vertical bars represent ±2 S.E. of the mean.

Table 12. Comparison of mean QO_2 of mantle samples from different salinities

Day	Salinity groups compared (‰)	Value of *P*
0	10 vs. 20	NS*
	10 vs. 30	NS
	20 vs. 30	NS
5	10 vs. 20	0.001
	10 vs. 30	0.02
	20 vs. 30	0.01
10	10 vs. 20	0.001
	10 vs. 30	0.001
	20 vs. 30	NS
15	10 vs. 20	0.001
	10 vs. 30	0.001
	20 vs. 30	NS
20	10 vs. 20	0.001
	10 vs. 30	0.001
	20 vs. 30	NS

*NS = not significant.

Responses of mantle tissues to changes in salinity are illustrated in Fig. 5 and in Table 12. After salinity was increased QO_2 of experimental tissues exhibited a significant increase by day 4. However, this increase proved to be transient in nature. By day 5 tissues from animals in dilute sea water exhibited a significantly higher QO_2 than mantle samples from the other two groups. An elevated level of oxygen consumption was then maintained throughout the duration of the experiment.

In neither of the above two tissues was there evidence of a return to "control" values so long as the animals were maintained in a dilute sea water environment.

As shown in Fig. 6 QO_2 of muscle tissues exhibited no tendency to increase or decrease in either dilute or concentrated sea water throughout the 21 day experimental period.

DISCUSSION

Several bivalve species have been shown to exhibit various levels of acclimation in the face of altered environmental conditions (Prosser & Brown, 1961; Kennedy & Mihursky, 1972; Bayne, 1973; Ansell & Sivadas, 1974).

In this study excised gill, mantle and muscle tissues of *Crassostrea virginica* exhibited diverse responses to changes in temperature and salinity. Gill tissue appears to have the greatest ability to compensate for temperature alterations and this ability is more pronounced in cold than in warm surroundings. The shift of the rate–temperature curve to the left following cold acclimation, and to the right following warm acclimation is a common response pattern in isolated gills of intertidal bivalves (Prosser, 1973; Vernberg *et al.*, 1963).

Mantle tissue showed little compensatory ability and muscle virtually none. An interesting phenomenon shown by the latter tissue is the low Q_{10} values over the lower part of the temperature range. This is a common ecological pattern shown by animals subjected to regular, cyclical temperature changes such as those associated with tidal ebb and flow (Newell, 1973).

The depression of respiration in cold-acclimated tissues at high temperatures (gill tissue, Fig. 1) parallels the metabolic depression noted by Kennedy and Mihursky (1972) in intact, cold-acclimated *Mya* and *Mulinia* when exposed to high (30°C) ambient temperatures.

The possible biochemical mechanisms for cellular acclimation to temperature have been reviewed extensively by Hochachka (1967), Hochachka & Somero (1973), Newell (1969, 1973), Newell & Pye (1971a,b) and Somero (1969).

In none of the tissues tested in the present study was there a significant rotational change in the rate–temperature curve, suggesting that reliance on alternate enzymatic pathways as a response mechanism was minimal. However, other studies have shown that the diversity of mechanisms for coping with temperature stress includes resorting to alternative, and possibly anaerobic, pathways (Dean & Goodnight, 1964; Hochachka & Hayes, 1962; Hochachka & Somero, 1973; Prosser, 1973).

Isolated gill and mantle tissues from animals acclimated to 20‰ and transferred to 10‰ salinity evidenced a significant and sustained increase in QO_2. A similar response has been noted in the gills of the blue crab (*Callinectes sapidus*) by Engel *et al.* (1975) and in gill tissues of the oyster (*Crassostrea virginica*) by Percy *et al.* (1971).

Muscle tissues showed no evidence of increased QO_2 when transferred to dilute sea water (10‰).

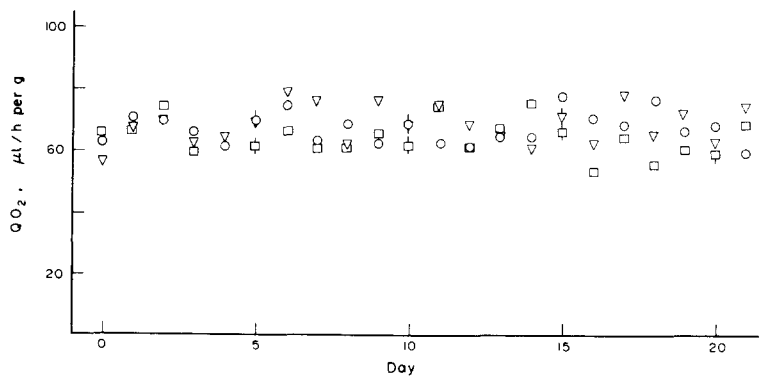

Fig. 6. Mean QO_2 of muscle tissues acclimated to 10‰ (triangles), 20‰ (circles) and 30‰ (squares) salinity during the 21 day experimental period. Vertical bars represent ±2 S.E. of the mean.

When animals were transferred to sea water of increased salinity (30‰) none of the three tissues exhibited a significant, sustained alteration in respiratory rate.

The responses of individual tissues do not always parallel those of intact animals. Bayne (1973) found that mussels (*Mytilus edulis*) recovered from reduced respiratory rates induced by diluting the sea water medium within 24–36 hr. Berger *et al.* (1970) noted that *Mytilus edulis* as well as two species of *Littorina* exhibited a decrease in respiration, following changes in salinity, which was only partially restored over a 10–14 day period.

Gilles (1973) postulated that the accelerated respiratory rates observed in animals transferred to dilute sea water media may be related to catabolism of free amino acids during the acclimation period. Utilization of intracellular free amino acids in osmotic regulation has also been described by Baginski & Pierce (1975) and by Pierce & Greenberg (1973).

The results of this study, along with those of others, show that the interactions between temperature or salinity and respiration may be quite complex and varied even in closely related species. The changes in respiratory rates produced by salinity variations may reflect energetic requirements for osmotic work, changes in water content of the tissues and even hormonal or enzymatic interactions within cells (Vernberg & Vernberg, 1972).

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