

## THE METABOLISM OF *PLOTOSUS ANGUILLARIS* (BLOCH) IN VARIOUS CONCENTRATIONS OF SALT AND OXYGEN IN THE MEDIUM

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### INTRODUCTION

A MATTER which is of interest in fisheries both from the industrial and scientific points of view is the ability of fish and other animals to migrate from sea to freshwater and *vice versa*. In spite of the attention which this field of research has claimed, much still remains to be explained. The nature of the adaptive processes that the individual undertakes is not fully known.

The relation between osmoregulation and metabolism with which this paper is concerned has been studied in a number of different animals by Sumner (1905), Schlieper (1929 and 1955), Beadle (1931), Keys (1931 and 1933), Raffy (1932 and 1933), Schwabe (1933), Krogh (1939), Wikgren (1953), Flemister and Flemister (1951), Lofts (1956), Gross (1957) and others. Of these one school believes that the increase in osmotic load is directly reflected in an increase in the metabolic rate. This is however denied by the other school which considers that the actual cost of osmotic regulation in terms of increased metabolic rate is relatively negligible. But whatever the position, it is universally believed that in the fish the gill is as important an organ of osmoregulation (excretion) as it is of respiration. As stated by Smith (1930 and 1953) the teleost can live in salt-water not because of any specialization of its kidney but because of the ability of its gills to retain the water and withdraw the salts from the blood and discharge them into the sea. This process according to the same author, requires, proportionate to the magnitude of work involved, valuable energy expenditure.

With the object of studying the problem further, a marine cat-fish: *Plotosus anguillarís* (Bloch) was used in the investigation. Even though no measurement of the osmotic changes of the blood of the fish was made, evidence is gathered from the study of the relationship of metabolism to changes

in salinity of the environment in order to demonstrate that the cost of regulation is indeed significant.

#### MATERIALS AND METHODS

*Plotosus anguillaris* (Bloch) occurs in this area from January to June in dense patches of some 300–500 individuals in a shoal. Each shoal is remarkably homogeneous regarding the size of the individuals constituting it. Probably the individuals of a shoal belong to a single brood. Fingerlings and adults of both sexes not actively breeding, and belonging to different shoals were employed in this work, and they weighed between 5 and 45 gm. Since none of the adult fish used was in the active breeding phase the effect of sex as noted by Keys (1931) in the killifish has been overlooked in this study. The fish on capture were held in 500 gal. cement tanks supplied continuously with sea-water. The average temperature in these holding tanks during the period of study was 29° C. and the experiments were also performed in that temperature. For obtaining salinities lower than that of sea-water the local tap-water of average salinity 0.4‰ and pH 7.5–8.0 was mixed with the former. The salinity in this case was maintained at any desired level by using a constant level mixer. For obtaining salinities higher than that of sea-water one lot of commercial sea-salt was used throughout, both for acclimation and experimental purposes. Following is the analysis of the salt used:—

Sodium chloride	..	..	97.77% M.F.B.
Calcium sulphate	..	..	1.04% M.F.B.
Magnesium sulphate	..	..	0.55% M.F.B.
Magnesium chloride	..	..	0.23% M.F.B.
Insolubles	..	..	0.84% M.F.B.
Moisture	..	..	7.84%

The water in the acclimation tanks for higher salinities was aerated throughout and the water itself renewed every week. The pH in these tanks ranged between 8.0 and 8.5. This procedure was adopted since it was not possible to maintain a continuous supply of high salinity water in the acclimation tanks. In all cases, however, the oxygen concentration was maintained at air saturation levels and the range of pH remained within 7.5–8.5.

The active metabolism and the standard metabolism were measured in the apparatus described by the author (Job, 1955 and 1957), but with the addition of a constant level mixer to the standard metabolism apparatus.

While the diluted sea-water cannot be compared with the concentrated 'sea-water' particularly with regard to the ionic composition the results presented are not without significance in that all the fish were subjected to the same conditions and their responses were studied (*cf.*, Quigley, 1928). Because of the difficulties involved and on the strength of Young's findings (Young, 1938), the use of commercial sea-salt was found to be the closest one could get to normal concentrated sea-water.

In the acclimation tanks the raising or lowering of the salinity was gradually done at the rate of 1-2.5‰ in 48 hours and maintained constant at the two other levels 40.0 and 12.5‰ besides sea-water, for not less than 20 days. The salinity values given in this work represent always the average values, the fluctuations being kept within 1.5‰ in any one level.

In the experiments the initial concentration of oxygen was brought to an average partial pressure ( $pO_2$ ) of 150 mm. Hg by bubbling air or oxygen through air diffusers made from coral stones (*Millipora* sp.) suitably ground and adapted in the laboratory. By using groups of five fish at a time (average weight 7.26 gm.) some preliminary experiments were performed to estimate the tolerance range of salinity for this species. It was found that fish held in sea-water at 29° C. tolerated from 2-52‰ and that salinities on either side of this range proved lethal in 48 hours. In the same way preliminary experiments on the active oxygen consumption indicated that the level of oxygen at which the fish died (lethal level of oxygen) was lower when tested in salinities of 12.5 and 40.0‰ than in any other salinity including its own natural habitat salinity of 30.4‰. In order to examine whether these two other salinities had any significance, fish were acclimated to 12.5 and 40.0‰ and experiments performed with them as well as with those held in sea-water (S.W.) of 30.4‰ salinity.

Except in the experiments in distilled water no adjustment of the pH of the experimental medium was attempted. Quigley (1928) found that buffers used for this purpose had a toxic effect upon the fish. The active metabolism of S.W. fish was measured in test salinities of 0.0, 0.4, 12.5, 30.4, 44.2 and 60.0‰; and those of fish acclimated to 12.5‰ in test salinities of 0.4, 12.5, 30.4 and 44.2‰; and those acclimated to 40.0‰ in test salinities of 0.4, 12.5, 18.0, 40.0 and 60.0‰. The measurement of standard metabolism was however restricted to two salinities only where the acclimation and test salinities were the same and were respectively 12.5 and 30.4‰. In the experiments the initial and final pH were always measured and the range was between 8.5 and 7.5 respectively.

## RESULTS

The data of oxygen consumption in relation to weight and under  $pO_2$  ranging from 150–25 mm. Hg for the respective salinities were submitted to the same statistical treatment as earlier (Job, 1957) and are presented in a double logarithmic grid in Figs. 1, 2 and 3 for fish acclimated to salinities of 30.4, 40.0 and 12.5‰ respectively.

As the experiments were carried through to the point of death in the active metabolism chambers, the data collected on these lethal levels were also treated as above. These, together with the data on standard metabolism measured for fish of average weight 7.26 gm., will be presented and discussed later.

## DISCUSSION

The basic data presented in Figs. 1–3 were used in obtaining the mean rates of oxygen consumption for the three weights 5, 15 and 45 gm., as this would facilitate comparison of the responses of the respective sizes to environmental stress. The main features of the experiments are: (1) The standard metabolism was measured at the two acclimation levels 12.5 and 30.4‰ with the oxygen concentration in the medium being maintained in the region of air saturation values during the entire period of the experiments; (2) the active metabolism was measured both in the three acclimation levels 12.5, 30.4 and 40.0‰ as well as in other test salinities, but with the oxygen tension in the medium falling from an average initial level of 150 mm. Hg to the lethal level.

The difference between the active metabolism in the acclimated salinities and that in the test salinities in any series is that in the former the falling oxygen tension in the medium is the only important extraneous factor; whereas in the latter the sudden impact of an entirely different salinity to which the fish was never exposed before, is an additional factor it must reckon with. Therefore, where the metabolism of fish is measured in the salinity to which they were already acclimated the term 'normal' metabolism is employed and where the acclimation and test salinities are not the same the metabolic response is termed 'instant' metabolism, purely as descriptive terms.

*Active Metabolism, Size and Salinity*: Data presented in the preceding three figures were used for obtaining the responses of a 5, 15 and 45 gm. fish to various salinities. Figure 4 illustrates the above, where the log. of oxygen consumption is shown against the salinity in a semi-logarithmic grid. Panel A shows the maximum rate of oxygen consumption when the  $pO_2$

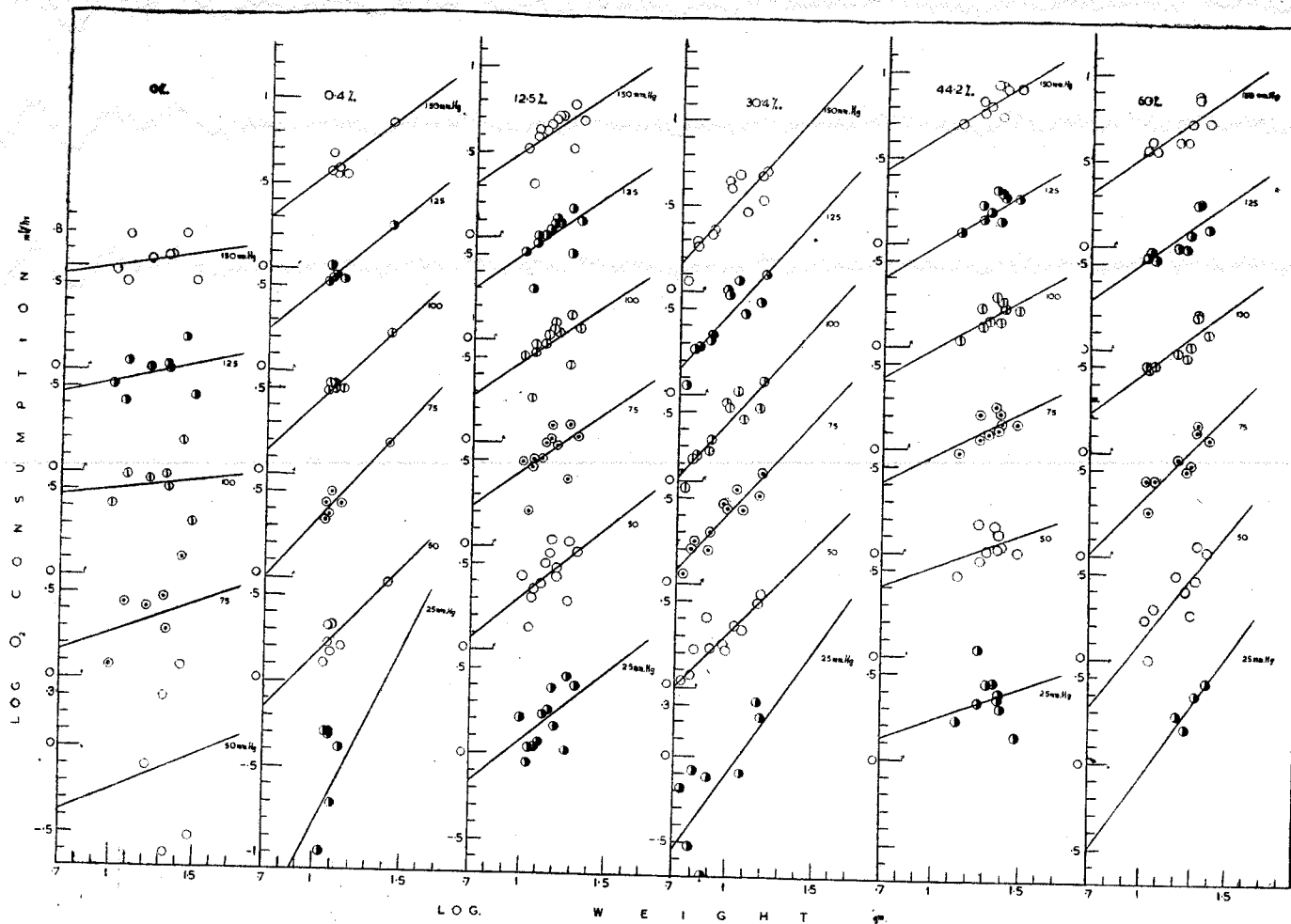


FIG. 1. Data of active metabolism at 29° C. of fish acclimated to that temperature and to sea-water salinity of 30.4‰. The partial pressures of oxygen are indicated at the upper end of each line. The test salinities are indicated in the respective compartments at the top. The lines of best fit were drawn according to the principle of least squares. The arrows along the ordinate indicate, in this double logarithmic plot, the corresponding cycle of the line immediately opposite.

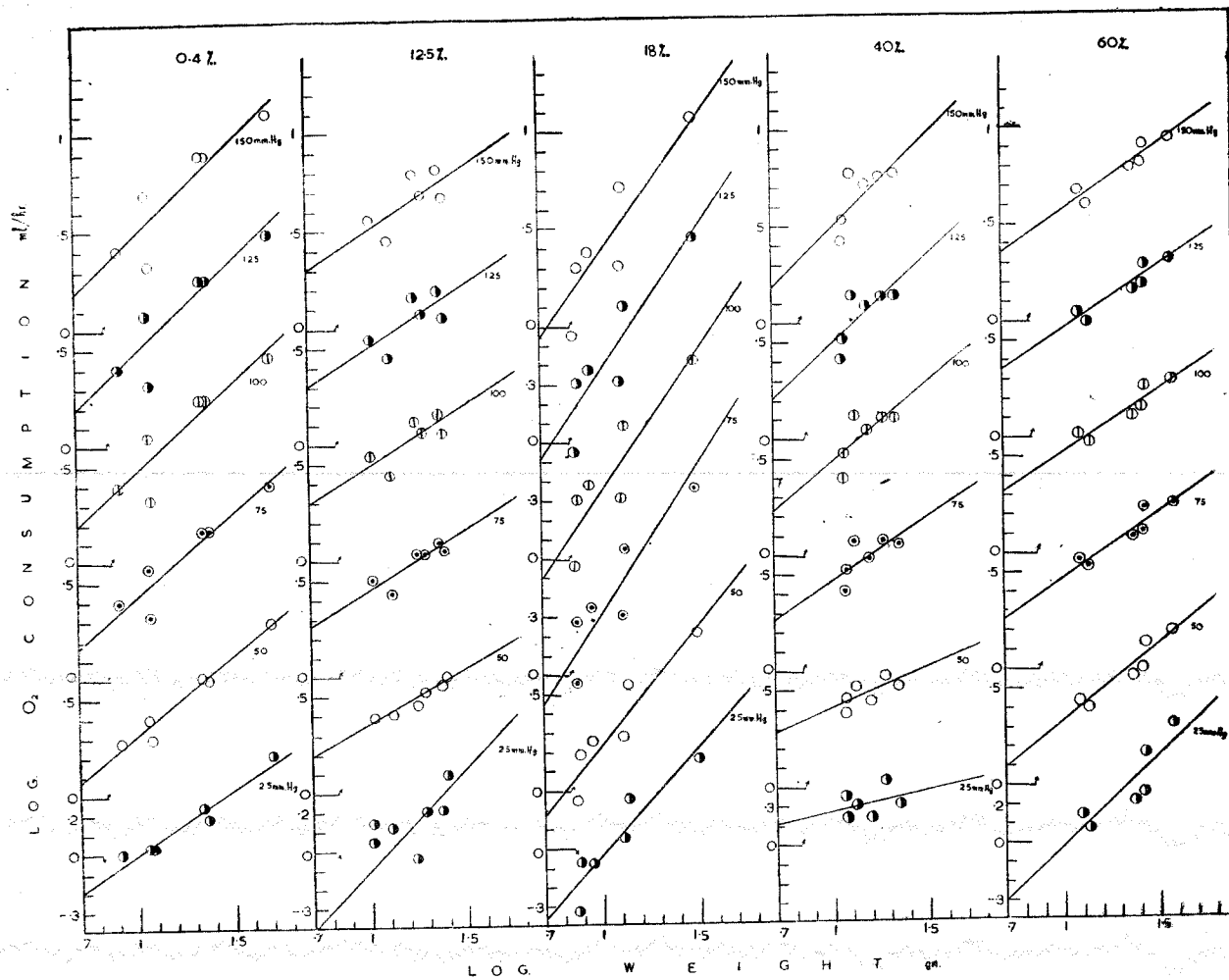


FIG. 2. Data as in Fig. 1, but for fish acclimated to 40.0‰ salinity.

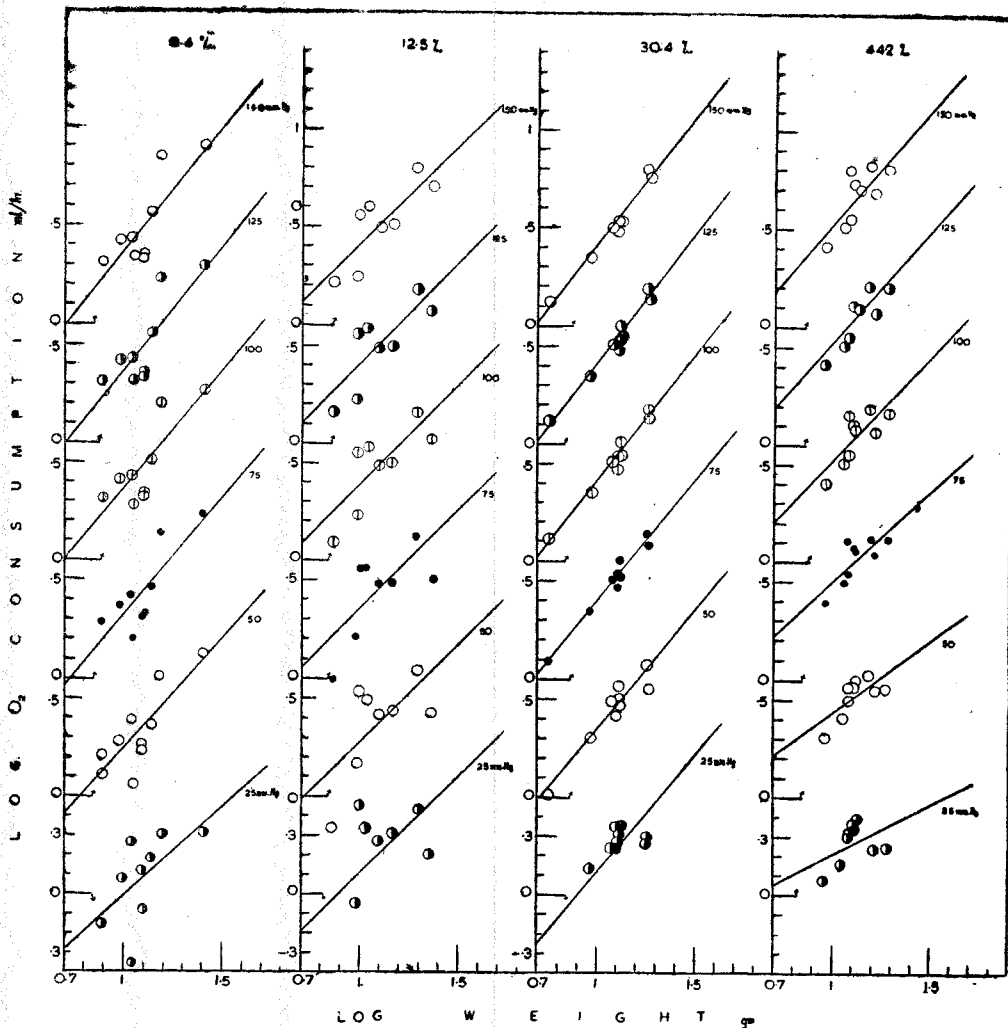


FIG. 3. Data as in Fig. 1, but for fish acclimated to 12.5‰ salinity.

is 25 mm. Hg in the medium and Panel B when it is 150 mm. Hg. The most striking feature of the figure is the variability of the responses particularly of the two extreme sizes which show almost opposite reactions, appearing to be mirror images of each other.

However in spite of these differences, within the respective weight groups the response of fish acclimated to sea-water and those acclimated to 12.5‰ are proportionately similar over the range of 0.4-44.2‰ salinity, and the curves follow the same pattern more or less. While the value of curve for

the 5 gm. fish in 12.5‰ is lower than that for the same weight in S.W. acclimation, the comparison between the respective curves of the 45 gm. fish shows the opposite trend. But the difference is relatively small except beyond 35‰ when the low salinity curve diverges and becomes more widely separated. The curves for the fish acclimated to 40‰ follow the same course as those acclimated to S.W. except that the instant rates in 18‰ are such as to produce a marked fall or rise in the rates of the 5 and 45 gm. fish, thus distorting the curve in that region in relation to the others.

The curves in Panel A belong to the section that follows. But they are of interest here merely to indicate the trends in the uptake of oxygen as the  $pO_2$  approaches the lethal point. Comparison of the curves in the two panels for the respective weights indicates that the 12.5‰ acclimated fish are least affected by the fall in the oxygen pressure probably because of the absence of other interfering factors besides  $pO_2$ . The greater fluctuations in the other two sets of curves could therefore indicate that as the fish approach the asphyxial levels of oxygen, the salinity of the medium reveals the extent of its interference with the uptake of oxygen. Even in this aspect the intrinsic differences between the 5 and 45 gm. fish are such that in the latter the 30.4 and 40‰ acclimation curves show greater similarity in the trend than does the 12.5‰ curve for that weight with either of the two curves. In the 5 gm. fish the closeness of the respective curves to one another is however limited only to certain sections of the range of salinity tested. The actual values for the curves are given in Table I. From Table I it can be said that the different sizes of fish respond differently to changes in the salinity of the medium, with reference to their active metabolism. If this is not entirely a metabolic response brought about by osmotic regulation, it will surely be granted that changes in salinity do impose a significant load on the gills or respiration so that the ability to utilise oxygen is measurably affected, almost instantaneously. The above conclusion is proved further in the relation of size and oxygen consumption, where the  $pO_2$  in the medium restricts its uptake by the fish, the subject of the following section.

*Size, Oxygen Tension and Salinity:* In Fig. 5 is illustrated the metabolism of the fish of the three sizes as the oxygen tension in the medium falls from 150–25 mm. Hg and in different salinities.

*Fish acclimated to 30.4‰ (S.W.).*—The normal rates of oxygen uptake of fish tested in this salinity showed that they were proportionately the same for all sizes under all partial pressures of the gas. In Fig. 5 A, this is clearly indicated by the broken line representing the curve of the 45 gm. fish reduced



TABLE I

Maximum uptake of oxygen (ml./hr.) by 5, 15 and 45 gm. fish acclimated to 30.4, 40.0 and 12.5‰ and under 25 and 150 mm. Hg pO<sub>2</sub> and in different test salinities. Figures in parenthesis are lethal oxygen levels (ml./lit.)

Test sal ‰	5 gm. Fish			15 gm. Fish			45 gm. Fish		
	Leth O <sub>2</sub> ml./lit.	O <sub>2</sub> uptake ml./hr. when pO <sub>2</sub> is		Leth O <sub>2</sub> ml./lit.	O <sub>2</sub> uptake ml./hr. when pO <sub>2</sub> is		Leth O <sub>2</sub> ml./lit.	O <sub>2</sub> uptake ml./hr. when pO <sub>2</sub> is	
		25	150		25	150		25	150
		mm. Hg	mm. Hg		mm. Hg	mm. Hg		mm. Hg	mm. Hg
For fish acclimated to 30.4‰									
30.4	(0.70)	0.29	1.45	(0.51)	1.41	5.01	(0.38)	2.69	17.18
0.0	(3.60)	*0.42	3.63	(2.31)	*0.66	4.27	(1.49)	*1.04	5.01
0.4	(0.55)	0.04	1.95	(0.87)	0.30	4.66	(1.38)	3.16	11.31
12.5	(0.20)	0.70	2.08	(0.22)	1.66	4.41	(0.25)	3.96	9.40
44.2	(0.24)	1.35	2.69	(0.30)	1.99	5.37	(0.36)	2.95	10.84
60.0	(1.91)	0.32	2.09	(0.64)	1.48	4.47	(0.28)	6.82	9.64
For fish acclimated to 40.0‰									
40.0	(0.43)	1.29	1.51	(0.33)	1.66	4.45	(0.26)	2.09	13.03
0.4	(0.39)	0.64	1.51	(0.39)	1.32	4.42	(0.38)	2.69	12.88
12.5	(0.45)	0.39	2.02	(0.44)	1.23	4.12	(0.43)	3.83	8.51
18.0	(0.47)	0.43	0.87	(0.35)	1.41	3.94	(0.26)	4.79	20.68
60.0	(0.28)	0.50	2.27	(0.33)	1.41	4.84	(0.35)	4.03	10.47
For fish acclimated to 12.5‰									
12.5	(0.03)	0.63	1.32	(0.08)	1.91	3.89	(0.15)	7.18	11.75
0.4	(0.86)	0.51	1.00	(0.38)	1.40	3.95	(0.16)	3.80	15.85
30.4	(0.50)	0.55	1.05	(0.31)	2.07	4.39	(0.18)	7.67	18.54
44.2	(0.34)	1.14	1.18	(0.30)]	2.00	5.42	(0.26)	3.50	19.32

\* at 50 mm. Hg.

by a factor and drawn over that of a 5 gm. fish, in order to display the closeness of proportionate rates of uptake between 150 and 25 mm. Hg pO<sub>2</sub>. However, this proportionality is not strictly maintained in the instant rates in other salinities with the exception of the instant rates in 0.0 and 12.5‰ of which the former is a lethal medium and the latter is not. The two sets of curves indicate some degree of proportionality with the difference that in the former, there is greater dependency on the pO<sub>2</sub> than in the latter case.

In 12.5 and 44.2‰ all the sizes display a relatively greater ability to utilize oxygen in the lower  $pO_2$ . This ability is probably related to the incipient lethal level because at 25 mm. Hg  $pO_2$  the fish is close to the lethal point. Assuming this to be the case, then, it can be stated that a higher rate of uptake or greater ability to utilize oxygen in the lower concentrations of oxygen in the medium is, within the viable range of salinity, related to a reduction in the standard metabolism; because the latter and the incipient lethal level are closely linked (Fry, 1947). In regard to the instant rates in 60‰ the irregularity of the curves are exaggerated and the size differences are more obvious. The flatness of the curve of the 45 gm. fish in 60‰ may again indicate that on this size the effect of the concentrated medium is not much and the fish is able to use oxygen with greater ease even in the lower  $pO_2$ . As was demonstrated with reference to the milk fish (Job, 1957) the curves reviewed here also reflect the intensity of metabolism of the different sizes and it appears that within the viable range of salinity and where no

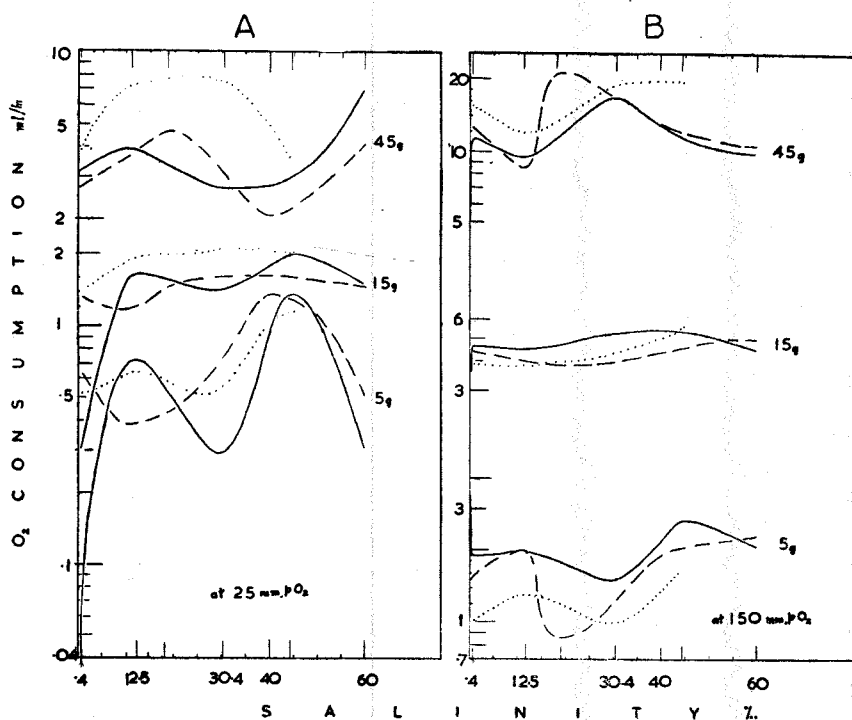


FIG. 4. Active metabolism of a 5, 15 and 45 gm. fish acclimated to salinities of: 30.4‰ (continuous line); 40.0‰ (broken line) and 12.5‰ (dotted line). Panel A displays the rate of oxygen consumption when the  $pO_2$  is 25 mm. Hg and Panel B when it is 150 mm. Hg. The respective weights for the three sets of curves are indicated against each set on the right.

accessory factor interferes, the proportionate ability to utilise oxygen remains the same for all sizes in the range tested, under all  $pO_2$  in the medium.

*High salinity acclimation (40‰):* Figure 5 B contains the curves for the dependent rates of respiration when fish were acclimated to a salinity higher than that in S.W. In this acclimation both the normal rates and the instant rates show the greatest variations. The normal rates show that there is no proportionality of response under all partial pressures of oxygen. The critical point (where the curve drops sharply) for the 5 gm. fish lies near 50 mm. Hg  $pO_2$  whereas it is even at 150 mm. Hg for the 45 gm. fish. This indicates the intensity of metabolism in the two sizes. The bigger fish has to expend energy and consume oxygen at a faster pace in order to maintain osmotic balance with the environment, whereas the 5 gm. fish has been able to do the same even as the  $pO_2$  reached 50 mm. Hg. The curves of the instant rates in the other salinities further reveal the inherent size differences. However, it must be mentioned that the instant rates in 18‰ showed that the 45 gm. fish consumed oxygen when the  $pO_2$  was 150 mm. Hg, at a rate which was the highest recorded for the entire series exceeding the normal rates in 12.5 and 40‰ by a wide margin and the normal rates in S.W. by a narrower margin. On the contrary the 5 gm. fish recorded the opposite trend in that salinity. As in the previous case of S.W. acclimated fish, the extremes of salinity appear to obliterate size differences in the proportionate ability to utilize oxygen under all  $pO_2$  so that the curves appear similar to one another.

*Low salinity acclimation (12.5‰):* The most striking feature of the curves in this series is the close resemblance of the respective curves of the normal and instant rates. The closeness of the proportionate ability to utilize oxygen under all  $pO_2$  for all sizes is once again illustrated in the normal rates displayed in Fig. 5 C, by the curve of the 45 gm. fish (broken line) overlapping the curve for the 5 gm. fish. As can be seen from the figure in this acclimation all sizes share to the same degree the ability to consume oxygen at a greater rate when the  $pO_2$  is 25 mm. Hg than in any other acclimation, indicating that when fish are acclimated to this salinity they are able to utilize oxygen with greater ease even in low concentrations of the gas in the medium. Further, within the series the 5 gm. fish displays proportionately a greater advantage over the 45 gm. fish in this ability.

As in the previous case the relatively higher rate of uptake in the lower  $pO_2$  of oxygen can only be accounted for in the corresponding change in the incipient lethal level which is in turn definitely related to the standard metabolism. Viewed from this point then, of the three acclimation levels 12.5‰

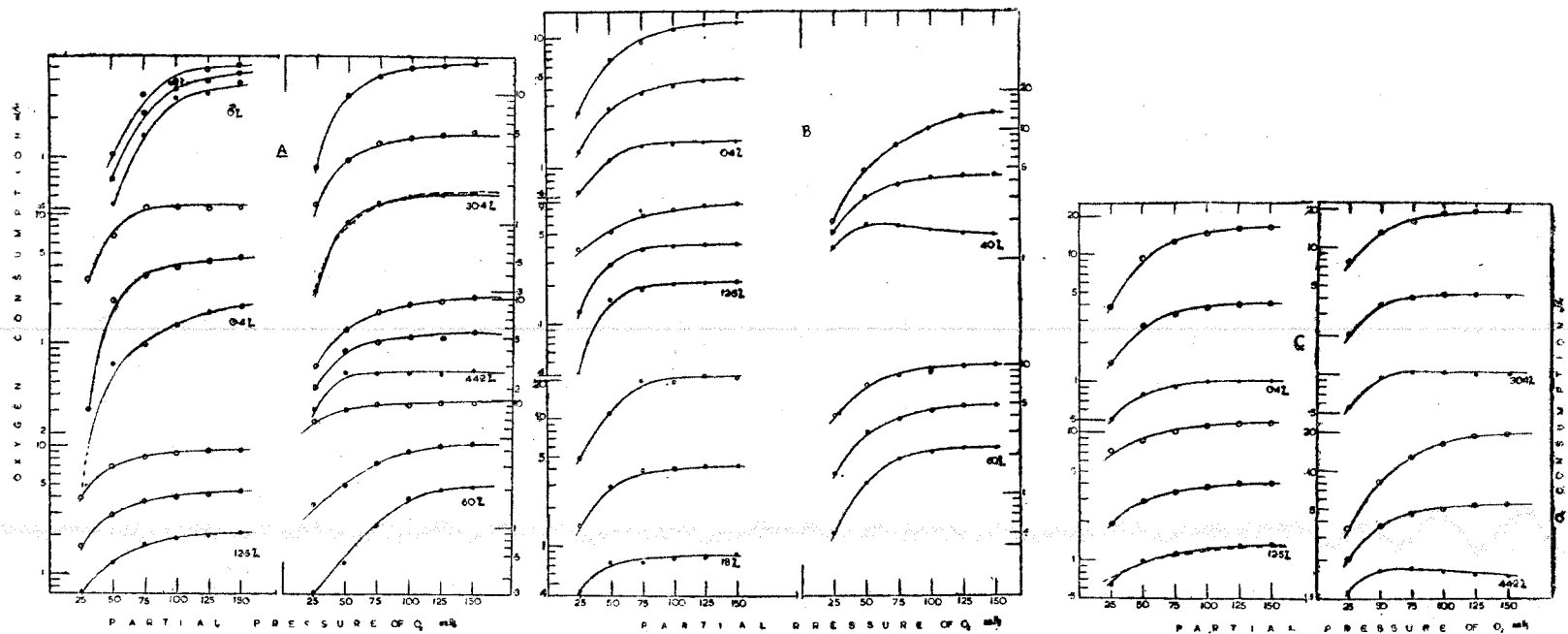


FIG. 5. Active metabolism of a 5, 15 and 45 gm. fish under various  $pO_2$  ranging from 150–25 mm. Hg at 29° C. The open circles are the rates of a 45 gm. fish, the half-closed circles those of a 15 gm. and the closed circles those of a 5 gm. fish. The salinity of the experimental medium is indicated on the right below each set of curves. A—for fish acclimated to 30.4‰, B—to 40‰ and C—to 12.5‰ salinity.

is a medium which confers on the fish of all sizes and particularly the 5 gm. fish greater ability to accommodate salinity change. It is somewhat like a neutral medium in which the regulating mechanism itself is not geared to any particular kind of osmotic work but stays in a plastic condition capable of adjustment to higher or lower salinity within the viable range, with equal facility and ease. Partial confirmation of the above is to be found in the following section where the correlation between the different acclimation salinities and the lethal level of the different sizes of fish is traced.

*Salinity and Lethal Oxygen Levels:* The values obtained for this series of measurements are given in parenthesis in Table I, and are displayed in Fig. 6. The lethal levels of fish acclimated to 30.4‰ (S.W.) and tested in

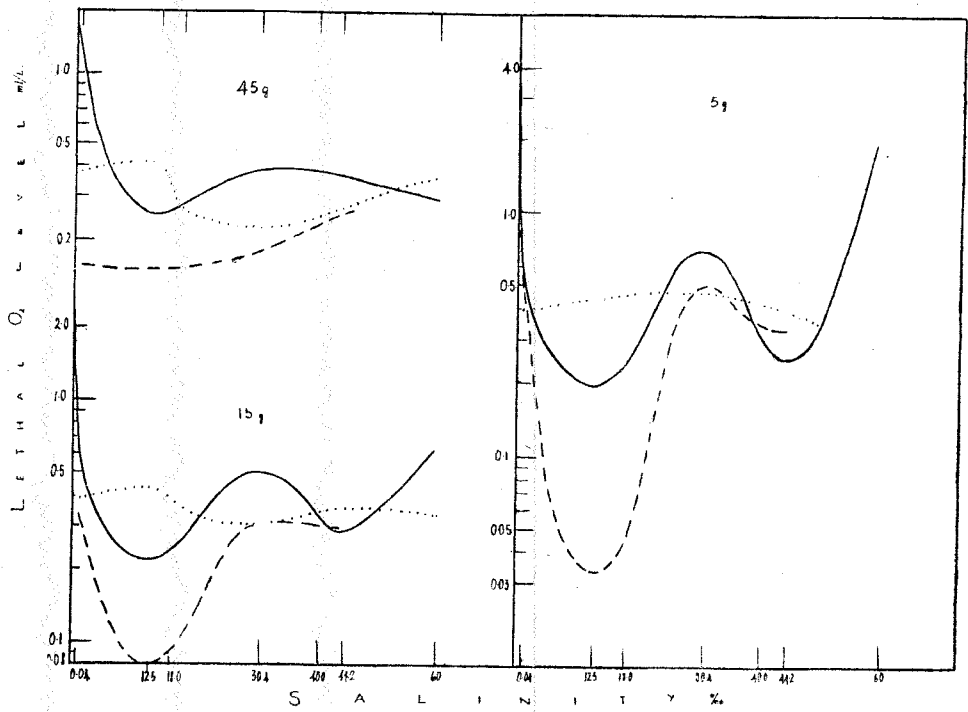


FIG. 6. Data illustrating the salinity-lethal level of oxygen relationship in a 5, 15 and 45 gm. fish acclimated to 29° C. and to salinities of 30.4‰ (continuous line), 40.6‰ (dotted line) and 12.5‰ (broken line) and tested at that temperature and in different salinities. The weights are indicated above the respective sets of curves.

the various salinities is shown by the unbroken line in the figure. One common feature shared by fish of all sizes is that the lethal level in 12.5‰ is depressed below even that in the natural environment of 30.4‰. It has been pointed out already that the ability to utilize oxygen even in the lower partial pressures of the gas in the medium was relatively high for all sizes in 12.5‰

and now the close relationship between that ability and the depression in the lethal level is clear.

Further close examination would indicate that there are inherent differences in the curves according to size in that the 5 and 15 gm. fish displayed a second depression in the lethal level in the region of 40‰ but not the 45 gm. fish. A comparison between the lethal levels in S.W. and those in 12.5 and 44.2‰ in the series shows that in the 5 gm. fish it falls from 0.7-0.2 and 0.24 ml./lit. and in the 45 gm. fish from 0.38-0.25 and 0.36 ml./lit. respectively; the difference being proportionately reduced as the size increases.

The curves indicated by the dotted line (Fig. 6) correspond to the lethal levels obtained for the three sizes when acclimated to a salinity of 40‰. In this series the highest lethal level for the 5 gm. fish was 0.47 ml./lit. in 18‰ and the lowest 0.28 ml./lit. in 60‰. The 15 gm. fish showed the highest value of 0.44 ml./lit. in 12.5‰ and the lowest in 40 and 60‰ amounting to 0.33 ml./lit. For the 45 gm. fish on the other hand the levels were 0.43 ml./lit. in 12.5‰ and 0.25 ml./lit. in 40‰. The most remarkable feature is that the effect of acclimation to the higher salinity (40‰) does not confer any special advantage to any of the three different sizes especially when these levels are compared with the corresponding levels in the salinity of their natural environment: S.W. 30.4‰. At the higher acclimation, the lethal level of the 5 gm. fish was reduced by 38.6%, that of the 15 gm. fish by 35.3% and that of the 45 gm. fish by 34.2% over the corresponding levels in S.W., for fish acclimated to that salinity. Thus the effect of acclimation to higher salinity appears to be uniform for all sizes as far as the lethal level is concerned.

In Fig. 6 the broken lines represent the curves for the lethal level of the three sizes when acclimated to a salinity of 12.5‰. Under this acclimation the 5 and 15 gm. fish displayed the same general trend that was noticed in the S.W. acclimated fish but the depression or elevation in the lethal level varied in intensity. Confirming what was already noted in S.W. fish the lethal level in this acclimation was lower for all sizes than in any of the other two cases of acclimation; and the depression in 12.5‰ itself was the lowest recorded in the entire series. When compared with the S.W. acclimated fish the lethal levels in 12.5‰ fell by 85%, 64% and 4% in the 5, 15 and 45 gm. fish respectively over the corresponding values in S.W. Thus the greatest effect according to size was noticed in the smallest fish. Another aspect of the figure is that the curves for the different sizes in this acclimation have a tendency to be lower in value in relation to the corresponding levels of S.W. fish in the zone of salinities below 30.4‰ and to be higher in the salinities above that, within the ranges tested.

From the above information and from the table given by Black (in Hoar *et al.*, 1951), it is possible to piece together an interesting correlation. In that table (p. 53) she has given the freezing point depression values for the blood of teleosts with the corresponding equivalents in salinity values. By re-plotting the data graphically a straight line relationship was obtained and it was possible from it to estimate that the blood of teleost will be in osmotic equilibrium in salinities between 7.5 and 14‰ approximately. It is to be expected then, that in these salinities naturally, the cost of regulation will be least in terms of the energy expended. Further, it will be more advantageous for a fish whose powers of regulation are limited or are still in the formative stages and is relatively 'poikilosmotic' to remain in a medium where it is in osmotic balance with the environment than for a fish whose powers of regulation are well organised and which is relatively 'homoiosmotic' since the latter will have to expend energy in any case to keep its regulating mechanism running. As the former will behave for all practical purposes like an osmometer the metabolic stress due to osmoregulation will be least when there is osmotic balance between the inner and the outer media (*cf.* Schlieper, 1929). This concept could be extended in interpreting the differences seen according to size in the lethal level of a 5 gm. fish whose survival value in 12.5‰ is greatly enhanced probably because that salinity is in osmotic balance with its internal *milieu*. The advantage for the 5 gm. fish is quite out of proportion to that of the 45 gm. fish which is capable of regulation and is actively regulating and expending energy and no marked change in its lethal level is seen. The advantage gained is in the order of 85% for the 5 gm. fish and only 4% for the 45 gm. fish.

It is thus possible that by adjusting the salinity of the water in which fish fry are transported to such levels when the osmotic stress on the fish will be least, their survival value particularly with reference to oxygen requirements could be enhanced. For *Plotosus* and perhaps for most marine species salinity of the order of 12.5‰ would appear to meet that condition. Fish fry dealers in Japan and in China have been using a slightly saline water for transporting fish fry apparently without knowing the precise physiological implications (Sproston, personal communication). Various chemicals (Srinivasan *et al.*, 1955) and drugs (McFarland, 1954) have also been used in this type of investigation and the results indicate that the chemicals intended to alleviate one condition impose upon the fish their own effects and create a complex situation, defeating the very purpose for which they were meant.

The advantage of the above method of reducing the osmotic stress on the animal and through it the respiratory metabolism has the distinct advantage of having no ill-effects on the animal and of being easily reversible

so that they can become normal when returned to the original medium. However, a precise knowledge in this regard of the requirements of the individual species employed on a large scale in fish culture would greatly add to the success of transportation or planting of the fry of the species.

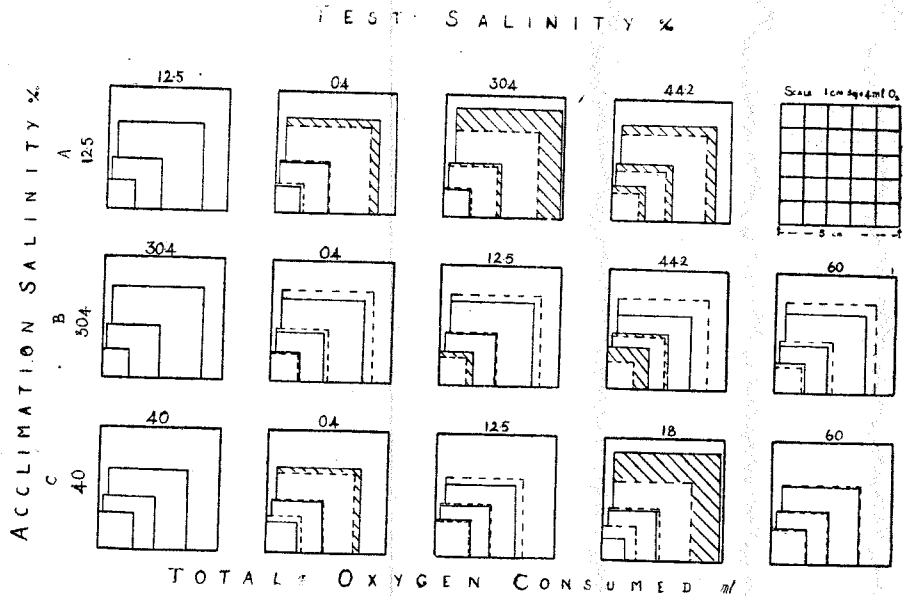


FIG. 7. Histogram illustrating the absolute and reciprocal effects of different salinities for fish weighing 5, 15 and 45 gm. For details please see text.

The relation of salinity and oxygen consumption dealt with above will remain incomplete without reference being made to the standard metabolism in the different salinity acclimations. The following section presents that aspect of the study.

*Standard Metabolism and Salinity:* The standard metabolic rate was measured at 29° C. with fish of average weight of 7.26 gm. acclimated to that temperature and to salinities of 12.5‰ and 30.4‰ and tested in the respective media. The S.W. (30.4‰) fish gave an average standard metabolic rate of 1.104 ml./hr. and the low salinity (12.5‰) fish an average rate of 0.177 ml./hr. The percentage of fall in the standard rate in the dilute medium works out to be nearly 84%. Though not strictly comparable in weight the fall in the lethal level of a 5 gm. fish acclimated to and tested in 12.5‰ when compared with that in S.W. (30.4‰) amounts to nearly the same percentage. Similar reductions in the metabolic rate have also been recorded in other animals by Lofts (1956) and by Potts (1954).



These figures prove convincingly that the cost of osmotic (ionic) regulation is quite significantly manifested in the metabolism of the fish. The statement made earlier that the lethal level of oxygen and the standard metabolism are directly proportional to each other is equally well established especially with reference to the particular size and the salinity in question.

Hayes (1930) suggested that the different responses in oxygen uptake in relation to salinity of the environment are positively related to changes in the standard metabolism. Such a change according to Fry (personal communication) can be brought about by a true reduction in the cost of osmotic regulation or a change in the blood ionic content sufficient to alter the standard metabolism. Even though it might be rather difficult to separate the one from the other, at least in regard to the smaller fish it has been definitely indicated that there is a substantial reduction in the standard metabolism especially in 12.5‰ salinity when isotonicity is probably reached between the fish and its environment. Veselov (quoted by Black, 1951) recorded a 60% reduction in the oxygen uptake in 10-15‰ salinity in the gold fish which indicates the proximity of the range between a marine and freshwater fish.<sup>1</sup>

It is evident from the above that there is a positive relationship between osmotic regulation and metabolism. But at the same time it is equally uncertain how if osmotic work turned out by the fish could be a function of the surface area of the gills or of the body, the curves relating metabolism to salinity (Figs. 1-3) could exceed the  $\frac{2}{3}$  proportionality of surface rule or the slopes relating growth of the gill to the growth of the entire animal (see review by Fry in Brown, 1957).

*Absolute and Reciprocal Effects of Salinity:* In the various sections dealt with earlier much of the subject related to a qualitative estimate of the response in the metabolism to the different salinities. The data lend themselves to a quantitative estimate also being made of that relationship. The rates of oxygen consumption are converted into total oxygen consumed during the experiment and the values obtained are presented in Table II and in Fig. 7. In the figure the largest of the squares represent the total oxygen available to the fish and is equal to 100 ml. The three remaining squares in each set represent the actual total oxygen consumed by the fish: the smallest representing the 5 gm., the intermediate square the 15 gm. and the next bigger the 45 gm. fish. The scale is indicated in the square at the right-hand top corner of the figure. The series A, B and C represent the normal

<sup>1</sup> Low salt concentration therefore does not always "stimulate" the basal metabolism as suggested by Wikgren (1953) (p. 74).

TABLE II

*Normal and instant metabolism of a 5, 15 and 45 gm. fish*

Exp. sal ‰	Total oxygen consumed (ml.) by a fish		
	5 gm.	15 gm.	45 gm.
For fish acclimated to sea-water (30.4‰)			
30.4	5.50	19.15	57.18
00.4	5.75	16.18	47.55
12.5	7.93	18.15	49.00
44.2	12.30	22.00	38.18
60.0	6.65	16.50	44.50
For fish acclimated to 40‰			
40.0	8.25	18.50	42.50
00.4	6.50	18.12	48.43
12.5	8.62	17.25	34.93
18.0	3.50	16.68	79.25
60.0	8.81	18.93	40.87
For fish acclimated to 12.5‰			
12.5	5.52	16.88	50.68
00.4	4.56	16.50	60.18
30.4	4.70	19.56	78.62
44.2	8.35	21.75	62.32

metabolism of fish acclimated to 12.5, 30.4 and 40‰ salinity respectively and the others read horizontally against the three, the corresponding instant metabolism in the respective test salinities indicated therein. The broken lines in the instant metabolism squares indicate the respective normal rates drawn for the sake of comparison. Wherever this area is shaded it indicates the extent to which the instant exceeds the normal and where it is left unshaded the opposite condition is indicated.

In brief the data indicate that (1) the normal metabolism of the 5 gm. fish is more or less the same in both 12.5 or 30.4‰ but it is not true of the other two sizes; (2) that the change in the instant metabolism when compared with that in the normal indicates opposite trends between the 5 and 45 gm. fish and the rise or fall in either case being remarkably close, *i.e.*, +44.18 and -14.8% in the 5 gm. and -14.25 and +55.1% in the 45 gm. fish; (3) the intrinsic changes of the 5 gm. on transfer from 12.5-30.4‰ is lower than that in the opposite direction and *vice versa* in the 45 gm. fish; (4) similar comparison between the values obtained between salinities of 12.5 and 40‰ indicates true reciprocity in the case of the 45 gm. fish (-18 and +22.9%) while the 5 gm. fish registers an increase either way, the change from the lower to the higher salinity being ten times higher than that in the opposite direction.

In this context the observations on the killifish (Keys, 1931 and 1933) and on young migrating (elver) and adult eel (Raffy, 1933) are of interest. Within the same species the effect of salinity varies in relation to size and in this particular species within the size range studied there is no true reciprocity in all cases. The greater powers of increasing the metabolic rate by the smaller fish is probably a compensating device for the absence of a more efficient osmoregulatory system. Likewise the greater ease of adaptation to a lower salinity (12.5‰) as against the higher (40‰) is also related to the fact that isotonicity being probably reached in the former case, the smaller fish expends proportionately less oxygen for osmoregulation. This capacity has already been demonstrated in the standard metabolism of the smaller fish and has been indicated in the flounder by Chaisson (1931) and in other animals by Raffy (1932) and Topping and Fuller (1942). It is also seen that the ability to utilize oxygen when the latter is near the lethal zone (25 mm. Hg) is of a higher order in the 5 gm. fish than in the 45 gm. fish amounting to 51.1 and 34.7% of the rates at 150 mm. Hg  $pO_2$  respectively as calculated from the data presented in Fig. 5.

This is an important factor in the distribution of the species in that while the adults have never been observed in the creeks and lagoons of this area when the salinity is low, the young ones did occur there.

#### SUMMARY

The metabolism of *Plotosus anguillar* (Bloch) a marine cat-fish weighing from 5-45 gm. was studied at 29° C. after they were acclimated to that temperature and to salinities of 12.5, 30.4 (S.W.) and 40‰. The salinity of the experimental medium ranged from 0.4-60‰ in the active metabolism

experiments and were 12.5 and 30.4‰ in the standard metabolism experiments.

When the active metabolism of the three sizes is related to different salinities (at  $pO_2$  of 150 mm. Hg) the 5 and 45 gm. fish showed opposite trends. In spite of the intrinsic differences both the 'normal' and 'instant' metabolism follow the same general pattern for fish acclimated to 12.5 and 30.4‰ and that the proportional ability to utilize oxygen also remains the same for all sizes at all  $pO_2$  as far as the normal rates are concerned in those two salinities. However this is not so in 40‰ salinity.

The lethal oxygen level showed distinct size-salinity differences in that the lowest level was obtained in 12.5‰. A 5 gm. fish showed an 85% reduction in its lethal level over that in S.W. and no comparable reduction was noted in that of the 45 gm. fish. There was no change in the lethal level of fish of either size when acclimated to 40‰ and similarly compared.

It is demonstrated through appropriate experiments that the ability of the fish to utilize oxygen at greater rates even in low (25 mm. Hg)  $pO_2$  within the viable range of salinity is related to a lowered standard metabolic rate and through it to a lowered lethal level especially in 12.5‰ salinity.

The absolute and reciprocal effects of salinity on total oxygen consumed shows that the smaller fish displays greater tolerance to low salinity and greater flexibility in changing its metabolic intensity as with changing environmental salinity. The absence of this ability in the 45 gm. fish is probably made up by a more efficient osmoregulatory system.

The distribution of the species according to size and salinity and the problem of live-fish transport are discussed.

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