SOME ASPECTS OF ADAPTATION IN *CHANOS CHANOS* (FORSKÅL)

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Introduction

The possibilities of culturing marine species of fishes in coastal belts containing salt or fresh-water have received much attention in recent years, following the lead given by Far Eastern countries in the culture of *Chanos*. In India, *Chanos* as well as species of mullets could be cultured on a large scale and a beginning in this direction has already been made in certain South Indian centres. A study of the mechanism of adaptation in these species is an essential prerequisite to any rational system of culture, since it will help in determining the optimal values of salinity and temperature of the environment for the various species.

Although presenting a general pattern of similarity in their osmotic relations, teleostean fishes differ considerably in the manner in which they adapt themselves to external salinity. In the adult eel, for instance, the gills do not appear to be able to extract chloride from fresh-water (Krogh, 1937) and the osmotic regulation is brought about by practically complete reabsorption of the chloride by the excretory system combined with a low permeability of the tissues to water (Keys, 1933). In *Fundulus heteroclitus*, on the other hand, certain cells in the gills have been considered responsible for salt regulation by the excretion of chloride in sea-water and the absorption of chloride from fresh-water (Copeland, 1948). These differences probably relate to the evolutionary history of the species, depending on whether the mechanisms of adaptation have been perfected for fresh or salt water. Specialisation has no doubt taken place even among groups and forms primarily belonging to the saline or fresh-water environment. But, as indicated by Krogh (1939), the functional peculiarities of adaptation in euryhaline teleosts are still not clear in spite of several studies on the problem.

The interspecific differences are very pronounced particularly as regards the lower limits at which fishes are able to extract chlorides. The ecological significance of this aspect of hypertonic regulation in fishes has been discussed by Krogh (1939) who has pointed out the possibilities of fresh-waters being low enough in chloride content to present difficulties to certain species. Black (1951) finds that calcium is effective in diminishing the exchange of salts and water by *Fundulus heteroclitus* transferred to fresh-water. It is, therefore, quite likely that the power of absorbing chlorides and the lower limits of salt concentration are modified by the calcium content of the water. The importance of these factors in the adaptation of fishes to estuarine conditions has been discussed by Panikkar (1950).

Other aspects of the influence of ambient salinity relate to the size of the fish and the condition of the endocrine organs. The eel is relatively stenohaline between the time of hatching and entrance into the new environment (Krogh, 1939). In the stickleback, the power of osmotic regulation is significantly diminished during the breeding season (Gueylard, 1924) and sexually mature sticklebacks prefer brackish or fresh water, this effect being simulated when thyroid is administered to the fish *via* the alimentary tract (Koch and Heuts, 1942; Heuts, 1943).

In contrast to the salinity, the temperature of the environment provokes more passive responses in fishes which are poikilotherms. Here, the possibility has, however, to be taken into consideration of a combined influence of salinity and temperature such as has been indicated in the case of certain invertebrates (Broekhuysen, 1936; Panikkar, 1940; Broekema, 1942). Heuts (1945) has found that the geographical distribution of two subspecies of *Gasterosteus aculeatus* corresponds with the results on the effects of temperature on salt balance. It is obvious then that descriptions of heat death, cold death and optimal temperatures are incomplete without reference to the salinity of the medium employed.

In the present paper, observations are reported on the adaptive responses of *Chanos chanos* to variations in the salinity and temperature of the external medium and on the probable influence of calcium ions on adaptation. *Chanos* is a coastal species the fry of which are observed in large numbers in many places on the Madras coast (Ganapati *et al.*, 1950). It is usually reported euryhaline but reliable experimental or field data on the limits of salinity and temperature tolerance of the species are lacking.

MATERIAL, METHODS AND RESULTS

Blood serum chloride in Chanos *fingerlings*

Fingerlings of *Chanos* (65–140 mm. long) were acclimatised to the experimental media (tap water, sea-water diluted with tap water or sea-water to which common salt had been added) for a minimum of 48 hours before blood samples were taken from the heart or caudal blood vessels by the use of glass cannulæ. The samples were kept in the frigidaire for the separation of serum from clot. For the determination of chloride, the method of Wigglesworth (1937) was used. In Fig. 1, the mean values of serum chloride of 3–6 specimens of *Chanos* are plotted against chloride in the external medium.

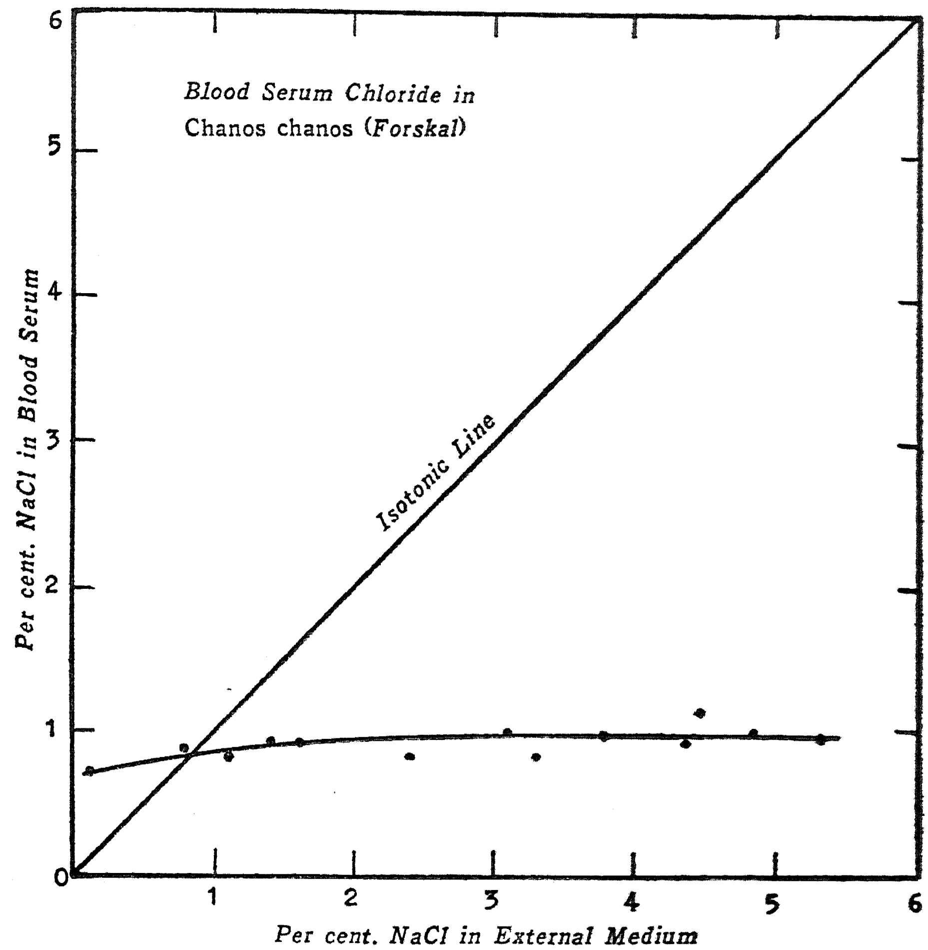

FIG. 1. Blood serum chloride in *chanos chanos* (Forskål)

Survival of Chanos *fingerlings in low salt concentrations*

In Tables I and II are given the results obtained by experiments with *Chanos* fingerlings in media containing low concentrations of salts. The external media were prepared by diluting tap water with glass-distilled water and by dissolving pure sodium chloride, calcium chloride and mixtures of these chlorides in glass-distilled water. The chloride and calcium content

TABLE I

Survival and Serum Chloride Values for Chanos *Fingerlings in Tap Water (Cl, 475 p.p.m. Ca, 112 p.p.m.) diluted with Glass-Distilled Water*

Date of experiment	% tap water in medium	Length of fish (mm.)	% NaCl in fish blood serum	Survival (hrs.)
26–12—49	100	70	0·969	*72
5–12—49	10	..	..	*24
7–12—49	1	..	..	*24
23–12—49	1	84	0·609	*72
9–12—49	0·1	..	..	*24
21–12—49	0·1	..	0·490	*24
17—1—50	0·1	123	..	19
4—4—50	0·1	..	..	*48
10–12—49	0·01	..	..	24
24—1—50	0·01	..	..	*48
19–12—49	0·008	96	0·372	*24
20–12—49	0·008	123	0·541	19
14–12—49	0·007	..	..	24
16–12—49	0·007	62	..	6
17–12—49	0·007	65	..	11
12–12—49	0·002	76	..	5
11–12—49	0·001	72	..	3

* The fish were alive at this stage but further observations were not made.

of tap water (and the fresh-waters listed in Table VII) were estimated by standard methods described in books on water analysis. Before transfer to the experimental media, the fingerlings were given a brief washing in glass-distilled water. One litre of medium was used per fingerling.

Thermal resistance of Chanos

The fish used were preacclimatised to the experimental media for a day.

(*a*) *Resistance to heat.—Chanos* fry, 10–20 in number, were kept in 1·5 l. of water contained in a glass cylinder (14 cm. ht.; 14 cm. diam.) and the temperature of the water was steadily raised by heating it in an air oven. The rate of increase of temperature was adjusted to about 4° C. per hour. The required temperature was maintained by switching the current on and off at intervals of 15 minutes. In experiments with the fingerlings, the fish was kept in 2·5 l. of water contained in a glass vessel (8″ diam.; 4″ ht.). The temperature of the water was raised over a closed type of electric hot plate (1,000 watts; 230 v.). The current was switched on initially for 1 minute and then at the rate of 1 minute for every 0·5 hr. till the temperature of water was 33° C. After this the rate of passing the current was increased to 1 minute per 15 minutes till the water attained the temperature of 35° C.

TABLE II

Survival and Serum Chloride Values for Chanos *Fingerlings in 'Artificial' Media: Figures within brackets in column 3 refer to calculated values of ionic concentrations, expressed in p.p.m.*

Expt. No.†	Date of expt.	Medium	Length of fish (mm.)	% NaCl in fish blood serum	Survival (hrs.)
(i) 1	25–11—49	Glass-distilled water	83	0·733	1·1
(i) 2	25–11—49	do	79	0·710	2·1
(ii) 3	20–10—52	do	76	..	*192
(ii) 4	20–10—52	do	97	..	*192
(ii) 5	20–10—52	do	89	..	192
(iii) 6	30–10—52	do	71	..	*24
7	16—8—50	NaCl (0·008)	87	..	1·3
8	4–12—49	do (84)	..	..	11
9	11—4—50	do (421)	..	..	*7
10	26—4—50	do (421)	..	..	*48
11	16—8—50	NaCl + $CaCl_2$ (Na, 0·003; Ca, 0·00002)	100	..	*48
12	9—2—50	do (Na, 0·177; Ca, 0·088)	..	..	5
13	4—2—50	do (Na, 0·177; Ca, 0·088)	71	..	*48
14	1—2—50	do (Na, 0·177; Ca, 0·883)	..	..	*48
15	29–11—49	$CaCl_2$ (14)	..	..	3·5
16	30–11—49	do (29)	83	..	*7
17	2–12—49	do (72)	..	..	7
(i) 18	28–11—49	do (144)	71	0·946	*48
19	28—3—50	do (144)	90	1·009	*48
20	28—3—50	do (144)	97	1·042	*48
21	28—3—50	do (144)	93	1·042	*48

* The fish were alive at this stage but further observations were not made.

† (i) Direct transfer from water of salinity 1·44% NaCl.

(ii) Fish preacclimatised for 4 months to fresh-water in a pond (salinity 1 part per 1000).

(iii) Fish preacclimatised for 4 months to fresh water and then for 42 hours to sea-water.

In experiments Nos. 7–17 and 19–21, the fish were collected from a saline pond and preacclimatised to tap water for 24 hours.

The subsequent maintenance of temperature at the required level was carried out by switching on the current for 0·5 or 0·75 minute at intervals of 15 minutes. Tables III and IV contain the results of experiments on the resistance of *Chanos* fry and fingerlings to heat.

(*b*) *Resistance to cold*.—The proportions of the number of fry (or fingerlings) and the volume of the medium were the same as in the experiments on the resistance of the fish to heat. The container was placed in a frigidaire for bringing down the temperature of the water. Tables V and VI contain the results of experiments on the resistance of *Chanos* fry and fingerlings to cold.

TABLE III

Resistance of Chanos *Fry* to Heat*

Date of expt.	Medium	No. of fry used	Temp. of water† (deg. C)		Time (Hours)										
			Initial	Test temp.‡	1	2	3	4	5	6	7	8	9	10	
25–4–50	Sea-water	20	30·6	39·7±0·1	100	100	90	85	..	..	..	..	..	..	Per cent. survival of fry
26–4–50	do	20	29·8	41·1±0·3	100	95	95	95	95	95	..	..	..	..	
27–4–50	do	20	29·6	41·6±0·2	75	65	55	55	..	50	50	50	..	..	
3–5–50	do	20	31·7	42·9±0·1	50	..	..	..	..	..	..	..	..	..	
1–5–50	do	20	30·0	42·9±0·2	60	55	40	..	25	..	20	15	5	0	
5–5–50	do	20	31·0	43·0±0·2	80	40	35	25	25	20	..	15	..	..	
3–5–50	do	20	29·6	43·1±0·4	20	0									
13–6–50	½ (sea-water)	10	29·9	39·2±0·2	100	100	100	100	100	..	..	..	..	..	
4–7–50	do	10	29·4	40·1±0·0	70	70	60	50	50	50	50	50	..	..	
5–7–50	do	10	30·0	41·4±0·4	100	80	80	70	70	70	..	..	..	..	
6–7–50	do	10	30·6	42·2±0·2	90	90	80	70	50	50	40	40	..	..	
29–5–50	Tap water	10	30·6	40·1±0·3	100	100	100	100	100	100	100	100	..	..	
30–5–50	do	10	30·0	40·8±0·4	100	100	100	100	100	..	..	..	..	..	
31–5–50	do	10	31·0	42·0±0·4	100	100	100	100	..	..	..	..	..	..	
1–6–50	do	10	29·8	43·0±0·3	10	..	..	..	..	..	..	..	..	..	

* Size of the fry used in the experiments : No. of fry whose lengths were measured 115; range, 13–30 mm.; mean, 17·4 mm.; & s.d., 3·3 mm.

† The rate of increase of temperature was about 4° C. per hour.

‡ Temperatures were recorded at intervals of 15 minutes and the figures correspond to (mean ± s.d.) of these readings.

The Analysis of variance of the data in Table III is given below:—

Source of variation	S.S.	D.F.	Variance	F by calcn.	F for P=0·05
Total ..	12124·3952	95	..	..	..
Temperatures, T ..	1161·1240	3	387·0413	4·08	2·83
Hours, H ..	2183·2593	7	311·8942	3·29	2·24
Salinities, S ..	40·2255	2	20·1127	4·72	19·5
Interaction : TS	314·4630	6	52·4105	1·81	3·8
Interaction : HS	621·2329	14	44·3738	2·14	2·3
Interaction : HT	3817·5219	21	181·7867	1·92	1·7
Residual ..	3986·5686	42	94·9183	..	..

At the P = 0·05 level, the only significant sources of variation are temperature, hour and interaction HT.

TABLE IV

Resistance of Chanos *Fingerlings to Heat*

Expt. No.	Date of expt.	Medium	Temperature of water (deg. C.)		Rate of increase (deg. per min.)	Survival (Hrs.)	Length of fish (mm.)
			Initial	Test temp.*			
1	22–12–50	Sea-water	26·2	(34–35)	8·6/191	‡6	117
2	12–12–50	do	25·8	34·6±0·3	8·9/195	3	135
3	16–12–50	do	25·7	35·2±0·1	9·5/234	‡7	..
4	5–12–50	do	28·5	(37–38)	8·2/20	‡10	..
5	8–12–50	do	25·9	(38–39)	11·6/198	‡3·3	..
6	20–7–50	do	27·9	(40·5—41·2)†	13·3/155	0·3	128
7	19–12–50	Tap water	27·6	(34·5–35)	7·6/157	‡6	133
8	13–12–50	do	26·7	35·4±0·2	8·7/195	‡3·5	..
9	6–12–50	do	28·3	(40·5—40·9)	11·3/223	§0·2	..

* In experiment Nos. 2, 3 & 8, the test temperatures correspond to (mean of temperature readings at 15 minute intervals ± s.d.). In all the other experiments, only the range of temperature has been indicated.

† By the procedure described for the fry.

‡ The fish were alive at this stage but further observations were not made.

§ The fish was in distress and jumped out of tank when the temperature was raised to 41° C.

TABLE V

Resistance of Chanos *Fry* to Cold*

Date of expt.	Medium	Temperature of water (deg. C.)			% Survival of fry
		Initial	Test temp.	Rate of fall of temp. (deg. per min.)	
12—5—50	Sea-water	29·0	21·8	7·2/390	100
15—5—50	do	30·1	18·0	12·1/525	100
13—5—50	do	28·5	13·8	14·7/600	58
		28·5	13·2	15·3/660	33
30—5—50	½ (Sea-water)	29·1	14·0	15·1/255	70
		29·1	14·0	15·1/285	50
		29·1	14·0	15·1/345	40
15—5—50	Tap water	33·2	20·7	12·5/335	93
		33·2	20·3	12·9/365	83
		33·2	18·1	15·1/485	75

* Size of fry : No. of fry whose lengths were measured ; 52.
Range ; 15-25 mm, Mean ; 19·4 mm. S.D. : 2·5 mm,

TABLE VI

Resistance of Chanos *fingerlings to cold*

Date of expt.	Medium	Temperature of water (deg. C.)			Length of fish (mm.)	Remarks
		Initial	Test temp.	Rate of fall of temp. (deg. per min.)		
22—11—50	Sea water	28·5	15·1	13·4/220	22	
30—11—50	do	25·2	12·3	12·9/190	122	Fish in distress
1—12—50	Tap water	27·6	14·4	13·2/165		
24—10—50	do	25·9	13·9	12·0/535		
17—10—50	do	25·8	13·6	12·2/285		Fish in distress
29—10—50	do	26·5	11·2	15·3/485	75	Fish comatose

DISCUSSION

For a variation in the external salinity corresponding to 5% NaCl, the change in the blood serum chloride of *Chanos* fingerlings is only of the order of 0·3% NaCl (Fig. 1). The chloride values for fingerlings kept in 1% and lower dilutions of tap water (equivalent to 5 p.p.m. of chloride and less) indicate a progressive breakdown of the osmoregulatory mechanism extending over a long period during which the loss of salt is more than what could be made good by the active uptake of ions.

The results with the 'artificial' media containing both sodium and calcium chlorides agree with those obtained with tap water of about equal ionic concentrations (Tables I and II). Since tap water differs from glass-distilled water chiefly in its content of chloride, sodium and calcium, the results obtained with the two sets of media may be taken to correspond to the effects of these ions. It is also seen that the survival of the fingerlings in glass-distilled water improves with prolonged preacclimatisation to fresh-water or by the addition of chloride, either as NaCl or $CaCl_2$, to the medium. For the same survival time, it would appear that less of either chloride suffices in a mixture than when the chlorides are used separately. Of special significance is the response of the fingerlings to solutions of calcium chloride, the mean serum chlorides (1·01% NaCl) in this case being considerably higher than what would be expected (0·7% NaCl, Fig. 1) in a medium of tap water of similar salinity. Evidently, the retention of blood chloride is facilitated by the presence of calcium in the external medium, more so in the absence of the antagonistic ion, sodium. In the light of this protective action of calcium, statistics on the survival and growth of *Chanos* in hard and soft natural waters would afford interesting material for comparative study.

The observations on the survival of *Chanos* in low salt concentrations further suggest the necessity for a more precise definition of 'fresh waters' used in *Chanos* culture than is at present available. Fresh-waters vary widely in their content of chloride and calcium (*cf.* Table VII). The possibility has to be borne in mind that certain fresh-waters especially those in the interior (*cf.* Krogh, 1939), may contain these ions in amounts lower than the critical limits. Under these conditions, *Chanos* may be losing blood salts to the surroundings and continue to live only till the minimum threshold value for blood salts is reached. Such waters, however, would not be suitable for large-scale culture of fish.

TABLE VII

Chloride and Calcium Content of Some Natural Fresh-Waters

Fresh-water	Cl. p.p.m.	Ca p.p.m.	Date
Upputeru (opp. Jungampadu) ..	72	28	19–11–47
Kondangi ..	79	25	17–11–47
Madras (Univ. Zool. Lab. tap water) ..	..	17	5–3 –48
Mandapam tap water ..	475	112	17–12–49
Akividu well water ..	3397	361	17–11–47

The time of exposure to the test medium and the rates of increase or decrease in temperature are likely to influence the heat death and cold death temperatures (Heilbrunn, 1947). Data on these have therefore been included in Tables III–VI. On the basis of the standard time of exposure of 30 minutes suggested by Heilbrunn, the heat death temperatures of *Chanos* fry and fingerlings are about 43° C. and 39° C. respectively which are higher than those recorded for marine fishes (Heilbrunn, *loc. cit.*). The upper lethal temperatures of fishes have been correlated with habitat by Hart (1951) who finds that species from base level streams and marshes have the highest lethal temperatures. It is interesting to note that the temperature of the water in the tidal flats from which *Chanos* fry were collected was on two occasions as high as 36° C. and 38·5° C., suggesting that in the present instance the high heat death temperature is possibly the result of adaptation to the natural habitat. From the analysis of variance of the data in

Table III, it is also seen that variations in the salinity of the water are not significant in respect of the per cent. survival of the fry under conditions of heat death.

In regard to the resistance to cold, the results in Tables V and VI indicate that *Chanos* fry and fingerlings can withstand momentary exposures to 18° C. and 14° C. respectively, both in sea-water and in tap water. In the absence of facilities for the maintenance of low and steady temperatures, the cold death temperatures and the influence of salinity on these could not be studied in detail.

SUMMARY

1. A study has been made of the adaptive responses of *Chanos* to variations in external salinity and temperature. In external salinities varying from 0·1% NaCl (hard tap water) to 5·3% NaCl (sea-water *plus* common salt), *Chanos* fingerlings retain their blood serum chloride values fairly constant over a narrow range of 0·3% NaCl. In dilutions of tap water corresponding to a chloride content of 5 p.p.m. and less, the serum chloride values suggest a breakdown of the osmoregulatory mechanism.

The period of survival of *Chanos* fingerlings in glass-distilled water is prolonged if they have been preacclimatised to fresh-water or if sodium or calcium chloride has been added to the medium. The serum chloride values for fingerlings kept in solutions of calcium chloride (144 p.p.m. of Ca) indicate that calcium in the external medium aids the fish in the retention of blood salts.

The chloride and calcium contents of natural fresh-waters appear to be of critical significance when *Chanos* is transferred direct from high salinities.

2. The heat death temperatures of *Chanos* fry and fingerlings (43 and 39° C. respectively) are higher than those reported for marine fishes. The heat death temperature of the fry is not significantly influenced by the salinity of the medium, the per cent. survival of the fry at any temperature being dependent only on the temperature of the water and the time of exposure.

The fry and fingerlings are able to withstand momentary exposures to 18° C. and 14° C. respectively, both in sea-water and in tap water.

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References

Black, V. S. .. *Some Aspects of the Physiology of Fish*, University of Toronto Biological Series, No. 59, 1951, 75.

Broekema, M. M. M. .. "Seasonal movements and the osmotic behaviour of the shrimp, *Crangon crangon* L.," *Arch. Néerl. Zool.*, 1942, **6**, 1–100.

Broeckhuysen, G. J. .. "On the development, growth and distribution of *Carcinides maenas* (L.)," *ibid.*, 1936, **2**, 257–400.

Copeland, D. E. .. "The cytological basis of chloride transfer in the gills of *Fundulus heteroclitus*," *J. Morph.*, 1948, **82**, 201–28.

Ganapati, S. V., Chacko, P. I., Srinivasan, R. and Krishnamurthy, B. "On the acclimatisation, transport and culture of some salt water fishes in inland waters of the Madras State," *Ind. Geog. Jour.*, 1950, **25**, 1–15.

Gueylard, F. .. "De l'adaptation aux changements de salinité. Recherches biologiques et physico-chimiques sur l'epinoche (*Gasterosteus leiurus* Cuv. et Val.)," Thése, Paris, 1924.

Hart, J. S. .. "Interspecific and intraspecific variation of lethal temperatures in fish," *Federation Proceedings*, U.S.A., 1951, **10**, No. 1, Part 1.

Heilbrunn, L. V. .. *An Outline of General Physiology*, Philadelphia, 1947, 419–32.

Heuts, M. J. .. "La règulation osmotique chez l'épinochette (*Pygosteus pungitius* L.)," *Ann. Soc. Roy. Zool. Belgique*, 1943, **74**, 99-105.

——— .. "La règulation minérale en fonction de la température chez *Gasterosteus aculeatus*. Son importance au point de vue de la zoogeographique de l'espèce," *ibid.*, 1945, **76**, 88–99.

Koch, H. J. and Heuts, M. J. "Influence de l'hormone thyroidenne sur la régulation osmotique chez *Gasterosteus aculeatus* L. forme *gymnurus* Cuv.," *ibid.*, 1942, **73**, 165–72.

Krogh, A. .. "Osmotic regulation in fresh-water fishes by active absorption of chloride ions," *Z. vergl. Physiol.*, 1937, **24**, 656–66.

——— .. *Osmotic Regulation in Aquatic Animals*, Cambridge University Press, 1939, 147, 200–204.

Keys, A. B. .. "The mechanism of adaptation to varying salinity in the common eel and the general problem of osmotic regulation in fishes," *Proc. Roy. Soc.*, Lond., 1933, **112** B, 184–99.

Panikkar, N. K. .. "Influence of temperature on osmotic behaviour of some Crustacea and its bearing on problems of animal distribution," *Nature*, 1940, **146**, 366.

——— .. "Physiological aspects of adaptation to estuarine conditions," *Indo-Pacific Fisheries Council Proceedings*, 2nd Meeting, Cronulla, N.S.W., Australia, 1950, 168–75.

Wigglesworth, V. B. .. "Volumetric determination of chloride in 0·3 microlitre of tissue fluid," *Biochem. J.*, 1937, **31**, 1719–23.