

AN ANALYSIS OF THE THYROID ROLE IN JUVENILE STEELHEAD
(SALMO GAIIRDNERI RICHARDSON) AND FACTORS RESPONSIBLE FOR
ITS SEASONAL FLUCTUATION IN ACTIVITY

by

JOHN GEOFFREY EALES

B.A., Oxford University, 1959

M.Sc., University of British Columbia, 1961

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in the Department of
Zoology

We accept this thesis as conforming to the
required standard

Members of the Department
THE UNIVERSITY OF BRITISH COLUMBIA
April, 1963

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Zoology

The University of British Columbia,
Vancouver 8, Canada.

Date April, 1963

PUBLICATIONS

A comparative study of thyroid function in
migrant juvenile salmon. Can. J. Zool.,
41: (in press). 1963.

The thyroid gland and low temperature re-
sistance of goldfish. Can. J. Zool.,
41: (in press), with W.S. Hoar. 1963.

The University of British Columbia

FACULTY OF GRADUATE STUDIES

PROGRAMME OF THE

FINAL ORAL EXAMINATION

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

of

JOHN GEOFFREY EALES

B.A., Oxford University, 1959

M.Sc., The University of British Columbia, 1961

TUESDAY, May 7, 1963 at 9:30 A.M.

IN ROOM 3332, BIOLOGICAL SCIENCES BUILDING

COMMITTEE IN CHARGE

Chairman: F.H. Soward

J.R. Adams	W.N. Holmes
D.H. Chitty	W.D. Kitts
I. McT. Cowan	V.J. Okulitch
W.S. Hoar	N.J. Wilimovsky

External Examiner: E.J.W. Barrington

Department of Zoology,

Nottingham University

AN ANALYSIS OF THE THYROID ROLE IN JUVENILE STEELHEAD
(SALMO GAIRDNERI RICHARDSON) AND FACTORS RESPONSIBLE
FOR ITS SEASONAL FLUCTUATION IN ACTIVITY

ABSTRACT

Investigation of factors controlling seasonal changes in thyroid activity of juvenile steelhead trout (Salmo gairdneri Richardson) in fresh water revealed positive correlations between temperature and radioiodine assessments of thyroid activity. Temperature and thyroid epithelial height, however, showed negative correlations. These correlations were verified experimentally.

In yearlings, the thyroid was refractory to increasing photoperiod (January to July) but two-year-old potential migrants showed a positive response at the same season. Thus, the high smolt thyroid activity is induced by the combined influence of rising temperature and increasing spring photoperiod.

Body mass (logarithm) was inversely related to various I^{131} parameters (logarithm), so that small fish had higher thyroid activities than large fish. Precocious sexual maturation of two-year-old male parr, increased swimming exercise and increased salinity were associated with higher thyroid activity. Increase in ambient I^{127} depressed thyroid activity and indicated that the activity of the gland (assessed by current methods) is partly a compensation for low I^{127} availability. The high thyroid activity of the smolt may be due partly to endemic goitre.

Since low temperature and 8-hour day length inhibited the thyroid activity of potential migrants but did not prevent silvering, the role of thyroxin in guanine deposition under natural conditions is doubted. Possible radiohormone catabolic sites were located in metabolically active tissues including gut, kidney, liver and brain. These findings suggest a general rather than a tissue-specific role of thyroxin in metabolism. It is concluded that thyroxin may have no stimulatory role in smoltification but reflects instead the total metabolic demands on the tissues.

GRADUATE STUDIES

Field of Study: Zoology

Quantitative Methods in Zoology	P.A. Larkin
Comparative Physiology	W.S. Hoar
Comparative Ethology	W.S. Hoar
	M.D.F. Udvardy
Comparative Invertebrate	
Embryology	C.V. Finnegan
Biology of Fishes	C.C. Lindsey
Endocrinology	W.N. Holmes
Marine Field Course	P.A. Dehnel
Fisheries Seminar	Staff

Other Studies:

Biochemistry	Faculty of Medicine
--------------	---------------------

ABSTRACT

Investigation of factors controlling seasonal changes in thyroid activity of juvenile steelhead trout (Salmo gairdneri Richardson) in fresh water revealed positive correlations between temperature and radioiodine assessments of thyroid activity. Temperature and thyroid epithelial height, however, showed negative correlations. These correlations were verified experimentally.

In yearlings the thyroid was refractory to increasing photoperiod (January to July) but two-year-old potential migrants showed a positive response at the same season. Thus, the high smolt thyroid activity is induced by the combined influence of rising temperature and increasing spring photoperiod.

Body mass (logarithm) was inversely related to various I^{131} parameters (logarithm), so that small fish had higher thyroid activities than large fish. Precocious sexual maturation of two-year-old male parr, increased swimming exercise and increased salinity were associated with higher thyroid activity. Increase in ambient I^{127} depressed thyroid activity and indicated that the activity of the gland (assessed by current methods) is partly a compensation for low I^{127} availability. The high thyroid activity of the smolt may be due partly to endemic goitre.

Since low temperature and 8-hour daylength inhibited the

thyroid activity of potential migrants but did not prevent silvering, the role of thyroxin in guanine deposition under natural conditions is doubted. Possible radiohormone catabolic sites were located in metabolically active tissues including gut, kidney, liver and brain. These findings suggest a general rather than a tissue-specific role of thyroxin in metabolism. It is concluded that thyroxin may have no stimulatory role in smoltification but reflects instead the total metabolic demands on the tissues.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Professor W. S. Hoar, F. R. S. C., Department of Zoology, for his stimulation, criticism and enthusiastic guidance throughout this project. I am also indebted to Professors J. R. Adams, W. N. Holmes, N. J. Wilimovsky (Department of Zoology) and Professor W. D. Kitts (Animal Science) for their constructive criticism. I further wish to acknowledge the several graduate students from whom I gained so much during informal discussions.

Dr. K. A. Evelyn of the Strong Laboratory, Vancouver General Hospital, supplied the radioiodide. Messrs. S. B. Smith and J. G. Terpenning (British Columbia Fish and Game) arranged for the provision of steelhead from the Cultus Lake Hatchery, where the experimental fish were tended for several months by Mr. Pat Martin and his staff. A great number of histological preparations were meticulously made by Miss S. Tabata. The statistical analyses were facilitated by use of IBM Computing Centre, University of British Columbia and the co-operation of the staff there was greatly appreciated. To all these people I wish to express my deepest appreciation for their various services; without them this study would have been impossible.

Financial assistance in the form of a Fisheries Research Board Studentship enabled me to carry out this study.

TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
MATERIALS AND METHODS	4
1. Living Materials	4
2. Radioiodide Injection	4
3. Blood Sampling and Separation of Protein-bound and Inorganic Radioiodine	6
4. Body and Thyroid	8
5. Thyroid Histology	9
6. Statistical Treatment	11
RESULTS	12
I. COMPARISON OF YEARLING STEELHEAD WITH DIFFERENT RATES OF RADIOIODINE METABOLISM	12
1. Extrathyroidal and Thyroidal Clearance of Plasma I ¹³¹	13
2. Experimental Measurement of Thyroid I ¹³¹ Accumulation	21
3. Estimation of Radiohormone Output Using the Conversion Ratio	27
II. SEASONAL CHANGES IN RADIOIODINE METABOLISM AND THE HISTOLOGICAL APPEARANCE OF THE THYROID IN JUVENILE STEELHEAD HELD IN FRESH WATER	30
1. I ¹³¹ Excretion	30
2. Thyroid Uptake	34
3. Conversion Ratio	34
4. Histological Changes	35

TABLE OF CONTENTS (Continued)

	PAGE
III. EXPERIMENTAL INVESTIGATION OF THE INFLUENCE OF TEMPERATURE ON THE HISTOLOGICAL APPEARANCE OF THE THYROID AND RADIOIODINE METABOLISM IN YEARLING STEELHEAD PARR	36
IV. EXPERIMENTAL INVESTIGATION OF TEMPERATURE AND PHOTOPERIOD INFLUENCE ON THYROID CELL HEIGHT AND RADIOIODINE METABOLISM OF STEELHEAD YEARLINGS (January to July)	44
V. EFFECT OF BODY MASS ON RADIOIODINE METABOLISM	55
VI. EXPERIMENTAL INVESTIGATION OF THE INFLUENCE OF SEASONAL CHANGE IN INCREASING PHOTOPERIOD AND INCREASING TEMPERATURE ON THE RADIOIODINE METABOLISM AND THYROID CELL HEIGHT OF TWO-YEAR-OLD STEELHEAD	61
1. I^{131} Excretion	62
2. Thyroid Uptake of I^{131}	64
3. Conversion Ratio	67
4. Cell Height	70
VII. INFLUENCE OF PRECOCITY OF MALE PARR ON THYROID ACTIVITY	70
VIII. INFLUENCE OF EXERCISE ON I^{131} METABOLISM OF STEELHEAD	72
IX. THE INFLUENCE OF CHEMICAL CHANGES (SALINITY AND IODINE LEVELS OF THE MEDIA) ON I^{131} METABOLISM OF STEELHEAD AND CHUM SALMON (<u>Oncorhynchus keta</u>)	74

TABLE OF CONTENTS (Continued)

	PAGE
X. THE RELATIONSHIP BETWEEN RADIOIODINE METABOLISM AND SILVERING	85
XI. PERIPHERAL SITES OF RADIOHORMONE CATABOLISM	89
DISCUSSION	95
I. COMPARISON OF PARAMETERS FOR MEASURING THYROID ACTIVITY AND THE EFFECT OF TEMPERATURE ON THESE PARAMETERS	95
II. FACTORS CONTRIBUTING TO SEASONAL CHANGES IN THYROID ACTIVITY IN JUVENILE STEELHEAD . . .	102
III. THE ROLE OF THE THYROID IN THE STEELHEAD. .	112
SUMMARY AND CONCLUSIONS	116
BIBLIOGRAPHY	119

LIST OF FIGURES

FIGURE	PAGE
1. Seasonal changes in water temperature in the laboratory and in two streams near Vancouver, British Columbia.	5
2. Plasma clearance following a single I^{131} injection in fish held at 5° C (○) and 10° C (●). Each point represents a mean of 5 to 7 fish. (Covariance analysis, Table I).. . . .	14
3. Total body clearance (○) and body excluding thyroid clearance (●) following a single I^{131} injection at 5° C (inactive thyroid) and 10° C (active thyroid). Equations calculated separately for each phase. Each point represents a mean of 5 to 7 fish (Covariance analyses, Tables II and III)	18
4. (A) Percentage accumulation of I^{131} by the thyroid following a single I^{131} injection at 10° C (active) and 5° C (inactive). Standard deviations shown. Each point represents a mean of 5 to 7 fish. (B) TUF values for the same fish as (A). (C) T/S values for the same fish as (A). The diphasic tendency is shown in the active state (Covariance analysis, Table IV).	23
5. Change in CR following a single I^{131} injection in fish at 10° C (active) and 5° C (inactive). Each point represents a mean of 5 to 7 fish.	28
6. Seasonal change in plasma I^{131} , % dose I^{131} in body, T/S, TUF, CR, mean epithelial cell height and percentage of follicles containing blue colloid in steelhead parr and smolts. Seasonal change in temperature is shown. Standard deviations are shown for certain parameters	32
7. Correlations between water temperature and four I^{131} parameters. All values drawn from seasonal data (Fig. 6). A (% dose in body); B(CR); C(T/S); D(TUF)	33

LIST OF FIGURES (Continued)

FIGURE		PAGE
8.	Experimental demonstration of the influence of temperature on percentage accumulation of I^{131} in the thyroid 4 and 8 days after a single I^{131} injection, on mean cell height (O) and on percentage of follicles containing blue colloid (●)	38
9.	Experimental demonstration of the influence of temperature on I^{131} levels in plasma and total body 4 days after a single I^{131} injection. Each point represents 4 to 7 individuals (Covariance analysis, Table VI).	40
10.	Experimental demonstration of the influence of temperature on T/S values 4 and 8 days after a single I^{131} injection. Each point represents a mean of 4 to 7 individuals.	42
11.	Experimental demonstration of the influence of temperature on CR values 4 and 8 days after a single I^{131} injection. Each point represents a mean of 4 to 7 individuals (Covariance analysis, Table VI)	43
12.	Experimental demonstration of the combined influence of temperature and photoperiod on plasma I^{131} levels and percentage dose in total body 4 days after a single I^{131} injection in 14 to 18-month steelhead. Each point represents a mean of 8 to 12 fish (Statistics, Table VIII). . . .	46
13.	Experimental demonstration of the combined influence of photoperiod and temperature on mean cell height and T/S ratios 4 days after a single I^{131} injection in 14 to 18-month steelhead. Cell height - each point represents a mean of 3 to 4 fish; T/S - each point represents a mean of 8 to 12 fish (Statistics, Table VIII).. . . .	48
14.	Experimental demonstration of the combined influence of photoperiod and temperature on plasma clearance following a single I^{131} injection in 18-month steelhead. Each point represents a mean of 5 to 7 fish (Covariance analysis, Table IX).	51
15.	Experimental demonstration of the combined influence of photoperiod and temperature on T/S values in 18-month steelhead following a single I^{131} injection. Each point represents a mean of 5 to 7 fish (Covariance analysis, Table IX).	52

LIST OF FIGURES (Continued)

FIGURE		PAGE
16.	Experimental demonstration of the combined influence of photoperiod and temperature on CR values following a single I^{131} injection in 18-month steelhead. Each point represents a mean of 5 to 7 individuals	53
17.	Influence of body mass on T/S values and percentate of I^{131} in the total body 84 hours after a single I^{131} injection in 19-month steelhead..	56
18.	Influence of body mass (gram) on plasma I^{131} levels and T/S values (4 days after injection) and CR values (8 days after injection). Means and standard deviations are shown for large, medium and small size groups. No significant difference was noted between medium and large size groups for any I^{131} parameter, but both differed from the small group for all parameters ($p < .01$).	59
19.	Relationship between the logarithm of body mass and the logarithm of various I^{131} parameters. T/S (Δ), $r = -0.57$; CR (\bullet), $r = -0.53$; % body (O), $r = -0.67$	60
20.	Experimental demonstration of the combined influence of temperature and photoperiod on plasma I^{131} levels and percentage dose in total body 4 days after I^{131} injection in 26 to 30-month steelhead (potential migrants). Each point represents a mean of 6 individuals (Statistics, Table XI).	63
21.	Experimental demonstration of the combined influence of temperature and photoperiod on T/S values 4 and 8 days after I^{131} injection in 26 to 30-month steelhead (potential migrants). Each point represents a mean of 6 individuals (Statistics, TABLE XI).	65
22.	Experimental demonstration of the combined influence of temperature and photoperiod on TUF values 4 and 8 days after I^{131} injection in 26 to 30-month steelhead (potential migrants). Each point represents a mean of 6 individuals (Statistics, Table XI).	66

LIST OF FIGURES (Continued)

FIGURE		PAGE
23.	Experimental demonstration of the combined influence of temperature and photoperiod on mean cell height and CR (8 days after I^{131} injection) in 26 to 30-month steelhead (potential migrants). Cell height - means (Δ ○) and individual values (Δ ○) shown; CR - each point represents a mean of 6 individuals.	68
24.	TUF (open bars) and CR (shaded bars) for precocious two-year-old male (○○) and immature (○○) steelhead in early March under 4 combined conditions of temperature and photoperiod	71
25.	Influence of increased swimming activity on various aspects of I^{131} metabolism in 19-month steelhead. Each point represents a mean of 6 individuals (Covariance analysis, Table XII). .	73
26.	Effect of transfer of 20-month steelhead to sea water (25o/oo saline) and iodide-reinforced fresh water on various aspects of I^{131} metabolism measured 4 and 8 days after I^{131} injection. Mean values represented (SW,X----; FW + I^{127} , Δ — —; FW,○— —).	79
27.	Effect of transfer of 30-month smolts and parr to sea water (25 o/oo saline) on various aspects of I^{131} metabolism. Mean values (bar) and individual values shown	81
28.	Effect of transfer of underyearling potential migrant chum salmon to sea water (25 o/oo saline) and iodide-reinforced fresh water. Mean values (bar) and individual values shown. $X \cong <0.05$; $XX \cong <0.01$	83
29.	Demonstration of the lack of a relationship between silvering and any aspect of I^{131} metabolism (8 days after injection). Mean values (bar) and individual values shown.	88
30.	Change in the tissue: blood radioactivity in a variety of tissues from 19-month steelhead. Each point represents a mean of 14 to 16 individuals	91

LIST OF FIGURES (Continued)

FIGURE	PAGE
31. Percentage change (relative to the value 3 days after injection) of tissue: blood ratios 6 and 9 days after injection in 19-month steelhead. .	93
32. Diagrammatic representation of the hypothesis that thyroxin utilization and production respond differently to temperature. x_5 and x_{10} represent the differences between utilization and production at 5 and 10° C. Since x_5 is greater than x_{10} it would imply greater release of TSH at low temperatures and also a greater cell height	99

LIST OF TABLES

TABLE	PAGE
I. Analysis of covariance for plasma I ¹³¹ (plasma I ¹³¹ Biological Concentration Coefficient/100) and time (hr) for active (10° C) and inactive (5° C) thyroid states	15
II. Analysis of covariance for I ¹³¹ clearance from the total body and time (hr); and body excluding thyroid and time (hr). Slope _{T+B} - Slope _B = Rate constant for thyroid I ¹³¹ uptake (Slope _T).	19
III. Analysis of covariance for I ¹³¹ clearance from total body and time (hr) and body excluding thyroid and time (hr) for active (10° C) and inactive (5° C) thyroid states. The analysis indicates the difference in excretion rates between the two temperatures in phase II	20
IV. Analysis of covariance for T/S and time (hr) for active (10° C) and inactive (5° C) thyroid states	24
V. A summary of radioiodine parameters and their significance	31
VI. Analysis of covariance for the relationship between various I ¹³¹ parameters and temperature (° C)	41
VII. Summary of the four combined temperature and photoperiod conditions. Symbols shown are used in figures	45
VIII. Summary of statistically significant differences for several I ¹³¹ parameters between different conditions of temperature and photoperiod in yearlings in June. White-Wilcoxon non-parametric ranking test used	49
IX. Analysis of covariance performed on plasma I ¹³¹ and time (hr), and T/S and time (hr) under four different photoperiod and temperature regimes.	54
X. Summary of injection details for size experiment	58

LIST OF TABLES (Continued)

TABLE		PAGE
XI.	Summary of statistically significant differences for several I^{131} parameters between different conditions of temperature and photoperiod in two-year-olds (potential migrants) in late May (White-Wilcoxon test)	69
XII.	Analysis of covariance for the relationship between various I^{131} parameters and time (hr) for parr held in fast and slow currents . .	75
XIII.	The effect of different temperature and photoperiod regimes on the percentage incidence of smoltification in two-year-old steelhead between 27 April and 29 May, 1962	87

INTRODUCTION

Seasonal changes in thyroid activity have been recorded by a variety of techniques in several teleost species. These include Misgurnus fossilis (Lieber, 1936); Anguilla anguilla (Hagen, 1936); Phoxinus phoxinus (Barrington and Matty, 1954); Esox lucius (Zaitzev, 1955); Salmo trutta (Swift, 1955, 1959); Plecoglossus altivelis (Honma, 1959); Fundulus heteroclitus (Berg, Gorbman and Kobayashi, 1959); and Platichthys stellatus (Hickman, 1962). Comprehensive reviews relating to these changes have been written by Hoar (1959), Matty (1960) and Swift (1960).

Most studied of all have been the seasonal changes in thyroid activity in anadromous salmonids. These fish hatch in fresh water but, except for spawning, spend adult life in the sea. At a certain period in development, young salmon descend the rivers to the sea and at this time undergo characteristic changes in morphology, behaviour and metabolism. The most obvious change, smoltification, is the loss of black parr marks and acquisition of the silvery guanine livery of the smolt. One of the most emphasized changes in the underlying physiology at smoltification is a marked thyroid hyperactivity first noted histologically in the Atlantic salmon (Salmo salar) by Hoar (1939). Since this initial discovery, more and more emphasis has been placed on the role of the thyroid during smoltification primarily by Fontaine and his co-workers, (Fontaine, 1954; Leloup and Fontaine, 1960). Robertson (1948) observed a similar hypertrophy and hyperplasia in the thyroid of smolting rainbow

trout (Salmo gairdneri) and also claimed that thyroxin stimulated guanine deposition (Robertson, 1949). Silvering, however, is not always dependent upon thyroxin. In Pacific salmon (genus Oncorhynchus), the thyroids of silvering coho and sockeye are moderately active at migration but those of pink, spring and chum are relatively inactive (Hoar and Bell, 1950; Eales, 1961, 1963). The role of the thyroid in the smoltification and migration of salmon is, therefore, far from established. Though certain speculations have been made, the control of these seasonal thyroid fluctuations is almost entirely uninvestigated. The matter is further complicated by the discrepancy between histological and radiochemical determinations.

With these unresolved points in mind, a comprehensive histological and radiochemical investigation of thyroid function was attempted in the steelhead trout (Salmo gairdneri Richardson). This species is anadromous and shows an easily recognizable parr-smolt transformation.

The investigation was divided into four interrelated areas of study:

(a) A preliminary comparative analysis of radioiodine metabolism in steelhead with low and high thyroid activity.

(b) Seasonal observations on histology and radioiodine metabolism from 17 months prior to migration up to seaward migration itself to determine seasonal changes.

(c) Experimental investigation of environmental factors and internal changes influencing these seasonal fluctuations.

(d) An investigation of possible sites of radiohormone catabolism in steelhead and the relationship between radioiodine

metabolism and silvering.

From these investigations it was hoped to determine the factors contributing to seasonal changes in thyroid activity and also to gain knowledge concerning the role of the thyroid, particularly at the time of migration.

MATERIALS AND METHODS

1. Living Materials

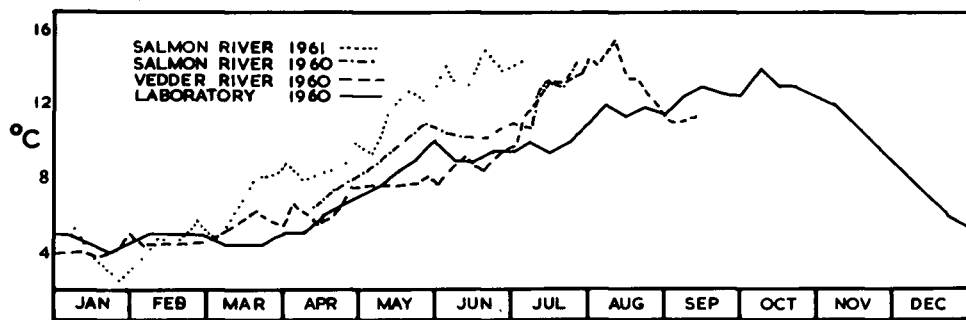
Steelhead were obtained as yearlings or two-year-olds from the British Columbia Fish and Game Hatchery at Cultus Lake, British Columbia, where they were fed on dried commercial food and kept in large concrete outdoor tanks. At intervals they were transported to the Department of Zoology, University of British Columbia. Here they were held indoors and fed on commercial fish food (J. R. Clark, Salt Lake City, Utah). They received an abundant fresh supply of dechlorinated tapwater varying markedly in seasonal temperature but with little diurnal change. The temperature was found to alter in a manner similar to that found in certain streams close to Vancouver known to contain the species under investigation (Fig. 1). Illumination was artificial (fluorescent lamps) and was controlled in length by an automatic switch regulated via a photoelectric cell exposed to the natural outside illumination. Thus, a changing seasonal daylength conforming to the outside photoperiod was imposed upon the fish. It departed from the natural condition in not being gradual in onset or offset (i.e. there was no twilight period) and also in general intensity.

Fish were removed from these conditions for a variety of experiments in which they were subjected to different physical and chemical environments. These conditions are many and varied and may be considered most conveniently with the results.

2. Radioiodide Injection

Carrier-free sodium iodide (NaI^{131}) was diluted with distilled

Fig. 1. Seasonal changes in water temperature in the laboratory and in two streams near Vancouver, British Columbia.



water and injected intraperitoneally via the dorsal muscle in a volume between 0.02 and 0.05 ml using a 0.25-ml tuberculin syringe and gauge 30 needle. Duplicates of the injected dose were diluted to 100 ml with 5% potassium iodide solution in 100-ml volumetric flasks and used as standards (1 ml = 1% of injected dose). The fish were not anaesthetized during injection but quickly lifted out of the water in a fine mesh net and then injected through the netting and immediately returned to the water. The entire operation took a matter of seconds. Unless otherwise stated, all fish were kept in running water after injection. Approximately $0.1 \mu\text{Ci } ^{131}\text{I}$ per gram body weight was administered to each fish.

3. Blood Sampling and Separation of Protein-bound and Inorganic Radioiodine

Blood samples were obtained by cutting through the caudal peduncle and drawing the blood by capillary action into a fine heparinized glass tube. Very large fish were anaesthetized for this procedure to facilitate handling; small fish were not anaesthetized. Within seconds of sampling all fish were killed by decerebration.

The technique for determining the organic and inorganic radioactive constituents of small samples of fish plasma has been described (Hickman, 1960; Hoar and Eales, 1963; Eales, 1963). However, in view of slight modification, the technique is given here in full.

On withdrawal of the blood sample, the heparinized capillary tube was plugged at one end with "plasticene" and the corpuscles

separated from the plasma by centrifugation. The plasma was separated from the corpuscles by breaking the tube at their junction. The tube plus plasma contents were weighed and the plasma blown into 4 ml of 12.5% trichloroacetic acid in a 12-ml thick-walled 'pyrex' centrifuge tube. The empty capillary tube was then reweighed to determine the plasma mass. The plasma protein precipitate was broken up with a glass rod, centrifuged and the supernate decanted off and saved. Two ml of 2.5% trichloroacetic acid were added and the precipitate again broken up and centrifuged. This procedure was repeated for a second time giving a total of 8 ml of supernate constituting the non-protein components of the plasma and including I^{131} . The remaining proteins were dissolved in 4 ml of 1.5 N sodium hydroxide (NaOH). This constituted the protein fraction of the plasma and included the hormonal or protein-bound radioiodine (PBI^{131}). The 4-ml sample of dissolved PBI^{131} was transferred to a glass counting tube and counted for 3000 counts. Half of the I^{131} wash derived from the same plasma sample was pipetted to a similar tube and counted for 3000 counts or longer depending on its activity. By doubling this count the total I^{131} content of the sample was found. Also counted for 30,000 counts were 4 ml (4%) of the standard solution. Thus either fraction could be expressed as a percentage of the injected dose. All counts were made in a Nuclear Chicago Well Counter (Model DS5 Versatile Scintillation Detector). From these data two parameters were determined:

(i) Conversion ratio, CR (Hickman, 1960, 1962; Eales, 1961, 1963).

$$\begin{aligned} \text{CR} &= \frac{\text{PBI}^{131} \text{ count/min} \times 100}{\text{PBI}^{131} \text{ count/min} + \text{I}^{131} \text{ count/min}} \\ &= \frac{\text{organic radioiodine(hormonal)}}{\text{total plasma radioactivity}} \end{aligned}$$

(ii) Biological concentration coefficient for I^{131} in the plasma. This is derived from the biological concentration coefficient, which expresses plasma I^{131} concentration as a percentage of the dose injected (Comar, 1955). It allows for the fact that the same dose put into a fish half the mass of another fish will have twice as much radioactivity per mass of plasma, and permits comparison of plasma I^{131} concentration between fish of different sizes.

$$\begin{aligned} \text{biological concentration coefficient} &= \\ \frac{\% \text{ of injected dose as } \text{I}^{131} \text{ in plasma sample} \times \text{body weight(g)}}{\text{mass of sample(g)}} \end{aligned}$$

The biological concentration coefficient has always been divided by 100.

4. Body and Thyroid

Immediately after death the fish was weighed and the basic branchial region cut out from the front of arch I (anterior) to the end of arch III. This region including the entire thyroid tissue was then dropped into a counting tube containing Bouin's fixative and counted for 30,000 counts in a Nuclear Chicago Well Counter. Two 4-ml standards (0.04% of the injected dose) were counted simultaneously and the percentage of the injected dose in the thyroid area was determined. The thyroids were counted in Bouin's fluid so that they could be sectioned later. Some discrepancy may have been introduced by counting essentially

a point source, the thyroid resting at the bottom of the counting well, against a 4-ml standard. However, comparison between undigested thyroids and the same thyroid digested with NaOH and then diluted to 4 ml revealed negligible differences when measured in the same counting system (Wiggs, 1962). This is probably due to the low absorption of the hard γ radiation of I^{131} . Thyroid uptake was expressed as the percentage accumulation of the injected dose.

The body lacking thyroid was sliced and the pieces arranged to form a 5-cm square whose thickness varied with the size of fish. By counting this "body" at a distance of 10 cm from an end-probe scintillation counter with a 45 mm diameter and 38.5 mm thick NaI (TI) crystal, an estimate of the total radioactivity left in the body could be made. To convert this radioactivity to a percentage of the injected dose, standards were counted at an identical distance and position from the inverted end-probe. These standards were formed by permeating piles of absorbent paper, cut to the same solid geometry as the samples, with 10 ml of standard solution. Each standard then represented 10% of the injected dose. Body retention of I^{131} was measured, therefore, as a percentage of the injected dose. This meant that physical decay, considerable in I^{131} with a half-life of 8.08 days, was entirely eliminated as both standards and samples decayed at the same rate.

5. Thyroid Histology

Thyroid tissues fixed in Bouin's fluid were decalcified, serially sectioned ($10\ \mu$) in the region of the second branchial

arch and then stained by the Azan technique (Gurr, 1953). Theoretically this stain renders the follicle colloid red in an inactive gland and blue in an active gland (Pickford, 1953, 1954; Barrington, 1963). Fifty unbroken follicles were examined and the lowest and the tallest cell heights ($\text{tallest} + \text{lowest} / 2 = \text{mean cell height}$) were measured using an ocular scale. The colloid was assigned to one of four categories - blue, red, mixture of red and blue or absent.

Since the thyroid of teleosts is variable, it was necessary to randomize the follicles measured. This was done by selecting tissue from at least three different areas of the second branchial region. If three slides were available from this area, 17 follicles would be examined on slide I, 17 on slide II and 16 on slide III. Randomization of follicles to be examined on any one section was achieved by centring the objective on the thyroid tissue under very low power magnification such that individual follicle structure was difficult to detect, and then switching to high power. The follicle closest to the ocular scale under the high power was then the first to be assessed. The next follicle to be examined was the one immediately to its lower left-hand margin. The follicle immediately to the latter's lower left was examined third and so on until the quota for the slide was exhausted. If this procedure resulted in exhaustion of follicles before the quota were reached, another section was treated similarly in the same slide but at least 150μ posteriorly. Since follicle diameters were usually far less than 150μ , repetitive measurement of follicles was avoided.

6. Statistical Treatment

In studies of rates of change of I^{131} fractions in various body compartments, linear regressions were calculated and either the slopes or adjusted means compared by analysis of covariance (Steel and Torrie, 1960). In comparing small samples (frequently below 12), which in many instances appeared to depart from a normal distribution, the Wilcoxon-White non-parametric ranking test was employed (Snedecor, 1956).

RESULTS

I. COMPARISON OF YEARLING STEELHEAD WITH DIFFERENT RATES OF RADIOIODINE METABOLISM

Reliable radioiodide estimates of thyroid activity are possible only when a thorough study of radioiodine metabolism has been made with the animal in various states of thyroid activity. Despite extensive use of radioiodine to study thyroid activity in teleosts, intensive studies of radioiodine metabolism in any one species are rare. Leloup and Fontaine (1960) have presented a comprehensive survey of radioiodine metabolism in lower vertebrates, but the number of species covered is so vast that it is difficult to reconstruct a complete picture of I^{131} metabolism for any one species. The work by Hickman (1959, 1962) on Platichthys stellatus is an exception. Since many estimates of radioiodine metabolism at different levels of activity have been made in the study, it is considered necessary to present a comparison between radioiodine metabolism in fish with active and inactive glands. In this instance the difference in I^{131} metabolism was induced with a 5° C temperature difference by holding fish at 5 and 10° C.

The object of this study was to follow the metabolism of a single dose of I^{131} for a period of several days. This could be done ideally by measuring the desired parameters on the same individuals. Use of the same individuals for the entire period presented the problem of keeping fish alive from one determination to the next. Besides the difficulty of counting the diffuse teleost thyroid in vivo in small fish, there was the

greater difficulty of extracting adequate blood samples repetitively without depleting the blood volume. To obviate these difficulties, 55 fish (8-25 g, av = 12 g) from each temperature regimen (5 and 10° C) were "simultaneously" injected with I¹³¹ and 5 to 7 fish from each group sampled at the required time intervals (12, 36, 60, 84, 108, 132, 156, 204 and 276 hr). Each fish was weighed and plasma I¹³¹ concentration, percentage of I¹³¹ dose in body, percentage of I¹³¹ dose in thyroid and conversion ratio recorded. By serially sampling from the populations in this manner and deriving the mean value for each sample at a given time under a given condition, it was possible to follow the changes in I¹³¹ concentrations in several compartments as an average of the whole population.

1. Extrathyroidal and Thyroidal Clearance of Plasma I¹³¹

Radioiodine injected intraperitoneally is rapidly absorbed into the blood stream (Hickman, 1959). The plasma level is then continually depleted due to loss via a variety of routes (Fig. 2). A small percentage of the dose is eventually accumulated by the thyroid but the greater part is excreted. Although plasma I¹³¹ was lost faster at the higher temperature, the difference was not statistically significant (Table I). All the mean values for 5° C fish were above those for 10° C fish, however, the adjusted means were statistically different ($p < 0.01$). This shows convincingly that the temperature elevation of 5° C had caused an increase in I¹³¹ loss from the plasma by one or more routes. Of further interest was the curvilinear tendency at the higher temperature. This is considered in greater

Fig. 2. Plasma clearance following a single I^{131} injection in fish held at 5° C (○) and 10° C (●). Each point represents a mean of 5 to 7 fish (Covariance analysis, Table I).

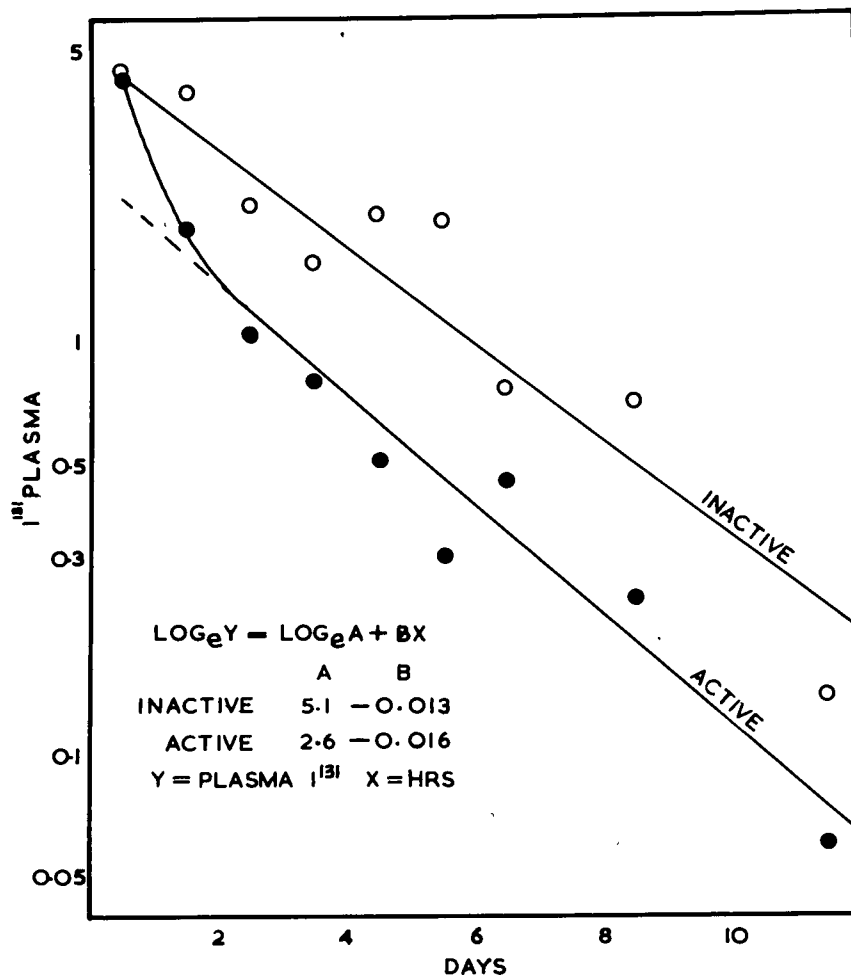


TABLE I

Analysis of covariance for plasma I^{131} (plasma I^{131} biological concentration coefficient/100) and time (hr) for active (10° C) and inactive (5° C) thyroid states

CONDITION	SLOPE	F_s	df	p	F_m	df	p
INACTIVE	-0.0127	2.08	1	0.05	39.3	1	0.01
ACTIVE	-0.0158		and 101			and 104	

F_s = Variance ratio for slopes.

F_m = Variance ratio for adjusted means.

detail below.

An equally reliable and perhaps more direct method of determining I^{131} excretion is to measure the percentage of injected dose remaining in the body (excluding thyroid) at various time intervals. This parameter and the percentage of the injected dose in the total body (including thyroid) are shown in Figure 3. Immediately evident is the curvilinear aspect of both parameters. To simplify the analysis, they have been approximated to diphasic linear plots with an inflexion at $3\frac{1}{2}$ days. Thus, for each parameter under both conditions, two relationships have been considered (Day 1-4 = phase I and Day 3-11 = phase II). The statistically significant differences between these relationships are given in Tables II and III.

Table II demonstrates the diphasic (curvilinear) nature of the excretion data. As yet this has no completely satisfactory explanation. According to Wiggs (1962), who observed a similar phenomenon in sticklebacks, it may be due to an initial and general rapid ion loss induced by "laboratory diuresis" as a result of experimental handling. Applied to these data it would suggest a significant diuresis for 2 to 3 days and then a stabilization. A more plausible explanation is suggested by Comar (1955) who demonstrated mathematically that a curvilinear relationship is expected if an isotope in one compartment is being diluted by movement to two or more compartments at markedly different rates. Thus the composite curvilinear relationship may be a combination of a rapid and a slow I^{131} loss via two different routes. According to Comar, the rapid removal would

predominate initially but would be replaced largely by the slower removal rate at low I^{131} levels. In considering I^{131} removal routes from the steelhead body, the gill could conceivably be the more rapid and the kidney the slower route since there is evidence to suggest that I^{131} may be removed predominantly via the gills (Hickman, 1959).

The diphasic nature of these curves does not influence the rate of thyroid I^{131} accumulation. Rate constants for thyroid I^{131} accumulation have been obtained by subtracting the percentage dose in body slope from the slope derived from the body and thyroid combined (Table II). At 5° C the constant is essentially the same over both phases of the curve (0.0054 and 0.0057; $\bar{a}v = 0.00555$) and the same applies at 10° C (0.0075 and 0.0077; $\bar{a}v = 0.0076$), although there are very marked differences between mean constants from the two temperatures (0.00555 and 0.0076). It is concluded that (i) temperature has a significant influence on the thyroid I^{131} accumulation rate constant and (ii) this constant is a reliable index of thyroid activity under different rates of I^{131} excretion.

Of further significance was the temperature influence on I^{131} excretion rates. Elimination appeared more rapid at the higher temperature but again it was difficult to demonstrate statistical differences between slopes (Table III). However, adjusted mean values showed a significant difference between the two regimes ($p=0.01$). This supports the earlier evidence that I^{131} loss from the body, like thyroid I^{131} accumulation, is more active at higher temperatures.

Fig. 3. Total body clearance (○) and body excluding thyroid clearance (●) following a single I^{131} injection at 5° C (inactive thyroid) and 10° C (active thyroid). Equations calculated separately for each phase. Each point represents a mean of 5 to 7 fish (Covariance analyses, Tables II and III).

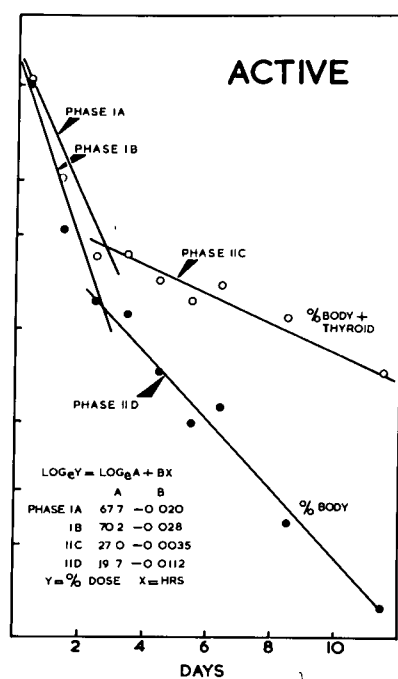
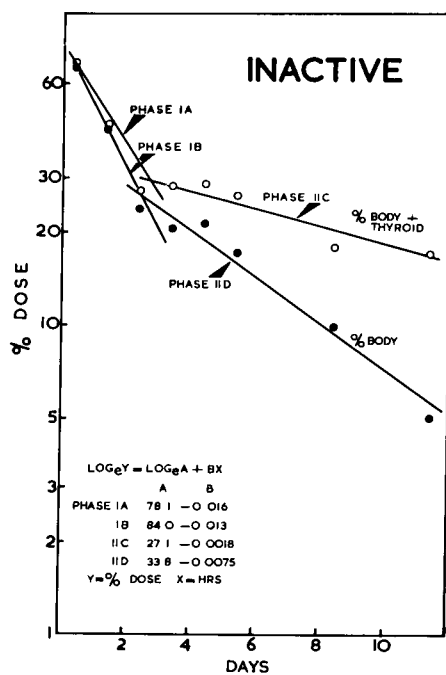


TABLE II

Analysis of covariance for I^{131} clearance from the total body and time (hr); and body excluding thyroid and time (hr). $\text{Slope}_{T+B} - \text{Slope}_B = \text{Rate constant for thyroid } I^{131} \text{ uptake } (\text{Slope}_T)$

Condition	Phase	% DOSE BODY AND % DOSE THYROID			% DOSE BODY			Slope $_T$
		Slope $_{T+B}$	F	df	Slope $_B$	F	df	
INACTIVE	I	-0.0161	9.92**	1	-0.0215	18.5**	1	0.0054
	II	-0.0018		and 56	-0.0075		and 62	0.0057
ACTIVE	I	-0.0204	14.17**	1	-0.0279	9.80**	1	0.0075
	II	-0.0035		and 57	-0.0112		and 58	0.0077

** Denotes statistical difference between compared slopes ($p = 0.01$).

TABLE III

Analysis of covariance for I^{131} clearance from total body and time (hr) and body excluding thyroid and time (hr), for active (10°C) and inactive (5°C) thyroid states. The analysis indicates the difference in excretion rates between the two temperatures in phase II

PHASE	CONDITION	% DOSE BODY				% DOSE BODY + % DOSE THYROID			
		SLOPE	df	F_s	F_m	SLOPE	df	F_s	F_m
I	INACTIVE	-0.0161	1	0.697		-0.0215	1	0.80	
	ACTIVE	-0.0204	40			-0.0279	42		
II	INACTIVE	-0.0018	1	1.175	58.50**	-0.0075	1	3.89?	29.87**
	ACTIVE	-0.0035	72			-0.0112	68		

F_s = Variance ratio for slopes.

F_m = Variance ratio for adjusted means.

** denotes highly significant difference ($p < 0.01$).

? Almost shows significance between slopes ($p = 0.05$).

Other "F" values show no difference between slopes.

2. Experimental Measurement of Thyroid I¹³¹ Accumulation

Ideally, all these I¹³¹ movements would be represented in terms of rate constants. In small fish, where serial sampling for determination of regressions for rate constants would involve many fish, an attempt was made to determine the extent of excretion and thyroid uptake on the basis of single terminal measurements. For comparison these samples were always taken at the same time after injection.

Due to the influence of temperature on extrathyroidal removal of I¹³¹, mere measurement of the percentage of the dose accumulated in the thyroid would be unreliable (Fig. 4A). Relative to the standard deviations the differences between the thyroid uptake means are very slight and are eventually reversed. At a slightly faster rate of extrathyroidal I¹³¹ clearance in fish with the active gland, the percentage of the dose accumulated in the thyroid would have been the same or lower than that in the inactive gland. Thus thyroid uptake of I¹³¹ measured in this way is very dependent on the extrathyroidal clearance rate, and in any single terminal assessment of thyroid uptake this blood loss of I¹³¹ must be taken into account.

Allowances for this have been made in a variety of ways. Hoar and Eales (1963) measured the Thyroid Uptake Factor (TUF) where

$$\text{TUF} = \frac{\% \text{ dose thyroid}}{\% \text{ dose thyroid} + \% \text{ dose body}} \times 100$$

It measures at death that percentage of the non-excreted I¹³¹ that has been partitioned from the body compartment into the

thyroid. TUF values for the inactive and active thyroids are shown in Figure 4B. A far better separation is now shown than that obtained from thyroid uptake values. It is to be noted that whereas the linear relationship was maintained until 12 days after injection in the fish with the slower rate of I^{131} metabolism, it fell markedly after 6 days in fish with the active thyroid. Further attention is drawn to this point below. Unlike other I^{131} parameters studied, it is arithmetically rather than exponentially related to time after injection. A theoretical explanation for this has not been attempted. It may also be noted at this stage how applicable the TUF measure is to in vivo studies providing adequate screening of extra-thyroidal tissue can be obtained.

Leloup and Fontaine (1960) have used the thyroid/serum or T/S ratio ($\frac{\text{thyroid radioactivity/g}}{\text{serum radioactivity/g}}$) for measuring thyroid uptake.

In assessing thyroid I^{131} concentration relative to serum concentration, this method is similar to thyroid clearance (Hickman, 1959; Baggerman, 1960). T/S is a direct measure, at a certain time after I^{131} injection, of the I^{131} accumulation in the thyroid relative to that in the serum. In this study it was found convenient to use a ratio very similar to the usual T/S ratio; it is referred to as the T/S ratio in this study. It is expressed as

$$= \frac{\frac{\text{thyroid uptake}}{\text{plasma } I^{131} \text{ concentration}}}{\frac{\% \text{ of injected } I^{131} \text{ in thyroid}}{\text{plasma } I^{131} \text{ biological concentration coefficient}}}$$

100

Fig. 4. (A) Percentage accumulation of I^{131} by the thyroid following a single I^{131} injection at 10° C (active) and 5° C (inactive). Standard deviations shown. Each point represents a mean of 5 to 7 fish.

(B) TUF values for the same fish as (A).

(C) T/S values for the same fish as (A). The diphasic tendency is shown in the active state (Covariance analysis, Table IV).

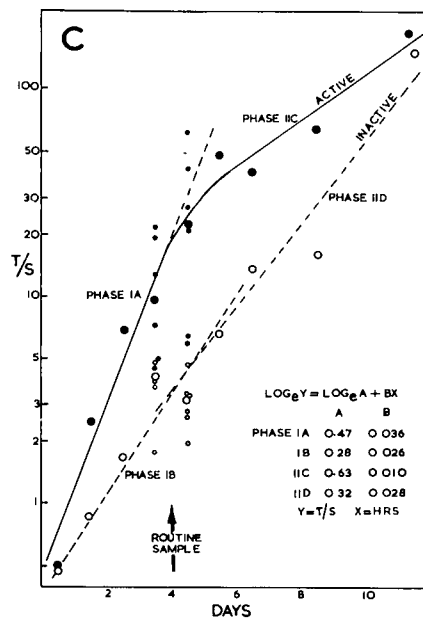
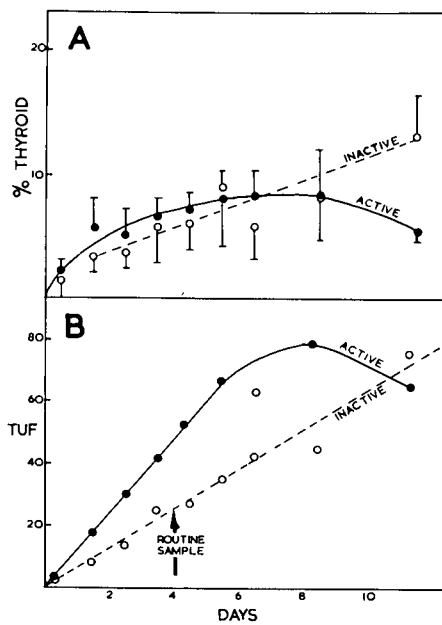


TABLE IV

Analysis of covariance for T/S and time (hr) for active (10° C) and inactive (5° C) thyroid states

CONDITION	PHASE	SLOPE	df	F	df	F
INACTIVE	I	0.0255	1	1.44	1	22.5**
	II	0.0281	56		54	
ACTIVE	I	0.0362	1	26.44**	1	9.06**
	II	0.0100	50		52	

** Denotes significant difference between slopes (p < 0.01).

Absolute accumulation relative to the plasma was higher in the more active group over the entire period of study and therefore showed a more complete separation of the two thyroid levels than did the TUF (Fig. 4C). Most information can be gained, however, by a mathematical analysis of the curves since, while the inactive gland showed a strictly exponential rise with time, the active gland showed a curvilinear or diphasic relationship. In the active gland an inflexion of the curve was apparent between 4 and 5 days after injection and consequently two regressions were compared separately for both the active and inactive states. Thus the exponential relationships between 12 and 108 hours (phase I) and 108 and 276 hours (phase II) were computed for each temperature (Table IV). The mathematical analysis revealed (i) a statistically significant reduction in slope in the active gland from the first to the second phase, but no change in the inactive gland between phases, and (ii) that phase I of the active gland had a slope statistically different from phase I of the inactive gland.

The difference in slope during phase I of the two holding conditions is considered due to greater build-up of I^{131} in the thyroid relative to that in the blood of the fish with more active glands. The change in slope of the active gland in phase II could mean either that the affinity of the thyroid for iodine had been reduced or that there had been a loss of radioactivity from the gland. The former seems unlikely unless the thyroid uptake activity had suddenly changed in the middle of the experiment. Reference to Figure 4A, however, shows a

significant drop in the percentage of the dose in the thyroid between 8 and 12 days in the active but not in the inactive gland. It is tempting to identify this loss of radioactivity with radiohormone output from the active gland.

There is, however, another possibility. Although the net result is an accumulation of iodine by the thyroid, there are fluxes of I^{127} and I^{131} both into and out of the gland. Initially the influx of I^{131} is high since the ratio between plasma I^{131} and plasma I^{127} is also high. The efflux, however, is low since there is little I^{131} in the gland. As the $I^{131}:I^{127}$ ratio builds up in the thyroid and becomes lowered in the plasma, the situation is reversed and the net tendency for I^{131} to come out of the gland is increased. This could account for the change in slope. The reduced increase in the T/S ratio is, however, partly due to an output of radioactive hormone as shown by studies of the plasma PBI^{131} .

From these data and theoretical consideration it seems evident that a T/S or TUF measurement taken at some fixed time after injection (4 days or in some instances 8 days) should give a reliable indication of the efficiency of the thyroid "iodide pump" and that these indices are far more reliable than thyroid uptake alone. In this study most thyroid measurements were done at 4 days after injection as shown in Figure 4C. In this same figure the individual values of T/S for 84 and 108 hours are shown for both the active and inactive states. At both time intervals the active and inactive T/S values were statistically different ($p < 0.01$, Wilcoxon ranking test), and there is every reason to believe that single terminal assessments

of T/S or TUF factors at 4 days give an accurate indication of the overall thyroid I^{131} accumulating efficiency.

3. Estimation of Radiohormone Output Using the Conversion Ratio

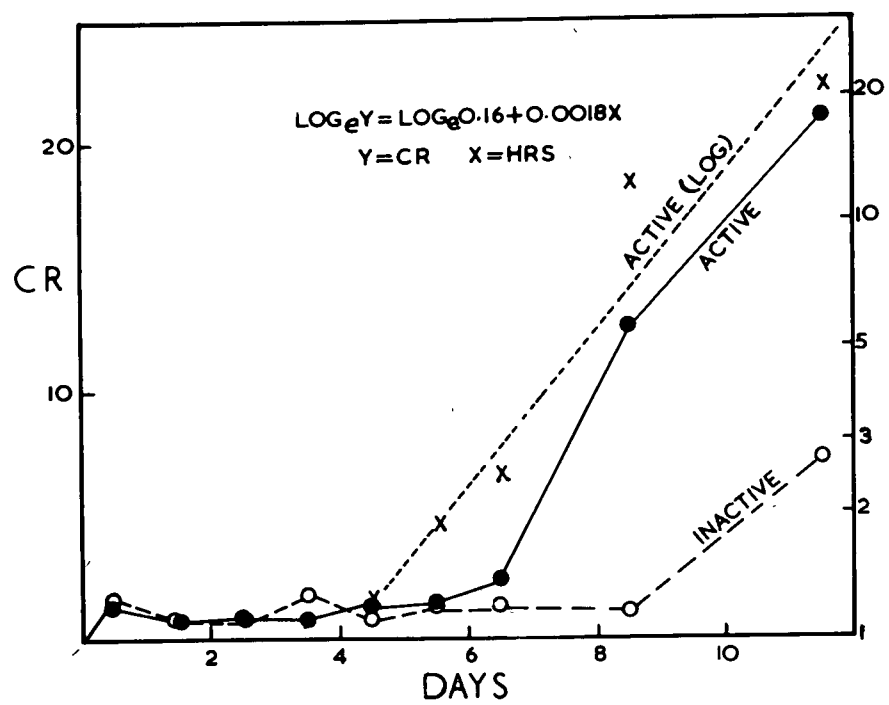
As indicated above, it would be logical to expect a significant build up in radiohormone (PBI^{131}) in the plasma after about 5 days in the fish with more active thyroid gland. Figure 5 shows the change in the conversion ratio with time where,

$$\text{Conversion ratio} = \frac{PBI^{131} \text{ count/min} \times 100}{PBI^{131} \text{ count/min} + I^{131} \text{ count/min}}$$

This ratio does indeed ascend steeply at 4 days in the active gland and after 4 days the rise conforms to an exponential relationship. That the exponential relationship only becomes evident after 4 days is probably an artifact caused by (i) the very small amounts of radioactive hormone liberated by the gland over the initial period and (ii) a technical limitation due to the fact that there is always imperfect washing of I^{131} from the PBI^{131} fraction such that the conversion ratio (CR) is rarely below 0.5%. Thus at low levels of radiohormone output the true PBI^{131} level is masked by I^{131} contamination of the PBI^{131} fraction, the exponential nature of the PBI^{131} build-up is obscured and a false picture of a lag phase is suggested.

This exponential rise of the CR is influenced by two factors- PBI^{131} output from the gland and PBI^{131} removal from the blood due to peripheral utilization and excretion of hormone. The latter effect would cause the CR exponent to flatten with time. This was not evident during the 12 day observation. Very little

Fig. 5. Change in CR following a single I^{131} injection in fish at 10° C (active) and 5° C (inactive). Each point represents a mean of 5 to 7 fish.



is known of the rate of removal of hormone from the blood in fish and until this has been determined its effect on the CR cannot be assessed. It can be pointed out, however, that until relatively large quantities of PBI^{131} have built up the ratio of $\text{PBI}^{131}:\text{PBI}^{127}$ in the blood would be so small that the chance of PBI^{131} being depleted due to peripheral utilization would be proportionately small. Thus the initial phase of the CR curve would represent largely the rate of radiohormone production.

As with other aspects of I^{131} metabolism, change in plasma PBI^{131} levels could probably be represented most precisely in terms of rate constants. In this entire study, however, the extent of PBI^{131} production was assessed by killing the fish 8 days after injection and then measuring the CR of a plasma sample. At this time PBI^{131} levels are becoming readily detectable in the plasma and also reveal the very marked difference between the active and inactive thyroids (Fig. 5).

It is important to note that the CR only indicates the rate of radiohormone production and not the rate of stable hormone production. To indicate the latter, the I^{131} must attain equilibrium with I^{127} throughout the body. This requires repetitive injections to maintain a constant I^{131} level and is certainly not achieved in these studies as a result of a single injection.

In summary, by making routine measurements of I^{131} levels in several body compartments at fixed time intervals, a great deal can be learned about radioiodine metabolism and thyroid function. As a result of these preliminary investigations it

was decided to determine routinely the parameters shown in Table V at 4 and 8 days after injection of I^{131} .

II. SEASONAL CHANGES IN RADIOIODINE METABOLISM AND THE HISTOLOGICAL APPEARANCE OF THE THYROID IN JUVENILE STEELHEAD HELD IN FRESH WATER

Following the preliminary investigation of I^{131} metabolism certain of the parameters shown in Table V were determined seasonally. Mean cell height and colloid colour were also measured. These data, together with seasonal changes in water temperature, are summarized in Figure 6.

1. I^{131} Excretion

Plasma I^{131} (4 days) and percentage of dose in total body (4 days), revealed definite seasonal trends. From May of the pre-migrant year until smoltification there was a general negative correlation between these two parameters and temperature, indicating more rapid removal of I^{131} at higher temperatures. This negative correlation is demonstrated in Figure 7 where mean seasonal values for total I^{131} retention have been plotted against temperature ($r = -0.77$, 26df, $p < 0.001$). It is important to note that these seasonal changes in I^{131} rate of excretion were not only demonstrated by total plasma clearance but also by extrathyroidal clearance (Fig. 7). This indicates that the loss of I^{131} from the plasma both to the thyroid and to the outside medium was increased at higher temperatures. These observations support the data from the preliminary investigations.

TABLE V

A summary of radioiodine parameters and their significance

PARAMETER	ABBREVIATION USED	SIGNIFICANCE
I Plasma I ¹³¹ biological concentration coefficient/100	Plasma I ¹³¹	Measures plasma I ¹³¹ concentra- tion and allows estimates of total blood clearance of I ¹³¹
II % injected I ¹³¹ dose in body (excluding thyroid)	% body	Measures total clearance of I ¹³¹ from the body
III % injected I ¹³¹ dose in whole body (including thyroid)	% body + % thyroid	Measures the % of injected dose in whole body. 100 % - % in whole body = % loss due to extrathyroidal clearance (excretion)
IV $\frac{\% \text{ injected I}^{131} \text{ dose in thyroid}}{\text{Plasma I}^{131}}$	T/S	Assesses build-up of I ¹³¹ in the thyroid relative to plasma I ¹³¹ .—measures the affinity of the thyroid for I ¹³¹ and allows for excretion rate
V $\frac{\% \text{ dose thyroid}}{\% \text{ dose body} + \% \text{ dose thyroid}}$	Thyroid uptake factor TUF	Measures that % of the unexcreted I ¹³¹ that is parti- tioned into the thyroid
VI Conversion ratio $\frac{\text{Plasma PBI}^{131} \times 100}{\text{Plasma I}^{131} + \text{plasma PBI}^{131}}$	CR	Assesses extent of conversion of I ¹³¹ to PBI ¹³¹

Fig. 6. Seasonal change in plasma I¹³¹, % dose I¹³¹, T/S, TUF, CR, mean epithelium cell height and percentage of follicles containing blue colloid in steelhead parr and smolts. Seasonal change in temperature is shown. Standard deviations are shown for certain parameters.

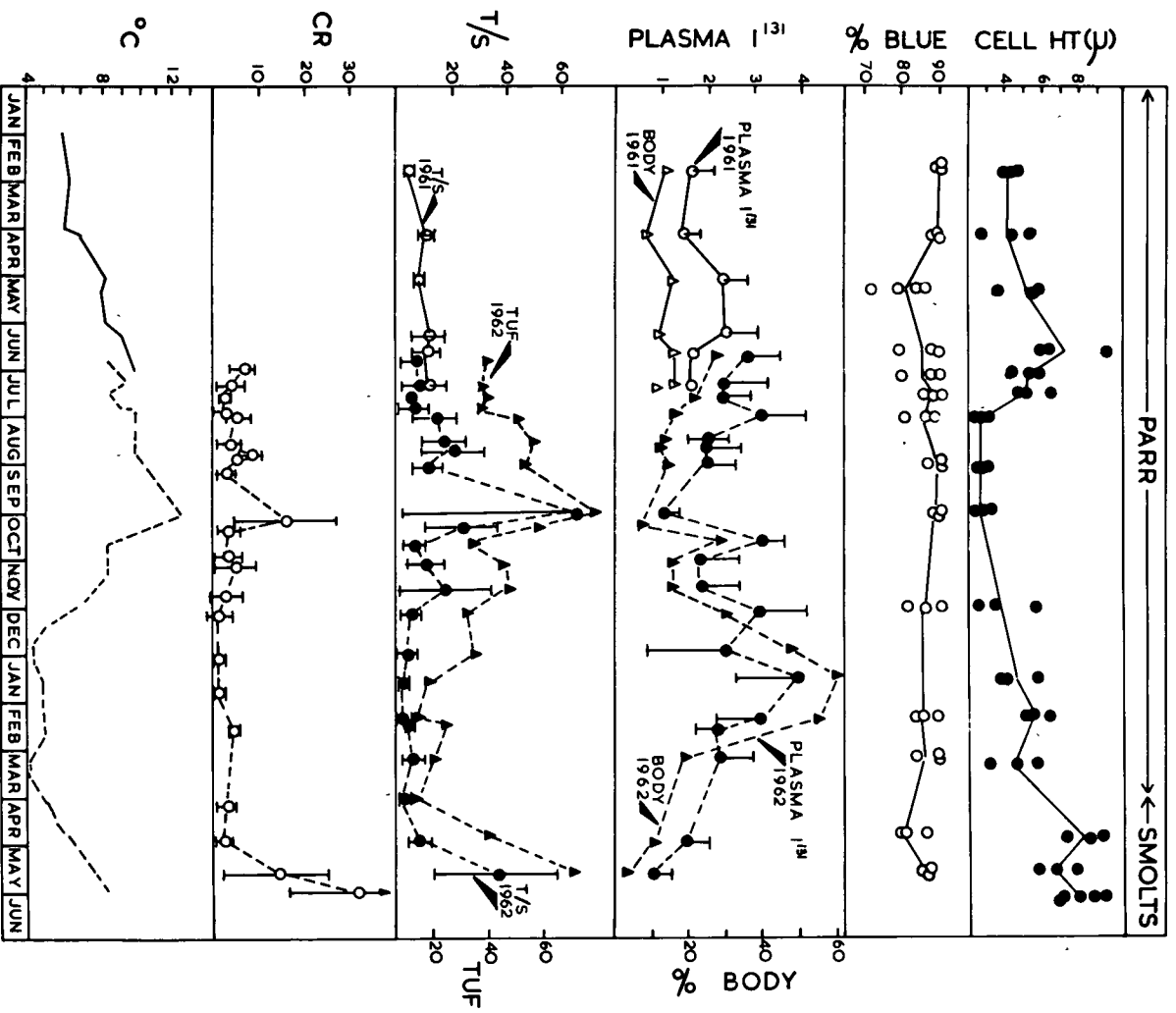
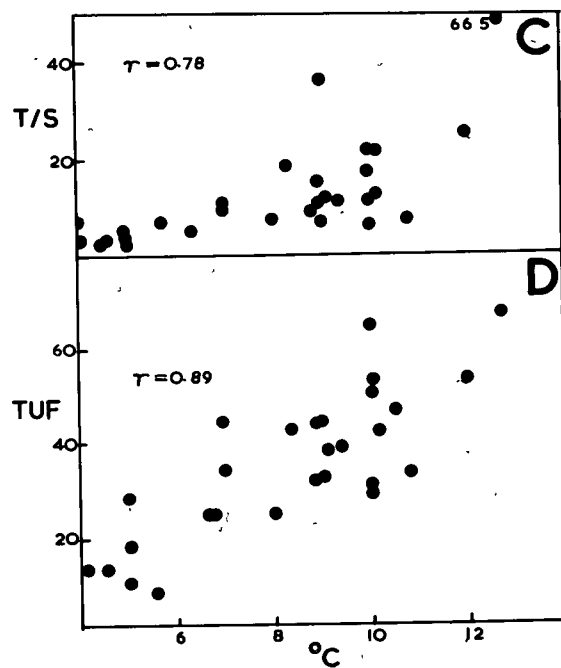
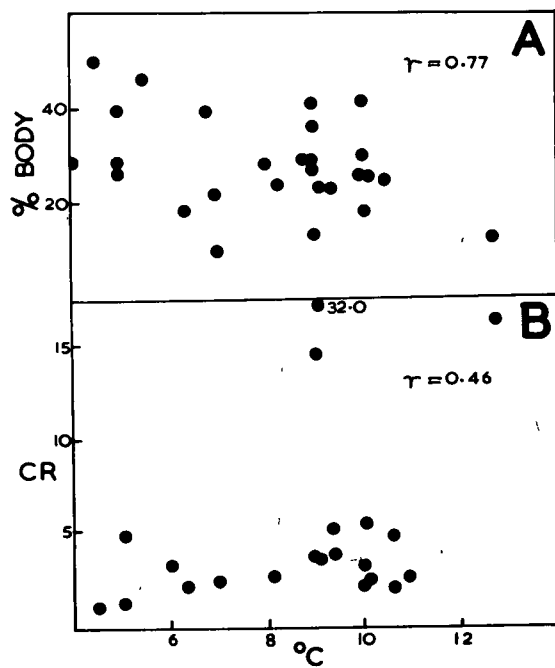


Fig. 7. Correlations between water temperature and four
1131 parameters. All values drawn from seasonal data (Fig. 6).
A (% dose in body); B (CR); C (T/S); D (TUF).



Of further significance was the departure of certain groups of fish from the general correlation. These fell into two categories - fish below 20 g in weight (usually 18 months or younger) and smolts (30-31 months). In the younger and smaller fish, the low retention of I^{131} indicated differences that could be attributable to size or growth. These fish are reconsidered later. Over the period of smoltification, however, both the total clearance and the extrathyroidal clearance were more rapid than at any other time of year. At the time of anticipated migration in May the water temperature was 8 to 9° C and at this temperature the plasma and body I^{131} levels were much lower than the plasma and body I^{131} retentions at 12 to 13° C in pre-migrants in September of the previous year. This would indicate that some factor other than temperature might be influencing the I^{131} excretion rate in steelhead at smoltification.

2. Thyroid Uptake

T/S and TUF values also showed temperature correlations but with the same two exceptions (small fish and smolts) (Fig. 6). The thyroid I^{131} uptake correlated positively with temperature over the seasonal range of 4 to 13° C at highly significant statistical levels (Fig. 7) (T/S, $r = 0.78$, 26df, $p < 0.001$; TUF, $r = 0.89$, 26df, $p < 0.001$).

3. Conversion Ratio

CR values showed a general increase with temperature rise ($r = 0.46$, 18df, $p < 0.05$), but the effect was not as marked as with thyroid uptake (Figs. 6 and 7). Of particular note were

the very high CR values of smolts at 8 to 9° C. These values were considerably higher than any recorded during the entire study. No CR estimates were available on yearling fish below 20 grams.

4. Histological Changes

Whereas all I^{131} parameters were positively correlated with temperature and suggested a possible temperature dependence, the cell height parameter indicated no such general correlation (Fig. 6). It was conclusively demonstrated that in yearling steelhead the lowest cell heights occurred at the highest and not the lowest temperature, and much of the seasonal data indicated a tendency for higher cell height at lower temperatures. In other words, the radioiodine and cell height parameters showed opposite trends with respect to temperature. Of some importance, however, was the significant negative correlation between mean values for cell height and mean values for percentage of follicles containing blue colloid ($r = -0.66$, 11df, $p < 0.05$). This indicated that the cell height data were not in agreement with the data obtained from colloid colour, since increasing cell height is associated with an active gland, and absence of blue colloid indicates decreased thyroid activity.

It is evident from this brief survey of seasonal changes in I^{131} metabolism and histological change in steelhead that there is no absolute picture of what might be affecting the thyroid. Temperature seems to be correlated with changes in I^{131} metabolism. But, is there a causal relationship between thyroid activity and changing temperature? Furthermore, the

smolts and small fish showed departures from this general trend, while the cell height data revealed a correlation possibly in the reverse direction, and showed a trend opposite to that obtained by the colloid staining. There are also other important seasonal variables including photoperiod. Cyclical changes in radioiodine metabolism independent of environmental influence might also be envisaged. The roles of various factors influencing the thyroid therefore appear uncertain and confused. In the remainder of this study, an attempt has been made to isolate experimentally the various components that might influence the thyroid and to explain as fully as possible the seasonal changes described above.

III. EXPERIMENTAL INVESTIGATION OF THE INFLUENCE OF TEMPERATURE ON THE HISTOLOGICAL APPEARANCE OF THE THYROID AND RADIOIODINE METABOLISM IN YEARLING STEELHEAD PARR

In the seasonal study of thyroid function correlations between radioiodine metabolism and temperature were established. From such correlations it might be predicted that temperature causes changes in radioiodine metabolism. In this aspect of the study it was hoped to test this point by holding fish under conditions which were different in temperature but similar in all other respects.

To assess experimentally the effects of temperature on thyroid function, 12 fish were put into an open system of running water at temperature regimes of 6, 9, 12, 15 and 18° C, all held to $\pm 0.05^\circ$ C. The holding temperature prior to the acclimation

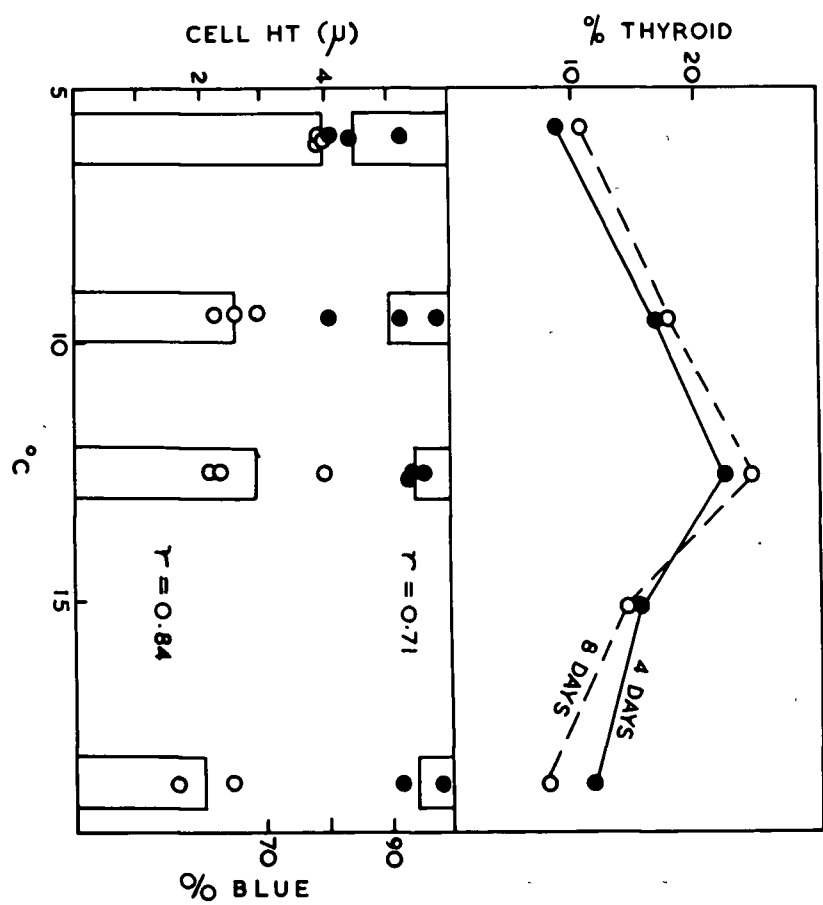
was $9 \pm 0.5^{\circ}$ C and the acclimation temperature was not imposed suddenly but built up over a period of several hours. On 18 July, 14 days after initiation to the regimes, all the fish were injected with I^{131} . Six were killed from each condition at four days and 6 at eight days. The results are shown in Figures 8 to 11 and in Table VI.

The effect of temperature on cell height and radioiodine metabolism of Salmo gairdneri has been investigated by Olivereau (1955a, b). Thyroid activity was found to be highest between 9 and 12° C using radiochemical techniques. With histological criteria, greatest activity was implied at the lower temperatures. At 20° C the thyroid activity was low by both radiochemical and histological criteria.

In the present study, cell height showed a trend identical to that claimed by Olivereau. By this criterion, the gland was significantly more active at lower temperatures ($r = -0.84$, 9df, $p < 0.01$) (Fig. 8). However, there was a statistically significant positive correlation between increasing blueness of colloid and increasing temperature, indicating a more active thyroid at higher temperatures by this criterion ($r = 0.71$, 9df, $p < 0.02$) (Fig. 8).

In regard to the uptake of radioiodine (Fig. 8), both 4 and 8 day estimates showed a very similar pattern with maximum uptake at 12° C. Olivereau (1955a) measured I^{131} uptake by autoradiography, which is essentially the same technique, and obtained a similar result. It has already been pointed out, however, that I^{131} uptake by the thyroid is only a valid measure

Fig. 8. Experimental demonstration of the influence of temperature on percentage accumulation of I^{131} in the thyroid 4 and 8 days after a single I^{131} injection, on mean cell height (○) and on percentage of follicles containing blue colloid (●).



of the thyroid affinity for iodine if allowance is made for the rate of extrathyroidal I^{131} clearance. Thus the above conclusions on maximal I^{131} uptake at 12° C could be invalid as an indication of thyroid activity.

Figure 9 shows the effect of temperature on I^{131} removal from the body as a whole and from the plasma at different temperatures (4 and 8 days). I^{131} retention falls exponentially with temperature. Some attempt at an analysis of these curves has been presented (Table VI). It would appear that both the 4 and 8 day estimates of I^{131} retention indicate slopes that do not differ significantly from each other. However, the high correlation coefficients emphasize a significant increase in I^{131} loss with temperature.

Figure 10 shows the approximate exponential increase in T/S with temperature. The CR values for the same fish showed a similar exponential change (Fig. 11). High correlation coefficients were found for the relationship and no statistical difference was evident between the 4 and 8 day slopes (Table VI).

It is concluded that temperature accelerates all aspects of I^{131} metabolism between 6 and 18° C and that radiochemically the thyroid is not maximally active at 9 to 12° C as Olivereau suggested. Where histological criteria are concerned, the reverse seems to be true since the cell height indicated greater gland activity at lower temperatures. The colloid colour, however, conformed with the radiochemical estimates. An attempt has been made to resolve these very important anomalies in the general discussion.

Fig. 9. Experimental demonstration of the influence of temperature on I^{131} levels in plasma and total body 4 days after a single I^{131} injection. Each point represent 4 to 7 individuals (Covariance analysis, Table VI) .

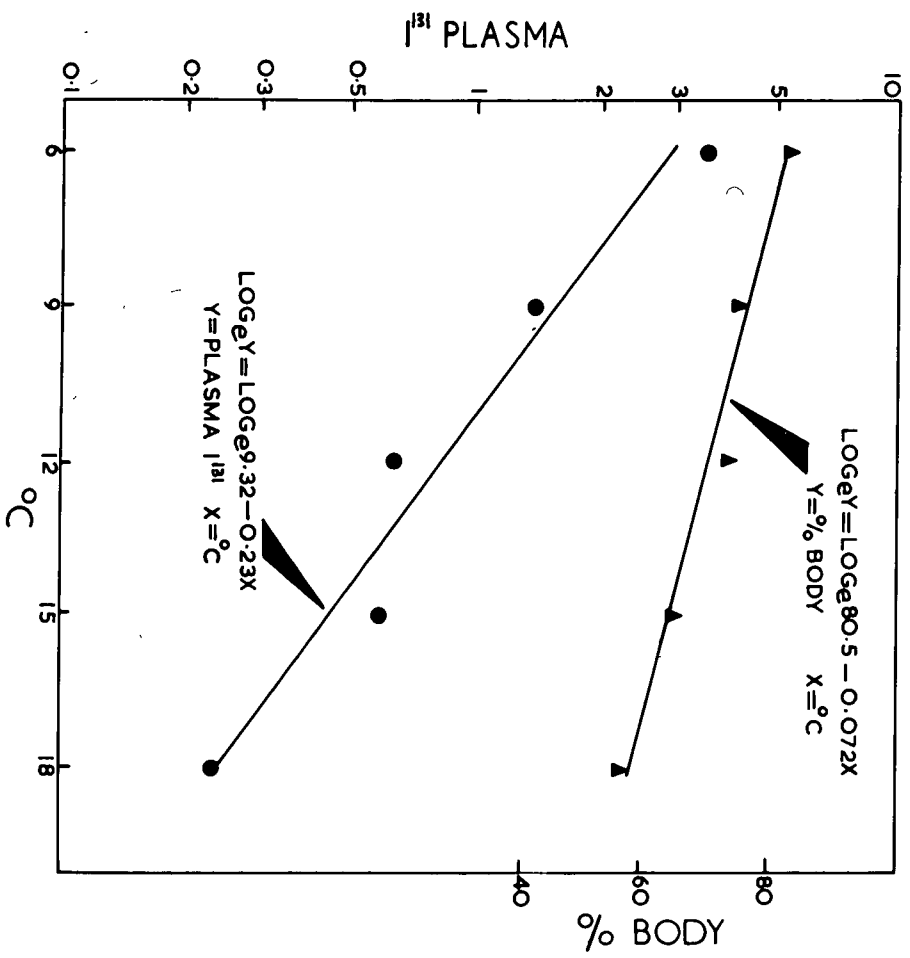


TABLE VI

Analysis of covariance for the relationships between various I^{131} parameters and temperature ($^{\circ}C$)

Parameter	Time (Days)	Slope	df	F	Correlation coefficient
CR	4	0.130	1	1.14	0.797**
	8	0.172	and 46		0.710**
I^{131} PLASMA	4	-0.226	1	1.08	-0.813**
	8	-0.285	and 46		-0.790**
% BODY + % THYROID	4	-0.0723	1	0.12	-0.665**
	8	-0.0818	and 46		-0.584**

** Denotes significance ($p < 0.01$).

Fig. 10. Experimental demonstration of the influence of temperature on T/S values 4 and 8 days after a single I^{131} injection. Each point represents a mean of 4 to 7 individuals.

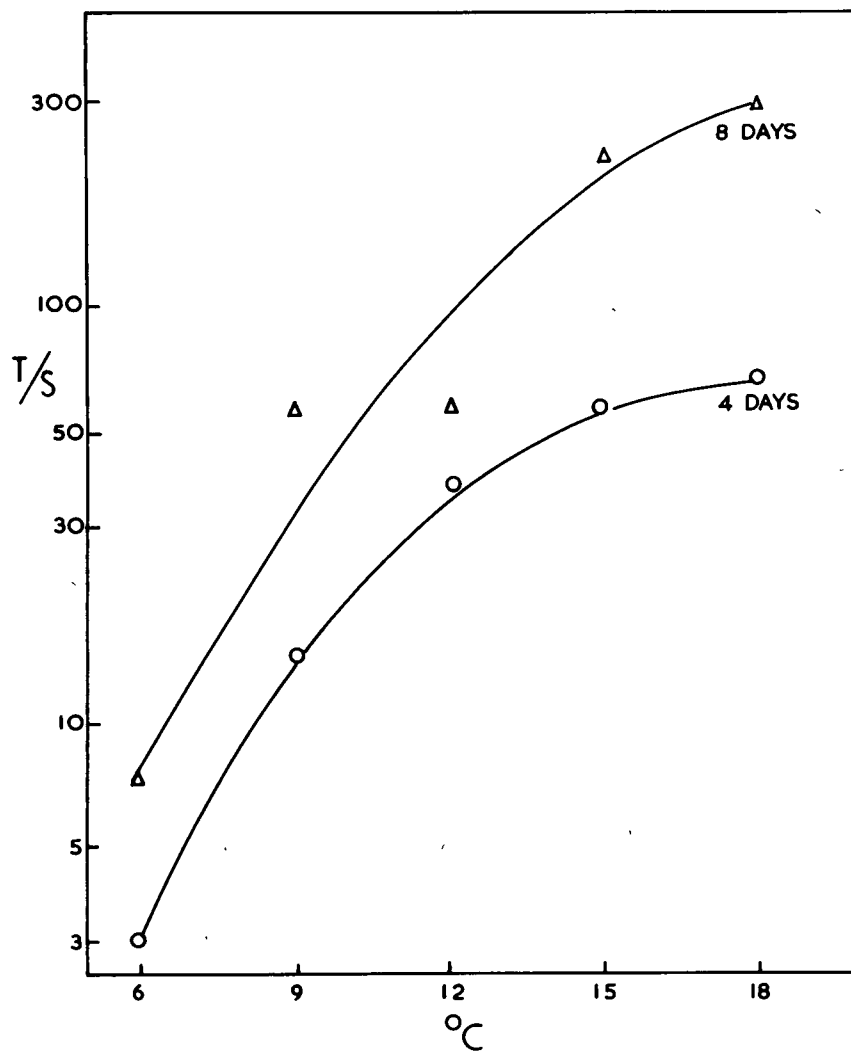
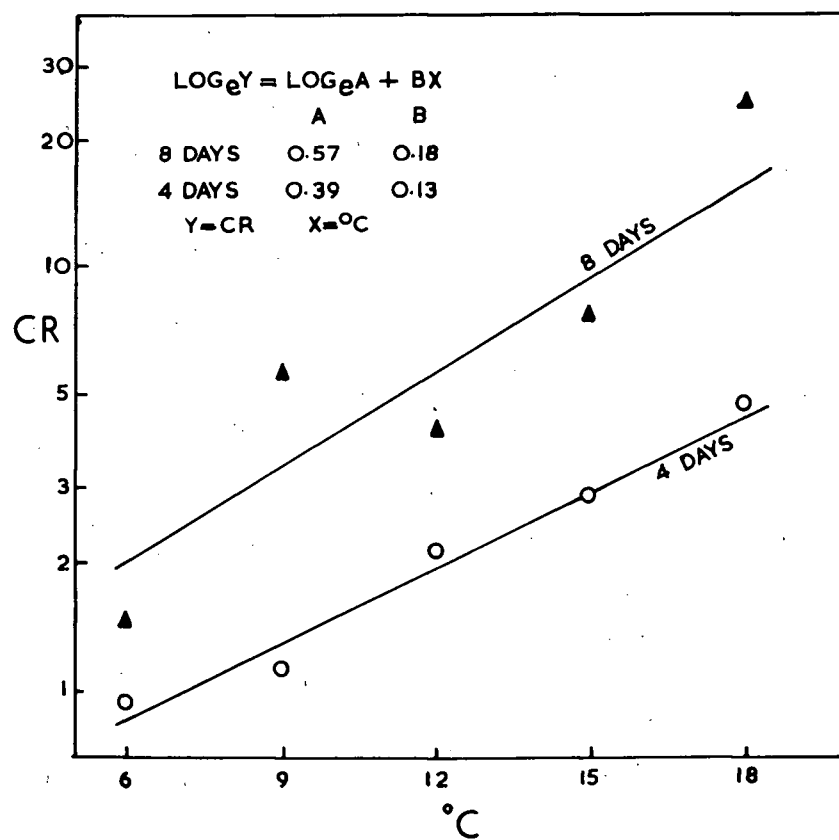


Fig. 11. Experimental demonstration of the influence of temperature on CR values 4 and 8 days after a single I^{131} injection. Each point represents a mean of 4 to 7 individuals (Covariance analysis, Table VI).



IV. EXPERIMENTAL INVESTIGATION OF TEMPERATURE AND PHOTOPERIOD
INFLUENCE ON THYROID CELL HEIGHT AND RADIOIODINE METABOLISM
OF STEELHEAD YEARLINGS (January to July)

The previous seasonal observations and experiments suggest that temperature is a major environmental factor influencing the thyroid. This does not exclude, however, the influence of other variables, prominent among which could be the changing photoperiod. Photoperiod is important in controlling certain endocrine changes and the spring increase in radioiodine metabolism in yearling and two-year-old steelhead could be influenced by the increasing spring daylight hours. To test this possibility, an experiment was conducted from late January to early July.

Fish were held under 4 experimental regimes in 2 m x 50 cm x 30 cm concrete troughs with a continually replenished supply of dechlorinated water. In two of the troughs the water temperature was not controlled and approximated to the outside conditions. In the other two tanks it was pre-cooled and maintained between 5 and 6° C. All 4 tanks were covered with light-proof boxes illuminated by fluorescent bulbs. Two of them were maintained on an 8-hour day and two on a natural daylength using the control mechanism mentioned in methods (Table VII). Fish were sampled routinely for histological and radioiodine determinations at approximately monthly intervals.

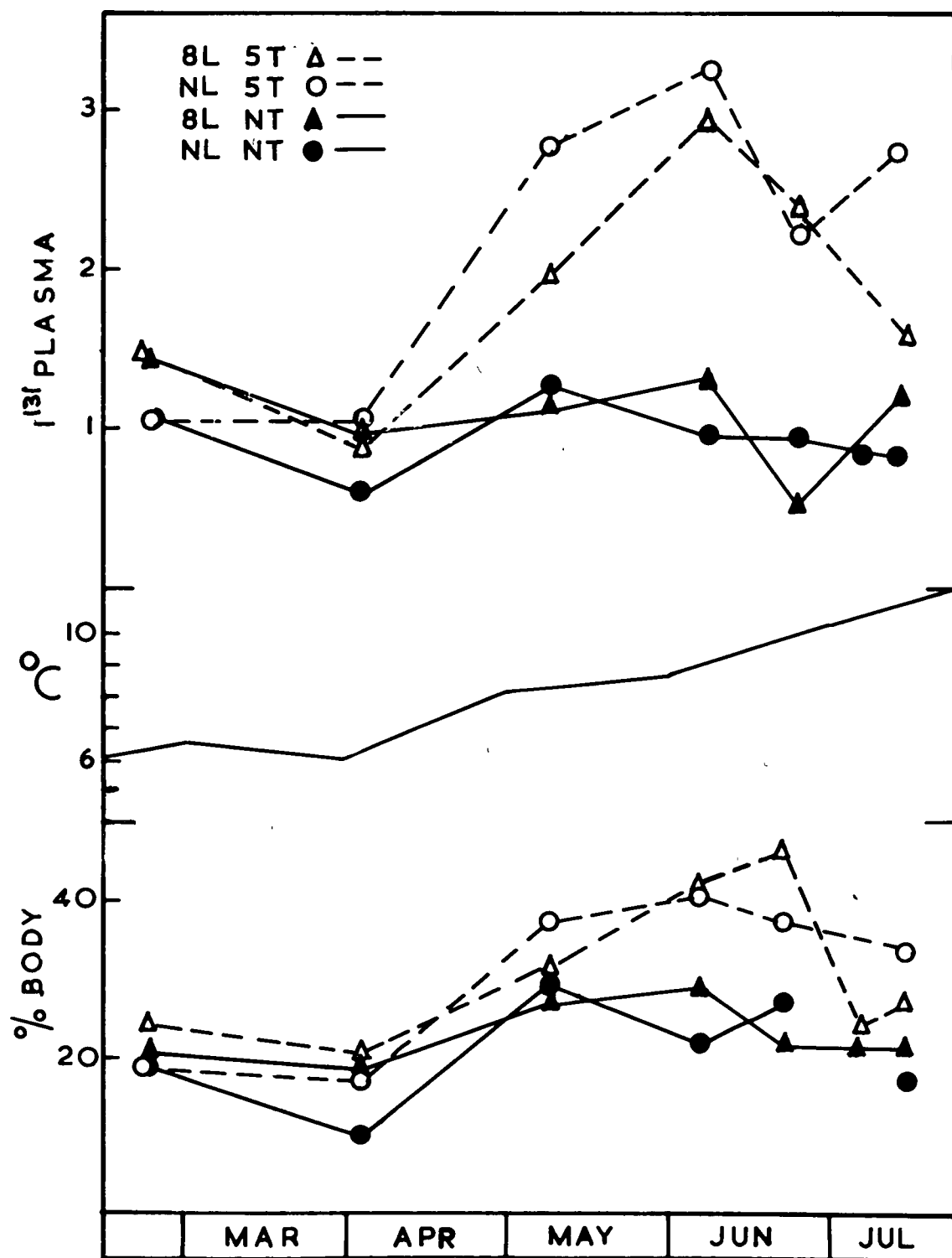
The I^{131} plasma levels showed a clear separation on the basis of temperature as did the extrathyroidal clearance (Fig. 12). There was no noticeable suggestion of a photoperiod influence.

TABLE VII

Summary of the four combined temperature and photoperiod conditions. Symbols shown are as used in figures

TANK	SYMBOL	PHOTOPERIOD	TEMPERATURE
1	●	Natural	Natural
2	▲	8 hours	
3	○	Natural	Controlled
4	Δ	8 hours	(5 - 6° C)

Fig. 12. Experimental demonstration of the combined influence of temperature and photoperiod on plasma I¹³¹I levels and percentage dose in total body 4 days after a single I¹³¹I injection in 14 to 18-month steelhead. Each point represents a mean of 8 to 12 fish (Statistics, Table VIII).



The T/S showed a similar increased response to rising temperature but showed no response to increasing photoperiod (Fig. 13). By comparing the four conditions in June when the greatest divergence between the treatment prevailed, it was not possible to demonstrate a significant effect of photoperiod, but a very significant influence of temperature on I^{131} metabolism was shown (Table VIII). In support of the earlier findings, however, the cell height did not agree with the radioiodine parameters (Fig. 13) and in general the fish held at the lower temperature had the higher cell height. In fact, ignoring photoperiod which did not appear to be influencing the cell height significantly, the means of all five seasonal samples of cold-adapted fish had a higher cell height than those subjected to the increasing temperature. Colloid colour estimates have not been included. They were extremely varied and revealed no apparent trend through the four conditions. Conversion ratios were not available from these experiments since at the time they were conducted it was not realized how slowly the PBI^{131} was produced and no 8-day readings were taken.

So far, estimates of thyroid activity have been based solely on single terminal measurements and do not include the CR. In order to reach more definite conclusions concerning the effects of photoperiod and temperature on yearling fish, serial investigations were carried out from these same conditions in early July (high temperature and long photoperiod under the natural conditions). Fifty to 55 fish from each condition were injected with I^{131} and 6 from each group killed at the following intervals:

Fig. 13. Experimental demonstration of the combined influence of photoperiod and temperature on mean cell height and T/S ratios 4 days after a single I^{131} injection in 14 to 18-month steelhead. Cell height - each point represents a mean of 3 to 4 fish; T/S - each point represents a mean of 8 to 12 fish (Statistics, Table VIII).

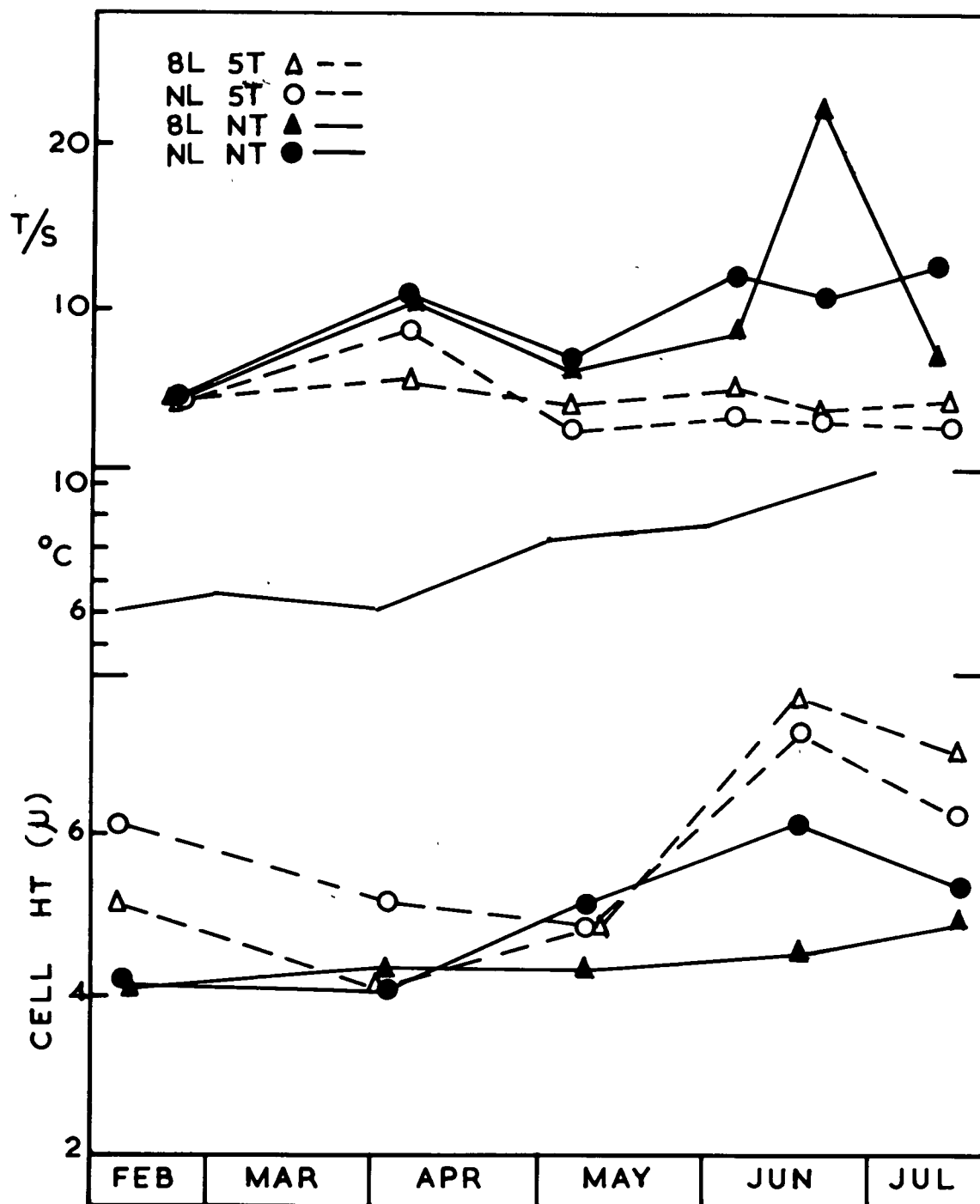


TABLE VIII

Summary of statistically significant differences for several I^{131} parameters between different conditions of temperature and photoperiod in yearlings in June. White-Wilcoxon non-parametric ranking test used

	HIGH TEMPERATURE		LOW TEMPERATURE	
	Photoperiod normal	Photoperiod 8 hours	Photoperiod normal	Photoperiod 8 hours
I^{131} plasma	_____	_____	_____	_____
	_____	_____ + _____	_____	_____
		_____ ++ _____		_____
% dose body + % dose thyroid	_____	_____	_____	_____
	_____	_____ ? _____	_____	_____
		_____ ++ _____		_____
T/S	_____	_____	_____	_____
	_____	_____ ++ _____	_____	_____
		_____ + _____		_____

- Two groups not statistically different ($p < 0.05$).
- ? Two groups almost statistically different ($p = 0.05$).
- + Two groups significantly different ($p < 0.05$).
- ++ Two groups significantly different ($p < 0.01$).

12, 36, 60, 84, 108, 132, 156, 204 and 276 hr after injection. Data are shown in Figures 14 - 16 inclusive; Table IX summarizes the statistics. The results obtained by the single 4-day measurements were confirmed. Photoperiod at this time of year exerted no influence on plasma I^{131} clearance nor on thyroid uptake, though temperature had a definite effect (Figs. 14 and 15). At the higher temperature, the characteristic diphasic thyroid uptake curve was evident. A similar interpretation is derived from consideration of the CR (Fig. 16). There was a distinct separation between the effects of the two temperature conditions but no differential response to photoperiod.

It is concluded therefore that in yearling steelhead (i) increasing temperature stimulates radioiodine metabolism and yet causes a decrease in cell height, (ii) there is no influence of increasing photoperiod on the steelhead thyroid under the conditions investigated and (iii) although the fish under the increasing temperature show a higher rate of I^{131} metabolism than fish at the lower temperature, the increasing temperature regime induces relatively little change. The divergence between the effects of the two regimes was not due only to changes in I^{131} metabolism in the fish under the variable temperature. In fact, with I^{131} excretion data, the greater changes were found in the fish held under constant temperature. This points to some change within the fish not governed by temperature and photoperiod. What was common to all the fish used over this period was their rapidly increasing size. One possibility is that this is a phase of rapid growth requiring

Fig. 14. Experimental demonstration of the combined influence of photoperiod and temperature on plasma clearance following a single I^{131} injection in 18-month steelhead. Each point represents a mean of 5 to 7 fish (Covariance analysis, Table IX).

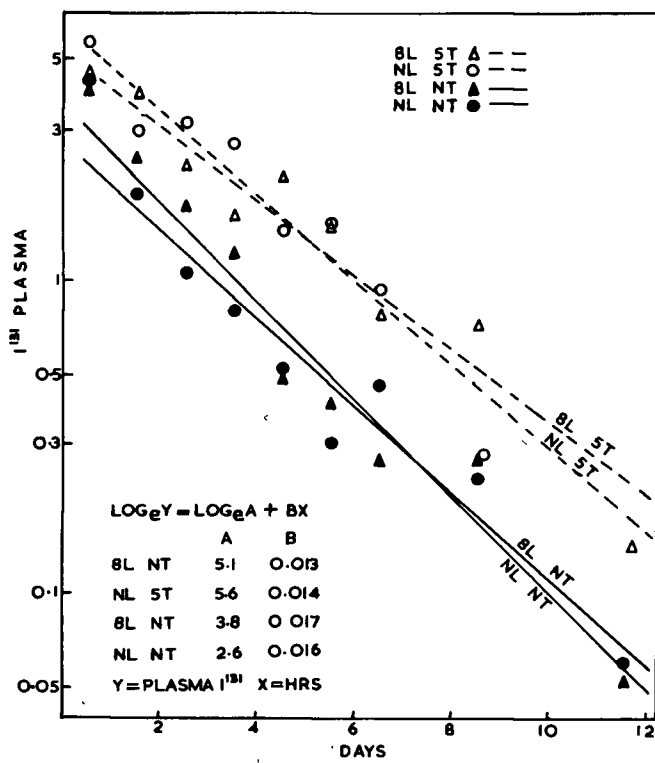


Fig. 15. Experimental demonstration of the combined influence of photoperiod and temperature on T/S values in 18-month steelhead following a single I^{131} injection. Each point represents a mean of 5 to 7 fish (Covariance analysis, Table IX).

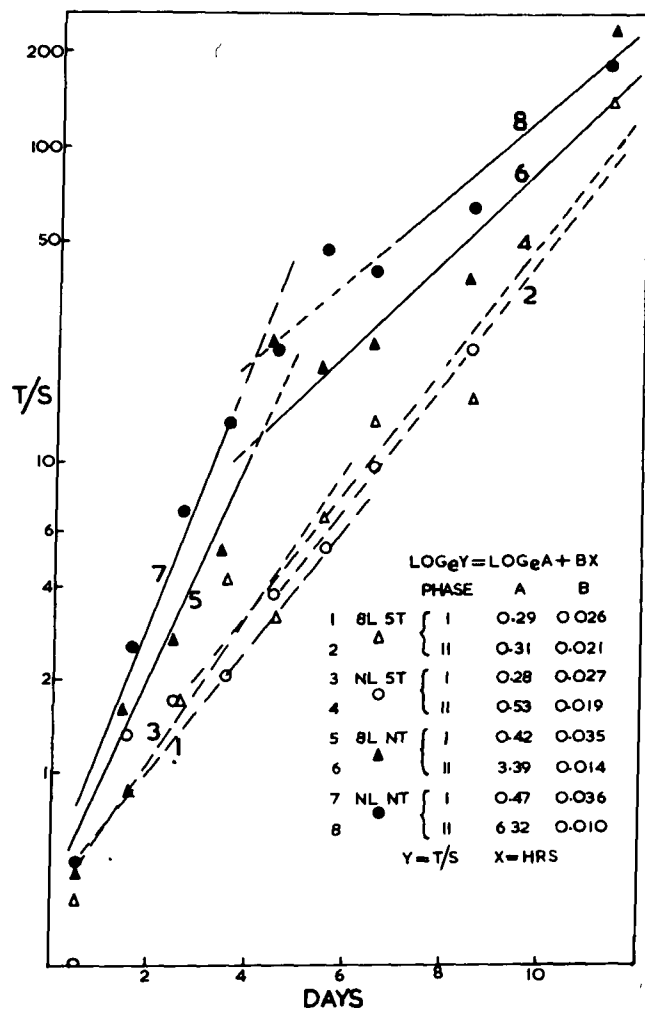


Fig. 16. Experimental demonstration of the combined influence of photoperiod and temperature on CR values following a single I^{131} injection in 18-month steelhead. Each point represents a mean of 5 to 7 individuals.

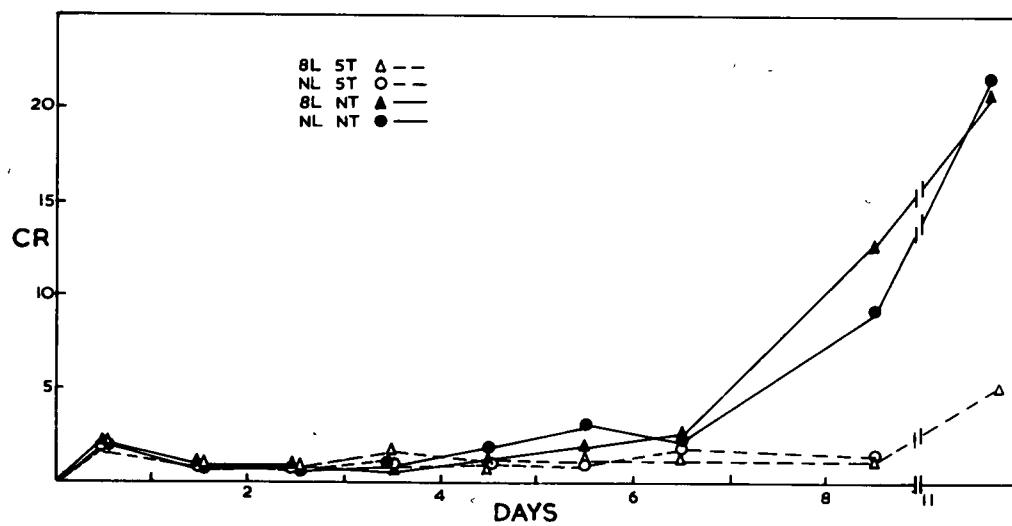


TABLE IX

Analysis of covariance performed on plasma I¹³¹ and time (hr), and T/S and time (hr) under four different photoperiod and temperature regimes

PLASMA I ¹³¹									
Temp. ° C	Photo- period	Phase	Slope	df	F _s	df	F _m	df	F _s
5	8	-	-0.013	1	0.02	1	0.44	1	6.67*
	16	-	-0.014	and 97		and 99		and 105	
10	8	-	-0.017	1	0.34	1	1.65	1	0.63
	16	-	-0.016	and 102		and 104		and 94	
				df	F _s	T/S df	F _s	df	F _s
5	8	I	0.026	1	1.44	1	0.07	1	4.48*
		II	0.021	and 56		and 56		and 55	
	16	I	0.027	1	3.08				
		II	0.019	and 48					
10	8	I	0.035	1	26.46**	1	0.24	1	2.58
		II	0.014	and 58		and 55		and 54	
	16	I	0.036	1	26.40**				
		II	0.010	and 50					

F_s = Variance ratio for slopes.

F_m = Variance ratio for adjusted means.

* Significant difference (p < 0.05).

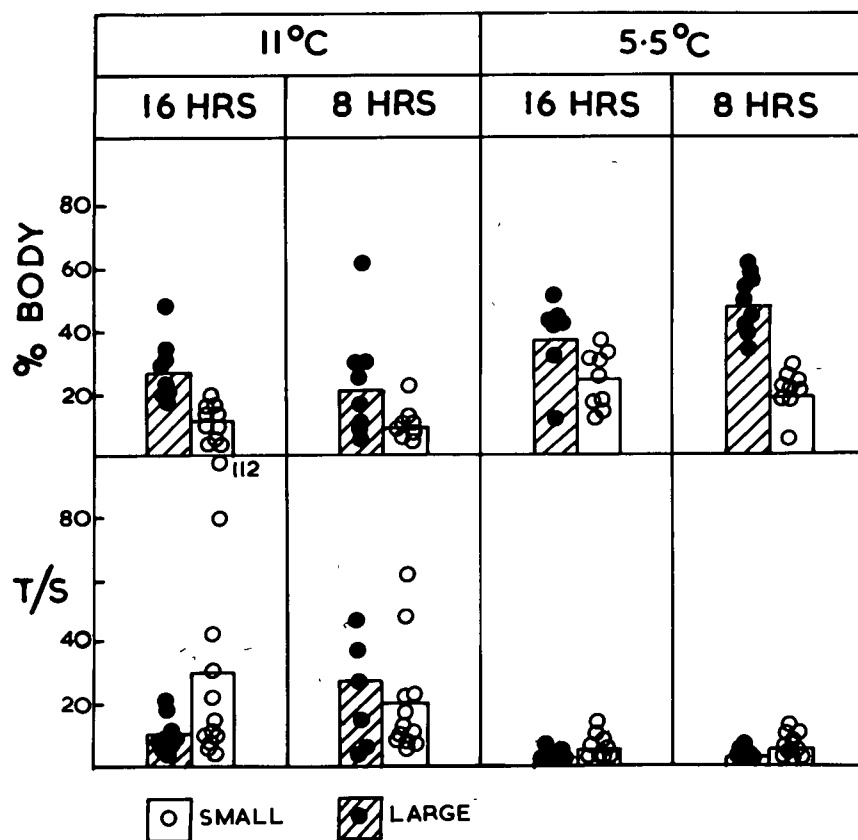
** Significant difference (p < 0.01).

a greater thyroxin demand, but an equally plausible hypothesis is that the changes in I^{131} metabolism under constant conditions were simply a function of increasing size.

V. EFFECT OF BODY MASS ON RADIOIODINE METABOLISM

To test the effect of body mass on I^{131} metabolism, two groups of yearling steelhead of the same age were taken in mid-June from the four holding conditions of combined temperature and photoperiod used above. One group from each condition consisted of 12 large fish (11-65 g, av = 19.0 g); these were injected with 0.02 ml of I^{131} (3 μ c). The other group consisted of 12 small fish (3.2-8.0 g, av = 5.4 g) and these were injected with 0.01 ml (1 μ c). All fish were killed 96 hours after injection. The percentage of the injected dose retained in the entire body was consistently different between the small and large fish, although both showed the expected dependence on temperature and lack of response to photoperiod (Fig. 17). The fact that large fish tended to retain injected I^{131} longer than small fish explains why fish held under constant conditions showed changes in iodine metabolism as the season progressed and the average mass increased. The tendency for increasing temperature to increase the rate of I^{131} loss was approximately balanced by the tendency for a growing fish to retain more iodine due to slower excretion. Thus the apparent anomaly in the yearling fish is explained on the basis of a change in body mass. At a low constant temperature, it induced a change that would otherwise not occur and under the increasing temperature the size

Fig. 17. Influence of body mass on T/S values and percentage of I^{131} in the total body 84 hours after a single I^{131} injection in 19-month steelhead.



effect almost exactly cancelled the stimulus of increasing temperature.

Measurement of T/S ratios in general supported the idea that I^{131} metabolism was more active in a smaller fish. As with I^{131} excretion, the independence of photoperiod and dependence on temperature were evident. With the exception of one condition, small fish had higher T/S ratios than large fish. Since no fish were held to 8 days, no CR values were available.

To verify some of the above findings, another experiment was conducted on 3 groups of yearling steelhead in September at 11° C (Table X). All fish were killed 8 days after injection to obtain CR values, lacking from the previous determinations. Again the rate of excretion and I^{131} accumulation were affected in the same way by body mass but two further important points emerged (Fig. 18): (i) the CR was also markedly stimulated in the smaller size range and (ii) with each of the 3 parameters of I^{131} metabolism there was a great size effect between 8 and 16 grams and little effect between 16 and 30 grams. The phenomenon may be explained in terms of a log body mass - log thyroid activity relationship. Both T/S and CR showed significant negative correlations when the data were transformed in this manner (Fig. 19). Such a transformation is characteristically used to show dependence of metabolic rate on mass and the fact that radioiodine parameters of thyroid activity conform to this relationship strongly suggests a general metabolic role of the thyroid. The excretion rate also showed a similar relationship with body weight (Fig. 19). Since extrathyroidal excretion

TABLE X

Summary of injection details for size experiment

No. of fish	Mass (g)	Vol. injected (cc)	I ¹³¹ (μ c)	μ c/g
13	8.17(4.40-10.50)	.02	1	0.122
4	15.87(12.72-20.00)	.04	2	0.126
9	29.28(19.00-41.20)	.08	4	0.136

Fig. 18. Influence of body mass (gram) on plasma I^{131} levels and T/S values (4 days after injection) and CR values (8 days after injection). Means and standard deviations are shown for large, medium and small size groups. No significant difference was noted between medium and large size groups for any I^{131} parameter, but both differed from the small group for all parameters ($p < .01$).

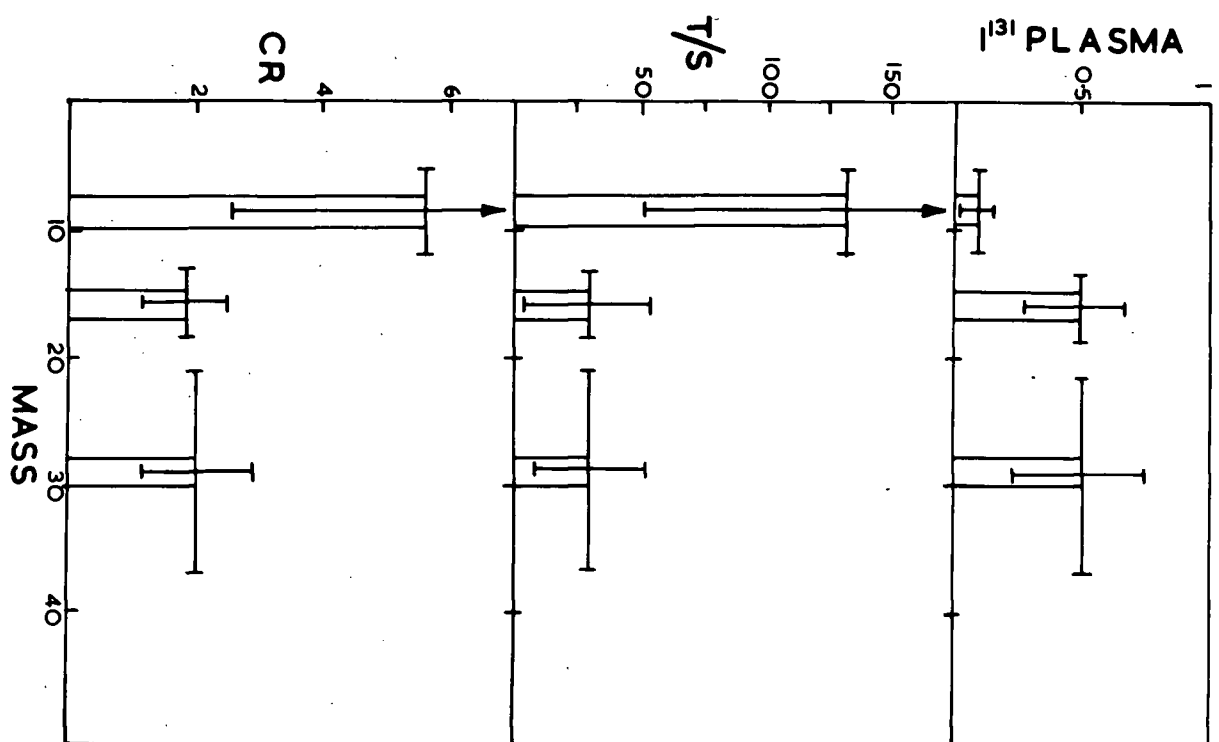
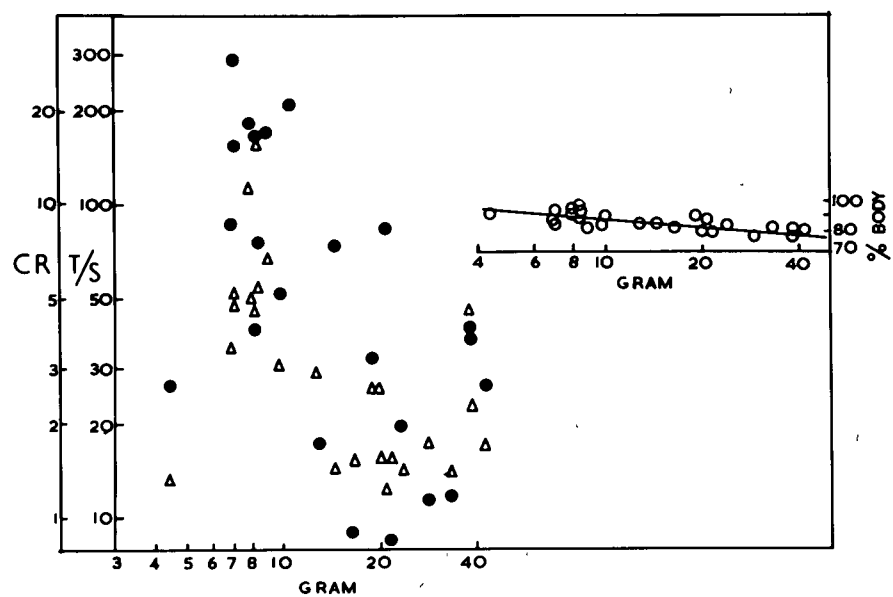


Fig. 19. Relationship between the logarithm of body mass and the logarithm of various ^{131}I parameters. T/S (Δ), $r = -0.57$; CR (\bullet), $r = -0.53$; % body (\circ), $r = -0.67$.



rate of I^{131} was independent of thyroid activity, its relationship to body size confirms that mass causes an overall change in metabolism. A similar dependence of I^{131} excretion on mass has been shown in Platichthys stellatus (Hickman, 1959) and in Gasterosteus aculeatus (Wiggs, 1962).

The apparent stabilization of the mass effect above about 16 grams was probably a result of the double logarithmic relationship whereby over this range a relatively great change in mass must be present for significant metabolic change to be observed. In smaller fish the reverse is true. A size exceeding 20 grams is therefore very desirable for experimental work as one is now working in an essentially mass-stable range.

VI. EXPERIMENTAL INVESTIGATION OF THE INFLUENCE OF SEASONAL CHANGE IN INCREASING PHOTOPERIOD AND INCREASING TEMPERATURE ON THE RADIOIODINE METABOLISM AND THYROID CELL HEIGHT OF TWO-YEAR-OLD STEELHEAD

By both histological and radiochemical criteria thyroid activity is very high in steelhead at the time of smoltification (Fig. 6) and appears far more active than would be anticipated on the basis of a temperature change alone. It is possible that this surge in thyroid activity is caused by the influence of increasing photoperiod. An attempt was made to test this hypothesis by repeating on two-year-olds the experiment previously carried out on yearlings under various photoperiod and temperature regimes (Table VII). The experiment was performed from late January until the beginning of June, a period covering the main

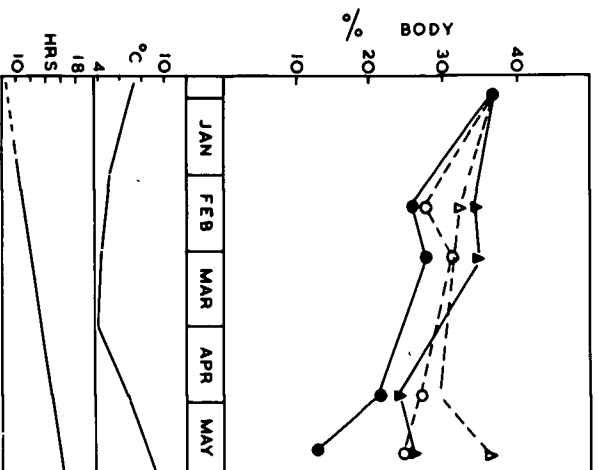
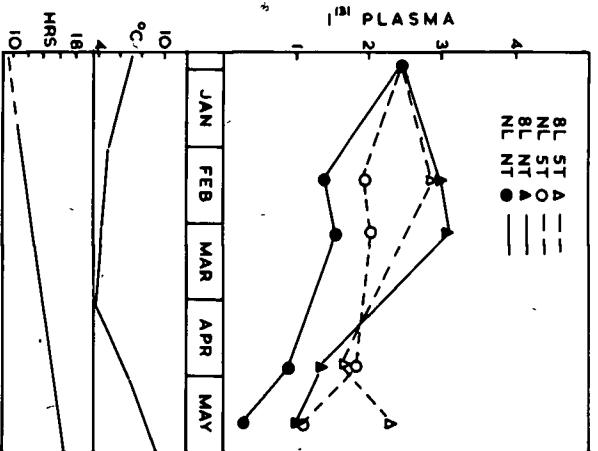
extent of increasing photoperiod and also the time of smoltification, which normally occurs in April and May. The usual I^{131} and histological parameters were measured.

1. I^{131} Excretion

This has been assessed by the percentage of the injected dose in body (4 days) and I^{131} plasma concentration (4 days). From the seasonal data (Fig. 6) it is seen that significant changes in I^{131} metabolism became evident towards the end of March. If photoperiod were having any effect on I^{131} metabolism it might be expected from this time onwards. From the end of March to the end of May the excretion parameters from the 4 conditions did conform to a general scheme (Fig. 20). With but one exception (not statistically significant) fish subjected to progressively increasing temperature showed a faster I^{131} loss than fish at the constant temperature of 5° C. In addition, fish subjected to the increasing photoperiod showed a significantly faster rate of I^{131} removal at any given temperature than those held under the constant 8-hour daylength (Table XI). The influence of the photoperiod on I^{131} excretion was not really as a trigger in the strict sense since it did not cause a sudden rise in excretion rate at a particular season. From early February onwards, the photoperiods were quite separable with the longer photoperiod causing the more rapid I^{131} elimination. The sudden rise in excretion rate in April and May was attributed to the influence of temperature which rose quite rapidly at this period and superimposed its influence on that of the photoperiod. As far as the excretion

4

Fig. 20. Experimental demonstration of the combined influence of temperature and photoperiod on plasma I^{131} levels and percentage dose in total body 4 days after I^{131} injection in 26 to 30-month steelhead (potential migrants). Each point represents a mean of 6 individuals (Statistics, Table XI).



rate is concerned it would appear that both temperature and photoperiod combined to induce the high rate of I^{131} loss characteristic of smolting fish. There was no indication of a synergism between temperature and photoperiod. By late May the effect of increasing photoperiod on fish held at $5^{\circ}C$ appeared equal to the effect of increasing temperature on fish from a constant 8-hour regime.

2. Thyroid Uptake of I^{131}

This was measured by T/S and TUF parameters (Figs. 21 and 22 respectively). For each parameter both 4-day and 8-day values were recorded. Since the fish sampled at 4 days were different individuals from those sampled at 8 days, this gives a "replication". As pointed out in the preliminary study of I^{131} metabolism the 4-day estimate is more reliable, as measurements later than this may be influenced by I^{131} loss from the thyroid as radiohormone.

In every instance for a particular temperature, the increasing photoperiod induced a higher thyroid uptake value than the constant 8-hour day. The data also showed that divergence between photoperiod influence became greater as the photoperiod differential increased; in May the T/S and TUF indices were high (Table XI). It may be concluded, therefore, that increasing photoperiod caused a progressive increase in thyroid uptake of I^{131} and that rising temperature superimposed a further increase. As with I^{131} excretion data, the condition of constant temperature and increasing photoperiod brought about a change in thyroid I^{131} uptake comparable to that in fish held under a

Fig. 21. Experimental demonstration of the combined influence of temperature and photoperiod on T/S values 4 and 8 days after I^{131} injection in 26 to 30-month steel-head (potential migrants). Each point represents a mean of 6 individuals (Statistics, Table XI).

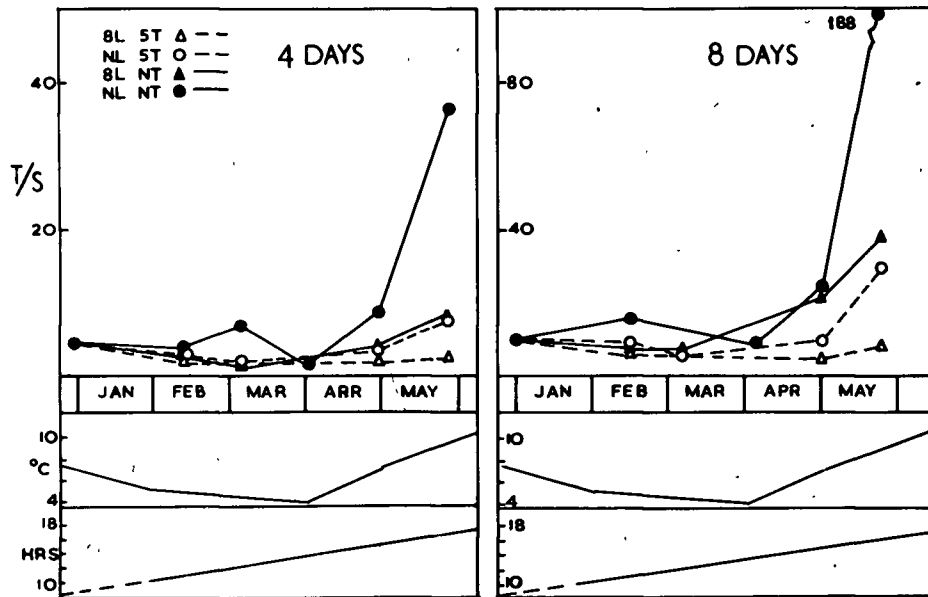
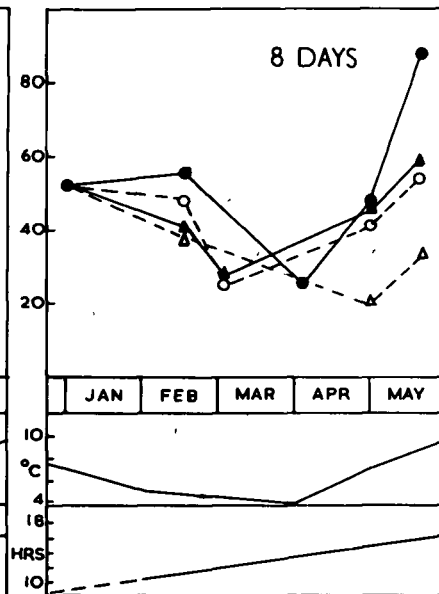
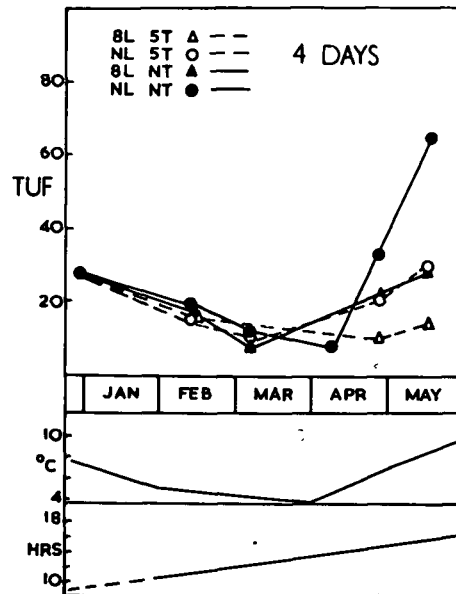


Fig. 22. Experimental demonstration of the combined influence of temperature and photoperiod on TUF values 4 and 8 days after I^{131} injection in 26 to 30-month steel-head (potential migrants). Each point represents a mean of 6 individuals (Statistics, Table XI).



constant 8-hour daylength but with rising temperature.

3. Conversion Ratio

This was measured 8 days after injection (Fig. 23). Although there were a few exceptions early in the year, the fish under increasing photoperiod for any given temperature showed a higher cell height than those under 8 hours. By May the separation was very evident and extremely high CR values were obtained as a result of rising temperature and increasing photoperiod. These differences were highly significant (Table XI).

I conclude from the I^{131} data on combined temperature and photoperiod influence on two-year-old steelhead, that increasing photoperiod stimulates progressive increase in rate of I^{131} excretion, I^{131} uptake by the thyroid and CR. It does not necessarily have a sudden action but is influencing the I^{131} metabolism over a long period. What appears to be a sudden change in I^{131} metabolism in April and May is due to the added stimulation by a temperature rise. Radioiodine metabolism is exponentially dependent upon temperature (Figs. 9 to 11). This relationship would tend to emphasize the response of the thyroid to rapidly rising temperature, and might give the impression of a synergism under the condition of increasing temperature and photoperiod. There is no definite evidence, however, for a synergism between these two important environmental factors. It must be noted that fish under constant 8-hour photoperiod and held at 5° C showed no tendency at all to change in any aspect of I^{131} metabolism. It is finally emphasized that the influence of increasing photoperiod on potential migrants was

Fig. 23. Experimental demonstration of the combined influence of temperature and photoperiod on mean cell height and CR (8 days after I^{131} injection) in 26 to 30-month steel-head (potential migrants). Cell height - means (○▲) and individual values (●▲) shown; CR - each point represents a mean of 6 individuals.

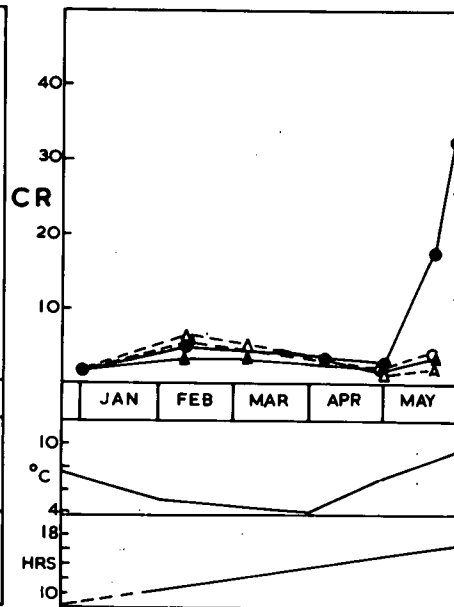
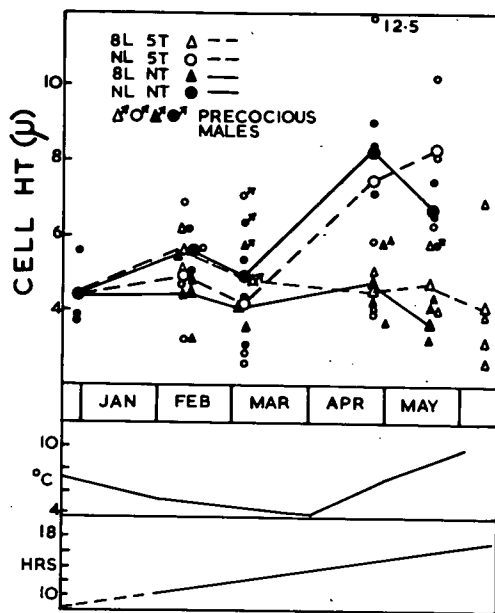


TABLE XI

Summary of statistically significant difference for several I^{131} parameters between different conditions of temperature and photoperiod in two-year-olds (potential migrants) in late May (White-Wilcoxon test)

	HIGH TEMPERATURE		LOW TEMPERATURE	
	Photoperiod increasing	Photoperiod 8 hours	Photoperiod increasing	Photoperiod 8 hours
I^{131} plasma (4 days)	— + —	— — —	— ? —	— — —
	— ++ —			
% dose body + % dose thyroid (4 days)	— + —	— — —	— — —	— — —
	— ++ —			
TUF (4 days)	— ++ —	— — —	— + —	— — —
	— ++ —			
T/S (4 days)	— + —	— — —	— ++ —	— — —
	— ++ —			
CR (8 days)	— ++ —	— — —	— + —	— — —
	— ++ —			

- Two groups not statistically different ($p = 0.05$).
- ? Two groups almost statistically different ($p = 0.05$).
- + Two groups significantly different ($p < 0.05$).
- ++ Two groups significantly different ($p < 0.01$).

completely absent in yearling non-migrant fish.

4. Cell Height

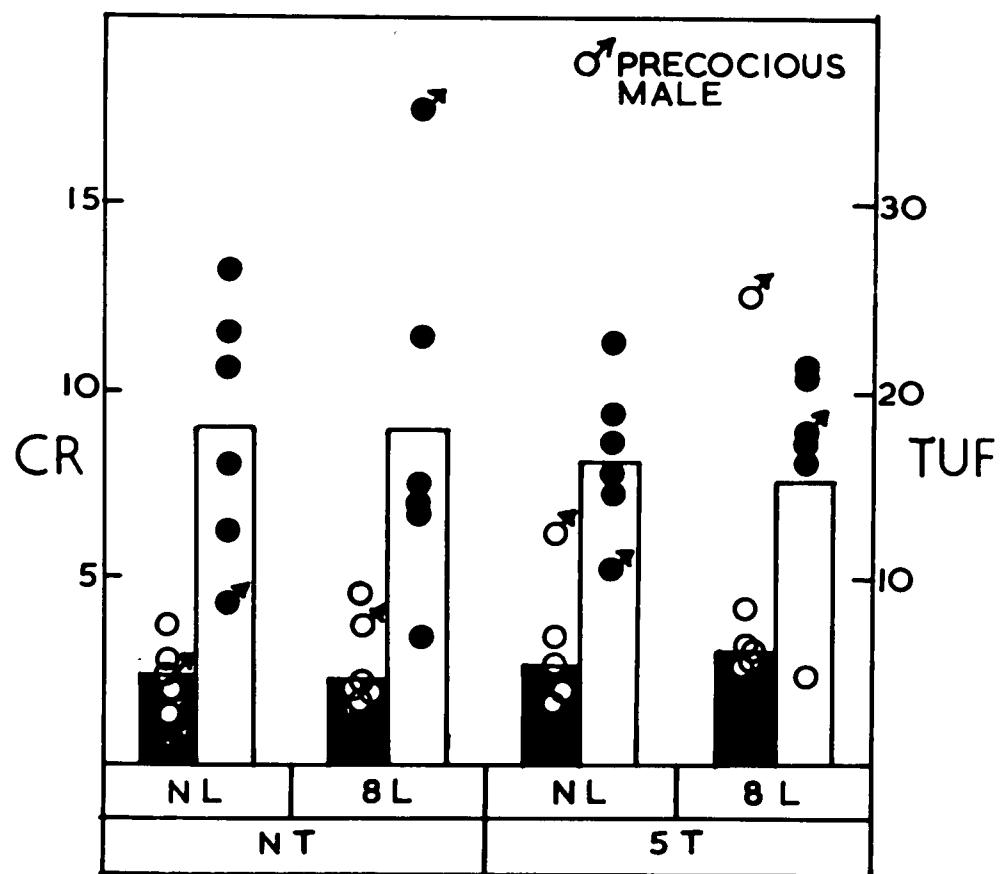
Measurement of cell height, though samples were small, showed a definite separation due to photoperiod between April and May (Fig. 23). No clear-cut effect of temperature on cell height could be demonstrated although in 8-hour photoperiod fish the lower temperature was generally associated with a taller cell height than the higher temperature. This effect of low temperature on cell height confirms earlier findings. In terms of colloid colour, it was difficult to recognise any differences at all between fish from the conditions.

VII. INFLUENCE OF PRECOCITY OF MALE PARR ON THYROID ACTIVITY

It was noted in early spring that up to 13% of the two-year-old steelhead sampled were precocious sexually mature males. These never showed the tendency towards silvering and preserved a modified parr coloration characteristic of spawning members of the species. Histological studies of thyroids of these parr revealed that cell heights were generally higher than in non-mature individuals (Fig. 23). This was particularly noticeable in early March and suggested that sexual maturation could be another variable affecting the thyroid activity. There are many instances in the literature associating increased thyroid activity with sexual maturation in teleosts. These have been summarized by Pickford and Atz (1957).

To investigate this hypothesis further, an examination was made of the TUF and CR data available from these same sexually

Fig. 24. TUF (open bars) and CR (shaded bars) for precocious two-year-old male (♂●) and immature (●●) steelhead in early March under 4 combined conditions of temperature and photoperiod.



mature individuals (Fig. 24). Although no definite conclusions could be drawn, certain sexually mature male parr did have thyroid activities considerably higher than the other members of their group. It is tentatively suggested that precocious sexual maturation in male parr is correlated with increasing thyroid activity. The causal aspects of this correlation were not investigated.

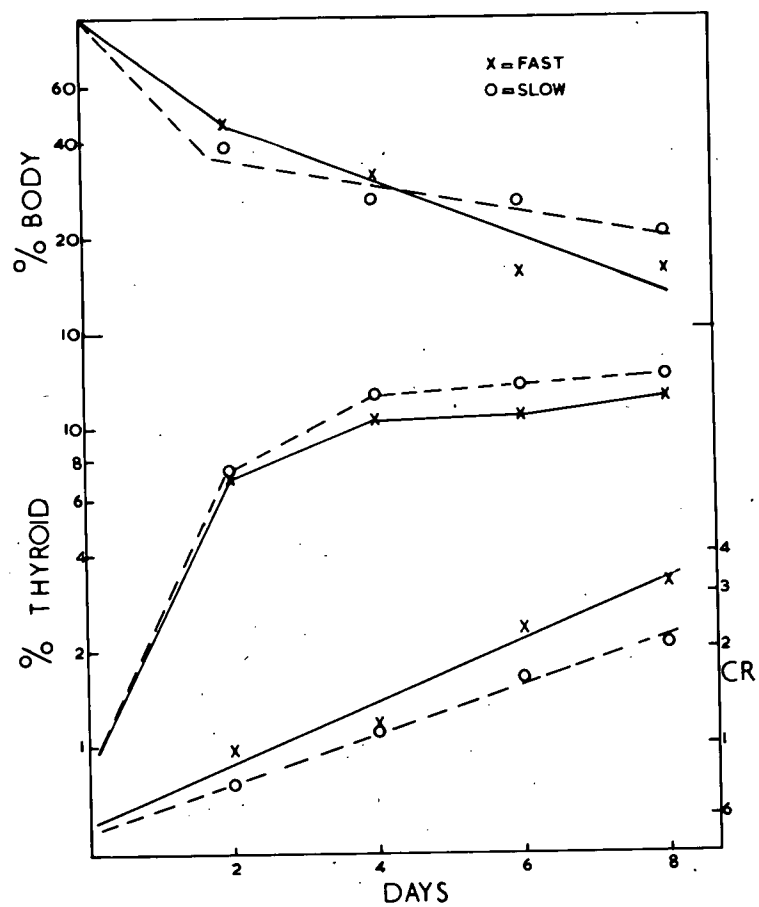
VIII. INFLUENCE OF EXERCISE ON I^{131} METABOLISM OF STEELHEAD

Mass, increasing photoperiod, temperature and possibly state of sexual maturation have been shown to influence annual cycles in thyroid activity. One other factor that could be contributing to cyclical changes in the natural environment is the water current. Fluvial fish, in general, maintain their position by rheotaxis. In a fast river the energy expenditure for maintaining position is presumably greater in a sluggish river and extra-metabolic demands could be made on the animal and reflected by increase in thyroid activity. Such an increase in thyroid activity in fast currents has been claimed for *Salmo gairdneri* (Fontaine, 1959).

In this experiment 48 fish were injected with I^{131} and immediately 24 of these were placed in a fast current and 24 in a slow current; the slow current was approximately 20% of the fast current. In both tanks the temperature was 10 to 10.5° C. At 2, 4, 6 and 8 days, 6 fish were killed from each condition (Fig. 25).

Three parameters were measured - percentage of the dose in

Fig. 25. Influence of increased swimming activity on various aspects of I^{131} metabolism in 19-month steelhead. Each point represents a mean of 6 individuals (Covariance analysis, Table XII).



total body, percentage of the dose in the thyroid and CR. They all showed significant differences between treatments (Table XII). The increased swimming caused an increased rate of extrathyroidal I^{131} excretion, a decrease in the total I^{131} uptake by the thyroid and an increase in CR. The increased CR indicates that radiohormone production is augmented, despite a decreased I^{131} uptake by the thyroid. The reduced accumulation of I^{131} in the thyroid is probably due to loss of radioactivity as hormone and would add support to the contention that muscular activity induces an increased demand for thyroxin. These findings agree with the general conclusions of Fontaine (1959).

IX. THE INFLUENCE OF CHEMICAL CHANGES (SALINITY AND IODINE
LEVELS OF THE MEDIA) ON I^{131} METABOLISM OF STEELHEAD
AND CHUM SALMON (*Oncorhynchus keta*)

Apart from the effects of physical aspects of the environment on thyroid activity and the influence of body mass and possibly sexual maturation, there is also the chemical environment to consider in these anadromous teleosts. Hoar (1959) surveyed the chemical factors that possibly influence the thyroid and concluded that the salinity and iodine concentration were most important. The influence of salinity was particularly pertinent here, in view of the various theories that have been put forward to explain the role of the thyroid in a potential migrant. The previous observations suggested the influence that various factors have on the thyroid, but it is now necessary to consider what this increased thyroid activity means in terms of

TABLE XII

Analysis of covariance for the relationship between various I^{131} parameters and time (hr) for parr held in fast and slow currents

Parameter	Condition	Slope	df	F_s	F_m
% dose total body	Calm	-.0031	1 and 48	7.5**	4.1*
	Torrent	-.0084			
CR	Calm	.0064	1 and 48	1.4	12.8**
	Torrent	.0083			

F_s = Variance ratio for slopes.

F_m = Variance ratio for adjusted means.

* Significant difference between slopes ($p < .05$).

** Significant difference between slopes ($p < .01$).

metabolism. This involves consideration of the various thyroid roles suggested for a migrating fish:

(i) Thyroxin stimulates certain changes in metabolism, such as silvering and salinity preference that occur at smoltification (Fontaine, 1954; Baggerman, 1960, 1963).

(ii) A fish prior to seaward migration tends to become demineralized. Such demineralization has been described for several species (reviewed by Hoar, 1959) and may be due to a variety of factors including the increased production of metabolic water or the functioning of certain salt elimination mechanisms prior to the actual entrance to the sea. Olivereau (1960) has shown a great increase in adrenocortical volume at this time of smoltification in Salmo salar. Hoar (1952, 1959) suggested that such changes in the internal environment could promote increased metabolic demands resulting in increased thyroid activity.

(iii) The final possibility stems from the demineralization described above. Namely, that under conditions of elimination of body salts the availability of iodine to the thyroid could become reduced, especially if the surrounding medium were deficient in iodine. Under conditions such as these, the fish would not be in iodine equilibrium. The thyroxin supply for the body would have to be produced despite progressively lower levels of available I^{127} . Active transport mechanisms would be relied upon even more for building up the iodine gradient between thyroid and plasma. If the gland were compensating for low plasma I^{127} levels in this way then it could appear active

by both histological and radiochemical criteria despite the fact that the actual output of hormone into the plasma had not really increased. In other words, the high thyroid activity of the smolt may be in part an artifact and merely a reflection of low available I^{127} induced by a rapid rate of I^{127} excretion. This would be supported by the data showing very rapid I^{131} elimination in steelhead smolts and other species at migration (Leloup and Fontaine, 1960).

In an attempt to evaluate these possibilities fish were kept in (i) fresh water, (ii) sea water (250/00 saline) and (iii) fresh water containing the same amount of I^{127} as 25 o/oo sea water (36 $\mu\text{g/l}$). By comparing the fresh-water and sea water fish the net effect of sea water on the the thyroid could be assessed. By comparing fresh water with I^{127} against sea water, the osmotic influence on the thyroid could be isolated since the iodine concentration would be the same in both media. As Hickman (1959) has pointed out, the I^{127} levels have to be made the same in both media since the level of I^{127} can alter considerably the metabolism of I^{131} . Finally, comparison of fish in fresh water with fish in fresh water containing iodine might indicate, by the response of the thyroid, whether the fish was living in iodine deficiency. These tests were carried out on both yearlings (parr) and fish of smolting age (smolts and parr).

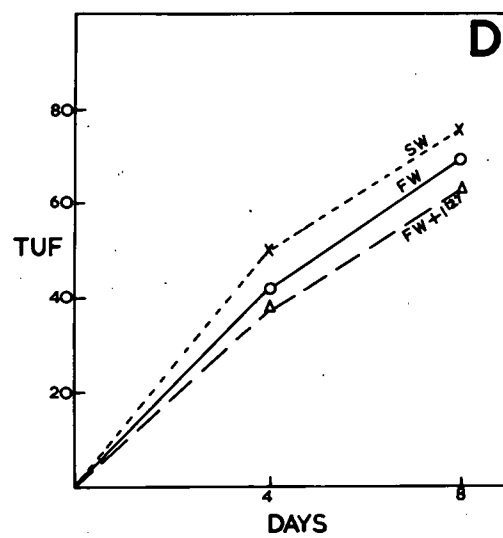
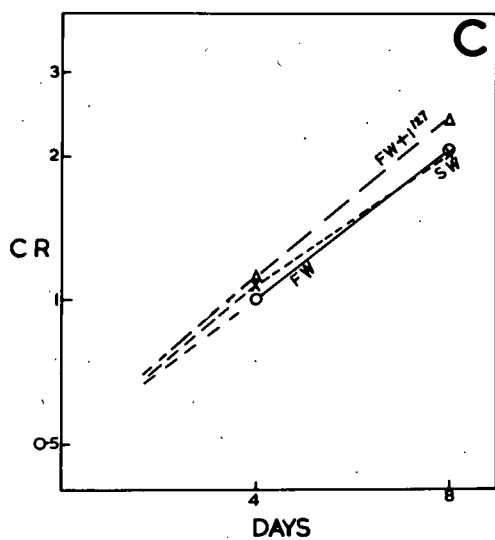
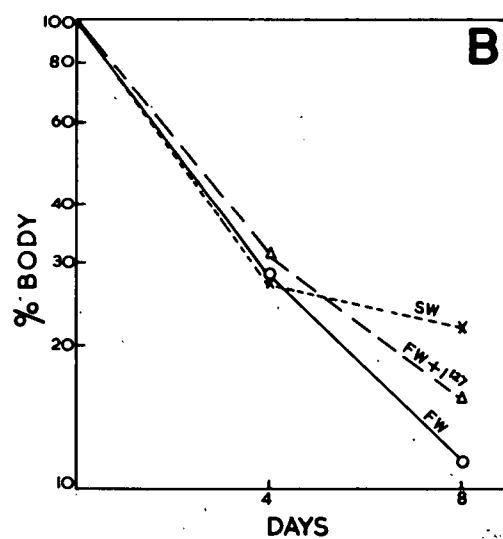
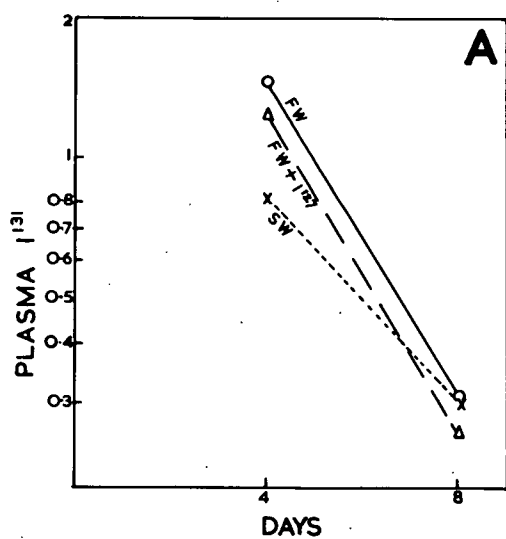
Yearling fish, all with very pronounced parr marks and no silvering, were placed in the three media in September at a water temperature of 13.6°C . They survived the transition whether

the introduction was direct or via intermediate salinities. After 10 days they were injected with I^{131} . Half of the fish were killed at 4 days; half were killed at 8 days.

A curvilinear relationship for I^{131} clearance was apparent in fish from all three media. The initial rates of loss of plasma I^{131} from fish held in iodine deplete and iodine replete fresh water did not differ. In sea water, on the other hand, this rate of loss was greater. In the second phase, the rates of loss were again similar in both fresh-water media but the sea water adapted fish showed a pronounced decrease in the rate of plasma I^{131} removal (Fig. 26). A similar difference between fresh water and sea water was noted with I^{131} elimination from the whole body. The more diphasic curves in sea water suggest that there is more than one mechanism of I^{131} elimination in the steelhead. Furthermore, since the curvilinear form was more pronounced in sea water, it is possible that the extrarenal excretory pathway is better developed in this medium. Possibly transfer to sea water increased general salt loss through the gills and reduced further the role of the kidney mechanism. The addition of I^{127} into the water itself does not affect the loss of I^{131} .

Addition of I^{127} appeared to reduce the thyroid I^{131} uptake (TUF) by a small amount and relative to this reduced activity in iodine reinforced fresh water, the enhanced TUF values in the sea water suggested that increased osmotic pressure may have stimulated the "iodine pump". The conversion ratio, however, showed no marked difference between the three conditions.

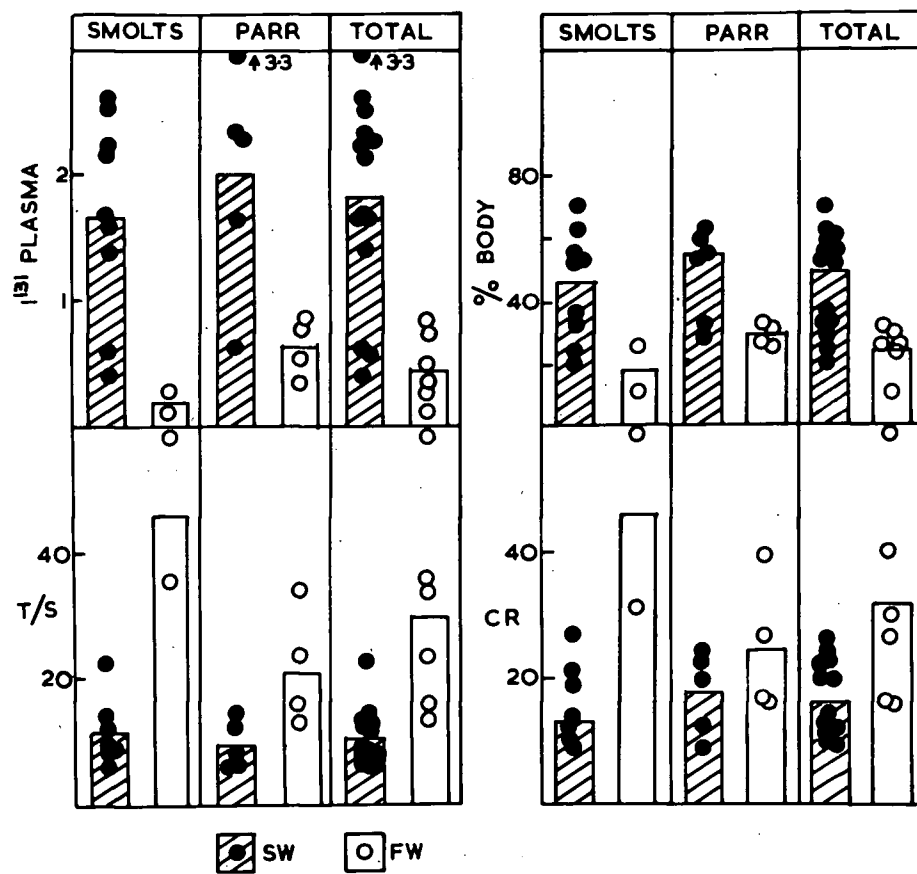
Fig. 26. Effect of transfer of 20-month steelhead to sea water (25 o/oo saline) and iodide-reinforced fresh water on various aspects of I^{131} metabolism measured 4 and 8 days after I^{131} injection. Mean values represented (SW, X----; FW + I^{127} , Δ --; FW, O—).



In late May the experiment was repeated at 8.5° C on potential migrants kept under the increasing photoperiod and increasing temperature regime. Unfortunately the temperature fell to a very low level (1° C) in the iodine control tank (F.W. + I¹²⁷) and in view of the temperature influence on I¹³¹ metabolism these data were discarded. Comparison could only be made, therefore, between fresh dechlorinated water and sea water (25 o/oo saline) (Fig. 27). For comparison, smolts have been treated separately from parr, though this breakdown considerably reduced the sample size and made a final critical examination difficult.

Certain important trends appear from the data. First, the extent of I¹³¹ loss, both total and extrathyroidal, was reduced in sea water while the parameters measuring the activity of gland, T/S and CR were lower than in fresh water. No difference between silvered or silvering fish and parr was noted. It was concluded that introduction into sea water of potentially migrating fish (parr or smolt) possessing a high thyroid activity considerably lowered thyroid hyperfunction and also reduced the former rapid rate of I¹³¹ excretion. It is inferred either that stabilization of plasma iodine at a higher level has inhibited the thyroid I¹³¹ accumulating efficiency, or that sea water itself lowered thyroid activity. A third possibility is that higher iodine levels in themselves reduced thyroid activity but that this trend was masked by the tendency for the increased osmotic pressure to stimulate a real increase in hormone output. Without controls in iodine reinforced water

Fig. 27. Effect of transfer of 30-month smolts and parr to sea water (25 o/oo saline) on various aspects of ^{131}I metabolism. Mean values (bar) and individual values shown.



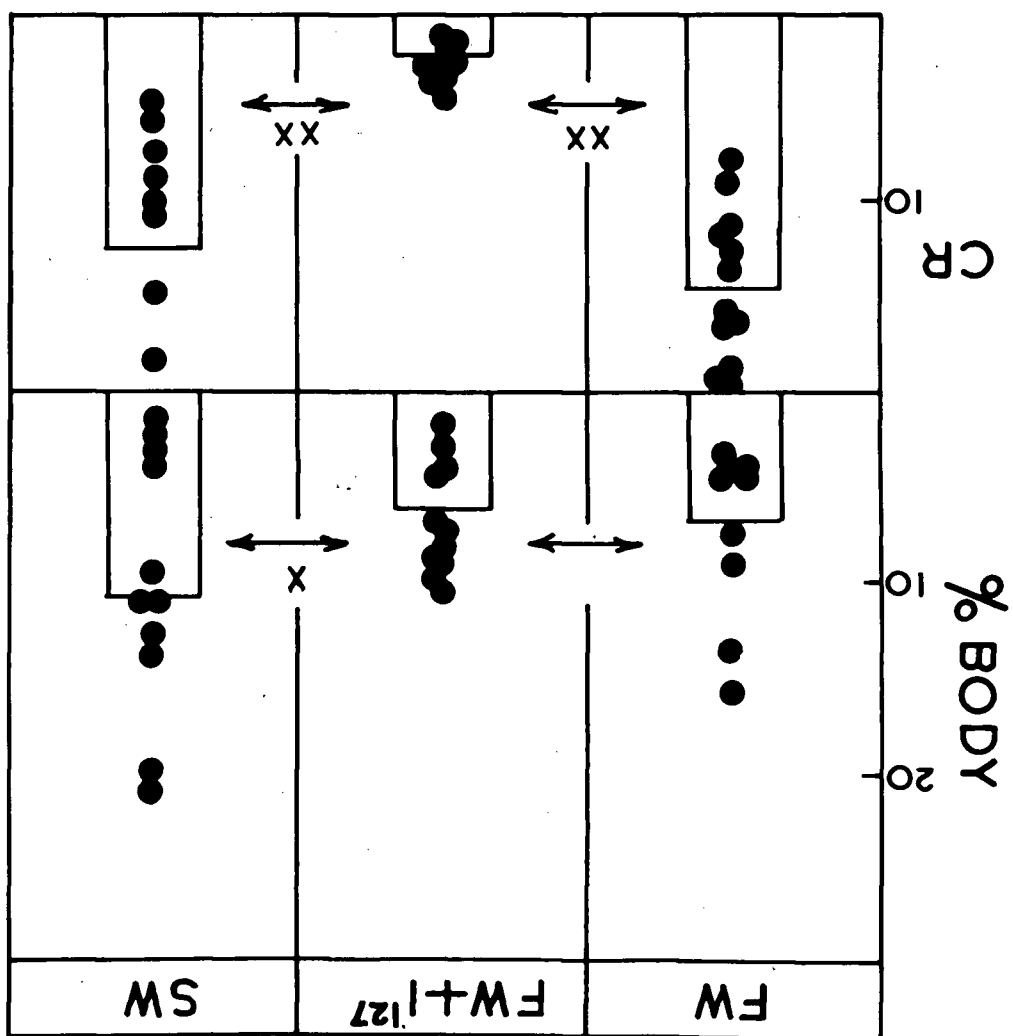
This issue cannot be resolved.

At this point in the proceedings the stocks of steelhead of migrant age were exhausted so the experiment could not be repeated. Chum salmon were available, however, in a more or less identical physiological condition. By mid-summer under-yearling chum salmon (which should have migrated in the spring) show very high rates of I^{131} metabolism when retained in fresh water. The biological half-life for the loss of serum I^{131} drops as low as 23 hours (Eales, 1961), while high thyroid uptake and the rapid production of very high CR values (60% or over in some cases) also indicates a very rapid I^{131} metabolism via thyroid routes. It has been suggested by Hoar (1952, 1959) and Eales (1961, 1963) that these fish may show in an exaggerated form the thyroid hyperactivity usually exhibited in a smolting fish. It was decided therefore to investigate the influence of sea water and change in iodine concentration on the chum salmon thyroid.

Twelve fish possessing thyroid activities of this kind were put into each of the following: (i) sea water (25 o/oo saline) (ii) fresh water with the same I^{127} content as 25 o/oo sea water and (iii) fresh water itself. The water temperature was 18° C. The fish were held under these conditions for 6 days and then injected with I^{131} and killed 108 hours after injection. At this time the extrathyroidal excretion and CR were measured (Fig. 28).

Stable iodine reinforcement, as with yearling steelhead, did not alter the I^{131} rate of excretion but lowered the CR

Fig. 28. Effect of transfer of underyearling potential migrant chum salmon to sea water (25 o/oo saline) and iodide-reinforced fresh water. Mean values (bar) and individual values shown $X = < 0.05$; $XX = < 0.01$.



greatly compared to the strictly fresh-water situation. In addition, salinity depressed I^{131} loss relative to either fresh water or iodine reinforced controls. This suggests that the reduced I^{131} excretion observed in sea water was due to the overall osmotic effect and not due to high ambient I^{127} . The conversion ratio in sea water was less than in fresh water although the influence was not as pronounced as with the migrant steelhead.

In these data on chum, the effects of iodine and osmotic change become much clearer. It appears that increased iodine levels caused a reduction in the CR. The hyperactivity of the thyroid as measured in fresh water by radiochemical methods could be abolished by a high ambient I^{127} level, even though the rate of iodine elimination from the body had not been altered. In sea water, however, thyroid activity was increased over the iodine controls and this revealed response to the osmotic change. As with steelhead yearlings, there was definite stimulation of the thyroid in a medium of high salt content.

If the migrant steelhead responded similarly to the chum in the iodine reinforced medium, it would be concluded that (i) the high level of I^{131} loss is reduced when the steelhead moves into the sea (ii) the high thyroid activity is partly due to a state of iodine deficiency and (iii) when the fish enters the sea its thyroid may again be activated due to the increased salinity. Whether the change from fresh to sea water produces an overall increase or decrease in thyroid activity would be due to several factors prominent among which would be the blood

level of I^{127} at the time of transition and also the response to the salt concentration.

Thus, although the net result of transfer from fresh water to sea water may be a drop in thyroid activity, analysis of the iodine and salinity influences generally show a depression in thyroid activity due to the increase in I^{127} level but a stimulation due to the salinity elevation. If the transition involves a net increase in thyroid activity then it can be argued that the stimulatory effect of salt water outweighs the response due to the increased iodine level.

The two groups of steelhead would show, therefore, some difference in this respect. In the yearlings, there was very little response to either medium but the iodine slightly depressed T/S ratios and the sea water caused slight stimulation. In the smolts, however, the net result was a depression of thyroid activity by all parameters and a marked thyroid inhibition by high iodine levels is suggested. This implies that the high thyroid activity in the smolt is partly due to an iodine deficiency.

X. THE RELATIONSHIP BETWEEN RADIOIODINE METABOLISM AND SILVERING

Thyroid hyperfunction in juvenile salmon is correlated with silvering and smoltification. Though certain experiments confirm this belief, there is considerable disagreement regarding the role of thyroxin. As noted previously, while thyroxin may stimulate subepidermal guanine deposition in the laboratory it may not be required under natural conditions. This seemed to be indicated by certain data obtained in this investigation. It

was noted that fish kept under an 8-hour daylength and at a temperature of 5 to 6° C showed no seasonal increase in thyroid function either with radioiodine or histological methods and yet certain of them silvered. Between the period 27 April to 29 May when investigations of thyroid function were being made of smolting fish from the four photoperiod and temperature regimes outlined in Table VII, a record was made of the state of smoltification of each fish (Table XIII).

Of the 4 categories (smolts, parr-smolts, parr and sexually mature males) considered, smoltification was not favoured by any one environmental regime. It is still possible that the smolts under any one condition might tend to have a higher thyroid activity than the parr under the same condition. To investigate this more fully, two-year-olds from the increasing photoperiod and low temperature regime were considered in greater detail as these constituted a larger sample of examined fish. Each aspect of I^{131} metabolism was treated separately in relation to the state of smoltification (Fig. 29). It is concluded that there was no consistent trend relating any aspect of I^{131} metabolism to silvering, and the causal relationship between thyroxin and subepidermal guanine deposition is therefore questioned.

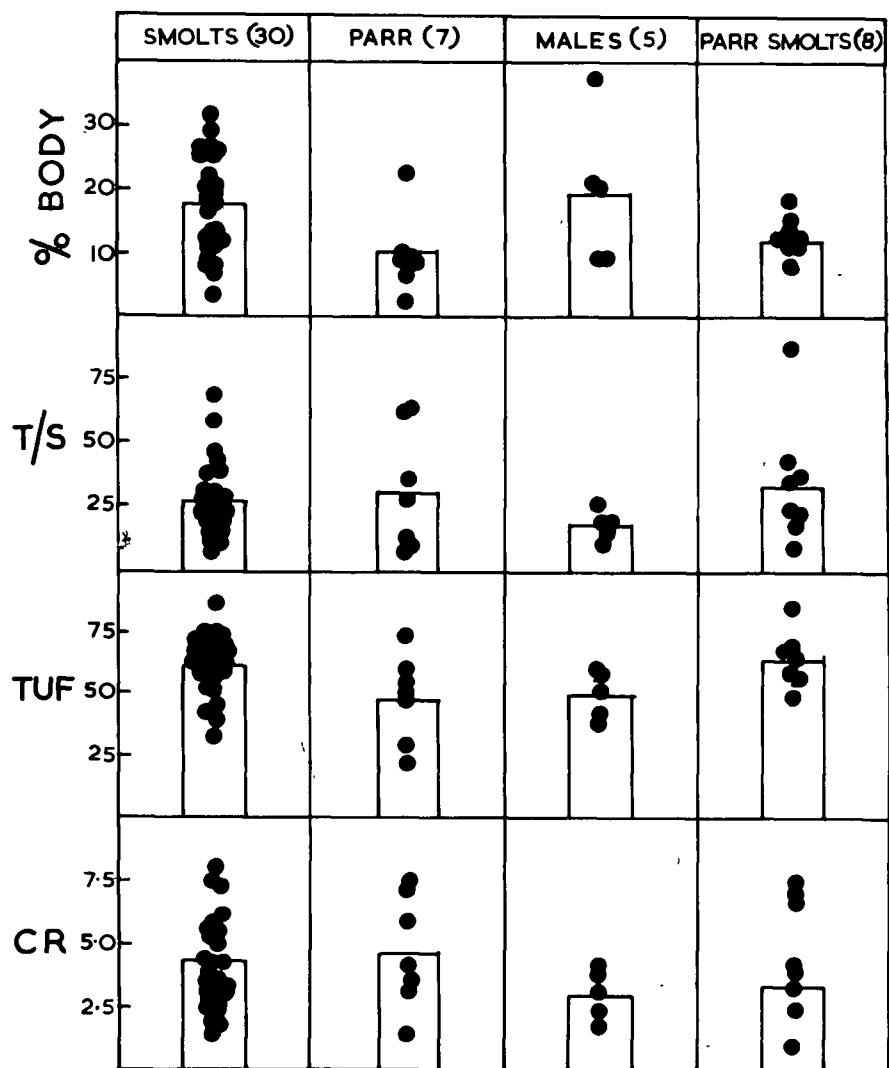
Of further interest was the constancy between the 4 holding conditions in the total number of steelhead showing any tendency to silver (smolts + parr-smolts). This was always between 65 and 75% of the total number examined in any one condition (Table XIII). According to Maher and Larkin (1954), approximately 60% of young steelhead trout from the Chilliwack River, British Columbia,

TABLE XIII

The effect of different temperature and photoperiod regimes on the percentage incidence of smoltification in two-year-old steelhead between 27 April and 29 May, 1962

	HIGH TEMPERATURE				LOW TEMPERATURE			
	Photoperiod increasing		Photoperiod 8 hours		Photoperiod increasing		Photoperiod 8 hours	
	No.	%	No.	%	No.	%	No.	%
Smolts (no parr marks)	24	40	7	28	39	58	7	30
Parr-smolts (Silver but with parr marks)	18	30	9	36	13	19	11	48
Parr (no silver)	10	17	7	28	10	15	3	13
Sexually mature male parr	8	13	2	8	5	8	2	9
TOTALS	60	100	25	100	67	100	23	100

Fig. 29. Demonstration of the lack of a relationship between silvering and any aspect of I^{131} metabolism (8 days after injection). Mean values (bar) and individual values shown.



migrate as two-year-olds at an average length of 16.49 cm. Both the length of the migrating fish and the percentage showing smoltification agreed favourably with the data from the laboratory-held fish considered above.

XI. PERIPHERAL SITES OF RADIOHORMONE CATABOLISM

Any attempt to clarify the role of thyroxin in teleosts will depend on knowledge of its site of action. So far the target organs of thyroxin in fish are unknown. One method would be to inject a sample of radioactive hormone and then follow its route into the various tissues. No pure radioactive thyroxin was available in the present study and so a less direct technique was employed.

When I^{131} is injected, the inorganic ion (I^{131}) very quickly reaches an equilibrium between the various body compartments. Thus as far as inorganic I^{131} is concerned, the ratio of

$$\frac{\text{tissue count/min/g}}{\text{blood count/min/g}}$$
 should remain constant for as long as there

is I^{131} in the body. Owing to the relatively rapid diffusion of the ion, departure from this ratio would only be noted in those tissues such as the thyroid which actively concentrate iodide.

It may also be argued that since I^{131} is the predominant radioactive constituent in the blood that the same ratio should hold between the total radioactivity in the tissues and blood, i.e.

$$\frac{\text{tissue } I^{131} \text{ count/min/g}}{\text{blood } I^{131} \text{ count/min/g}} = \frac{\text{total tissue radioactivity count/min/g}}{\text{total blood radioactivity count/min/g}}$$

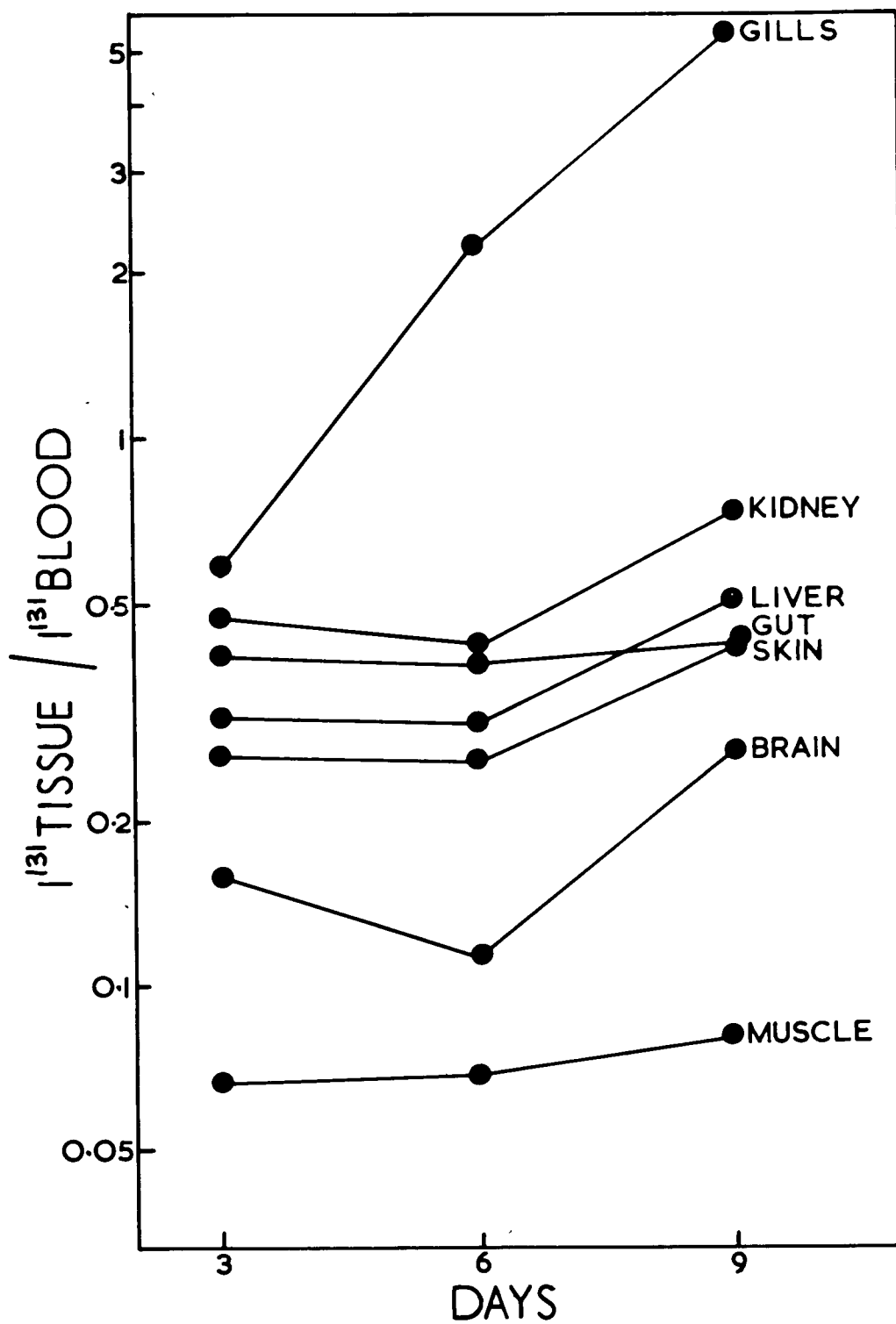
If this ratio based on total radioactivity in tissue and blood should change, it would imply a change between blood and tissue

of an organic I^{131} fraction. A rise in the ratio would mean accumulation of organically bound I^{131} in the tissues - presumably an accumulation of radiohormone. Thus by measuring the ratio of radioactivity of tissue to blood at various times after I^{131} injection some indication might be gained of sites of peripheral metabolism of radiohormone.

This was the theoretical basis for the experiment carried out below in which 48 fish (10-40 g) were injected with 4 uc I^{131} and one-third of this total killed at 3, 6 and 9 days. From each fish a small whole blood sample was removed in the usual manner and blown into a tared planchet. The planchet was reweighed and the whole blood digested for 2 days with one ml of 2N NaOH and counted at a distance of 4 cm from the end-probe scintillation counter for 3,000 counts. Weighed pieces of gill, kidney, skin, liver, gut, brain and muscle were digested and counted in a similar manner. In each fish the radioactivity per gram tissue was expressed relative to the radioactivity per gram blood. At each time period, 16 values for each ratio were obtained and means calculated (Fig. 30).

With the exception of the gill tissue, there was little change in the ratio between 3 and 6 days. No significance is placed on the absolute levels of radioactivity in the various tissues as this was undoubtedly due to greater vascularity of the tissue - if the tissue were completely vascular it would possess a ratio of exactly 1. Any increase in radioactivity above 1 means an active accumulation of iodine from the blood. This is found only in the gill tissue. For other tissues, the constancy of the ratio in general between 3 and 6 days would bear out the initial

Fig. 30. Change in the tissue: blood radioactivity in a variety of tissues from 19-month steelhead. Each point represents a mean of 14- to 16 individuals.

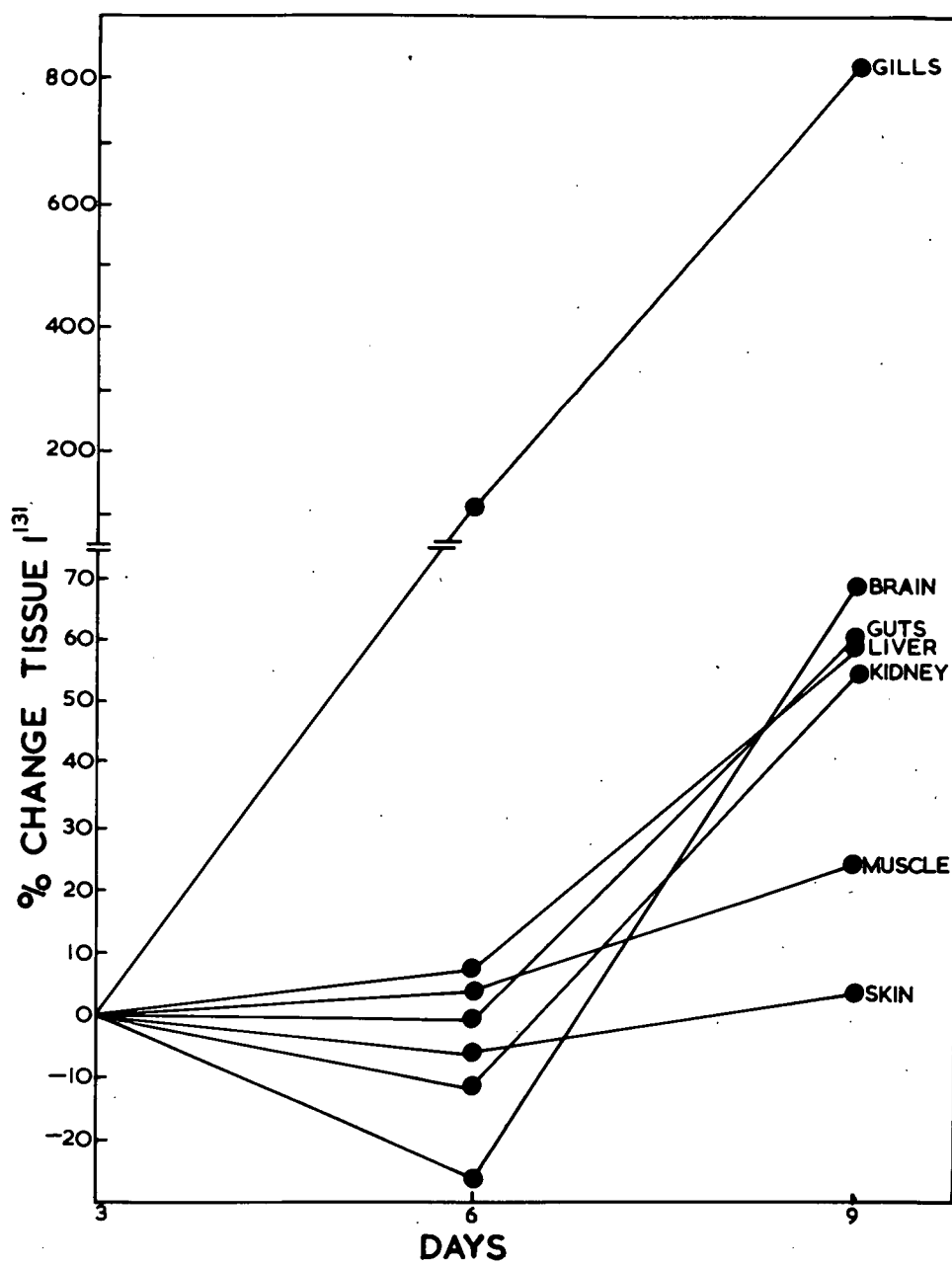


assumption that I^{131} moves rapidly between body compartments and provides support for the suggestion that any later change in the ratio would be due to change in distribution of organic fractions, which even at 6 days are only present in small quantities in the blood (Figs. 5 and 16).

The change in the ratio in the gill tissue is interesting and, in view of the small amount of PBI^{131} usually encountered in blood at 6 days, presumably involves an accumulation of I^{131} . This was also found by Hickman (1959) in the starry flounder and it was concluded by Hickman that the high I^{131} concentration there could be reconciled with the known function of the gills in salt excretion. However, while the data of Hickman showed a steady ratio between gill and blood 24 hours after injection, these data on steelhead indicate a progressive accumulation of radioactivity in the gill with time. No satisfactory explanation for this phenomenon can be given, unless the later high values were due to accumulation of I^{131} in a form other than the freely diffusible ion.

The tissue:blood I^{131} ratio changed between 6 and 9 days in all tissues, but was consistently most pronounced in the very metabolically active tissues, i.e. brain, gut, liver and kidney. Skin showed only a slight percentage increase and muscle only a moderate percentage change from 6 to 9 days (Figs. 30 and 31). That the rise in the tissue:blood ratio between 6 and 9 days is due to an accumulation of radiothyroxin is borne out by the observation that over this period, detectable amounts of PBI^{131} are released from the gland (Figs. 5 and 16).

Fig. 31. Percentage change (relative to the value 3 days after injection) of tissue: blood ratios 6 and 9 days after injection in 19-month steelhead.



It is therefore concluded that if change in

$$\frac{\text{total tissue count/min/g}}{\text{blood count/min/g}}$$

is indicative of build up of radioactive hormone in the tissue, then the highest accumulation was observed in brain, liver, kidney and gut. In all these tissues energy demands are probably high. In less active tissues such as muscle and skin, the PBI¹³¹ accumulation was less. This suggests that the thyroid hormone is not utilized in specific areas but probably required by any metabolically active system. It would argue against a specifically localized role of the thyroid in teleosts and implies a general metabolic role.

DISCUSSION

I. COMPARISON OF PARAMETERS FOR MEASURING THYROID ACTIVITY AND THE EFFECT OF TEMPERATURE ON THESE PARAMETERS

Several discrepancies between techniques for measuring thyroid activity may be cited. Swift (1959), simultaneously using histology and radiochemistry to measure seasonal change in thyroid activity in brown trout, demonstrated that the two techniques were not necessarily correlated. Olivereau (1955) showed that high temperature caused no histological change in the eel, but Leloup and Fontaine (1960) have shown a very significant stimulation of the thyroid by temperature with I^{131} techniques. Eales (1963) obtained some conformity between histological and radiochemical methods in two species of juvenile Oncorhynchus, coho and sockeye, but in two other species, pink and chum there was a complete disagreement. Other instances of disagreement have been cited by Matty (1960). It is emphasized that in general these discrepancies relate either to comparisons of thyroid activity at different temperatures or to seasonal changes probably induced by temperature differences. That disagreement between histological and radioiodine measurements may only occur when temperature is the main variable is very important for the explanation that follows.

In the present study, estimation of thyroid activity based on percentage I^{131} accumulation, can be entirely eliminated from further consideration as it does not allow for change in extra-thyroidal I^{131} loss. This disadvantage is eliminated by TUF and T/S which show close correspondence both in seasonal data

and in experimental temperature treatments. The same is true of the CR which conforms with the TUF and T/S index. Thus the behaviour of radioiodine at various levels of thyroid activity seems consistent but it does not necessarily correlate with the histological observations. This is strikingly evident where the experiment on temperature is concerned (Figs. 8 to 11). The CR was correlated positively with temperature while the cell height assessments showed a negative correlation. The colloid colour showed a positive correlation similar to the I^{131} data. Comparable discrepancies were demonstrated in the seasonal data, especially in late summer. The main question to be resolved is why the radiochemical data and colloid data should show increase in thyroid activity with rising temperature and the cell height decreasing activity with rising temperature. Is it possible to reconcile these two conflicting observations, bearing in mind that with respect to other environmental influences, cell height and radioiodine assessments generally agree?

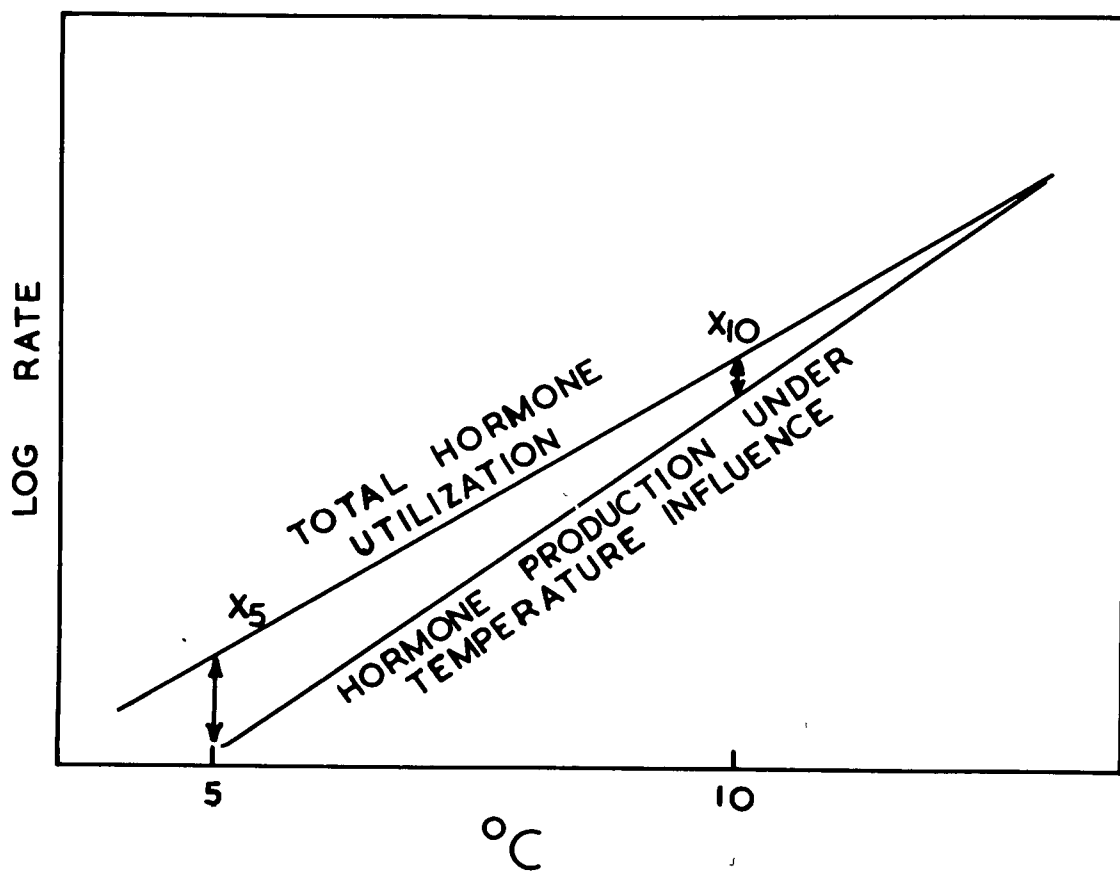
The following attempt to do this, rests on 3 assumptions - (i) cell height is controlled solely by TSH (ii) assuming no control from higher nervous centres, the release of TSH is controlled by a feedback mechanism that is dependent upon the blood hormone (thyroxin and probably triiodothyronine levels) and (iii) that with increasing temperature there is a greater need by the tissues for thyroxin. The latter is not an unreasonable assumption if thyroxin is involved in some basic aspect of cellular metabolism whose rate increases with temperature.

Consider a fish acclimated to a low temperature. It is a poikilothermic animal and the rate constants for many biochemical reactions would tend to be low. As the temperature is raised so is the thyroxin requirement. Due to the postulated increased peripheral metabolism of hormone, the blood levels would be depleted and the output of TSH augmented via the feedback. The cell height would become greater and so would the activity of the gland as measured radiochemically. However, in a poikilotherm one must assume that rate constants for all the body reactions will be increased by rising temperature and, rate constants determining the rate of operation of the thyroid will also be stimulated. If the stimulatory effect on the peripheral catabolism of thyroxin were simultaneously balanced by an identical stimulation of synthesis, then there would be no fall in blood levels and no increased release of TSH from the pituitary. If the same relationship were to hold over the entire temperature range then the increasing tissue demands for thyroxin would be completely compensated by the stimulation of its synthesis. Thus one would expect no change in histology with temperature but a marked stimulation where radiochemical techniques were concerned. As Oliverreau (1955a,b,c) has shown the following species reveal no histological change to temperature - Cyprinus carpio, Tinca vulgaris, Anguilla anguilla, Mugil auratus and Scyllium canicula. In at least one of these species, Anguilla anguilla, however, Leloup and Fontaine (1960) have shown by I^{131} methods an extremely marked positive response to rising temperature. All these examples would conform, therefore,

with the above theory that thyroxin production and consumption respond to temperature to the same degree.

Though such an ideal and economical temperature control of iodine metabolism may operate, it need not hold over the entire temperature range, nor for all species. For instance, there is evidence to suggest that thyroxin demands may be greater than thyroxin output at low temperature in goldfish (Hoar, 1958, 1959; Hoar and Eales, 1963). Also the data on Salmo gairdneri imply more TSH production at low temperatures when the cell height is greatest. This indicates that the enzymes controlling the thyroxin production and secretion are inhibited more by the temperature reduction than those governing its peripheral catabolism. Under these conditions the serum level of thyroxin would drop, TSH would be released and cell heights stimulated. This has been hypothetically represented (Fig. 32). From this figure, at 5° C, a high cell height (proportional to x_5) might be expected since, due to the effect of temperature alone, the level of hormone production is considerably less than its use. At 10° C, however, both output and peripheral utilization have increased, but to different degrees. Under these conditions thyroxin would build up in the plasma, and TSH production would be inhibited with a resulting reduction in cell height (proportional to x_{10}). Thus one would expect a greater cell height at lower temperatures, because the thyroxin producing system is inhibited more than the thyroxin utilization system. The ensuing low blood thyroxin levels would cause a production of TSH and increased cell height. In other species it is possible that blood thyroxin levels might

Fig. 32. Diagrammatic representation of the hypothesis that thyroxin utilization and production respond differentially to temperature. x_5 and x_{10} represent the differences between utilization and production at 5 and 10° C. Since x_5 is greater than x_{10} it would imply greater release of TSH at low temperatures and also a greater cell height.



drop at higher temperatures. These species would show increasing cell height with increasing temperature as demonstrated by Phoxinus phoxinus (Barrington and Matty, 1954).

The above hypothesis may also account for the anomaly reported by Hoar and Eales (1963). They showed that although thyroid activity measured radiochemically was much lower at low temperatures, TSH was still of importance in preventing cold death in goldfish. This could be explained by assuming that at low temperatures (i) thyroxin is needed in adequate amounts and (ii) due to a different effect of cold on thyroxin production and peripheral metabolic sites, thyroxin was being removed from the blood at a greater rate than it was being replenished. In addition, Hochachka and Hayes (1962) and Hochachka (1962) have shown that thyroxin treatment directs carbohydrate metabolism via the pentose shunt in Salvelinus fontinalis. They have shown also that, at low temperatures, the pentose phosphate shunt becomes more prominent. This suggests there may also be extra thyroxin demands at low temperatures in goldfish (Hoar and Eales, 1963). Under these conditions anything that stimulated the activity of the thyroid would enhance survival and the action of TSH is immediately understandable.

Both cell height and radioiodine are important in estimating thyroid activity under different temperature regimes. While the I^{131} consistently allows a dynamic analysis of thyroid activity, the cell height is dependent on TSH secretion. This TSH secretion may be stimulated via the hypothalamico-hypophyseal axis or by the plasma feedback mechanism. In the latter case this can give

rise to discrepancy between histological and radioiodine criteria. Although the histological change may measure relative differences between hormone production and consumption it can be unreliable for assessing the general activity of the gland.

Why the colloid should show changes paralleling the radiochemical assessments rather than cell height is not clear. From what is now known of thyroxin biosynthesis, it appears that apart from the initial uptake of iodine the remainder of the metabolic pathway is pursued in the colloid itself (Gorbman and Bern, 1962). The role of the cells is uncertain but they probably produce the colloid thyroglobulins. Since the colloid appears to be the main centre for iodination of tyrosine and release of the hormone, it is perhaps not surprising that its state should conform more closely with the radiochemical estimates.

Finally, I emphasize that the effect of temperature on iodine metabolism is probably unique among all the factors considered. It may be the only environmental factor that directly affects the thyroid tissue. All the remaining factors - sexual maturity, photoperiod and salinity might be expected to act more indirectly, presumably by changing TSH levels either via the feedback or via the hypothalamico-hypophyseal axis. Thus, in all instances except temperature stimulation, one would expect agreement between histological and I^{131} criteria. In general the literature reveals this to be true.

Before concluding the discussion on temperature, reference must be made to the work of Swift (1955, 1959) who concluded that in brown trout (Salmo trutta) (i) radioiodine assessments

of thyroid activity paralleled the cell height measurements and (ii) thyroid activity was inversely related to temperature. These conclusions directly oppose my own and since they are derived from a species very closely related to the steelhead they require closer examination.

Swift's conclusions based on cell height could be unreliable indications of thyroid activity if the thesis developed here on temperature influence is correct. Furthermore, Swift based his I^{131} estimates on the rate of loss of I^{131} from the head region between 6 and 72 hours. Yet, from my studies on the steelhead thyroid, maximal thyroid uptake of I^{131} is only achieved at 6 days. This is also the time at which detectable amount of PBI^{131} first appear in the blood. It is possible that Swift was measuring predominantly rate of extrathyroidal I^{131} loss, from the vascular head region and for this reason not measuring thyroid activity at all.

II. FACTORS CONTRIBUTING TO SEASONAL CHANGES IN THYROID ACTIVITY IN JUVENILE STEELHEAD

Seasonal change in thyroid activity has been interpreted solely in terms of radiochemical data. The important influence of temperature has been discussed at considerable length, but size, sexual maturation, photoperiod, iodine level of the environment and salinity also modify thyroid activity.

Size: The effect of size on I^{131} metabolism agrees with the work of Hickman (1959) and Wiggs (1962) and indicates a more rapid rate of radioiodine metabolism in smaller fish. That

the increased metabolism is due to the small size itself and not due to a phase of increased growth is indicated by several factors. Prominent among these is the qualitative conformity of the body mass and thyroid data to the double logarithmic relationship of body mass and tissue metabolism. This would suggest that the thyroid may have a very general role in metabolism and be related to the higher O_2 demands of smaller fish. This has been demonstrated in Platichthys stellatus by Hickman (1959). Further evidence against the higher thyroid activity of small fish being associated with periods of more active growth is the similar age of small and large fish used in these studies. This would mean that the small fish were stunted relative to the large ones and had a slower growth rate. If stunted fish had a higher thyroid activity than more rapidly growing fish, this would contradict the findings of Barrington (1963) that thyroxin stimulates growth in Salmo gairdneri. It lends further support to the idea that it is a change in body mass and not growth alone that is influencing thyroid activity in this investigation.

The influence of body mass, particularly on small fish, could explain the seasonal changes observed in certain species of juvenile Oncorhynchus where there was considerable departure from a temperature correlation (Eales, 1963). It could also have some bearing on the discrepancy noted by Swift (1955, 1959) between brown trout of different age classes.

Sexual Precocity in Male Parr: The relationship between sexual maturation and thyroid activity is one of the least controversial aspects of thyroid function. Thyroid activity,

with few exceptions, increases either by histological or radiochemical criteria over the period of sexual maturation. The most recent of many appraisals of this aspect of thyroid function is by Matty (1960).

This study supports the conclusion that the thyroid and gonadal activity are related. In two-year-old steelhead, irrespective of the immediate temperature or photoperiod condition, certain extremely high histological and radiochemical assessments of thyroid activity were noted in sexually mature male individuals. This additional influence on the thyroid activity must be recognised, but only at certain times of the year. Metabolic demands during sexual maturation would tend to be higher, and it is possible that the demands for thyroxin would also be increased. There is no evidence favouring the theory that thyroxin stimulates the onset of sexual maturity in precocious males.

Photoperiod: It seems conclusively demonstrated in this study that increasing photoperiod (January to June) induces no change in the thyroid physiology of yearling steelhead, but has a very definite effect on larger members of the same species precisely one year older (potential migrants). Whether size or age is important in potentiating response to photoperiod has not been revealed by this study. This would indicate that the increasing photoperiod is of importance in causing metabolic changes within the fish prior to migration. However, the exact status of the thyroid in the metabolic sequence has not been resolved. One possibility is that the hypothalamico-hypophyseal

systems receiving impulses from pineal or optic centres release TSH which stimulates thyroxin production which in turn causes changes in the smolt. Evidence for this is negligible and an equally realistic explanation is that increasing photoperiod induces increased metabolic demands (perhaps due to increased general activity) that secondarily bring about increase in thyroid activity via the feedback. A third possibility is that photoperiod acting via the pituitary and adrenal cortex (shown by Oliverreau in 1960) to be active in migrating smolts) affects mineral water metabolism to the extent that iodides are lost faster than they are replaced, so that the blood iodine level available to the thyroid is reduced. This possibility is considered in detail below.

Iodine Availability: As Hickman (1962) has emphasized, before valid comparisons can be made between I^{131} determinations, the fish must be in iodine balance with its environment, i.e. the amount of I^{127} entering the body and thyroid is equal to that leaving the body and thyroid. One situation in which this condition may not be satisfied is in an iodine deficient environment, where plasma I^{127} levels could be considerably reduced. As Leloup and Fontaine (1960) have shown, marine teleosts generally have a higher plasma I^{127} than fresh-water forms. This is attributed to the different amounts of iodine present in the two media. Robertson and Chaney (1953) demonstrated in Salmo gairdneri that the amount of ambient iodine directly determined the plasma iodine levels.

Since the thyroid uptake of I^{127} is directly dependent on

the plasma supply of I^{127} , any condition tending to lower plasma levels becomes limiting. If a certain minimal uptake of I^{127} is required by the thyroid to produce an adequate hormone supply, in order to maintain this level in the gland, the I^{127} gradient between thyroid and plasma becomes greater and more demands are placed on active transport mechanisms. This is the theoretical basis of the iodine deficiency goitre described for Salvelinus fontinalis (Marine and Lenhart, 1910), Salmo gairdneri (Robertson and Chaney, 1953) and for Salmo salar (La Roche, 1952). Under these conditions of low plasma iodine the gland increases its iodine concentrating efficiency by hypertrophy and hyperplasia. As the above authors demonstrated, this hyperplasia and hypertrophy can be completely removed by providing the fish with adequate iodine.

The same response to changing iodine levels could conceivably occur in less stringent circumstances. The higher the level of available iodine the less efficient the "iodine pump" has to be. None of the methods currently used in determining thyroid activity will distinguish true and apparent increases in thyroid activity. In the former condition, the net output of hormone is increased while under conditions of low environmental iodine, the iodine accumulation is more efficient but the net hormone production is not augmented.

There is evidence to suggest that many fresh-water fish are compensating for some measure of iodine deficiency. This can easily be demonstrated by observing the histological or radiochemical response by the thyroid when the available stable

iodine is increased. If these parameters do not change at all then adequate iodine is available; if they are reduced then this is indicative that part of what was originally being measured as thyroid activity was really only an adaptation to low iodine levels. There are many instances in the literature of iodine levels reducing thyroid activity in conditions not generally considered as goitrous. Hickman (1959) observed, in the starry flounder, that increase in the iodine level of the water decreased thyroid uptake and Berg, Gorbman and Kobayashi (1959) have demonstrated this for several fresh-water species. In no instance has addition of iodine increased either the histological or radiochemical activity of the gland.

In the present study, underyearling steelhead showed only a mild reduction in thyroid uptake on the addition of high levels of iodine to the medium. It is therefore concluded that at this state of development the ambient iodine level was more or less adequate. The same species tested at the time of smoltification showed a very significant depression of thyroid activity when introduced into sea water despite the fact that this treatment generally makes higher demands on the thyroid. It is concluded, therefore, that the smolt has very definite problem in obtaining adequate iodine. The experimental work on the chum salmon would support this. Since the water available to both non-migrant and smolting fish came from the same reservoir (Cleveland Dam) and the external availability of iodine was probably unchanged one must therefore investigate endogenous physiological changes. A change in efficiency of

I^{127} assimilation from the food could also have some effect, but this has not been considered further. A significant point to emerge was the extremely rapid elimination of injected I^{131} in steelhead at the time of migration. This was higher than at any other time of the year. Such a rapid loss of I^{131} was also found in Atlantic salmon smolts by Leloup and Fontaine (1960) and in several species of Pacific salmon (Eales, 1961). It is considered that this loss of I^{131} is a consequence of the general demineralization that has been observed in many diadromous species immediately prior to descent to the ocean (reviewed by Hoar, 1959). A fish living in waters already low in iodine could show a greater loss from the body than uptake from the medium i.e. plasma iodine levels would be low and thyroid compensation for this would occur as outlined above. Leloup and Fontaine (1960) contend that the rapid I^{131} loss is merely a reflection of a more active I^{131} metabolism via the thyroid. This study shows that this is not the case and that the bulk of the I^{131} lost from the body is via extrathyroidal routes.

On this basis one would anticipate low levels of I^{127} both in the thyroid and in the blood of a migrating salmon. Leloup and Fontaine (1960) have made a comprehensive study of the distribution of stable iodine in the various body compartments of the Atlantic salmon smolt and parr. While the thyroid iodine levels are much lower in smolts than in parr, the blood iodine in the smolt is actually higher. At first sight this argues against the above hypothesis, but Leloup and Fontaine have also shown that most of this I^{127} is loosely bound to protein. (This

is a binding different from that formed in PBI^{131} , since it is split by trichloroacetic acid). This effectively makes the I^{127} molecule much larger and less diffusible and would explain why although the injected non-bound I^{131} is rapidly lost, the smolt still has high plasma I^{127} levels. According to Leloup and Fontaine this binding mechanism, also present in steelhead, permits a store of iodine to be built up in the blood at smoltification. This is claimed to be of selective advantage due to the high thyroid activity at this time when the demands for iodine would be considerable. It is argued here, however, that a binding of I^{127} to plasma proteins does not necessarily increase the I^{127} plasma levels available for thyroid uptake. The very mechanism that renders I^{127} bound to proteins and therefore reduces its rate of extrathyroidal elimination may also render it unavailable for uptake by the thyroid. In other words the binding of proteins to iodide may decrease the free iodide available for thyroid uptake and may accentuate any tendency towards goitre.

One experiment to test this hypothesis would be to investigate the uptake of I^{131} from a pool of protein-bound I^{131} in the blood. If the hypothesis of Leloup and Fontaine were correct then (i) there would be a reduced rate of extrathyroidal loss of the protein-tagged I^{131} and (ii) because this I^{131} was being lost so slowly from the blood it would be taken up in increased amounts by the thyroid. Such a experiment was performed by Chavin (1956) on Carassius auratus. He injected one group of goldfish with I^{131} and one group with I^{131} bound to albumin. The excretion rate of the protein-tagged I^{131} was very slow but

the thyroid accumulation of radioactivity was no higher than with free I^{131} . This supports the present theory that binding of I^{127} to proteins does not necessarily augment the pool of iodine available to the thyroid. One can therefore conclude that the smolt may be in an iodine deficient condition despite the apparent abundance of I^{127} in its body. The very low levels of I^{127} , either organically bound or free, in the thyroid itself would support this. The binding of iodine to protein is possibly a fortuitous change occasioned by an increase at smoltification of a blood protein fraction that has an exceptional affinity for iodides.

It is concluded, therefore, that the fresh-water teleost thyroid activity can be influenced markedly by the plasma iodine level, and that this level in turn is dependent on the net result of both the uptake of I^{127} from the medium via the food or the water and on its total rate of loss from the body. It is suggested that the altered physiology of the smolt renders less iodine available to the thyroid and that this, to some yet unascertained degree, contributes to the high thyroid "activity" at smoltification.

Salinity: The principle object of the entire investigation was an analysis of factors contributing to seasonal change in I^{131} metabolism and thyroid function during the fresh-water phase of development. On this basis investigation of the effect of salinity appears inconsistent, as fish in fresh water (by definition) would never be exposed to such a situation. A study of response to salinity, however, could help to elucidate the

change in iodine metabolism at smoltification. It has already been indicated that this can be explained on the basis of iodine deficiency but the extent of this effect is not known and other theories put forward to explain this increase cannot be excluded immediately.

One suggestion, referred to earlier, is that a smolting fish possibly due to increased amounts of metabolic water and to changes in its ion retaining mechanisms prior to movement to the sea, has problems associated with salt and water balance (Hoar, 1952, 1959; Eales, 1961). Consequently, transfer to sea water should alleviate the osmotic problems encountered by the fresh-water smolt. Thus thyroid activity would be high in the smolt in fresh water where the metabolic demands are presumably great, but reduced in sea water. The data from both yearling steelhead and post-migrant chum where metabolic demands might be high in fresh water, however, show increased thyroid activity in sea water. This agrees with the findings of Hickman (1959) on the euryhaline flounder, Platichthys stellatus, where salinity increased both oxygen consumption and thyroid activity. It does not support the theory that thyroxin demands of the smolt are greater in fresh water due to osmotic stress.

In conclusion, many factors affect thyroid activity and contribute to the seasonal cycle of the steelhead. Universal among these is temperature and at all seasons the thyroid responds to this important variable. Superimposed on the basic response to temperature are the effects of other factors. Of these, body size is probably always exerting some effect. Thyroid activity

is logarithmically related to body mass. As a result of this relationship, appreciable effects on the thyroid are noted only over the lower size range and become negligible above 20 grams. At a restricted time of year, the state of sexual maturation (as shown here in precocious male parr) can place an extra demand on the thyroid. In general, the thyroid appears refractory to changes in photoperiod but potential migrants show a very definite response that is superimposed on a positive response to temperature in the spring. The physiological route by which the increasing photoperiod progressively exerts its effect is unknown. Finally, certain of these changes in thyroid may be apparent rather than real. Due to the effects of different factors on plasma I^{127} levels, the availability of iodine to the thyroid may be altered. It is considered that this effect may be particularly important in the smolt but negligible in the parr.

III. THE ROLE OF THE THYROID IN THE STEELHEAD

Any comprehensive theory concerning the thyroid role in steelhead must take into account that rising temperature, small size, sexual maturation and exposure to higher salinity all exert greater demands on the thyroid. Without exception all these factors have been associated with higher rates of metabolism in poikilotherms. This suggests that the thyroid is involved in some basic metabolic role. There is no evidence from this study to implicate a specific role of the thyroid in a diadromous teleost although several such roles have been suggested in the

literature. These relate to the role of thyroxin in inducing salinity preference (Baggerman, 1960, 1963), silvering (Robertson, 1949) and changes in glycogen metabolism (Fontaine and Hatey, 1950). Data presented earlier showed the independence of silvering on increased thyroxin output and I concluded that the stimulation of guanine deposition is a pharmacological effect of thyroxin, since increased thyroxin is not required in nature. Furthermore, thyroxin administration does not always produce silvering and thyroid extracts containing mainly iodine and thyroglobulin are often more potent. The same is true where experiments with thyroxin and thyroid extracts have been used to stimulate mobilization of glycogen characteristic of the smolt. In addition, though Baggerman (1963) obtained positive results with the effect of TSH on salinity preference and an inhibition of salinity preference with thyroid inhibitors in juvenile Oncorhynchus, she could obtain no change in salinity response with thyroxin alone (Baggerman, 1960).

Although such specific roles of thyroxin cannot be entirely eliminated, consideration of the better established general metabolic roles of thyroxin might be more profitable. Such a general metabolic role is in fact suggested by the data on sites of peripheral catabolism of radioactive hormone. All the metabolically active tissues showed a similar rise in hormone accumulation suggesting a systemic rather than an organ or tissue-specific action. Muscle with a lower metabolism than kidney, liver, brain or probably gut had a much lower accumulation of presumed hormonal radioactivity, while skin the most

metabolically inactive of all showed the least rise. It is concluded therefore that thyroxin is probably important to all cells of the body but that quantitative requirements of each will be geared to their rate of metabolism.

One of the principle reasons for not accepting a general role of thyroxin in metabolism, similar to that described in mammals, is the almost complete inability of thyroxin to stimulate oxygen consumption in poikilotherms. There are a few notable exceptions and the positive results obtained by Muller (1953) in the goldfish are best known. However, merely because thyroxin will not stimulate a change in metabolic rate does not mean that it has no metabolic role. It is possible that thyroxin plays a similar role at the biochemical level in both warm and cold-blooded vertebrates. In the former not only does thyroxin take part in the reaction but it is presumably a limiting factor to certain reactions and for this reason controls them. In the latter, however, though thyroxin may serve exactly the same subcellular role, it may be rarely the sole limiting factor. It is speculated that in such a situation increase in thyroxin level might not be able to cause an increase in metabolism because other vital substances were also lacking. However, under certain rather rare experimental conditions thyroxin might be the sole limiting factor. If this were the case (as could conceivably be true with Muller's work) then administration of thyroxin would allow metabolism to proceed at a faster rate and oxygen consumption would increase.

Such a passive role of thyroxin would probably be accompanied

by an equally passive form of control i.e. via the plasma feedback method postulated earlier. It is proposed that as a result of all the thyroxin-demanding processes (rise in body temperature, small size, sexual maturation, increased salinity and possibly increased exercise) plasma levels of hormone would be depleted and then reinstated via the hypophyseal feedback system. Where the effect of photoperiod is concerned it is tempting to suggest that this stimulates the thyroid directly via the hypothalamico-hypophyseal system. But there is no direct evidence for this and it is equally likely that it could either induce demineralization with secondary effects on the thyroid due to iodine deficiency. A third possibility is that lengthening photoperiod stimulates the smolt to higher levels of general activity that secondarily cause increased thyroxin production.

The general speculative conclusion is that thyroxin may subserve a very similar biochemical role in both poikilotherms and homiotherms. While in the latter, however, there are distinct advantages to its controlling metabolism by being a limiting factor in certain reactions, in poikilotherms this is not the case. Instead, the production of thyroxin may be governed solely by the demands of the tissues and its production controlled to a large extent, or entirely by the hypophyseal feedback mechanism.

SUMMARY AND CONCLUSIONS

- I. Reliable estimates of thyroid activity can be made using single terminal assessments of TUF, T/S or CR at 4 or 8 days after injection in juvenile steelhead.
- II. Seasonal changes in radioiodine metabolism revealed some correlation with temperature but smolts departed from this rule in having higher than anticipated values. Smolts had a histologically active gland but, in general, cell height showed a negative correlation with temperature.
- III. Experimental investigation of temperature on thyroid activity revealed a direct dependence of all aspects of I^{131} metabolism on temperature from 4 to 18° C. Histologically, however, the gland was more active at low temperatures.
- IV. Photoperiod exerted no effect on cell height or radioiodine metabolism of steelhead yearlings from January to July.
- V. Body mass had an important effect on radioiodine metabolism with small fish having the higher activity. The logarithmic dependence of thyroid activity on mass permitted neglect of this factor in fish over 20 grams.
- VI. Potential migrant steelhead examined from January to June showed a marked positive response to both increasing temperature and increasing photoperiod. The high smolt value was due to summation of the photoperiod and temperature response. There was no evidence

for a synergism between temperature and photoperiod influences.

- VII. Precocious sexually mature male parr in March frequently had a higher thyroid activity both by histological and radiochemical techniques (than immature forms).
- VIII. Exercise slightly increased the conversion ratio.
- IX. Salinity generally caused an increase in thyroid activity. Parr in mid-summer were only slightly sensitive to increased iodine levels in the medium but smolts and chum salmon showed a great reduction in thyroid activity when the iodine concentration of the medium was raised. This indicates that part of the high activity of the smolt is due to an iodine deficiency.
- X. Potential migrant steelhead held under conditions of low temperature and 8-hour daylength had unchanging thyroid activity. A normal percentage of these fish silvered and the role of thyroxin in smoltification is therefore questioned.
- XI. "Radiohormone" was shown to build up in several tissues but especially in those that were metabolically most active. Gill showed an accumulation of I^{131} that could be indicative of a role in ion exchange with the surrounding medium.

Integration of these findings suggests that environmental temperature and mass of fish always play a role in determining thyroid activity. However, in fish over 20 grams the latter can be discounted as an important variable. It is believed that

temperature is unique amongst the variables affecting the thyroid in that it may alter thyroid function without the mediation of TSH. This could explain discrepancies between histological and radiochemical data, observed in this study and in the literature.

Superimposed on the size and temperature influences may be thyroid responses to gonad maturation, increased exercise, salinity, photoperiod and iodine concentration of the water. It is suggested that gonad maturation, exercise and increased osmotic pressure all require metabolic work and an increase in thyroid activity would be anticipated. The response to increased iodine concentration is, however, an indication that part of what is often considered to be the activity of the gland is really a compensation whereby the thyroid "iodine pump" becomes more efficient at low iodine levels. In the smolt such a compensation seems very apparent. It is postulated that photoperiod could cause ionic imbalance in the fish and that low iodine levels develop at smoltification which lead to an apparent increase in thyroid activity.

In view of the lack of a specific role in silvering and its general accumulation in actively metabolizing tissues, it is concluded that thyroxin subserves a very fundamental biochemical role and that its production is probably largely governed by the demands of the tissues acting via a feedback mechanism.

BIBLIOGRAPHY

- Baggerman, B. 1960. Salinity preference, thyroid activity and the seaward migration of four species of Pacific salmon (Oncorhynchus). J. Fish. Res. Bd. Canada, 17: 295-322.
- Baggerman, B. 1963. The effect of TSH and antithyroid substances on salinity preference and thyroid activity in juvenile Pacific salmon. Can. J. Zool., 41: 307-319.
- Barrington, E. J. W. 1961. Metamorphic processes in fishes and lampreys. Am. Zoologist, 1: 97-106.
- Barrington, E. J. W. 1963. An introduction to general and comparative endocrinology. Clarendon Press, Oxford.
- Barrington, E. J. W. and Matty, A. J. 1954. Seasonal variation in the thyroid gland of the minnow, Phoxinus phoxinus L., with some observations on the effect of temperature. Proc. Zool. Soc. London, 124: 89-95.
- Berg, O., Gorbman, A. and Kobayashi, H. 1959. The thyroid hormones in invertebrates and lower vertebrates. Symposium on Comparative Endocrinology, edited by A. Gorbman. John Wiley and Sons, New York, pp. 302-319.
- Chavin, W. 1956. Thyroid distribution and function in the goldfish, Carassius auratus L. J. Exptl. Zool., 133: 259-279.
- Comar, C. L. 1955. Radioisotopes in biology and agriculture. McGraw-Hill Book Co., Toronto.
- Eales, J. G. 1961. A comparative study of iodine metabolism in juvenile Oncorhynchus. M.Sc. Thesis, Univ. of British Columbia.
- Eales, J. G. 1963. A comparative study of thyroid function in migrant juvenile salmon. Can. J. Zool., 41: (in press).
- Fontaine, M. 1954. Du determinisme physiologique des migrations. Biol. Rev., 29: 390-418.
- Fontaine, M. 1959. Influence de la nage a contre-courant sur le metabolisme de l'iode et le fonctionnement thyroïdien chez la Truite arc-en-ciel (Salmo gairdnerii Rich). C. R. Soc. Biol., Paris, 249: 343-347.
- Gaylord, H. R. and Marsh, M. C. 1912. Carcinoma of the thyroid in salmonid fishes. Bull. U. S. Bur. Fish., 32: 367-524.
- Gorbman, A. and Bern, H. A. 1962. A textbook of comparative endocrinology. John Wiley and Sons, New York.

- Gurr, E. 1953. A practical manual of medical and biological staining techniques. Leonard Hill Limited, London.
- Hagen, G. 1936. Die wichtigsten Endokrinen des Flusssaals. Thyroidea, Thymus, und Hypophyse im Lebenszyklus des Flusssaals (Anguilla vulgaris) vebst einigen Untersuchungen uber das chromophile und chromophobe Kolloid der Thyroidea. Zool. Jb., Abt. 2 Anat. Ontog., 61: 467-538.
- Hickman, C. P. 1959. The osmoregulatory role of the thyroid gland in the starry flounder, Platichthys stellatus. Can. J. Zool., 37: 997-1060.
- Hickman, C. P. 1961. The conversion ratio as a discriminatory test for thyroid activity in fish. Nature, 189: 1012-1013.
- Hickman, C. P. 1962. Influence of environment on the metabolism of iodine in fish. Gen. Comp. Endocrinol., Suppl., 1: 48-62.
- Hoar, W. S. 1939. The thyroid gland of the Atlantic salmon. J. Morphol., 65: 257-295.
- Hoar, W. S. 1952. Thyroid function in some anadromous and land-locked teleosts. Trans. Roy. Soc. Canada, 46: 49-53.
- Hoar, W. S. 1959. Endocrine factors in the ecological adaptation of fishes. Symposium on Comparative Endocrinology, edited by A. Gorbman, John Wiley and Sons, New York, pp. 1-23.
- Hoar, W. S. and Bell, C. M. 1950. The thyroid gland in relation to the seaward migration of Pacific salmon. Can. J. Res., 28: 126-136.
- Hoar, W. S. and Eales, J. G. 1963. The thyroid gland and low temperature resistance of goldfish. Can. J. Zool., 41: (in press).
- Hochachka, P. W. and Hayes, F. R. 1962. The effect of temperature acclimation on pathways of glucose metabolism in the trout. Can. J. Zool., 40: 261-270.
- Hochachka, P. W. 1962. Thyroidal effects on pathways for carbohydrate metabolism in a teleost. Gen. and Comp. Endocrinol., 2: 499-505.
- Honma, Y. 1959. Studies on the endocrine glands of a salmonid fish, ayu, Plecoglossus altivelis. 1. Seasonal variation in the endocrines of the annual fish. J. Fac. Sci. Niigata Univ. (Ser. II), 2: 225-233.
- La Roche, G. 1952. Effets de preparations thyroïdiennes et d'iodures sur le goitre ("pseudo-cancer) des salmonides. Revue Canadienne de Biologie, 11: 439-445.

- Leloup, J. and Fontaine, M. 1960. Iodine metabolism in lower vertebrates. Ann. N. Y. Acad. Sci., 86: 316-353.
- Lieber, A. 1936. Jahreszyklus der Schilddrüse von Misgurnus fossilis. Z. wiss. Zool., 148: 364-400.
- Maher, F. P. and Larkin, P. A. 1954. Life history of the steel-head trout of the Chilliwack River, British Columbia. Trans. Am. Fish. Soc., 84: 27-38.
- Marine, D. 1914. The rapidity of the involution of active thyroid hyperplasias of brook trout following the use of fresh sea fish as food. J. Exptl. Med., 19: 376-382.
- Marine, D. and Lenhart, C. H. 1910. Observations and experiments on the so-called carcinoma of Salmo fontinalis and its relation to ordinary goitre. J. Exptl. Med., 12: 311-337.
- Matty, A. J. 1960. Thyroid cycles in fish. Symp. Zool. Soc. London, 2: 1-15.
- Muller, J. 1953. Über die Wirkung von Thyroxin und Thyreotropem Hormon auf den Stoffwechsel und die Färbung der Goldfisches. Z. Vergl. Physiol., 35: 1-12.
- Olivereau, M. 1955a. Influence de la temperature sur le fonctionnement thyroïdien de la Truite etudie a l'aide de l'iode radioactif. C. R. Soc. Biol., Paris, 149: 536-539.
- Olivereau, M. 1955b. Influence de la temperature sur l'histologie thyroïdienne de divers teleosteens. Bull. Soc. Zool. Fr., 80: 43-52.
- Olivereau, M. 1955c. Temperature et fonctionnement thyroïdienne chez les poissons. J. Physiol., Paris, 47: 256-258.
- Olivereau, M. 1960. Etude volumetrique de l'interrenal anterieur au cours de la smoltification de Salmo salar L. Acta Endocrinologica, 33: 142-156.
- Pickford, G. E. 1953. A study of the hypophysectomized male killifish, Fundulus heteroclitus. Bull. Bingham Oceanogr. Coll., 14: 5-41.
- Pickford, G. E. 1954. The response of hypophysectomized male killifish to prolonged treatment with small doses of thyrotropin. Endocrinology, 55: 589-592.
- Pickford, G. E. and Atz, J. W. 1957. The physiology of the pituitary gland of fishes. N. Y. Zool. Soc., New York.
- Robertson, O. H. 1948. The occurrence of increased activity of the thyroid gland in rainbow trout at the time of transformation from parr to silvery smolt. Physiol. Zool., 21: 282-294.

- Robertson, O. H. 1949. Production of the silvery smolt stage in rainbow trout by intramuscular injection of mammalian thyroid extract and thyrotropic hormone. J. Exptl. Zool., 110: 337-355.
- Robertson, O. H. and Chaney, A. L. 1953. Thyroid hyperplasia and tissue iodide content in spawning rainbow trout: a comparative study of Lake Michigan and California sea-run trout. Physiol. Zool., 26: 328-340.
- Snedecor, G. W. 1956. Statistical methods. The Iowa State College Press, Ames, Iowa.
- Steel, R. G. D. and Torrie, J. H. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., New York.
- Swift, D. R. 1955. Seasonal variations in the growth rate, thyroid gland activity and food reserves of brown trout (Salmo trutta L.). J. Exptl. Biol., 32: 751-764.
- Swift, D. R. 1959. Seasonal variation in the activity of the thyroid gland of yearling brown trout, Salmo trutta L. J. Exptl. Biol., 36: 120-125.
- Swift, D. R. 1960. Cyclical activity of the thyroid gland of fish in relation to environmental changes. Symp. Zool. Soc. London, 2: 17-27.
- Wiggs, A. J. 1962. Some factors affecting radioiodide metabolism in the threespine stickleback. M.Sc. Thesis, Univ. of British Columbia.
- Zaitzev, A. V. 1955. A histological investigation of the annual changes of the thyroid gland of the pike, and the neurosecretory activity of the hypothalamic nuclei in the seasonal change of the thyreotropic function of the hypophysis. Dokl. Akad. Nauk, U. S. S. R., 104: 315-318 (In Russian, quoted from Pickford and Atz, 1957).