BLOOD VOLUME OF TWO ANTARCTIC FISHES

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Abstract. Measurements were made of the blood volume of the haemoglobin-free species *Chaenocephalus aceratus* and the red-blooded species *Notothenia neglecta*, using the Evans blue-dye dilution method. Mean values obtained were: *C. aceratus* 9·2 ml. per 100 g. body weight, *N. neglecta* 5·7 ml. per 100 g. body weight. The large blood volume of *C. aceratus* is considered a respiratory compensation for lack of haemoglobin; the blood volume of *N. neglecta* is larger than most teleost fish and may represent a parallel though less extreme condition to *C. aceratus* in view of its low haemoglobin concentration.

Measurements were also made of the rates of flow of blood of both species through fine glass capillaries. *C. aceratus* blood was found to have a significantly higher rate of flow than *N. neglecta* blood and this is attributed to its lower viscosity as a result of the absence of erythrocytes.

The physiology of the haemoglobin-free Antarctic Chaenichthyidae has been investigated by several workers since this condition was first reported by Ruud (1954). Recent workers have found a number of physiological adaptations which appear to compensate for the lack of an oxygen-transport pigment, and are not typical of other teleost fishes (Ruud, 1965; Ralph and Everson, 1968; Hemmingsen and Douglas, 1970; Holeton, 1970). All of these workers noted the large heart size and probable large blood volume of *Chaenocephalus aceratus*, and Hemmingsen and Douglas (1970) reported a mean blood volume of 7·6 per cent body weight in five *C. aceratus*.

The purpose of this study was to determine the blood volumes of *C. aceratus* and the red-blooded Antarctic fish *Notothenia neglecta*. Simple experiments were also conducted to verify the predicted lower viscosity of the erythrocyte-free blood of *C. aceratus* compared with blood of *N. neglecta*. This was a part of a larger study carried out by the author on aspects of the physiology and ecology of *C. aceratus* between December 1968 and March 1971 at the British Antarctic Survey station at Signy Island, South Orkney Islands. Some notes are included on the behaviour of *C. aceratus* which conflict with an earlier report by Holeton (1970).

Material and Methods

The fish used for this study were obtained from Normanna Strait and Borge Bay, Signy Island (lat. 60°43′S., long. 45°36′W.), during the austral summers 1968–69 and 1969–70. The experimental work was carried out at the British Antarctic Survey laboratory on Signy Island.

The two species, *Chaenocephalus aceratus* and *Notothenia neglecta*, are common benthic fish. *C. aceratus* are found at depths greater than 50 m. and *N. neglecta* from 0 to 40 m. during the austral summer. The fish were caught in trammel nets which were laid from a drifting boat during the morning and recovered the same evening. The fish were carefully removed from the nets, placed in polythene tanks filled with fresh sea-water and transported to the laboratory. Experimental fish were kept in glass aquaria supplied with sea-water at −0·5° to +2·0° C by an open circulation system (Ralph and Everson, 1968; Holeton, 1970). No attempt was made to feed the fish as they were used for experiments within 4–5 days of capture.

Trammel nets as a means of capture were found to be very effective, particularly for *C. aceratus* which had previously proved difficult to obtain in quantity. These fish do not enter traps and are taken infrequently on long lines (Ralph and Everson, 1968). This species was caught at 60–100 m. in Normanna Strait and the specimens taken were mainly mature females (90–95 per cent); few males and immature specimens were obtained. The preponderance of mature female *C. aceratus* at the depths fished has been well established by the author, and it is thought to be a pre-spawning movement into the area. *N. neglecta* were taken in large numbers at depths between 10 and 20 m. in Borge Bay, over rough ground colonized extensively by macro-algae (Everson, 1970).

A large proportion of both species suffered damage to skin and gills in the nets and could not be used for experimental purposes. *C. aceratus* showed a high post-capture mortality in the aquaria and special care was taken to use only healthy specimens. A good criterion of condition in *C. aceratus* was found to be their behaviour in aquaria. Healthy fish raised the

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head and abdomen off the bottom on extended pelvic fins, whereas fish in poor condition lay unsupported on the bottom of the aquaria. Fish in the former group were used experimentally and fish in the latter group were discarded. Whilst *N. neglecta* proved to be a more hardy species which could be maintained in aquaria for long periods, diuresis, an indication of stress, was occasionally observed (unpublished work of the author).

Blood volumes

Experimental fish were acclimatized in aquaria for at least 24 hr. They were then anaesthetized in sea-water containing 1/20,000 M.S. 222 (weight/volume) and transferred to an operating table where the gills were irrigated with sea-water. Experiments could be maintained for approximately 1 hr. in this way, but for longer experiments, anaesthesis was maintained by irrigation of the gills with 1/40,000 M.S. 222 sea-water. The method used for blood-volume determinations with Evans blue dye (T.1824) was modified from Conte and others (1963). Cannulation of the ventricle and dorsal aorta for dye injection and blood sampling was achieved as follows. The ventricle was cannulated by insertion of a short bevel 21 gauge needle attached to 25 cm. of pp60 Portex polyethylene tubing, maintained with a syringe containing 0·5 ml. of heparinized saline (after Holeton, (1970), 50 i.u./ml.). The point of entry of the needle was the ventral surface of the cleithrum, anterior to the pelvic fins; direct injection into the ventricle by opening the pericardial cavity (Conte and others, 1963) could not be used, owing to the extensive vascularization of this region in both species. The dorsal aortal cannulation was made using the same equipment, the needle being inserted with artery forceps into the roof of the oesophagus posterior to the pharyngeal teeth. This vessel was difficult to locate and maintain, and a number of experiments had to be terminated due to failures of this nature.

With both cannulae in place, a small blood sample was withdrawn from the dorsal aorta for determination of packed cell volume (haematocrit) by centrifugation at 3,000 r.p.m. for 20 min. Heparin (5,000 i.u./ml.) was then injected into the ventricle at a dosage of 2,500 i.u. per animal for *N. neglecta* and 5,000 i.u. for *C. aceratus*, and allowed to circulate for 5 min. A blood standard was removed from the dorsal aorta. The dye was then injected over a period of 30 sec. into the ventricle at a dosage of 2,000 μg./1,000 g. body weight for *N. neglecta* and 4,000 μg./1,000 g. body weight for *C. aceratus*. The Evans blue dye used for the experiments was made up in saline (Holeton, 1970) at 2 mg./ml. concentration. Care was taken to ensure quantitative delivery of dye and heparin. This was achieved by flushing the cannula with 2 ml. of blood removed prior to the dye injection. Exclusion of air bubbles from the cannulae was achieved by lowering or raising the tube against blood pressure.

After the dye injection, blood samples of 0·5 ml. were taken from the dorsal aorta at timed intervals, transferred to tubes and made up to 5 ml. with heparinized saline. Before each sample was taken, 1 ml. of blood was withdrawn into a separate syringe to clear the dead space in the cannula, and this was re-injected after sampling. The blood samples were centrifuged at 3,000 r.p.m. for 20 min. to spin down cellular material, and optical density measured against the blood standard at 610 nm. in a Unicam 600 spectrophotometer. Length from snout to base of caudal fin, sex, gonad weight and total weight were measured after each experiment.

Capillary flow

Blood samples from a male *N. neglecta*, haematocrit 31 per cent (369 g.) and a female *C. aceratus* (1,242 g.) were withdrawn with a 5 ml. syringe and transferred to tubes dusted with powdered heparin. The *C. aceratus* blood was coloured with a few grains of T.1824 dye. Distilled water, also coloured with T.1824 dye, was used to obtain some comparative measurements of capillary flow. Soda glass tubing was drawn out into fine capillaries and the external diameter measured with a micrometer. Groups of three capillary tubes were matched for length and taper since it was not possible to make capillary tubing of uniform diameter.

Flow experiments were carried out on a clear Perspex sheet mounted on white paper and marked transversely at 2·5 cm. intervals. Each capillary tube was placed at right-angles to the markings, mounted on short pieces of capillary (an essential precaution to prevent seepage between capillary and Perspex). Each sample of blood or water was placed over one end of a capillary tube and the time taken by the fluid to flow through the capillary over each 2·5 cm. section was measured. Two experiments were carried out for each species.

RESULTS

Blood volume

Complete mixing of injected dye was found to take about 20 min. Plasma volume was calculated by extrapolation to time of injection, using the serial samples taken between 20 min. and 1 hr. after injection. Several fish were sampled for up to 3 hr. and dye concentration was found to decrease by up to 10 per cent/hr. The values for blood volume of 15 *C. aceratus* expressed as ml./100 g. body weight ranged from 7·85 to 10·98, mean value 9·16 (Table I).

TABLE I. BODY SIZE, ORGAN SIZE AND PLASMA VOLUME OF *C. aceratus*

Fish (number)	*Sex*	*Length* (mm.)	*Weight* (g.)	*Ventricle weight*		*Plasma* (ml.)	*Volume* (ml./100 g.)
				(g.)	(per cent body weight)		
3	♀	510	1,134	3·76	0·33	94·50	8·33
7	♀	560	1,431	4·09	0·29	112·34	7·85
8	♀	520	1,339	4·39	0·33	130·50	9·75
14	♀	515	1,194	4·02	0·34	129·19	10·82
15	♀	515	1,275	3·91	0·31	120·29	9·44
21	♀	600	2,388	6·66	0·28	199·53	8·36
24	♀	515	1,160	3·71	0·32	105·73	9·12
28	♀	530	1,425	3·51	0·25	125·58	8·81
30	♀	490	1,082	2·59	0·24	95·00	8·78
31	♀	505	1,155	3·66	0·32	116·67	10·10
34	♀	475	933	2·56	0·27	79·96	8·57
35	♀	530	1,501	3·81	0·25	164·85	10·98
37	♂	420	753	2·11	0·29	60·62	8·05
38	♀	500	1,223	3·65	0·30	114·20	9·34
40	♂	390	582	1·82	0·31	63·66	10·94
Mean					0·294		9·16*
Standard deviation					±0·033		±0·97

* Hemmingsen and Douglas (1970) plasma density of 1·04 mean body volume as per cent body weight = 9·53 per cent.

The values for 15 *N. neglecta* ranged from 4·54 to 6·97, mean value 5·69 (Table II).

Capillary flow

Graphs of the data obtained in a typical experiment are given in Fig. 1. The method used [illegible]as not deemed sufficiently accurate to calculate the true viscosities of the blood samples. However, the final time data may be expressed as viscosities relative to water. *N. neglecta* blood had relative viscosities of 5·46 and 4·40, and *C. aceratus*, 3·0 and 2·3 in the experiments.

DISCUSSION

Physiology

The mean blood volume of 15 *C. aceratus* (Table I) was found to be 9·2±1·0 ml./100 g. or 9·5 per cent body weight. This is appreciably larger than the value of 7·6 per cent reported by Hemmingsen and Douglas (1970) for five *C. aceratus*. The higher value found in this study may be due to the larger sample size, the different technique of dye injection and sampling, and the shorter time from capture to the experiments. The use of Evans blue-dye (T.1824) dilution technique has been shown by Conte and others (1963) to give higher values than those obtained by labelled erythrocyte and albumin techniques in *Salmo gairdnerii*. However, the blood volume of *C. aceratus* is larger than the reported values of other osteichthyes (3·0 per cent body

TABLE II. BODY SIZE, ORGAN SIZE AND PLASMA VOLUME OF *N. neglecta*

Fish (number)	*Sex*	*Length* (mm.)	*Weight* (g.)	*Ventricle weight* (g.)	(per cent body weight)	*Plasma volume* (ml.)	(ml./100 g.)	*Haematocrit* (per cent)	(vol. ml.)	*Blood volume* (ml.)	(ml./100 g.)
8	♀	325	827	0·97	0·12	31·02	3·75	37·50	18·61	49·63	6·00
9	♂	350	887	0·73	0·08	25·27	2·85	47·50	22·86	48·13	5·43
11	♀	315	565	0·66	0·12	18·98	3·36	40·00	12·65	31·36	5·55
14	♀	380	1,242	1·20	0·10	50·67	4·08	33·25	25·24	75·91	6·11
19	♀	345	799	0·86	0·11	34·88	4·37	29·00	14·25	49·13	6·15
24	♀	335	859	0·63	0·07	33·53	3·90	34·50	17·69	51·19	5·96
30	♀	365	996	1·12	0·11	36·15	3·63	38·00	22·16	58·31	5·85
31	♀	360	1,063	1·00	0·09	31·54	2·97	35·50	17·36	48·90	4·60
34	♀	380	1,099	1·13	0·10	48·45	4·41	36·75	28·15	76·60	6·97
36	♀	355	944	0·81	0·09	32·90	3·49	40·50	22·39	55·29	5·86
38	♀	360	1,120	0·94	0·08	36·33	3·24	28·50	14·48	50·81	4·54
39	♀	315	720	0·78	0·11	23·96	3·33	39·50	15·64	39·60	5·50
41	♀	330	818	0·76	0·09	28·68	3·51	36·25	16·31	44·99	5·50
42	♂	350	888	0·89	0·10	30·38	3·42	40·00	20·25	50·63	5·70
44	♀	380	1,065	1·16	0·11	41·68	3·91	35·75	23·19	64·87	6·09
Mean					0·098		3·59	36·91			5·69
Standard deviation					±0·015		±0·469	±4·86			±0·615

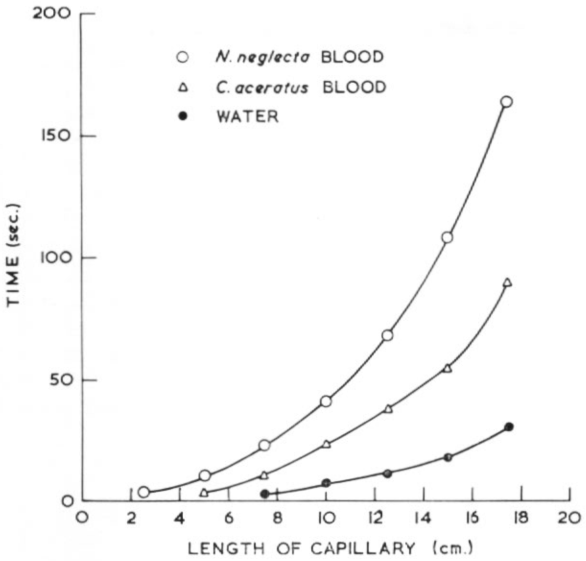

Fig. 1. Graph to show rates of flow of fish blood and water through glass capillaries.

weight; Thorson, 1961). As was suggested by Ruud (1965) and Hemmingsen and Douglas (1970), the large blood volume of *C. aceratus* is the major physiological adaptation enhancing the oxygen capacity of its haemoglobin-free blood.

The mean blood volume of 15 *N. neglecta* (Table II) was found to be 5·7±0·6 ml. per 100 g. body weight. The significance of the relatively large blood volume of this benthic red-blooded fish is of interest and it would be useful to ascertain if this is a feature of other nototheniids. *N. neglecta* blood was shown by Everson and Ralph (1968) to have a low haemoglobin level in common with the blood of other Antarctic and some Arctic fishes (Scholander and van Dam, 1957; Tyler, 1960; Kooyman, 1963; Grigg, 1967). The low levels of haemoglobin in these fish and the absence of haemoglobin in the Chaenichthyidae may be considered in part to result from the high oxygen content of the cold water in which they live. High oxygen tension reduces the amount of blood required to be pumped through the gills to extract oxygen for metabolism (Ruud, 1954, 1959, 1965; Holeton, 1970). Whilst this explanation is quite plausible and haemoglobin levels can be related to the activity of fish (Hall and Gray, 1929), it provides no positive explanation for reduction of haemoglobin other than functional redundancy which should not be accompanied by the compensation of a larger blood volume.

Hemmingsen and Douglas (1970) pointed out the advantage of the inferred reduced viscosity of erythrocyte-free blood of *C. aceratus*, and the results of two experiments carried out by the author show that the viscosity of *C. aceratus* blood is approximately half that of *N. neglecta* blood. Fig. 1 illustrates this point. The experiments were designed to compare the blood of these two species but are insufficiently accurate to give absolute viscosity values since sedimentation would be likely to occur in the horizontal capillary tubes used. This would result in lowered viscosity values for *N. neglecta* blood. The lower viscosity of *C. aceratus* blood would clearly reduce the energy required for blood circulation, but this is offset by the very much larger blood volume which this fish must circulate. Holeton (1970) concluded from measurements of blood pressure in *C. aceratus* that it had a large-bore capillary network, since it was able to circulate blood at a low pressure differential. This was supported by the earlier work of Steen and Berg (1966), who found significantly larger blood capillaries in the gill lamellae of *C. aceratus* than in other teleost fishes. Jakubowski and others (1969) and Jakubowski and Byczkowska-Smyk (1970), using a single specimen of *Chaenichthys rugosus* (*C. rhinoceratus*; Hureau, 1964) injected with Indian ink, found extensive vascularization of the skin and fins, and a mean capillary bore of 17 μm. compared with a bore of 10 μm. in other fish they have studied. Holeton (1970) pointed out the advantage of large-bore capillaries which greatly reduce vascular resistance in accordance with the Poiseuille equation. The predicted large capillary bore and extensive vascularization of *C. aceratus* (Walvig, 1960) are reflected in its large blood volume. These adaptations have probably evolved to compensate for the reduction of haemoglobin in the red-blooded ancestors of this fish. Walvig (1960), Jakubowski and others (1969), and Jakubowski and Byczkowska-Smyk (1970) found extensive vascularization of the skin and fins of *C. aceratus* and *C. rhinoceratus*, respectively, and predicted cutaneous gas exchange. Hemmingsen and Douglas (1970) found cutaneous gas exchange in the trunk of *C. aceratus* but Holeton (personal communication) considers that cutaneous exchange is a relatively unimportant secondary feature, since the skin and fins are undeveloped for this function and receive only a small portion of the total blood circulated through the gills. It is interesting to note that Everson and Ralph (1968) found very low haemoglobin and erythrocyte counts in the bathydraconid, *Parachaenichthys georgianus*, and the erythrocyte size of this fish was 20 by 14 μm. which is comparatively large and suggests that it may have large-bore blood capillaries. Clearly, study of the blood volume, circulatory characteristics and capillary size of this and other red-blooded Antarctic fish may clarify the evolution of the haemoglobin-free condition of the *Chaenichthyidae*.

Behaviour

It has been suggested by Ruud (1965) and Holeton (1970) that, based on the sluggish behaviour of this fish when captured and its usually empty stomach, *C. aceratus* is probably an inactive benthic fish which feeds only sporadically. Occasionally *C. aceratus* are taken with their mouths and stomachs filled with *Euphausia* spp. or single large fish (Holeton, 1970). Studies by the author suggest that these assumptions are somewhat erroneous.

When captured, *C. aceratus* usually adopt a defence posture with mouth wide open and opercula extended and remain motionless, in contrast to most other fish which flap vigorously when first caught. In aquaria, *C. aceratus* also remain relatively inactive. To assess its activity and obtain photographs under natural conditions, the author released a female *C. aceratus* (length 60 cm., weight 1,320 g.) at a depth of 7 m. in Factory Cove on 19 February 1970, and observed its behaviour, using aqualung equipment. The fish proved capable of swimming 50 m. across the sea bed before recapture, at approximately its own length per second (36 m./min.). Propulsion was provided by the rowing action of the expanded pectoral fins, as has been observed in *Pagetopsis macropterus* (Robillard and Dayton, 1969), and the trunk musculature appeared to play no part in swimming (Fig. 2). Whilst this fish was undoubtedly under stress, its swimming performance appeared to be both strong and economical and it made no attempt to rest. Use of the pectoral fins for primary locomotion has also been observed by the author in *N. neglecta*, but this species when disturbed on the sea bed uses its trunk musculature to swim rapidly, a feature not seen in the released *C. aceratus*.

Support for the more active habits of *C. aceratus* has also been found in a fishing survey carried out by the author each month at four stations between 25 and 100 m. in Borge Bay (Signy Island) and Normanna Strait. During the summer the numbers of *C. aceratus* taken overnight in trammel nets laid on the sea bed varied considerably from month to month. Occasionally, large numbers were taken which had been feeding on *Euphausia* spp. (Holeton, 1970). This shoaling habit, coupled with the variation in numbers taken from monthly nets, suggests that *C. aceratus* search actively for food, and concentrate in shoals where food such as *Euphausia* spp. is plentiful. The empty stomach phenomenon described by Holeton (1970) and Olsen (1955) probably results from the habit of voiding the stomach when captured, for both line-caught and net-caught fish have been observed to disgorge their stomach contents as lines and nets are hauled to the surface. Only when the stomach is distended with a large fish or euphausiids does disgorging of food become more difficult (unpublished work of the author). It would therefore seem more accurate to describe *C. aceratus* as a moderately active predacious fish.

Conclusion

Perhaps it is wrong to ask the question, how do the chaenichthyids survive without haemoglobin? Clearly they are well adapted and widespread in Antarctic waters and do not

Fig. 2. Released *Chaenocephalus aceratus* swimming over sea bed, depth 7 m. Pectoral fins are used to propel the fish by "rowing" action, and the trunk region appears to act only as a rudder.

live a marginal inactive existence (Hemmingsen and Douglas, 1970). The significance of evolved physiological and anatomical adaptations which appear to compensate for lack of an oxygen-transport pigment may imply that haemoglobin is functionally inefficient under cold conditions. Grigg (1967) showed that the haemoglobin of *Trematomus* spp. at McMurdo Sound was both cold-adapted and extremely sensitive to temperature change. Clearly, the haemoglobin of these fish has evolved to function efficiently in their cold stenothermal environment.

By contrast, the Chaenichthyidae have evolved a haemoglobinless condition, compensated by a modified vascular system and oxygen transport in physical solution. This is perhaps an alternative to low-temperature haemoglobin. It is possible that the precursors of the present-day Antarctic Nototheniiformes had haemoglobin which was not low-temperature efficient and adaptations representing at least two divergent physiological mechanisms have evolved. Clearly, further work is required to substantiate this suggestion.

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REFERENCES

CONTE, P. F., WAGNER, H. H. *and* T. O. HARRIS. 1963. Measurement of blood volume in the fish (*Salmo gairdneri gairdneri*). *Am. J. Physiol.*, **205,** No. 3, 533–40.

EVERSON, I. 1970. The population dynamics and energy budget of *Notothenia neglecta* Nybelin at Signy Island, South Orkney Islands. *British Antarctic Survey Bulletin*, No. 23, 25–50.

————. *and* R. RALPH. 1968. Blood analyses of some Antarctic fish. *British Antarctic Survey Bulletin*, No. 15, 59–62.

GRIGG, G. C. 1967. Some respiratory properties of the blood of four species of Antarctic fishes. *Comp. Biochem. Physiol.*, **23,** No. 1, 139–48.

HALL, F. G. *and* I. E. GRAY. 1929. The haemoglobin concentrations of the blood of marine fishes. *J. biol. Chem.* **81,** No. 3, 589–94.

HEMMINGSEN, E. A. *and* E. L. DOUGLAS. 1970. Respiratory characteristics of the haemoglobin-free fish *Chaenocephalus aceratus*. *Comp. Biochem. Physiol.*, **33,** No. 4, 733–44.

HOLETON, G. F. 1970. Oxygen uptake and circulation by a haemoglobinless Antarctic fish (*Chaenocephalus aceratus* Lonnberg) compared with three red-blooded Antarctic fish. *Comp. Biochem. Physiol.*, **34,** No. 2, 457–71.

HUREAU, J. C. 1964. Sur la probable identité des deux especes du genre *Chaenichthys* de la famille des Chaenichthyidae. *Bull. Mus. natn. Hist. nat., Paris*, Sér. 2, **36,** No. 4, 450–56.

JAKUBOWSKI, M. *and* W. BYCZKOWSKA-SMYK. 1970. Respiratory surfaces of white-blooded fish *Chaenichthys rugosus* Regan (Perciformes). *Polskie Archwm Hydrobiol.*, **17,** 273–81.

————., ————. *and* Y. MIKHALEV. 1969. Vascularization and size of respiratory surfaces in the Antarctic white-blooded fish *Chaenichthys rugosus* Regan (Percoidei, Chaenichthyidae). *Zoologica Pol.*, **19,** Fasc. **2**, 303–17.

KOOYMAN, G. L. 1963. Erythrocyte analysis of some Antarctic fishes. *Copeia*, 1963, No. 2, 457–58.

OLSEN, S. 1955. A contribution to the systematics and biology of chaenichthyid fishes from South Georgia. *Nytt Mag. Zool.*, **3,** 79–93.

RALPH, R. *and* I. EVERSON. 1968. The respiratory metabolism of some Antarctic fish. *Comp. Biochem. Physiol.*, **27,** No. 1, 299–307.

ROBILLARD, G. A. *and* P. K. DAYTON. 1969. Notes on the biology of the chaenichthyid fish *Pagetopsis macropterus*. *Antarct. Jnl U.S.*, **4,** No. 6, 304–06.

RUUD, J. T. 1954. Vertebrates without erythrocytes and blood pigment. *Nature, Lond.*, **173,** No. 4410, 848–50.

————. 1959. Vertebrates without blood pigment: a study of the fish family Chaenichthyidae. *Proc. 15th Int. Congr. Zool., London, 1958*, Sect. 6, Paper 32, 526–28.

————. 1965. The ice fish. *Scient. Am.*, **213,** No. 5, 108–14.

SCHOLANDER, P. R. *and* L. VAN DAM. 1957. The concentration of haemoglobin in some cold water Arctic fishes. *J. cell. comp. Physiol.*, **49,** No. 1, 1–4.

STEEN, J. B. *and* T. BERG. 1966. The gills of two species of haemoglobin-free fishes compared to those of other teleosts—with a note on severe anaemia in an eel. *Comp. Biochem. Physiol.*, **18,** No. 3, 517–26.

THORSON, T. B. 1961. The partitioning of body water in osteichthyes: phylogenetic and ecological implicatio[illegible] in aquatic vertebrates. *Biol. Bull. mar. biol. Lab., Woods Hole*, **120,** No. 2, 238–54.

TYLER, J. C. 1960. Erythrocyte counts and haemoglobin determinations for two Antarctic nototheniid fishes. *Stanford ichthyol. Bull.*, **7,** No. 4, 199–201.

WALVIG, F. 1960. The integument of the icefish *Chaenocephalus aceratus* (Lonnberg). *Nytt Mag. Zool.*, **6,** No. 1, 31–36.