OXYGEN CONSUMPTION BY <u>SPHAERIUM</u> <u>SIMILE</u>

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by

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INTRODUCTION

Since 1914 information has been accumulated concerning metabolic adaptions of poikilotherms to their environments (for reviews see Bullock, 1955; Prosser, 1955; Prosser and Brown, 1961). There has been a growing interest since 1935 in the respiratory rate fluctuations of molluscs in response to environmental factors such as temperature, lowered ambient oxygen tension and radioisotope pollution. Knowledge of freshwater species respiratory rates has been limited since most studies have centered primarily on intertidal species reactions.

The usual pattern of adaptation for marine species is that oxygen consumption tends toward some constant rate independent of environmental temperature, within certain limits (Read, 1962). Bayne (1967) working with _Mytilus perna_ and Coleman (1973) with _M. edulis_ found those organisms to be insensitive to temperature changes. Marine poikilotherms seem to compensate for temperature by a homeostatic mechanism (Read, 1962). However, this mechanism, or adaptation, has not been extensively studied in freshwater molluscs.

Available evidence indicates that some lamellibranchs can regulate oxygen consumption under reduced oxygen tensions. Prosser and Brown (1961) termed these animals "regulators". All marine species studied had this ability, including _Mya arenaria_ (van Dam, 1935), _Pecten grandis_ (van Dam, 1954),

Ostrea edulis (Gaarder and Eliasson, 1954), Mytilus perna
(Bayne, 1967), Arctica islandica L., Mytilus edulis L.,
Laevicardium crassum Gmeim (Bayne, 1971), Geloina ceylonica
and Anadora granosa (Bayne, 1972). Little information is
available on oxygen tension effects on freshwater bivalves.
Experimental data has been collected largely from Anodonta
cygnea L. (Salanki and Lukacsovias, 1967), Pleurobema
coccineum Conrad (Badman and Chin, 1973) and Dreissenia gill
tissue (Wernstadt, 1944).

The potential for small amounts of radiation to leak
into the freshwater system has become greater with increased
use of radiosiotopes. Few studies have attempted to measure
what effect this radiation might have upon aquatic organisms.
Price (1965) observed radionuclide uptake and accumulation
in the hardclam (Mercenaria mercenaria Linne), the oyster
(Crassostrea virginica Gmelin), and the bay scallop
(Aequipecten irradians Say). Following exposure to small
amounts of gamma irradiation, Mix (1972) found chronic gill
degeneration in the Pacific oyster (Crassostrea gigas).
Angelovic and Engel (1968) reported alteration of respiration
rates after irradiation in the brine shrimp (Artemia salina)
nauplii. As more radioisotopes are used, primarily in energy
production, effects of exposure should also be studied on
freshwater organisms.

Although Sphaeriidae are abundant in streams, rivers,
ponds and lakes, their physiology has not been extensively

studied. Brooks and Herrington (1944) and Foster (1932) studied life histories, but no published reports on the respiratory responses of Sphaeriidae to their environment were found.

The following study was designed to measure the oxygen consumption of the fingernail clam, <u>Sphaerium</u> <u>simile</u> Say (Burch, 1972), under the following conditions: change of temperature, lowered oxygen tension, and gamma radiation exposure. Badman and Chin (1973) and Coleman (1973) reported extreme variation in whole organism rates of <u>Pleurobema</u> <u>coccineum</u> and <u>Mytilus</u> <u>edulis</u>, while excised gill tissue rates were more consistent. Pennak (1953) stated that respiration exchange was greatest in the gill and mantle tissue of molluscs generally. Therefore, this study also compared excised gill and mantle tissue respiratory rates to that of the whole organism.

METHODS AND MATERIALS

The _Sphaerium_ _simile_ used in this study were collected
from the Neosho River at Neosho Rapids, Kansas, and
Gladfelter Pond, Ross Natural History Reservation, Lyon
County, Kansas. Clams averaged 15 mm in size. They were
acclimated in aquaria filled with pond water at 25 C for a
minimum of one week before measurements were made.

There are many methods for the measurement of oxygen
in an aqueous system. The Winkler method, a well-known
procedure, is subject to errors in standardization and
titration (Carritt and Carpenter, 1966). Microgasometric
determination of dissolved oxygen (Scholander et al., 1955)
presents problems in the physical handling of the gas (Teal,
1971). Although the Warburg respirometer has been used in
many studies, research grade equipment was not readily
available for this study. Perhaps the best way to measure
respiration is by using an electrode which gives a continuous
recording, rather than samples at intervals (Teal, 1971).
Carey and Teal (1965) suggest that the oxygen electrode is
the easiest probe to work with generally. The membrane
covered solid electrode was chosen for this study.

Respiration measurements were made using the Model 53
Biological Monitor System (Yellow Springs Instrument Co.),
including a Model 5331 oxygen probe and Model 5350 membranes.

A Bausch and Lomb Model 10 Strip Chart and a Hitachi Perkin-Elmer Model 159 Recorder were used for continuous rate monitoring.

The Haake Type FE Constant Temperature Water Circulator was placed in a constant temperature box (10±3 C) to cool the water for the low temperature runs. The incoming water was passed through a coiled tubing in an ice water bath and then warmed to the desired temperature.

The Model 5302 Macro Bath attachment (Yellow Springs Instrument Co.) was used for the whole organism rate determination. Excised gill and mantle tissue rates were determined using the Model 5301 Standard Bath Assembly.

Clams were cleaned of algae and placed in 20 ml of distilled water (Macro Bath attachment). Five to 10 clams were used per run. All runs were made in the morning to cancel any diurnal rhythm effects. Using a full range of oxygen tensions (160 to 0 mm Hg), runs were conducted at 15, 20, 25, and 30 C (five runs per temperature). Wet weights were recorded following each run.

Oxygen consumption of the gill and mantle tissue was measured similarly to that of whole clams. The gills and mantle were extracted rapidly and young clams, if present in excised gill tissue, were removed. The gill and mantle tissue was placed in 4.0 ml of distilled water (pH 6.5) on a net screen to prevent homogenation by the stirrer (Standard Bath Assembly). Wet weights were recorded following each run.

Clams were transported to Kansas State University, Department of Nuclear Engineering, where they were exposed to gamma radiation from a Co-60 irradiator. The clams were given a 5 krad $\pm$ 5 % dose at a dose rate of 1600 rad sec^{-1} at room temperature. Oxygen consumption of the whole organism and excised gill and mantle tissue was measured as previously outlined three to seven days after exposure.

The Student t-test at $p=0.05$ was used to determine significant differences among all data sets.

RESULTS AND DISCUSSION

Whole Organism

Results of whole organism respiration rate determinations
were variable (Table 1). Such variation has been reported
in other recent studies. Anodonta exhibits frequent "rest
periods" during which oxygen consumption drops to near zero
(Salanki and Lukacsovias, 1967). Badman and Chin (1973)
reported that variation was due to tissue weight differences
in animals (Pleurobema coccineum) of the same shell size.
They also noticed individual differences in activity, as did
Coleman (1973) in working with Mytilus edulis. The rate
variations recorded with S. simile were within the variation
limits reported by others.

Temperature Effects: Generally, with an increase in
temperature (from 15 to 30 C), there was an increase in
oxygen consumption (Fig. 1). The respiration rate is expressed
as ml O_2 g^{-1} hr^{-1} corrected to standard pressure. The
difference in respiration rates between 15 and 20, 20 and
25, and 15 and 25 C were not significant (Student t-test,
p=0.05). The increased oxygen consumption at 30 C was
significantly different from the other temperatures.

The Q_{10} value is a measure of temperature sensitivity.
Over a specified temperature range, a value of one indicates
that the rate is virtually independent of temperature; a

Table 1. Normal respiration rates of whole organisms with high and low values indicating variation. Q_{O_2} values are expressed in ml O_2 g^{-1} hr^{-1} X 10^{-2}.

| Temperature (C)	Oxygen Tension (mm Hg)	Q_{O_2} Mean	Low	High
15	160-144	3.96	1.86	6.78
	144-128	1.38	1.08	2.16
	128-112	1.26	0.24	1.98
	112- 96	1.86	1.20	3.24
	96- 80	1.74	1.50	2.12
	80- 64	1.44	1.14	1.74
	64- 48	1.32	1.02	1.74
	48- 32	1.26	0.90	1.86
	32- 16	1.44	0.90	1.86
	16- 0	0.90	0.78	0.96
20	160-144	4.50	2.52	6.66
	144-128	3.66	1.86	5.16
	128-112	2.16	1.20	3.72
	112- 96	1.92	0.78	3.12
	96- 80	1.56	0.84	2.46
	80- 64	1.44	0.60	2.04
	64- 48	1.44	0.54	2.10
	48- 32	1.50	0.54	2.16
	32- 16	1.80	1.14	2.22
	16- 0	0.96	0.78	1.08
25	160-144	5.34	2.10	10.86
	144-128	4.44	2.10	10.86
	128-112	4.20	1.02	13.62
	112- 96	4.38	1.08	13.62
	96- 80	3.42	0.96	9.12
	80- 64	2.46	0.96	4.56
	64- 48	2.52	1.14	3.90
	48- 32	2.46	1.32	3.90
	32- 16	2.46	1.68	4.14
	16- 0	0.84	0.48	1.38
30	160-144	7.50	4.20	9.48
	144-128	7.86	4.80	11.52
	128-112	7.50	6.66	8.94
	112- 96	7.44	4.32	10.08
	96- 80	7.56	2.64	10.08
	80- 64	7.38	2.16	10.08
	64- 48	6.96	2.34	10.26
	48- 32	7.14	2.34	11.16
	32- 16	6.84	2.34	11.16
	16- 0	2.22	1.80	2.70
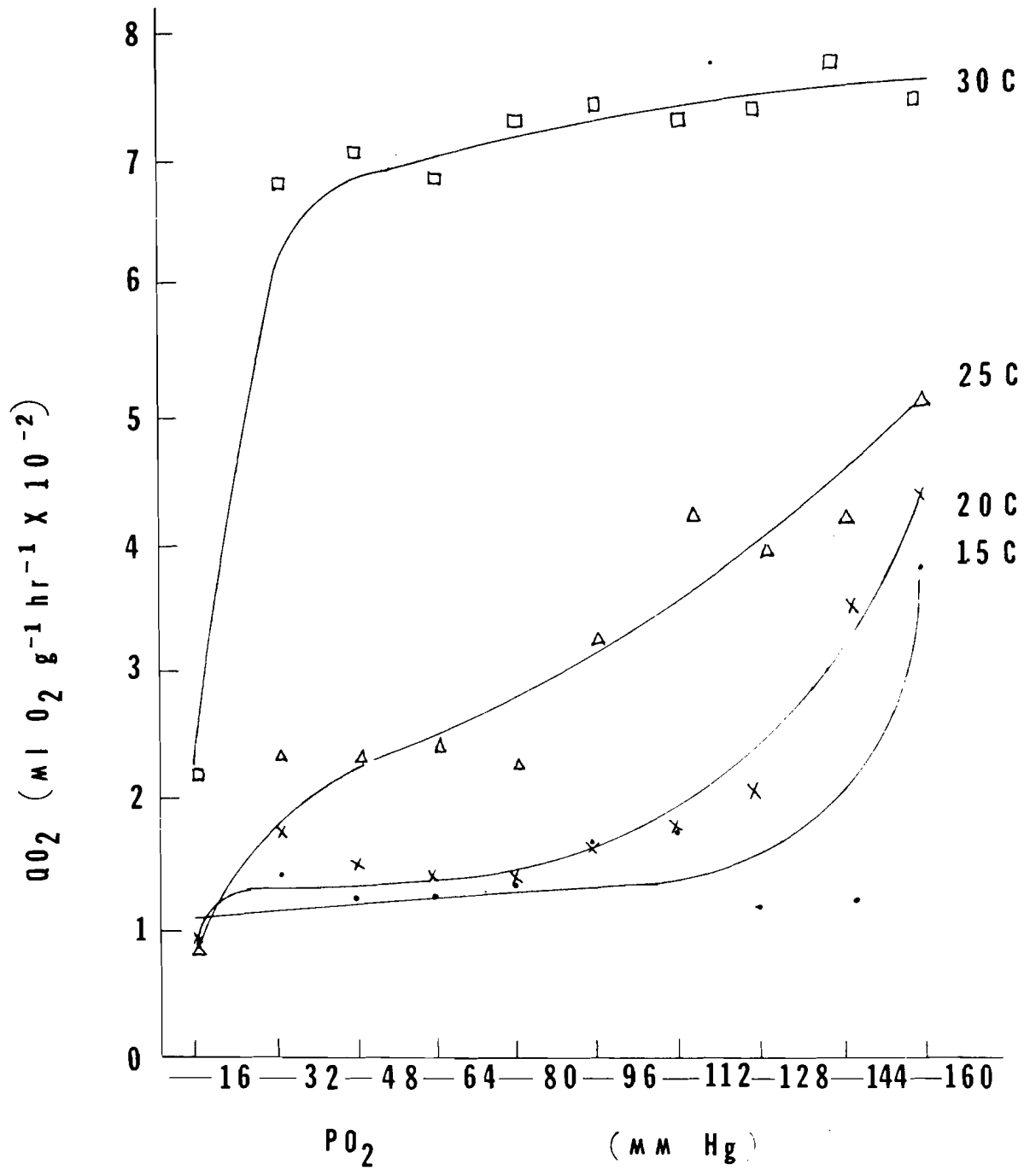
Fig. 1. Mean rate of oxygen consumption by whole organisms.

higher Q_{10} indicates a higher degree of dependency (Coleman,
1973). Q_{10} values for S. simile were calculated for 5 C
temperature-steps. These values increased with temperature
at any given oxygen tension with the exceptions noted in
Table 2. For any given temperature range, there is no
pattern of Q_{10} values throughout the range of oxygen tensions,
a situation also reported by Huebner (1973) for Polinices
duplicatus.

Although Q_{10} values may indicate some sensitivity (Q_{10}
values greater than one) to temperature at 15 to 25 C, there
were no significant differences among oxygen consumption
values. At 30 C, the Q_{10} and Q_{O_2} increased significantly
as compared to the rates at the other temperatures, indicating
a degree of environmental stress at that temperature.

The information accumulated on other poikilotherms
suggests a compensation or homeostatic mechanism may operate
for different environmental temperatures (Bullock, 1955;
Prosser, 1955; Prosser and Brown, 1961). Within the temperature
range of 15 and 25 C, the rate of oxygen consumption might
be attributed to this postulated homeostatic mechanism since
the differences between rates were not significant. At 30 C
the organism's ability to cope with temperature stress was
exceeded, and repiration rates increased rapidly.

The low temperature range (0 to 10 C) was not studied.
Initial attempts at 10 C were fruitless when problems were
encountered with the oxygen probe's sensitivity, and the

Table 2. The Q_{10} values for whole organisms before and after irradiation. Measurements of irradiated organisms were made three to seven days after exposure.

Temperature (C)	Oxygen Tension (mm Hg)	Q_{10}	
		Normal	Irradiated
15-20	160-144	1.30	2.13
	144-128	7.02*	2.25
	128-112	2.89	1.64
	112- 96	1.06	0.88
	96- 80	0.81	0.62
	80- 64	0.00	0.81
	64- 48	1.19	0.79
	48- 32	1.42	0.62
	32- 16	1.56	0.77
	16- 0	1.15	0.77
20-25	160-144	1.42	1.56
	144-128	1.49	1.23
	128-112	3.80*	2.62
	112- 96	5.20*	3.50
	96- 80	4.75	5.02
	80- 64	2.93	4.00
	64- 48	3.06	4.67
	48- 32	2.82	4.75
	32- 16	1.88	4.58
	16- 0	0.77*	2.72
25-30	160-144	1.99	1.64
	144-128	3.13	2.50
	128-112	3.21	2.82
	112- 96	2.89	3.20
	96- 80	4.88	2.86
	80- 64	9.00	3.88
	64- 48	7.62	3.03
	48- 32	8.35	4.00
	32- 16	7.73	2.62
	16- 0	7.02	0.92

* indicates the exceptions to the trend of increased Q_{10} values with temperature for the normal organisms.

relative inactivity of the clams.

Effects of Oxygen Tension: Fig. 1 represents the
results of the experiments relating oxygen consumption to
oxygen tension. The clams exhibited a relatively constant
uptake of oxygen through the range of 32 to 128 mm Hg for
the temperatures 15 and 20 C, and from 32 to 160 mm Hg for
30 C. At 20, 25 and 30 C there was a significant decrease
in respiration rates from 32 to 16 mm Hg (Student t-test,
p=0.05). This value, according to Prosser and Brown (1961),
is the critical pressure (P_c). Critical pressure is that
oxygen tension below which the organism's oxygen consumption
declines rapidly. At 15 C there was no sharp drop in
oxygen consumption.

In oxygen regulating animals, the metabolism-oxygen
curve is often hyperbolic (Tang, 1933), of the form:

$$Q_{O_2} = \frac{pO_2}{K_1 + K_2(pO_2)}$$

in which Q_{O_2} represents ml O_2 g^{-1} hr^{-1}; pO_2 is in mm Hg;
K_1 and K_2 are constants. When pO_2/Q_{O_2} is plotted against
pO_2, a straight line results and K_1 is the intercept and
K_2 is the slope.

Plots of pO_2/Q_{O_2} were made for 15, 20, 25 and 30 C
(Fig. 2). The line was placed using the method of least
squares. From these plots, K_1 and K_2 of Tang's equation
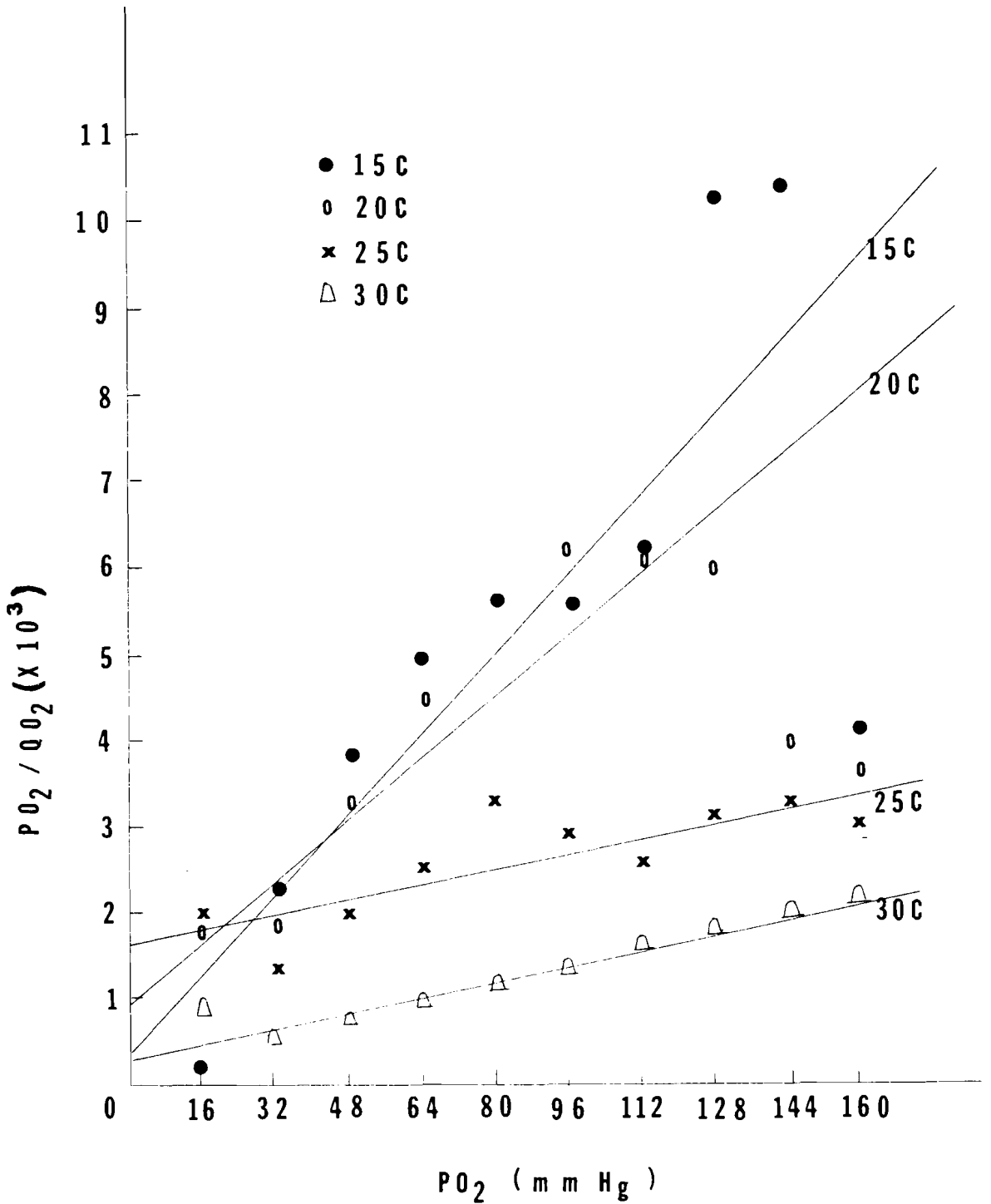were derived.

Fig. 2. pO_2/Q_{O_2} vs pO_2 to obtain K_1 and K_2 values for the oxygen dependency index for whole organisms.

Bayne (1971) derived an index of dependence of oxygen consumption on oxygen tension using the ratio K_1/K_2. A high value for this ratio indicates a greater dependence on oxygen tension or a decreased ability to regulate consumption. Lower values represent lesser dependence. K_1/K_2 values for S. simile (Table 3) indicated increasing values to 25 C and a decrease at 30 C. According to the index, S. simile was most dependent on oxygen tension at 25 C.

Prosser and Brown (1961) term animals that regulate oxygen consumption down to the critical pressure as "regulators"; other animals are termed "conformers" because their oxygen consumption increases with oxygen concentrations. S. simile would be considered a "regulator" except at 25 C where it approaches the status of "conformer" within the range from 48 to 160 mm Hg.

The fact that S. simile is a "regulator" indicates, again, that some homeostatic mechanism may be functioning in the whole organism. Why S. simile is sensitive to oxygen tension at 25 C and not at 30 C is not known. At 30 C, the temperature stress may play a part in the different response to oxygen tension.

Effects of Gamma Irradiation: Three to seven days after exposure to 5 krads of gamma irradiation, the S. simile appeared to have a higher death rate in the holding tanks than did nonirradiated organisms. This increased mortality

Table 3. K_1/K_2 indices of oxygen dependence for the whole organism before and after irradiation. Measurements for the irradiated organisms were made three to seven days after exposure.

Temperature (C)	K_1/K_2	
	Normal	Irradiated
15	5.34	30.0
20	17.8	23.75
25	144.5	34.05
30	23.9	0.0009

rate was not quantified.

Oxygen consumption after irradiation (Fig. 3) when compared with normal oxygen consumption (Fig. 1) is slightly variant. According to the Student t-test (p=0.05), changes in oxygen consumption from normal to irradiated organisms at a given temperature are not significant, with the exception of the value at 16 mm Hg which occurred at 30 C being significantly lower after irradiation.

Q_{10} values (Table 2) for the 15 to 20 C temperature-step indicated a decreased sensitivity to temperature for irradiated organisms for most of the ranges of oxygen tension (values below 1.0). No pattern between irradiated and nonirradiated organisms was present for the 20 to 25 C temperature-step. Q_{10} values for the 25 to 30 C temperature-step were lower after irradiation with only one exception (value for 112 to 96 mm Hg). After irradiation the pattern of increasing Q_{10} values with temperature at any given oxygen tension range were destroyed.

Not only were the Q_{10} values of irradiated clams lower than those of normal clams at the 25 to 30 C temperature-step, but the Q_{O_2} values for 30 C were consistently lower also after irradiation. This trend may indicate that temperature stress plus cell damage due to irradiation results in decreased ability to withdraw oxygen and hence a lowered respiration rate. Angelovic and Engel (1968) found _Artemia salina_ nauplii respiratory rates significantly lower at
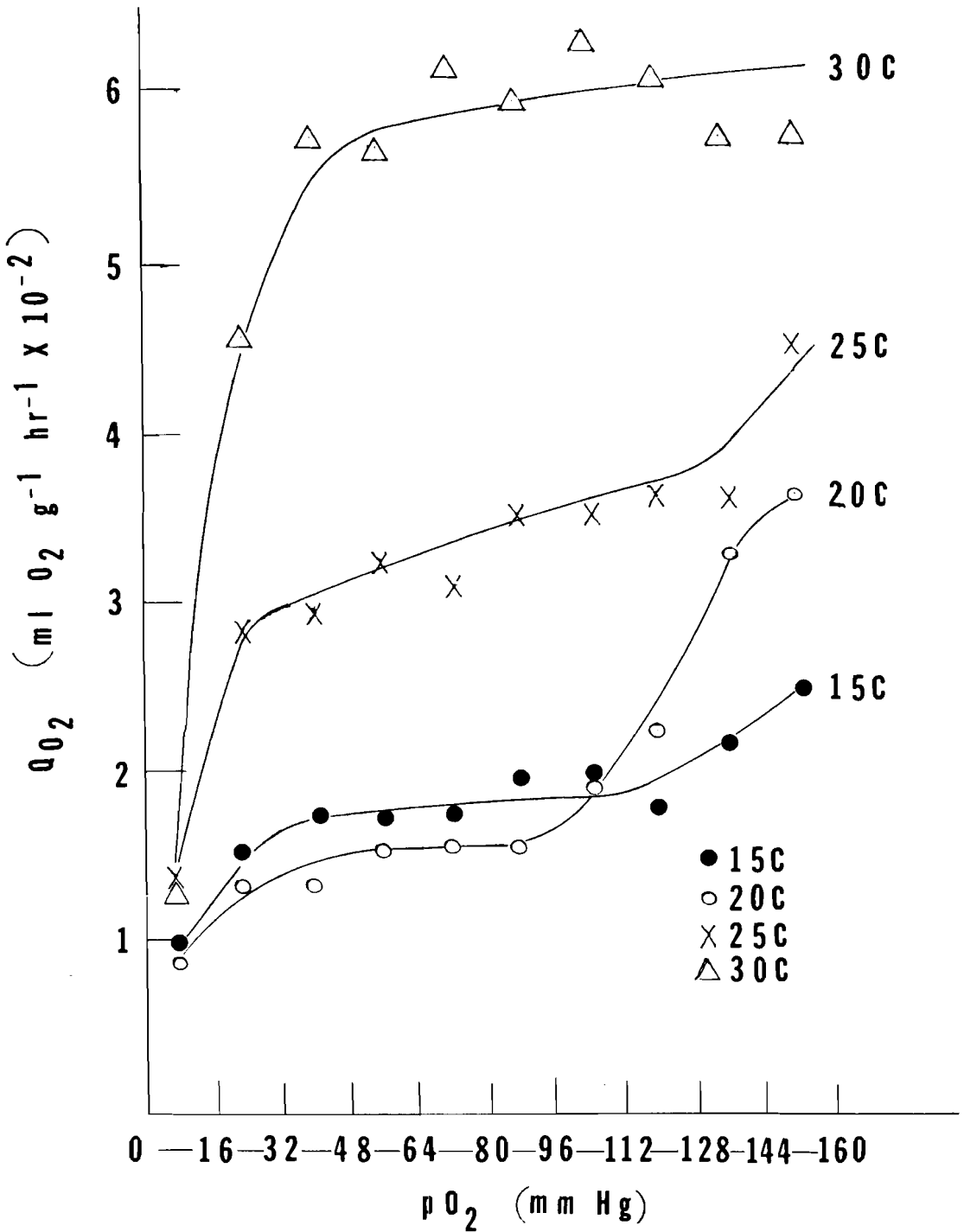
Fig. 3. Mean oxygen consumption in the whole organism three to seven days after irradiation.

certain salinities after irradiation. They speculated that the radiation damaged ion transport by organelles in the gill and thus decreased their osmoregulation ability.

Fig. 3 illustrates that a critical pressure (P_c) is present at all four temperatures studied. Critical tension for 15, 20 and 25 C is approximately 16 to 32 mm Hg, which is the same critical tension that nonirradiated organisms had at 20, 25 and 30 C. However, the critical pressure for 30 C has been shifted to approximately 48 mm Hg as compared to 16 to 32 mm Hg for normal animals. This shift means that metabolism is limited by oxygen tension at an earlier stage than nonirradiated individuals.

At 25 C, the oxygen consumption curve changed from approximately a straight line response in normal organisms to a plateau response in irradiated organisms (Figs. 1 and 3). The irradiated organism responded more as a "regulator" rather than as a "conformer" as defined by Prosser and Brown (1961).

Using Bayne's (1971) oxygen dependence index (K_1/K_2), the values, derived from the plots shown in Fig. 4, increased for irradiated organisms at 15 and 20 C over the values for normal individuals (Table 3). However, at 25 and 30 C, the K_1/K_2 values were lower. After irradiation, oxygen tension may be more of a limiting factor on metabolism at the lower temperatures. However, at the higher temperatures the radiation damage may limit metabolism more than oxygen tension.
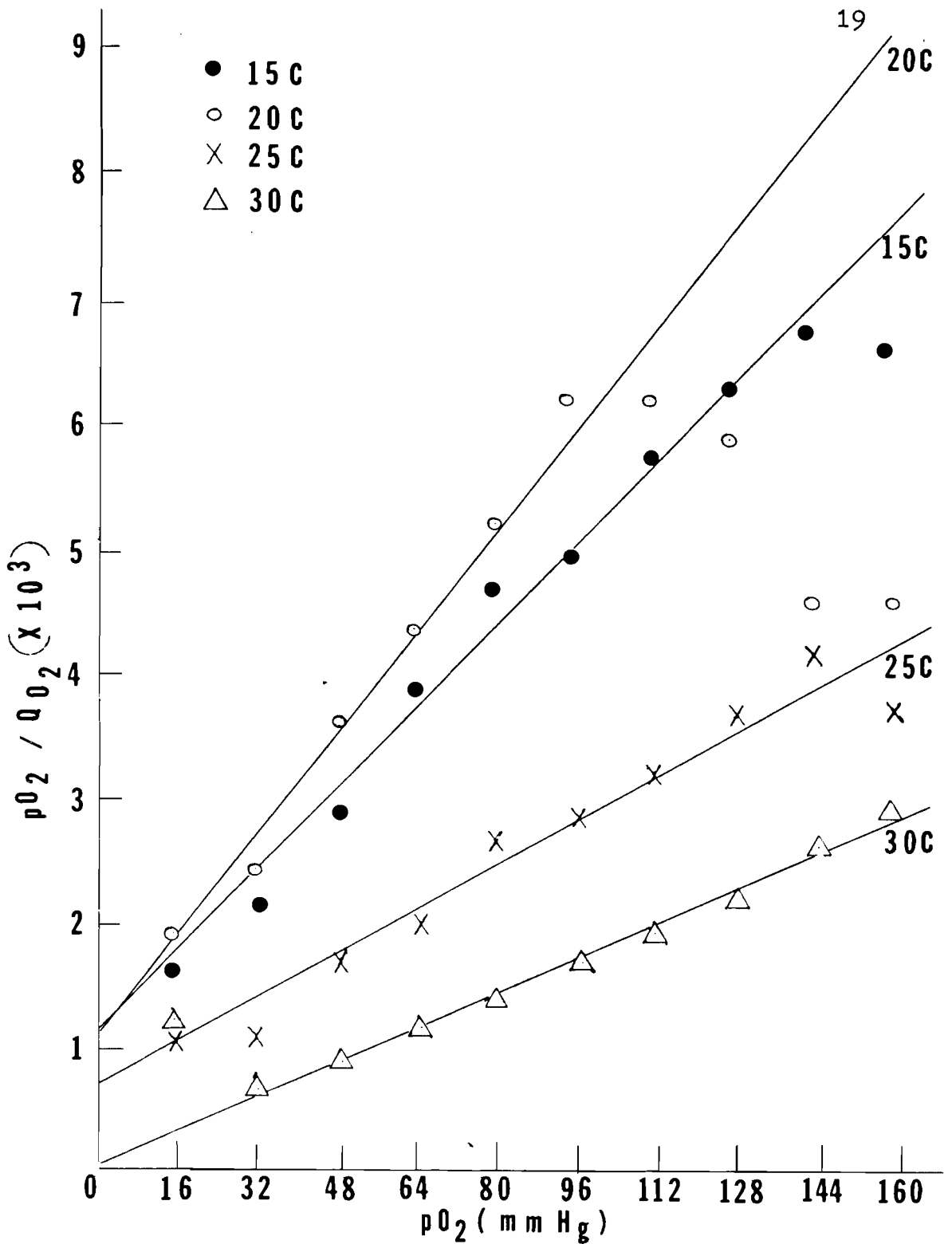
Fig. 4. pO_2/Q_{O_2} vs pO_2 to obtain the K_1 and K_2 values for the oxygen dependency index for whole organisms three to seven days after exposure.

Excised Gill and Mantle Tissue

Gill and mantle tissue rates were less variable than those of the whole organism (Table 4). These data confirm the previous work of Wernstedt (1944) on Dreissenia gill tissue and Badman and Chin (1973) on Pleurobema coccineum.

Temperature Effects: With each increase in temperature from 15 to 25 C, there was a significant increase in oxygen consumption (Fig. 5). Each rate was almost double the rate at the next lower temperature. The rate at 30 C was significantly lower than the rate at 25 C.

Q_{10} values also indicated that gill and mantle tissue rates were sensitive to temperature (Table 5). The tissue exhibited a peak Q_{10} value at 25 C, with lower values at both higher and lower temperatures. This pattern was reported by Read (1962) for Mytilus edulis and Brachidontes demissus plicatulus.

Respiratory rates were entirely temperature dependent. Stress was indicated at 30 C, with a reduction of oxygen consumption. At 30 C the gill and mantle tissue may be suffering from cell, cilia or enzyme damage that inhibits oxygen withdrawal from the water at that temperature.

Effects of Oxygen Tension: Fig. 5 indicates that the excised gill and mantle tissue was totally independent of oxygen tension. The excised tissue exhibited a constant oxygen uptake throughout the tests.

Table 4. Normal respiration rates of excised gill and mantle tissue with high and low values indicating variance. Q_{O_2} values are expressed in ml O_2 g^{-1} hr^{-1} X 10^{-2}.

Temperature (C)	Oxygen Tension (mm Hg)	Q_{O_2} Mean	Low	High
15	160-144	7.74	6.30	12.12
	144-128	7.68	6.30	12.12
	128-112	7.68	6.24	12.12
	112- 96	7.68	6.30	12.12
	96- 80	7.62	6.30	12.12
	80- 64	7.62	6.30	12.12
	64- 48	7.68	6.30	12.12
	48- 32	7.68	6.30	12.12
	32- 16	7.68	6.30	12.12
	16- 0	7.74	6.30	12.12
20	160-144	13.98	11.88	16.44
	144-128	13.92	11.10	16.44
	128-112	13.92	11.10	16.44
	112- 96	13.92	11.10	16.44
	96- 80	14.10	11.88	16.44
	80- 64	13.92	11.10	16.44
	64- 48	13.92	11.10	16.44
	48- 32	13.80	11.10	16.44
	32- 16	13.80	11.10	16.44
	16- 0	13.80	11.10	16.44
25	160-144	30.66	25.98	38.04
	144-128	29.94	25.98	38.04
	128-112	29.94	25.98	38.04
	112- 96	27.72	22.08	38.04
	96- 80	30.66	25.98	38.04
	80- 64	31.56	25.98	38.04
	64- 48	29.94	25.98	38.04
	48- 32	27.72	22.08	38.04
	32- 16	28.32	22.08	38.04
	16- 0	29.88	22.08	38.04
30	160-144	22.86	16.80	28.20
	144-128	22.56	18.00	28.20
	128-112	22.32	18.00	28.20
	112- 96	23.16	18.00	28.20
	96- 80	22.56	18.00	28.20
	80- 64	23.40	18.00	28.20
	64- 48	23.40	18.00	28.20
	48- 32	22.80	18.00	28.20
	32- 16	23.64	18.00	28.20
	16- 0	23.64	18.00	28.20

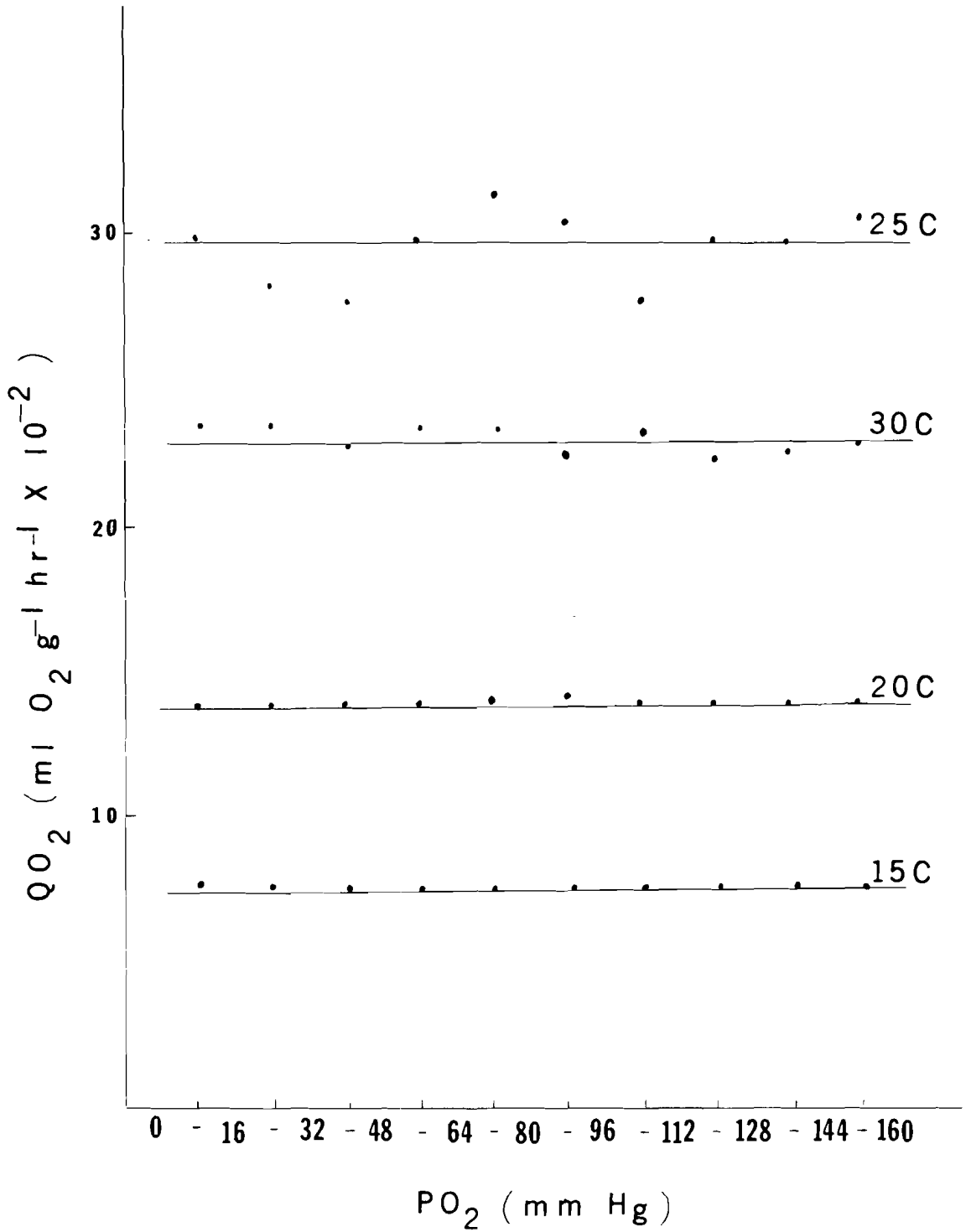Fig. 5. Mean oxygen consumption in excised gill and mantle
         tissue.

Table 5. Q_{10} values for excised gill and mantle tissue before and after irradiation. Q_{10} values for irradiated tissue were determined three to seven days after exposure.

Temperature-Step (C)	Q_{10}	
	Normal	Irradiated
15-20	3.28	3.42
20-25	4.54	5.24
25-30	0.60	0.30

Using Bayne's (1971) index, K_1 and K_2 values were
derived from the plots shown in Fig. 6. The K_1/K_2 indices
are shown in Table 6. According to the index values, the
excised tissue is virtually independent of oxygen tension,
confirming the Q_{O_2} values. The value at 25 C was the
highest for the four temperatures tested.

Excised gill and mantle tissue exhibited no regulation
of oxygen consumption at oxygen tensions. It appeared to
consume oxygen at a steady maximum rate until all the
oxygen was exhausted.

The K_1/K_2 value for 25 C indicates that the gill and
mantle tissue is more sensitive to oxygen tension at that
temperature. This sensitivity may result from temperature
effects on the respiratory pigments.

Effects of Gamma Irradiation: In comparison to
nonirradiated excised tissue, the irradiated tissue oxygen
consumption rates did not vary significantly at 15, 20 and
25 C (Fig. 7). However, at 30 C oxygen consumption after
irradiation was significantly lower than the consumption of
the nonirradiated tissue.

The Q_{10} values (Table 5) were slightly higher after
irradiation for the 15 to 20 C and 20 to 25 C temperature-
steps. According to Coleman (1973), an increased Q_{10} value
indicates a greater temperature sensitivity. The excised
tissue, then at these temperatures, may be more sensitive
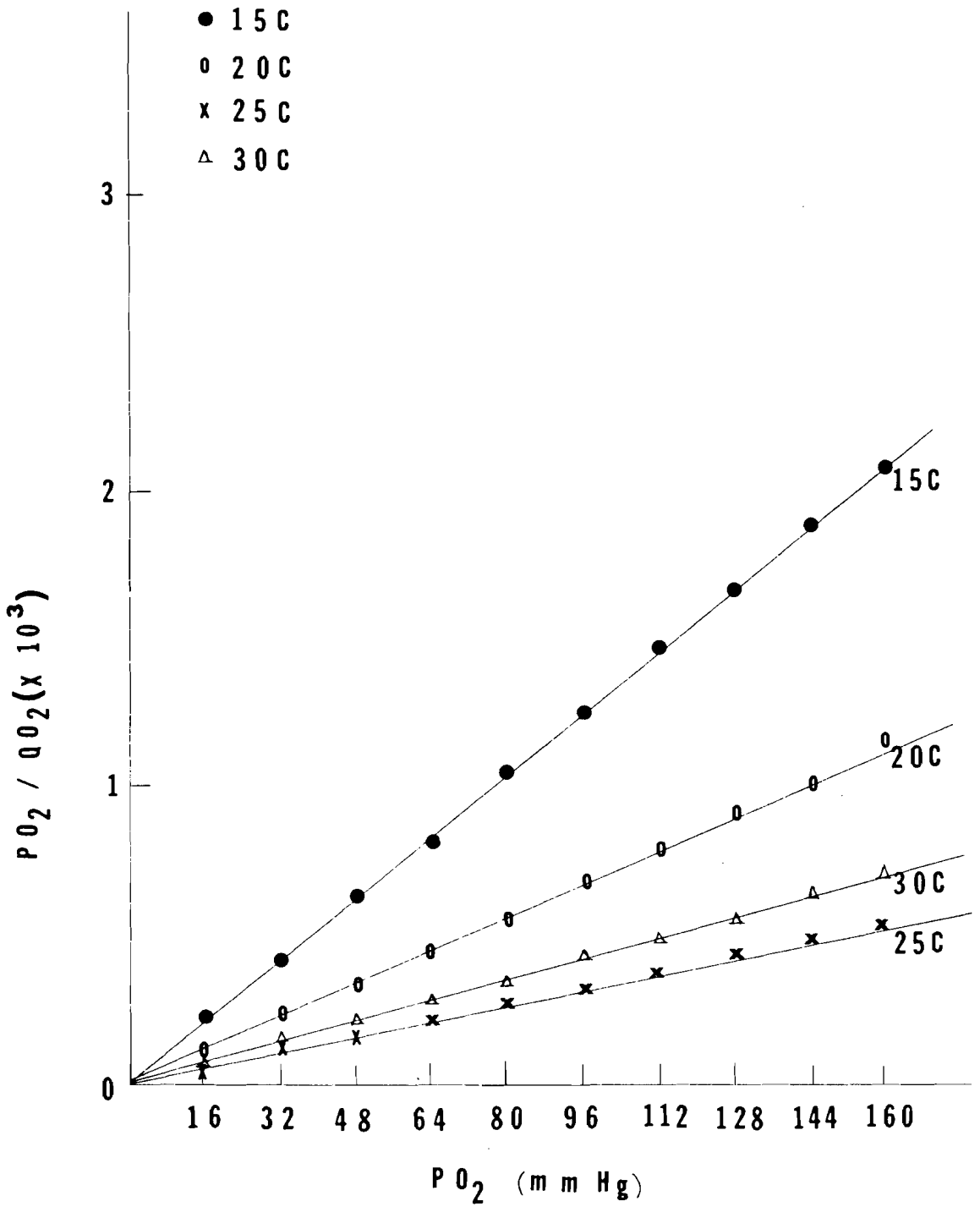to temperature after irradiation.

Fig. 6. pO_2/Q_{O_2} vs pO_2 for excised tissue to obtain K_1 and K_2 values.

Table 6. K_1/K_2 indices of oxygen dependence for excised gill and mantle tissue before and after irradiation. Measurements for the irradiated tissue were made three to seven days after exposure.

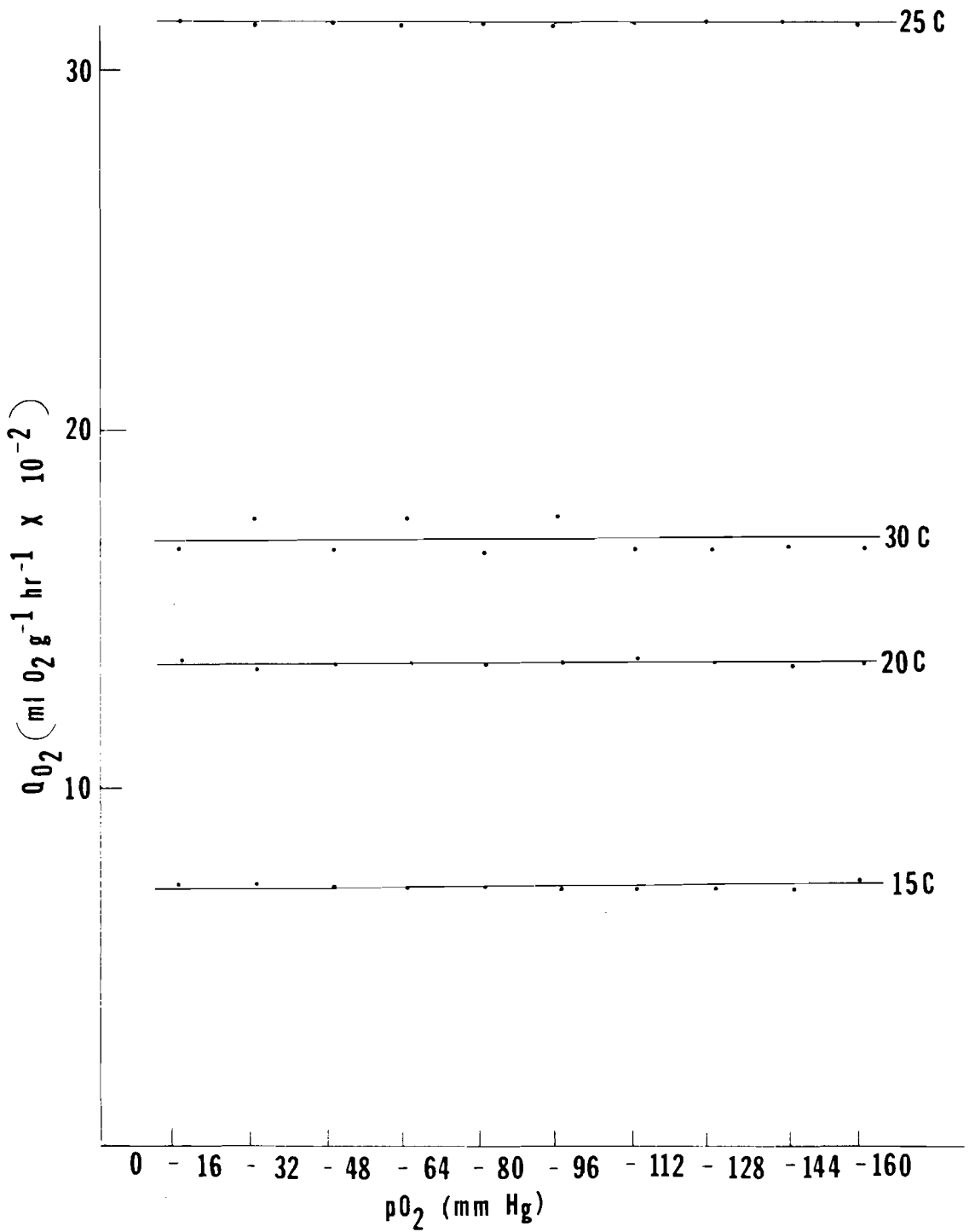| Temperature (C)	K_1/K_2	
	Normal	Irradiated
15	0.38	3.85
20	2.00	4.14
25	11.00	6.00
30	8.00	2.17

Fig. 7. Oxygen consumption (mean) in excised gill and mantle
tissue three to seven days after irradiation.

At the 25 to 30 C temperature-step, the Q_{10} values were approximately one-half that of the normal reflecting the lower Q_{O_2} values at 30 C. In the Pacific oyster (Crassostrea gigas), after irradiation there was an acute tissue response observed one to nine days after exposure, followed by a chronic tissue degeneration 10 to 180 days later (Mix, 1971). The acute tissue response was characterized by leucocytic infiltration and necrotic cells with abnormal nuclei. If S. simile have the same response to irradiation, the inflammation and cell damage under the temperature stress may result in decreased ability to withdraw oxygen.

The excised tissue after irradiation still gives a straight line response to varying oxygen tensions (Fig. 7). Oxygen consumption of the excised tissue remained independent of oxygen tension.

The K_1/K_2 values, derived from the plots of Fig. 8, for the 15 and 20 C temperatures increased over the normal values. The increased value indicates an increased sensitivity to oxygen tension at those temperatures (Bayne, 1971). The values for 25 and 30 C decreased over the normal values, or a decreased sensitivity to oxygen tension. At the higher temperatures, perhaps the irradiation damage is a greater limiting factor that oxygen tension.
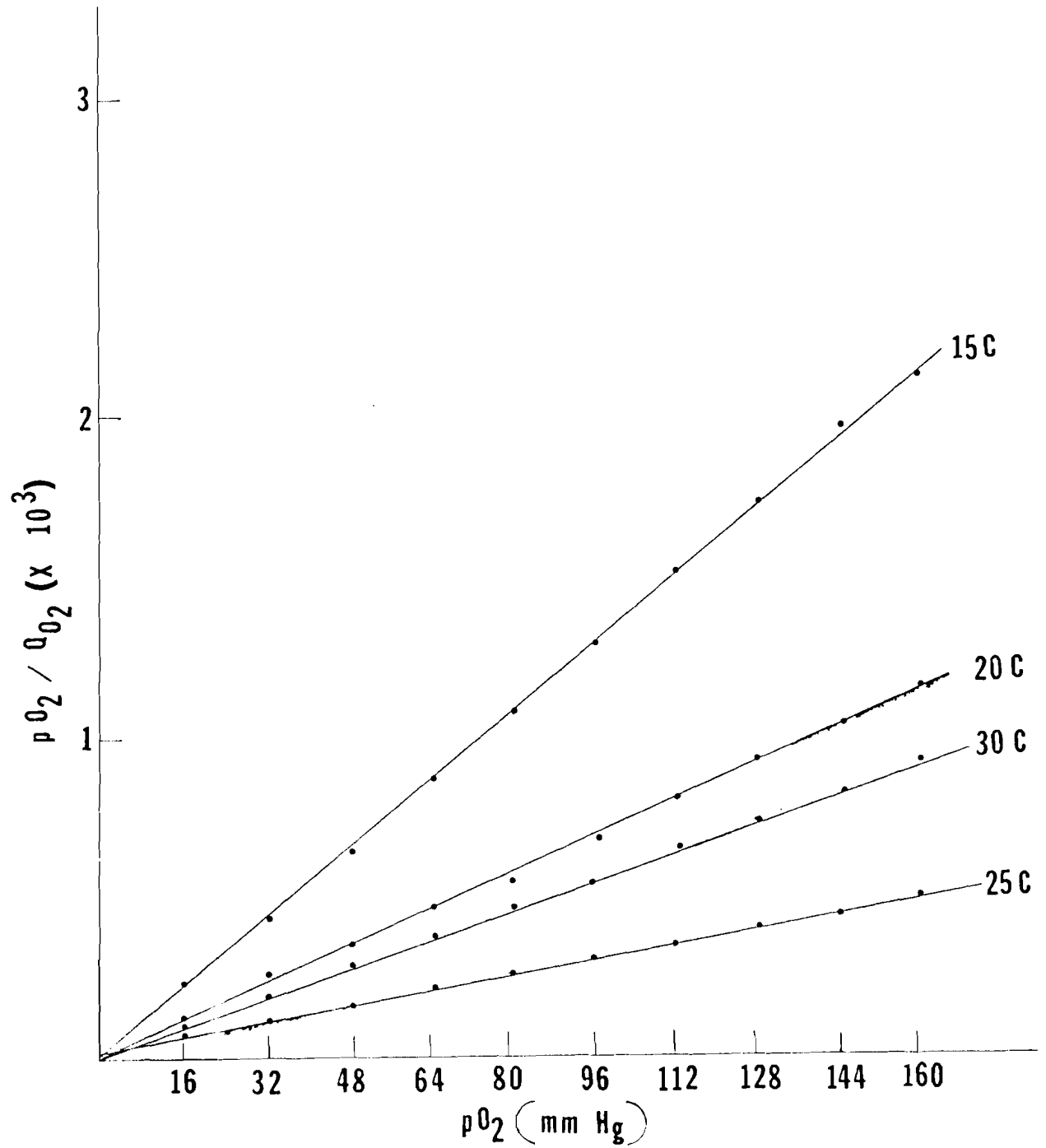
Fig. 8. pO_2/Q_{O_2} vs pO_2 for excised tissue after irradiation to obtain K_1 and K_2 values for the oxygen dependency index.

Comparison of Whole Organism

to Excised Gill and Mantle Tissue

Temperature Effects: Although gill and mantle tissue oxygen consumption increased significantly over the temperature range of 15 to 25 C, the whole organism rates for those temperatures were not significantly different from each other. The difference between the excised tissue and the whole organism may indicate a control mechanism operating in the whole organism to maintain a relatively constant physiological state despite environmental temperature changes. However, when the tissue is removed, the mechanism is destroyed.

Maximal oxygen consumption in excised gill and mantle tissue occurred at 25 C and then dropped at 30 C. In the whole organism at 30 C there was a significant increase in oxygen consumption over 25 C. If 30 C is a stressful situation, and the gill and mantle tissue is being damaged, there may be a mechanism in the whole organism to compensate for this damage. This compensation may be increased withdrawal efficiency or increased water volume passing through the mantle (Bayne, 1967). I doubt that there was an increased withdrawal efficiency if the gill tissue was damaged from the temperature increase. It would be more likely that the whole organism is passing more water through the mantle cavity to obtain the necessary oxygen requirements.

Effects of Oxygen Tension: Whole organism rates, when compared with excised gill and mantle tissue rates, indicated a control mechanism may be present. S. simile appeared to be "regulators", as termed by Prosser and Brown (1961), under varying oxygen tensions, with the exception at 25 C when they approached conformity to oxygen tension. However, when the gill and mantle tissue was excised, the ability for regulation was lost.

K_1/K_2 values showed a greater degree of dependence on oxygen tensions for both whole organisms and excised tissue at 25 C. This sensitivity appears to be within the tissue itself and may be related to the enzyme system.

Effects of Gamma Irradiation: In comparing whole organism and excised tissue consumptions, damage from irradiation becomes apparent at 30 C and not at the other three temperatures. It appears that the gill and mantle tissue suffered radiation damage possibly at the cellular level as described by Mix (1972). The high temperature, already applying stress to the oxygen removal system, in combination with the radiation damage significantly lowered oxygen consumption. This tissue damage became evident in the decreased Q_{O_2} and Q_{10} values.

According to the K_1/K_2 values for the whole organism and excised tissue and the P_c for whole organisms, at 15 and 20 C oxygen tension is becoming more of a limiting factor (Table 6). The irradiation may be damaging not only

the mantle and gill tissue, but also the postulated homeostatic control mechanism.

At 25 C, the K_1/K_2 values decreased for both whole organisms and excised tissue (See Table 6). Oxygen tension dependence decreased, or radiation effects may have become the limiting factor before oxygen tension variation.

At 30 C, P_c for the whole organism after irradiation shifted to approximately 48 mm Hg. However, K_1/K_2 values decreased for both excised tissue and whole organism. Tissue damage due to stress and radiation seems to limit the organism's ability to regulate oxygen consumption at the lower oxygen tensions. The damage and stress may actually prove to be the limiting factor on metabolism through the mid-range of oxygen tensions, decreasing the impact of oxygen tension on the tissue and organism.

This study of gamma effects is limited. Initially the study was to also include a follow-up of radiation effects 18 to 22 days after exposure. However, adverse weather conditions prevented collection of needed S. simile. The study does seem to indicate that small doses of radiation damage the gill's and mantle's ability to function. Further studies should be conducted to determine if the condition is chronic or if there is recovery by the tissue. Experiments should also be implemented to test dosage effects and determine LD_{50}. If S. simile is sensitive to radiation in small amounts, they might be used as environmental indicators of radioisotope leakage into the freshwater system.

SUMMARY

Oxygen consumption by Sphaerium simile was determined
as a function of temperature, oxygen tension and gamma
irradiation. Studies were conducted on both whole organism
and excised gill and mantle tissue.

For the whole organism, the increase from 15 to 25 C
did not significantly increase oxygen consumption; however,
at 30 C there was a significant increase. Under changes of
oxygen tension S. simile was found to be a "regulator",
except at 25 C when they approached oxygen conformity. It
was postulated that a homeostatic mechanism may be in operation.
The temperature of 30 C may be a stressful environmental
condition to the organism.

The whole organsim, after irradiation, had a lower
oxygen consumption at 30 C. The irradiation coupled with
the temperature stress may have decreased the ability of the
organism to withdraw oxygen. The clams were dependent on
oxygen tension at 15 and 20 C more after exposure to
irradiation; they were less dependent on oxygen tension
at 25 and 30 C.

Oxygen consumption of excised tissue was dependent on
temperature and independent of oxygen tension. At 30 C there
was a significant decrease in the respiration rate from
25 C, indicating tissue damage.

After irradiation, the excised tissue remained independent

of oxygen tension. Oxygen consumption decreased significantly
at 30 C from the norm.

Further studies should be conducted to determine the
extent of irradiation on S. simile in the freshwater system.
Suggested studies include: varying dosages, long-term
follow-ups, and LD_{50} determination.

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