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Title SUCCINIC DEHYDROGENASE ACTIVITY IN MESOPELAGIC OCEANIC FISHES

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The purposes of this investigation were to measure the relative levels of enzymatic and respiratory metabolism of some vertically migrating mesopelagic fishes collected from the ocean waters off Oregon and to modify and adapt an enzyme method for use as a measure of metabolic activity at sea with a minimum of equipment. A knowledge of the quantitative measure of the respiratory and metabolic rates of these organisms could be usefully applied to several problems of interest; oxygen minimum layers, vertical migration, and vertical distribution.

Ten species of fishes were studied with regard to their respiratory potential as measured by succinic dehydrogenase activity. Oxygen consumption for two species was measured at various temperatures for comparative purposes.

The metabolic rates calculated from the enzyme data fell within the limits of the standard metabolic rate - temperature relationships

compiled from the data of several authors for fishes of the same size from various environments. The fish from the greatest depths had low metabolic rates comparable to those reported for some Antarctic and Arctic species. Metabolic rates of species of mesopelagic fish differed significantly and declined with the general depth and temperature at which the species was found.

SUCCINIC DEHYDROGENASE ACTIVITY IN MESOPELAGIC OCEANIC FISHES

by

JOHN FRANKLIN KARINEN

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Typed by Lucinda M. Nyberg

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I. INTRODUCTION

Vertical Migration and Respiratory Rates

The respiratory rates of pelagic, oceanic organisms have relevance to a number of questions concerning the phenomenon of diurnal vertical migration and rates of energy transfer in marine populations. It is known that many mesopelagic organisms, defined as those distributed between depths of 200 meters and 1000 meters during daylight, undertake extensive, diel, vertical migrations during which they are subjected to great changes in temperature and pressure (35, p. 835-839; 28, p. 767-784; 7, p. 158-192; 11, p. 1-8). Knowledge of the respiratory rates of these organisms would be helpful when applied to some specific problems, such as: What are the energy requirements of these extensive vertical migrations? Does the rate of respiration of these organisms while at depth differ considerably from that when the same organisms are at or near to the surface? Are there significant differences in respiratory rates between deep-living species as compared to species which come to or near the surface during their vertical migration? Can the respiratory rates of these organisms account in part for the oxygen minimum layers which have been observed to sometimes coincide with the vertical

distribution of these organisms? Is it possible for these organisms to live for extended periods within these oxygen minimum layers? What are the rates of energy transfer in mesopelagic, populations? Are there both eurythermal and stenothermal fish species among mesopelagic fishes?

Although many studies have been conducted on the respiratory and metabolic rates of organisms from cold and warm habitats, (8, p. 945-955; 22, p. 213-224; 30, p. 67-92; 26, p. 33-44) few studies have been made on the respiratory rates of mesopelagic, oceanic organisms and the "in situ" effects of temperature and pressure on these rates. Napora (27, p. 1685) has recently reported a study describing respiration and the excretion of phosphate and ammonia by the prawn Systellaspis debilis in relation to temperature and pressure at depths of 1100 to 1300 meters in the North Atlantic Ocean. The temperature responses and tissue respiration in some Antarctic crustacea taken from the Wedell Sea have been recorded by McWhinnie (15, p. 63-72).

Wohlschlag (41, p. 287-292; 42, p. 11-18; 43, p. 1050-1051; 44, p. 589-587; 46, p. 33-62) has extensively studied the respiratory metabolism, growth, swimming activity and cold adaptation of some pelagic and benthic fishes of McMurdo Sound, Antarctica.

However, I am not aware of any study on the respiratory metabolism of the vertically migrating, mesopelagic fishes of the temperate

oceans. The need for such a study is apparent if we wish to begin to determine the answers to the questions previously proposed.

In most studies on respiration, the respiratory rates of whole organisms are determined. However, some comparisons of the temperature influence on the respiratory rates of excised tissues taken from organisms in different climates have been made (9, p. 275-282; 5, p. 330-338; 22, p. 213-224). A recent contribution is that of McWhinnie (15, p. 63-72), cited above. Manometric techniques have been employed in most studies of this kind.

It is also possible to study respiratory metabolism on the molecular level, that is by measuring the activities of enzymes of enzyme systems which are involved in cellular respiration. Measurements of the activities of specific enzymes of deep water organisms would be of interest to the study of pressure effects on enzyme systems. Several contributions have been made in the study of pressure effects on enzyme systems (14, p. 226-236; 17, p. 251-255; 18, p. 1341-1346; 10, p. 113-120; 12, p. 243-248). In most of these studies, however, microorganisms were used as the source of biological material.

Curl and Sandberg (6, p. 123-138) have measured respiratory metabolism at the molecular level by developing a method in which the activity of one of the enzyme systems, of the chain of respiratory enzymatic reactions interposed between the oxidation of a substrate

and the assimilation of oxygen, is measured and correlated with the oxygen consumption of the whole organism. The parameter measured by this method has been called, "respiratory potential"; which may be defined as the maximum rate of cellular respiration which a tissue homogenate is capable of maintaining under a given set of conditions with unlimited substrate. Because of the applicability and convenience of this method for use on a small oceanographic research vessel, it was selected for the present study.

The objectives of the present study were to determine the "respiratory potential" of muscle tissue of some mesopelagic fishes, ascertain whether differences in "respiratory potential" exist between mesopelagic species of fish, and apply the data to as many of the previously mentioned questions as possible.

II. SELECTION OF A METHOD

The standard procedure for determining respiratory rates and metabolic activity is to measure oxygen consumption by whole organisms, tissue slices or homogenates. Oxygen consumption is usually measured by the Winkler method, an oxygen electrode, or by manometry (36, p. 1-90).

The usual method for measuring "in vivo" respiratory rates of small fishes and organisms is to confine them in a container and measure the reduction in oxygen concentration occurring during a measured time interval. The initial and final oxygen concentration is usually measured by the Winkler method. A control is also used so that changes due to microscopic plants and bacteria may be applied to the values obtained.

There are some problems in the use of these procedures for measurement of respiratory rates of mesopelagic organisms. First, it is difficult to obtain specimens of deep-water species which are in suitable condition. The nets used to capture these organisms combined with the long journey to the surface tends to mutilate most of the specimens considerably, and although they are "alive" when they reach the surface, their condition is not conducive to measurements of respiration of the intact organisms. The warmer water at the surface has a detrimental effect on the survival of species from depth.

Extreme, rapid changes in pressure are also detrimental to the survival of organisms, especially those fishes with swim bladders.

Ideally, to make measurements of whole fish respiration or tissue respiration of mesopelagic fish one should either never remove the fish from the conditions of temperature and pressure of its environment or return it to the temperature and pressure of its environment and maintain it under these conditions until the effects of the changes of pressure and temperature occurring during capture may have been nullified. One method might be to use a constant pressure and temperature apparatus in which the fish could be incubated at the proper temperature and pressure during the measurement of whole fish respiration or immediately prior to tissue respiration studies. Another method would be to confine the fish to a non-rigid walled container and send it back to various depths for the period of measurement of oxygen consumption. However, this method presents some technical difficulties in that the fish would respire during the lowering and raising of the container to the desired depth, thereby making the oxygen consumption a function of several different pressures and temperatures. One might overcome this difficulty by measuring oxygen consumption by means of an oxygen electrode, but many engineering problems would be involved.

Since the primary objective of the present study was to compare the respiratory rates of various mesopelagic fishes, and because of

the difficulties encountered in obtaining enough suitable specimens for whole-fish respiratory studies by the capturing techniques employed, it was decided to measure respiration at the enzyme level.

It is possible to measure one or more of the enzyme systems associated with tissue respiration, particularly enzymes of the Krebs cycle and of the electron transport chain in mitochondria. The activity of an enzyme system closely associated with the electron transport chain will be proportional to gaseous respiration, since molecular oxygen is the ultimate acceptor of this system. Two logical selections are to measure either the enzymes succinic dehydrogenase or cytochrome oxidase. For the present study the measurement of the activity of succinic dehydrogenase was chosen because of the availability of a relatively simple assay procedure which utilized a comparatively stable substrate. Oxygen consumption measurements on a limited number of individual fish of two species were made for comparative purposes.

The enzymatic method used in the present study is actually the application of a method described by Curl and Sandberg, (6, p. 123-138) for the estimation of respiratory potential in marine organisms. They showed that the measured activity of the enzyme system associated with the oxidation of succinate to fumurate in the Krebs cycle by homogenates from the marine minnow Menidia menidia (Linn.) 1766 is directly related to routine oxygen consumption (2, p. 48).

Therefore, by measuring the activity of a specific respiratory enzyme or enzyme system, it is possible to obtain an estimate of the relative "respiratory potential" of an organism and to calculate a theoretical rate of oxygen consumption provided that there is a constant relationship between gaseous respiration and enzymatic activity.

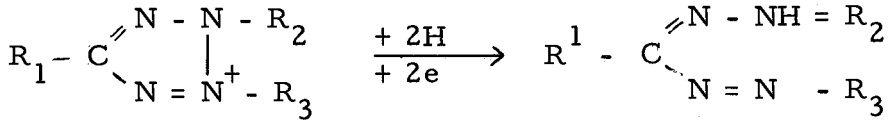
Considerable variation of Q_{O_2} is apparent among animal tissues of various types within an organism (38, p. 378-79).

White states that, "It is not possible, precisely, to define the factors which determine the respiratory rate of a given tissue. Since, however, the bulk of this respiration in all tissues appears to occur over the cytochrome chain and, presumably, is phosphate-linked, it may be assumed that it is the demand for energy-rich phosphate which conditions the particular respiratory rate of each tissue." Each kind of tissue will have a characteristic enzyme activity so that the total potential gaseous respiration will depend upon the sum total of the potential respiration of each and all tissues which make up the organism. In the present study muscle tissue was used for all the assays. According to White et al. (38, p. 379) the Q_{O_2} for muscle tissue is considerably less than the Q_{O_2} for most other tissues, i. e. kidney, liver, spleen; being seven times less than that of kidney and about six times less than that for liver. In the rat the Q_{O_2} of muscle is about 1/3 of the Q_{O_2} for the whole organism. Muscle Q_{O_2} of the prawns Pandalus borealis and P. montagui were about 1/3 to 1/4 of

the whole organism Q_{O_2} (9, p. 281). Therefore, we would expect the "potential respiration" of muscle tissue as measured by this method to be considerably less than the total "potential respiration" of the organism as a whole. However, it is believed that in this method oxidative phosphorylation is uncoupled from the electron chain which would increase the enzymatic rate and possibly make the calculated "respiratory potential" of the muscle tissue approach the average Q_{O_2} of the whole organism. Comparisons between calculated Q_{O_2} from enzyme measurements and Q_{O_2} determined from actual oxygen consumption measurements were made.

III. METHODS

The method is based upon the fact that colorless, water-soluble, tetrazolium salts can be reduced, under aerobic conditions, by the electron transport system of cells to form water-insoluble colored formazans (1, p. 953-54).



Tetrazolium Salt Formazan

The colored formazan may be extracted in an organic solvent and the concentration determined spectrophotometrically.

There is no agreement as to where the tetrazolium salts are intercepting the hydrogen ions but it has been suggested that a flavoprotein must be present before reduction will take place (1, p. 954). According to Slater (33, p. 27) it is not the dehydrogenase itself that reacts in the succinic dehydrogenase assay but rather a factor which mediates the transport of hydrogen from the dehydrogenase to cytochrome C. Actually, the present method might be called a measure of the succinic oxidase system rather than a measure of succinic dehydrogenase.

Reagents

Reagents were prepared as described by Curl and Sandberg (6,

p. 125-127). The tetrazolium salt used was p-iodonitrotetrazolium violet, (INT) purchased from Sigma Chemical Co. and Nutritional Biochemical Corp. An INT solution was prepared by dissolving 100 mg. of the reagent in 50 ml of distilled water. Two or three drops of 3N sodium carbonate solution (pH 7. 5) were added to increase the solubility of the INT. The solution was then filtered and refrigerated at 1 or 2° C until use.

Sodium succinate was prepared as a 0. 4 M stock solution in distilled water with a few drops of 3N sodium carbonate added as a preservative.

Phosphate buffer solutions of pH 7. 7 and 0. 2 M were prepared from KH_2PO_4 and NaOH solutions. Buffer solutions of pH 12. 0 and 0. 2 M were prepared from K_2HPO_4 and NaOH for use in the preparation of standard curves.

Cysteine HCl solutions of 0. 005 M and ascorbic acid solutions of 0. 0025 M were prepared for standard curve determination.

The solvent used to dissolve the formazan was a 1:1. 5 v/v mixture of tetrachloroethylene:acetone. It was prepared in one liter quantities.

The INT, sodium succinate and phosphate buffer solutions were prepared shortly before each cruise. They were all frozen, either separately or mixed in the proper proportions, shortly before the cruise departure and remained in that condition until immediately

prior to use.

Biological Materials

Specimens of mesopelagic fishes were collected from the offshore waters of Oregon with an Isaacs-Kidd mid-water trawl during regular nekton cruises of the R. V. Acona of the Department of Oceanography, Oregon State University. The cruises varied in length from three to five days. Eight cruises were made on the dates shown in Table I.

TABLE I. SCHEDULE OF CRUISE DATES.

Experiment Number	Dates of Cruise
1	10 July - 13 July 1962
2	17 July - 19 July 1962
3	8 August - 10 August 1962
4	17 September - 21 September 1962
5	5 July - 8 July 1963
6	26 August - 31 August 1963
7	26 February - 29 February 1964
8	27 July - 1 August 1964

Data from experiment number four was very limited and is not included.

Mid-water trawls were made from about 200 meters to the surface at night and from about 1000 meters to the surface during the

daylight hours. Some tows were also made from 400-500 meters to the surface. (See Pearcy(20, p 85) for location of tows.) Most specimens for this work were collected at the station 50 nautical miles west of Newport, Oregon. Although the greater number of specimens came from a non-closing mid-water trawl, some were collected from different depths during the 1963 cruises, when a Lamont multiple plankton sampler was used as an opening and closing codend collecting unit on the mid-water trawl (21, p. 263-264). Specimens from the non-closing, mid-water trawl were generally not as mutilated as specimens obtained with the multiple plankton sampler.

Only specimens which were active were selected for enzymatic respiratory measurements and only those which appeared unharmed were used in oxygen consumption measurements.

Shipboard Procedure

Organisms were removed from the net and transferred to a bucket of sea water taken from the surface (14 - 16° C). Live fishes were used for enzyme analysis as quickly as possible.

Oxygen respiratory rates over 30 to 60 minute periods were measured on two species, Tarletonbeania crenularis (Jordan and Gilbert) 1880 and Poromitra crassiceps (Gunther) 1878. These specimens were acclimated for 30 minutes at the temperature of the experiment before measurements commenced. Measurements of oxygen uptake

were made in the dark as well as in the light in aspirator bottles of 250 ml capacity with controls to correct for the respiration of micro-organisms. Aliquots of 60 ml were taken for oxygen determinations using the Winkler method. The rate of oxygen uptake was independent of the respiratory period and the results were averaged for all experiments. These two species reacted differently to confinement. Tarletonbeania crenularis was actively swimming both at the beginning and at the end of the experiments. Poromitra crassiceps lay on its side or back in some cases or remained upright but moved only its pectoral fins and opercula throughout the experiment, giving either a moribund or "basal rate".

Organisms to be used in enzyme analysis were measured for standard length. Two muscle tissue samples of the same size (about 250 mg wet weight) were cut from the area of the caudal peduncle. One was preserved by freezing for use in determining wet weight, dry weight, ash-free dry weight and ash-free, lipid-free dry weight. The other was homogenized with a ground glass tissue grinder cooled with ice water. The size of samples and replicates were determined by displacement. A volume change of about 0.3 ml were equivalent to approximately 250 mg of wet weight tissue. Some whole fishes were frozen for length and weight determinations.

The homogenate was made up to 10 ml, with distilled water, and centrifuged for about three minutes to provide a supernatant fluid

devoid of large visible particles. One ml of this homogenate fluid was added to 4 ml of reaction mixture consisting of 2 ml phosphate buffer, 1 ml of 0.4 M sodium succinate and 1 ml of 0.2% INT solution in a screwcapped, 15 ml, graduated centrifuge tube. This mixture was usually incubated at 35° C in the dark for 20 minutes, however, some other temperatures were tried for comparative purposes.

The reaction was stopped by adding two drops of concentrated HCl and the tubes were placed in an ice chest to cool. Eight ml of the TCE-acetone solvent was added and the mixture was shaken. The tubes were then stored in an ice chest in the dark until return to the shore laboratory. Duplicates were run in some of the experiments.

Blanks for the determination of endogenous color were prepared by adding one ml of the homogenate to 4 ml of distilled water and 8 ml of a TCE-(tetrachloroethylene)-acetone mixture. This type of blank was found to be necessary when working with crustaceans which contain high concentrations of carotenoid pigments, but interference from pigments in most fishes was negligible.

Blanks for endogenous respiration were prepared in some experiments to determine what part of the total observed activity was due to respiration of endogenous succinate or substrates other than succinate. These were prepared by adding one ml of homogenate to a mixture consisting of all of the components except succinate. Distilled water was substituted for the sodium succinate in this case. Reagent blanks

and blanks for endogenous activity were treated in the same manner as the regular activity measurements.

Laboratory Procedure

The reaction mixture-solvent emulsions were kept in the dark through the following operations.

Tubes containing homogenate and solvent were centrifuged at 6000-8000 g's for ten minutes. The clear top layer (acetone:water) was decanted and discarded. The colored bottom layer was then made up to 8 ml with solvent and the tubes were placed in the dark for about ten minutes to allow the solvent to extract the formazan from the lipid and protein present. Centrifugation was repeated and any clear acetone:water layer which appeared on top was decanted. If necessary the tubes were again made up to 8 ml with solvent. The solutions were then filtered through Whatman No. 1 filter paper using a polyethylene funnel. (Polyethylene was used because the somewhat fatty solution did not adhere to it.)

The absorbance of the formazan solution was measured at 490 $m\mu$ against an endogenous color blank or against TCE-acetone solvent in a Beckman DB spectrophotometer.

The absorption spectrum of the formazan formed upon the reduction of INT by ascorbate is shown in Figure 1.

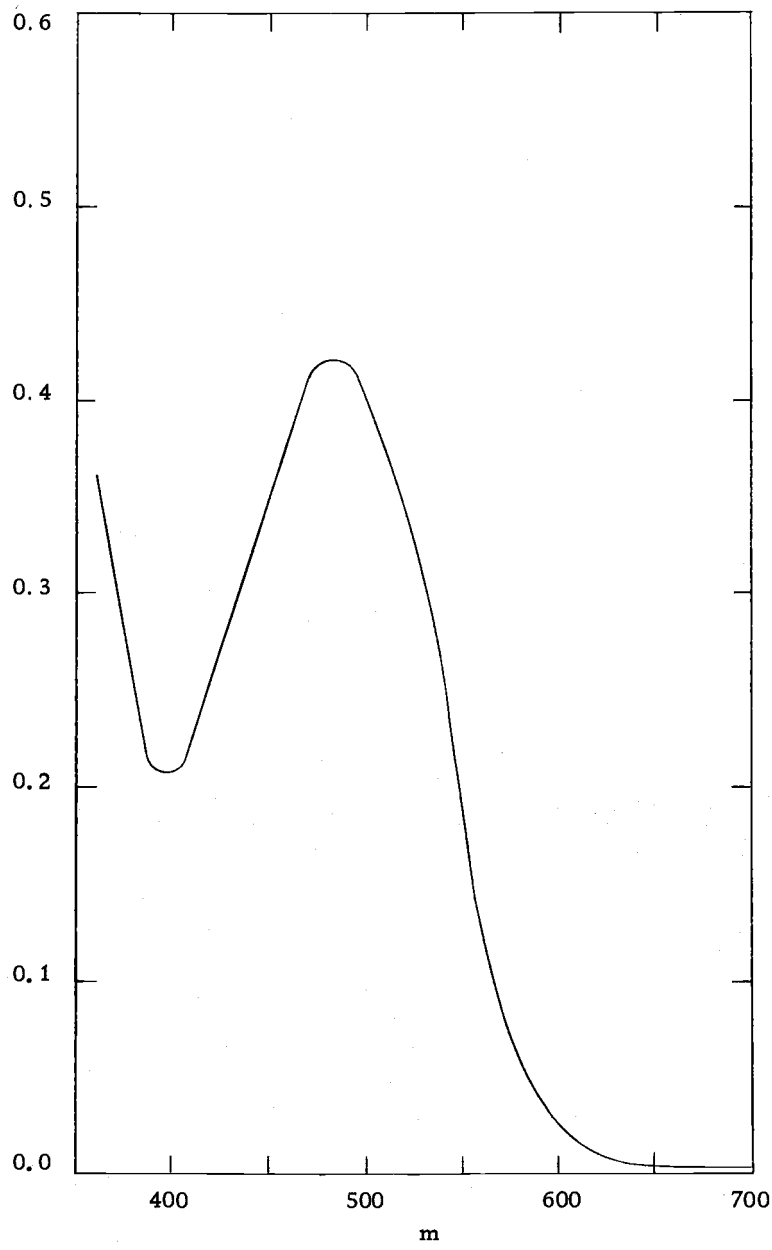


Figure 1. Absorption spectrum of formazan from INT.

Tetrazolium salt was reduced with ascorbate under aerobic conditions and the resulting formazan dissolved in TCE-acetone.

Weight Determinations

Duplicate tissue samples of those used to prepare the homogenates were weighed wet, dried at 65° for 72 hours and weighed again. Lipids were extracted with petroleum ether for 24 hours in a soxhlet apparatus. The samples were then transferred to a tared crucible and ashed for 24 hours at 450° C.

These procedures gave wet weights (W^W), dry weights (W^D), ash-free dry weights (W^{AFD}) and ash-free, lipid-free dry weights (W^{ALFD}) of samples to compare with enzyme activities. Protein determinations using the spectrophotometric method of Warburg and Christian (37, p. 400-403) were performed on the homogenates in connection with some of the experiments.

Standardization of INT Solutions

The reaction mixture was standardized before each cruise by incubating aliquots of a freshly prepared stock solution of cysteine . HCl (0. 005 M) with 1 ml of INT solution (0. 2%), 1 ml of 0. 4 M sodium succinate, 2 ml of phosphate buffer (pH 12. 0), and freshly boiled distilled water to make the volume up to 5 ml. Screw cap, 15 ml centrifuge tubes were used as containers. The mixture was allowed to react in the dark in a 35° C water bath until the reaction was complete. Time of incubation in the bath was 12-18 hours although 12 hours was

sufficient for the reaction to reach completion.

The stock solution of cysteine . HCl was prepared with freshly boiled distilled water which had been cooled in a closed container. This technique reduced the rate of oxidation of the solution during addition to the standards and prolonged the reducing ability of the cysteine solution.

Ascorbic acid was used in earlier trials but reproducible, linear standardization curves could not be obtained due to rapid oxidation by molecular oxygen. A pH of 12. 0 for the standardization procedure was selected, since pH 11. 5 to 12. 0 gave a linear relationship while pH's above or below gave non-linear curves. (Figure 2). At the lower pH's the rate of oxidation-reduction was so slow that the reaction did not go to completion. At very high pH's water itself causes the reduction of INT, making reduction a function of two factors rather than one. INT can be reduced by water if exposed to strong radiant energy of wavelength shorter than 450 $m\mu$, especially at high pH (19, p. 647).

At the conclusion of the incubation period the standards were cooled, and 8 ml of TCE-acetone was added. After shaking they were placed in the dark for 30 minutes. They were then centrifuged for ten minutes at 6000 to 8000 g's. The clear top layer was decanted and placed into another screwcap vial into which 2 ml of TCE-acetone was added. After extracting for 15 minutes in the dark, they were again centrifuged and the clear top layer was decanted and discarded.

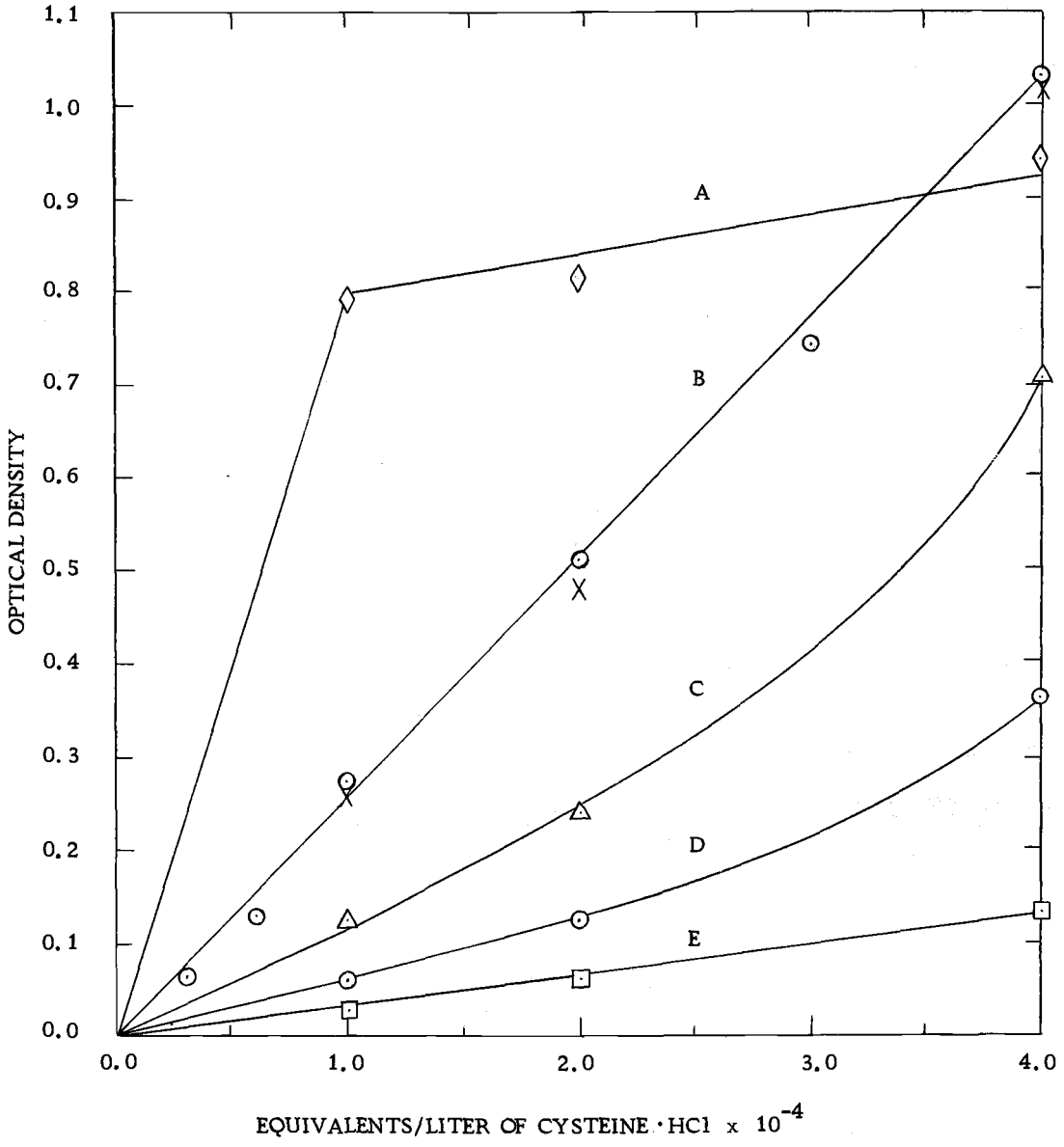


Figure 2. Effect of pH on standard curve.

A = pH 12.7 (PO_4 buffer)
B = pH 11.5 (x) and 12.0 ( ) (PO_4 buffer)
C = pH 11.5 (borate buffer)
D = pH 10.0 (PO_4 buffer)
E = pH 7.7 (PO_4 buffer)

The two colored extracts resulting from each concentration of cysteine were combined, made up to 8 ml with TCE-acetone and read at 490 mμ against a TCE-acetone blank.

Standardization curves were plotted for each experiment. Optical density at 490 mμ was plotted against concentration of cysteine; which is expressed as equivalents of cysteine per liter.

The reaction mixture from the first experiment showed considerably less activity than in subsequent experiments, due to a lower activity per unit weight of the INT used for this experiment. The activities of different lots varied significantly (Figure 3).

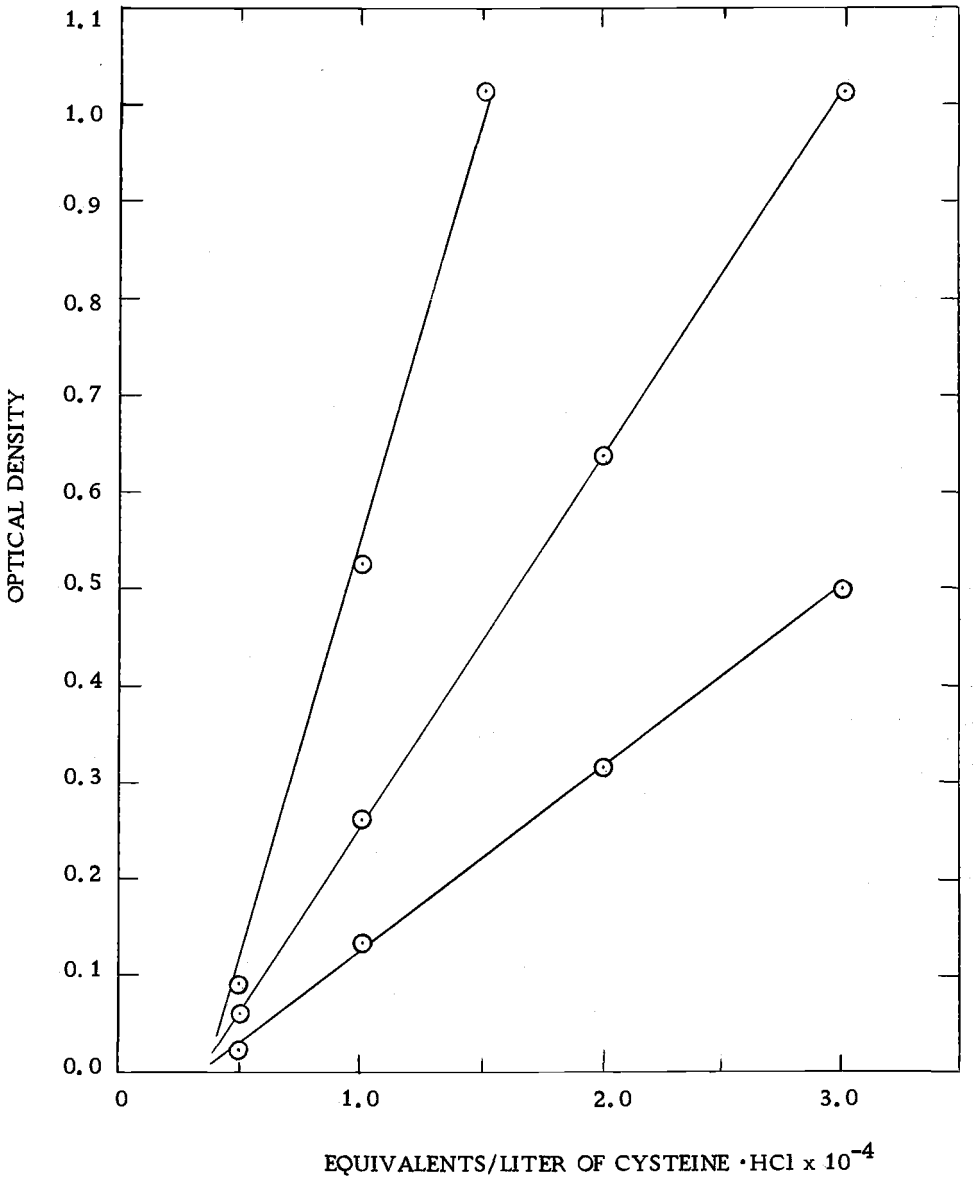


Figure 3. Typical standard curves showing variation in reducing capacity, optical density versus concentration of cysteine ·HCl.

IV. RESULTS

Enzyme Studies

A variable number of specimens of each of several species of mesopelagic fishes were used in this study. Some species occurred in the trawls infrequently so that only a few individuals of these species were available for assay purposes. Data for ten species, including the four dominant mesopelagic fish of this region (20, p. 88), are reported (Figures 4-7). These species are Tarletonbeania crenularis (Jordan and Gilbert) 1880, Diaphus theta (Eigenmann and Eigenmann) 1890, Lampanyctus leucopsarus (Eigenmann and Eigenmann) 1890, Tactostoma macropus (Bolin) 1939, Bathylagus milleri (Jordan and Gilbert) 1898, Bathylagus pacificus (Gilbert) 1890, Poromitra crassiceps (Gunther) 1878, Lampanyctus ritteri (Gilbert) 1890, Lampanyctus nannochir (Gilbert) 1890, and Lampanyctus regalis (Gilbert) 1891. Data for Bathylagus milleri and Bathylagus pacificus were combined and reported as Bathylagus spp.

Data for tissue respiration in the ten species were converted to units of hydrogen ion equivalents transferred per unit of weight per time interval. The means, standard deviations, and ranges of the potential respiratory activity were determined for each species and are expressed in various categories; wet weights, ash-free dry weight, ash-lipid-free dry weight and weight of protein. These data

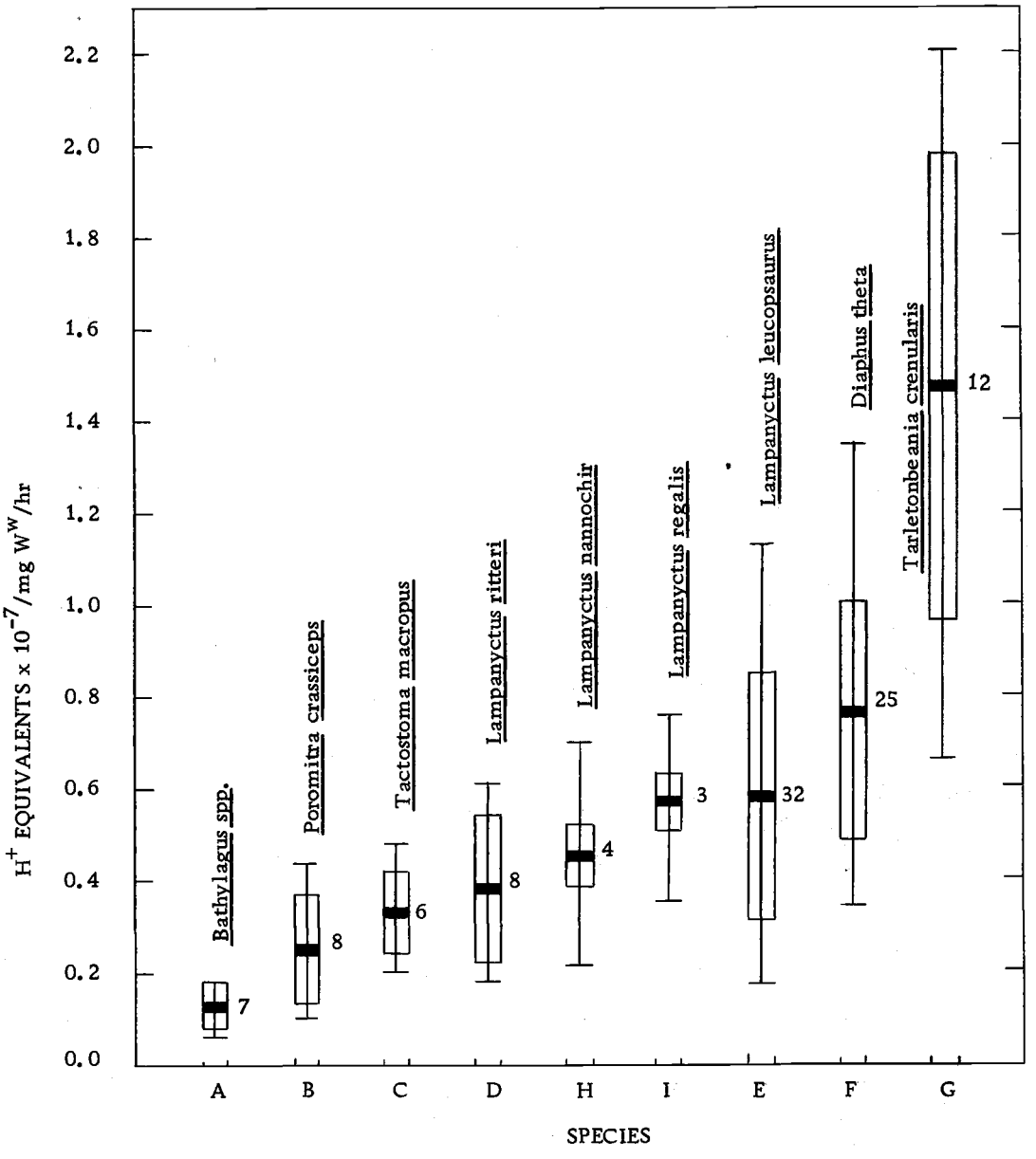


Figure 4. Tissue respiratory activity of various species of mesopelagic fishes.

The number of hydrogen ion equivalents transferred per milligram of wet weight of skeletal muscle tissue per hour is plotted for each species. The numbers beside the bars indicate the number of specimens assayed. The rectangular figures with the heavy lines across the centers (means) indicate the standard deviation from the means. The vertical center lines with the horizontal lines at the ends indicate the ranges.

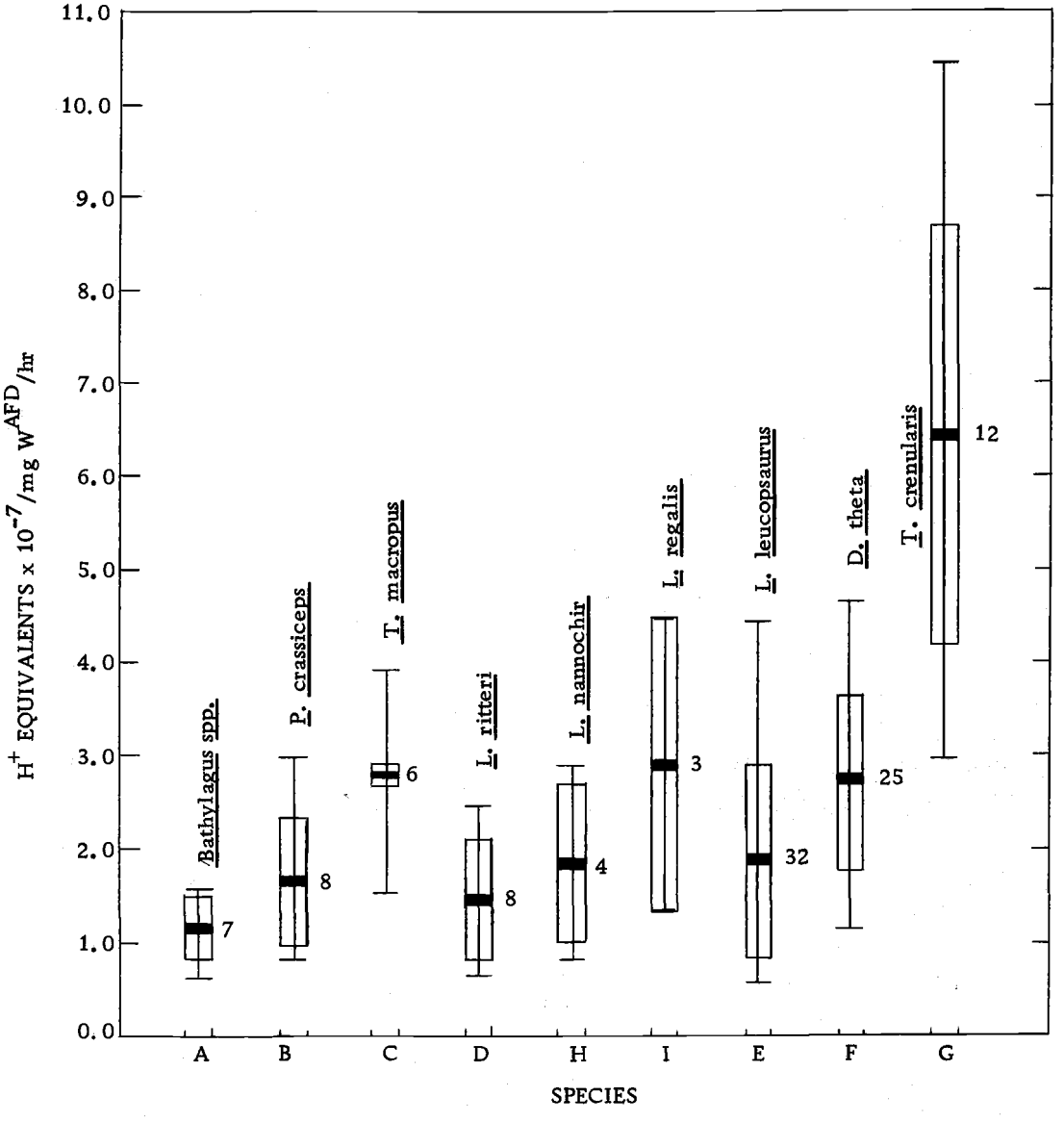


Figure 5. Tissue respiratory activity of various species of mesopelagic fishes.

The number of hydrogen ion equivalents transferred per milligram of ash-free dry weight of skeletal muscle tissue per hour is plotted for each species. See the caption of Fig. 4 for a description of the presentation.

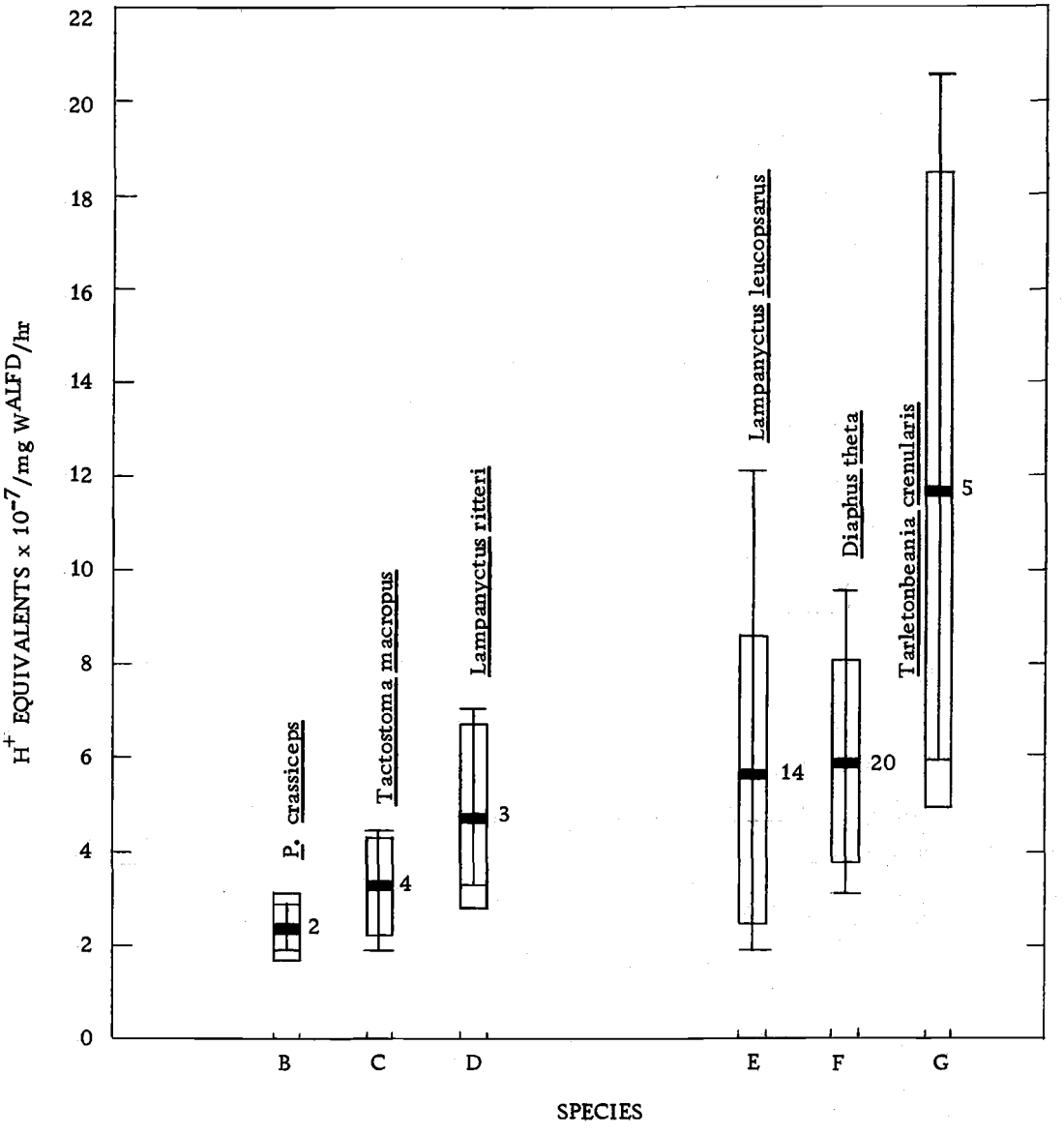


Figure 6. Tissue respiratory activity of various species of mesopelagic fishes.

The number of hydrogen ion equivalents transferred per milligram of ash-free, lipid-free dry weight of skeletal muscle tissue per hour is plotted for various species. See the caption of Fig. 4 for a description of the presentation.

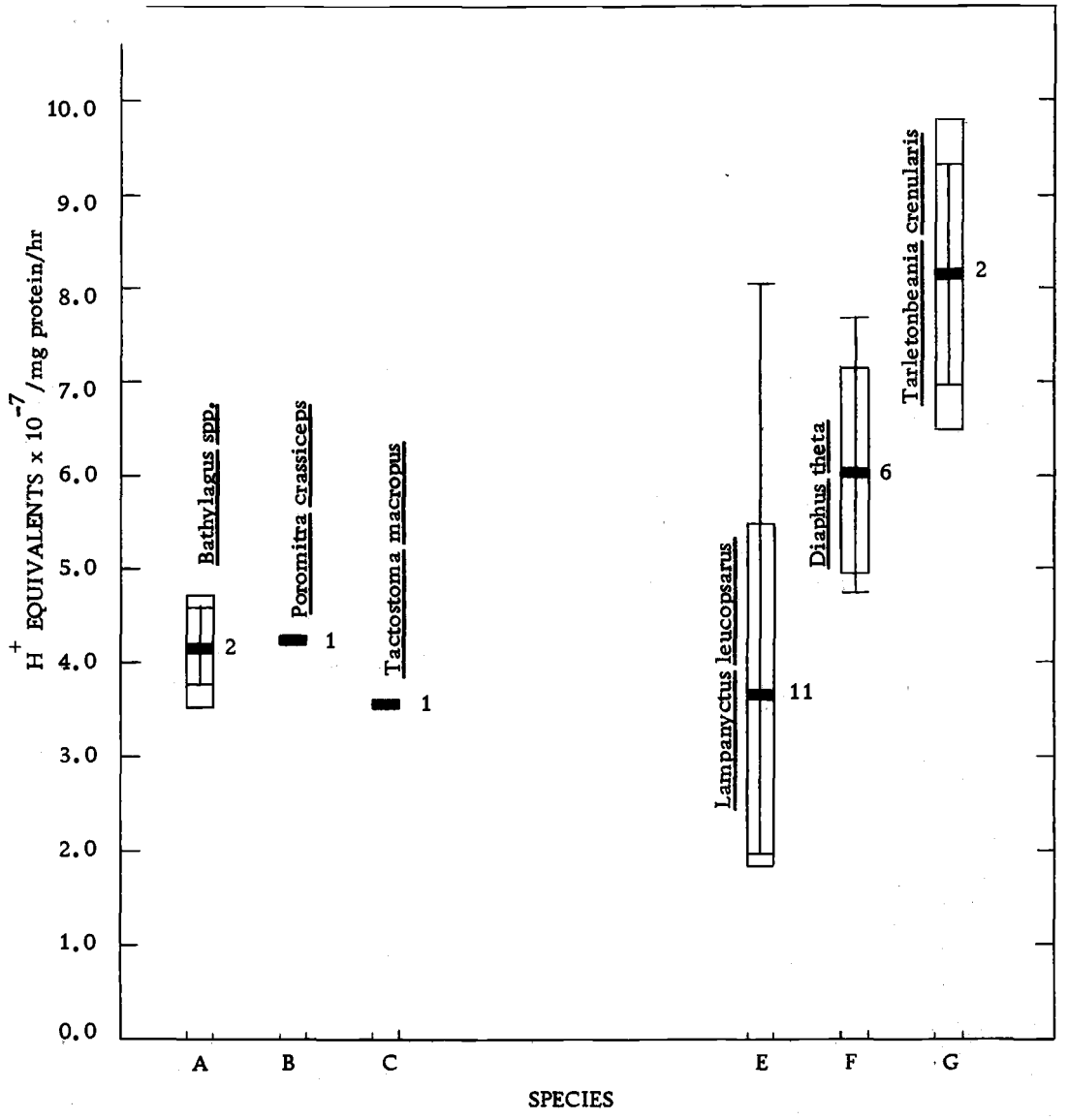


Figure 7. Tissue respiratory activity of various species of mesopelagic fishes.

The number of hydrogen ion equivalents transferred per milligram of protein per hour is plotted for each species. See Fig. 4 for the description of the presentation.

are not corrected for size differences between species. It is customary to show differences in metabolic rate or respiratory rate between organisms of varying weight by plotting the logarithm of the wet weight versus the log activity of metabolic or respiratory rate (30, p. 72; 47, p. 1-11). The general formula relating respiratory rate to body size is: ml O_2 = K W^x, where K is a species constant, W is the wet weight in grams, x is a regression exponent which varies according to the kind and size of organism, and ml O_2 is the milliliters of oxygen consumed per hour by a single organism of W weight. If oxygen consumption is exactly related to surface area, x is 0. 66, however a great range of values of x have been reported for fishes from various environments.

Wohlschlag (46, p. 42) reports that the weight regression exponent (x) for Antarctic fishes is somewhat higher than 0. 80 - 0. 85, which is the value commonly observed for temperate and arctic species. He reports values from . 42 to as high as 1. 4 but discounts values below about . 70 as due to differences in response to temperature by fishes of different sizes of differences due to sex. The relationship of weight to oxygen consumption was found to be significantly different between females and males or immature females as shown in Wohlschlag's Figure. The lumping of both sexes together increases the scatter as is indicated by line (a) in his figure. This difference in metabolic rate between sexes may account, in part, for

the scatter of data in the present study.

The general formula relating metabolic rate and body size is similar to that for respiratory rate except that the metabolic rate is expressed per unit weight, i. e. $\text{ml } \frac{O_2}{g} = KW^{-x}$. In this case, however, the value of x will be negative and the equation will express a line with a negative slope. The metabolic rate (ml O_2/g/hr) is sometimes referred to as Q_{O_2}. This equation may also be written in the logarithmic form as:

$$\log Q_{O_2} = \log K - X \log W.$$

The logarithm of the metabolic rates of two fish species from the present study, Tarletonbeania crenularis and Poromitra crassiceps are plotted in Figure 8 with respect to the logarithm of the wet weight of the specimens. In this case, the metabolic rate is expressed by the enzyme activity rather than by oxygen consumption per unit weight. It appears that each of the species shows some metabolic heterogeneity, possibly due to sexual differences as noted earlier by Wohlschlag (45, p. 48). In the case of T. crenularis, however, the two distinct groups may be partially explained by experimental procedure. In most instances, tissue sections were taken from the caudal peduncle, however, with very small specimens of this species, it was not possible to obtain enough material in this manner. Therefore, in a few instances either a whole fish or one half of the fish was

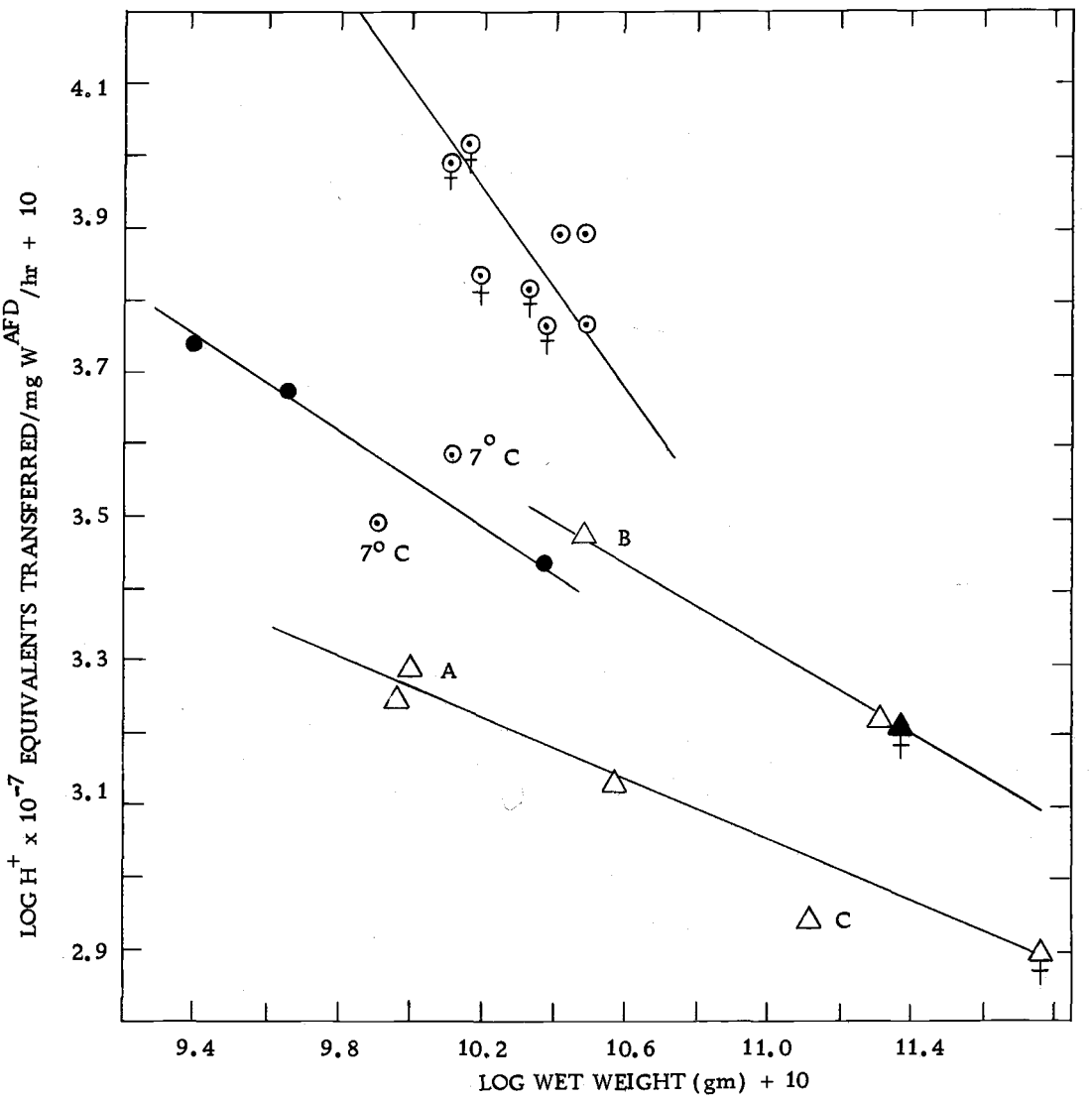


Figure 8. Logarithm of enzyme activity versus the logarithm of the whole fish wet weight.

T. crenularis
- ♀ = females,
- ● = whole fish,
- ⊙ = other

P. crassiceps
- ♀ = mature female,
- ▲ = immature female,
- △ = other

used. The metabolic rate measured in this manner was apparently significantly less than when only muscle tissue was used. The two specimens marked 7.0° C in Figure 8 also represent a change in experimental procedure. These are two specimens which were used in gaseous respiratory measurements and held at 7.0° C during these tests and immediately prior to the enzyme measurements. The eight remaining points in the upper group represent specimens which were held at 14.4 to 18.0° C immediately prior to enzyme analysis. In order to conform to uniformity of procedure the data from only these eight specimens of T. crenularis will be considered in later calculations, although all the specimens were included in the calculations for Figures 4-7. Unfortunately, no attempt was made, originally in this experiment, to systematically determine the sex of the individual specimens, therefore the data for both sexes and various stages of maturity for a species are combined. A few chance observations had been noted and recorded, however, and some of the specimens had been preserved so it was possible to determine the sex of a small portion of the specimens. Only T. crenularis and P. crassiceps were sexed, as shown in Figure 8. No males were positively identified in either species, however five of the eight T. crenularis represented by the points in the upper group proved to be females. The apparent heterogeneity of the P. crassiceps specimens could not be correlated with either holding temperature or sex, since it was possible to

determine the sex of only two individuals. The regression lines were eye-fitted to the points and although two lines were drawn for P. crassiceps there is no distinct reason for doing so except to show the heterogeneity of the group. The letters A, B, and C designate individual specimens of P. crassiceps which were also used in oxygen uptake measurements (Figure 14).

Calculation of Oxygen Consumption Rates from the Enzyme Data

It is desirable for comparative purposes to express the metabolic rates as the rate of oxygen consumed per unit weight per unit time. Since the activity of the enzyme system measured by this method is believed to be directly proportional to the oxygen consumption rate (6, p. 137), it is possible by considering the metabolic reactions involved, to make a direct calculation to obtain an estimate of the oxygen consumption rate which a specific enzyme rate indicates. A sample calculation using the average enzyme activity for T. crenularis will show how this was done.

T. crenularis had an average enzyme activity of 1.48×10^{-7} H^+ transferred per milligram of wet weight per hour. In the electron transport chain, which is the metabolic connection between the reactions of the Krebs cycle and the reduction of molecular oxygen within the cell, each 1/2 O_2 reduced requires two hydrogen ions.

$$2\,H^{+} + 1/2\,O_2 \text{ ——— } H_2O$$

Therefore, the activity in T. crenularis indicates an oxygen consumption rate of $.37 \times 10^{-7}$ moles of oxygen reduced/ mg W^w/hr or $.37 \times 10^{-4}$ millimoles of O_2/mg W^w/hr. Since one mmole of oxygen equals 22.4 ml, 22.4 ml times $.37 \times 10^{-4}$ mmoles O_2/mg W^w/hr equals .83 ml 0_2/g W^w/hr. The conversion factor is 5.6×10^{6}; that is 1.48×10^{-7} H^{+}/mg W^w/hr times 5.6×10^{6} equals .83 ml O_2/g W^w/hr.

All of the enzyme measurements for each of the species were converted to oxygen consumption rates in this manner and the logarithm of the metabolic activity has been plotted against the logarithm of the wet body weight in grams (Figures 9-11). The regression line of log metabolic rate on log wet body weight was determined for several of the species by the method of least squares (16, p. 212-231; 3, p. 123). In most cases not enough data was available so as to ensure a reliable regression line, however, the relative metabolic rates for fishes of the same size is indicated. No regression line was determined for Lampanyctus ritteri, Lampanyctus nannochir, and Lampanyctus regalis. The regression line drawn in Figure 10 is for Lampanyctus leucopsarus. In the regression equations Q_{O_2} is the metabolic rate, W is the whole fish wet weight, and r is the coefficient of correlation. Figure 12 shows three species plotted in a similar manner except that the metabolic rate is expressed on an ash-free, dry-weight basis.

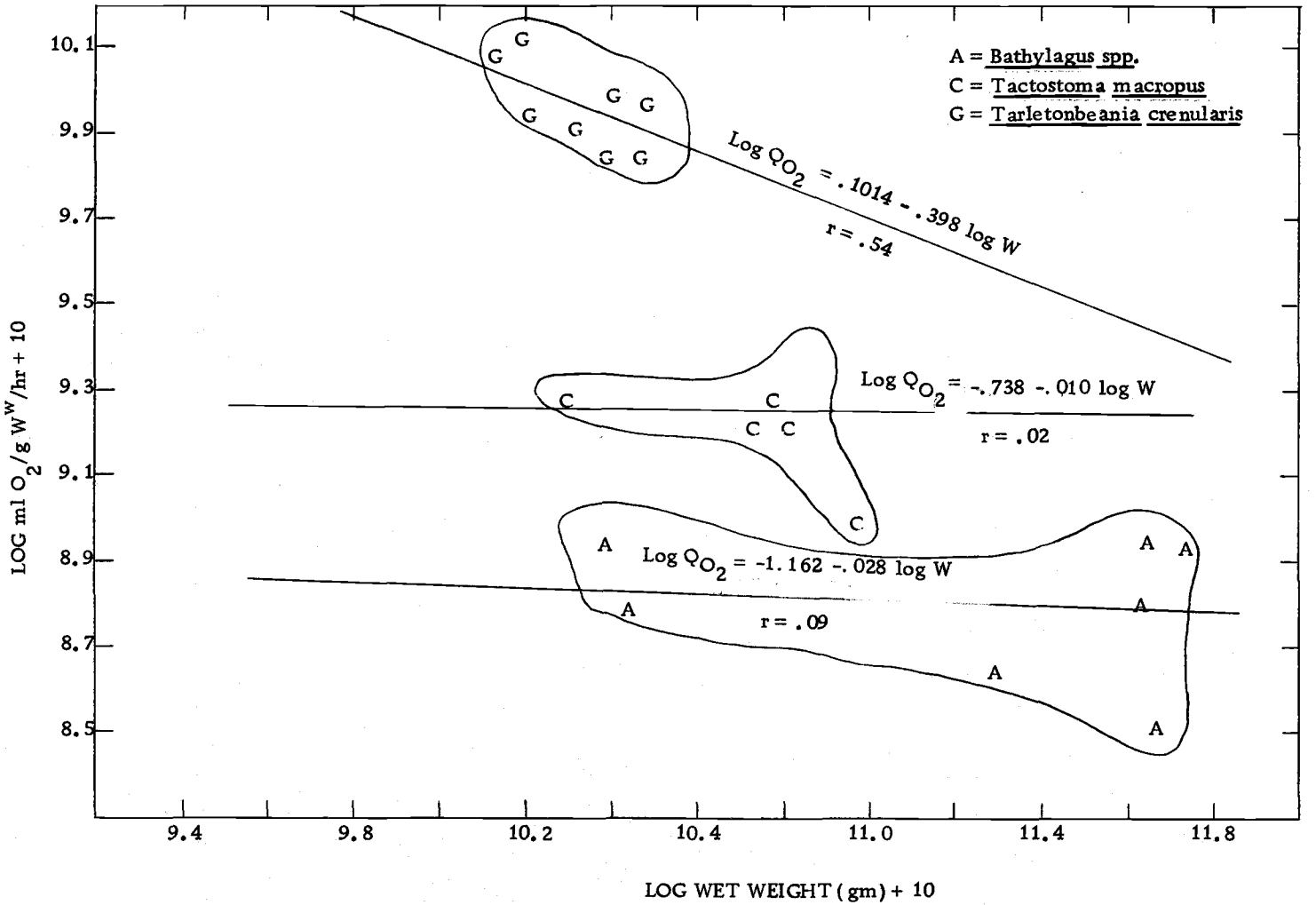


Figure 9. Log metabolic rate (wet weight basis) versus log wet weight of whole fish.

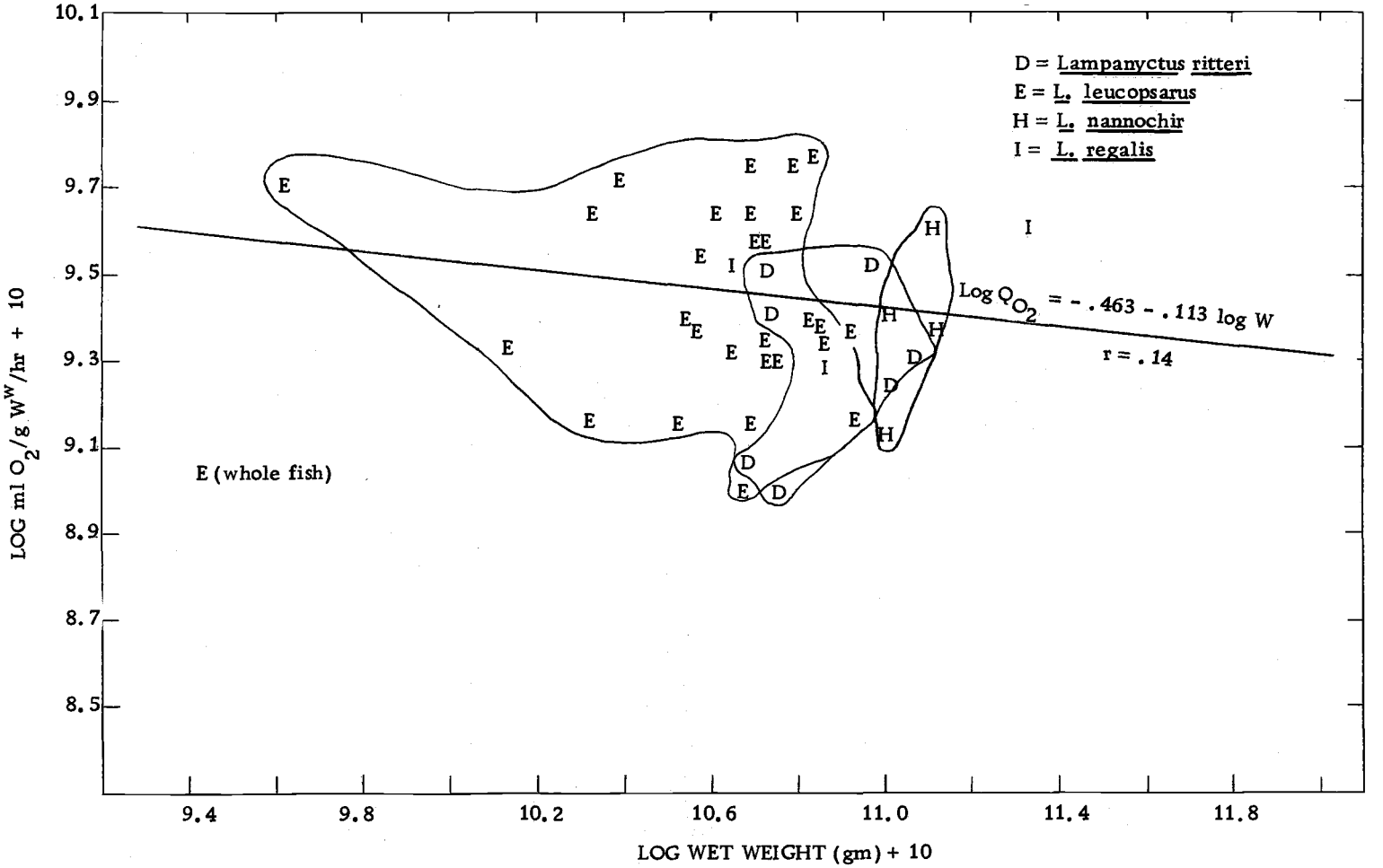


Figure 10. Log metabolic rate (wet weight basis) versus log wet weight of whole fish.

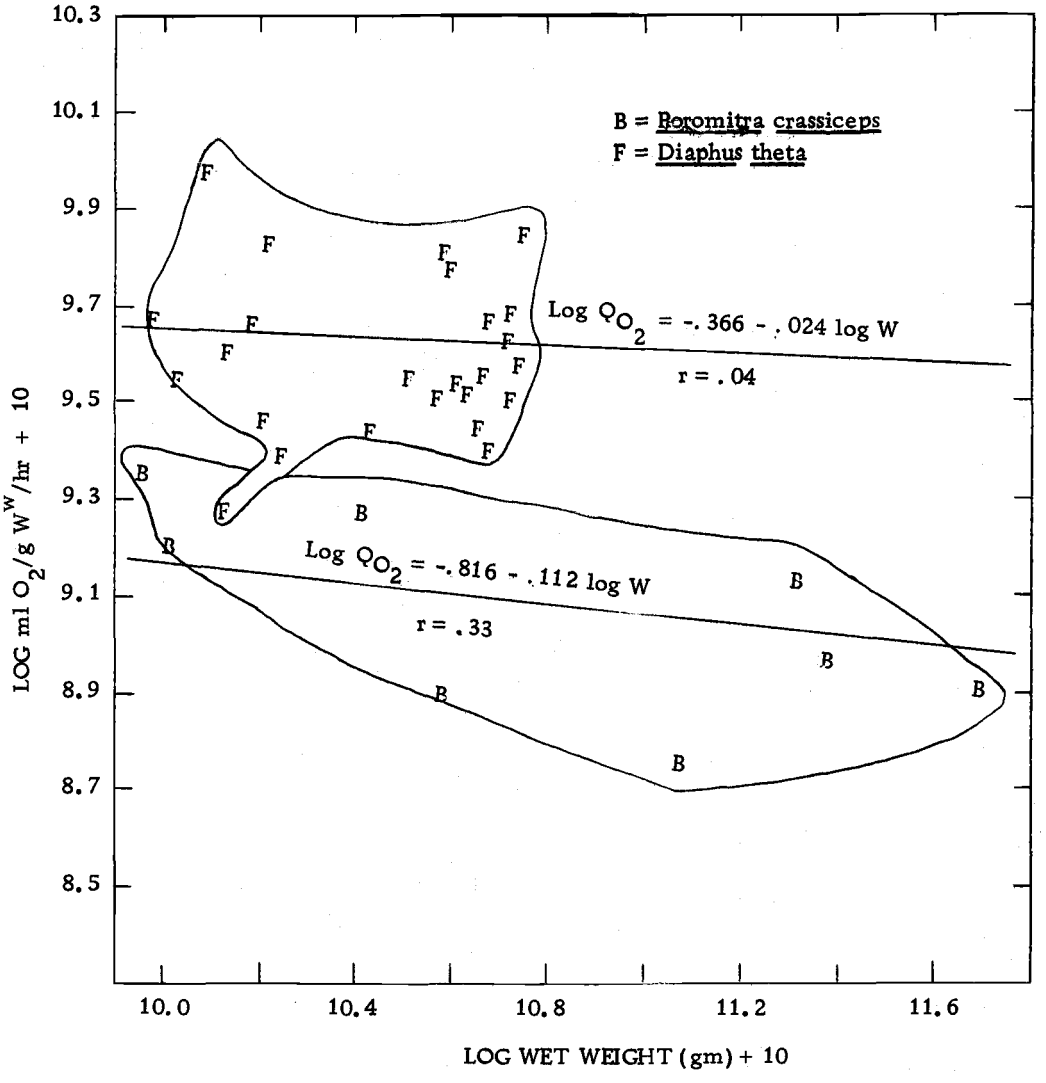


Figure 11. Log metabolic rate (wet weight basis) versus log wet weight of whole fish.

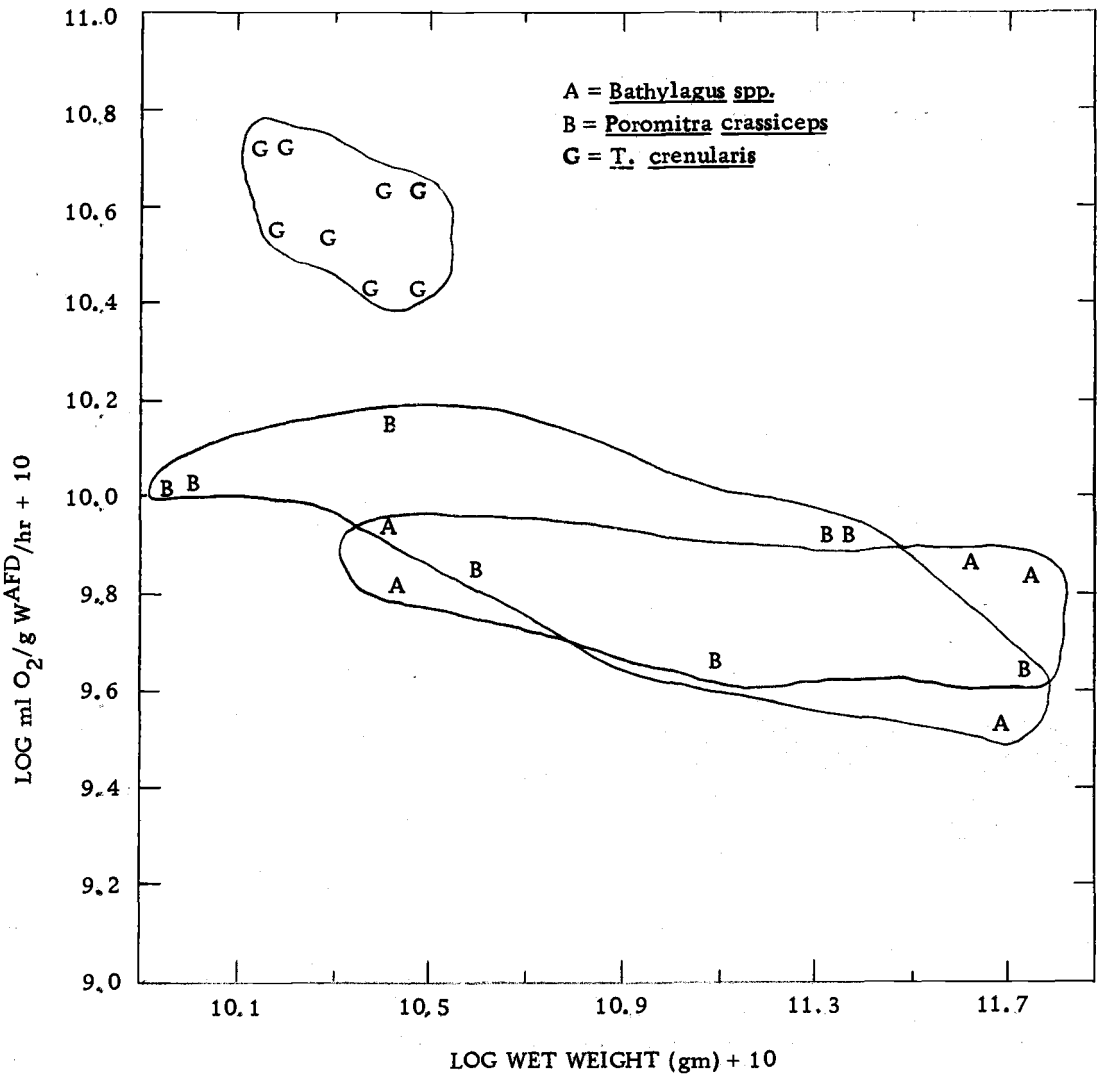


Figure 12. Log metabolic rate (ash-free, dry weight basis) versus log wet weight of whole fish.

On this basis the differences in metabolic rate are not as great, and all of the species except Tarletonbeania crenularis occur very close to the same position in Figures 5 and 12.

Since the metabolic rates appear to be essentially independent of the size of the organism, Figure 6 will suffice to show the relative magnitude of metabolic rate on an ash-free, lipid-free, dry weight basis.

Oxygen Uptake Measurements of Respiration

Gaseous respiration was determined for a limited number of individuals of the two species, T. crenularis, and P. crassiceps. Measurements were made during the month of July at various temperatures, both in the light and in the dark, (Figures 13, 14). An approximate Q_{10} has been calculated for each species. Cases in which the same specimen was used at more than one temperature are indicated by the "S" adjacent the points. The solid circles indicate that the measurement was made in the dark while the open circles indicate that the specimen respired in the light.

The T. crenularis used in these measurements varied in total wet weight from .90 to 3.20 grams. It is possible to draw an eye-fitted curve between the points indicating a Q_{10} of approximately 2.5 (Figure 13). This corresponds to a respiratory rate of about 2.0 ml O_2 per gram of wet weight per hour at 14° C which is close to the

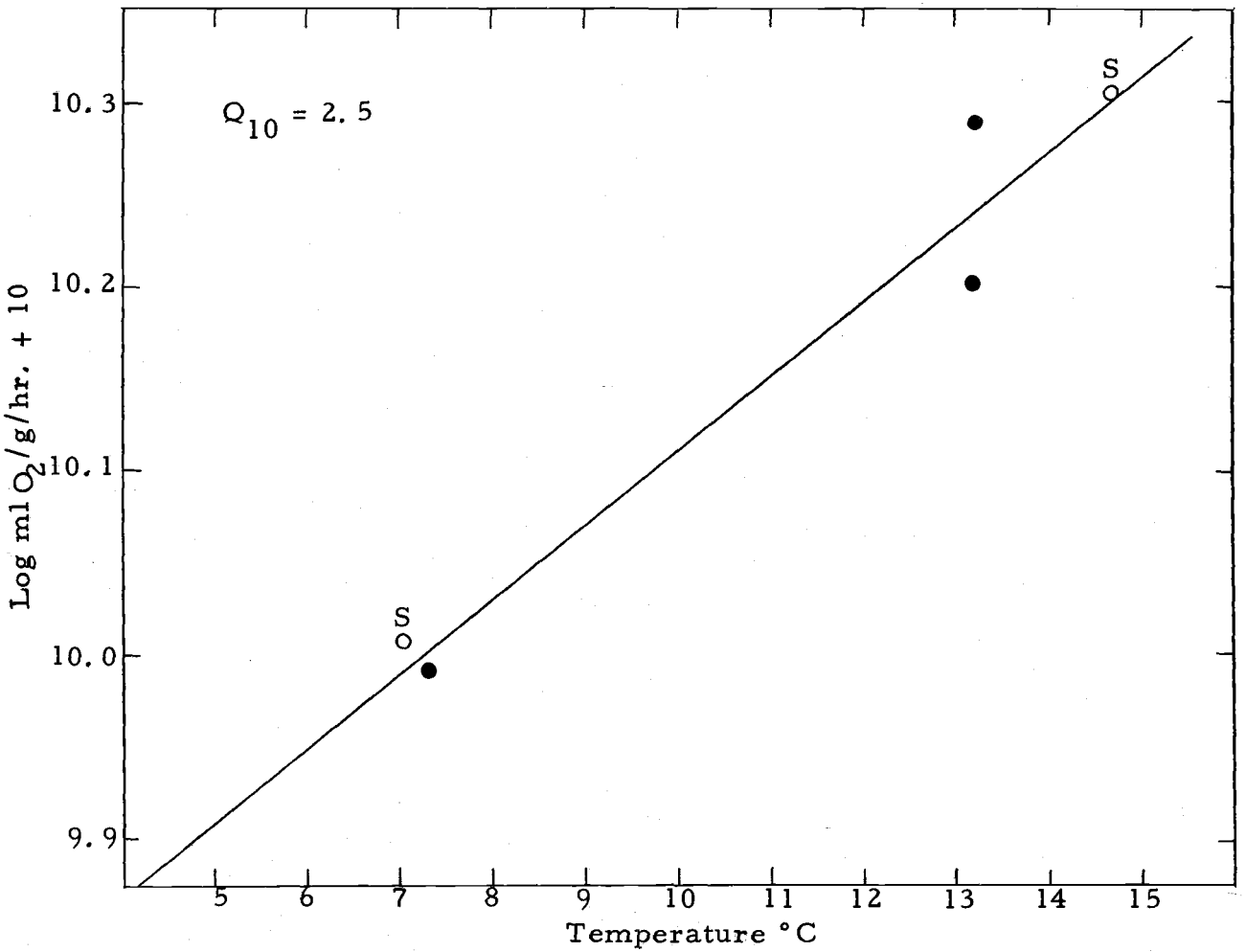


Figure 13. Gaseous respiration of T. crenularis using the modified Winkler method for oxygen analysis.

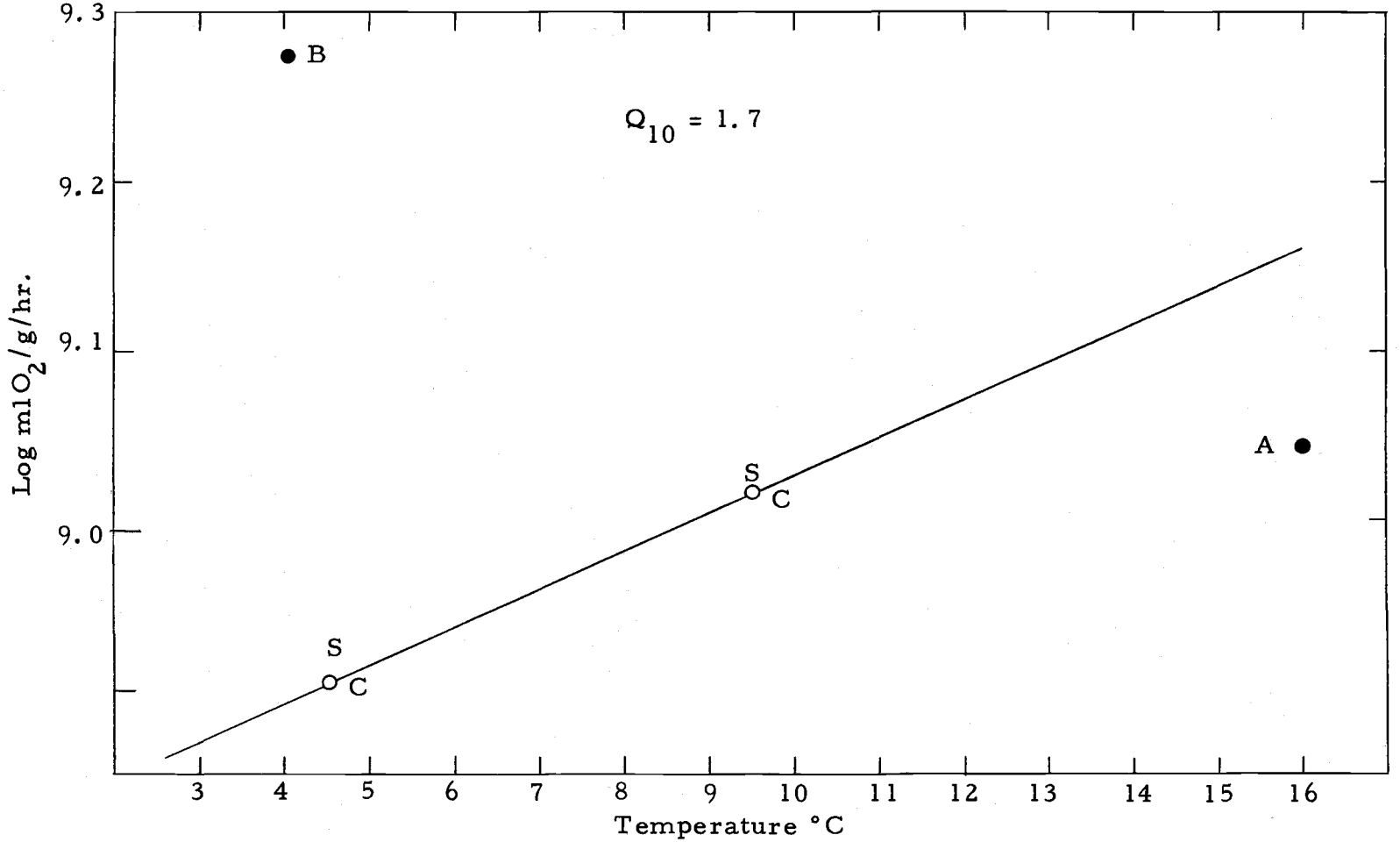


Figure 14. Gaseous respiration of Poromitra crassiceps using the modified Winkler method for oxygen analysis.

temperature of the normal environment for T. crenularis at the surface during the summer, while at 5° C the Q_{O_2} would be about 0.8 ml O_2/g W^w/hr. This species was extremely active while in the respiratory chamber and it appears that light had no effect on its respiratory rate.

The data for Poromitra crassiceps (Figure 14) is limited. One of the specimens, designated A and weighing 1.20 grams, was found to be almost dead at the termination of the measurement at the highest temperature. It was possible to revive this specimen, however, by returning to the 5° C bath, but no further measurements were made on this particular fish, except for enzyme measurements, which show (Figure 8) that data from this specimen (again designated by A) lie on the lower of the two regression curves plotted for this species. Potential respiration data for a specimen C (11.7 grams) is also close to this regression line (Figure 8) which agrees with its relative gaseous respiration, (Figure 14). Specimen B (2.6 grams) shows a higher respiratory and metabolic rate on both graphs. The agreement between these two methods suggests that the differences in respiration and the scatter of data in Figure 14 is real and is probably due to inherent physiological differences within the individual specimens rather than light conditions or experimental error. It is possible to estimate a Q_{10} of 1.7 by using the two points obtained from specimen C.

The gaseous respiration measurements agreed with the enzyme data in showing a considerably lower oxygen consumption rate for Poromitra crassiceps than for T. crenularis. The respiratory rate for P. crassiceps ranged from .08 ml O_2/g W^W/hr at 5° C to approximately .128 ml O_2/g W^W/hr at 15° C except for the exceptionally high value of .189 ml O_2/g W^W/hr at 4.5° C obtained with specimen B. The normal environmental temperature for this species is believed to be about 4-5° C.

V. DISCUSSION

Comparison of Metabolic Differences With Vertical Distribution of Species

The actual respiratory or metabolic activity of a fish will depend on several factors; i. e. recency of food consumption, motor activity, physiological condition, temperature of the environment to which it is adapted, sexual state, effects of handling, temporal rhythms, size, oxygen tension, light, age, growth rates, stage of acclimation to the existing environment, and social organization patterns (40, p. 45-59 and 2, p. 30-61). Because of this multitude of factors which can affect metabolic activity, the large standard deviations and ranges of metabolic activity noted in Figures 4-7 are not unexpected.

It is apparent from Figures 9-12 that there are significant differences in "respiratory potential" between species of mesopelagic fishes as measured by the method of Curl and Sandberg (6, p. 123-138). The significance and reasons for these differences, however, are not so apparent.

Some investigators have shown that poikilotherms differ in metabolic activity at the enzyme level according to their temperature history (24, p. 255; 4, p. 328). Stroganov (34, p. 3-21) and Bullock (4, p. 321-322) contend that fish become adapted to a temperature range over which they are able to regulate their metabolic rate as the

temperature changes. It is very possible that differences in temperature with increasing depth may in part account for the noted differences in metabolic rates of various species of mesopelagic fish, especially if they limit their vertical migrations to a specific temperature range. Wohlschlag (45, p. 48) has correlated seasonal changes of metabolic rate in an Antarctic Nototheniiform fish, Trematomus bernacchii, with very slight temperature changes (ca. 0.05° C). The changes in temperature with increasing depth within the environment of the mesopelagic fish used in the present study are sufficient to account for a two fold change in metabolic rate if we assume a Q_{10} of 2. This change is not sufficient of itself to explain the observed differences in metabolic rate. Table II compiled from data presented by Pearcy (20, p. 92) gives the temperature ranges for various depths during the summer, 50 miles off Newport, Oregon.

TABLE II. TEMPERATURE VERSUS DEPTH AT NH-50

Depth (meters)	Temperature Range (° C)
0 - 10	15
10 - 25	15 - 10
25 - 50	10 - 9.6
50 - 100	9.6 - 8.2
100 - 200	8.2 - 7.0
200 - 300	7.0 - 6.2
300 - 400	6.2 - 5.8
400 - 500	5.8 - 5.2
500 - 1000	5.2

The thermocline was located between 10 and 25 meters and the halocline at about 75 meters.

As of this time a detailed description of the vertical distribution of mesopelagic fishes off the Oregon coast is not available since studies with opening and closing nets have not been completed. However, Pearcy (20, p. 91-93) has reported some generalizations deduced from data obtained with non-closing nets fished to successive depths during periods of daylight and darkness. Figure 15, compiled from generalized depth distribution data as reported by Pearcy (20, p. 91-93) gives the approximate range of day and night vertical distribution as compared to temperature and depth for the seven mesopelagic species. It should be emphasized that this is only a very rough approximation based upon the frequency with which these species appeared in tows to various depths, therefore exact limits; if they exist, cannot be depicted and this generalized distribution applies only to the area of study. Adults of the four dominant mesopelagic species, Tactostoma macropus, Lampanyctus leucopsarus, Diaphus theta and Tarlotenbeania crenularis are known to undertake extensive diel vertical migrations into the upper 200 meters at night through the halocline. Diaphus theta and T. crenularis apparently migrate through the thermocline as well and T. crenularis is even found at the surface at night. On the other hand, Bathylagus spp. and Poromitra crassiceps were seldom taken in tows above 200 meters and usually in tows from

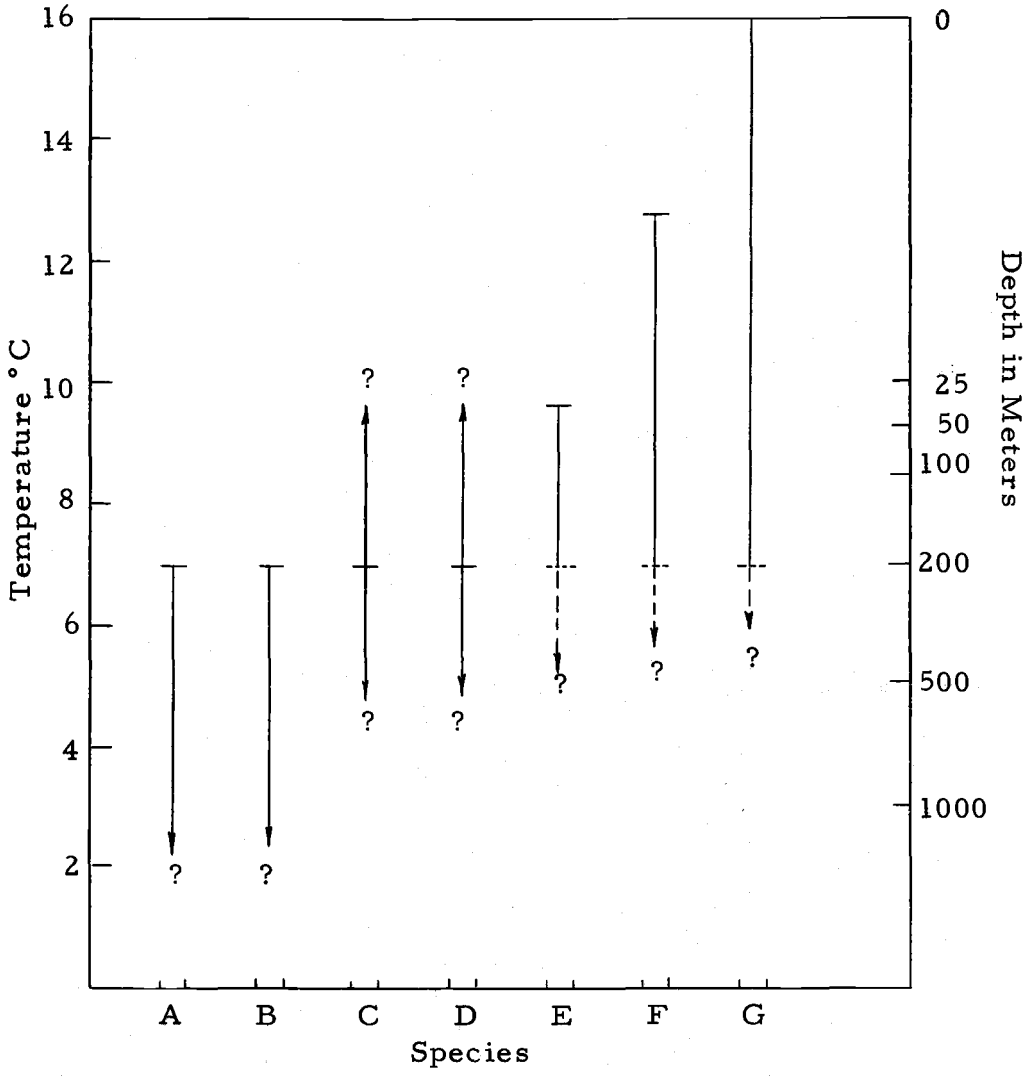


Figure 15. Approximate range of day and night vertical distribution with temperature of some mesopelagic fishes.(Compiled from data reported by Pearcy (20, p. 91-93).

0 - 1000 meters.

Upon examination of Figures 4-7 and 9-12 as compared to Figure 15 it is apparent that the relative order of the magnitude of mean metabolic rate between species is generally in the same relative order as their approximate vertical distribution within the water column when we consider fishes of the same size, i. e. 2. 5 grams or about log 0. 4 wet weight. When the "respiratory potential" is considered on an ash-free dry weight basis (Figure 5) the relative position of the species changes somewhat due to differences in water and lipid content. However on an ash-free, lipid-free dry weight basis the magnitudes of metabolic rate for all the species in Figure 6 retain the same general positions as when the metabolic activities of these species are presented on a wet weight basis, (Figure 4).

The noted correlation between metabolic activity and the approximate depth distribution of these seven species can hardly be accidental. Temperature differences alone do not appear sufficient to account for all of these metabolic differences, when we consider the Q_{10}'s which were obtained for Poromitra crassiceps and Tarlotonbeania crenularis (Figures 13 and 14). It is probable that the largest differences are truly genotypic metabolic differences which have evolved through natural selection and now limit the range of the environment acceptable to the species phenotype. Which environmental factors originally determined this selection are unknown.

Comparison of the Calculated Oxygen Consumption Rate from the Enzyme Data with the Measured Oxygen Consumption Rate

The oxygen consumption rate calculated for seven of the species is shown in Table III.

TABLE III. CALCULATED OXYGEN CONSUMPTION RATE FROM ENZYME ACTIVITY

Species	Average H^+ eq. x 10^{-7}/mg W^w/hr		ml O_2/g W^w/hr at 35° C	
	means for all fish	means for fish of 2.51 g	all fish	2.51 g
T. crenularis	1.48	1.56	.829	.872
D. theta	.76	.79	.426	.447
L. leucopsaurus	.58	.55	.325	.309
L. ritteri	.38	.45*	.213	.251
T. macropus	.33	.33	.185	.182
P. crassiceps	.25	.25	.140	.138
Bathylagus spp.	.13	.08	.070	.043

*Estimated.

These are mean values only. Because of the small coefficients of correlation (r) for most of the regression lines the standard deviations for these lines will be approximately the same as those shown in Figure 4 when converted to the same units. Considerable overlap between species may occur.

Examination of the Q_{O_2} calculated from the enzyme data as compared to the measured Q_{O_2} for the two species, T. crenularis

and P. crassiceps suggests that the enzyme method is not measuring the same proportion of the total measured gaseous respiration for each of these species, even if we correct for temperature by using the Q_{10}'s calculated from Figures 13 and 14.

If the Q_{O_2} measurement of gaseous respiration and the calculated Q_{O_2} from the enzyme data are corrected to 20° C by applying a Q_{10} of 2.5 and 1.7 (see Table III and IV) then the calculated Q_{O_2} for T. crenularis is only 8.4 % (i.e. $\frac{.244}{2.90}$) of the measured Q_{O_2} for this species. Similarly the calculated Q_{O_2} for P. crassiceps is 37.5% (i.e. $\frac{.064}{.173}$) of the measured Q_{O_2} if the calculated Q_{O_2} for specimen C is extrapolated to 20° C.

There are several possible reasons why the enzyme method appears not to be measuring the same percentage of the gaseous respiration for both species. One likely reason is the fact that the two species responded differently to the confinement in the respirometer and their motor activities were not standardized. The measurement for Poromitra crassiceps probably was nearer its "basal rate" than that for T. crenularis. Several authors have noted great differences in metabolic rate between moving and nearly quiet fish (2, p. 48; 39, p. 7; 46, p. 51). According to Fry (2, p. 46) the effect of temperature should be considered for the two extremes of metabolism, the active and the standard or basal rate. Intermediate to these is routine metabolism where the fish is exhibiting spontaneous activity.

The active metabolic rate in relation to temperature does not necessarily follow a course parallel to the curve for the standard rate which is usually close to linear, or even to the curve for routine metabolism; therefore, it is essential that the motor activities of the species be standardized before a comparison of the two methods can be made. If the enzyme method is measuring the standard metabolic rate then it may not necessarily measure the same proportion of the active metabolic rates of different species at the same temperature, because the difference between the standard and active metabolic rates when plotted against temperature is not always linear (2, p. 50). There is also a possibility that the two species differ as to the nature of the Krebs cycle in respiration or else the relative Q_{O_2}'s of their tissues differ. However, experiments showed that the measured enzyme activity between 5° C and 35° C was maximal for both species at 35° C.

More experiments are needed to determine what part of the gaseous respiration of each species the enzyme method measures.

The measured gaseous respiration of T. crenularis shows a higher rate than any reported for comparable sized fish by Scholander (30, p. 72) in both arctic as well as tropical forms. On the other hand the oxygen consumption rate for Poromitra crassiceps agrees quite closely with the rates found for Arctic cod, Boreogadus saida (Lepechin) at the same temperature and size (30, p. 72). This contributes additional weight to the hypothesis that the extreme activity

of T. crenularis during the measurements significantly increased its oxygen consumption rate above that of a "quiet state."

Comparison of the Q_{O_2} Calculated by Three Methods

Winberg (39, p. 1-11) made a general review of the data from many authors and found that the standard rate of oxygen consumption of freshwater and marine fishes of different species, when adjusted to a temperature of 20° C using "Krogh's normal curve," is proportional to the weight of the fish raised to a power close to 0.8. He has calculated an empirical expression by which it is possible to estimate an average expected metabolic rate of a practically motionless fish, of a given weight at 20° C. This equation may be written in the following two forms:

$$Q = 0.3\ W^{0.8} \qquad \text{or} \qquad \frac{Q}{W} = 0.3\ W^{-0.2}$$

where Q is the oxygen consumption of one fish in ml O_2 per hour at 20° C; W is the wet weight of the fish in grams; $\frac{Q}{W}$ is the metabolic rate in ml O_2 per gram per hour at 20° C.

Winberg states that this equation gives only an average metabolic rate and that the average level of metabolism of fishes belonging to different systematic groups can exhibit characteristic differences, apparently associated with the different ecological habits of these fishes. However, it can be of value in evaluating metabolic rates

obtained experimentally.

Table IV compares the Q_{O_2} rates calculated from the enzyme data with the $\frac{Q}{W}$ rate calculated from the average weight of each species utilizing Winberg's empirical equation. The columns designated by A are the mean values for each species while those designated B are the values for fish of 2.51 grams wet weight. Q_{10}'s of 2.5 and 1.7 were used for T. crenularis and P. crassiceps, respectively while 2.0 was used for all others. The measured gaseous respiratory rates for T. crenularis and P. crassiceps at 20° C were 2.90 ml O_2/g W^w/hr and .173 ml O_2/g W^w/hr, respectively.

TABLE IV. COMPARISON OF Q_{O_2} RATES.

Species	Q_{O_2} (ml O_2/g W^w/hr) (from enz. data)				$\frac{Q}{W} = .3W^{-0.2}$ (ml O_2/g W^w/hr)
	at 35° C		at 20° C		at 20° C
	A	B	A	B	
T. crenularis	.829	.872	.232	.244	.270
D. theta	.426	.447	.160	.168	.235
L. leucopsarus	.325	.309	.122	.116	.218
L. ritteri	.213	.251	.081	.094	.206
T. macropus	.185	.182	.070	.068	.191
P. crassiceps	.140	.138	.065	.064	.176
Bathylagus spp.	.070	.043	.027	.017	.152

There is a general agreement between the mean Q_{O_2} calculated from the enzyme data and the mean Q_{O_2} calculated from Winberg's empirical equation for some species. The agreement for the more

epipelagic species, T. crenularis, is quite good; however, the values of Q_{O_2} calculated from the enzyme data increasingly depart from the Winberg values as we proceed from the more epipelagic species toward those species found at greater depths. The mean metabolic rate of Bathylagus spp. appears to be only about 1/5 or 1/6 of the rate expected for a fish of its size.

Wohlschlag (46, p. 53) reported that, Trematomus bernacchii and Trematomus hansoni, two species of fish from a darkened environment 45 to 180 meters beneath the permanent ice at Heald Island, McMurdo Sound, Antarctica; showed a comparatively very low metabolic rate. He also noted that T. bernacchii from deep open waters in summer had a lower respiratory metabolism than those from shallow waters and that the T. bonnbergi and the Rhigophila dearboni (a Zoarcidae) from 680 meters had a lower metabolism than the other Trematomus species from shallower waters. Two explanations are offered by Wohlschlag. One is that fish permanently confined to darkness have an inherently low metabolism that is retained after their transfer to laboratory conditions. The other is that exposure to light has a depressing effect on the metabolism of dark-adapted fish.

Data from the present study agrees with Wohlschlag's observation that species from deep waters have a lower respiratory metabolism than species from shallow waters. In addition, the limited data on oxygen consumption measurements in the light and in the dark (Figure

14) suggest that light does not have a depressing effect on the metabolism of dark-adapted fish. It is clear from Figure 13 that light has no depressing effect on the respiration of Tarletonbeania crenularis.

Using the data of Pearcy (20, p. 91-93) on the general vertical distribution of the seven species and the Q_{O_2} calculated from the enzyme data it is possible to calculate the Q_{O_2} of each species at the estimated maximum temperature which each encounters during the diel vertical migration (Table V). A Q_{10} of 2.0 was estimated for each of the species except for T. crenularis and Poromitra crassiceps which had Q_{10}'s of 2.5 and 1.7, respectively.

TABLE V. Q_{O_2} FOR THE SEVEN SPECIES AT THE TEMPERATURE OF THE ENVIRONMENT CALCULATED FROM THE ENZYME DATA.

Code	Species	Approximate Maximum T° C	Average Q_{O_2} ml O_2/g W^w/hr	
			for fish of 2.51 g	for all fish
G.	T. crenularis	15° C	.140	.166
F.	D. theta	13° C	.101	.151
E.	L. leucopsaurus	10° C	.058	.061
D.	L. ritteri	8° C	.041	.037
C.	T. macropus	10° C	.034	.035
B.	P. crassiceps	7° C	.032	.033
A.	Bathylagus spp.	5° C	.005	.009

With this data it is possible to compare the actual Q_{O_2} value

obtained by Wohlschlag (46, p. 42) for Trematomus bernacchii at a temperature of -1.8° C with the average Q_{O2} value obtained for Bathylagus spp. at 5° C.

For Trematomus bernacchii Wohlschlag obtained the following regression equation:

$$\overline{Y} = -2.0307 + 1.3117\ X_w$$

where $\overline{Y}$ = expected log mg O_2/hr

and X_w = log weight in grams.

With a weight of 30.1 grams, which was the average weight of Bathylagus spp., this equation gives a Q_{O_2} value of .017 ml O_2/g/hr. This value agrees fairly well with the average Q_{O_2} value of .009 ml O_2/g/hr obtained for Bathylagus sp. in the present study (Table V). The fact that Wohlschlag's value for a lower temperature is higher also agrees with his findings that at very low temperatures, 0° C to -1.9° C, there is an upward displacement of metabolic levels because of cold adaptation of polar fishes. This displacement is shown schematically in Figure 16 adapted from Wohlschlag (46, p. 56-58).

Wohlschlag explained this phenomenon as follows:

> ... the lower limits of metabolism are rather nicely described by Krogh's (1916) "standard" line over the temperature range from about 0° C to about 35° C. Below 0° C in marine waters the metabolic range is displaced upward by reason of cold adaptation of fishes in polar marine waters. The upper level of the

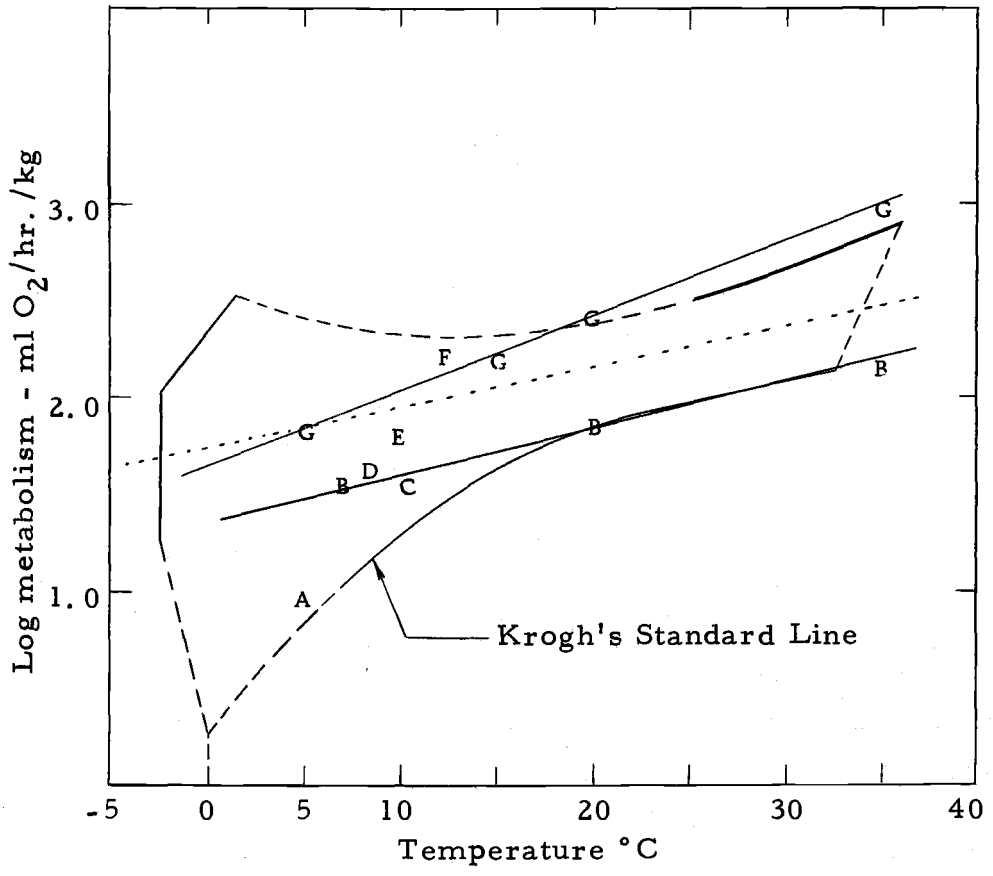


Figure 16. Schematic diagram of metabolism-temperature relationships of fishes adapted to various environments. This figure shows the limits of metabolism noted for thermally adapted fishes of about 1 to 30 grams. Metabolic rates of the seven species of this study are indicated by their respective letter code. The dotted line shows the predicted metabolic response of thermally adapted fish in general (46, p. 58).

> relationship in these figures tends to remain relatively near the same level for cold-adapted polar species and for more temperate and tropical species. As a consequence there is 'metabolic range attenuation' at very low temperatures for cold-adapted polar fishes (Wohlschlag - 1960) ...
>
> The metabolic response to temperature appears to be high for individual species at low temperatures, and the response declines at higher temperatures in accordance with Krogh's (1916) original concept; but the metabolic response for thermally adapted fishes in general is much lower and more linearly uniform from polar to tropical temperatures, ...
>
> The empirical metabolism - temperature relationship (for thermally adapted fish) is
>
> $$Y_1 = 1.75 + 0.02\ X_t$$
>
> where Y_1 is the expected log mg O_2 hr^{-1} kg^{-1}, X_t is the natural adaptation temperature, and the constant is pertinent to fish of the order of 5 grams. The equation applies to fishes that are genotypically adapted and not phenotypically acclimated; for phenotypically acclimated fishes the relationship proposed by Bullock (1955 Figure 5) applies.

Figure 16 was compiled from data of numerous species of fish from less than 1 gram to about 30 grams in weight, therefore the Q_{O_2} values calculated for the seven species in this study should fall within or close to the limits of this diagram. The values of Q_{O_2} for each of the species from Table V are designated by the respective letter code for each of the species and are shown in Figure 16. Lines G and B designating the metabolic rates of T. crenularis and Poromitra crassiceps were calculated from their respective Q_{10}'s. The Q_{10}'s for the remainder of the species are not known so only an

average point has been indicated, assuming a Q_{10} of 2.0.

It is apparent that the values of Q_{O_2} for these species at the estimated maximum temperature of their environments generally fall within the boundaries of this schematic diagram. Values of Q_{O_2} which do not occur within the limits are in most cases at temperatures believed to be above that of the species natural environment.

The line for Poromitra crassiceps has approximately the same slope as Wohlschlag (40, p. 502-515) noted for an Arctic fish, Coregonus sardinella. That is, it is comparatively stenothermal polar, according to his classification and appears to be thermally adapted to its cold environment. On the other hand, the line for T. crenularis is more similar to those obtained for temperate or some tropical fishes and exhibits more of a eurythermal character (46, p. 56). In fact, the mean metabolic rates calculated from the enzyme data for T. crenularis of 2.51 grams agree very well with the metabolic rates reported for a temperate fresh water fish adapted to approximately the same temperature range, 11 to 14° C (34, p. 90). In Table VI the metabolic rate of the female mosquito fish, Gambusia affinis holbrooki, from southern Russia is compared with that of T. crenularis (mostly females) at various temperatures.

TABLE VI. COMPARISON OF METABOLIC RATES.

G. affinis holbrooki		T. crenularis	
Temperature in ^{o} C	ml O_2/g/hr	Temperature in ^{o} C	ml O_2/g/hr
2.3	.046		
6.0	.059	5.0	.056
10.3	.093	10.0	.098
13.4	.137	15.0	.140
17.9	.202	20.0	.244
22.8	.301	25.0	.349
32.9	.706	35.0	.872

Significance of Slopes of Weight Regression Lines and Scatter of the Data

The data presented in Figures 9-12 shows considerable scatter and in some cases almost no correlation of metabolic activity to the size of the organism. In most cases there is simply not enough data to determine a reliable weight regression line, however, even so it is apparent that there are large differences in metabolic rate between the species with the lowest and the highest rates. The data for Diaphus theta and Lampanyctus leucopsarus is particularly scattered. Attempts were made to correlate this scatter with several factors; day or night capture, surface temperature at the time of capture, depth of the net tow, etc. but no correlation could be found. Expressing the activity on an ash-free dry weight or on an ash-free, lipid-free dry weight basis did not appear to improve the scatter and in some cases appeared to increase it.

The lumping of the data for both sexes and immature specimens might account for a large part of the scatter but no attempt was made to separate the sexes for these two species since most specimens were not available. The possibility that immature small fish may reside at a different or a more or less stationary level in the water column while the mature fish migrate above and below this level might contribute to such a scatter of data. The scatter may be attributable to differences in degree of stress to which the specimens have been exposed during capture and to quantitative differences in the action of endocrines on their physiology, although there is not strong evidence that adrenaline increases the metabolic rate of muscle itself other than by increasing its temperature and work output (29, p. 165). It does, however, increase the oxygen consumption of the intact organism. Thyroxine has been shown to increase the respiration of both the whole fish and of muscle in some cyprinids (23, p. 140). Of course the prime suspect is experimental error, but more laboratory experiments are necessary to evaluate the reproducibility of this method.

VI. CONCLUSIONS

The enzymatically determined, mean metabolic rates of seven species of mesopelagic fishes, when examined with respect to temperature and size, fell within the limits of the standard metabolic rates reported for fishes of similar size from various habitats. Significant differences between the mean metabolic rates of most of these seven species were noted. The mean metabolic rates of these seven species, when corrected for size, differed in varying magnitudes from the expected average standard metabolic rates as calculated by Winberg's empirical equation. When the species were arranged in their estimated vertical distribution according to available data from net tows to successive depths, it was observed that their mean metabolic rates assumed a descending order. The species which migrated to the surface of the water column had the highest metabolic activity while the species which is believed to occur at the greatest depth had the lowest metabolic activity. A positive correlation between the estimated relative depth of occurrence of these species and their mean metabolic rates is apparent.

Several factors which change with depth may in part contribute to the metabolic differences of these species and to this apparent correlation. These factors are temperature, pressure, oxygen concentration, carbon dioxide concentration, hydrogen ion concentration,

light penetration and, possibly, salinity. Many of these factors have known effects on the active and standard metabolic rates of fishes (2, p. 23-55). The magnitude and direction of the effects of these factors vary and the combined effect of two or more factors may differ considerably from the effect of any one factor. Therefore it may be difficult to show a correlation with any one factor.

Differences in temperature at various depths may account, in part, for the observed mean differences in metabolic rate. However, data in Tables IV and V make it clear that, even if we assume a Q_{10} of 2 or 3, different temperatures alone will not explain all of the observed differences in metabolic rates between species. Some of these differences in metabolic rate may be related to factors not directly related to depth. For example, T. crenularis has a gas-filled swim bladder while several of the other species which migrate close to the top of the water column have fat-filled swim bladders. Such a difference in anatomy and physiology may explain the higher metabolic rate observed for T. crenularis. It is apparent (Figures 4-6 and 9-12) that water and lipid content are responsible for a part of the differences in metabolic rate noted when the rates are compared on a wet weight basis.

Regardless of the reasons for these differences in metabolic rate between vertically migrating species it is of significance that the order and magnitude of these differences appears to coincide with

the upper limits of the known vertical distribution of the adults. Such a vertical distribution would serve to reduce competition between similar species, even if it is only a partial separation. If future experiments prove this distribution to be true then the method may be applicable in ascertaining the vertical distribution of mesopelagic fishes.

The data from this study has shown that there are significant differences in metabolic rates between deep-living pelagic fish species as compared to species which come to or near the surface during their vertical migration. The metabolic rates of these species appear to coincide with the differences observed between some temperate and polar species.

The calculated respiratory rates from the enzyme data increasingly depart from the values calculated from Winberg's empirical equation as we proceed from the more epipelagic species toward those species found at greater depths. This departure from the expected average metabolic rate with increasing depth and pressure, and decreasing temperature as well as oxygen content of the environment may prove to be of adaptive significance to these species.

There do appear to be both eurythermal and stenothermal fish species among mesopelagic fishes.

In order to determine whether the respiratory rate of these organisms can significantly account in part for the oxygen minimum

layers we must determine whether pressure has any positive effect on the low metabolic rate of the deep-living species, what the biological concentrations of these organisms are and what the dynamics of oxygen exchange are in these areas. Because of very low metabolic rates for the deepest species, here studied, it may be possible for these organisms to live within these oxygen minimum layers. In order to answer questions on energy requirements of vertical migrations and energy transfer within mesopelagic populations more information is needed on resting versus swimming metabolic rates and also metabolic rates on species from other trophic levels.

Evaluation of Techniques

The metabolic rates calculated from the data obtained by this method fall within the accepted limits for the sizes of fish investigated. The method appears to have great potential application, however some refinements must be made.

First, we must determine just what part of the resting, gaseous respiration the method measures and whether this is constant for different species. This will involve removing the effects of spontaneous activity of the specimen in some manner. Next the reproducibility of the method should be determined.

It may be possible to refine the technique somewhat by basing the enzyme activity on the protein content of the homogenate sample.

Summary

Studies on the levels of enzymatic and respiratory metabolism were conducted on a number of oceanic mesopelagic fishes collected from stations 50 to 65 nautical off the Oregon coast during the period from July 1962 to July 1964. Ten species of mesopelagic fishes were studied with regard to their respiratory potential as measured by succinic dehydrogenase enzyme activity. Oxygen consumption rates for two species were measured at various temperatures for comparative purposes using the modified Winkler method. Methods were modified and adapted for use at sea with a minimum of equipment.

The relative magnitudes of the metabolic rates calculated from the enzyme data were comparable to the relative measured oxygen consumption rates. The metabolic rates calculated from the enzyme data fell within the limits of the metabolic - temperature relationships compiled from the data of several authors for fishes of the same size from various environments.

Metabolic rates of species of mesopelagic fish differed significantly and declined with the general depth and temperatures at which the species was found. One species which migrates close to the top of the water column exhibited a eurythermal metabolism characteristic of temperate fishes.

A deep-water species from an environment of little or no light

and low temperatures exhibited very low metabolic rates which were stenothermal and similar in magnitude to polar species.

The origin of these metabolic differences are discussed as to whether environmental factors or physiological adaptations are their cause.

It is suggested that the descending order and magnitude of the metabolic rates of these mesopelagic species corresponds to their relative position in the water column.

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