

AN ABSTRACT OF THE THESIS OF

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Title RESPIRATORY ADAPTATIONS OF TWO MACRUROUS-  
ANOMURAN MUD SHRIMPS, CALLIANASSA CALIFORNIENSIS AND  
UPOGEBIA PUGETTENSIS ( DECAPODA, THALASSINIDEA)

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Callianassa californiensis Dana and Upogebia pugettensis (Dana) inhabit estuarine mudflats and are subjected to the hypoxic conditions that prevail during tidal exposure.

Metabolic responses for both these species, as indicated by critical oxygen tension, metabolic rate, and tolerance to anoxia were determined. An oxygen macro-electrode and a physiological gas analyzer in conjunction with the sealed jar method were used in all metabolic experiments. The survival time under anoxia was measured. A procedure is also described for counting heart rates of Callianassa. Field samples were analyzed for oxygen content by a micro-Winkler procedure.

The ghost shrimp, Callianassa, and the blue mud shrimp, Upogebia, show metabolic adaptations for living in the mudflat biotope. Both species are good metabolic regulators. Callianassa,

however, has a considerably lower critical oxygen tension (10-20 mmHg) than Upogebia (45-50 mmHg). The mean metabolic rates, within the independent range of respiration, are significantly different at the one percent level of probability. Callianassa has a metabolic rate of 0.024 mls O<sub>2</sub>/gm wet wt/hr compared to 0.050 mls O<sub>2</sub>/gm wet wt/hr for Upogebia. Data are also presented which suggest that postmolt Upogebia are relatively more oxygen dependent than intermolt shrimp and hence temporarily lose their regulatory ability. Heart rates of Callianassa subjected to diminishing oxygen concentrations show a regulatory pattern similar to that of the metabolic rate, with bradycardia occurring at low oxygen tensions. Both species survive anoxia for astonishing periods of time. The mean survival time for Callianassa is approximately 5.7 days and for Upogebia 3.3 days.

It is proposed that the quantitative differences in the metabolic requirements of the two species reflect the availability of oxygen in their respective niches. Despite the fact that muds are generally more anoxic than sandy substrates Upogebia, in fact, has more oxygen available owing to a firmly constructed burrow system. Callianassa burrows are not firmly constructed and the upper reaches of the burrows tend to collapse during ebb tide. Hence, the ghost shrimps are most likely exposed directly to hypoxic interstitial waters. Preliminary field data and ecological observations support

this conclusion. Upogebia burrow water has a mean concentration of 0.58 mls O<sub>2</sub>/l and interstitial water probably less.

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(DECAPODA, THALASSINIDEA)

by

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(DECAPODA, THALASSINIDEA)

INTRODUCTION

Various aspects of respiratory physiology have been extensively investigated in numerous species of crustaceans. The influence of oxygen tension, salinity, body weight, and temperature on metabolic rate have been determined for various members of the Macrura and some astacurans (Wolvekamp and Waterman, 1960; Prosser and Brown, 1961). Crustaceans have been shown to be conformers or regulators of metabolic rate with respect to oxygen tension.

Attempts to correlate the respiratory demands of crustaceans to the availability of oxygen in their habitat have not often been made. However, the metabolic rates of insect nymphs have been shown to correlate with the availability of oxygen in their environments (Fox et al., 1933, 1935, 1937; Walshe, 1948).

There are few studies of anaerobiosis in the higher crustacea. Those few species which have been subjected to experimental conditions of anoxia survive a relatively short period of time (von Brand, 1946).

Published accounts on the respiration of members of the Thalassinidea are virtually non-existent. This is not particularly

surprising since their physiology in general has been neglected. The majority of the extant literature pertains to their taxonomy, paleontology, distribution, and ecology. The ecology of the West coast species Callianassa californiensis and Upogebia pugettensis, is fairly well known due to the significant contributions of Mac Ginitie (1930, 1934, 1935, 1949).

It was the overall intention of the present investigation to understand to what degree ecological factors might correlate with the respiratory physiology of two thalassinid shrimps. It is well substantiated that mudflats are primarily an anaerobic "reducing" environment, and that oxygen availability is presumably a limiting factor to mudflat inhabitants (Krogh, 1941; Brafield, 1964; Pearse, Humm and Wharton, 1942). Hence, it was the purpose of the present investigation to determine the effect of oxygen tension on the metabolic rate of two mudflat shrimps, Callianassa californiensis and Upogebia pugettensis. Tolerance to anoxia and post-anoxic metabolic response were also investigated with the intention of determining their importance in metabolic adaptation.

## MATERIALS AND METHODS

### Collection and Maintenance of Animals

All the experiments on Callianassa and Upogebia were done at the Oregon State University Marine Science Laboratory, Newport, Oregon, from April through August, 1966.

Ghost shrimps, Callianassa californiensis Dana (Stevens, 1928) were collected from the south side of Yaquina Bay directly east of the Oregon State University Marine Science Laboratory. Callianassa californiensis (hereinafter, Callianassa) generally were found between the plus one foot and zero tide levels in predominately sandy substrates. The shrimps could not be readily obtained by digging with a shovel, due to the shifting nature of the sandy substrate. A "shrimp gun", a plastic cylinder (31 x 3 inches) equipped with a plunger, proved most effective in getting the shrimps out of their burrows and the sand. Although the depth at which the shrimps were located was variable, the average depth seemed to be between two and three feet below the surface. A "yabby pump", which operates on the same principle, was used successfully by Hailstone and Stephenson (1961) to collect Callianassa australiensis in Australia.

Blue mud shrimps, Upogebia pugettensis (Dana) (Stevens, 1928) were collected from the north side of Yaquina Bay between Coquille Point and Sally's Slough. Upogebia pugettensis (hereinafter, Upogebia)

were found at a lower tide level than Callianassa, approximately the zero to minus one foot level. The substrate in this area was a mud-clay composite. The Upogebia burrows (Figures 1 and 2) are well constructed tubes with an apparent lining, and in this respect differ from the Callianassa burrows. The latter, when present, are loosely constructed and are not lined. The majority of the blue mud shrimps, especially the larger ones, were located two to three feet below the surface. The "shrimp gun" proved ineffective in dislodging Upogebia from their burrows. Sufficient suction could not be attained with the "shrimp gun". Hence, the blue mud shrimps were obtained by digging.

Immediately after collection the shrimps were taken to the laboratory. General observations as to the number collected, size, sex, and reproductive state of the shrimps were recorded at this time. The stages of the molt cycle could not be accurately determined. No criteria for staging shrimp in the super-family Thalassinidea could be found in the literature. However, postmolt animals could be singled out by their light color, white hairs, and softness of exoskeleton. When possible, postmolt shrimps were excluded from the experiments.

All the shrimps used in the experiments were males, except when otherwise indicated, and were kept in containers previously filled with sand or mud obtained from the respective collecting areas. Typically groups of twelve Callianassa males of approximately the

same size ( 4-7 gms) were placed in rectangular glass aquaria 11 x 7 x 6 inches which were filled with sand to a depth of approximately five inches. Upogebia, on the other hand, are pugnacious and if kept together tend to tear off each other's appendages. Thus, Upogebia (4-8 gms) were placed individually into culture dishes or plastic food freezer containers containing a mud-clay mixture several inches deep. These aquaria and containers were maintained in large tanks of running, aerated sea water.

The salinity and the temperature of the sea water system in the laboratory were measured at least twice daily. A modified Schales method (Schales and Schales, 1941) was used for all salinity determinations. The salinity of the salt water in the laboratory tanks during April, May and June, 1966, ranged from 25.6 ‰ to 34.6 ‰ with a median value of 31.9 ‰. The temperature range in the laboratory tanks during April-May and July-August was from 9° C. to 13° C. The temperature range was slightly higher in June ( 10 -13° C.).

The shrimp were not fed. The unfiltered sea water contained sufficient organic detritus to maintain two aquaria of Callianassa in apparently good condition for as long as three months. The presence of faecal pellets around burrow openings as well as observations that the guts were full (easily noted due to the relative transparency of the exoskeleton) indicated the ghost shrimp were feeding.

Upogebia were not maintained in the laboratory for as long a period; hence, no statement can be made about their survival time under these conditions. Upogebia are not transparent; however the presence of faecal pellets indicated that the blue mud shrimps were also feeding. My observations support MacGinitie's (1930, 1934) conclusion that both species are detritus-feeders, Callianassa sifting the sand, and Upogebia straining the water. No attempts were made to starve the animals. None of the shrimps used for experimental purposes were ever under laboratory conditions over two weeks.

#### Metabolic Rate — Oxygen Tension Measurements

There are several approaches to investigating the effect of oxygen tension on the respiration rate of aquatic animals. The procedure usually employed involves measuring either the oxygen consumption by an animal at predetermined oxygen tensions or measuring the amount of dissolved oxygen remaining in a volume of water as the animal respire (Keys, 1930). The Winkler method has been generally used in such experiments.

In the present study an oxygen macro-electrode (Beckman catalog No. 325814) was used in conjunction with a Beckman Physiological Gas Analyzer Model 160 to determine the oxygen tension in water. This electrode and analyzer system was used to follow the drop in oxygen tension affected by the metabolic rate of an individual shrimp

in a tightly sealed jar. Each shrimp thus served as its own control. The depletion of oxygen as a function of time resulted in a time-tension curve from which the metabolic rate of the whole animal could be calculated.

Essentially, the macro-electrode consists of a fine platinum wire cathode and a silver wire anode electroplated with silver chloride. Electrical contact is established by presence of a KCl gel electrolyte which is in turn held in place by a poly-propylene membrane (0.025 mm thick) permeable only to oxygen. The membrane is tightly stretched over the tip of the electrode and held in place by a rubber O-ring. A new membrane was put on at the beginning of every experiment. The rate of diffusion of oxygen through the membrane will differ to some extent with each new membrane depending on how tightly stretched it is across the electrode tip. Thus, the oxygen electrode and the analyzer were calibrated before each experiment.

Calibration consists of establishing two known reference points on the analyzer panel meter. One point is for zero oxygen and the other point refers to a known oxygen concentration at the high end of the anticipated range of measurements. The oxygen-160 scale was used throughout all the experiments. Calibration solutions were made with sea water whose salinity was identical to that used throughout each experiment. Since the electrode performance varies widely

with temperature, calibration was made at the temperature at which the sample measurements were to be made. All experiments were carried out in a large cylindrical water bath maintained at  $10^{\circ}\text{C.} \pm 0.2^{\circ}\text{C.}$  (Figure 3).

A zero oxygen calibration solution was obtained by using Oxsorbent (Burrell Corp. Catalog No. 39-710), a compound which absorbs oxygen completely from gases and aqueous solutions. An attempt was also made to obtain a zero calibration solution by bubbling with nitrogen gas. However, the chemical method proved easier and gave more consistent results than nitrogen-bubbled water. Compressed air was used to obtain a saturation calibration solution. After establishing the zero reference point, the electrode was placed in the saturated sea water solution and the meter needle adjusted to a previously calculated setting based on the following formula:

$$\text{meter setting in mmHg} = \frac{\% \text{ of calibration O}_2}{100} \times \left( \frac{\text{barometric pressure} - \text{partial pressure of water vapor } 10^{\circ}\text{C.}}{\text{barometric pressure} - \text{partial pressure of water vapor } 10^{\circ}\text{C.}} \right)$$

A value of 20.95% was used as the amount of oxygen in air. This method of calibration results in relative and not absolute readings on the panel meter scale.

Since there is no direct conversion of oxygen tension (mmHg) to milliliters of oxygen per liter (mls O<sub>2</sub>/l), empirical calibration is necessary to yield an absolute scale. Hence, a small water sample

was taken from the jar of saturated sea water and analyzed for oxygen content by a micro-Winkler method, the procedure for which was developed in this laboratory. The procedure is described in detail in a later section. The equivalence values of oxygen tension to the oxygen concentration in mls/l at saturation were then used in a simple proportion to calculate the number of mls O<sub>2</sub> equivalent to the number of mmHg used during a given time interval. The following proportion was employed when calculating the metabolic rate from the oxygen time-tension curves:

$$\begin{array}{l} \text{(A)} \quad \frac{\text{mls O}_2/\text{l at saturation}}{\text{calculated meter setting at saturation in mmHg}} \\ \text{(B)} \end{array} \quad = \quad \begin{array}{l} \text{(X)} \quad \frac{\text{O}_2 \text{ consumed in mls/l in given time interval}}{\text{mmHg consumed in given time interval}} \\ \text{(Y)} \end{array}$$

Thus:  $X = A/B \text{ times } Y$

And:  $X \text{ times volume of respirometer jar minus volume of shrimp in liters equals mls O}_2 \text{ consumed by a shrimp in given time interval}$

The following conditions were observed in all experiments.

Only adult male shrimps, ranging from 3.4- 8.6 gms and in apparent good condition, were used. Shrimps were removed directly from the sand or mud substrate in the laboratory and acclimated to either a 0.20 liter or 0.40 liter jar, depending on the size of the shrimp, for one to two hours before the beginning of a run. During acclimation the water was kept at or near saturation by aeration. The salinity of the water used ranged from 31-35 ‰.

Within the jar the animal rested on a circular plastic screen supported by a leucite cylinder one inch high in the center of which was a magnetic stirring bar (Figure 4). A water driven underwater stirrer (Bronwill Co.) was found necessary to prevent stratification of oxygen from occurring within the jar as the shrimp respired. The performance of this particular oxygen electrode was independent of the stirring rate which was, therefore, kept at as low a rate as possible. The activity of individuals, although not monitored, was observed and in most cases was minimal in spite of the presence of the slowly moving stirring bar.

After acclimation of the shrimp, the experimental jar was sealed with a rubber stopper through which the previously calibrated electrode and a bleeder line had been inserted. As the shrimp depleted the oxygen the drop in oxygen tension was recorded from the analyzer at 15 minute or one-half hour intervals, whichever was convenient. Wet weights were taken on a Mettler balance at the completion of the run.

All experiments were started around noon and lasted until the individual shrimp had lowered the oxygen tension to zero, a period lasting 12-24 hours. It was beyond the scope of the present work to investigate metabolic rhythms. However, some measurements were made on both species using the micro-Winkler in conjunction with the sealed jar method. Preliminary results suggested a

metabolic rate maximum at the time of high tide. For this reason it was decided to commence all the experiments at approximately the same time.

All experiments were run under constant light conditions. A limited number of measurements on both species indicated that the metabolic rate was essentially the same in both "dark" and "light" conditions.

#### Heart Rate Measurements

The effect of slowly decreasing oxygen tension on the heart rate of Callianassa was measured. The heart is located in the thorax just anterior to the first abdominal segment. The heart can be seen through the relatively transparent carapace and hence the heartbeat can be counted quite easily. Upogebia heart rates could not be determined by this method because of the opaque nature of the carapace. Only heart rates of Callianassa were determined in this study.

A rectangular leucite box (Figure 5) was constructed in order to facilitate regulation of the oxygen tension of sea water flowing over the shrimp. To minimize movement the shrimp was confined within the box by a glass tube whose open ends were covered with pieces of plastic screen. Full strength sea water (34-35 ‰) was siphoned through this "heart counting chamber" at an approximate

rate of 25 mls/min from a three gallon reservoir of sea water. A temperature controlled circulating water bath (Forma-Temp Jr.) was used to keep the reservoir and the accessory water bath in which the counting chamber was placed at 10°C., the temperature at which all heart rates were counted. The experimental animal was placed in the chamber one to two hours before counting commenced. Heartbeats were counted under a dissecting microscope; the time to complete ten heartbeats was measured (Figure 6). After heart rates were obtained at full oxygen saturation, the oxygen tension was lowered slowly by bubbling nitrogen gas through the three gallon reservoir of sea water. Periodically a micro-Winkler sample was taken from the box and the heart rate was measured immediately thereafter. This procedure was repeated until anoxic conditions were obtained.

#### Survival Time Under Anoxic Conditions

Callianassa and Upogebia were kept under anoxic conditions until death occurred. Anoxic conditions were obtained by bubbling full strength sea water with nitrogen gas for 1-2 hours. A micro-Winkler sample at the end of this time usually indicated that a small amount of oxygen remained in the water (Average 0.011 mls/l). This amount was considered negligible and was most likely consumed sometime during the experiment because a micro-Winkler sample at the termination of the experiment yielded essentially zero oxygen. The weight

and sex of each shrimp was recorded. Except for one group experiment (12 shrimps/gal jar), the shrimps were individually put into the jars of anoxic water. The jars were sealed with two layers of Parafilm and a screw cap or glass plate. They were then placed in lab tanks of running sea water ( $10^{\circ}\text{C.} \pm 5^{\circ}\text{C.}$ ).

#### Post-Anoxic Respiration Rates

The respiration rate after various periods of anoxia was obtained for both Callianassa and Upogebia. The oxygen macro-electrode and physiological gas analyzer were again used and the experimental protocol was essentially the same as previously described for the oxygen tension—metabolic rate experiments. Pre-anoxia values were measured at full oxygen saturation for one to two hours.

After pre-anoxia measurements of respiration rate the shrimp was carefully placed in a 0.4 liter jar of nearly anoxic sea water, the preparation of which was previously described (p. 12). All the shrimps weighed between five and eight grams. The jar was securely sealed with Parafilm and a screw cap, and then placed in a lab tank for either 12 or 36 hours.

At the end of the anoxic period a rubber stopper through which was inserted the pre-calibrated electrode and two bleeder lines, was quickly introduced into the experimental jar. The anoxic water was flushed out and replaced with saturated sea water. Oxygen uptake

measurements were started immediately after flushing the system. Oxygen tension measurements were taken every five minutes for a total of 15 minutes at the following designated recovery time intervals: 0, 0.5, 1, 2, 4, 8, and 12 hours after anoxia. The wet weight of each shrimp was obtained at the end of the experiment.

#### Burrow and Interstitial Water Sampling

Water samples were obtained from Upogebia burrows and analyzed for dissolved oxygen. To obtain the water samples soft plastic tubing (5mm diameter) was carefully threaded into the firm burrows (12-20 mm diameter) to a minimum depth of 12 inches. Approximately 10 mls of sample water were drawn up into a glass syringe in such a fashion that no air bubbles were introduced. Cocktail toothpicks, which served as excellent standard taper stoppers, were used to seal the syringes. The syringes were placed on ice until they could be taken to the laboratory for analysis by the micro-Winkler procedure. To keep large amounts of sand and/or mud from entering the syringe, the end of the sampling tube was covered with cheesecloth.

In the micro-Winkler procedure the reagents,  $\text{MnSO}_4$  and  $\text{KI-KOH}$ , were injected into the water sample within the 10 ml syringe. After mixing and allowing the precipitate to settle, concentrated  $\text{H}_2\text{SO}_4$  was injected and the released iodine in a 10 ml aliquot was titrated with standardized sodium thiosulfate using a micro-buret.

Most burrows were sampled only once; however, to check the procedure some duplicate samples were taken. In one field study selected burrows were sampled for two consecutive hours as the tide ebbed. Callianassa burrow water was unobtainable due to their highly collapsible burrows and the shifting nature of the substrate. Thus, interstitial water samples were obtained from sandy areas where only ghost shrimp occurred. All samples were taken in areas between burrow openings. An interstitial water sampler (37 x 3/4 inches) was constructed of hard, clear, plastic tubing. A solid pointed end enabled the sampler to penetrate one to two feet into the substrate. Small holes, drilled around the circumference for a distance of six inches from the bottom, allowed the interstitial water to enter the sampler. A 10ml sample of interstitial water was collected and treated as described above for burrow samples.

With the apparatus as described above, one interstitial water sample was obtained from the Upogebia collecting area. For exceptionally muddy or fine substrates it will be necessary to improve the sampling technique.

## RESULTS

Oxygen Tension vs Metabolism

Oxygen tension in the respirometer jars was plotted as a function of time. A resulting time-tension curve (drawn by inspection) typical for Callianassa appears in Figure 7. A linear relationship is seen except at very low oxygen tensions. Deviation from linearity first occurs around 10 mmHg. A typical curve for Upogebia (Figure 8) shows a deviation from the linear relationship at a higher oxygen tension, 45-50 mmHg.

The amount of oxygen consumed in mmHg per ten or 15 minute interval was determined from these time-tension curves. The formula on page nine was used to calculate the number of mls O<sub>2</sub> consumed by an individual shrimp per given time interval. These values were then corrected to one hour, divided by the wet weight of the animal and expressed as mls O<sub>2</sub> consumed/gm wet body weight/hour. The latter were plotted against the average oxygen tension for each time interval selected.

The oxygen consumption rate of Callianassa, as shown by each of four shrimp, is independent of the external oxygen concentration until the oxygen tension is lowered to approximately 10-20 mmHg (Figure 9). Below this the metabolic rate rapidly declines. Although the oxygen consumption rates for the four animals obviously are

converging towards the origin (zero oxygen tension and zero oxygen consumption), the rates in the range of "independence" were sufficiently different for the four individuals to warrant a separate line for each set of points. The oxygen tension at which the metabolic rate ceases to be independent is called the critical oxygen tension or  $T_c$  (Prosser, 1955). For Callianassa this was 10-20 mmHg. For comparative purposes 100% air-saturated sea water will be considered equivalent to 160 mmHg or 6 mls  $O_2/l$  at 10°C. Thus, the critical oxygen tension corresponds to 0.4-0.8 mls  $O_2/l$ , or 6.2-12.5% air-saturation.

The oxygen consumption data from four Upogebia were averaged in a curve drawn by inspection (Figure 10). The metabolic rate is independent of the external oxygen concentration as the tension is lowered from saturation to approximately 50 mmHg. The  $T_c$  range of 45-50 mmHg corresponds to 1.7-1.9 mls  $O_2/l$ , or 28.1-31.3 % air-saturation. Below this point the metabolic rate appears dependent on the external oxygen concentration, the slope of "dependent" respiration being much less than in Callianassa.

The data obtained for a postmolt Upogebia were significantly different from that for intermolt mud shrimps. Figure 11 shows the time-tension curve from which the relationship between oxygen uptake and oxygen tension (Figure 12) was determined. In this postmolt animal the metabolic rate appears dependent on the external

oxygen concentration over the entire range of oxygen tensions.

Within the range of respiratory "independence" it appears that the metabolic rate of Upogebia is considerably higher than that of Callianassa. A comparison was made of mean metabolic rates for both species within the independent range (110-150 mmHg) of the oxygen tension-metabolic rate relationship, using animals from 4-7gms in weight. The average metabolic rate for Callianassa (n=11) is 0.024 mls O<sub>2</sub>/gm wet wt/hr. On the other hand Upogebia (n=8) has a mean metabolic rate of 0.050 mls O<sub>2</sub>/gm wet wt/hr, twice that of Callianassa. The difference between the means is significant at the 1% level (  $t = |6.47| \geq t_{.01} = 2.90$  ).

#### Heart Rates vs Oxygen Tension

The purpose of these experiments was to determine the effect of lowering oxygen tension on the heart rate of Callianassa. Heart rates are reported for three individual shrimp in Figure 13. The data for two of the shrimp tested are quite similar so one line has been drawn (by inspection) through the points. The heart rate of the third shrimp is considerably lower at all oxygen tensions; thus, a separate line has been drawn.

Heart rates varied directly with the activity of the shrimp. Even though the glass tube minimized body movements the walking legs and pleopods often moved sporadically. If the latter activity was noted

while counting the heartbeat, the shrimp was considered active. A shrimp was considered inactive when the only visible movement was that of the scaphognathite (gill bailer). The points plotted for a single shrimp were all obtained under the same activity conditions.

Owing to the unfortunate lack of points in the seemingly critical area of 0.75-1.5 mls O<sub>2</sub>/l, the graph may be interpreted by two methods. Two lines were drawn (by inspection) through the experimental data and the resulting slopes were extended to the point of intersection. According to this method the heart rate of Callianassa remains relatively constant from full oxygen saturation down to a T<sub>c</sub> of approximately 0.5-1.0 mls O<sub>2</sub>/l, or 13-27 mmHg. Below this range the heart rate drops off sharply with further lowering of the oxygen tension in a manner quite similar to the metabolic rate. In the second method a dashed line was drawn through the critical area where values were lacking. The resulting curvilinear relationship indicates a less sharply defined critical oxygen tension than that shown for metabolic rate.

#### Survival Time Under Anoxic Conditions

The survival times for individually tested shrimp are found in Table 1. The average survival time for Callianassa (138 ± 23 hrs) is 1.7 times greater than the average survival time for Upogebia (81 ± 13 hrs). All the shrimp tested were believed to be in intermolt

except for a few postmolt Upogebia (indicated by asterisks). The average survival time for postmolt Upogebia is  $32 \pm 14$  hrs in contrast to an average of  $81 \pm 13$  hrs for the intermolt animals. The survival times for Callianassa tested in groups of 12 are in the same order of magnitude as the values obtained for individually tested shrimp (Table 2).

Inspection of Table 1 and Table 2 indicates that survival time under anoxia is independent of both sex and body weight. The possible effect of waste products and the pH were not determined at the end of the test period. It was noted that shrimps under anoxic conditions were not lethargic but appeared to remain relatively active until a few hours before death.

#### Post-anoxic Respiration Rates

To determine if a compensatory increase in metabolic rate occurred after subjection to anoxia, a base or pre-anoxia oxygen uptake rate was first determined for each of six shrimp, two Callianassa and four Upogebia. The oxygen uptake values obtained after 12 or 36 hours of anoxia were then expressed as percent of the base rate and plotted against hours after anoxia (recovery time). The data obtained after 12 hours of anoxia are seen in Figure 14 and after 36 hours in Figure 15.

The maximum observed oxygen uptake rate for all the animals is seen immediately following the anoxic period. For both test periods

Callianassa shows the greatest post-anoxia increase above the base rate.

The oxygen uptake of Callianassa at time zero is greater following 12 hours of anoxia (537%) than after 36 hours (347%). Also, the recovery data after 12 hours of anoxia seems to indicate a more rapid return to the base rate. In fact after 12 hours recovery from a 36 hour anoxia exposure, oxygen consumption is still elevated about 260% above the pre-anoxia base rate.

The data for Upogebia separate into two distinct patterns. Two animals (shown by □ symbols on graphs) show a small increase in oxygen uptake immediately after both 12 and 36 hours of anoxia and a rapid return to the base rate. On the other hand, two animals (shown by ∇ symbols on graphs) show a relatively large increase in oxygen uptake after both 12 and 36 hours of anoxia. The recovery patterns shown by these latter animals are more erratic, but roughly similar to those shown by Callianassa. The oxygen uptake rate of the mud shrimps subjected to 12 hours of anoxia returns almost to the pre-anoxia base level after 12 hours of recovery, whereas the mud shrimps subjected to 36 hours anoxia exhibit an elevated metabolic rate throughout the recovery period.

#### Burrow and Interstitial Water Samples

The concentration of dissolved oxygen in the water samples

obtained from Upogebia burrows (Table 3) at the time of low tide ranged from zero to 0.91 mls/l with an average of 0.58 mls/l. The average depth at which the samples were obtained was  $23.5 \pm 1.6$  inches. The average temperature within the burrows at a 12 inch depth was  $12.8 \pm 1.3^{\circ}\text{C}$ .

Using each burrow water temperature and assuming an average salinity of 33 $^{\circ}$ /oo, the oxygen solubility in sea water (mls/l) was determined from a nomogram by Richards and Corwin (1956). This value was taken to be 100% air-saturation. The prevailing salinity of burrow water samples taken throughout the summer was 33 $^{\circ}$ /oo and showed little or no variation (Thompson, 1967). Calculation of the percent oxygen saturation of burrow water yielded an average of 9.8%. Surface water gave an average value of 70.3% saturation.

Two consecutive samples separated by a one hour interval were taken from the same Upogebia burrow as the tide ebbed (Table 4). The oxygen concentration of the water in each burrow decreased significantly. According to the little data available the percent change of dissolved oxygen in the burrow appears to be proportional to the burrow diameter.

Interstitial water samples taken at low tide from both Callianassa and Upogebia collecting areas contained less than 1.0 ml O<sub>2</sub>/l (Table 5). The values are within the same order of magnitude as those reported above for Upogebia burrow water.

## DISCUSSION

One of the most serious problems a mudflat inhabitant encounters is exposure to low levels of oxygen. Mud and sand substrates are generally hypoxic and, except for animal burrows, allow little circulation of oxygen even when covered with water. Exposure of the mudflats during ebb tide further increases the degree of hypoxia, aerobic exchange being negligible except at the surface. Because of the daily bi-tidal cycle on the Pacific coast the mudflats within an estuary may be exposed for a maximum of 18 hours or more in a 24 hour period (Mac Ginitie, 1935).

The amount of oxygen remaining in the interstitial water during low tide depends on several factors (Brafield, 1964). The most significant are the amount of drainage, the amount of sand blackened from the presence of ferrous sulphide and the percentage of fine particles in the substrate. In view of these criteria mud should be relatively more oxygen deficient than sand, although both have been shown to be hypoxic. Another factor which contributes greatly to the formation of anaerobic conditions within the mudflat is bacterial respiration (Zo Bell and Feltham, 1942). The amount of oxygen in interstitial water also decreases with increasing depth (Pearse, Humm, and Wharton, 1942). Interstitial water obtained from a sandy beach at depth of six inches or below contained no oxygen whereas

small amounts (0.3-2.0 mls/l) were detected nearer the surface.

Even though interstitial water from sand contains relatively more oxygen than from mud, the following ecological and experimental observations led me to conclude that Upogebia has more available oxygen in its environment than does Callianassa.

Upogebia builds relatively permanent burrows in a mud-clay substrate. The burrows are basically U-shaped, with side branches and may extend downward to a depth of 2-3 feet. Each burrow has a minimum of two openings which are constricted near the surface and often surrounded by faecal pellets. These observations in general agree with those made by Mac Ginitie (1930).

Upon close examination the burrows are lined with a reddish-brown lining 1-3 mm thick. Microscopic examination of this lining reveals that it is an amorphous mass and is not diatomaceous. In the laboratory the deposit only occurred in containers in which Upogebia had burrowed into the mud. Hence, it does not appear to be a function of the substrate alone. Conceivably, the red-brown color could result from oxidative processes. Pohl (1946) described a similar dark rust-brown lining (3-7 mm) in Callianassa major burrows. However, cursory analyses for iron in the lining were negative. Pearse (1945) briefly mentioned that Upogebia affinis burrows have a firm coating like that of C. major. Mac Ginitie and Mac Ginitie (1949) did not mention a lining in the burrows which they

examined but reported that the walls of the burrows are smooth owing to body movement and that an adhesive secretion from the mouth region is used to repair the burrow walls. However, the exact nature of the lining and its formation is unknown and is certainly in need of further investigation.

In view of the preceding discussion of the burrow lining it seems appropriate at this time to speculate about its possible purposes. The permanent nature of the burrow might in part be due to a strengthening effect of the lining. Upogebia uses its pleopods to keep a current moving through the burrow. Perhaps the smooth walls help reduce the friction incurred by the moving water column, or help promote laminar flow. This in turn would tend to reduce the amount of energy expended by the shrimp for this purpose. The lining might also prevent oxygen from following a concentration gradient and diffusing outward into the interstitial water.

Upogebia burrows by the very nature of their location (zero to -1 ft) are exposed by the tide for less time than Callianassa burrows (zero to +1 ft). The ghost shrimp burrows in the high intertidal area are often uncovered during a low high tide whereas Upogebia burrows are not affected.

Callianassa burrows differ significantly from Upogebia burrows. They are not firmly constructed and a lining is not readily apparent. During low tide the burrow openings are often occluded and attempts

to trace the natural configuration of the burrow with narrow flexible tubing are to no avail. I feel that the burrows are probably kept open by hydrostatic pressure during high tide. When the water table drops during ebb tide, the upper reaches of the burrows tend to collapse and the animals may migrate downward. This is suggested by the observation that ghost shrimp collecting is usually the easiest just as the water is leaving or returning to the burrow openings. Mac Ginitie (1935) also reported that the tides seemed to influence the position of the shrimps in their burrows. It has also been recently shown (Thompson, 1967) that within the Yaquina Bay region Callianassa is an osmo-conformer and Upogebia is an osmo-regulator. The lack of a relatively permanent burrow system might be adaptive to the survival of ghost shrimps during seasons of fresh water runoff into the estuary.

In contrast to Upogebia, the ghost shrimp does not depend on a water current in its burrow for food but instead sifts the substrate for detritus. Callianassa has its head stuck in the sand, burrowing continuously in order to feed. In this respect Callianassa is analogous to an earthworm. Hence, in the absence of a permanent burrow system ghost shrimps must be fairly tolerant of hypoxia since they are constantly exposed to the hypoxic interstitial waters.

Experimental observations also support the conclusion that burrow water contains relatively more oxygen than does the

surrounding interstitial water. Jones (1955), using a modified Winkler method, reported a mean concentration of 0.50 mls O<sub>2</sub>/l at 15°C. in water obtained from exposed burrows of the lugworm Arenicola and a mean concentration of 0.25 mls O<sub>2</sub>/l at 15°C. in interstitial water obtained from the neighboring substrate. Hence, the burrow water contained twice as much oxygen as the interstitial water. In the present study the mean concentration of oxygen in water from exposed Upogebia burrows was 0.58 mls O<sub>2</sub>/l at 13°C. (Table 3). This value is surprisingly close to that obtained by Jones for Arenicola burrow waters. Due to the shortcomings of the previously described sampling procedure (p. 15) only one interstitial water sample from the Upogebia collecting area was analyzed (0.15 mls O<sub>2</sub>/l). No major conclusion can be drawn except to say that the oxygen content is low. Brafield (1964) reported an average oxygen concentration of 0.26 mls/l (17°C., depth 5 cm) for interstitial water obtained at mid-tide level from a sandy, poorly drained flat with a black layer and a high percentage of fine sand. Thus, interstitial water from silty and fine sandy areas contains less oxygen than water from burrows in the same area.

The differences in the nature and location of burrows, in the respiratory and feeding habits of the two shrimps, and the preliminary field data support the conclusion that Upogebia has more oxygen available in its habitat than does Callianassa. This fact will be

considered later in discussing the adaptive significance of the shrimps metabolic responses.

The effect of oxygen tension in the external medium on the oxygen consumption or metabolic rate of various organisms has been investigated since early in the 20th century. Early work on invertebrates was reviewed by Hyman (1929) and the recent work on crustaceans by Wolvekamp and Waterman (1960). In general, this area has not been intensively researched since the 1940's.

From the early work developed the classic idea that aquatic poikilotherms are either regulators or conformers with respect to oxygen concentration in the external medium. An organism is considered a regulator if its oxygen consumption is independent over a wide range of external oxygen concentrations down to a critical tension ( $T_c$ ) below which oxygen consumption is dependent on the oxygen concentration of the medium. Conformers, on the other hand, are directly dependent on the external oxygen tension up to high oxygen concentrations. In the latter case, if a  $T_c$  occurs, it is usually not sharp and is above 155 mmHg or air-saturation (Prosser, 1955).

Both regulators and conformers have been found in the crustacea. Amberson, Mayerson and Scott (1924) were the first to demonstrate that higher crustaceans such as the lobster Homarus americanus and the blue crab Callinectes sapidus are in fact metabolic conformers. Before this time many researchers sided with Henze (1910) who

hypothesized regulation for all the higher invertebrates owing to their more efficient circulatory and respiratory systems. Later, other decapod crustaceans such as Homarus vulgaris, and the woolly-handed crab Eriocheir sinensis were also shown to be conformers (Thomas, 1954; Chen, 1932).

In the present study it was found that both C. californiensis and U. pugettensis are metabolic regulators. Callinassa however can regulate its oxygen consumption to a lower  $T_c$  range (10-20 mmHg) than Upogebia (45-50 mmHg).

The critical oxygen tension has apparently not been determined for other members of the Thalassinidea. However, there are reports of other decapod crustaceans which are regulators with a  $T_c$  below air-saturation. Amberson et al. (1924) gave no data but mentioned that preliminary experiments on Palaemonetes vulgaris had revealed a  $T_c$  of 50% air-saturation (ca. 80 mmHg). The critical oxygen tensions reported for several other decapods are as follows:

Cambarus virilis, 40 mmHg (Hiestand, 1931); C. clarki, 120 mmHg (Maloeuf, 1937); Pugettia producta, 50-70 mmHg (Weymouth et al., 1944); Uca pugnax and U. pugilator, 4 mmHg (Teal, 1959). Helff (1928) reported critical oxygen tensions of 20%, 30%, and 40% air-saturation for Cambarus immnis weighing 4.3, 9.0, and 17.1 grams. Maloeuf (1936) severely criticized Helff's work, establishing that the crayfish C. bartoni does not secrete oxygen at low oxygen

tensions as Helff had implied, nor is it incapable of removing the last traces of oxygen from the medium. Henze (1910) reported that Carcinus maenas and Scyllarus latus were regulators over the entire range tested. However, he only tested from 12.8 to 2.5 mls O<sub>2</sub>/l, hence the T<sub>c</sub> would probably lie somewhere below 2.5 mls O<sub>2</sub>/l (ca. 45% air-saturation).

Several investigators have shown that metabolic pattern is altered by the experimental conditions. Hiestand (1931) reported that Cambarus virilis, normally a regulator, shows a conformer pattern if the jar-animal volume ratio is too small or if the experiment commences at less than air-saturation. Hence, in the present experiments ample consideration is given to this problem. Wiens and Armitage (1961) found that another crayfish, Orconectes immunis regulates at 16°C. but conforms at 35°C. (probably approaching a lethal temperature). Egusa (1961) demonstrated dependent respiration for actively digging Penaeus japonicus and independent respiration (T<sub>c</sub> = 1.0 mls O<sub>2</sub>/l) when the shrimp is under standard (i. e. resting) conditions. Temperature and activity effects on the oxygen consumption were not measured in the present investigation. Thus, the rates measured should probably be considered as "routine" metabolic rates (as defined by Fry, 1957). However, in view of the research reported above, if the temperature were increased above 10°C. or if active conditions were induced, both Callinassa and

Upogebia would be expected to show a higher  $T_c$  and tend toward greater metabolic dependence.

A criticism of the sealed jar method used in the present study involves the possible effect of the accumulation of carbon dioxide and nitrites as the animal depletes the available oxygen. The fact that shrimps were shown to survive in a sealed jar from three to six days (Table 1) led me to believe that accumulation of waste products in ten to 20 hours probably had little effect on the animals. In the present study the pH of the medium was not measured at the end of an experiment. However, other workers have found that increasing carbon dioxide, hence decreasing pH, did not affect the oxygen consumption of the crustaceans which they studied (Amberson et al., 1924; Marshall, Nicholls, and Orr, 1935; Helff, 1928). The presence of nitrites affects the oxygen concentration results obtained by the unmodified Winkler method (Allee and Oesting, 1934). Since the oxygen electrode monitored the decrease of oxygen in the sealed jar, the possible accumulation of nitrites is regarded as insignificant.

The mean metabolic rates, within the range of respiratory independence, of Callianassa (0.024 mls  $O_2$ /gm/hr) and of Upogebia (0.050mls  $O_2$ /gm/hr) are in the same order of magnitude as those listed for several macrurans and anomurans at 15°C. (Wolvekamp and Waterman, 1961). The mean metabolic rates of the shrimps also compare favorably to the metabolic rates of other mud dwelling

forms such as the polychaete Arenicola, the oligochaete Enchytraeus and the echiuroid Urechis, 0.031, 0.030, and 0.012 mls O<sub>2</sub>/gm wet wt/hr respectively (Prosser and Brown, 1961). However, such comparisons have limited significance since experimental conditions in the various investigations differ greatly. In searching the literature I found only one obscure reference to respiration rates of thalassinids. Montuori (1913) reported respiration rates of 0.132 and 0.368 mls O<sub>2</sub>/gm wet wt/hr respectively for Callianaxa subterranea and Gebia (= Upogebia; Borradaile, 1903) litoralis. He used smaller animals (1.5 gms), a higher temperature (25°C.), and activity state was not recorded, making comparison difficult.

Bradycardia is one of the mechanisms which enable diving mammals, birds, and turtles to tolerate hypoxic conditions. The slowing of heart rate in conjunction with decreased circulation to the muscles serves to conserve the amount of oxygen in the blood stream (Hoar, 1966). A decrease in the heart rates of mosquito larvae subjected to low oxygen concentrations has been reported (Jones, 1956). Few attempts, however, have been made to measure the effects of hypoxia on the heart rate of crustaceans. Larimer (1962) reported that bradycardia developed in the crayfish Procambarus simulans as the oxygen tension diminished. Earlier Larimer and Gold (1961) showed that this species is a metabolic conformer. Hence, both the heart rate and the metabolic rate decrease as the oxygen tension is lowered.

In the present study the heart rates of Callianassa show a regulatory pattern roughly similar to that seen for the metabolic rate (Figure 13). Bradycardia did not occur until the oxygen tension had dropped below 27 mmHg which is near the  $T_c$  range (10-20 mmHg) reported here for Callianassa. Regardless of which graphic interpretation is chosen (see p.19) bradycardia did not develop until after an extended period of regulation. Perhaps the maintenance of a constant heart rate and presumably constant cardiac output enables the shrimp to regulate its metabolic rate as the external medium becomes increasingly hypoxic.

In contrast to the independent relationship seen for intermolt shrimp, the single postmolt Upogebia studied appears to be a metabolic conformer (Figure 12). Thomas (1954) reported similar data for Homarus vulgaris (Figure 16 A).

Exponential time-tension curves similar to that seen for the postmolt Upogebia in Figure 11 have been reported for Homarus americanus, and Eriocheir sinensis (Amberson et al., 1924; Chen, 1932). When these workers made a log-plot of their oxygen concentration data a linear relationship resulted similar to that seen in Figure 16 B. For the postmolt Upogebia it is interesting to note that a plot of the log of oxygen tension against time does not yield a linear relationship. Perhaps this curve is a reflection of partial regulation and/or activity. This loss of regulation is most likely because the shrimp's oxygen demands are greater than normal. Hence, from this data the postmolt

mud shrimp appears to be intermediate between a classical regulator and conformer.

The investigation of survival time under anoxic conditions also seems to indicate that postmolt Upogebia are more oxygen dependent than intermolt shrimp (Table 1). Although these postmolt shrimp all had soft exoskeletons, the length of time after molt could not be determined accurately. Mc Leese (1956) and Egusa (1961) briefly noted that molting lobsters and prawns seemed to be more sensitive to low oxygen concentrations than those in the intermolt stage. It is not too surprising that the oxygen demands of a shrimp would increase during ecdysial and postecdysial stages since tissue growth and mobilization of materials to harden the exoskeleton are supposedly taking place during this time.

Information about the effect of molting on the respiration of crustaceans is sparse. Roberts (1957) reported that metabolism increases two-fold in Pachygrapsus crassipes two weeks prior to and during ecdysis. Newly molted Crangon armillatus shows a similar elevation (Darby, 1938). Bliss (1953b) reports that respiration rates of eye-stalkless Gecarcinus lateralis are maximal at the time of ecdysis. I feel that proecdysial, ecdysial and postecdysial metabolic patterns should be further investigated.

Von Brand (1946) reviewed anaerobiosis in invertebrates and pointed out that crustaceans in general, particularly decapods, show

little tolerance for experimentally induced anoxic conditions. However, a few species of copepods, cladocerans, and ostracods, which usually inhabit low oxygen waters, prove very resistant to anoxia (e. g. 14-90 days). The average survival time under anoxia for Callianassa (ca. 5.7 days) is 1.7 times greater than for Upogebia (ca. 3.3 days). Also the observation from the present study that shrimps under anoxic conditions are not lethargic contradicts Mac Ginitie (1935) who claims that all activity ceased under anoxic conditions. It seems quite evident that both species of this study, in particular Callianassa, are remarkably resistant to anoxia and that this tolerance is of obvious adaptive significance in the mudflat biotope. The question of the mechanism involved in anoxic tolerance naturally arose next.

Aerobic metazoans under hypoxic or anoxic conditions can often temporarily rely on anaerobiosis as a source of energy (vonBrand, 1946). If the oxygen consumption of a "stressed" animal is greater than normal upon restoration to oxygen-saturated conditions, the classical interpretation has been to suggest anaerobic pathways. The extra oxygen used i. e. "oxygen debt" is presumably used to oxidize the intermediate end products produced during anaerobiosis. It is significant that in both mud shrimp the oxygen consumption following anoxia increased above the pre-anoxic rate (Figures 14 and 15).

Glycolysis, generally resulting in pyruvate or lactate, is a well

known anaerobic metabolic pathway and is widespread among the vertebrates. Glycolysis has also been reported in crustaceans, particularly in muscles (Vonk, 1960). In view of this, glycolytic involvement in mud shrimp tolerance to anoxia should be investigated.

In connection with "oxygen debt" one would expect a greater increase in oxygen uptake above the base level at time zero after the longer exposure time to anoxia. This however was not the case for either Callianassa or Upogebia. It has been shown in other mudflat inhabitants such as Nereis, and Urechis that all the acid products of anaerobiosis are excreted and hence no "oxygen debt" exists (vonBrand, 1946). Perhaps after so many hours of anoxia the shrimps start to excrete anaerobic end products. A mechanism of this type would decrease the apparent magnitude of the post-anaerobic oxygen consumption and would enable the shrimps to survive anoxia for a longer period of time.

Why two blue mud shrimps should show a small elevation in oxygen uptake immediately after both 12 and 36 hours of anoxia and a rapid return to base rate while the other two shrimps show a large increase in oxygen uptake and erratic recovery patterns, is not clear. Perhaps this is simply a reflection of differences in activity levels during post-anaerobic measurement of oxygen uptake.

These very preliminary experiments on a small number of animals provide indirect evidence that anaerobiosis is used by the shrimps

during anoxic stress. However, the nature of the anaerobic pathway, the magnitude of the "oxygen debt", if any, after longer periods of anoxia, and the type of organic acids produced remains to be elucidated. An obvious starting point would be to ascertain whether lactate is produced by these shrimps and whether increased carbohydrate utilization occurs during anoxic stress. At any rate, the problem requires further study.

Metabolic requirements, indicators of which are  $T_c$ , metabolic rate and tolerance to anoxia, generally reflect the availability of oxygen in the environment. Animals inhabiting high oxygen environments usually have greater metabolic requirements than those inhabiting low oxygen environments. Fox and Simmonds (1933) reported that isopods, ephemeropterid nymphs and trichopteropterid larvae from swift streams have greater metabolic rates than the same or related species from ponds. Fox, Simmonds, and Washbourn (1935) confirmed their earlier results and in addition reported that ephemeropterid nymphs from stagnant water survive longer in low oxygen water than nymphs from swift streams. Bovbjerg (1952) found that Cambarus fodiens, a pond mud-burrowing crayfish, survived anoxic conditions four times longer than C. propinquus, a swift stream inhabitant. It is known that oxygen concentration in ponds and waters with high organic content is lower and more variable than in streams (Krogh, 1941). Earlier in the discussion it was emphasized that the amount

of oxygen available in the burrow of Upogebia is appreciably greater than in the "collapsible" burrow of Callianassa. It is suggested that the significantly higher metabolic rate of Upogebia in the zone of respiratory independence reflects the relatively greater availability of oxygen in its niche.

Fox, Wingfield and Simmonds (1937) showed that the ephemerid nymph Baetis (swift stream) is a conformer with a critical oxygen concentration actually above air saturation ( $T_c = 12$  mls  $O_2/l$ ). Whereas Leptophlebia (lake) and Cloeon (pond) are both regulators with critical oxygen concentrations of 2.5 and 2.0 mls  $O_2/l$ , respectively. Thus, the metabolic regulation pattern can be correlated with the degree of hypoxia in the habitat, animals from low oxygen environments having lower  $T_c$ 's. Ephemera (pond) reported as a conformer by Fox et al. (1937) was later shown by Eriksen (1963) to be a regulator. Walshe (1948) reported that stream chironomid larvae have higher metabolic rates, and are less resistant to hypoxia than those from ditches. Correspondingly, stream larvae are metabolic conformers and the ditch species, regulators. Based on field data, Walshe emphasizes that the differences in metabolic requirements of the two species reflect the amount of oxygen normally available in their habitats.

There is further evidence that metabolic responses are indicative of physiological adaptation and not of phylogenetic position. Closely

related species from different habitats have been shown to have metabolic responses which correlate with the availability of oxygen in their immediate environments. Cambarus virilis (found under stones in Yahara River) and Penaeus japonicus (normally buried in sand) are metabolic regulators (Hiestand, 1931; Egusa, 1961), whereas Cambarus bartoni (swift streams) and Penaeus indicus (free swimming in estuary) are metabolic conformers (Maloeuf, 1936; Subrahmanyam, 1962).

In conclusion, emphasis is placed on the adaptive significance of the respiratory responses reported for mud-dwelling shrimps in the present study. Callianassa and Upogebia live in mudflats, an environment relatively low in oxygen. Both show the following physiological mechanisms: 1) low metabolic rates; 2) metabolic regulation with a critical oxygen tension below 50 mmHg; and 3) survival in anoxia for at least three days. These mechanisms correlate well with a hypoxic habitat and are therefore considered adaptive. Closer analysis reveals quantitative differences in their respiratory responses. Upogebia has a greater metabolic rate, higher  $T_c$  range, and is less able to tolerate anoxia than Callianassa. Despite the paradoxical situation of living in a substrate whose interstitial waters are poorer in oxygen, Upogebia probably has in fact more oxygen available in its specific niche environment within the mudflat than does Callianassa; hence, metabolic requirements

of Upogebia are greater and regulatory features are less pronounced than in ghost shrimps. The present study supports the generality that metabolic requirements reflect the availability of oxygen in the environment.

## SUMMARY

1. The respiratory responses of two species of mud shrimp from Yaquina Bay, Newport, Oregon were measured.
2. Determination of the influence of oxygen tension on the metabolic rate revealed that both species are metabolic regulators. The critical oxygen tension for Callianassa is 10-20 mmHg (6.2-12.5% air-saturation) and for Upogebia is 45-50 mmHg (28.1-51.3% air-saturation).
3. Callianassa has a mean metabolic rate within the independent range of respiration of 0.024 mls O<sub>2</sub>/gm wet wt/hr compared to a value for Upogebia of 0.050 mls O<sub>2</sub>/gm wet wt/hr. These differences are statistically significant ( $P < .01$ ).
4. Heart rates of Callianassa subjected to diminishing oxygen tensions show a regulatory pattern similar to the metabolic rate. Significant bradycardia occurs after 27 mmHg tension is reached.
5. Both species are remarkably tolerant to anoxia. Callianassa survives approximately 5.7 days and Upogebia 3.3 days under such conditions.
6. Post-anoxia respiration rates increase above the normal rate after 12 and 36 hours of anoxia for both species. The presence of some anaerobic pathway is proposed.
7. The metabolic pattern of a postmolt Upogebia is that of a conformer.

Upogebia believed to be in a postmolt condition survive anoxia only 2.5 days. These data suggest that postmolt Upogebia are relatively more oxygen dependent than intermolt mud shrimp.

8. The mean concentration of oxygen in water obtained from exposed Upogebia burrows is 0.58 mls O<sub>2</sub>/l. Interstitial water samples, when obtained, contained less than 1.0 mls O<sub>2</sub>/l.
9. Callianassa in contrast to Upogebia does not construct firm burrows and is probably directly exposed to hypoxic interstitial waters.
10. Both species have respiratory adaptations for survival in a hypoxic environment. It is proposed that the quantitative differences in the metabolic requirements of the two species may reflect the availability of oxygen in their respective niches.

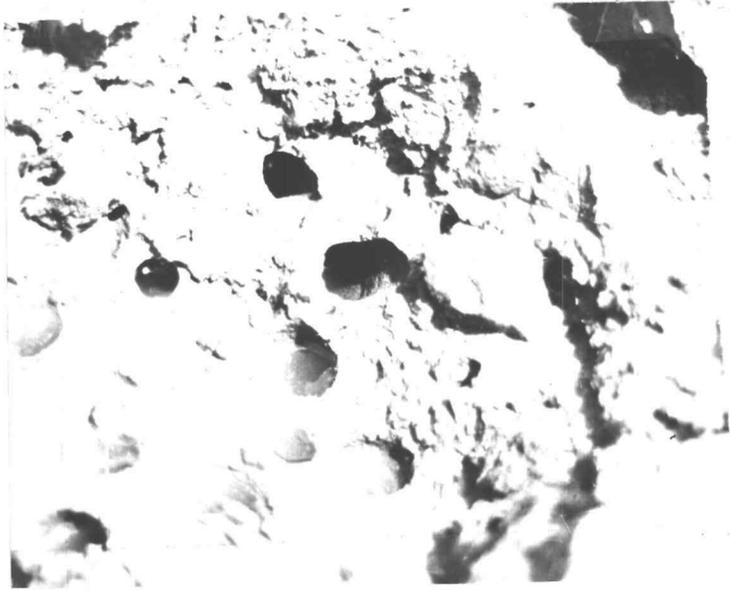


Figure 1: Near surface view of Upogebia burrow openings after top few inches of substrate have been removed. Picture taken between Sally's Slough and Coquille Point (Yaquina Bay).



Figure 2: Longitudinal section of Upogebia burrows. Note the smooth and substantial nature of the burrow walls.

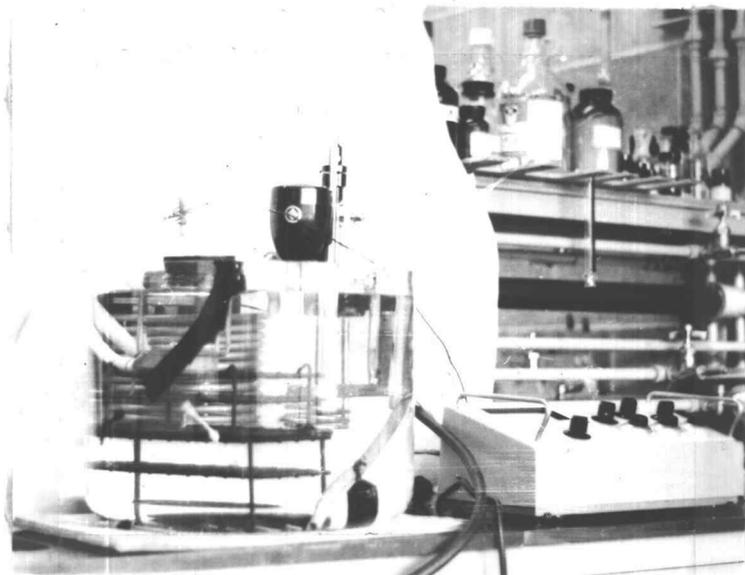


Figure 3: Experimental set up used in obtaining the oxygen consumption data. Includes the water bath, heating and cooling units, supporting platform, underwater stirrer and physiological gas analyzer.

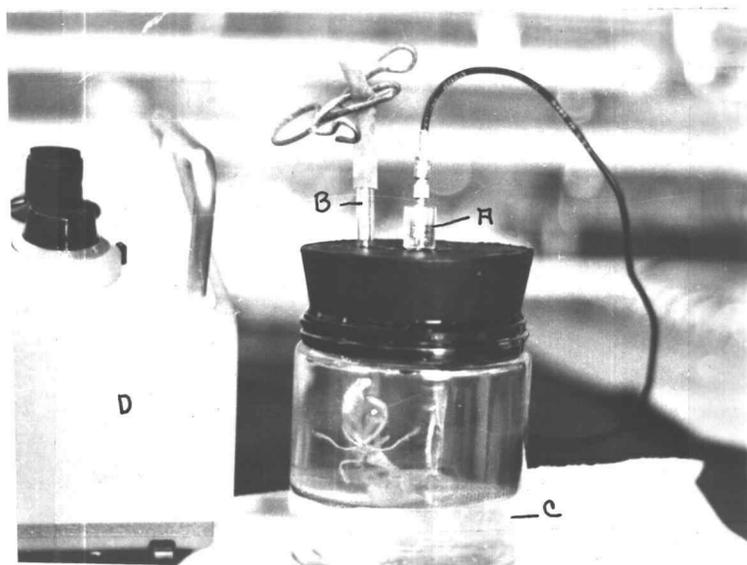


Figure 4: The experimental jar used in obtaining time-tension curves and metabolic rates. A-oxygen macro-electrode; B-bleeder line; C-leucite cylinder covered with plastic screen; D-physiological gas analyzer.

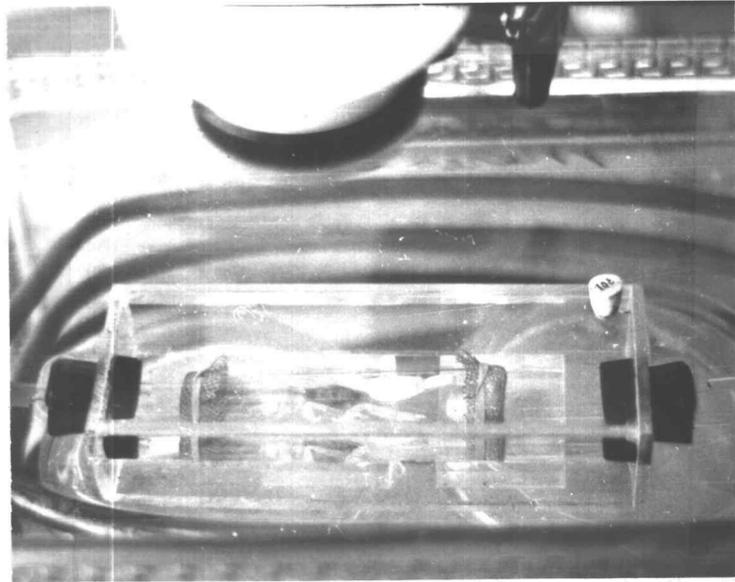


Figure 5: A close up view of the heart rate "counting chamber" within the constant temperature water bath.

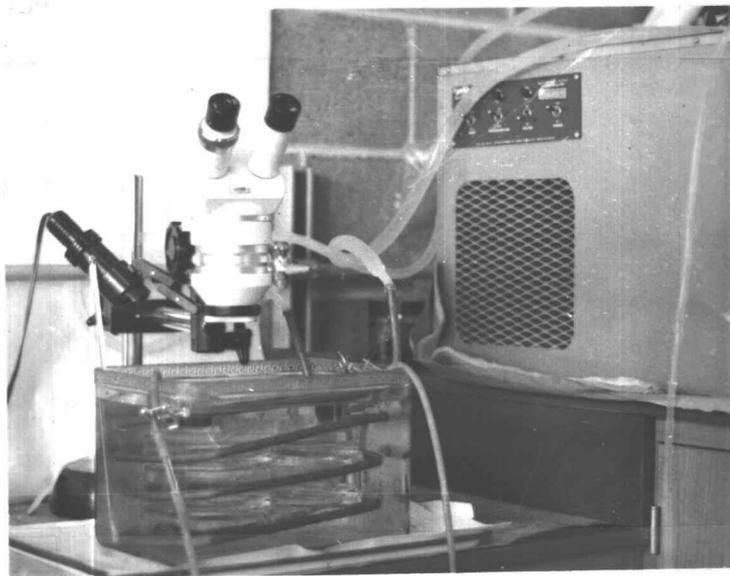


Figure 6: Experimental set up used for counting heart rates of Callianassa californiensis.

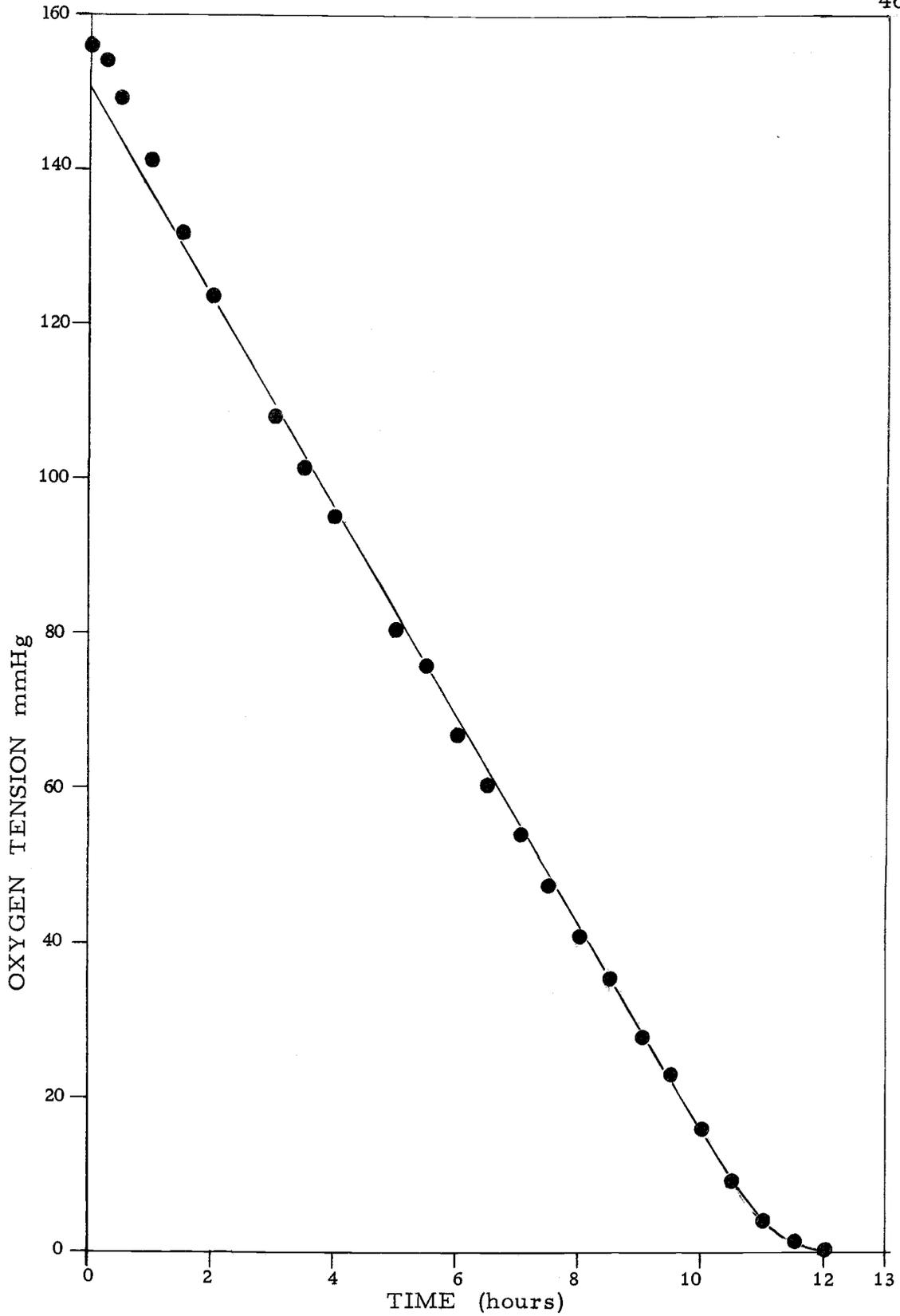


Figure 7: The rate of oxygen depletion by Callianassa californiensis (5.7 gms)

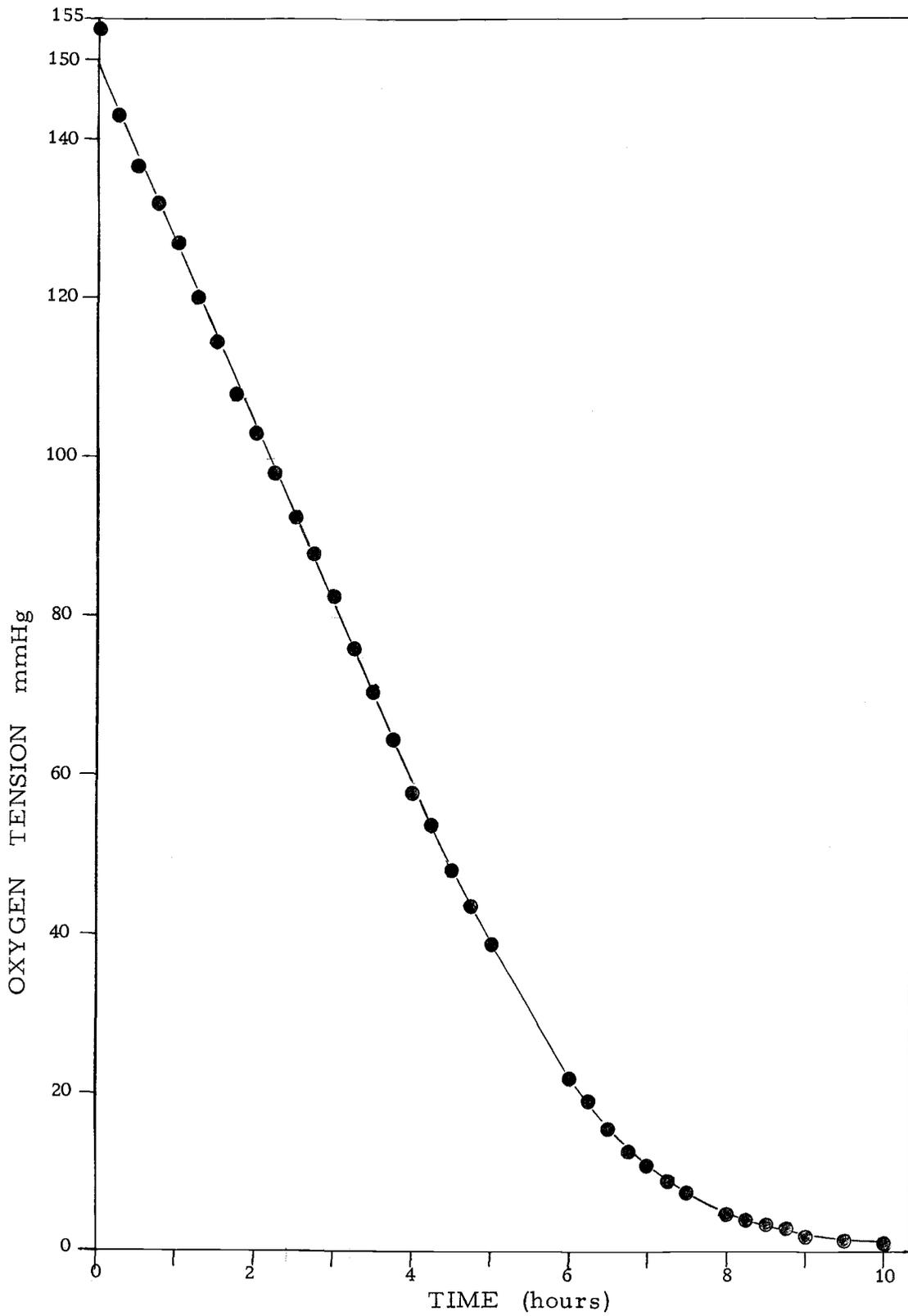


Figure 8: The rate of oxygen depletion by *Upogebia pugettensis* (5.9 gms).

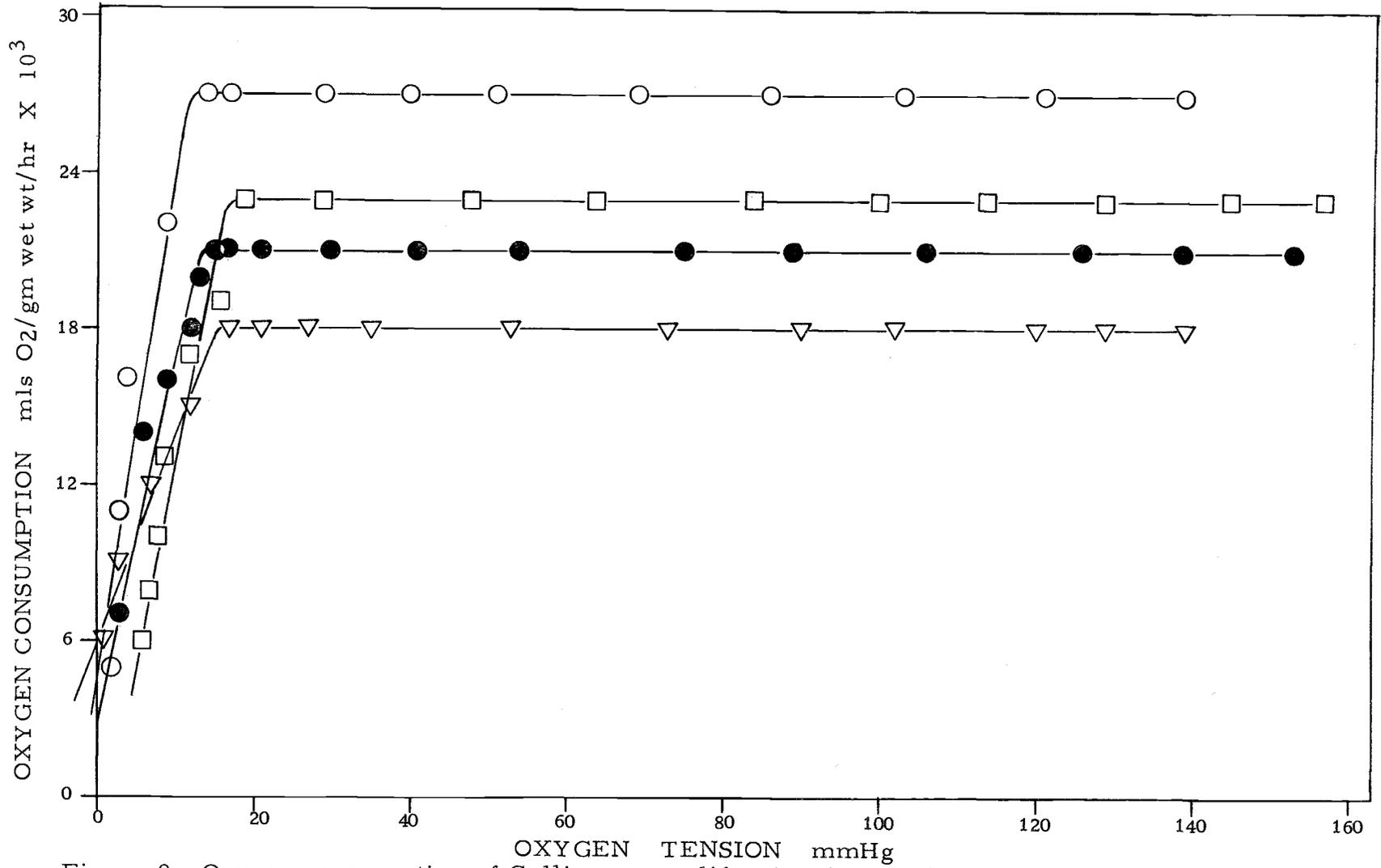


Figure 9: Oxygen consumption of *Callinassa californiensis* as a function of oxygen tension (○ 7.3 gms; □ 8.7 gms; ● 5.7 gms; ▽ 5.3 gms).

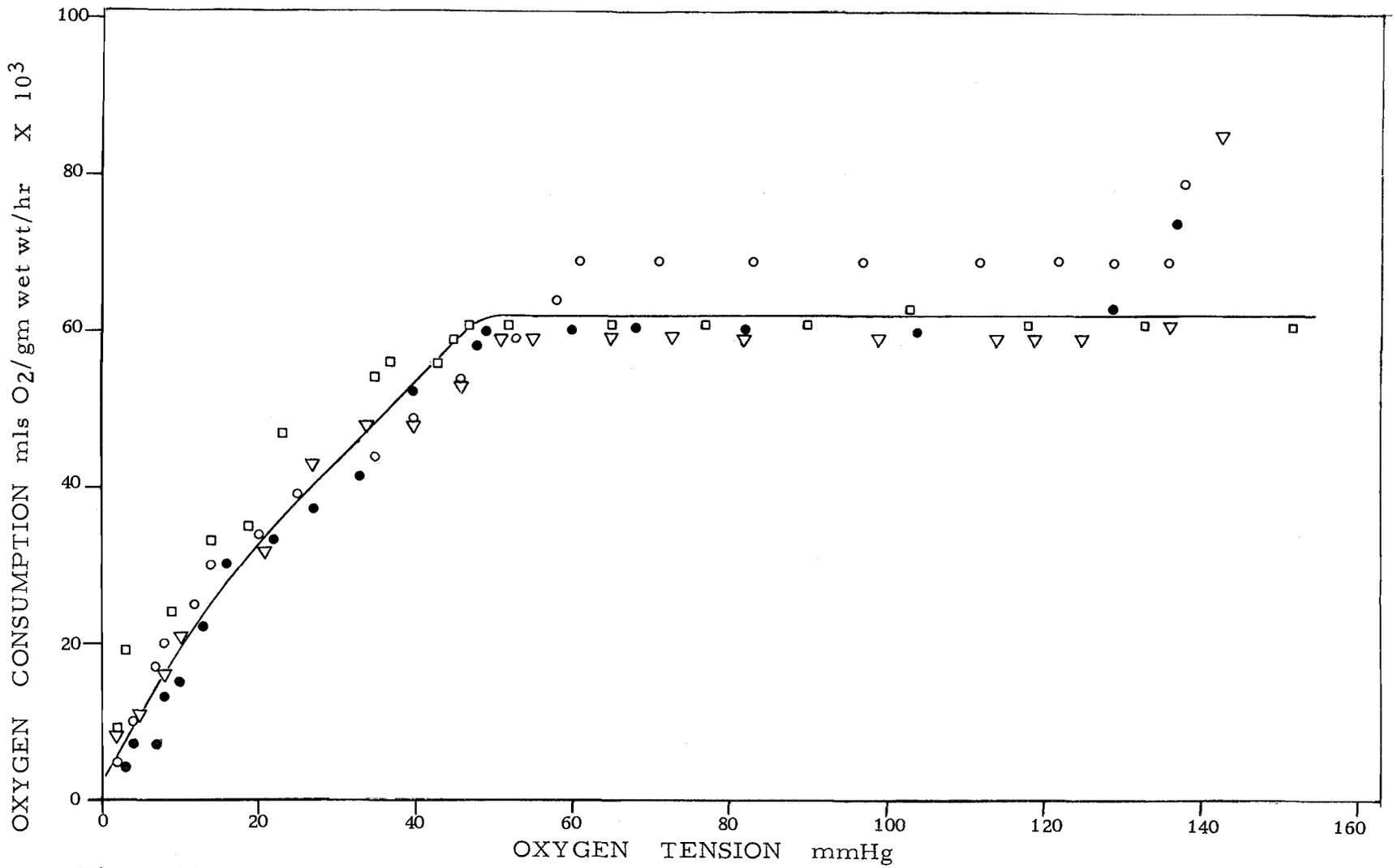


Figure 10: Oxygen consumption of *Upogebia pugettensis* as a function of oxygen tension (○ 6.5 gms; □ 3.4 gms; ● 8.2 gms; ▽ 5.9 gms).

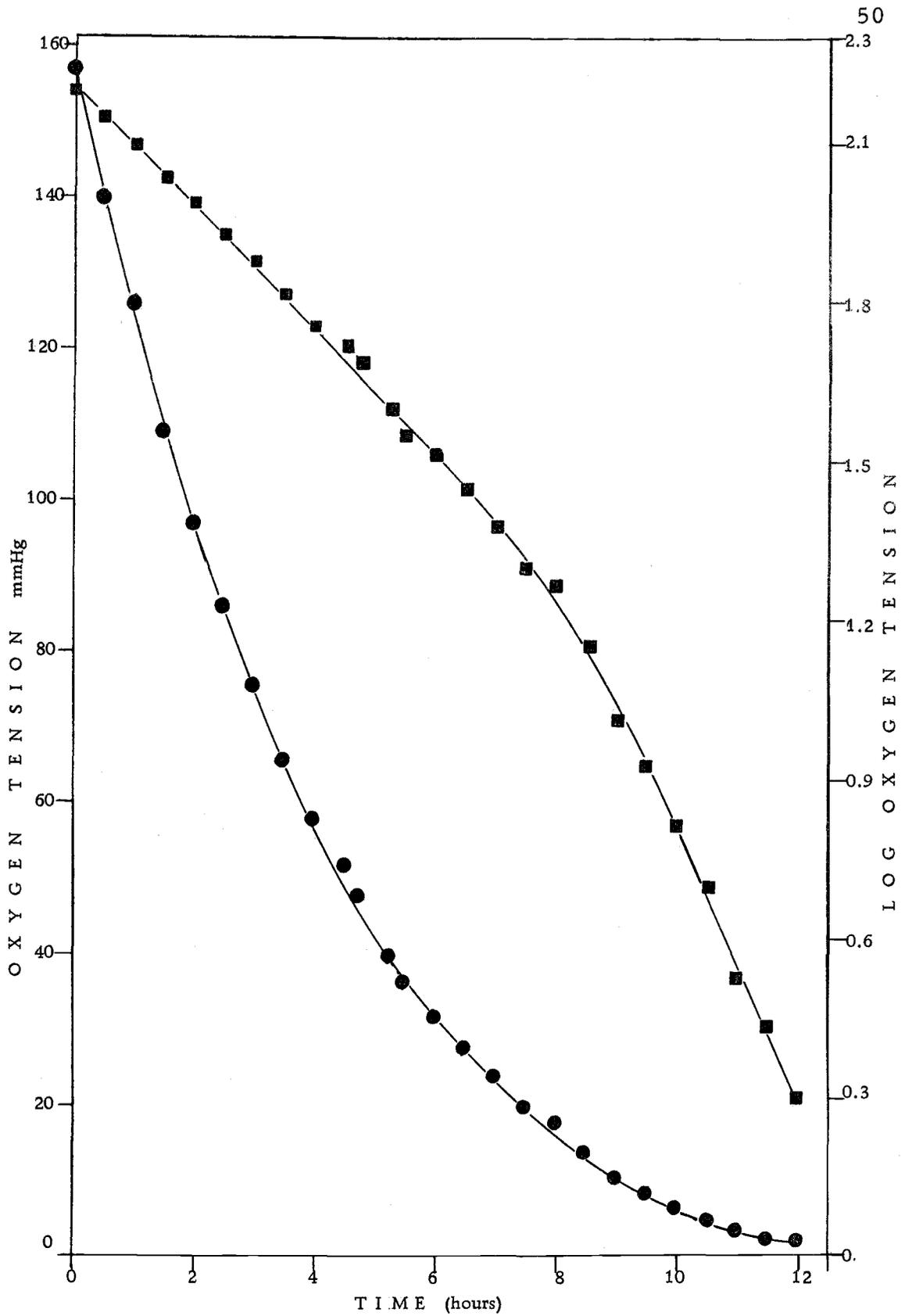


Figure 11: The rate of oxygen depletion by a postmolt *Upogebia pugettensis*, ● oxygen tension (mmHg); ■ log of oxygen tension.

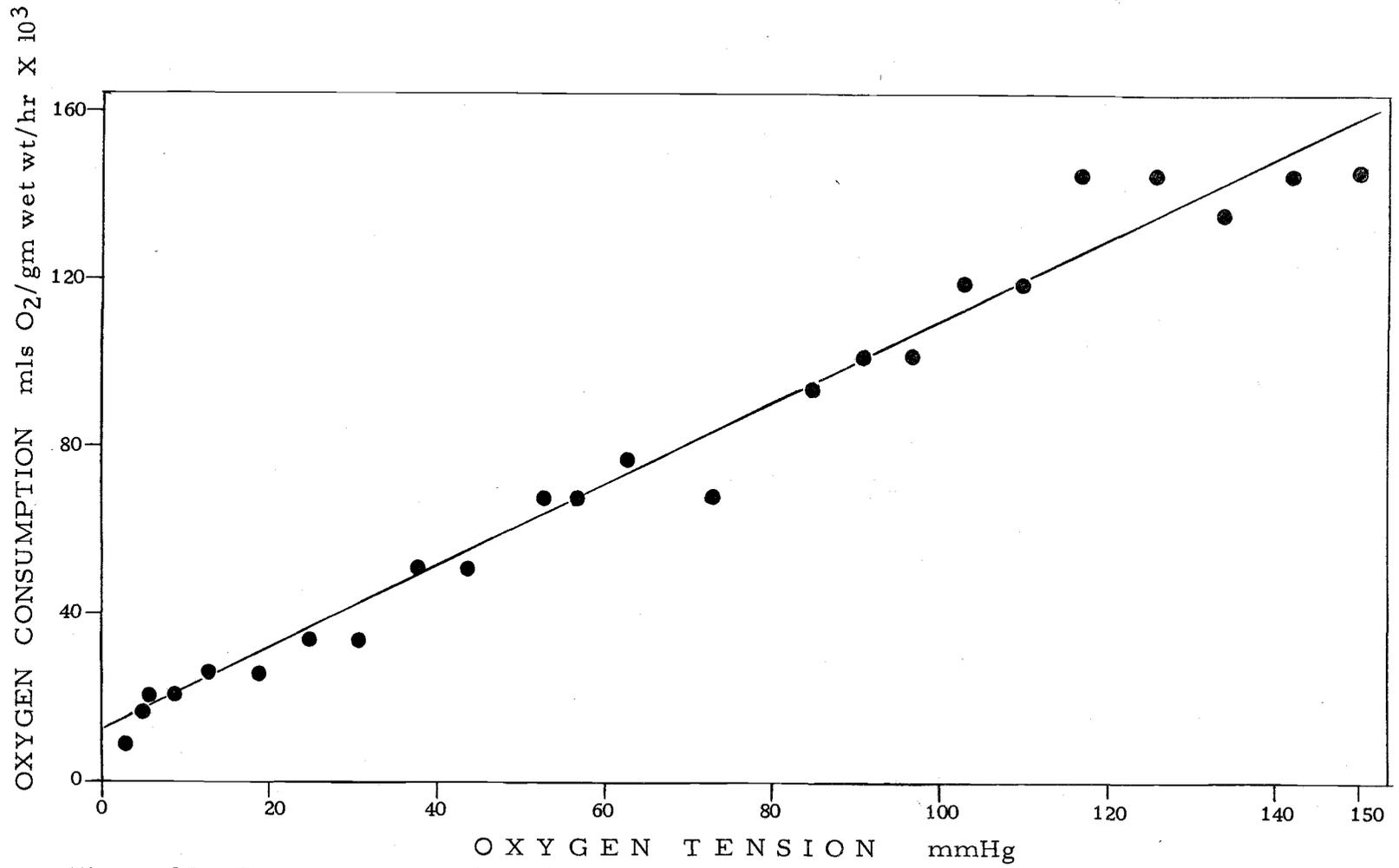


Figure 12: Oxygen consumption of a postmolt Upogebia pugettensis as a function of oxygen tension (3.7 gms).

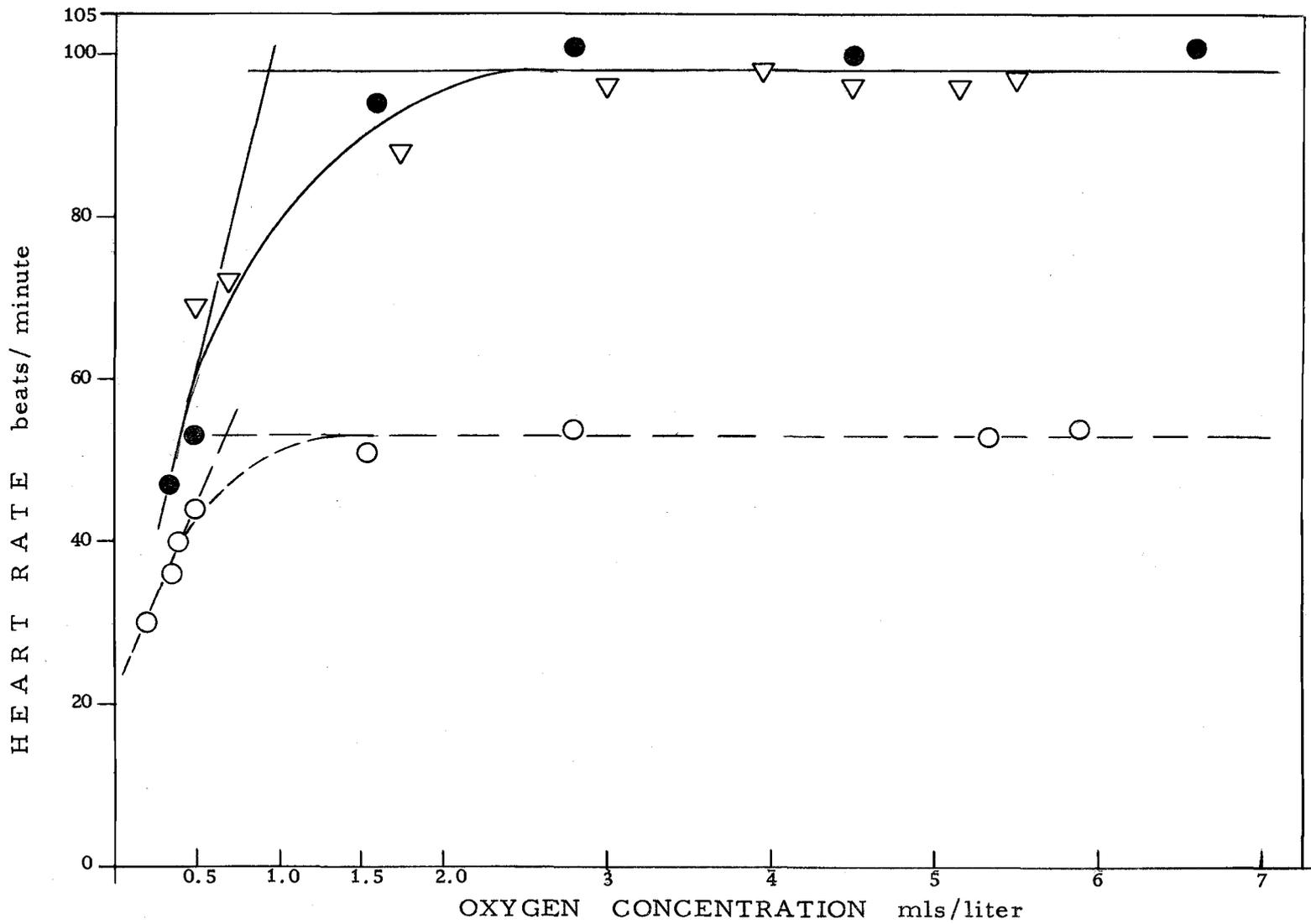


Figure 13: Heart rate of Callinassa californiensis in beats per minute at various oxygen tensions; inactive shrimp ●,○; active ▽; solid line represents two shrimps.

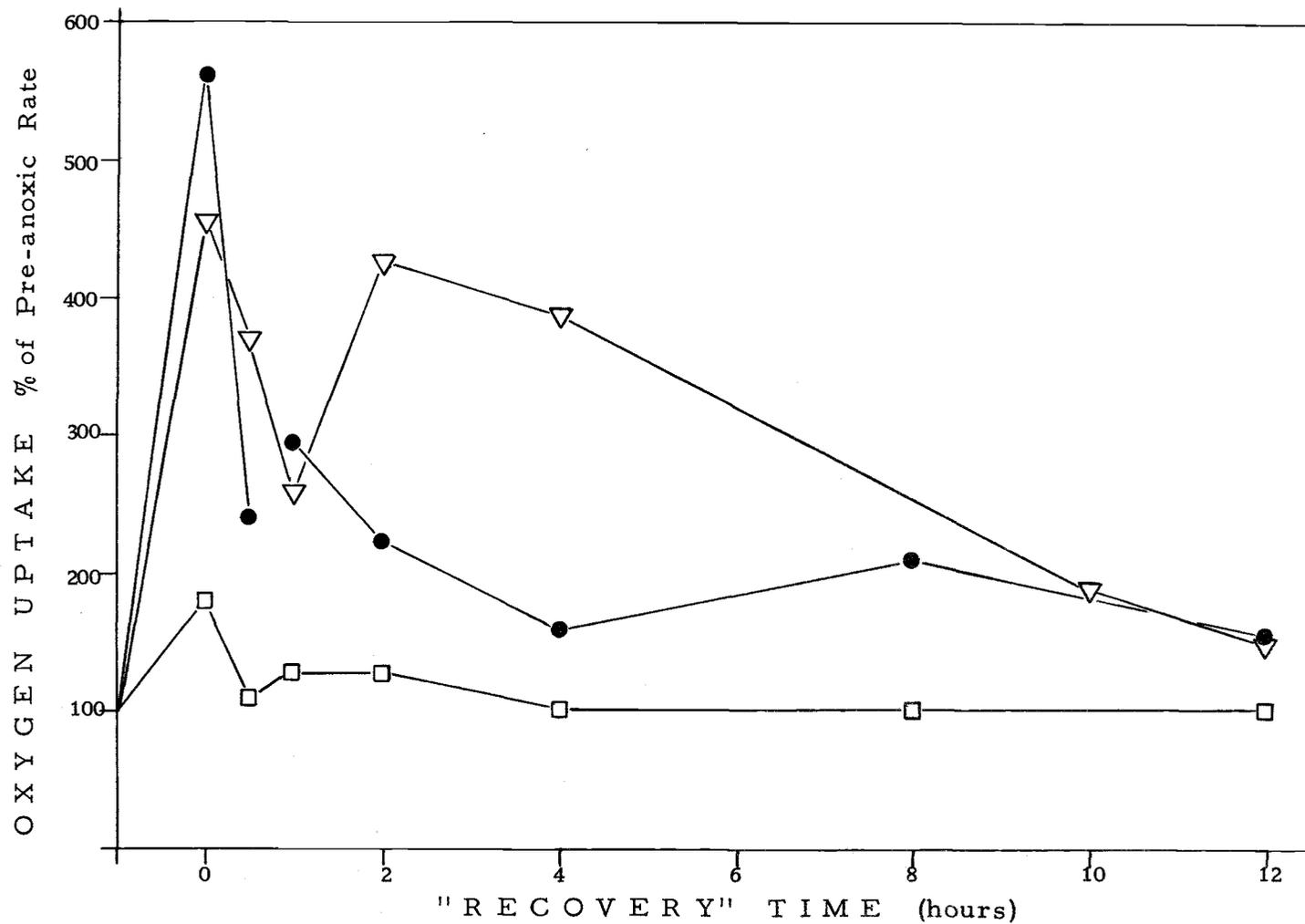


Figure 14: Changes in levels of oxygen uptake following 12 hours of anoxia. The first point on the left is the pre-anoxic or control value. All values are expressed as percent of pre-anoxic rate. Callianassa ●; Upogebia ▽, □.

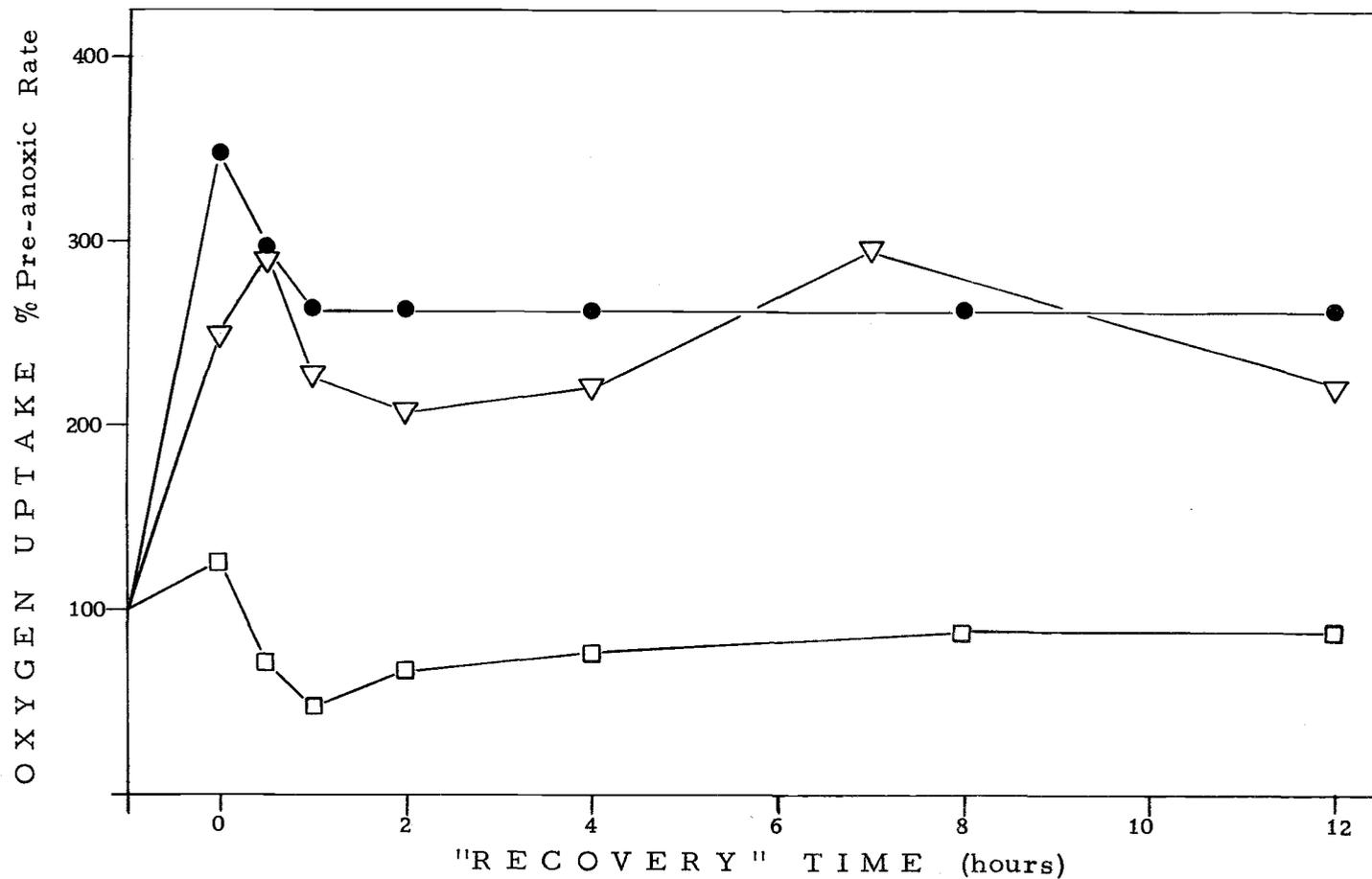


Figure 15: Changes in levels of oxygen uptake following 36 hours of anoxia. The first point on the left is the pre-anoxic or control value. All values are expressed as percent of pre-anoxic rate. Callianassa ●; Upogebia △, □.

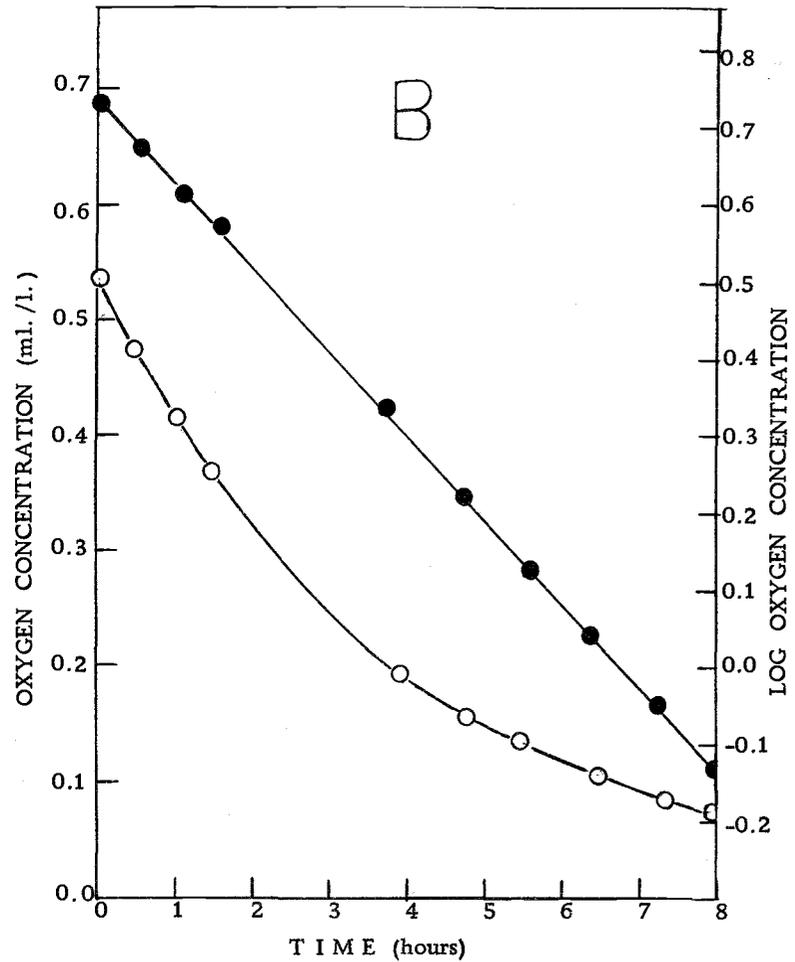
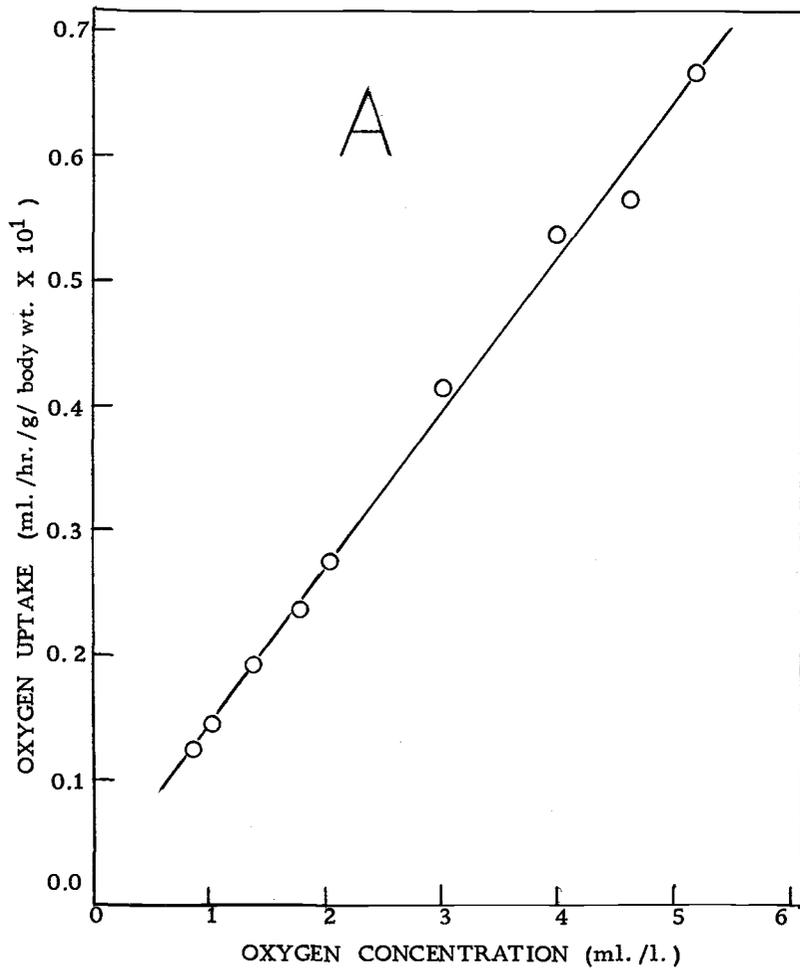


Figure 16: From Thomas (1954). A - Oxygen consumption of Homarus vulgaris (325 g.) at 15°C. as a function of oxygen concentration in the external medium. B - Rate of oxygen depletion by same crayfish (A); O, oxygen concentration, ●, log oxygen concentration.

Table 1: Survival time in hours for Callianassa californiensis and Upogebia pugettensis individually subjected to anoxic conditions. Averages are presented with the standard deviation.

<u>Callianassa californiensis</u>				<u>Upogebia pugettensis</u>			
Animal number	Sex	Wet weight grams	Survival time hours	Animal number	Sex	Wet weight grams	Survival time hours
1	♀	2.8	156	1	♂	1.9	95
2	♂	4.0	111	2	♀	3.7	92
3	♂	4.1	128	3	♂	3.8	72
4	♂	4.3	156	4 *	♀	5.5	12
5	♂	4.8	108	5 *	♂	5.6	42
6	♂	5.0	187	6	♀	5.9	64
7	♂	5.3	129	7*	♂	7.4	43
8	♂	5.6	126				
9	♂	6.2	132				
10	♂	6.4	179				
11	♂	7.2	110				

Average:  $138 \pm 23$  hours

Average:  $60 \pm 23$  hours

Average Postmolt Animals:  $32 \pm 14$  hours

Average Intermolt Animals:  $81 \pm 13$  hours

Table 2: Survival time in hours for Callianassa californiensis subjected to anoxic conditions in groups of 12 shrimp per gallon jar.

<u>Group Study A</u>				<u>Group Study B</u>			
Animal number	Sex	Wet weight grams	Survival time hours	Animal number	Sex	Wet weight grams	Survival time hours
1	♀	1.5	138	1	♂	2.0	179
2	♀	1.5	138	2	♂	2.1	78
3	♂	1.8	126	3	♀	2.1	129
4	♂	1.9	138	4	♀	2.2	141
5	♂	2.0	126	5	♀	2.3	179
6	♂	2.5	176	6	♀	2.6	129
7	♂	2.7	126	7	♀	3.0	129
8	♂	2.7	126	8	♂	3.2	78
9	♂	2.8	126	9	♂	3.2	78
10	♂	2.8	126	10	♀	3.3	179
11	♂	3.2	176	11	♂	3.4	129
12	♂	3.7	138	12	♂	3.6	78

Average:  $138 \pm 12$  hours

Average:  $126 \pm 32$  hours

Table 3: Amount of dissolved oxygen (mls/liter) in water samples obtained from Upogebia pugettensis burrows located between Coquille Point and Sally's Slough. Data were obtained on three different days at the time of low tide. Averages are given with the standard deviation.

Date	Burrow number	Depth inches	Temp. °C. at 12 inches	mls O <sub>2</sub> /liter	100% Saturation mls O <sub>2</sub> /liter	% Saturation of burrow water
5/11/66	1 a	20	12.0	0.77	5.95	12.9
	1 b	26	12.0	0.77	5.95	12.9
	2	26	12.5	0.88	5.88	14.9
	3	26	12.5	0.41	5.88	6.9
	4	20	12.0	0.59	5.95	9.9
5/24/66	5	26	12.5	0.88	5.88	14.9
	6	24	13.0	0.70	5.80	12.1
	7	24	12.0	0.75	5.95	12.6
	8	24	11.0	0.91	6.70	13.5
	9	24	12.5	0.31	5.88	5.2
6/24/66	10	24	11.0	0.62	6.70	9.2
	11	24	12.0	0.67	5.95	11.2
	12	19	--	0.00	--	00.0
	13	22	16.0	0.39	5.48	7.1
	14	24	16.0	0.31	5.48	5.6
	15	24	16.2	0.39	5.45	7.1
Average:		23.5 ± 1.6 inches	12.8 ± 1.3 °C.	0.58	5.92	9.8
Surface Water			70.3 % air-saturated			
Aerated Water in Laboratory			97.5 % air-saturated			

Table 4: Amount of dissolved oxygen (mls/liter) in water samples taken from selected Upogebia pugettensis burrows on May 26, 1966. Each burrow was sampled twice as the tide ebbed. Average sampling depth (inches) was  $24.8 \pm 1.0$  and the average temperature at a depth of 12 inches was  $10.6 \pm 0.4$  °C.

Burrow number	Burrow diameter inches	Time A. M.	mls O <sub>2</sub> /liter	Percent change
1	0.75	9:00	2.70	72.2
		10:00	0.75	
2	0.50	9:15	2.18	100.0
		10:15	0.00	
3	0.75	9:30	2.44	55.3
		10:30	0.99	
4	1.00	9:40	1.56	46.1
		10:40	0.83	
5	0.75	9:50	1.14	68.4
		10:50	0.36	

Table 5: Interstitial water samples number 1, 2, and 3 are from the Callianassa collecting area, whereas number 4 is from the Upogebia collecting area. The samples were obtained at low tide.

Sample number	Sampling depth inches	mls O <sub>2</sub> /liter
1	18	0.46
2	13	0.58
3	16	0.81
4	32	0.15

## BIBLIOGRAPHY

- Allee, W. C. and R. Oesting. 1934. A critical examination of Winkler's method for determining dissolved oxygen in respiration studies with aquatic animals. *Physiological Zoölogy* 7(4):509-541.
- Amberson, W. R., H. S. Mayerson and W. J. Scott. 1924. The influence of oxygen tension upon metabolic rate in invertebrates. *The Journal of General Physiology* 7(1):171-176.
- Bliss, Dorothy E. 1953b. Endocrine control of metabolism in the land crab, Gecarcinus lateralis (Fréminville). I. Differences in the respiratory metabolism of sinusglandless and eyestalkless crabs. *The Biological Bulletin* 104(3):275-296.
- Borradaile, L. A. 1903. On the classification of the Thalassinidea. *The Annals and Magazine of Natural History*, ser. 7, 12(71):534-551.
- Bovbjerg, Richard V. 1952. Comparative ecology and physiology of the crayfish Orconectes propinquus and Cambarus fodiens. *Physiological Zoölogy* 25(1):34-56.
- Brafield, A. E. 1964. The oxygen content of interstitial water in sandy shores. *The Journal of Animal Ecology* 33:97-116.
- Chen, Tse-Yin. 1932. The effect of oxygen tension on the oxygen consumption of the Chinese fresh-water crab, Eriocheir sinensis. *The Chinese Journal of Physiology* 6(1):1-12.
- Darby, Hugh H. 1938. Moulting in the crustacean Crangon armillatus. (Abstract) *Anatomical Record* 72(4;Suppl.):78.
- Egusa, Syuzo. 1961. Studies on the respiration of the "Kuruma" prawn Penaeus japonicus Bate. II. Preliminary experiments on its oxygen consumption. *Bulletin of the Japanese Society of Scientific Fisheries* 27(7):650-659.
- Eriksen, Clyde H. 1963. Respiratory regulation in Ephemera simulans Walker and Hexagenia limbata (Serville) (Ephemeroptera). *The Journal of Experimental Biology* 40(3):455-467.
- Fox, H. Munro, B. G. Simmonds and R. Washbourn. 1933. Metabolic rates of aquatic arthropods from different habitats. *The Journal of Experimental Biology* 10(1):67-74.

- Fox, H. Munro, B. G. Simmonds and R. Washbourn. 1935. Metabolic rates of ephemerid nymphs from swiftly flowing and from still waters. *The Journal of Experimental Biology* 12(2):179-184.
- Fox, H. Munro, C. A. Wingfield and B. G. Simmonds. 1937. The oxygen consumption of ephemerid nymphs from flowing and from still waters in relation to the concentration of oxygen in the water. *The Journal of Experimental Biology* 14(2):210-218.
- Fry, F. E. J. 1957. The aquatic respiration of fish. In: *The physiology of fishes*, ed. by Margaret E. Brown. Vol. 1. New York, Academic Press. p. 1-63.
- Hailstone, T. S. and W. Stephenson. 1961. The biology of Callianassa (Trypaea) australiensis Dana 1852 (Crustacea, Thalassinidea). University of Queensland at Brisbane, Department of Zoology Papers 1(12):259-285.
- Helff, O. M. 1928. The respiratory regulation of the crayfish, Cambarus immunis (Hagen). *Physiological Zoölogy* 1(1):76-96.
- Henze, Martin. 1910. Über den Einfluss des Sauerstoffdrucks auf den Gaswechsel einiger Meerestiere. *Biochemische Zeitschrift* 26:255-278.
- Hiestand, William A. 1931. The influence of varying tensions of oxygen upon the respiratory metabolism of certain aquatic insects and the crayfish. *Physiological Zoölogy* 4(2):246-270.
- Hoar, William S. 1966. *General and comparative physiology*. New Jersey, Prentice-Hall. 815p.
- Hyman, Libbie H. 1929. The effect of oxygen tension on oxygen consumption in planaria and some echinoderms. *Physiological Zoölogy* 2(4):505-534.
- \_\_\_\_\_ 1932. Relation of oxygen tension to oxygen consumption in Nereis virens. *The Journal of Experimental Zoölogy* 61(2):209-221.
- Jones, Jack Colvard. 1956. Effects of different gas concentration on heart rates of intact Anopheles quadrimaculatus Say larvae. *The Journal of Experimental Zoölogy* 131(3):257-265.

- Jones, J. D. 1955. Observations on the respiratory physiology and on the haemoglobin of the polychaete genus Nephtys, with special reference to N. hombergii (Aud. Et. M. -Edw.). The Journal of Experimental Biology 32(1):110-125.
- Keys, A. B. 1930. The measurement of the respiratory exchange of aquatic animals. The Biological Bulletin 59(2):187-198.
- Krogh, August. 1941. The comparative physiology of respiratory mechanisms. Philadelphia, University of Pennsylvania. 172p.
- Larimer, James L. 1962. Responses of the crayfish heart during respiratory stress. Physiological Zoölogy 35(2):179-186.
- Larimer, James L. and Alvin H. Gold. 1961. Responses of the crayfish, Procambarus simulans, to respiratory stress. Physiological Zoölogy 34(2):167-176.
- Mac Ginitie, G. E. 1930. The natural history of the mud shrimp Upogebia pugettensis (Dana). The Annals and Magazine of Natural History, ser. 10, 6(31):36-44.
- \_\_\_\_\_ 1934. The natural history of Callianassa californiensis Dana. The American Midland Naturalist 15(2):166-177.
- \_\_\_\_\_ 1935. Ecological aspects of a California marine estuary. The American Midland Naturalist 16(5):629-765.
- Mac Ginitie, G. E. and Nettie Mac Ginitie. 1949. Natural history of marine animals. New York, McGraw-Hill. 473p.
- Mc Leese, Donald W. 1956. Effects of temperature, salinity and oxygen on the survival of the American lobster. Journal of the Fisheries Research Board of Canada 13(2):247-272.
- Maloeuf, N. S. Royston. 1936. Quantitative studies on the respiration of aquatic arthropods and on the permeability of their outer integument to gases. The Journal of Experimental Zoölogy 74(3):323-351.
- \_\_\_\_\_ 1937. Studies on the respiration (and osmoregulation) of animals. I. Aquatic animals without an oxygen transporter in their internal medium. Zeitschrift für Vergleichende Physiologie 25(1):1-28.

- Marshall, S. M., A. G. Nicholls and A. P. Orr. 1935. On the biology of Calanus finmarchius. Part VI. Oxygen consumption in relation to environmental conditions. *Journal of the Marine Biological Association of the United Kingdom* 20(1):1-27.
- Montuori, A. 1913. Les processus oxydatifs chez les animaux marins en rapport avec la loi de superficie. *Archives Italiennes de Biologie* 59(2):213-234.
- Pearse, A. S. 1945. Ecology of Upogebia affinis (Say). *Ecology* 26(3):303-305.
- Pearse, A. S., H. J. Humm and G. W. Wharton. 1942. Ecology of sand beaches at Beaufort, N. C. *Ecological Monographs* 12(2):135-190.
- Pohl, Marian Evelyn. 1946. Ecological observations on Callianassa major Say Beaufort, North Carolina. *Ecology* 27:71-80.
- Prosser, C. Ladd. 1955. Physiological variation in animals. *Biological Reviews of the Cambridge Philosophical Society* 30:229-262.
- Prosser, C. Ladd and Frank A. Brown, Jr. 1961. Comparative animal physiology. 2d ed. Philadelphia, Saunders. 688p.
- Roberts, John L. 1957. Thermal acclimation of metabolism in the crab Pachygrapsus crassipes Randall. I. The influence of body size, starvation, and molting. *Physiological Zoology* 30(3):232-242.
- Schales, Otto and Selma S. Schales. 1941. A simple and accurate method for determination of chloride in biological fluids. *The Journal of Biological Chemistry* 140(3):879-884.
- Stevens, Belle, A. 1928. Callianassidae from the West Coast of North America. *Publications of the Puget Sound Biological Station* 6:315-369.
- Subrahmanyam, C. B. 1962. Oxygen consumption in relation to body weight and oxygen tension in the prawn Penaeus indicus (Milne Edwards). *Proceedings of the Indian Academy of Sciences, ser. B*, 55(3):152-161.

- Teal, John M. 1959. Respiration of crabs in Georgia salt marshes and its relation to their ecology. *Physiological Zoology* 32(1):1-14.
- Thomas, H. J. 1954. The oxygen uptake of the lobster (Homarus vulgaris Edw.). *The Journal of Experimental Biology* 31(2):228-251.
- Thompson, Lawrence C. 1967. Osmotic adaptation in Callianassa californiensis and Upogebia pugettensis (Crustacea, Thalassinidea). Master's thesis. Berkeley, University of California. 45 numb. leaves.
- Richards, F. A. and N. Corwin. 1956. Some oceanographic applications of recent determinations of the solubility of oxygen in sea water. *Limnology and Oceanography* 1(4):263-267.
- von Brand, Theodor. 1946. Anaerobiosis in invertebrates. Normandy, Mo., Biodynamica. 328p.
- Vonk, H. J. 1960. Digestion and metabolism. In: *The physiology of crustacea*, ed. by Talbot H. Waterman. Vol. I. New York, Academic Press. p. 291-316.
- Walshe, Barbara M. 1948. The oxygen requirements and thermal resistance of chironomid larvae from flowing and from still waters. *The Journal of Experimental Biology* 25(1):35-44.
- Weymouth, F. W. et al. 1944. Total and tissue respiration in relation to body weight. A comparison of the kelp crab with other crustaceans and with mammals. *Physiological Zoology* 17(1):50-71.
- Wiens, A. Wayne and Kenneth B. Armitage. 1961. The oxygen consumption of the crayfish Orconectes immunis and Orconectes nais in response to temperature and to oxygen saturation. *Physiological Zoology* 34(1):39-54.
- Wolvekamp, H. P. and Talbot H. Waterman. 1960. Respiration. In: *The physiology of crustacea*, ed. by Talbot H. Waterman. Vol. I. New York, Academic Press. p. 35-100.
- ZoBell, Claude E. and C. B. Feltham. 1942. The bacterial flora of a marine mud flat as an ecological factor. *Ecology* 23(1):69-78.