THYROID STRUCTURE AND FUNCTION IN TWO ANTARCTIC FISHES

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ABSTRACT. The histological structure and location of thyroid follicles of *Notothenia coriiceps neglecta* (Nototheniidae) and *Chaenocephalus aceratus* (Channichthyidae) are described and compared.

A seasonal cycle of activity has been detected in the follicle cells and analysed in relation to reproduction and seasonal changes of temperature and photoperiod.

Serum samples from both species were assayed for total thyroxine (TT4) by a competitive protein-binding technique giving a mean value for *N. c. neglecta* of 23·9 μg./100 ml. and for *C. aceratus* of 3·4 μg./100 ml., the former being much higher than previously described for teleosts.

AT Signy Island (lat. 60°43′S., long. 45°38′W.) the sea temperature in the shallow-water environment rarely varies by more than 3° C annually, and for 4–5 months of the year the sea temperature remains near freezing point. Such a narrow seasonal temperature range is typical of the Southern Ocean and at higher latitudes the overall range is less, the sea temperature remaining at freezing point for most of the year (Dearborn, 1965).

Within the Antarctic zone 65 per cent of total fish species belong to the sub-order Notothenioidei (DeWitt, 1971), an endemic group of the order Percoidei. Recent research on these fish has shown several examples of a high degree of adaptation to their cold environment.

Cold adaptation (Wohlschlag, 1960; Somero and DeVries, 1967).
Blood fortified with antifreeze (DeVries, 1970).
Temperature-sensitive haemoglobin (Grigg, 1967).
High thermal sensitivity (Crawshaw and Hammel, 1971).

There has, however, been only one previous study of the endocrine glands of these fish. Hureau (1963, 1970), working on *Trematomus bernacchii*, *T. hansoni* and *Notothenia coriiceps neglecta* from Terre Adélie, found the thyroid follicles of these fish localized in an area just anterior to the ventral aorta. He noted their relatively large size and compact structure, and also showed that the thyroid underwent a seasonal cycle of activity. Hureau also studied thyroid samples of *Notothenia cyanobrancha*, *N. rossii* and *N. macrocephala* from Iles Kerguelen and found these to be generally less extensive than the thyroids of Terre Adélie fish. *N. cyanobrancha* thyroids were hyperactive, and *N. rossii* and *N. macrocephala* hypoactive at the time of sampling (January–February).

The present study was made to complement ecological and physiological investigations on fish at Signy Island.

MATERIALS AND METHODS

Samples for histological examination were taken from fish caught on lines or in traps (Everson, 1970*a*). Because the precise location and extent of the thyroid tissue in *Notothenia corriceps neglecta* was unknown, large samples of the floor of the mouth were removed. The ventral end of each gill arch on both sides was cut, and transverse cuts were made anterior to the first gill arch and posterior to the heart. The block of tissue containing the ventral aorta and afferent branchial arteries was removed and fixed in Bouin's fluid. After 24 hr. the tissue was transferred to 70 per cent alcohol for storage. The initial large pieces of tissue were trimmed to leave the aorta and a small amount of tissue around and anterior to it. Large amounts of bone necessitated decalcification for 3 days in formol–nitric acid (Mahoney, 1966) prior to embedding in 58° C melting-point wax. Sections 6 μm. thick were stained in haematoxylin and eosin, and the follicular cell height measured with an eyepiece graticule and converted to μm. Ten measurements of follicular cell height were made from follicles on a single section and used to calculate mean follicular cell height for bimonthly groups of fish. For comparison, samples from the ventral aortic region of three formalin-fixed specimens of *Chaenocephalus aceratus* (Channichthyidae) were examined in the same way.

A sample of fish caught in trammel nets (Twelves, 1972) was maintained 3–4 hr. in running sea-water aquaria. They were killed by severing the spinal cord and blood samples were withdrawn from the conus arteriosus using disposable syringes. The blood was allowed to clot for ½ hr., centrifuged at 3,000 r.p.m. and the serum collected and stored deep frozen (−20° C) for later analysis in the United Kingdom. Total serum thyroxine (TT4) was estimated by the competitive protein-binding technique using Thyopac-4 kits (code I.M.64; Radiochemical Centre, Amersham, England) and the results were expressed as μg. thyroxine per 100 ml. of serum.

Results

Photomicrographs of vertical sections through the median thyroid regions of *Notothenia coriiceps neglecta* and *Chaenocephalus aceratus* are shown in Figs. 1 and 2, respectively. Although the scales are slightly different, it is apparent that the thyroid follicles of *N. c. neglecta* are more extensive than those of *C. aceratus*. The follicles of the former are localized to an area around and below the ventral aorta, whilst those of the latter species are localized to an area around two small blood vessels below the ventral aorta.

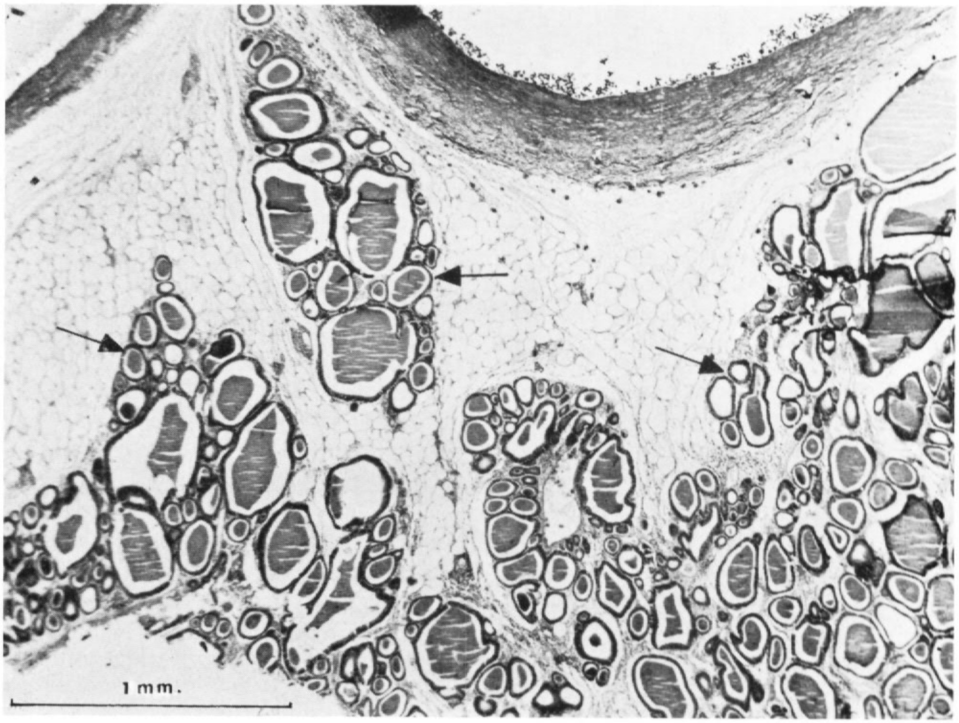

Fig. 1. Low-power photomicrograph of a vertical section through the thyroid region of a male *Notothenia coriiceps neglecta* (weight 1,050 g.; July 1965); 6 μm. section stained with haematoxylin and eosin.

Photomicrographs of sections through active and inactive thyroid follicles of *N. c. neglecta* are shown in Figs. 3 and 4, respectively. The reduction in colloid content and follicle size, coupled with increased follicular cell height and vascularization can be seen when active follicles are compared with inactive follicles. This variation in thyroid follicle activity can also be seen by plotting the mean follicular cell height and standard error of bimonthly samples of *N. c. neglecta* as shown in Fig. 5. To examine the possible influence of intrinsic and extrinsic seasonal cycles on thyroid activity, graphs of relative gonad size (from Everson, 1970*b*), annual variation in sea-water temperature (from Everson, 1970*a*) and day length (from Giles, 1971) at Signy Island are shown as graphs below the graph of follicular cell height in Fig. 5. It is apparent that maximum thyroid activity is synchronous with both the onset of spawning and

Fig. 2. Low-power photomicrograph of a vertical section through the thyroid region of a female *Chaenocephalus aceratus* (weight 1,370 g.; February 1970); 8 μm. section stained with haematoxylin and eosin.

also maximum sea-water temperature. To find whether there was any correlation between thyroid activity and gonad development, the mean follicular cell height was plotted against relative gonad size for each fish (Fig. 6). The correlation coefficients for both females and males are low ($r = 0 \cdot 322$; $r = 0 \cdot 063$), suggesting that the thyroid and gonad cycles are independent.

Because of the marked seasonality of the temperature cycle, the fish may be classed in three discrete groups depending on the sea temperature when caught—"winter group" (less than $-1 \cdot 5°$ C), "intermediate group" ($-0 \cdot 3$ to $-0 \cdot 4°$ C) and "summer group" (greater than $-0 \cdot 4°$ C). A series of *t* tests was used to compare the mean follicular cell height of immature and mature fish and between each relevant category. The results are shown in Table I. Although the data are incomplete because no summer immature fish were studied, the fact that no significant differences were found between immature and mature fish in either the intermediate or winter groups suggests little interaction between reproduction and thyroid activity, confirming the results derived from direct comparison with the gonads. The differences between

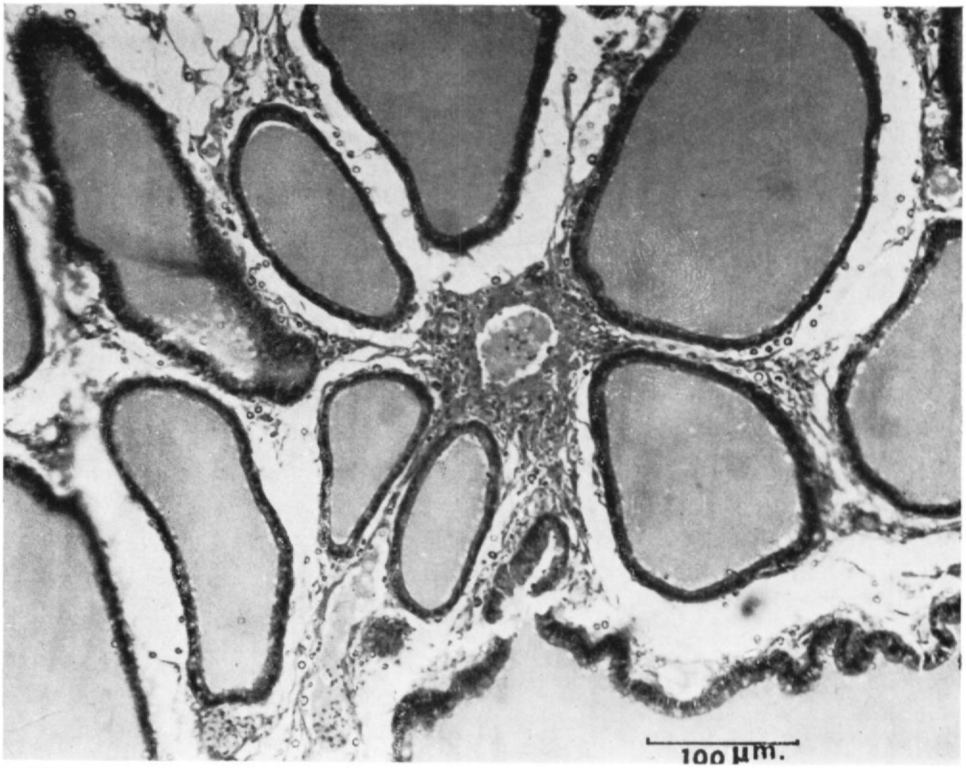

Fig. 3. High-power photomicrograph of inactive thyroid follicles of a female *Notothenia coriiceps neglecta* (weight 986 g.; November 1966); 6 μm. section stained with haematoxylin and eosin.

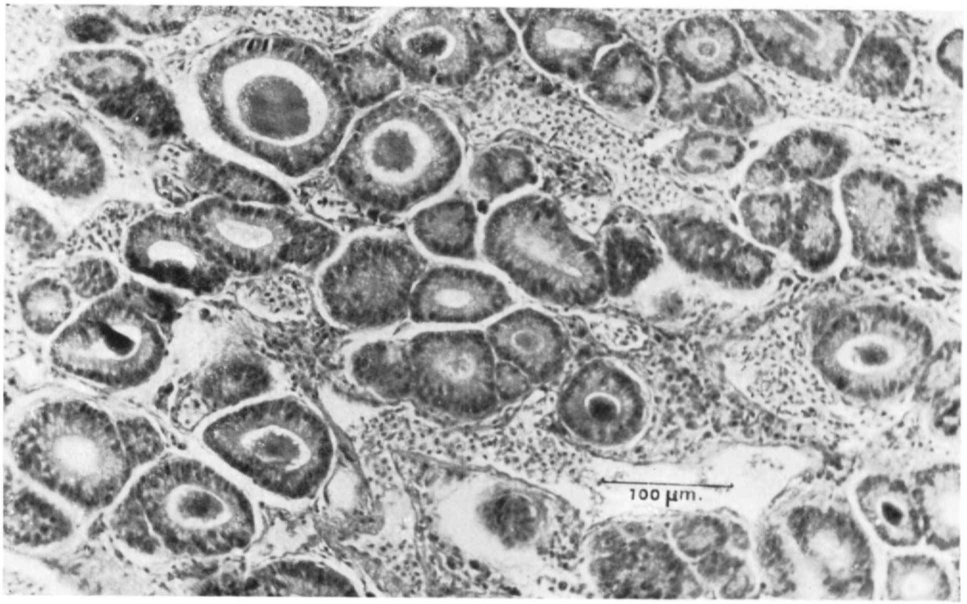

Fig. 4. High-power photomicrograph of active thyroid follicles of a female *Notothenia coriiceps neglecta* (weight 1,248 g.; March 1966); 6 μm. section stained with haematoxylin and eosin.

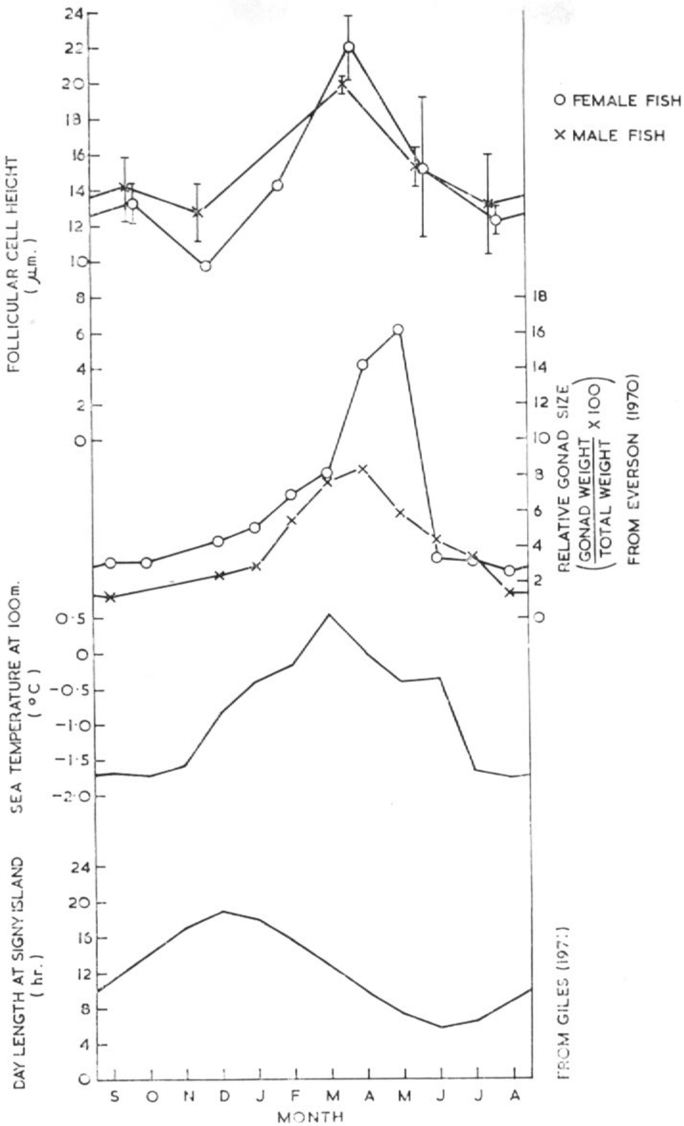

Fig. 5. Graphs of thyroid and reproductive cycles of *Notothenia coriiceps neglecta* with annual variation in sea-water temperature and day length at Signy Island.

summer and winter mature fish, and summer and intermediate mature fish, were both significant, indicating synchrony between sea-water temperature and thyroid cycle, and suggesting that temperature change between the intermediate and summer groups stimulated thyroid activity.

The influence of photoperiod on thyroid activity cannot be determined from this study. Annual variation in day length at lat. 60°S. is considerable as can be seen in Fig. 5 and it is possible that increasing or maximum day length could "trigger" the increase in thyroid activity via the pituitary.

Serum thyroxine levels of *N. c. neglecta* and *C. aceratus* are listed in Table II. At the t ime of

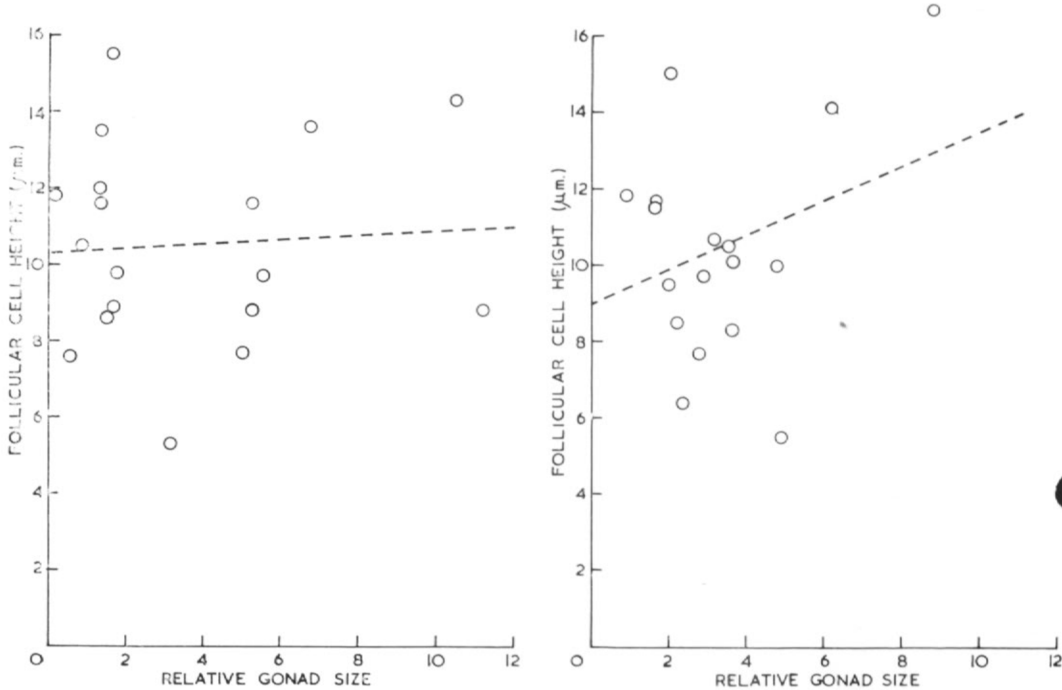

Fig. 6. Graphs of follicular cell height (FCH) against relative gonad size (RGS) for mature male and female *Notothenia coriiceps neglecta*.

TABLE I. TABLE OF STUDENT'S *t*-TEST ANALYSIS OF FOLLICULAR CELL HEIGHT OF MATURE AND IMMATURE *Notothenia coriiceps neglecta* IN SUMMER, INTERMEDIATE AND WINTER GROUPS

		Summer	*Intermediate*		*Winter*
		Mature	*Mature*	*Immature*	*Mature*
Winter	Immature	N.A.	N.A.	1·658 (9) $0·05<P<0·1$	1·098 (37) N.S.
	Mature	4·432 (32) $P = 0·001$	0·062 (33) N.S.	N.A.	
Intermediate	Immature	N.A.	0·406 (4) N.S.		
	Mature	2·001 (6) $0·025<P<0·05$			

N.A. Not applicable. N.S. Not significant.

sampling, *N. c. neglecta* had a considerably higher level of thyroxine than *C. aceratus*. This supports the histological findings of this study in that *C. aceratus* has a much smaller thyroid than *N. c. neglecta* and at the time of sampling the thyroid of *N. c. neglecta* would be approaching maximum activity. *C. aceratus* also has a larger blood volume (Twelves, 1972) and a lower resting oxygen consumption (Holeton, 1970) than *N. c. neglecta* which could affect circulating thyroxine levels.

TABLE II. SERUM THYROXINE (TT4) OF *Notothenia coriiceps neglecta* AND *Chaenocephalus aceratus* AT SIGNY ISLAND

Fish number	*Date*	*Species*	*Sex*	*Weight* (g.)	*Standard length* (mm.)	*Gonad weight* (g.)	*Total serum T4* (μg./100 ml.)
1	31.3.71	*N. neglecta*	♀	2,197	480	308	21·2
2	31.3.71	*N. neglecta*	♀	1,670	410	203	23·8
3	31.3.71	*N. neglecta*	♀	1,333	405	18	27·4
4	31.3.71	*N. neglecta*	♀	1,560	430	228	24·1
5	31.3.71	*N. neglecta*	♀	1,336	400	119	20·6
6	1.4.71	*N. neglecta*	♀	1,239	390	85	19·2
7	1.4.71	*N. neglecta*	♀	771	350	12	22·9
8	1.4.71	*N. neglecta*	♀	1,154	350	16	23·5
9	1.4.71	*N. neglecta*	♀	1,280	385	124	26·8
10	1.4.71	*N. neglecta*	♂	1,110	380	98	29·9
1	31.4.71	*C. aceratus*	♀	1,608	576	163	6·8
2	31.4.71	*C. aceratus*	♀	1,420	541	283	1·3
3	31.4.71	*C. aceratus*	♀	1,768	577	307	2·0
4	31.4.71	*C. aceratus*	♀	1,165	593	271	3·5

N. c. neglecta mean 23·94. S.E. 1·043.
C. aceratus mean 3·4. S.E. 1·22.

DISCUSSION

Hureau (1963, 1970) produced diagrams of the distribution of thyroid in *Trematomus bernacchii* and *T. hansoni* from Terre Adélie, which showed that in both species the thyroid follicles were concentrated and localized to an area from around the ventral aorta to well beyond the first afferent branchial artery. By comparison, the thyroid follicles of *Notothenia coriiceps neglecta* from Signy Island were similarly concentrated but found only between the ventral aorta and the first afferent branchial artery. The thyroid follicles of *Chaenocephalus aceratus* were much less numerous and were not associated with the ventral aorta and afferent branchial arteries.

The cycle of thyroid activity described by Hureau (1970) in *T. bernacchii*, *T. hansoni* and *N. c. neglecta* showed that maximum activity occurred during the austral summer (December–February). Results for *N. c. neglecta* at Signy Island showed that maximum thyroid activity occurs during the austral autumn (March–April). Hureau (1970) considered that the seasonal thyroid cycles of the nototheniids from Terre Adélie were controlled by the hypophysis, which in turn was influenced by seasonal variation in salinity, state of nutrition and photoperiod, the latter showing greatest annual variation at high latitude. Results from the present study suggest that seasonal variation in temperature and photoperiod are likely to influence thyroid activity via the hypophysis. It is interesting to note that maximum thyroid activity in the nototheniids at Terre Adélie also occurs during the period of highest annual sea-water temperature. Woodhead and Woodhead (1965) found a seasonal cycle of thyroid activity in *Gadus morhua* and noted that the peak of thyroid activity coincided with highest sea-water temperature and was least active at lowest sea-water temperature. They were, however, unable to discern a clear relationship between temperature and thyroid activity, but did suggest that changing photoperiod could be a controlling factor. Although Blanc and Buser (1949) showed activation of the thyroid of *Ictalurus melas* with increase in temperature, and reduced activity with decrease in temperature, other studies (Olivereau, 1955) have failed to induce temperature response in the thyroid, or have induced a decrease in activity with increase in temperature (Barrington and Matty, 1954). Gorbman (1969) has reviewed a large amount of information on thyroid function and control in fish. There is evidence that both extrinsic factors, temperature and photoperiod, can affect the self-regulation intrinsic cycle of thyroid activity by neurosecretion from the hypothalamus. However, thyroid activity may also be stimulated by other substances and it has been shown that gonadotrophins and other glycoproteins of certain species may also increase thyroid activity. Controlled laboratory experiments would be required to demonstrate conclusively the effect of temperature and photoperiod on the thyroid activity of these Antarctic nototheniids.

There are few published records of circulating thyroxine levels in the serum of poikilotherms

(Jacoby and Hickman, 1966; Refetoff and others, 1970; Higgs and Eales, 1971). Commercially available competitive protein-binding techniques used by recent workers have shown that in temperate teleost fish total thyroxine (TT4) values range from 0·07 to 4·5 μg./100 ml. (Refetoff and others, 1970; Higgs and Eales, 1971). The serum TT4 values for *C. aceratus* (Table I) fall within this range with one exception. In contrast, the values for *N. c. neglecta* are considerably higher. From the cycle of thyroid activity described earlier, which shows a peak at the time when the samples were obtained, it is reasonable to expect that circulating TT4 levels would be high.

Total T4 includes protein-bound and free T4 and, since the former is not directly available for tissue metabolism (personal communication from S. Refetoff), TT4 does not give a realistic indication of biological activity. The separation of bound and free T4 would be of greater value in understanding the role of thyroxine in Antarctic fish. A more complete study is therefore planned for the future.

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