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THE EFFECT OF STRESS AND FOOD DEPRIVATION ON THE THYROIDAL STATUS OF PLAICE AND OF RAINBOW TROUT

by

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Summary

The changes in the concentrations of thyroid hormones in the blood plasma of the plaice and of the rainbow trout following the stress of their capture and adaptation to aquaria have been examined. It has been found that, in the plaice but not in trout, there occurs a transitory rise in the thyroxine concentration; in both species, a long lasting reduction ensues which is in excess of that expected to result from a stress induced dilution of the blood plasma. This reduction does not reflect any disfunction of the thyroid gland but arises as a result of a reduction in the output of thyroid stimulating hormone by the pituitary.

Food deprivation induces a reduction of thyroid hormone levels which is less marked for thyroxine (T_4) than for its metabolite triiodothyronine (T_3). The effect of such reduction in hormone levels is to make the fish relatively inactive and unresponsive to external stimuli. Administration of thyroid stimulating hormone (TSH) restores normal thyroid status and states of activity and arousal.

Introduction

Major changes occur in the blood chemistry of fish following their capture from the wild. These include increases in the concentrations of the fright and stress hormones, catecholamines and corticosteroids, together with increases in plasma glucose levels and in lactic acid.

Other changes take place which have little adaptational significance. Thus fish confined in tanks, post capture, may be resistant to further stimulation, failing to become hyperglycaemic when subjected to fright or to release lactic acid when exercised. Such fish are held to be stressed, (Wardle, 1972).

The experiments described in the present communication were designed to provide information on possible changes in thyroidal status in the plaice and in the rainbow trout following capture and tank adaption stress and on changes in hormone status resulting from food deprivation.

Materials and Methods

Biological material

Adult plaice Pleuronectes platessa were caught either by trawl or seine net off Aberdeen, and a small (0.5 ml) blood sample was taken immediately by syringe from the renal portal vein. The fish were then held in tanks on the fishing vessel and subsequently transferred to large tanks in the laboratory. They were offered a diet of chopped squid but it is doubtful whether all the fish fed.

Rainbow trout Salmo gairdneri, average weight 200 g, were taken from a local hatchery and blood samples taken immediately from (a) stressed fish, (b) fish anaesthetised with MS222 and (c) fish that were unanaesthetised. All samples were taken by syringe from the renal portal vein. The fish were then either left in a cage at the hatchery or brought back to a large laboratory tank in plastic bags, the head spaces of which were filled with oxygen. They were maintained on a diet of commercial trout pellet and sampled at further intervals, with and without anaesthesia, by the syringe technique. To assess the effects of starvation on thyroid hormone levels in the rainbow trout two groups of fifteen trout from the same hatchery were held in identical aquaria. One group received no food, the others were fed ad libitum with trout pellets. Blood samples were taken, by syringe, after stunning, at the end of the experiment (70 days).

Miscellaneous techniques

Plasma samples were analysed for the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) by the chemically specific, gas chromatographic procedure described earlier (Osborn and Simpson, 1973).

Haematocrits were measured by centrifugation for 10 min. in a Hawksley micro-haematocrit centrifuge.

Bovine TSH (Koch Light Laboratories) was injected into the renal portal vein in a diluent of composition: Phenol 15 mg; Glucose 250 mg in water 5 ml water pH 2.1.

Results

Plaice

Two identical experiments were carried out in 1969 and 1972, the results of which are shown in Figs 1, 2 and 3.

It is apparent that T_3 levels fell within 2-3 days to 25-40% of their values immediately after capture and remained depressed. In some cases, noticeably in the second experiment, there was a slight rise in T_3 values before the inevitable fall.

The results for T_4 were rather more variable. Although in all the fish studied T_4 levels finished at approximately 25% of their initial value post capture, in some fish there was a transitory rise in T_4 levels shortly after capture. It is significant that in those fish in which T_3 levels were also raised at this time, the rise in T_4 levels was relatively large.

Haematocrits determined in the second experiment showed a fall from a mean value of 36 at zero time to means of 20 and 13 after 24 hours and 72 hours respectively. Applying the correcting formula (Simpson, unpublished)

$$\frac{P_n}{P_o} = \frac{(x_1 V_o - x_1 v_1 - x_2 v_2 \ldots\ldots - x_n v_n)(100 - x_{n+1})}{V_o\ (100 - x_1)\ x_{n+1}}$$

where V_o and P_o are respectively the whole blood and plasma volumes at time 0, x_n and v_n are the haematocrit and volume of blood removed at the nth sampling while P_n is the plasma volume achieved after equilibration following the nth sample, the plasma volume is calculated as having increased by factors of 1.44 and 3.7 after 24 hours and 72 hours respectively.

Rainbow Trout

In this experiment, one group of fish was stunned immediately after capture (zero time, control group) and a blood sample taken. A second group was sampled under anaesthesia and a third group without anaesthesia. Half the fish in the second and third group were left in a cage at the hatchery and the remaining fish brought back to the laboratory (Figs 4 and 5). Thyroid hormones fell rapidly to a low level and showed no signs of recovery in both the fish left at the hatchery and those brought to the laboratory (Figs 4 and 5). There was no statistical difference between the levels of T_4 or T_3 in anaesthetised and untreated fish, or between the zero time levels of the control group and the hatchery or laboratory held fish. The mean plasma concentration of T_4 5 days post capture, of the hatchery maintained fish (0.7 µg/100 ml) was significantly higher ($p < 0.05$) than that of the laboratory held fish (0.4 µg/100 ml) sampled at the same time.

The haematocrit values of the laboratory held fish fell from an initial mean value of 52 to a mean of 34 after 24 hours, declining further to a mean of 28 after 40 days. These changes are calculated using the formula above, as corresponding to increases in the plasma volume by factors of 2.1 and 2.8 respectively. The haematocrits of the fish maintained at the hatchery fell to a mean value of 40, representing a 1.4 fold expansion of the plasma volume, after 8 days, but were fully restored to the zero time values after 40 days.

<u>The effect of thyroid stimulating hormone (TSH)</u>

The effect of injected bovine TSH on tank held plaice, 40 days after capture, was to raise the mean T_4 values from 0.4 µg/100 ml to a mean concentration of 1.3 µg/100 ml; the concentrations of T_3 were not statistically changed, nor were the concentrations of T_4 changed by injections of the medium from which TSH had been omitted.

The effect of injections of TSH on the T_4 and T_3 concentrations in rainbow trout are shown in Figs 4 and 5. These indicate that, as in the plaice, the thyroid of tank held rainbow trout releases T_4 but not T_3.

The effect of TSH treatment on both plaice and trout was to increase swimming activity and responsiveness to external stimuli.

The effect of food deprivation in rainbow trout

During the period of the experiment, the fed fish registered a mean weight gain of 32%, while the starved fish had lost 28% of their initial weight. T_4 levels in the starved fish (mean 0.14 µg/100 ml) were statistically lower ($p < 0.05$) than the mean levels in fed fish (0.26 µg/100 ml). T_3 concentrations were even more dramatically reduced ($p < 0.005$) from a mean 0.28 µg/100 ml to 0.06 µg/100 ml.

The starved fish were unmistakably less active and less responsive to visual stimuli than the fed fish.

Discussion

Following the capture of plaice from the wild and of rainbow trout from the hatchery, and during the subsequent periods of adaptation to unfamiliar environments, thyroid hormone levels fall to a low value and remain depressed. There are some differences in the pattern between plaice and trout. In the plaice, there seems in certain fish to be an initial release of T_4, and in these fish T_3 levels may also be elevated for a short period, presumably as a result of the deiodination of T_4 (unpublished data). There is some evidence to suggest that the extent of depression of plasma thyroid hormone levels is a function of the severity of the post capture stress. Thus, the levels in the trout maintained in a cage at the hatchery were higher than those in fish transported to the laboratory and placed in unfamiliar tanks.

From an examination of the haematocrit data (assuming that the number of red cells, apart from those removed during sampling, remains the same) it is clear that although a considerable dilution of the plasma occurs post capture, its extent is insufficient to account for the whole reduction in T_4 and T_3 concentrations. Moreover, after a period of time, the haematocrit values of the hatchery held trout were restored to their initial levels although hormone concentrations remained depressed.

The possible explanation that T_4 and T_3 levels encountered immediately after capture are artificially high as a result of a release of TSH, induced by stress, seems implausible since the zero time control group of rainbow trout was sampled within one minute of capture and did not differ in T_4 or T_3 levels from more leisurely sampled fish. Moreover, TSH has earlier been found to have a latency of at least 12-24 hours; its release seems to be the most likely explanation for the transitory elevation of hormone levels in plaice during the first two days after capture. Side reactions of the anaesthetic MS222 are clearly not a significant factor in the depression of thyroid hormone levels.

The fall in hormone levels is most plausibly explained by a reduction in the output of the thyroid gland. Since the fish showed normal responses to TSH, this reduction must result from a reduction in the output of the pituitary, either effected directly or resulting from a lack of normal responsiveness in the hypothalamus. In mammals, adrenocortical hormones are known to reduce the secretion of TSH (Brown-Grant, 1966). In the fish species Astyanax (Rasquin and Atz, 1952) and Carassius (Chavin, 1956) cortisone treatment has been shown to reduce thyroid cell height.

An alternative explanation is that the depression of thyroid hormone levels is the result of a reduction in the synthesis of the binding proteins with a consequent rise in the concentration of "free" hormones. The effect of stress on such changes in man has been reviewed by Oppenheimer (1968).

Small reductions in thyroid activity in starving mammals have been reported (Yousef and Johnson, 1968; Lerner, 1970). The large reduction noted in rainbow trout, in the present study, is not unexpected, since there is a clear survival value in the ability of organisms which are, in their normal environment, subjected to extended periods of food deprivation, to reduce their activity, appetite and metabolism.

The reduction in the responsiveness and in the spontaneous activity of plaice and trout as a result of tank stresses and of food deprivation was unmistakable. It has been shown (Osborn and Simpson, 1973) that there is a significant negative correlation between plasma T_4 levels and the catch/unit fishing effort for one stock of plaice, and it has been speculated that this is the result of thyroid induced changes in swimming behaviour or responsiveness to external stimuli. It seems likely that the minimum T_4 levels encountered in spring resulted from a reduction of food intake, experienced earlier. If so, the present results indicate that the required behavioural effects of reducing swimming activity and raising response thresholds would undoubtedly ensue.

The results of these experiments, though clearly requiring extension to provide quantitative data on the stress effects associated with adaption to aquaria of different size and type, serve to warn again of the dangers in extrapolating research on tank held fish to the wild stock.

References

Brown-Grant, K 1966 in The Pituitary Gland Volume 2 Edited by G W Harris and B T Donovan London Butterworths:?, 255.

Chavin, W 1956 "Thyroid distribution and function in the goldfish Carassius auratus L." J. exp. Zool., 133: 259-279.

Lerner, L J 1970 "Endocrine and nutritional interrelations." Protein Metab. Biol. Function. 61-70.

Oppenheimer, J H 1968 "Role of plasma proteins in the binding distribution and metabolism of the thyroid hormones." New England J. Med., 278: 1153-1162.

Osborn, R H and Simpson, T H 1973 "A possible relationship between thyroidal status and the ease of capture of fish." ICES. CM 1973 Gear and Behaviour Cttee, Doc. no. B23.

Rasquin, P and Atz, E H 1952 "The effects of ACTH and cortisone on the pituitary, thyroid and gonads of the teleost Astyanax mexicanus." Zoologica, 37: 77-87.

Wardle, C S 1972 "The changes in blood glucose in *Pleuronectes platessa* following capture from the wild: a stress reaction." J. mar. biol. Ass. UK, 52: 635-651.

Yousef, M V and Johnson, H D 1968 "Effects of heat and feed restriction during growth on thyroxine secretion rate of male rats." Endocrinol., 82: 353-358.

Figure 1   Changes in thyroxine concentration in Plaice plasma with time after capture.

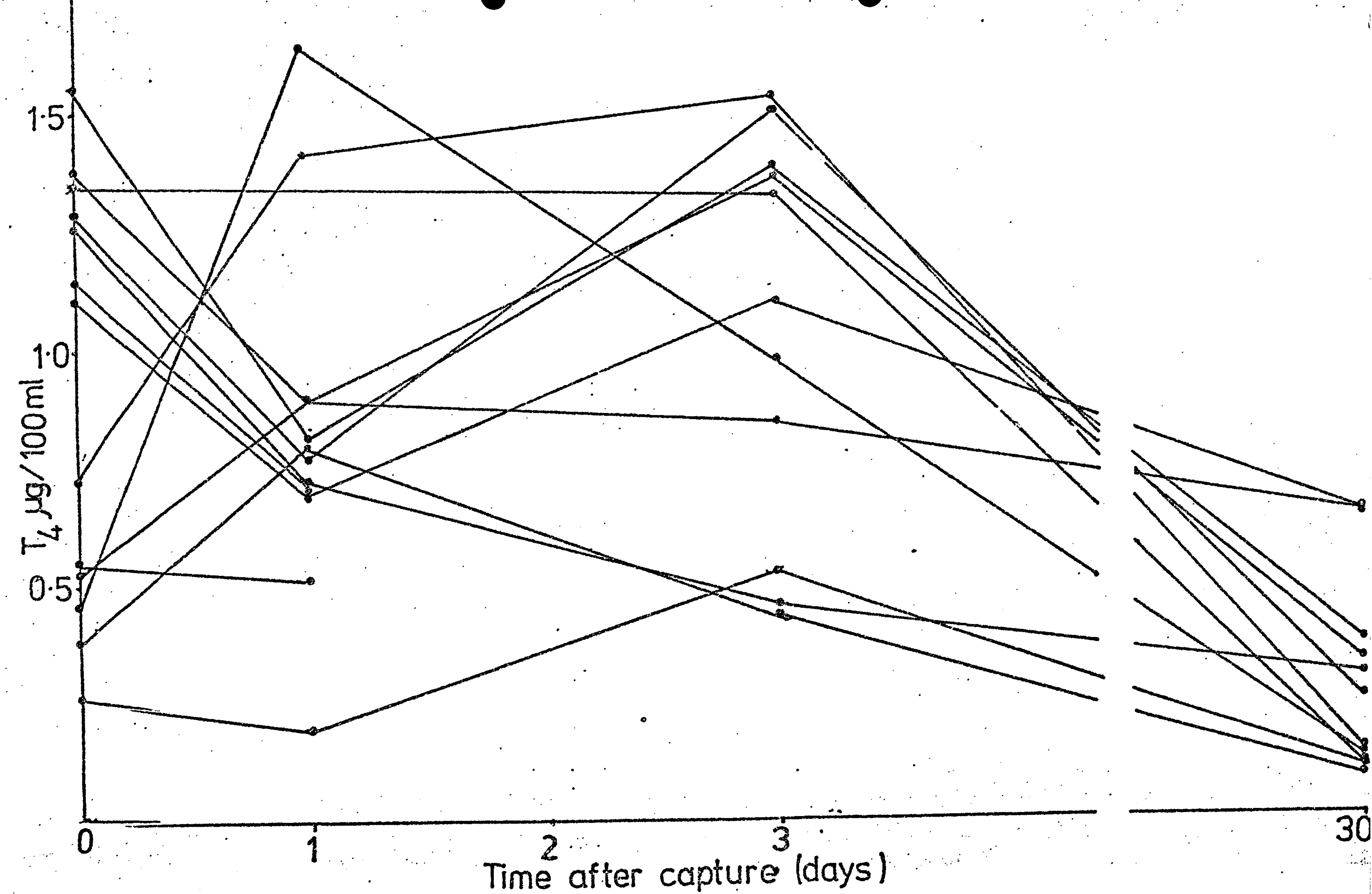


Figure 2 Changes in Triiodothyronine concentration in Plaice plasma with time after capture.

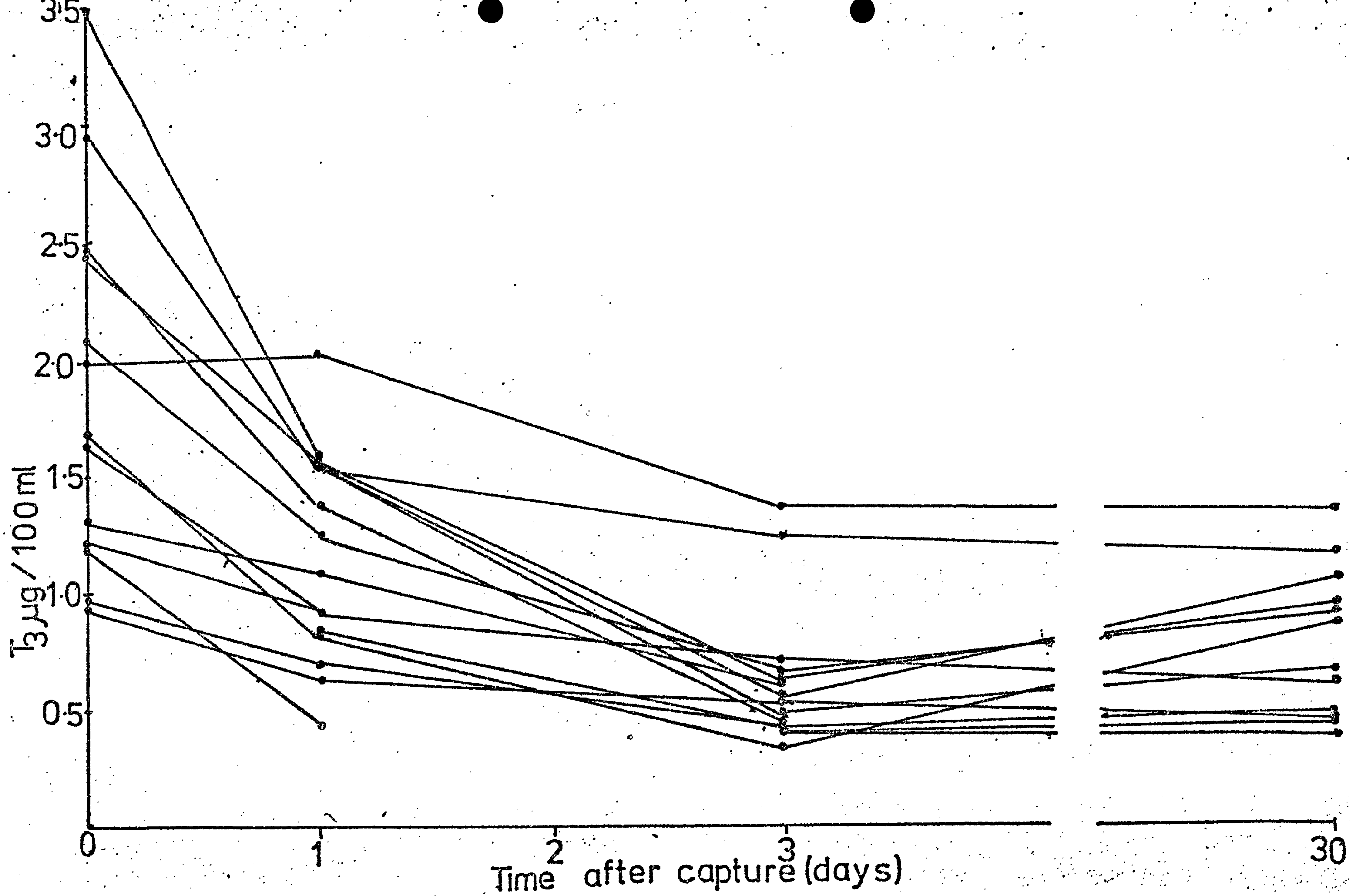


Figure 3 Change in thyroid hormone levels in Plaice plasma

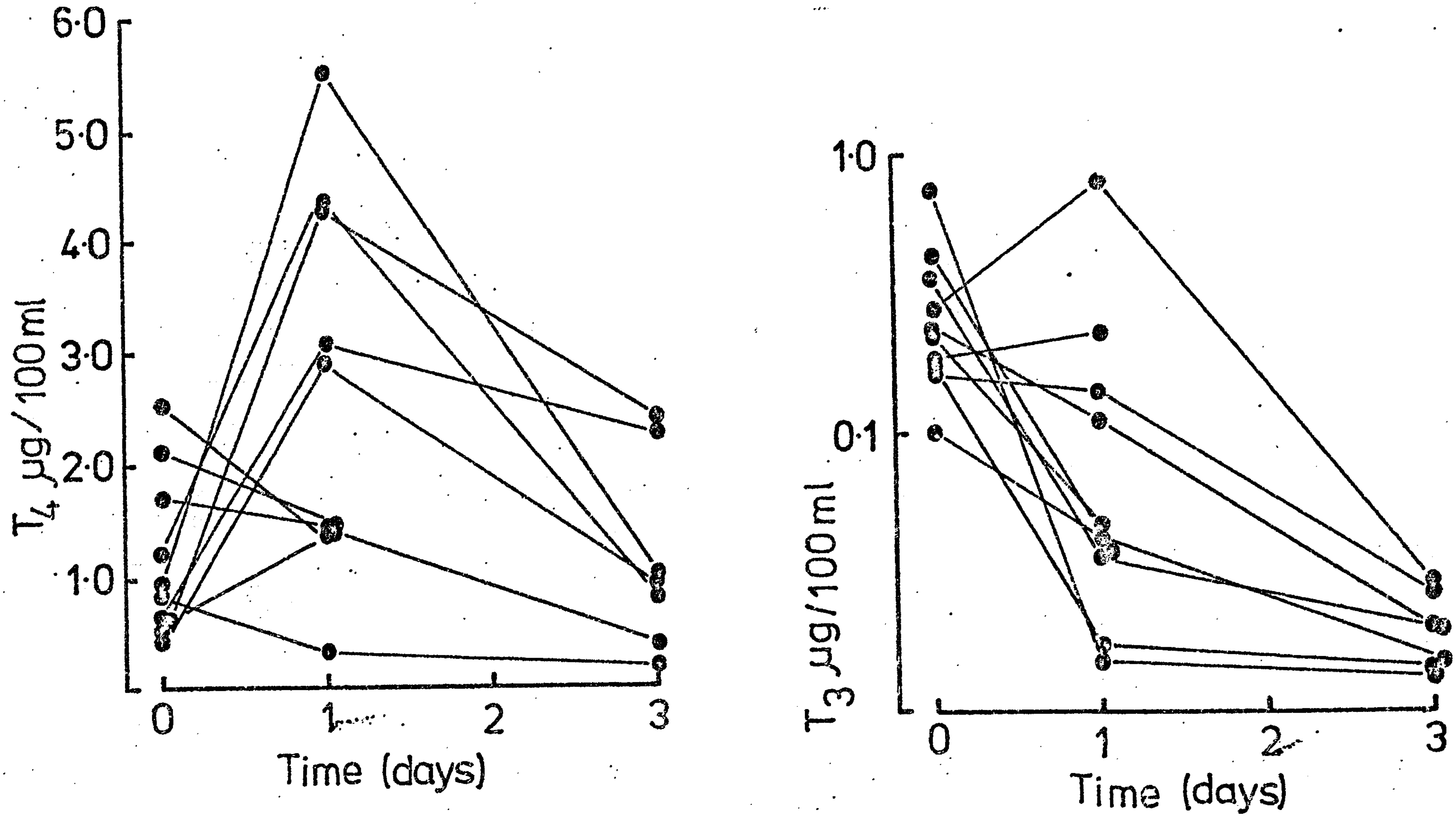
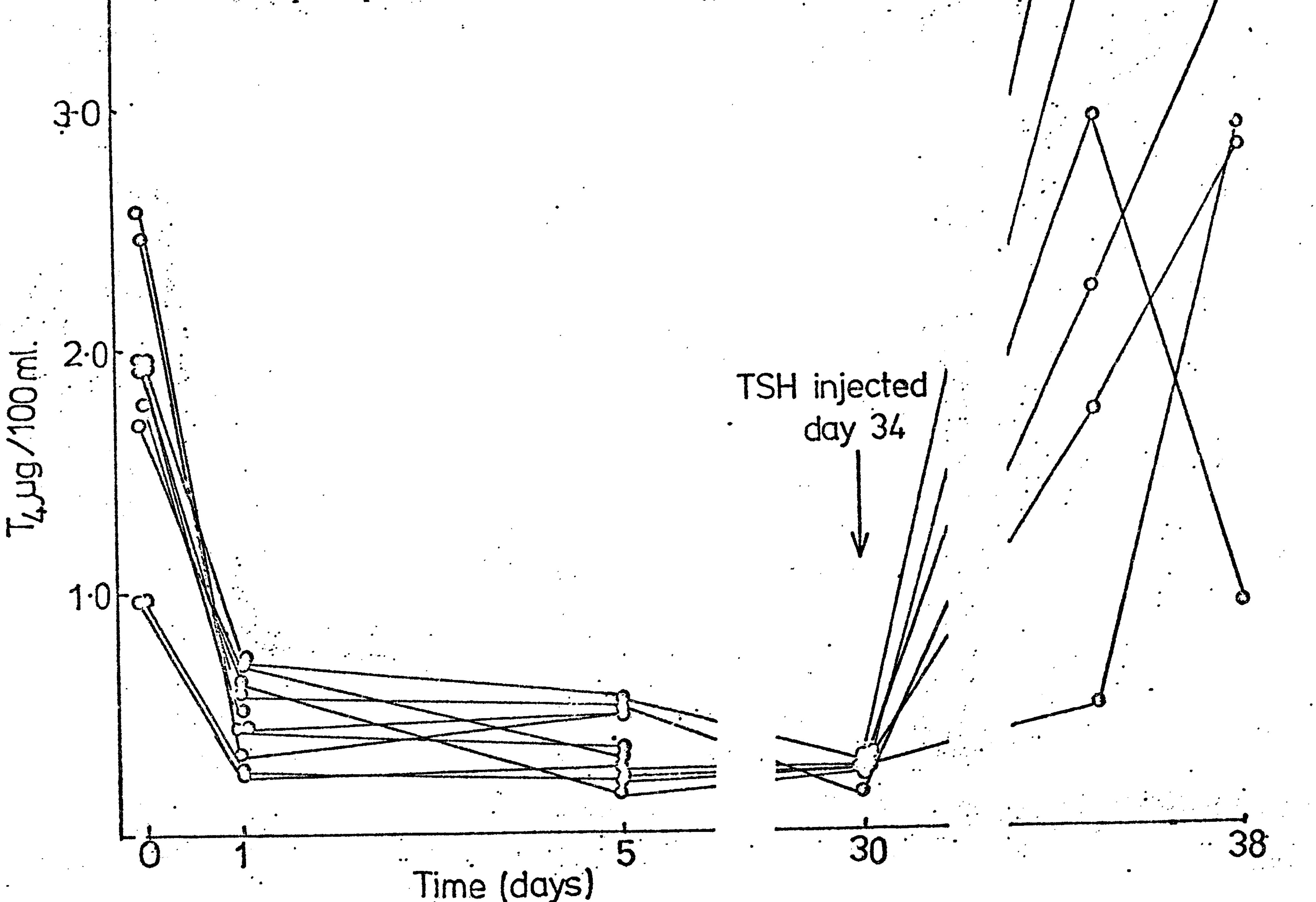


Figure 4. Changes in thyro[illegible]ne concentrations in Rainbow Trout p[illegible]sma post capture.



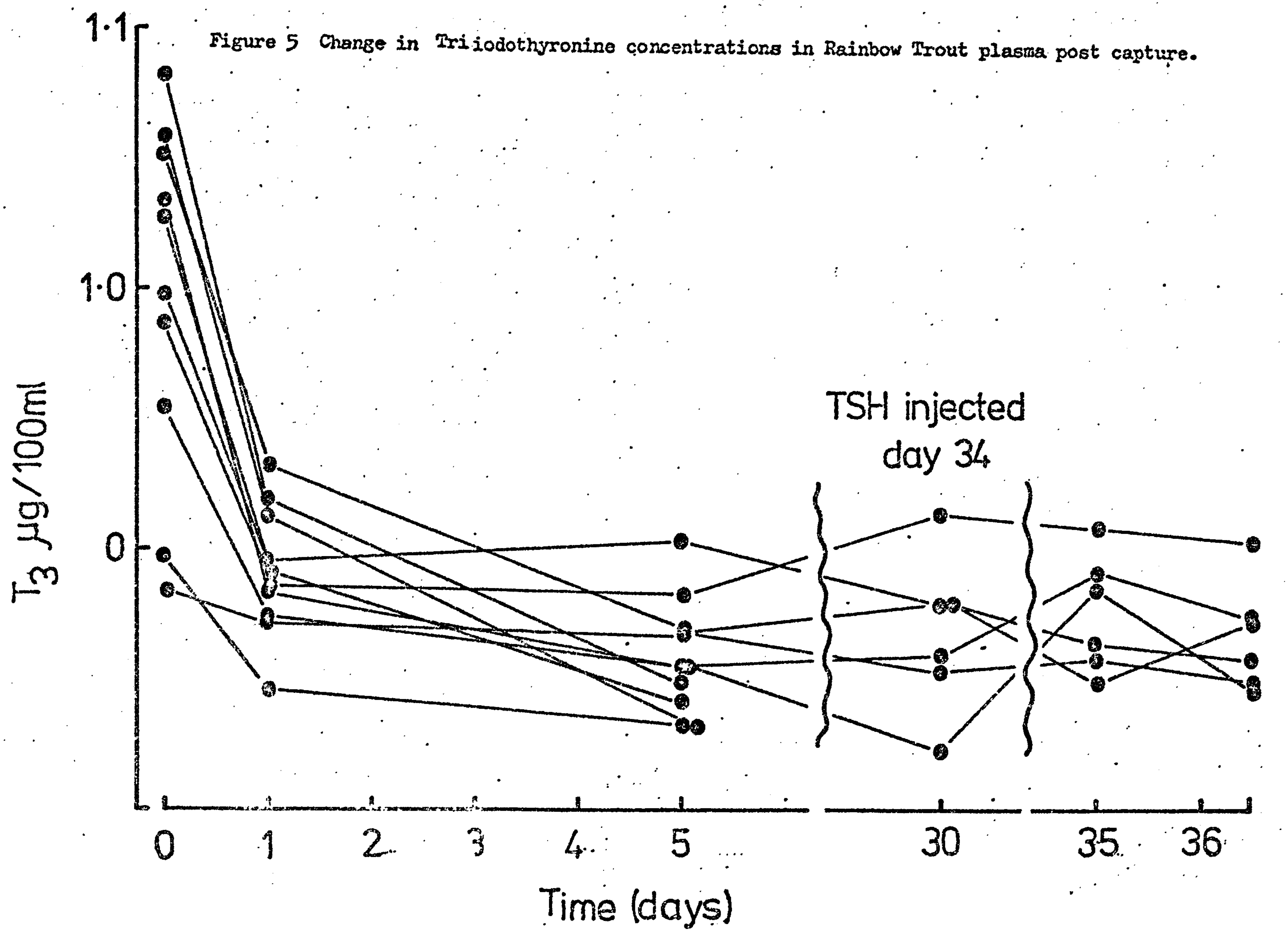


Figure 5 Change in Triiodothyronine concentrations in Rainbow Trout plasma post capture.