THE UNIVERSITY OF MANITOBA

DIEL VARIATIONS IN PLASMA THYROID HORMONE LEVELS IN THE RAINBOW TROUT, *SALMO GAIRDNERI*: EFFECTS OF FEEDING AND ALTERATION IN PHOTOCYCLE

by

Robert Frederick Cook

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ABSTRACT

In rainbow trout held at 10-13°C under a 12L(0700h-1900h):12D photocycle and fed 1% body weight once during the photophase, there was a diel change in plasma L-thyroxine (pT_4) and a less consistent and less marked change in plasma 3,5,3'-triiodo-L-thyronine (pT_3). Feeding, either under a 12L:12D photocycle or under continuous darkness elevated pT_4 and pT_3, and elicited or amplified diel changes in pT_4. Both a change in feeding regime and a phase-shift of the 12L:12D photocycle modified diel patterns in pT_4. The diel pattern of pT_3 was altered by both variables, but less consistently. In starved trout, reversal of a 12L(0700h-1900h):12D photocycle depressed pT_4. There was an interaction between the effects of feeding regime and photocycle on diel changes in both pT_4 and pT_3. Light intensity over the range of 36-162 lux, had no significant effect on pT_4 or pT_3. In starved trout held under continuous darkness, there was no diel change in pT_4 or pT_3, indicating no spontaneous endogenous rhythm. It is concluded that in laboratory-held trout, photocycle and feeding regime interact in a complex manner to determine diel changes in pT_4 and pT_3, and that the feeding effect is somewhat more pronounced. This contrasts with previously published work on goldfish which showed that light cycle rather than food was more influential in setting diel changes in plasma levels of thyroid hormones.

INTRODUCTION AND LITERATURE SURVEY

Diurnal variations and circadian patterns in endocrine systems have been noted in animals for many years. Recently, studies of diurnal patterns in thyroid hormones and thyroid activity have been made in several vertebrate groups with somewhat inconclusive results.

Mammals

Most studies on mammals have involved humans or rats. In humans, plasma levels of both L-thyroxine (T_4) and 3,5,3'triiodo-L-thyronine (T_3) are highest in the morning and lowest at night (Lucke et al., 1977; DeCostre et al., 1971). Short-term fluctuations in plasma T_4 levels (pT_4) and T_3 levels (pT_3) are also apparent (Lucke et al., 1977; O'Connor et al., 1974; Pekary et al., 1976), but are not related to the sleep-wake cycle (O'Connor et al., 1974).

Studies on rats show inconsistent results. Fukuda et al. (1975) and Rookh et al. (1979) found no significant daily rhythm in pT_4, but Rookh et al. (1979) demonstrated a significant though variable rhythm in pT_3. Azukizawa et al. (1975) found that individual rats demonstrated diurnal variations in pT_4 and pT_3, but the time of day at which maximum and minimum levels occurred varied between animals. Increments in pT_3 and pT_4 always occurred after peak levels of thyroid stimulating hormone (TSH).

Jordan et al. (1980) and Ottenweller and Hedge (1981, 1982) found that plasma levels of both thyroid hormones reached a peak in the light period of a light-dark cycle and decreased in the dark, but Pallardo et al. (1976) demonstrated a pT_4 peak in the daytime and a pT_3 peak at night. The light cycle may be the entraining factor for both rhythms, as reversal of the light cycle reversed the rhythm (Ottenweller and Hedge, 1981, 1982). However, Ortiz-Caro et al. (1984) demonstrated that significant changes in pT_4 and pT_3 during the light period were eliminated by food restrictions.

In Syrian hamsters, Vriend (1983, 1984) found highly significant variations in both pT_3 and pT_4, with highest levels at 2115 hours (h) and lowest levels at 0515h (lights on at 0630h and off at 2030h). Woods et al. (1966) estimated mean cycles of thyroid activity in cats to be 24.8h. Calves have circadian rhythms which may be phased by light cycle and outside activity (Curtis and Abrams, 1977). Kemppainen and Sartin (1984) found no evidence of a pT_4 circadian rhythm in cross-bred dogs though episodic variations were noticeable in all animals.

Birds

Birds show more apparent rhythms in thyroid hormones than mammals, probably due to a shorter half-life of T_4 and T_3 (3 hours), which are bound to albumin and prealbumin-like plasma binding protein rather than globulin binding proteins, characteristic of

mammals (Sadovsky and Bensadoun, 1971; Klandorf et al., 1978). In chickens, changes in pT_4 and pT_3 generally follow significant sinusoidal curves (Newcomer, 1974; Klandorf et al., 1978; Decuypere and Kühn, 1984; Sharp et al., 1984; Kühn et al., 1982). Rhode Island red chickens demonstrated a pT_4 peak in either the early morning or just before noon and a pT_3 acrophase in the afternoon (Decuypere and Kühn, 1984; Kühn et al., 1982). Food deprivation caused a shift in the pT_4 acrophase to the afternoon, and after 2 days eliminated the pT_3 acrophase. Refeeding returned the rhythms to normal (Decuypere and Kühn, 1984).

Several studies have been done on white leghorn chicks. Newcomer (1974) found a pT_3 peak at 1616h and a pT_4 peak at 1200h when chicks were held under a 16L(0500h-0600h):8D photoperiod. Chicks held under a 12L(0600h-1800h):12D photoperiod showed a pT_3 peak at 1200h with a nadir at 0400h (Cogburn et al., 1983). The peak pT_4 was at 0400h with a nadir at 1200h. Klandorf et al. (1978) discovered that pT_3 acrophases occurred 8h after light onset on both a 14L:10D photoperiod and an 8L:8D photoperiod. A pT_4 peak under both light cycles occurred 8h after dark, but it was only significant under a 14L:10D regime. In white leghorn roosters, pT_3 was significantly higher at 0800h and 1600h (main peak) than at 2400h and 0400h. Plasma T_4 patterns were similar, but not as well defined (Sadovsky and Bensadoun, 1971).

Sharp et al. (1984) found in young broiler hens that under 7 out of 8 different photoperiods, pT_3 increased during the light period and decreased after darkness. Plasma T_4 levels in all groups reached a peak near the onset of light and fell during the light period. Continuous darkness abolished the pT_3 rhythm and dampened the pT_4 rhythm while continuous light dampened both rhythms.

In young domestic ducks, pT_4, free T_4(FT_4), and pT_3 all followed significant 24-h sinusoidal curves (Harvey et al., 1980). Both FT_4 and pT_4 had acrophases at 0252h and 0347h. Plasma T_3 reached an acrophase at 1750h and declined over the dark period. Free T_3 followed a 12h rather than a 24h rhythm, with acrophases at 0109h and 0309h. Pigeons exhibited peak pT_4 at night (0300h) and low levels in the light phase of a 14L:10D light cycle (0600h-2000h light) (Ami et al., 1982).

Amphibians

Several studies have demonstrated diurnal changes in poikilotherm thyroid systems. Kühn et al (1982, 1983) described fluctuations in glandular T_3 and T_4 levels in Rana ridibunda kept under natural temperature and photoperiod in October, and in hibernating R. ridibunda acclimated to 5°C in December. The T_4 acrophase occurred at 2100h (October) to 2300h (December), while the T_3 peak occurred at noon in October frogs, and at 1900h in December frogs. Plasma T_4 levels showed no diurnal variation at any time, but peak

pT_3 occurred at midnight in both October and December frogs (Kühn et al., 1982, 1983). The T_3/T_4 ratio (T_3/T_4) was also highest at midnight. In September, no diurnal patterns were evident in plasma or glandular T_4 or T_3. Temperature was important in altering the daily variation in T_4 and T_3 levels. High temperature (20°C) abolished the T_4 and T_3 thyroidal changes evident at 5°C and shifted the pT_3 acrophase from midnight (5°C) to morning. Feeding had no significant effect on warm-acclimated January frogs. In fact, starved frogs and hibernating frogs at this time demonstrated a diurnal variation which was absent in fed frogs. Kühn et al. (1983) suggested that the low glandular T_3 level in hibernating frogs, the high T_3/T_4, and the difference between thyroidal and pT_3 acrophases indicate that most pT_3 is derived from peripheral monodeiodination of T_4.

Fish (non-salmonid)

Several fish species demonstrate diel variation in plasma levels of thyroid hormones. Spieler and Noeske (1979) demonstrated diurnal variations in pT_4 and pT_3 in goldfish. Both hormones showed a peak at 1600h, and a nadir between 0400h and 0800h, in fish kept under a 12L(0800h-2000h):12D light cycle. Diel variations in both hormones were significantly correlated, but the pT_3 exceeded the pT_4 at 2000h. Fish were last fed 12h before the initial 24-h cycle so the variations could not have been caused by food (Spieler and Noeske, 1979), unless food set the original cycle.

Light cycle is the most important factor affecting daily pT_4 changes in goldfish. Noeske and Spieler (1983) demonstrated that under either a 12L(1200h-2400h):12D light cycle or a 16L(0800h-2400h):8D light cycle, goldfish demonstrated a significant daily pT_4 variation, with a peak at light onset and nadir when darkness began. Under a 12L(1200h-2400h):12D photoperiod, an additional peak occurred 4h before light onset. However, under neither an 8L(0400h-1200h):16D light cycle nor a reversed 12L:12D cycle (2400h-1200h light) was a diurnal pattern evident. In three experiments, groups of fish fed at the same time of day but kept under from 2 to 6 staggered 12L:12D photoperiods always showed peak pT_4 during the light period (Spieler and Noeske, 1984).

Feeding regime may alter diel thyroid patterns in goldfish to some extent, since Spieler and Noeske (1981) found that fish maintained on a 12L(0800h-2000h):12D light cycle and fed at either 0800h or 1600h had significantly higher pT_4 at 1600h than at any other time of day. However, in the early-fed fish the level at 1200h was also significantly higher than all lower mean values. Late-fed fish had significantly higher pT_3 at 1600h and 2000h than at other sampling times. Fish fed at 0800h had no significant pT_3 variation, but the peak level was at 1600h. Also, fish in groups fed from 4 to 6h after light onset had lower mean pT_4 than in fish fed earlier or later (Spieler and Noeske, 1984). MacKenzie (unpublished data, 1985)

found modest diel pT_4 changes throughout the year in goldfish acclimated to 21°C but not in goldfish acclimated to 12°C. On the basis of the above, it appears that the light cycle in goldfish is the most important factor entraining daily thyroid variations, but food or temperature may modify a pattern set by photoperiod.

White suckers (Catastomus commersoni) sampled for pT_4 and pT_3 diel changes during the 1981 spawning season (from prespawning to late spawning) in Mission Creek, Alberta, showed varying results depending on sex and spawning condition (Stacey et al., 1984). Female suckers demonstrated no diel changes in pT_4 at any reproductive stage. Prespawning males had significantly higher pT_4 at 1500h than at 0300h, but spawning males had significantly higher levels at 1500h than at any other time of day. Late-spawning males showed no significant variation in pT_4. No significant pT_3 changes were noted in either sex at any time. In 1982, fish were sampled at only 2 times a day and no changes in pT_4 were seen.

Fish (salmonid)

Several studies have demonstrated daily pT_4 and pT_3 changes in salmonid fish. White and Henderson (1977) found that pT_4 in brook trout (Salvelinus fontinalis) sampled in June under seminatural conditions was lowest at 0530h, but increased significantly at 1130h and remained constant until 2030h. The pT_3 was also lowest in the early morning, but increased gradually from

morning to evening, rather than showing an abrupt change as in pT_4. The pT_3 was not significantly different over the three sampling times. McCormick and Naiman (1984) also found that pT_4 in brook trout was highest during the light period, but declined prior to dusk, and reached low values during darkness.

Rydevik et al. (1984) demonstrated diel variations in pT_4 in Atlantic salmon (Salmo salar). In May, continuously-fed fish kept at a natural water temperature (9°C), and under natural photoperiod (0200h-2120h), demonstrated a large diurnal variation in pT_4 with a peak at 2000h and a nadir at 0800h. In March, salmon kept at 0.04°C, and under a natural photoperiod (0530h-1800h), did not feed, but demonstrated a significant though damped pT_4 variation. The peak was at 2400h and the nadir was at 0800h. The overall mean pT_4 in May was significantly higher than in March. In March, pT_3 was stable, and in May the only significant change was a decrease between 0400h and 0800h (Rydevik et al., 1984). The water temperature, the degree of feeding, and the light cycle all changed between March and May, so one or more of these factors could be involved.

Several studies have been done on diurnal variations in pT_3 and pT_4 in rainbow trout (Salmo gairdneri). Starved trout demonstrated no diurnal variation in pT_4 or pT_3 after either 3 days (Brown et al., 1978; Eales et al., 1981) or 13 days

(Flood and Eales, 1983) of food deprivation, but hormone levels in fed trout did vary over time. Eales et al. (1981) found that pT_4 in rainbow trout fed at 0930h under a 12L(0800h-2000h):12D light cycle, and sampled every 4h over 2 days, was significantly greater at 0900h on day 1 and at 1300h on day 2 than in starved trout. Plasma T_3 levels were significantly greater at 1300h and 0100h on day 1, and at 1300h on day 2 than starved fish. The 0100-h elevation in pT_3 may have represented a greater overall pT_3 in fed fish, but significant differences in pT_3 between fed and starved fish were not found at other times (Eales et al., 1981). Flood and Eales (1983) found significantly higher pT_4 at 0400h than at 1100h in trout fed at 1200h. The 1100-h value was similar to that of starved trout, suggesting that feeding sets the pT_4 pattern (Flood and Eales, 1983).

Eales et al. (1981) demonstrated that feeding regime could affect the pT_4 and pT_3 patterns to some extent. In trout fed at 1630h under a 12L(0800h-2000h):12D photoperiod, the pT_4 pattern was similar to that of early-fed fish (0930h) with a peak between 0900h and 1300h, and low values during the dark period. However, rather than dropping abruptly as in early-fed trout, the pT_4 remained significantly elevated until 2300h. The pT_3 also remained significantly elevated until 2300h, rather than dropping abruptly as in early-fed trout. In trout held at 11°C or 5°C and fed at 1600h, the pT_4 and pT_3 were greater early in the dark phase (2300h), than

during the initial period of the light phase (0900h). With the exception of pT_4 at 11°C differences were significant (Eales et al., 1981). The late feeding did not seem to shift the pT_4 or pT_3 peak, so the pT_3 and pT_4 elevation may not be a direct result of feeding time, but may require food for it to occur (Eales et al., 1981).

Rainbow trout, sampled under natural conditions in April and September in Scotland, also show significant differences in mean pT_4 between different time periods (Osborne et al., 1978). In September, the pT_4 reached a maximum 1h after sunset, remained high until just before sunrise, and dropped to a nadir 5h after sunrise. In March, maximum levels occurred at 5h after sunset and minimum levels were at 9h after sunrise (Osborne et al., 1978). This suggests that there is a shift in the pattern over the course of a year. This would not be due to light cycle since it is the same in March as in September. It could be due to the ambient temperature, which changed from 15-16.5°C in September to 5-6°C in April.

Only one paper does not support diel variations in pT_4 in salmonids. Leatherland et al. (1977) found that rainbow trout held at 18°C, and fed ad libitum quantities at either 1,3,6, or 9h after photoperiod onset, and sampled at 1,6, or 11h after the onset of light, showed no variation in pT_4.

Influence of Food in Salmonids

Feeding may be an important factor in the diurnal variation of thyroid hormones in rainbow trout. In several studies on brook trout and rainbow trout, feeding or starvation has been shown to influence thyroid hormone kinetics. Starvation in yearling brook trout caused a slower turnover rate of protein bound radioiodide (PB*I), and a lower metabolic clearance rate for (^{125}I) T_4 ($*T_4$), (^{125}I) T_3 ($*T_3$), and ^{125}I ($*I$). It also lowered the extent of $*T_4$ deiodination, and lowered conversion of $*T_4$ to $*T_3$ (Higgs and Eales, 1977, 1978). There was also slower loss of liver radioactivity and depressed fecal loss of $*T_4$ and $*T_3$ in starved trout (Higgs and Eales, 1977). Higgs and Eales (1977) found that pT_4 was not significantly different between fed and starved trout (12 days), but Higgs and Eales (1978) found that starved trout had significantly lower pT_4 than fed trout.

In rainbow trout, Eales (1979) found that 3 days starvation reduced the plasma and tissue T_4 pools, the flow rates between the pools, T_4 degradation rate, and T_4 deiodination, and decreased conversion of T_4 to T_3. Biliary-fecal T_4 excretion was not altered. In contrast to brook trout, starvation did not alter pT_3 or T_3 degradation rate (Eales, 1979), though the brook trout were starved for a longer period (12-37 days). Flood and Eales (1983) found significantly lowered pT_4 after 10 and 20 days starvation, and significantly lowered pT_3 after 6 or 10 days starvation. Milne et

al. (1979) found that pT_4 in rainbow trout was significantly lowered only after 40 to 65 days starvation and that pT_3 did not change significantly. Leatherland et al. (1977) found that rainbow trout starved for 40 days had significantly lowered pT_4.

Feeding may cause a pT_4 or pT_3 increase at only a specific time of day. Flood and Eales (1983) found that fed trout had elevated pT_4 at 0400h but not at 1100h when compared to starved trout. However, at both 0400h and 1100h, pT_3 was elevated in fed trout. Refeeding of fish starved for 6, 10, 16 or 19 days caused an elevated pT_4 4h after refeeding (significant for all but 16 day fish), but refeeding caused no change at either 2.7h (5-day starved trout) or 8h (10-day starved trout) after feeding (Flood and Eales, 1983). The 4-h pT_4 elevation could be due to increased T_4 release from the thyroid, stimulated by food or feeding behaviour (Flood and Eales, 1983).

The size of the food ration also can modify thyroid hormone levels and kinetics. A food ration of 3.2 or 4.4% of body weight elevated pT_3 to a greater extent than a 1 to 1.1% ration in rainbow trout (Flood and Eales, 1983). Large trout injected with $*T_4$ after 15 or 19 days starvation, showed an increase in $*T_3$ and $*I$, 5h after being refed a 3.3% ration, but only showed an increase of $*I$ after being refed a 5% ration (Flood and Eales, 1983). In brook trout, high-fed groups had significantly higher pT_4 at most sampling times

(from June to December) than those fed lesser amounts (McCormick and Naiman, 1984). Brook trout fed a 5% ration had a higher fractional turnover of PB*I, but a smaller T_4 distribution volume, and a lower T_4 metabolic clearance rate than trout fed a 2.5% ration (Higgs and Eales, 1978). The $p*T_4$, $p*T_3$, the $*T_4/*T_3$ ratios, and the T_4 degradation rate, were greater in fish fed the higher ration.

Objectives

In summary, diel changes in one or both plasma thyroid hormones have been established in goldfish (Spieler and Noeske, 1979, 1981, 1984; Noeske and Spieler, 1983; McKenzie, 1985, unpublished), white suckers (Stacey et al., 1984), brook trout (White and Henderson, 1977; McCormick and Naiman, 1984), Atlantic salmon (Rydevik et al., 1984), and rainbow trout (Osborne et al., 1978; Eales et al., 1981; Flood and Eales, 1983). The first objective of this thesis is to confirm that diel changes in pT_4 and pT_3 occur in the rainbow trout.

Food is important in altering plasma thyroid hormone levels and kinetics in brook and rainbow trout (Higgs and Eales, 1977, 1978; Eales, 1979; Flood and Eales, 1983; Milne et al., 1979; Leatherland et al., 1977), and alters diel pT_4 changes in Atlantic salmon (Rydevik et al., 1984), and goldfish (Spieler and Noeske, 1981). In a preliminary study, Eales et al. (1981) showed that feeding regime could alter diel pT_4 patterns in rainbow trout.

Therefore, the second objective is to determine whether feeding acts to establish or alter possible diel patterns in pT_4 or pT_3 in rainbow trout by studying two feeding regimes concurrently and by using more sampling points.

Since phasing of the photoperiod is important in modifying the pT_4 pattern in goldfish (Noeske and Spieler, 1983), the third objective is to determine if phasing of the photoperiod establishes or alters diel patterns in pT_4 and pT_3 in rainbow trout.

Finally, Osborne and Simpson's (1978) results suggest that an endogenous rhythm may exist in pT_4 of semi-wild rainbow trout. The fourth objective is to determine if this occurs in pT_4 or pT_3 in laboratory-held rainbow trout.

MATERIALS AND METHODS

Rainbow trout of various strains were obtained from the Rockwood Experimental Hatchery at Balmoral, Manitoba. Before experimental use they were held in the Animal Holding Facility, Duff Roblin Building, University of Manitoba. The trout were maintained under a 12L(0700h-1900h):12D light cycle in 2700-litre fibreglass tanks, containing running, oxygenated and dechlorinated, Winnipeg city water at 12°C. They were fed 1% of body weight at 1300h-1330h each day. During

transfer to experimental rooms, the trout were anaesthetized in 2.4g tricaine methane sulfonate (TMS) dissolved in 36L of 12°C water. One or 2 trout were randomly assigned to each experimental tank. This procedure was continued until each tank contained the desired sample size (8-12 trout).

During the acclimation and experimental periods, the experimental rooms were kept at 12 ± 1°C and the tanks contained running, oxygenated and dechlorinated water, delivered at 2.5L/min. In Experiments 1 and 2, the trout were kept in 8 fibreglass tanks, filled to 48L and covered with transparent plastic tops. In Experiments 3-7, the trout were held in 12 (Experiment 3) or 16 rectangular, steel-rimmed, 68-L or 91-L aquaria, each filled to 57L. Each aquarium was covered on all sides with white, opaque, adhesive paper, and covered on top with a white translucent plastic cover. These features allowed light into the tanks but minimized disturbances. An individual plastic tap to each tank was used to regulate water flow. In any experiment in which fish were fed, the ration was 1% of body weight of Ewos 4P trout pellets.

During each sampling period, 1 tank from each experimental group was randomly selected, and TMS (3.6g in Experiments 1 and 2, 3.8g in Experiments 3-7) was added directly into the tank to minimize disturbance during capture. With the exception of Experiment 3, 1 tank from each experimental group, consisting of 8 tanks, was sampled at each of 8 equidistant sampling times, beginning at 0700h of one day

and ending at 0700h of the next day. In Experiment 3, 1 tank from each experimental group (6 tanks per group) was sampled at each of 6 equidistant sampling times, beginning at 0700h and ending at 0010h. The fish were netted and transferred to plastic tubs containing 1.2g TMS dissolved in 18L of 12°C dechlorinated and oxygenated water. During the dark period a minilux portable safelight, a yellow or red safelight, or no safelight (Experiment 7) was used to net the fish. Trout were bled from caudal vessels using heparinized, 18-gauge, 1½-inch needles and 3 ml syringes. The volume of blood removed was 2-3 ml per fish. It took approximately 20 min to sample blood from all fish at each time period. The blood was centrifuged and the plasma was pipetted and then stored at -20°C (Experiments 1 and 2) or -80°C (Experiments 3-7) in plastic, flat-bottomed vials, covered with Parafilm. A combined radioimmunoassay procedure for T_3 and T_4 was used to analyze samples (Omeljaniuk et al., 1984). In Experiments 3, 6, and 7, Isolab Quik-Sep radioassay columns, instead of Ames columns were used, and phosphate buffer (pH 7.4) rather than barbital buffer, was used to wash the T_3, T_4 and iodide fractions off the column, and to dissolve the T_3 antibody. In Experiment 6, the standard solutions were made with trout plasma (centrifuged with charcoal to remove thyroid hormones) rather than with .1N NaOH. To minimize interassay variation, samples within an experiment were randomly assigned between assay runs.

EXPERIMENTAL PROTOCOL

Experiment 1

To establish the effect of the time of day on pT_4 and pT_3 in fed rainbow trout (Sunndalsora stock), 96 trout were assigned to 8

tanks. They were then held for 30 days at 11.5-12°C on a 12L(0700h-1900h):12D light cycle. Between 1300h and 1310h each day, they were fed. On day 21 and day 28, all the tanks were cleaned. On the final day, 1 tank was sampled at every sampling period (8 equidistant time points over 24h, beginning and ending at 0700h). The mortality during the acclimation period was 4.2% but no fish died over the last 16 days.

Experiment 2

To establish the effect of the time of day on pT_4 and pT_3 in starved rainbow trout (Sunndalsora stock) the above procedure was used with the same number of fish, but with the following modifications: 1) The water temperature during the experiment varied from 11.3-12.8°C. 2) The fish were not fed, but were checked once per day. For the last 5 days they were checked at 1300h. 3) The experiment was concluded at 24 rather than at 30 days. The mortality during the acclimation period was 0%.

Experiment 3

In all experiments conducted in this study, some variation in light intensity occurred between tanks or groups. In Experiment 1 and 2 the light intensity was unregulated, but exceeded 200 lux. In Experiments 4-7 the light intensity was carefully adjusted (measured at the bottom of the tank), but some variation still existed due to the position of tanks in relation to the overhead lights. For these reasons, it was necessary to determine if a difference in light

intensity could cause differences in mean pT_3 or mean pT_4, or in the diurnal pattern in pT_3 or pT_4.

To test this, 132 rainbow trout (Tagworker stock) were held for 22 days in 6 tanks at 10.4-11.8°C under a 12L(0700h-1900h):12D photocycle at low light intensity (36-41 lux) or a high light intensity (104-109 lux), and were fed at 0730h. The low light intensity was much lower than the minimum level used in Experiments 4-7 (78 lux) while the high light intensity was greater than the maximum level used in Experiments 4-7 (103 lux). Fish from each group were sampled at each of 6 equidistant sampling periods beginning at 0700h and ending at 0100h. At the time most likely for a significant change in pT_4 to occur (1026h), an additional tank (10 fish) at a light intensity approaching that of Experiments 1 and 2 (162 lux) was sampled. The mortality during the acclimation period was 7.6% in the low-light intensity group and 3.0% in the high-light intensity group. There was no mortality in the high-light intensity group subsequent to the first 6 days of acclimation and only 1.6% in the low-light intensity group (1 fish).

Experiment 4

To determine the effect of the timing of the feeding regime on the diel variation of pT_4 and pT_3, 175 rainbow trout (Sunndalsora stock) were held in 16 tanks for 18 days at 12.0-13.0°C on a 12L(0700h- 1900h):12D photoperiod (light intensity 84-103 lux), and were fed at either 0730h (8 tanks) or 1800h (8 tanks). Tanks were

cleaned every day between 1130-1145h. One tank from each group was sampled at each of 8 equidistant time periods, beginning and ending at 0700h. The mortality during the acclimation period was 4.8% in both groups.

Experiment 5

To investigate the effect of a reversal of the 12L:12D light cycle on diel changes in pT_4 and pT_3, 192 starved rainbow trout (Isle of Mann stock) were held at 11.0-11.8°C for 21 days under either a 12L(0700h-1900h):12D photoperiod (8 tanks) or under a 12L(1900h-0700h):12D photoperiod (8 tanks) in two separate controlled environment rooms. The light intensity was 78-94 lux. All tanks were cleaned at 1900h on day 13 and 0700h on day 14. A group of fish from each room was sampled at each of 8 equidistant time periods, beginning and ending at 0700h. The mortality during the acclimation period was 3.2% under the normal photocycle and 2.1% under the reversed photocycle.

Experiment 6

To determine the effect of two different 12L:12D photoperiods on diel variations in pT_3 and pT_4 in fed rainbow trout, 163 rainbow trout (Nasqually stock) were held for 35 days at 11.0-13-8°C in 2 separate controlled environment rooms (each containing 8 tanks) and fed at 0800h. For the first 8 days, all trout were held under a 12L (0700h-1900h):12D photocycle (88-97 lux). For the final 27 days, 8 tanks were shifted to a 12L(2045h-0845h):12D photocycle, while the other 8 were kept under the original photocyle. The tanks were

cleaned at 0715h or 0730h every day. A tank from each room was sampled at each of 8 equidistant time periods, beginning and ending at 0700h. The mortality during the acclimation period was 1.2% in both groups.

Experiment 7

A preliminary experiment showed that rainbow trout would feed when held under continuous darkness. To investigate the effect of feeding and starvation in continuous darkness on diel variations in pT_4 and pT_3, 163 rainbow trout (Tagworker stock) were held in 16 tanks at 11.0-12.0°C for 39 days. For the first 5 days all the trout were fed at 1330h. For the remaining 34 days 8 tanks were starved, while 8 tanks were held on the original feeding regime. On the sixth experimental day the water and oxygen in the building were shut off due to technical problems. Several fish died and 4 fish were assigned (1 from each of 4 tanks) to the tank with the heaviest mortality (5 fish). This tank was subsequently used for the second 0700h sampling time of the fed group. On the final day, 1 tank from each group was sampled at each of 8 equidistant time periods, beginning and ending at 0700h. The mortality during the oxygen shut-down was 12.8% in the fed group and 1.2% in the starved group. No other mortality occurred during the acclimation period.

STATISTICAL ANALYSES

To test for a significant diel variation in pT_3, pT_4 or T_4/T_3 ratio (T_4/T_3), a 1-way ANOVA was used. If this was significant ($p<0.05$), the Student-Newman-Keul's test (SNK test) was used to compare mean levels between different sampling times.

A 2-way ANOVA was used to test for: i) differences between 2 experimental treatments, ii) differences between sampling times for pooled levels and iii) interaction between experimental treatment and sampling time. A 1-way ANOVA was used to compare pT_4, pT_3, or T_4/T_3 between treatments at each sampling time.

RESULTS

Experiment 1

Plasma T_4 levels showed a diel change (Fig. 1a) with peak levels from 1026h (4.0 ng/ml) to 1352h (4.2 ng/ml) which were higher than at any other time of day. The pT_3 did not exhibit a significant change (Fig. 1b). Diel change was evident in T_4/T_3 (Fig. 1c) with a peak at 1026h.

Experiment 2

The pT_4 in starved trout exhibited a modest diel change (Fig. 1a) with a peak at 1352h (1.6 ng/ml) and another peak (1.5 ng/ml) at 0700h (second sampling period). The lowest values occurred at 1026h (0.8 ng/ml) and at 0010h (0.7 ng/ml). A diel change in pT_3 was not

apparent (Fig. 1b). The T_4/T_3 exhibited a diel change with a peak value at 1352h (Fig. 1c).

Experiment 3

Both the high- and low-light intensity groups demonstrated diel changes in pT_4 (Fig. 2a) with a peak at 1026h (4.5 ng/ml and 3.5 ng/ml, respectively) and a nadir at 0010h (1.4 ng/ml). Neither group showed significant diel changes in pT_3 (Fig. 2b). In both groups a diel change in T_4/T_3 was evident (Fig. 2c). The low-light intensity profile showed a peak between 1026h and 1352h and troughs at 0700h and 0010h. The high-light intensity group showed a peak level at 1026h and a nadir at 0010h.

A 2-way ANOVA showed no difference in pT_4, pT_3, or T_4/T_3 due to light intensity. There was a pooled time-of-day effect for pT_4 and for T_4/T_3 but not for pT_3. There was no interaction between light intensity and time-of-day for pT_4, pT_3, or T_4/T_3. One-way ANOVA tests showed that pT_4, pT_3 and T_4/T_3 were not significantly different between light intensity groups at any sampling time.

The pT_4, pT_3, and T_4/T_3 for trout in the additional tank at 162 lux (1026h) were not significantly different from the corresponding points of the high- and low-light intensity groups.

Figure 1. Diel variations in pT_4 (a), pT_3 (b) and T_4/T_3 (c) in rainbow trout held at 11-13°C under a 12L(0700h-1900h):12D photocycle and either fed at 1300h (o—●; Expt. 1) or starved (□—■; Expt. 2). Each point represents the mean hormone level for a group of 9-12 trout (Expt. 1) or 12 trout (Expt. 2). Standard errors from the mean (±1) are indicated by vertical bars. ANOVA (1-way) indicated a diel variation for pT_4 in Expt. 1 ($p<0.0005$) and Expt. 2 ($p<0.05$), but no diel change for pT_3. A diel change was indicated for T_4/T_3 in Expt. 1 ($p<0.005$) and Expt. 2 ($p<0.025$).

★ : Higher than the 6 lowest values for fed trout ($p<0.005$ for the first 0700-h, 1718-h and 2044-h values; $p<0.001$ for the 0010-h, 0336-h, and second 0700-h values).

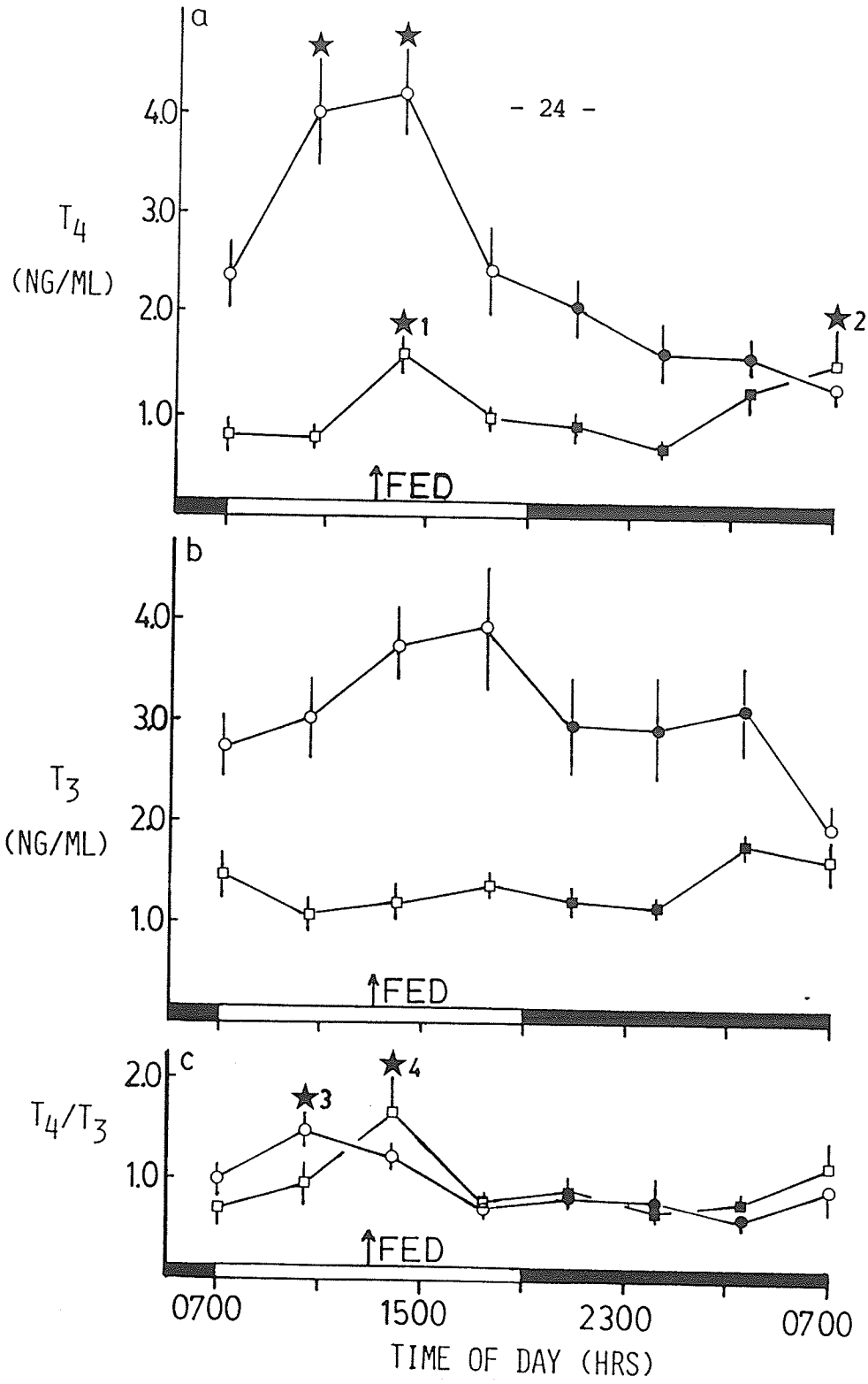
★1: Higher than the 1026-h ($p<0.05$) and 0010-h values ($p<0.025$) for starved trout.

★2: Higher than the 0010-h value for starved trout ($p<0.05$).

★3: Higher than the 1718-h and 0336-h values for fed trout ($p<0.05$).

★4: Higher than the 6 lowest values for starved trout ($p<0.025$ for the first 0700-h, 1718-h, 0010-h and 0336-h values; $p<0.05$ for the 1026-h and 2044-h values).

Data are shown in Appendix Table 1 (fed fish) and Table 2 (starved fish).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T3
(NG/ML)
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c
2.0
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T4/T3
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4
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0700
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2300
0700
TIME OF DAY (HRS)

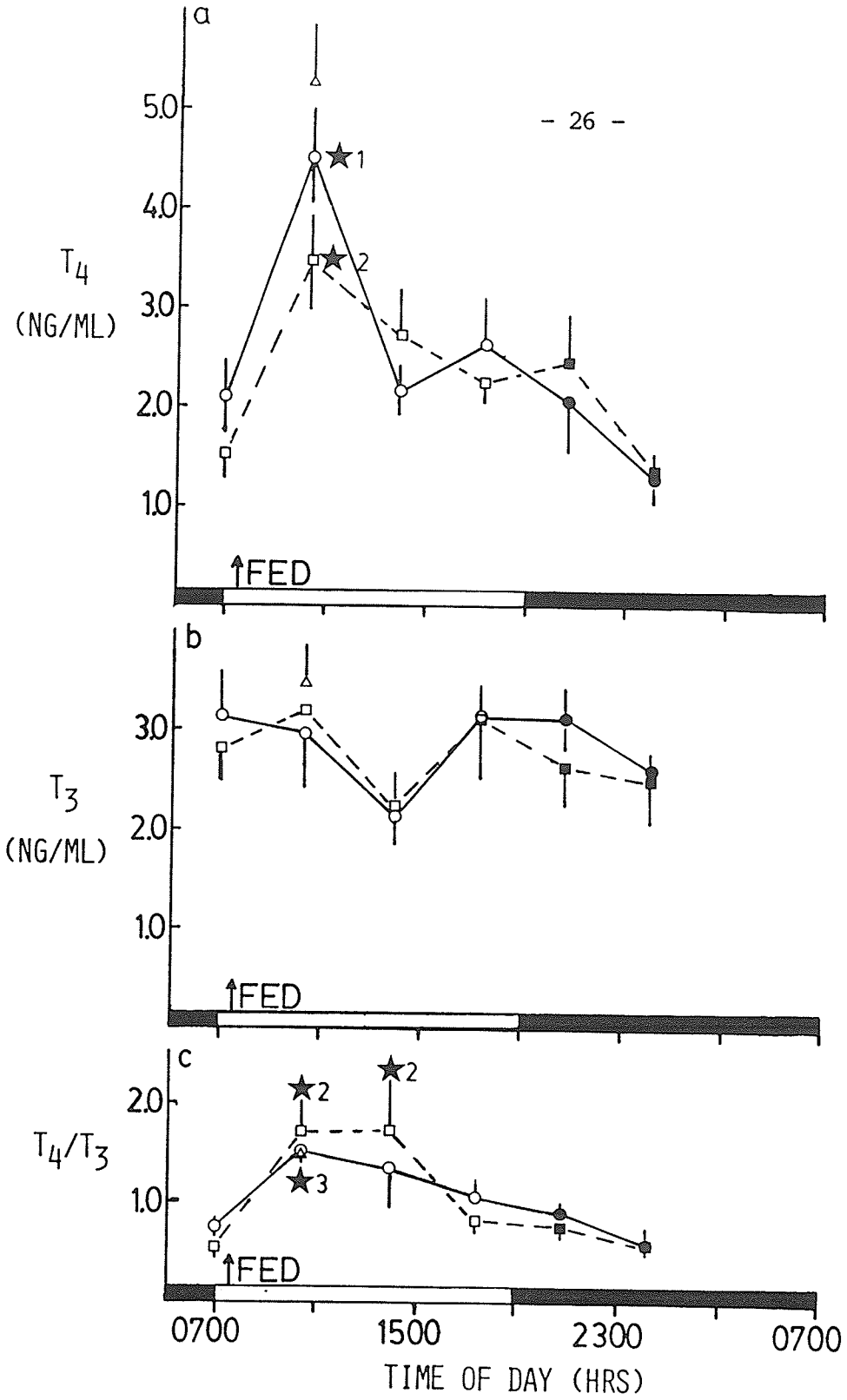
Figure 2. Diel variation in pT_4 (a), pT_3 (b) and T_4/T_3 (c) in rainbow trout held at 10.4-11.6°C under a 12L(0700h-1900h):12D photocycle at a light intensity of 104-109 lux (o——●) or 36-41 lux (□---■), and fed at 0730h (Expt. 3). At 1026h a single tank of trout was sampled, that had been held under identical conditions, but at a light intensity of 162 lux. Each point represents the mean hormone level for 9-11 trout. Standard errors from the mean (±1) are indicated by vertical bars. ANOVA (1-way) indicated a diurnal variation in pT_4 for both the high- ($p<0.0005$) and low-light ($p<0.0025$) intensity groups, and in T_4/T_3 for both the high- ($p<0.025$) and low-light ($p<0.01$) intensity groups, but not in pT_3 for either group. ANOVA (2-way) indicated: i) sampling time influenced pT_4 ($p<0.0005$) and T_4/T_3 ($p<0.0005$) but not pT_3, ii) neither pT_4, pT_3 nor T_4/T_3 differed with light intensity, iii) there was no interaction between light intensity and time of sampling for pT_4, pT_3, or T_4/T_3.

★1: Higher than all other sampling points at high-light intensity ($p<0.005$ for 1352h; $p<0.001$ for all other times).

★2: Higher than the 0700-h and 0010-h values at low-light intensity ($p<0.005$ for pT_4; $p<0.05$ for T_4/T_3).

★3: Higher than the 0010-h value ($p<0.05$) for the high-light intensity.

Data are shown in Appendix Table 3 (high-light intensity) and Table 4 (low-light intensity).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1.0
T_3
(NG/ML)
FED
c
2.0
1.0
T_4/T_3
2
2
3
FED
0700
1500
2300
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TIME OF DAY (HRS)

Experiment 4

pT_4

Early-fed trout demonstrated a diurnal variation in pT_4 (Fig. 3a). The peak level at 1026h (3.3 ng/ml) was higher than the values at 2044h (1.8 ng/ml) and at the second 0700-h sampling time (1.1 ng/ml). Another peak at 0336h (2.5 ng/ml) was not significant.

Late-fed trout also demonstrated a diel variation in pT_4 (Fig. 3a). There were 2 major peaks; one occurred between 1026h (3.0 ng/ml) and 1352h (2.8 ng/ml) and another occurred at 2044h (3.2 ng/ml). The nadir occurred at the second 0700-h sampling time (1.1 ng/ml).

The early- and late-fed pT_4 profiles corresponded closely with the exception of the 2044-h sampling period (Fig. 3a). A 2-way ANOVA showed no effect of feeding regime on mean pT_4. There was a pooled time-of-day effect, but no interaction between the feeding regime and time-of-day. An additional peak in the late-fed group (2044h) was higher than the corresponding level in the early-fed group (1-way ANOVA). This was the point immediately after late-feeding.

The late-fed pT_4 profile was shifted graphically to allow comparison of both groups with respect to corresponding times after feeding (Fig. 4a). When this was done the profiles closely resembled one another. A 2-way ANOVA showed that pooled pT_4 changed significantly in relation to the time-after-feeding, but showed no

interaction between photoperiod and time-after-feeding. However, at about 17h after feeding there was a higher pT_4 in the late-fed than in the early-fed group (1-way ANOVA).

$\underline{pT_3}$

Early-fed trout demonstrated a diurnal variation in pT_3 (Fig. 3b). The peak levels occurred between 0010h (1.5 ng/ml) and 0336h (1.8 ng/ml), but only the 0336-h value was significant. Late-fed trout demonstrated no significant diel variation in pT_3 (Fig. 3b).

The early- and late-fed pT_3 profiles did not resemble one another (Fig. 3b). One-way ANOVA tests showed that at 1352h and at 2044h, pT_3 was higher in the late-fed as compared to the early-fed group. The peak pT_3 level at 0336h in the early-fed group was higher than the corresponding level in the late-fed group. A 2-way ANOVA showed no pooled pT_3 change with the time of day, but showed an interaction between the feeding regime and the time-of-day. The feeding regime had no effect on mean pT_3.

The late-fed pT_3 profile was shifted as for pT_4 (Fig. 4b). A 2-way ANOVA showed a pooled time-after-feeding effect but showed no interaction between photoperiod and time-after-feeding. The curves were closely related but at 13h 15 min after feeding the late-fed group had a higher pT_3 than the early-fed group.

$\underline{T_4/T_3}$

Early-fed trout demonstrated a diel variation in T_4/T_3 (Fig. 3c). The ratios were elevated from 1026h to 2044h, but low for the remainder of the dark period. No point was significantly higher than any other. Late-fed trout showed no diel variation in T_4/T_3 (Fig. 3c).

A 2-way ANOVA showed a change in pooled T_4/T_3 with time of day, but showed no interaction between feeding regime and time-of-day. The feeding regime had no effect on mean T_4/T_3. At 0336h a 1-way ANOVA showed that the late-fed group had a higher T_4/T_3 than the early-fed group (Fig. 3c).

When the late-fed T_4/T_3 profile was shifted (Fig. 4c), a 2-way ANOVA showed no time-after-feeding effect and no photoperiod-time-after-feeding interaction. At about 17h after feeding, however, T_4/T_3 was higher in the late-fed than in the early-fed group (1-way ANOVA).

Experiment 5

$\underline{pT_4}$

Starved trout exhibited no significant diel changes in pT_4 under either photocycle (Fig. 5a). There was a high pT_4 value (2.3 ng/ml) under the normal 12L(0700h-1900h):12D photocycle at 2044h, that corresponded to, and was higher than (1-way ANOVA) the lowest point

Figure 3. Diel variations in pT_4 (a), pT_3 (b) and T_4/T_3 (c) in rainbow trout held at 12-13°C under a 12L(0700h-1900h):12D photocycle and fed at either 0730h (o—●) or 1800h (□—■). Each point represents the mean hormone level for a group of 9-11 trout. Standard errors from the mean (±1) are indicated by vertical bars. ANOVA (1-way) showed a diel variation in pT_4 for both groups ($p<0.005$), and in pT_3 ($p<0.005$) and T_4/T_3 ($p<0.01$) in the early-fed group. There was no diurnal variation in pT_3 or T_4/T_3 in the late-fed group. ANOVA (2-way) indicated: i) pT_4 ($p<0.0005$) and T_4/T_3 ($p<0.01$), but not pT_3 varied with the time of sampling, ii) neither pT_4, pT_3 nor T_4/T_3 was influenced by feeding time, iii) there was an interaction between sampling time and feeding time for pT_3 ($p<0.01$), but not for pT_4 or T_4/T_3.

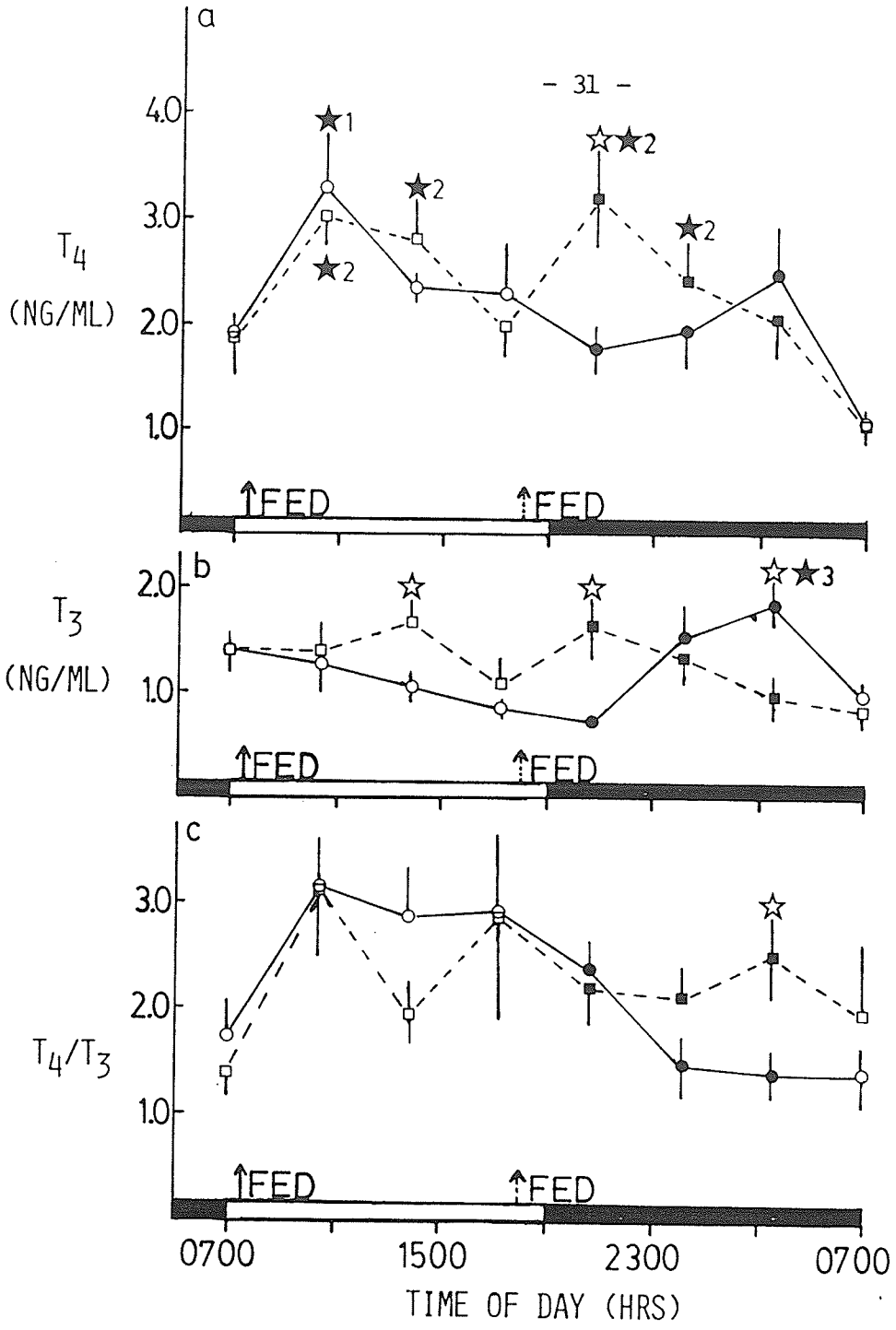
☆ : Higher than the corresponding point in the other group (graph a: $p<0.025$; graph b: $p<0.05$, $p<0.005$, $p<0.025$; graph c: $p<0.025$).

★1: Higher than 2044h ($p<0.05$) and 0700h ($p<0.001$-second sampling period) for early-fed trout.

★2: Higher than the value at the last sampling point in the late-fed group ($p<0.005$, $p<0.025$, $p<0.005$, $p<0.05$).

★3: Higher than at 1352h ($p<0.05$), 1718h ($p<0.025$), 2044h ($p<0.005$), and 0700h ($p<0.05$-second sampling time) for the early-fed group.

Data are shown in Appendix Table 5 (early-fed fish) and Table 6 (late-fed fish).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(NG/ML)
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c
3.0
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T4/T3
FED
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0700
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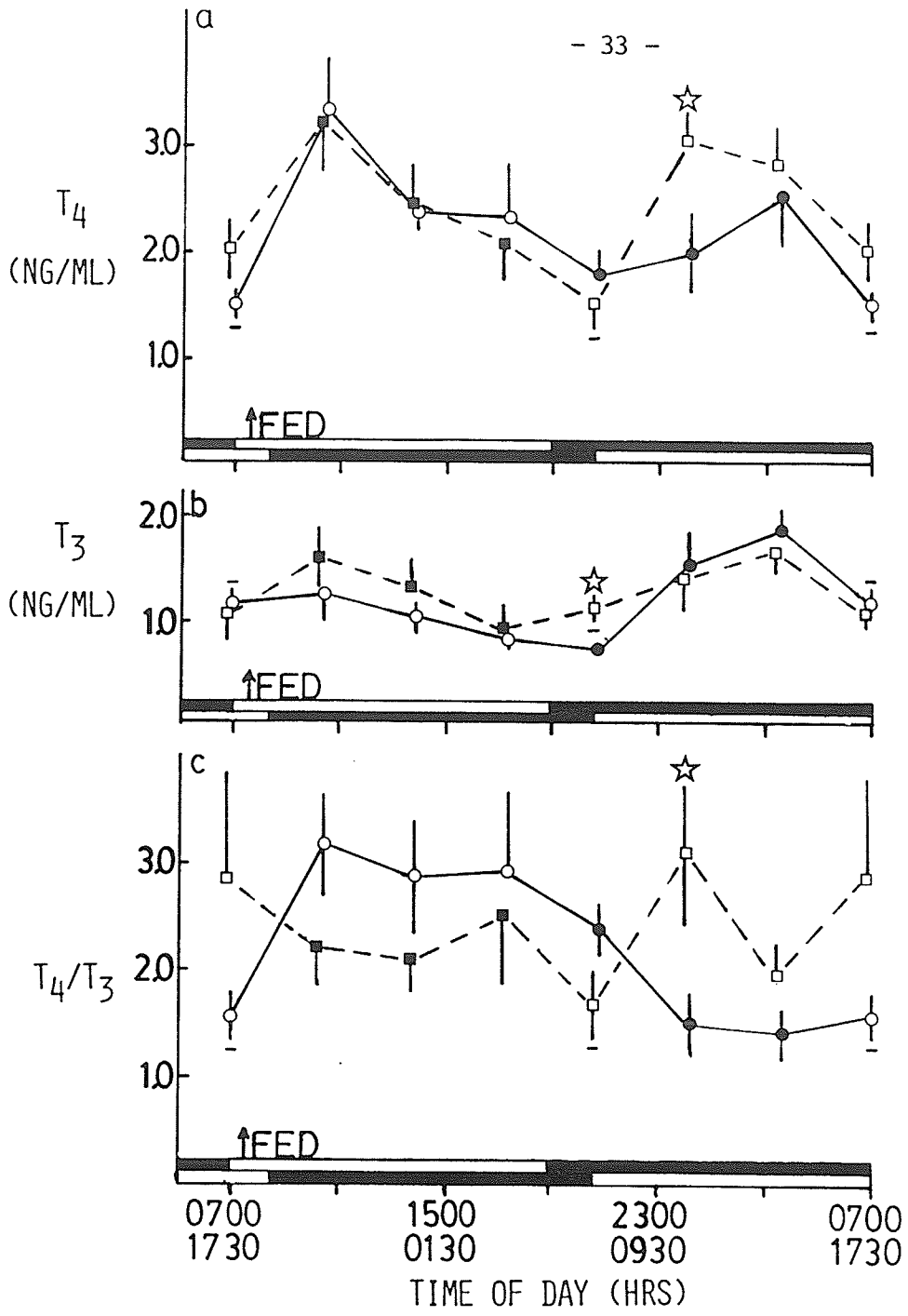
2300
0700
TIME OF DAY (HRS)

Figure 4. The same data as in Figure 3, except the late-fed profile was shifted graphically so the time of feeding coincided with that of the early-fed profile. The upper photocycle on the X-axis corresponds to the early-fed group (o—●), while the lower photocycle corresponds to the late-fed group (□—■). o or □ indicates the mean value for both 0700-h sampling times. ANOVA (2-way) indicated: i) pT_4 ($p<0.0005$) and pT_3 ($p<0.01$) changed with time after feeding, but T_4/T_3 did not, ii) there was no interaction between time after feeding and photocycle for pT_4, pT_3 or T_4/T_3.

☆ : Higher than the corresponding value in the other group ($p<0.05$).

Data are shown in Appendix Table 5 (early-fed fish) and Table 6 (late-fed fish).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(NG/ML)
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1.0
FED
c
T_4/T_3
3.0
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FED
0700
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2300
0930
0700
1730
TIME OF DAY (HRS)

under the reversed 12L(1900h-0700h):12D photocycle (0.9 ng/ml). A 2-way ANOVA showed no pooled pT_4 change with the time-of-day, and no interaction between light cycle and time-of-day. The mean pT_4 of the normal photocycle was higher than that of the reversed photocycle.

$\underline{pT_3}$

No diel variations in pT_3 were evident under either photocycle. The pT_3 profiles under both photoperiods corresponded closely (Fig. 5b). A 2-way ANOVA showed no pooled time-of-day effect, no interaction between photoperiod and time-of-day, and no effect of photoperiod regime on mean pT_3.

$\underline{T_4/T_3}$

No significant diel variations were evident in T_4/T_3 under either photoperiod (Fig. 5c). The T_4/T_3 did not differ significantly between photoperiod at any time-of-day. A 2-way ANOVA showed no pooled time-of-day effect and no interaction between photoperiod and time-of-day. The mean T_4/T_3 was not different between the 2 photoperiod groups.

Experiment 6

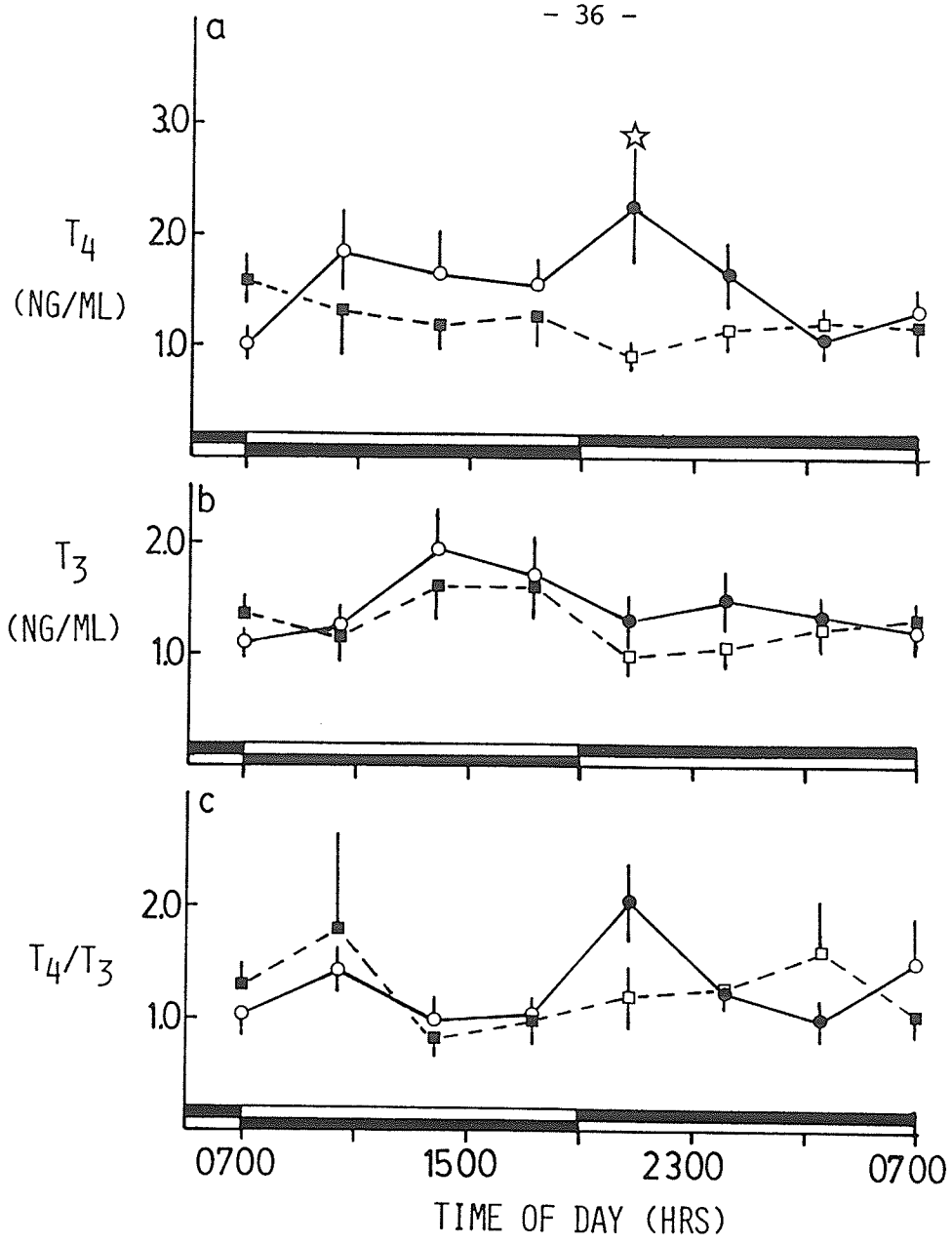
$\underline{pT_4}$

Rainbow trout held under the normal 12L:12D photoperiod exhibited a diel variation in pT_4 (Fig. 6a) with a peak at 1026h (5.2 ng/ml) and another between 0336h (4.9 ng/ml) and the last 0700-h sampling

Figure 5. Diel variation in pT_4 (a), pT_3 (b) and T_4/T_3 (c) in starved rainbow trout held at 11.0-11.8°C under a 12L(0700h-1900h): 12D photocycle (o---●) or a reversed 12L(1900h-0700h):12D photocycle (□---■). Each point represents the mean hormone level for a group of 10-12 fish. Standard errors from the mean (±1) are indicated by vertical bars. ANOVA (1-way) indicated no diel variation in pT_4, pT_3 or T_4/T_3 under either photoperiod. ANOVA (2-way) indicated: i) neither pT_4, pT_3 nor T_4/T_3 changed with sampling time, ii) pT_4 ($p<0.0005$), but not pT_3 or T_4/T_3 was influenced by photocycle, iii) there was no interaction between sampling time and photocycle for pT_4, pT_3 or T_4/T_3.

☆ : Higher than the corresponding point in the other group ($p<0.05$).

Data are shown in Appendix Table 7 (normal photoperiod) and Table 8 (reversed photoperiod).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period (5.1 ng/ml). The nadir occurred between 2044h (2.5 ng/ml) and 0010h (2.2 ng/ml).

Trout held under the shifted 12L(2045h-0845h):12D photoperiod exhibited a diurnal variation in pT_4 with a peak at 1026h (5.6 ng/ml) and another peak between 0010h (5.4 ng/ml) and 0036h (5.2 ng/ml) (Fig. 6a). The nadir occurred between 1718h (2.5 ng/ml) and 2044h (2.2 ng/ml).

The normal and shifted pT_4 profiles corresponded closely (Fig. 6a). A 2-way ANOVA showed a variation in pooled pT_4 with time-after-feeding (sampling time) and an interaction between photoperiod regime and time-after-feeding. There was no difference in mean pT_4 between photoperiod regimes. The pT_4 at 0010h under the shifted photoperiod was higher than the corresponding level under the normal photoperiod (1-way ANOVA).

The 12L(2045h-0845h):12D(0845h-2045h) profile was shifted graphically to compare both groups with respect to time-after-light onset (Fig. 7a). A 2-way ANOVA showed a change in pooled pT_4 with time-after-light onset, which demonstrated a photoperiod effect. An interaction between feeding regime and time-after-light onset was also evident, which indicated a feeding effect. At light onset and at 3h 24 min prior to light onset, the points under the normal photoperiod were higher than the corresponding points under the shifted

photoperiod (1-way ANOVA). At 13h 44 min after light onset, the level under the shifted photoperiod was higher than that under the regular photoperiod. This corresponded to the point immediately after feeding under the shifted photoperiod.

$\underline{pT_3}$

Rainbow trout held under the normal photoperiod showed a diel variation in pT_3 (Fig. 6b). The pT_3 was elevated from 1026h to 2044h. The 2044-h point (5.6 ng/ml) was higher than the first 0700-h point (3.8 ng/ml).

Rainbow trout held under the shifted photoperiod exhibited a diel variation in pT_3, with a peak at the first 0700-h sampling time (5.7 ng/ml) and a nadir at 2044h (3.8 ng/ml).

The pT_3 profiles for the 2 photoperiods did not resemble one another (Fig. 6b). A 2-way ANOVA showed no change with the time-after-feeding (sampling time) for pooled pT_3, but showed an interaction between photoperiod and time-after-feeding. This showed that the profile was altered by the photoperiod change. Mean pT_3 was not affected by the photoperiod. The 1352-h and the 2044-h values were higher under the normal photoperiod than the corresponding values under the shifted photoperiod (1-way ANOVA). The first 0700-h value under the shifted photoperiod was higher than the corresponding point under the normal photoperiod (1-way ANOVA).

When the 12L(2045h-0845h):12D profile was shifted graphically, to compare both groups with respect to time-after-light onset, the two pT_3 profiles resembled one another (Fig. 7b). A 2-way ANOVA showed a change in pooled pT_3 with time-after-light onset, but showed no interaction between the feeding regime and time-after-light onset. At no sampling time was pT_3 different between the two photoperiod groups.

$\underline{T_4/T_3}$

Rainbow trout held under the normal photoperiod exhibited a diel change in T_4/T_3. The ratio was elevated from 0336h to 1026h (Fig. 6c). The nadir occurred between 2044h and 0010h. Rainbow trout held under the shifted photoperiod also showed a diel change in T_4/T_3 (Fig. 7c). The T_4/T_3 was highest at 1026h and at 0010h. The nadir occurred between 1718h and 2044h.

The T_4/T_3 profiles resembled one another (Fig. 6c). A 2-way ANOVA showed a pooled time-after-feeding (sampling time) effect and an interaction between photoperiod and time-after-feeding. There was no effect of photoperiod regime on the mean T_4/T_3. A 1-way ANOVA showed that the T_4/T_3 under the shifted photoperiod was higher at 0010h than the corresponding ratio under the normal photoperiod.

When the 12L(2045h-0845h):12D, T_4/T_3 profile was shifted graphically, the profile differed at 3 points (Fig. 7c). At 13h 44

min after light onset and at 17h 10 min after light onset, the values for the shifted photoperiod were higher than those for the normal photoperiod (1-way ANOVA). At 3h 24 min before light onset, the level for the normal photoperiod was higher than that for the shifted photoperiod (1-way ANOVA). A 2-way ANOVA showed a change with time-after-light onset, but there was a more significant interaction between feeding regime and time-after-light onset.

Experiment 7

pT_4

Fed trout demonstrated a diel change in pT_4 (Fig. 8a). The pT_4 rose to a peak at 1352h (2.1 ng/ml) which was not significantly higher than the low levels between 0100h and 0700h (0.9-1.2 ng/ml). Starved trout showed no significant diel variation in pT_4 (Fig. 8a).

A 2-way ANOVA showed that mean pT_4 was higher in the fed trout than in the starved trout. There was a pooled time-of-day effect, but no interaction between feeding and the time-of-day. At no sampling time was pT_4 significantly different between the fed and starved groups (1-way ANOVA).

pT_3

Neither fed nor starved trout demonstrated a diel change in pT_3 (Fig. 8b). A 2-way ANOVA showed that mean pT_3 was higher in the fed group than in the starved group. The pooled time-of-day effect was

Figure 6. Diel variation in pT_4 (a), pT_3 (b) and T_4/T_3 (c) in rainbow trout fed at 0800h and held at 11.0-13.0°C, under either a normal 12L (0700h-1900h):12D photocycle (o---●) or a shifted 12L(2045h-0845h): 12D photocycle (□——■) (Expt. 6). Each point represents the mean hormone level for a group of 9-11 trout. Standard errors from the mean (±1) are indicated by vertical bars. The upper light cycle on the X-axis corresponds to the normal photocycle. The lower light cycle corresponds to the shifted photocycle. ANOVA (1-way) showed a diel variation in pT_4 ($p<0.001$ and $p<0.005$), pT_3 ($p<0.05$ and $p<0.025$), and T_4/T_3 ($p<0.0025$ and $p<0.001$) under normal and shifted photocycles, respectively. ANOVA (2-way) indicated: i) pT_4 ($p<0.0005$) and T_4/T_3 ($p<0.0005$), but not pT_3 varied with sampling time, ii) neither pT_4, pT_3 nor T_4/T_3 differed between photocycles, iii) pT_4 ($p<0.01$), pT_3 ($p<0.0005$) and T_4/T_3 ($p<0.01$) showed interaction between sampling time and photocycle.

☆ : Higher than the corresponding value in the other group (graph a: $p<0.0005$; graph b: $p<0.005$, $p<0.025$, $p<0.025$; graph c: $p<0.0005$).

★ : Higher pT_4 than at 2044h ($p<0.05$) and 0010h ($p<0.025$ for 1026h and 0336h; $p<0.01$ for the second 0700-h value) under normal photocycle.

★1: Higher pT_4 than at 1718h ($p<0.005$ for 1026h; $p<0.025$ for 0010h; $p<0.05$ for 0336h) and at 2044h ($p<0.005$ for 1026h; $p<0.01$ for 0010h; $p<0.025$ for 0336h) under the shifted photocycle.

★2: Higher pT_3 than at 0700h ($p<0.05$-first sampling value) under the normal photocycle.

Figure 6 cont'd....

★3: Higher pT_3 than at 2044h ($p<0.025$) under the shifted photocycle.

★4: Higher T_4/T_3 than at 2044h and 0010h ($p<0.025$ for 1026h; $p<0.05$ for 0336h; $p<0.025$ for second 0700-h value) under the normal photocycle.

★5: Higher T_4/T_3 than at 0700h ($p<0.05$-first sampling period), 1718h ($p<0.005$) and 2044h ($p<0.005$) under the shifted photocycle.

★6: Higher T_4/T_3 than at 1718h ($p<0.05$) and at 2044h ($p<0.05$) under the shifted photocycle.

Data are shown in Appendix Table 9 (normal photoperiod) and Table 10 (phase-shifted photoperiod).

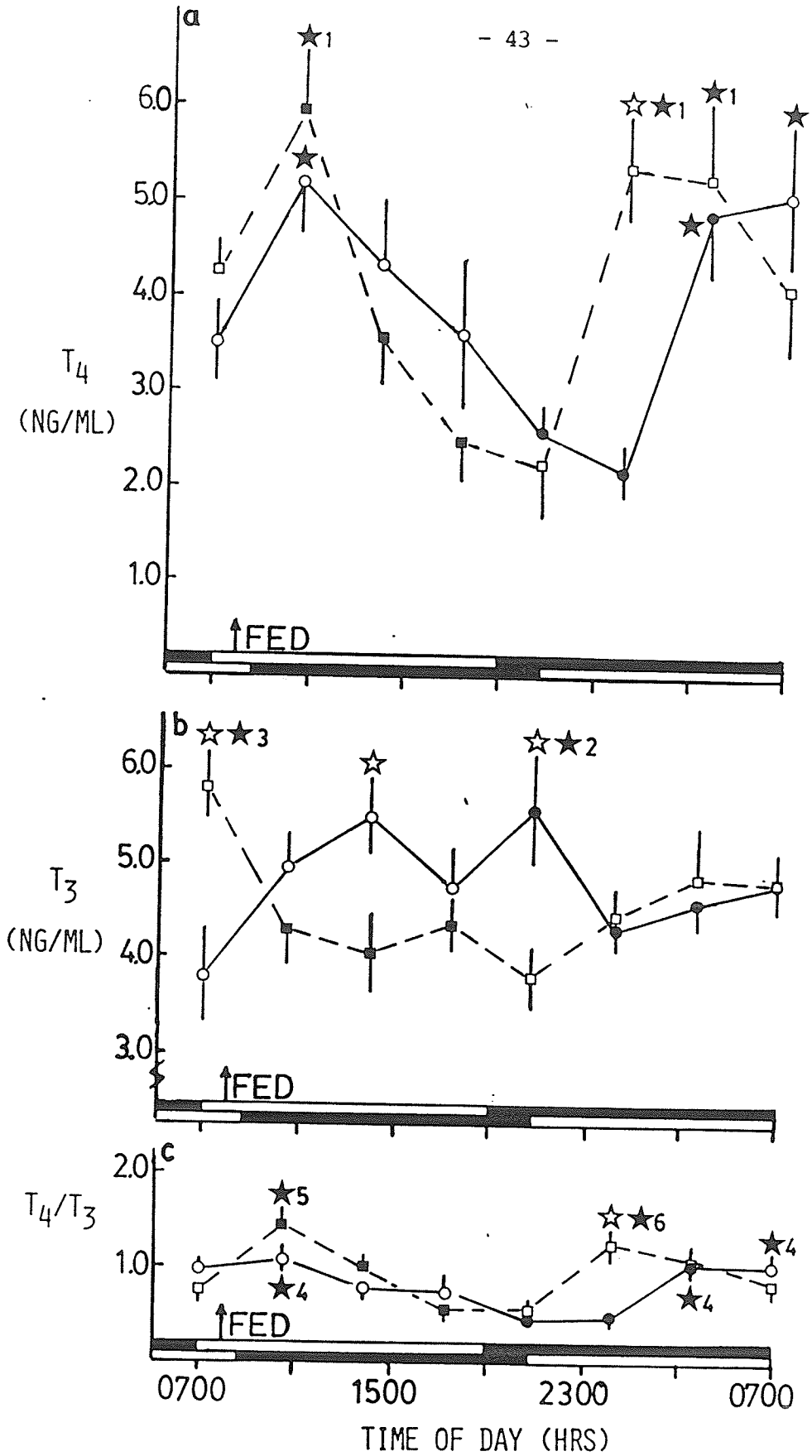
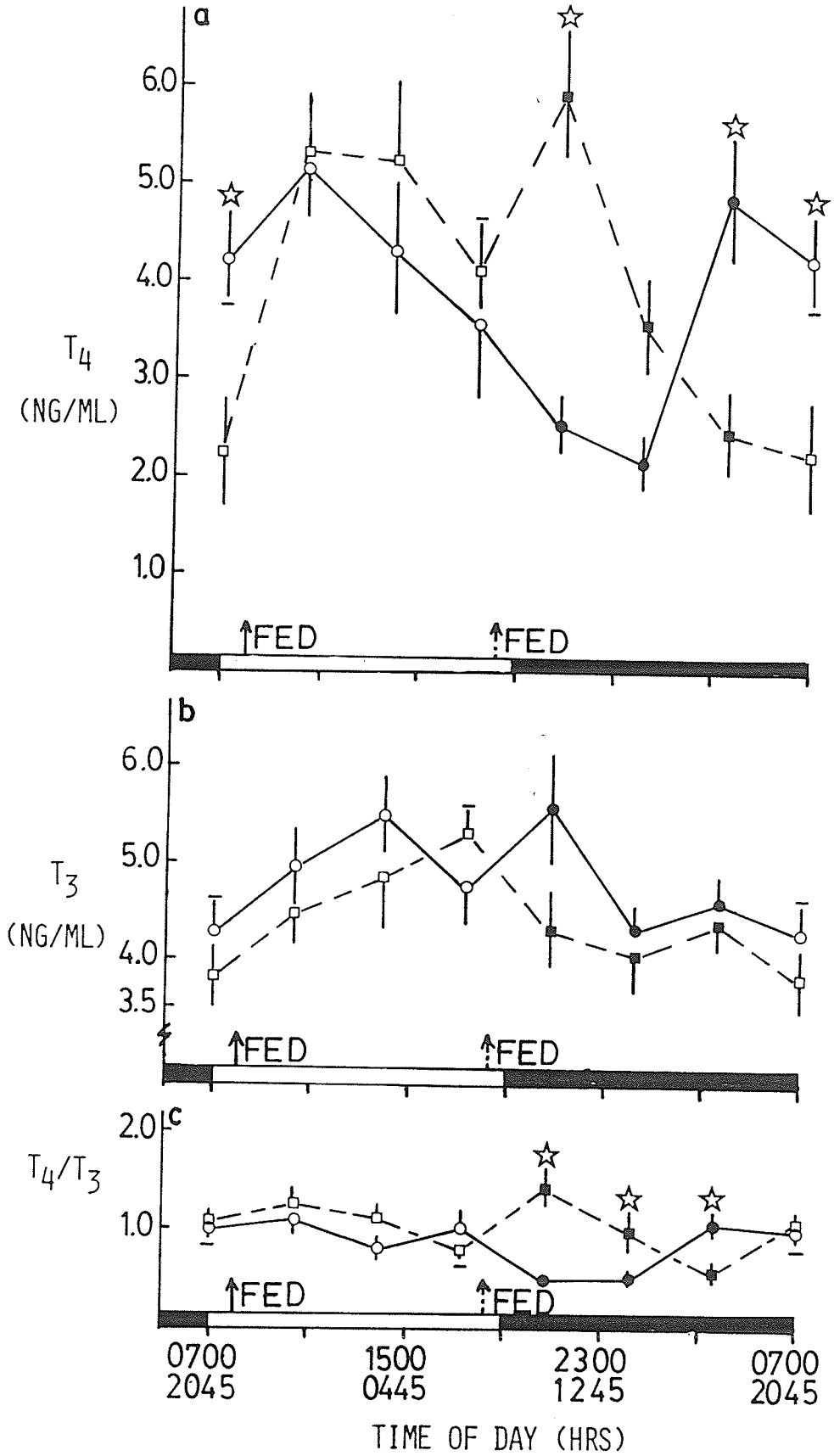

a
b
c
T_4 (NG/ML)
T_3 (NG/ML)
T_4/T_3
6.0
5.0
4.0
3.0
2.0
1.0
FED
0700
1500
2300
0700
TIME OF DAY (HRS)

Figure 7. The same data as in Figure 6 except the 12L(2045h-0845h): 12D photocycle profile was shifted graphically so the time of light onset coincided with that of the normal photocycle. o---● illustrates normal photocycle; □---■ illustrates shifted photocycle; o or □ indicates the mean value for both 0700h sampling times. ANOVA (2-way) indicated: i) pT_4 ($p<0.0025$), pT_3 ($p<0.01$) and T_4/T_3 ($p<0.05$) varied with time after light onset, ii) there was an interaction between time after light onset and feeding regime for pT_4 ($p<0.0005$) and T_4/T_3 ($p<0.0005$), but not for pT_3.

☆ : Higher than the corresponding value in the other group (graph a: $p<0.01$, $p<0.0005$, $p<0.01$, $p<0.01$; graph c: $p<0.005$, $p<0.05$, $p<0.025$).

Data are shown in Appendix Table 9 (normal photoperiod) and Table 10 (phase-shifted photoperiod).


a
6.0
5.0
4.0
3.0
2.0
1.0
T4
(NG/ML)
FED
FED
b
6.0
5.0
4.0
3.5
T3
(NG/ML)
FED
FED
c
2.0
1.0
T4/T3
FED
FED
0700
2045
1500
0445
2300
1245
0700
2045
TIME OF DAY (HRS)

significant, but there was no interaction between feeding and the time-of-day. At all sampling periods but the last one, pT_3 was significantly higher in the fed group than in the starved group (1-way ANOVA).

T_4/T_3

Neither fed nor starved trout demonstrated a diel change in T_4/T_3 (Fig. 8c). A 2-way ANOVA showed that the mean T_4/T_3 was higher in the starved group than in the fed group. There was no pooled time-of-day effect, and no interaction between feeding and the time-of-day. At 2044h, 0010h, and 0336h, the T_4/T_3 ratio was higher in the starved group than in the fed group (1-way ANOVA).

Reaction to Food Presentation

Upon food presentation, the fish could be heard splashing at the surface, presumably in response to the food stimulus. When sampled, all trout in the fed group had full stomachs or intestines.

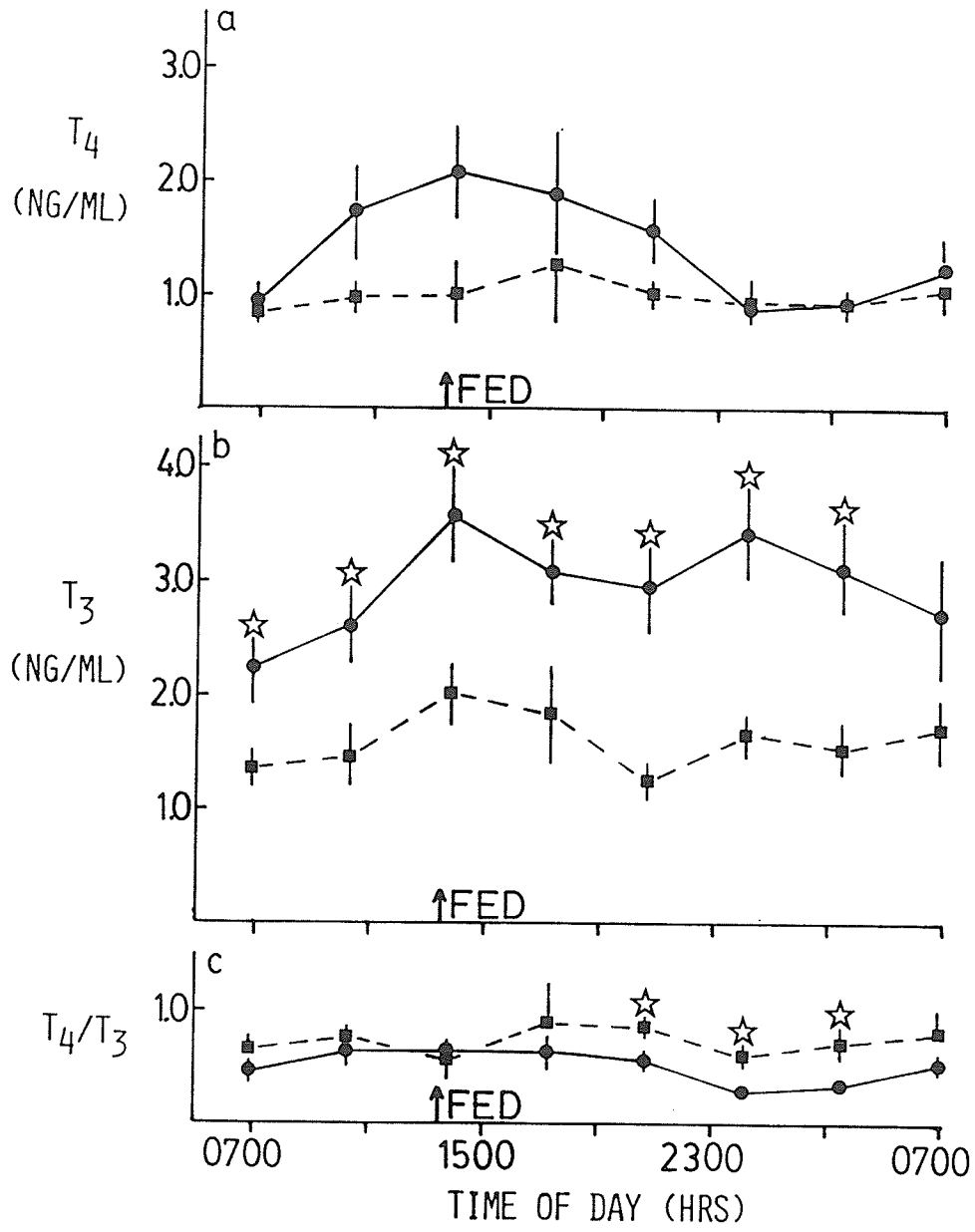
Analysis of Differences in Initial Mean Weights

In all experiments, trout were randomly assigned to tanks. This did not eliminate the possibility that there were differences in mean fish weights between sampling periods within experimental groups or between experimental groups. Therefore, ANOVA (1-way) was used to test for any differences in mean weights within or between experimental groups. At the beginning of Experiment 7, the fed group

Figure 8. Diel variations in pT_4 (a), pT_3 (b) and T_4/T_3 (c) in rainbow trout held at 11.0-12.0°C under continuous darkness and fed at 1330h (●---●) or starved (■——■) (Expt. 7). Each point represents the mean hormone level for a group of 8-12 trout. Standard errors from the mean (±1) are indicated by vertical bars. ANOVA (1-way) indicated a diel variation in pT_4 in fed trout ($p<0.05$), but not in starved trout. No diel variation was found for pT_3 or T_4/T_3 in starved or fed trout. ANOVA (2-way) indicated: i) diel change in pT_4 ($p<0.05$) and pT_3 ($p<0.05$), but not in T_4/T_3, ii) elevated pT_4 ($p<0.01$) and pT_3 ($p<0.0005$) in fed trout, and elevated T_4/T_3 in starved trout ($p<0.0005$), iii) no interaction between time of sampling and feeding in pT_4, pT_3 or T_4/T_3.

☆ : Higher than the corresponding point in the other group (graph b: $p<0.01$, $p<0.025$, $p<0.025$, $p<0.025$, $p<0.0005$, $p<0.0005$, $p<0.005$; graph c: $p<0.025$, $p<0.025$, $p<0.05$).

Data are shown in Appendix Table 11 (fed trout) and Table 12 (starved trout).


a
3.0
2.0
1.0
T_4
(NG/ML)
FED
b
4.0
3.0
2.0
1.0
T_3
(NG/ML)
FED
c
1.0
T_4/T_3
FED
0700
1500
2300
0700
TIME OF DAY (HRS)

had a significantly higher mean weight than the starved group. In no other experiments was there a difference in initial mean weights between experimental groups. There were no significant differences in mean trout weights between sampling periods in any experiment.

Effects of Gender and Maturity on pT_4 and pT_3

Upon sampling, the gender of each fish was determined whenever possible and maturity was assessed. In several cases there were uneven distributions of mature fish or of sexes either between sampling periods within a group or between experimental groups. In these cases, ANOVA (1-way) was used to test for differences in mean pT_4 and mean pT_3 between immature and mature fish or between sexes. To compare immature and mature trout, or to compare male and female trout in respect to pT_3 and pT_4, only those sample groups which had similar ratios of males to females or mature to immature fish were used. There were no differences between male and female fish in pT_4 or pT_3 in any experiment. In Experiment 1, mature trout had significantly higher pT_3 than immature trout. The only effect this could have had would be to prevent a borderline significant pT_3 change from showing up. In Experiment 7, mature starved fish had significantly higher pT_3 than immature starved fish. Except at 0336h, the mature fish were equally spread throughout the sampling periods. The F value ($F_{7,73} = 0.89$) was low, so it was unlikely that the difference between mature and immature fish made any

difference to the interpretation of the experiment. In Experiment 7, mature fed fish had significantly lower pT_4 than immature fed fish. This could not account for the significant pT_4 change in fed fish, as there were many mature fish in the group sampled at 1352h, when pT_4 was highest.

DISCUSSION

Diel Variation

pT_4

The first objective was to seek confirmation that diel changes in pT_4 and pT_3 occur in rainbow trout. In relation to this, fed trout in all experiments showed significant diurnal changes in pT_4, though the pattern varied with photoperiod and feeding regime. Diel changes in pT_4 have been noted before in rainbow trout (Osborne *et al.*, 1978; Eales *et al.*, 1981; Flood and Eales, 1983), though Leatherland *et al.* (1977) found no significant change in pT_4 with time. In Leatherland *et al.*'s study the high ambient temperature (18°C) could have been responsible. Also, as Eales *et al.* (1981) noted, the sampling was done only during the light period, and fish were fed *ad libitum* quantities at either 1, 3, 6, or 9h after light onset. Diurnal variations in pT_4 have been noted in Atlantic salmon (Rydevik *et al.*, 1984), brook trout (White and Henderson, 1977; McCormick and Naimon, 1984), goldfish (Spieler and

Noeske, 1979, 1981, 1984; Noeske and Spieler, 1983; MacKenzie et al., 1985, unpublished) and white suckers (Stacey et al., 1984).

Diurnal changes in pT_4 are probably due to surges resulting from TSH stimulation of the thyroid. A surge in the plasma levels of endogenous labelled T_4, and stable T_4 after TSH stimulation has been demonstrated in salmonid fish (Chan and Eales, 1975; Brown et al., 1978; Milne and Leatherland, 1978). In parrotfish, in vitro cultures of thyroid tissue showed T_4 release in response to bovine TSH stimulation (Grau et al., 1986).

pT_3

Significant pT_3 changes occurred in the late-fed trout in Experiment 4 and in the trout held under normal and shifted photoperiods in Experiment 6. The T_3 profiles in all experiments resembled somewhat those for T_4, but the changes were not as marked. Eales et al. (1981) also found a significant but modest pT_3 change in rainbow trout. Spieler and Noeske (1979, 1981) found significant pT_3 changes in goldfish, but Stacey et al. (1984) found no diurnal pT_3 changes in prespawning to late-spawning white suckers.

Plasma T_3 changes are not likely due to release of T_3 from the thyroid. During in vitro culture, the parrotfish thyroid

releases no detectable T_3 (Grau et al., 1986). Under TSH stimulation in salmonids, surges in endogenously labelled T_4 and stable T_4 are not accompanied by changes in T_3 (Chan and Eales, 1975; Brown et al., 1978; Milne and Leatherland, 1978). Diel changes in pT_3 are probably due to changes in extrathyroidal deiodination. In $*T_4$ injected salmonids, $*T_3$ is the main labelled organic product of deiodination, indicating a predominant T_4 monodeiodination pathway (Higgs and Eales, 1977; Eales et al., 1983). Pimlott and Eales (1983) demonstrated in vitro hepatic deiodination of $*T_4$ in rainbow trout, accompanied by $*T_3$ production. Eales (1977) showed that 59 to 69% of circulating T_4 was converted to T_3 in rainbow trout.

The modest changes in pT_3 as compared to pT_4 are probably due to the constancy of T_3 within the system. Rainbow trout challenged with T_4 show pT_4 values up to 50x those of control fish, with no significant changes in pT_3 (Blaschuk et al., 1982; Fok and Eales, 1984). The constancy is due partly at least, to a decrease in the proportion of T_4 peripherally deiodinated to T_3 (Fok and Eales, 1984). Therefore, the release of T_4 as a result of TSH stimulation causes a pT_4 surge, which is not likely to be paralleled by as strong a pT_3 surge due to deiodination of T_4.

T_4/T_3

Significant diel T_4/T_3 changes occurred in Experiments 1, 2, 3 and 6. The ratio, though used by previous workers, is hard to interpret since it is probably a reflection of T_4 output from the thyroid in relation to the extent of T_4 to T_3 monodeiodination. The T_4/T_3 changes tended to correspond more closely to the pT_4 profiles rather than the pT_3 profiles, so the ratio may be more a reflection on thyroidal T_4 output than T_4 to T_3 deiodination.

In summary, diel changes in pT_4 were confirmed in rainbow trout. Diel changes in pT_3 and T_4/T_3 were evident, but less consistent. The pT_4 pattern is probably regulated through thyroidal stimulation by TSH, while the pT_3 pattern is likely regulated by peripheral deiodination.

Effects of Starvation

Rainbow trout

The second objective was to determine whether feeding acts to establish or alter possible diel patterns in plasma thyroid hormone levels. In relation to this, starved fish showed no significant diel variation in pT_3 in any experiment. Diurnal variations in pT_4 and T_4/T_3 in starved trout were significant only in Experiment 2, and these changes were dampened in comparison to those of the fed trout in Experiment 1. A significant but suppressed diurnal pattern was previously observed in pT_4 and pT_3 in starved Atlantic salmon

(Rydevik et al., 1984). However, a significant change in pT_3 only occurred between 0400h and 0800h.

Apart from Experiment 2, the experiments on starved fish were consistent with previous studies on rainbow trout, which showed no significant diel variation in pT_3 or pT_4 after starvation for 3 days (Brown et al., 1978; Eales et al., 1981) or 13 days (Flood and Eales, 1983). Feeding, as shown in Experiment 2, does not necessarily establish the diel variation in pT_4. However, in all fed experiments there were significant daily changes in pT_4.

Starvation appears to suppress the levels of plasma thyroid hormones, which may partially account for abolishment of daily variations. In Experiment 2 and Experiment 5 the mean hormone levels were depressed in comparison with the levels for fed fish in other experiments. In Experiment 2 the pT_4 and pT_3 values were much lower than in Experiment 1. The experiments were done at different times, but the fish used in each experiment were selected from the same stock and group of fish. In Experiment 7 the starved fish had significantly lower pT_4 and pT_3 than fed fish. At 7 out of 8 sampling times the pT_3 in the starved group was significantly lower than in the fed group. This, and the observation that mean T_4/T_3 was significantly higher in the starved group than in the fed group, suggests that the effect of starvation on pT_3 is more dramatic than the effect on pT_4. These results are consistent with those of Flood

and Eales (1983). They found significantly lowered pT_4 in rainbow trout deprived of food for 10 or 20 days, and significantly lowered pT_3 after 6 or 10 days of starvation. At 0400h, starved trout had depressed pT_4 and pT_3 in relation to fed trout, and at 1100h, starved trout also had a lowered pT_3. In rainbow trout, 3 days of starvation also reduced the plasma and tissue T_4 pools, flow rates between pools, T_4 degradation rate and T_4 deiodination, and decreased conversion of T_4 to T_3 (Eales, 1979). However, pT_3 and the T_3 degradation rate were not lowered by 3 days of starvation. Milne et al. (1979) and Leatherland et al. (1977) found that starvation only lowered pT_4 significantly after 40 to 65 days. Plasma T_3 values did not change significantly with starvation (Milne et al., 1979).

Brook trout

Starvation had similar effects in depressing the thyroid system in yearling brook trout. Higgs and Eales (1978) found that starved brook trout had significantly lower pT_4 than fed brook trout, though an earlier study (Higgs and Eales, 1977) showed no significant difference between fed and starved trout. Starvation caused a slower turnover rate of PB*I, and a lower metabolic clearance rate for $*T_4$, $*T_3$, and *I (Higgs and Eales, 1977, 1978). It also lowered the extent of $*T_4$ deiodination and the conversion of $*T_4$ to $*T_3$. There was slower loss of liver radioactivity and depressed fecal loss of radioactivity in starved trout (Higgs and Eales, 1977).

In the salmonids studied, starvation tends to depress the thyroid system and refeeding activates it. Feeding can establish diel changes in pT_4 and pT_3 in rainbow trout, and consistently elevates the levels of both hormones.

Effects of Feeding Regime

pT_4

In further relation to the second objective, Experiments 4 and 6 showed that the feeding regime can alter the daily pattern of change in pT_4. Late-feeding in Experiment 4 resulted in a peak at 2044h, which was significantly greater than the corresponding level in the early-fed group. Otherwise the 2 profiles resembled each other closely. Experiment 6 showed that the normal and shifted photoperiod profiles corresponded closely and changed significantly with respect to the time-after-feeding. When the late-fed curve in Experiment 4 was shifted graphically so that both the late- and early-fed groups corresponded to time-after-feeding, the profiles corresponded closely (Fig. 4), and resembled those in Experiment 6 (Fig. 6). In addition, there was a significant time-after-feeding effect. The results represented in Figure 4 cannot be given too much weight by themselves, but when compared to those of Experiment 6, they emphasize that diurnal patterns in pT_4 are altered by changes in feeding regime. Feeding, as demonstrated in Experiment 7, can elicit a significant diurnal pattern in pT_4 in the absence of photoperiod. Nevertheless,

the changes under continuous darkness are dampened in relation to those exhibited in experiments run in the presence of a light-dark cycle.

$\underline{pT_3}$

Experiment 4 showed that changes in feeding regime can alter diel changes in pT_3. The early-fed and late-fed profiles did not resemble each other, and the significant interaction between time-of-day and feeding showed that the feeding regime was responsible for the differences. When the late-fed curve was shifted so that the profiles corresponded in respect to time-after-feeding (Fig. 4b), the curves resembled each other closely, and the time-after-feeding effect was significant. The results represented in Figure 4b cannot be taken as direct proof of diurnal changes in pT_3 in response to feeding, but they support the results represented in the unshifted profiles (Fig. 3b). On the other hand, Experiment 6 did not support these findings. There was no significant pooled time-after-feeding effect on diurnal changes in pT_3, and the normal and shifted photoperiod patterns did not resemble each other, suggesting that in this case it was photoperiod and not feeding regime which set the pattern.

$\underline{T_4/T_3}$

A change in feeding regime also altered the profile of T_4/T_3. Experiment 4 showed that a change in feeding regime did not significantly alter the overall T_4/T_3 profile, but it did result

in a significantly higher level at 0336h in the late-fed, as compared to the early-fed group. Experiment 6 showed that T_4/T_3 changed in relation to the time-after-feeding. Supporting evidence resulted from a comparison between the shifted profile and the normal profile with respect to the time-after-light onset. This showed an interaction between feeding regime and the time-after-light onset.

Eales et al. (1981) also found that a change in feeding regime could alter diurnal patterns in pT_4 and pT_3 in rainbow trout. In trout fed at 0930h, the peak pT_4 was at 0900h or at 1300h, but the levels dropped quickly after the peak. In trout fed at 1630h, the pattern was similar, except that the hormone levels remained significantly elevated until 2300h. The peak pT_3 values were at 1300h (on 2 consecutive days) and 0100h (only on day 1) in early-fed fish. The pattern was similar in late-fed fish, except that rather than dropping, the levels remained elevated until 2300h.

In summary, the time of feeding alters the diel pattern of change in pT_4 in rainbow trout. It can alter the daily patterns of pT_3 and T_4/T_3, but not as consistently.

Effects of a Photoperiod Shift

pT_4

The third objective was to determine if phasing of the photoperiod establishes or alters diel patterns in pT_4 and pT_3 in

rainbow trout. In relation to this, Experiments 4 and 6 showed that changes in photoperiod can alter diurnal patterns in pT_4. The results from both experiments showed that significant daily changes in pT_4 occurred with respect to the time-after-light onset. Experiment 6 showed that when a 12L:12D photoperiod was shifted, the overall pT_4 profile was significantly altered. The point immediately following light onset in the shifted profile was significantly higher than the corresponding point in the normal profile, which emphasized the change.

In starved trout (Experiment 5) a change in photoperiod did not alter the overall diel pT_4 pattern, but at 2044h the mean pT_4 in the group held under the normal photoperiod was significantly higher than the corresponding value in the photoperiod reversed group. Thus, even in the absence of food, a photoperiod change had altered the profile. A more dramatic effect was that the mean pT_4 was significantly lower under the reversed photoperiod regime than under the normal photoperiod regime. The reason for this difference is unclear.

$\underline{pT_3}$

Experiment 6 showed that a change in a 12L:12D photoperiod can cause changes in the diurnal pattern of pT_3. The normal and shifted pT_3 profiles did not resemble each other (Fig. 6b). A significant interaction between photoperiod and time-after-feeding showed that the change was due to a change in the photoperiod. When the 12L(2045h-

0845h):12D profile was shifted graphically, the curves corresponded closely with respect to time-after-light onset, and there was a significant change in pooled pT_3 with time-after-light onset. In contrast, Experiment 4 showed no indication of a change in pT_3 with the time-after-light onset. Instead, the feeding regime was the important factor in setting the pattern. In starved fish (Experiment 5) there was no indication that pT_3 pattern was changed in relation to photoperiod.

T_4/T_3

In both Experiments 4 and 6, a significant change in T_4/T_3 occurred with the time-after-light onset. This showed that photoperiod was responsible for causing diel changes in T_4/T_3. There was no indication in starved fish (Experiment 5) that a change in the T_4/T_3 profile occurred in relation to a change in the 12L:12D photoperiod.

In summary, when a 12L:12D photoperiod is phase-shifted, it alters the diel patterns of pT_4 and T_4/T_3. It can also modify the daily pattern of pT_3, but less consistently.

Relationship between Feeding and Photoperiod

Rainbow trout

Apparently, in rainbow trout, daily changes in plasma thyroid hormones in response to feeding and photoperiod are interrelated. In Experiment 7, feeding under continuous darkness caused significant

diurnal changes in pT_4, but the changes were modest in relation to experiments where fish were also exposed to a light cycle. There were no changes in pT_3 or in T_4/T_3. In Experiment 2, significant diel changes occurred in pT_4 and T_4/T_3 in starved fish, but these changes were also suppressed. In Experiment 5, neither starved group showed any diel changes in plasma thyroid hormone levels. Small diurnal changes in pT_4 or pT_3 occurred in the absence of one or the other variable, but when both variables were present, they seemed to complement each other to amplify the diel pattern of change.

Goldfish

Similar feeding and photoperiod experiments have been done with goldfish. Noeske and Spieler (1983) demonstrated that under either a 12L(1200h-2400h):12D light cycle or a 16L(0800h-2400h):8D light cycle, goldfish demonstrated a significant daily pT_4 variation with a peak at light onset and a nadir when darkness began. Under the 12L:12D cycle, there was an additional peak at 4h before light onset. No diurnal pattern was evident under either an 8L(0400h-1200h):16D light cycle or a reversed 12L:12D light cycle. In 3 experiments, groups of fish fed at the same time of day, but under from 2 to 6 staggered photoperiods, always showed peak pT_4 during the photoperiod.

Nevertheless, feeding in goldfish may alter the pattern set by photoperiod. Spieler and Noeske (1981) found that fish maintained on a 12L(0800h-2000h):12D light cycle, and fed at either 0800h or 1600h,

had significantly higher pT_4 at 1600h than at any other time of day. However, in early-fed fish the 1200h value was also significantly higher than all other mean values. Late-fed fish had significantly higher pT_3 at 1600h and 2000h than at other sampling times. Fish fed at 0800h, on the other hand showed no significant pT_3 variation, though there was a peak at 1600h. Goldfish in groups fed from 4-6h after light onset had lower mean pT_4 than fish fed earlier or later (Spieler and Noeske, 1984). However, goldfish deprived of food for 12h before a 24h sampling period still demonstrated significant diurnal variations in both pT_4 and pT_3 (Spieler and Noeske, 1979).

Rainbow trout and goldfish are similar in that photoperiod and feeding can alter diurnal changes in pT_4 and pT_3. The difference is that in goldfish, feeding appears to play a minor role and does not set diurnal patterns in plasma thyroid hormones. There could be several reasons for this difference. Rainbow trout, being predatory, may depend on a more fluctuating food supply than goldfish. When food is not available it may be beneficial to retard growth and development and "shut down" systems such as the thyroid, which are related to growth and development. When food is continuously available to rainbow trout, it is eaten in distinct meals rather than in a continuous fashion (Grayton and Beamish, 1977; Grove et al., 1978). Therefore, it may be important to set the thyroid system in response to feeding, so that food can be used more beneficially for growth and development. Goldfish, being omnivorous, have continuous

feeding habits (Rozin and Mayer, 1961, 1964), and thus may not depend on feeding to adjust diurnal thyroid changes.

Another possible reason for differences between trout and goldfish is that goldfish are a more domestic and inbred species than rainbow trout and over long periods have been bred under artificial conditions of feeding and light cycle. Thus, over time food may have become less important in adjusting, or setting diurnal changes in the thyroid system.

Endogenous Rhythm

The fourth objective was to determine if an endogenous rhythm in pT_4 occurs in laboratory-held rainbow trout. In relation to this, there was no evidence of a completely endogenous rhythm in the absence of photoperiod and food in Experiment 7. However, the possibility that there was an inherent system, established by feeding or photoperiod was not ruled out. In both Experiment 1 and Experiment 7, the rise in pT_4 in fed fish was not a direct result of feeding, because the level was near to maximal at 3h before feeding. In Experiment 2, the significant pT_4 peak in starved trout did not occur until 6.5h after light onset, and in Experiment 5, the peak level (though not significant) in starved trout, under a regular cycle did not occur until after darkness. Therefore, these patterns were not likely set directly by light onset either. In Experiment 6, an extra pT_4 peak at 0336h could not have been directly due to

photoperiod or feeding regime. Osborne and Simpson (1978) found that peak pT_4 in rainbow trout occurred at 1h after sunset in September, but occurred 5h after sunset in March. The minimum levels were at 5h after sunrise in September and 9h after sunrise in March. This suggests that there may be an inherent system which shifts in pattern over the course of a year. This could not be due to light cycle which is the same in March as September. It could be due to the ambient temperature which changed from 15-16.5°C in September to 5-6°C in April, or to a change in feeding habits over the course of the year.

Effects of Light Intensity Change

Light intensity changes (Experiment 3) caused no significant changes in pT_4, pT_3, or T_4/T_3. However, the progressive increase in pT_4 with increased light intensity at 1026h indicated that a significant change may have occurred over a greater light intensity range. A high light intensity may have been partially responsible for the significant changes in pT_4 and T_4/T_3 in Experiment 2, which was carried out at high light intensity (>200 lux). Leatherland et al. (1977) found no difference in pT_4 between rainbow trout kept under different light intensities (100 lux or 600 lux measured at the water surface), but these measurements were only taken at one time of day.

Pineal-Thyroid Interactions

Mammals

The pineal system has been linked to changes in levels of plasma thyroid hormones in the rat and the hamster in relation to the photoperiod. Relkin (1978) found that under diurnal lighting (10L:14D), pT_4 in pinealectomized rats was elevated in relation to sham-operated controls. Under dark exposure, pT_4 in intact rats was depressed. Pinealectomy prevented this effect. Gordon et al. (1980) found an increase in pT_3 and the free T_3 index (FT_3I) in rats after optic nerve sectioning. The increase was abolished by pinealectomy. The free T_4 index (FT_4I) and pT_4 were not affected by optic nerve sectioning.

In golden hamsters, blinding caused significant decreases in the FT_4I, and in pT_4, after 8-10 weeks (Vriend et al., 1977; Vriend and Reiter, 1977; Vriend et al., 1979; Vriend, 1984). Pinealectomy or superior cervical ganglionectomy reversed the effects of blinding, which suggested that the levels of the FT_4I and pT_4 were depressed by the dark-stimulated pineal gland (Vriend et al., 1977; Vriend et al., 1979; Vriend, 1984). Melatonin injections in most cases depressed pT_4 and the FT_4I in hamsters (Vriend et al., 1977; Vriend et al., 1982). Vriend et al. (1982) found that melatonin injections caused significant lowering of the FT_3I in hamsters kept under a 10L:14D photoperiod, but not in those held under

14L:10D conditions. Vriend (1984) found that pinealectomy in hamsters eliminated diurnal changes in pT_4 and FT_4I, but Brammer et al. (1979) found that pinealectomy did not abolish a diurnal change in pT_3. Brammer et al. (1979) also found that pinealectomy did not alter pT_3, the FT_3I, pT_4 or FT_4I.

Fish

The interrelationship between the pineal and thyroid systems that exists in the rat and the hamster may also exist in fish, but it has not yet been demonstrated. Changes in diel or circadian patterns in fish are often affected by pinealectomy. Pinealectomy caused significant alterations in free-running activity patterns in lake chub (Couesius plumbeus), and in burbot (Lota lota) (Kavaliers, 1979a, 1980). In the white sucker (Catastomus commersoni), pinealectomy caused the activity rhythm to split into 2 components and the fish showed a loss of stability and increased variability in activity onsets (Kavaliers, 1979b). In white suckers, pinealectomy also eliminated a significant diel rhythm in behavioural thermoregulation (Kavaliers and Ralph, 1980). Diurnal patterns of colour change are affected by the pineal system in several fish. In the killifish, Fundulus heteroclitus (Kavaliers et al., 1980) and in several species of lamprey ammocoete larvae (Young 1935; Eddy and Strahan, 1968; Joss, 1973), diurnal colour changes were eliminated by pinealectomy. In the guppy (Poecilia reticulata) (Nayadu and Hunter, 1979), and in pencilfish (Nannostomus sp.) (Reed, 1968,

1969), melatonin injections caused the fish to show their night colouration. In goldfish, pinealectomy abolished daily fluctuations of liver glycogen in July, August, and February and abolished daily glucose fluctuations in the autumn (Delahunty et al., 1978). Pinealectomy altered daily changes in plasma glucose levels in the summer, and in liver lipid levels during the autumn. Pinealectomy repressed a daily cycle in serum gonadotropin (GTH) in the goldfish under a 16L:8D photoperiod, but promoted a daily cycle in serum GTH under an 8L:16D photoperiod, which was not present in control fish (Hontela and Peter, 1980). However, Vodicnik et al. (1978) found that pinealectomized goldfish continued to exhibit a diurnal variation in gonadotropin under a long photoperiod. Gern et al. (1978a,b) found that melatonin varies diurnally in rainbow trout, with maximal levels occurring during the dark phase and minimal levels occurring during the light phase, so it is possible that it is involved in setting daily changes in other systems. After pinealectomy, there were lower circulating melatonin levels during the dark phase in relation to control trout, but not during the light phase. In pinealectomized trout there were still diurnal changes in plasma melatonin levels. Gern and Ralph (1979) found that the retina also synthesizes melatonin in rainbow trout, so it may be as important as the pineal organ in setting diurnal variations in fish.

In summary, the pineal system is often important in phasing circadian rhythms in fish, and the thyroid system is affected by the pineal system in at least 2 mammal species. It is possible that the

pineal is partially responsible for causing diurnal changes in pT_3 and pT_4 levels in response to photoperiod in rainbow trout. Experiments have yet to be designed to investigate this possibility.

Mechanism of Feeding Response

The response of the thyroid system to feeding could be by several mechanisms. The smell or sight of the food, stomach distension or weight of food in the stomach, or release of compounds into the bloodstream could all be responsible to varying degrees. Physical activity in itself is not likely a factor since there were no changes in plasma thyroid hormones in response to cleaning of the tanks.

Conclusions

Diel changes in pT_4 in rainbow trout were consistently demonstrated in this study. There were less marked diel changes in pT_3 and T_4/T_3. A change in either feeding regime or a 12L:12D photocycle modified the diel pattern of pT_4 and to a lesser extent pT_3. A change in the 12L:12D photocycle modified the diel pattern of T_4/T_3 more consistently than a change in feeding regime. There was no evidence of a completely endogenous rhythm in pT_4 or pT_3 and large diel changes occurred only when fish were fed and held under a 12L:12D photocycle. Feeding and photoperiod probably interact in a complex manner to elicit diel changes in plasma thyroid hormones but the effect of feeding is somewhat more pronounced.

Much work must be done to understand the internal mechanisms involved in diurnal changes in the thyroid system in rainbow trout and in other fish. In this study, diurnal changes in thyroid hormones were studied in relation to changes in a 12L:12D photoperiod and to changes in the feeding regime, which were both external factors. It is probable that other external factors, such as length of the photoperiod, amount of feeding, or temperature are involved in modifying the pattern. MacKenzie (1985, unpublished), for example, found that diurnal variations in pT_4 were present in goldfish held under 21°C conditions, but not under 12°C conditions. The diurnal patterns in pT_3 and pT_4 appear to vary between species, and a factor which is important in setting a pattern in one species, may not be as important in another species. Plasma T_4 and pT_3 are not necessarily equally affected by each factor, as the mechanisms involved in causing increments or declines in the level of each hormone are probably different.

LITERATURE CITED

Ami, M., Herbuté, S., Ramade, F., Bouillé, C., and Baylé, J.-D. (1982). Repercussions de lésions hypothalamiques antérieures sur les variations nycthémérales de la thyroxine et de la corticostérone plasmatiques chez la pigeon. C.R. Soc. Biol. 176, 670-674.

Azukizawa, M., Roohk, H.V., DiStefano, J.J., and Hershman, J.M. (1975). Diurnal rhythm of plasma TSH, T_3, and T_4 levels in rats. Clin. Res. 23, 92A.

Blaschuk, O.W., Jamieson, J.C., and Eales, J.G. (1982). Properties of hexosaminidases in cell-free extracts of rainbow trout livers and effects of thyroid hormones. Comp. Biochem. Physiol. 73B, 729-734.

Brammer, G.L., Morley, J.E., Geller, E., Yuwiler, A., and Hershman, J.M. (1979). Hypothalamus-pituitary-thyroid axis interactions with pineal gland in the rat. Amer. J. Physiol. 236, E416-E420.

Brown, S., Fedoruk, K., and Eales, J.G. (1978). Physical injury due to injection or blood removal causes transitory elevations of plasma thyroxine in rainbow trout, Salmo gairdneri. Canad. J. Zool. 56, 1998-2003.

Chan, H.H., and Eales, J.G. (1975). Identification of iodoamino acids in the plasma and bile of brook trout, Salvelinus fontinalis (Mitchill). Canad. J. Zool. 53, 97-101.

Cogburn, L.A., Wilson, S.J., and Freeman, R.M. (1983). Photo-thermal features of pineal and thyroid hormone rhythms. Federation Proceedings 42, N3, 576.

Curtis, R.J., and Abrams, J.T. (1977). Circadian rhythms in the concentration of thyroid hormone in the plasma of normal calves. Br. Vet. J. 133, 134-143.

DeCostre, P., Buhler, U., DeGroot, L.J., and Refetoff, S. (1971). Diurnal rhythm in total serum thyroxine levels. Metabolism 20, 782-791.

Decuypere, E., and Kühn, E.R. (1984). Effect of fasting and feeding time on circadian rhythms of serum thyroid hormone concentrations, glucose, liver monodeiodinase activity and rectal temperature in growing chickens. Domestic Animal Endocrinology 1, 251-262.

Delahunty, G., Bauer, G., Prack, M., and De Vlaming, V. (1978). Effects of pinealectomy and melatonin treatment on liver and plasma metabolites in the goldfish, Carassius auratus. Gen. Comp. Endocrinol. 35, 99-109.

Eales, J.G. (1977). Use of thyroxine- and triiodothyronine-specific antibodies to study thyroxine kinetics in rainbow trout, Salmo gairdneri. Gen. Comp. Endocrinol. 32, 89-98.

Eales, J.G. (1979). Comparison of L-thyroxine and 3,5,3'-triiodo-L-thyronine kinetics in fed and starved rainbow trout (Salmo gairdneri). Comp. Biochem. Physiol. 62A, 295-300.

Eales, J.G., Hughes, M., and Uin, L. (1981). Effect of food intake on diel variation in plasma thyroid hormone levels in rainbow trout, Salmo gairdneri. Gen. Comp. Endocrinol. 45, 167-174.

Eddy, J.M.P., and Strahan, R. (1968). The role of the pineal complex in the pigmentory effector system of the lampreys, Mordacia nordax (Richardson) and Geotria australis (Gray). Gen. Comp. Endocrinol. 11, 528-534.

Flood, C.G., and Eales, J.G. (1983). Effects of starvation and refeeding on plasma T_4 and T_3 levels and T_4 deiodination in rainbow trout, Salmo gairdneri. Can. J. Zool. 61, 1949-1953.

Fok, P., and Eales, J.G. (1984). Regulation of plasma T_3 levels in T_4-challenged rainbow trout, Salmo gairdneri. Gen. Comp. Endocrinol. 53, 197-202.

Fukuda, H., Greer, M.A., Roberts, L., Allen, C.F., Critchlow, V., and Wilson, M. (1975). Nyctohemeral and sex-related variations in plasma thyrotropin, thyroxine, and triiodothyronine. Endocrinology 97, 1424-1431.

Gern, W.A., Owens, D.W., and Ralph, C.L. (1978a). Plasma melatonin in the trout: day-night change demonstrated by radioimmunoassay. Gen. Comp. Endocrinol. 34, 453-458.

Gern, W.A., Owens, D.W., and Ralph, C.L. (1978b). Persistence of the nyctohemeral rhythm of melatonin secretion in pinealectomized or optic tract-sectioned trout (Salmo gairdneri). J. Exp. Zool. 205, 371-376.

Gern, W.A., and Ralph, C.L. (1979). Melatonin synthesis by the retina. Science 204, 183-184.

Gordon, J., Morley, J.E., and Herschman, J.M. (1980). Melatonin and the thyroid. Horm. Metab. Res. 12, 71-73.

Grau, E.G., Helms, L.M.H., Shimoda, S.K., Ford, C.A., LeGrand, J., and Yamauchi, K. (1986). The thyroid gland of the Hawaiian parrotfish and its use as an *in vitro* model system. Gen. Comp. Endocrinol. 61, 100-108.

Grayton, B.D., and Beamish, F.W.H. (1977). Effects of feeding frequency on food intake, growth, and body composition of rainbow trout (*Salmo gairdneri*). Aquaculture 11, 159-172.

Grove, D.J., Loizides, L.G., and Nott, J. (1978). Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. J. Fish Biol. 12, 507-516.

Harvey, S., Davison, T.F., Klandorf, H., and Phillips, J.G. (1980). Diurnal changes in the plasma concentrations of thyroxine and triiodothyronine and their binding to plasma proteins in the domestic duck (*Anas platyrhynchos*). Gen. Comp. Endocrinol. 42, 500-504.

Higgs, D.A., and Eales, J.G. (1977). Influence of food deprivation on radioiodothyronine and radioiodide kinetics in yearling brook trout, *Salvelinus fontinalis* (Mitchill) with a consideration of the extent of L-thyroxine conversion to 3,5,3'-triiodo-L-thyronine. Gen. Comp. Endocrinol. 32, 29-40.

Higgs, D.A., and Eales, J.G. (1978). Radiothyroxine kinetics in yearling brook trout, *Salvelinus fontinalis* (Mitchill) on different levels of dietary intake. Canad. J. Zool. 56, 80-85.

Hontela, A., and Peter, R.E. (1980). Effects of pinealectomy, blinding, and sexual condition on serum gonadotropin levels in the goldfish. Gen. Comp. Endocrinol. 40, 168-179.

Jordan, D., Rousset, B., Perrin, F., Fournier, M., and Orgiazzi, J. (1980). Evidence for circadian variations in serum thyrotropin, 3,5,3'-triiodothyronine, and thyroxine in the rat. Endocrinology 107, 1245-1248.

Joss, J.M.P. (1973). The pineal complex, melatonin, and color change in the lamprey, *Lampetra*. Gen. Comp. Endocrinol. 21, 188-195.

Kavaliers, M. (1979a). Pineal involvement in the control of circadian rhythmicity in the lake chub, *Couesius plumbeus*. J. Exp. Zool. 209, 33-40.

Kavaliers, M. (1979b). The pineal organ and circadian organization of teleost fish. Rev. Canad. Biol. 38, 281-292.

Kavaliers, M. (1980). Circadian locomotor activity rhythms of the burbot, Lota lota: seasonal differences in period length and the effect of pinealectomy. J. Comp. Physiol. 136, 215-218.

Kavaliers, M., Firth, B.T., and Ralph, C.L. (1980). Pineal control of the circadian rhythm of colour change in the killifish (Fundulus heteroclitus). Can. J. Zool. 58, 456-460.

Kavaliers, M., and Ralph, C.L. (1980). Pineal involvement in the control of behavioural thermoregulation of the white sucker, Catastomus commersoni. J. Exp. Zool. 212, 301-303.

Kemppainen, R.J., and Sartin, J.L. (1984). Evidence for episodic but not circadian activity in plasma concentrations of adrenocorticotrophin, cortisol, and thyroxine in dogs. J. Endocrinol. 103, 219-226.

Klandorf, H., Sharp, P.J., and Duncan, I.J.H. (1978). Variations in levels of plasma thyroxine and triiodothyronine in juvenile female chickens during 24- and 16-hr lighting cycles. Gen. Comp. Endocrinol. 36, 238-243.

Kühn, E.R., Decuypere, E., Colen, L.M., and Michels, H. (1982). Posthatch growth and development of a circadian rhythm for thyroid hormones in chicks incubated at different temperatures. Poultry Sci. 61, 540-549.

Kühn, E.R., Delmotte, N.M.J., and Darras, V. (1982). Circadian rhythmicity of thyroid hormones in hibernating frogs. Annales d'Endocrinologie 43, N3, 88A.

Kühn, E.R., Delmotte, N.M.J., and Darras, V.M. (1983). Persistence of a circadian rhythmicity for thyroid hormones in plasma and thyroid of hibernating male Rana ridibunda. Gen. Comp. Endocrinol. 50, 383-394.

Leatherland, J.F., Cho, C.Y., and Slinger, S.J. (1977). Effects of diet, ambient temperature and holding conditions on plasma thyroxine levels in rainbow trout (Salmo gairdneri). J. Fish. Res. Board Can. 34, 677-682.

Lucke, C., Hehrmann, R., von Mayersbach, K., and von zur Mühlen, A. (1977). Studies on circadian variations of plasma TSH, thyroxine, and triiodothyronine in man. Acta Endocrinol. 86, 81-88.

McCormick, S.D., and Naiman, R.J. (1984). Osmoregulation in the brook trout, Salvelinus fontinalis.- I. Diel photoperiod and growth related physiological changes in freshwater. Comp. Biochem. Physiol. 79A, 7-16.

MacKenzie, D.S. (1985). Thyroid function during reproductive activity in teleost fishes. Unpublished. 10th International Symposium of Comparative Endocrinology, Copper Mountain, Colorado, U.S.A.

Milne, R.S., and Leatherland, J.F. (1978). Effect of ovine TSH, thiourea, ovine prolactin, and bovine growth hormone on plasma thyroxine and triiodothyronine levels in rainbow trout, Salmo gairdneri. J. Comp. Physiol. 124, 105-110.

Milne, R.S., Leatherland, J.F., and Holub, B.J. (1979). Changes in plasma thyroxine, triiodothyronine and cortisol associated with starvation in rainbow trout (Salmo gairdneri). Env. Biol. Fish. 4, 185-190.

Nayadu, P.L., and Hunter, C.R. (1979). Cytological aspects and differential response to melatonin of melanophore based color mutants in the guppy, Poecilia reticulata. Copeia. 1979, 232-242.

Newcomer, W.S. (1974). Diurnal rhythms of thyroid functions in chicks. Gen. Comp. Endocrinol. 24, 65-73.

Noeske, T.A., and Spieler, R.E. (1983). Photoperiod and diel variations of serum cortisol, thyroxine, and protein in goldfish, Carassius auratus L. J. Fish Biol. 23, 705-710.

O'Connor, V.F., Wu, C.Y., Gallagher, T.F., and Hellman, L. (1974). The 24-hour plasma thyroxine profile in normal man. J. Clin. Endocrinol. Metab. 39, 765-771.

Omeljaniuk, R.J., Cook, R.F., and Eales, J.G. (1984). Simultaneous measurement of thyroxine and triiodothyronine in trout plasma using a solid-phase radioimmunoassay. Canad. J. Zool. 62, 1451-1453.

Ortiz-Caro, J., Gonzalez, C., and Jolin, T. (1984). Diurnal variations of plasma growth hormone, thyrotropin, thyroxine, and triiodothyronine in streptozotocin-diabetic and food-restricted rats. Endocrinology 115, 2227-2232.

Osborne, R.H., Simpson, T.H., and Youngson, A.F. (1978). Seasonal and diurnal rhythms of thyroidal status in the rainbow trout, Salmo gairdneri (Richardson). J. Fish Biol. 12, 531-540.

Ottenweller, J.E., and Hedge, G.A. (1981). Daily rhythms of plasma thyrotropin (TSH), thyroxine (T_4), and triiodothyronine (T_3) in rats are phase-shifted by inverting the light-dark cycle. Amer. Zool. 21, N4, 1026.

Ottenweller, J.E., and Hedge, G.A. (1982). Diurnal variations of plasma thyrotropin, thyroxine, and triiodothyronine in female rats are phase-shifted after inversion of the photoperiod. Endocrinology 111, 509-514.

Pallardo, L.F., Pericás, I., and Jolín, T. (1976). Thyroid iodine uptake, thyroid iodine secretion and plasma TSH levels in male rats during the day and night. Acta Endocrinol. 82, 517-529.

Pekary, A.E., Azukizawa, M., Hershman, J.M., and Parker, D.C. (1976). Plasma TSH, T_4 and T_3 relationship in man. Clinical Research 24, 100A.

Pimlott, N.J., and Eales, J.G. (1983). In vitro study of hepatic iodothyronine deiodination in rainbow trout, Salmo gairdneri. Canad. J. Zool. 61, 547-552.

Reed, B.L. (1968). The control of circadian pigment changes in the pencil fish: a proposed role for melatonin. Life Sci. 7, 961-973.

Reed, B.L., Finnin, B.C., and Ruffin, N.E. (1969). The effects of melatonin and epinephrine on the melanophores of freshwater teleosts. Life Sci. 8, 113-120.

Relkin, R. (1978). Use of melatonin and synthetic TRH to determine site of pineal inhibition of TSH secretion. Neuroendocrinology 25, 310-318.

Rookh, H.V., Azukizawa, M., DiStefano, J.J., Ogihara, T., and Hershman, J.M. (1979). Pituitary-thyroid hormone periodicities in serially sampled plasma of unanaesthetized rats. Endocrinology 104, 851-856.

Rozin, P., and Mayer, J. (1961). Regulation of food intake in the goldfish. Am. J. Physiol. 201, 968-974.

Rozin, P., and Mayer, J. (1964). Some factors influencing short-term food intake of the goldfish. Am. J. Physiol. 206, 1430-1436.

Rydevik, M., Lindahl, K., and Fridberg, G. (1984). Diel pattern of plasma T_3 and T_4 levels in Baltic salmon parr (Salmo salar L) during two seasons. Canad. J. Zool. 62, 643-646.

Sadovsky, R., and Bensadoun, A. (1971). Thyroid iodohormones in the plasma of the rooster (Gallus domesticus). Gen. Comp. Endocrinol. 17, 268-274.

Sharp, P.J., Klandorf, H., and Lea, R.W. (1984). Influence of lighting cycles on daily rhythms in concentrations of plasma tri-iodothyronine and thyroxine in intact and pinealectomized immature broiler hens (Gallus domesticus). J. Endocrinol. 103, 337-345.

Spieler, R.E., and Noeske, T.A. (1979). Diel variations in circulating levels of triiodothyronine and thyroxine in goldfish, Carassius auratus. Canad. J. Zool. 57, 665-669.

Spieler, R.E., and Noeske, T.A. (1981). Timing of a single daily meal and diel variations of serum thyroxine, triiodothyronine and cortisol in goldfish, Carassius auratus. Life Sci. 28, 2939-2944.

Spieler, R.E., and Noeske, T.A. (1984). Effects of photoperiod and feeding schedule on diel variations of locomotor activity, cortisol, and thyroxine in goldfish. Trans. Am. Fish Soc. 113, 528-539.

Stacey, N.E., MacKenzie, D.S., Marchant, T.A., Kyle, A.L., and Peter, R.E. (1984). Endocrine changes during natural spawning in the white sucker, Catastomus commersoni. I. Gonadotropin, growth hormone, and thyroid hormones. Gen. Comp. Endocrinol. 56, 333-348.

Vodicnik, M.J., Kral, R.E., and de Vlaming, V.L. (1978). The effects of pinealectomy on pituitary and plasma gonadotropin levels in Carassius auratus exposed to various photoperiod-temperature regimes. J. Fish. Biol. 12, 187-196.

Vriend, J. (1983). Pineal-thyroid interactions. Pineal Research Reviews 1, 183-206.

Vriend, J. (1984). Influence of the pineal gland and circadian rhythms in circulating levels of thyroid hormones of male hamsters. J. Pineal Res. 1, 15-22.

Vriend, J., and Reiter, R.J. (1977). Free thyroxine index in normal, melatonin-treated and blind hamsters. Horm. Metab. Res. 9, 231-234.

Vriend, J., Anderson, G.R., and Reiter, R.J. (1979). Effects of the pineal gland and melatonin on thyroid activity of male golden hamsters. Gen. Comp. Endocrinol. 38, 189-195.

Vriend, J., Sackman, J.W., and Reiter, R.J. (1977). Effects of blinding, pinealectomy, and superior cervical ganglionectomy on free thyroxine index of male golden hamsters. Acta Endocrinol. 86, 758-762.

White, B.A., and Henderson, N.E. (1977). Annual variations in the circulating levels of thyroid hormones in the brook trout, Salvelinus fontinalis, as measured by radioimmunoassay. Canad. J. Zool. 55, 475-481.

Woods, J.W., Wayt, H.J., and Baker, H.J. (1966). Cyclic fluctuations in I-131 content of thyroid glands of cats and monkeys. Proc. Soc. Exp. Biol. Med. 122, 211-214.

Young, J.Z. (1935). The photoreceptors of lampreys. II. The functions of the pineal complex. J. Exp. Biol. 12, 254-270.

APPENDIX

Table 1. Means, standard deviations and standard errors at 8 equidistant sampling times for plasma T_4 levels, plasma T_3 levels and T_4/T_3 in rainbow trout held at 11-13°C under a 12L (0700h-1900h):12D photoperiod and fed at 1300h (Experiment 1) (see Fig.1).

	T_4 (ng/ml)			T_3 (ng/ml)			T_4/T_3		
Time (h)	X	SD	SE	X	SD	SE	X	SD	SE
0700	2.4	1.2	0.35	2.7	1.1	0.32	0.99	0.66	0.19
1026	4.0	1.8	0.52	3.0	1.4	0.41	1.5	0.62	0.18
1352	4.2	1.4	0.41	3.7	1.3	0.39	1.2	0.44	0.13
1718	2.4	1.6	0.46	3.9	2.2	0.65	0.71	0.34	0.10
2044	2.1	0.98	0.30	3.0	1.6	0.47	0.83	0.36	0.11
0010	1.6	0.86	0.29	2.9	1.5	0.50	0.79	0.74	0.25
0336	1.6	0.65	0.19	3.1	1.4	0.41	0.61	0.25	0.073
0700	1.3	0.66	0.20	1.9	0.85	0.26	0.90	0.69	0.21

Table 2. Means, standard deviations and standard errors at 8 equidistant sampling times for plasma T_4 levels, plasma T_3 levels and T_4/T_3 in starved rainbow trout held at 11-13°C under a 12L(0700h-1900h):12D photoperiod. (Experiment 2) (see Fig.1).

	T_4 (ng/ml)			T_3 (ng/ml)			T_4/T_3		
Time (h)	X	SD	SE	X	SD	SE	X	SD	SE
0700	0.8	0.5	0.2	1.5	0.80	0.24	0.7	0.5	0.2
1026	0.8	0.4	0.1	1.1	0.61	0.18	1	0.7	0.2
1352	1.6	0.69	0.20	1.2	0.65	0.19	1.7	1.1	0.32
1718	1.0	0.46	0.13	1.4	0.55	0.16	0.78	0.32	0.093
2044	0.9	0.5	0.1	1.2	0.66	0.19	0.9	0.5	0.1
0010	0.7	0.3	0.08	1.2	0.39	0.11	0.7	0.4	0.1
0336	1.3	0.69	0.20	1.8	0.52	0.15	0.79	0.54	0.16
0700	1.5	1.2	0.35	1.6	0.75	0.22	1.1	0.96	0.28

Table 3. Means, standard deviations and standard errors for plasma T_4 levels, plasma T_3 levels and T_4/T_3 for 6 equidistant sampling times in rainbow trout held at 10.4-11.6°C under a 12L(0700h-1900h):12D photoperiod (light intensity at 104-109 lux) and fed at 0730h (Experiment 3) (see Fig.2).

	T_4 (ng/ml)			T_3 (ng/ml)			T_4/T_3		
Time (h)	X	SD	SE	X	SD	SE	X	SD	SE
0700	2.1	1.3	0.38	2.8	1.1	0.32	0.75	0.32	0.096
1026	4.5	1.6	0.47	3.2	1.2	0.35	1.5	0.61	0.18
1352	2.2	0.79	0.25	2.3	1.0	0.33	1.4	1.2	0.37
1718	2.6	1.6	0.49	3.1	2.0	0.60	1.1	0.64	0.19
2044	2.1	1.7	0.51	2.6	1.3	0.39	0.91	0.59	0.18
0010	1.3	0.79	0.25	2.5	1.3	0.41	0.60	0.51	0.16
1026@	5.2	2.2	0.70	3.5	1.2	0.39	1.5	0.37	0.12

Table 4. Means, standard deviations and standard errors for plasma T_4 levels, plasma T_3 levels and T_4/T_3 at 6 equidistant sampling times in rainbow trout held at 10.4-11.6°C under a 12L(0700h-1900h):12D photoperiod (light intensity at 36-41 lux) and fed at 0730h (Experiment 3) (see Fig.2).

	T_4 (ng/ml)			T_3 (ng/ml)			T_4/T_3		
Time (h)	X	SD	SE	X	SD	SE	X	SD	SE
0700	1.5	0.76	0.23	3.1	1.5	0.44	0.54	0.27	0.081
1026	3.5	1.4	0.47	3.0	1.6	0.54	1.7	1.4	0.45
1352	2.7	1.5	0.46	2.1	0.95	0.30	1.7	1.7	0.52
1718	2.2	0.72	0.22	3.1	1.1	0.34	0.83	0.45	0.14
2044	2.5	1.6	0.49	3.1	0.98	0.31	0.76	0.39	0.12
0010	1.4	0.55	0.17	2.6	0.56	0.18	0.57	0.32	0.095

Table 5. Means, standard deviations and standard errors for plasma T_4 levels, plasma T_3 levels and T_4/T_3 at 8 equidistant sampling times in rainbow trout held at 12-13°C under a 12L(0700h-1900h):12D photoperiod and fed at 0730h (Experiment 4) (see Figs. 3 and 4).

	T_4 (ng/ml)			T_3 (ng/ml)			T_4/T_3		
Time (h)	X	SD	SE	X	SD	SE	X	SD	SE
0700	1.9	0.56	0.19	1.4	0.70	0.23	1.7	1.0	0.35
1026	3.3	1.7	0.56	1.3	0.80	0.27	3.2	1.4	0.48
1352	2.4	0.52	0.16	1.1	0.49	0.15	2.9	1.8	0.53
1718	2.3	1.5	0.46	0.9	0.3	0.08	3	2	0.7
2044	1.8	0.75	0.23	0.7	0.1	0.04	2	0.8	0.2
0010	2.0	1.2	0.37	1.5	0.98	0.31	1.5	0.93	0.30
0336	2.5	1.4	0.45	1.8	0.71	0.22	1.4	0.77	0.24
0700	1.1	0.43	0.14	1.0	0.45	0.14	1.4	0.88	0.28

Table 6. Means, standard deviations and standard errors for plasma T_4 levels, plasma T_3 levels and T_4/T_3 at 8 equidistant sampling times in rainbow trout held at 12-13°C under a 12L(0700h-1900h):12D photoperiod and fed at 1800h (Experiment 4) (see Figs. 3 and 4).

	T_4 (ng/ml)			T_3 (ng/ml)			T_4/T_3		
Time (h)	X	SD	SE	X	SD	SE	X	SD	SE
0700	1.9	1.1	0.34	1.4	0.58	0.18	1.4	0.81	0.26
1026	3.0	0.87	0.28	1.4	0.89	0.28	3.1	2.1	0.65
1352	2.8	1.2	0.39	1.7	0.79	0.25	2.0	0.95	0.30
1718	2.0	0.91	0.29	1.1	0.83	0.26	2.9	3.0	0.95
2044	3.2	1.5	0.46	1.6	0.90	0.29	2.2	1.1	0.35
0010	2.4	1.2	0.35	1.4	0.84	0.25	2.1	1.0	0.30
0336	2.1	1.1	0.35	1.0	0.66	0.22	2.5	1.2	0.40
0700	1.1	0.53	0.18	0.8	0.5	0.2	2	2	0.7

Table 7. Means, standard deviations and standard errors for plasma T_4 levels, plasma T_3 levels and T_4/T_3 at 8 equidistant sampling times in starved rainbow trout held at 11.0-11.8°C under a 12L(0700h-1900h):12D photoperiod (Experiment 5) (see Fig.5).

	T_4 (ng/ml)			T_3 (ng/ml)			T_4/T_3		
Time (h)	X	SD	SE	X	SD	SE	X	SD	SE
0700	1.0	0.49	0.15	1.1	0.53	0.16	1.1	0.68	0.20
1026	1.9	1.2	0.39	1.3	0.51	0.16	1.4	0.62	0.20
1352	1.6	1.3	0.38	1.9	1.3	0.36	0.99	0.74	0.21
1718	1.6	0.76	0.22	1.7	1.2	0.34	1.1	0.50	0.14
2044	2.3	1.8	0.51	1.3	0.80	0.23	2.1	1.20	0.35
0010	1.6	0.99	0.30	1.5	0.86	0.26	1.2	0.85	0.26
0336	1.1	0.49	0.14	1.3	0.64	0.18	0.98	0.60	0.17
0700	1.3	0.76	0.22	1.2	0.76	0.22	1.5	1.4	0.40

Table 8. Means, standard deviations and standard errors for plasma T_4 levels, plasma T_3 levels and T_4/T_3 at 8 equidistant sampling times in starved rainbow trout held at 11.0-11.8°C under a 12L(1900h-0700h):12D photoperiod (Experiment 5) (see Fig.5).

	T_4 (ng/ml)			T_3 (ng/ml)			T_4/T_3		
Time (h)	X	SD	SE	X	SD	SE	X	SD	SE
0700	1.6	0.80	0.23	1.4	0.64	0.19	1.3	0.69	0.20
1026	1.3	1.3	0.37	1.2	0.76	0.22	1.8	2.8	0.82
1352	1.2	0.76	0.22	1.6	0.96	0.28	0.82	0.43	0.12
1718	1.3	1.0	0.30	1.6	1.0	0.30	0.96	0.67	0.19
2044	0.9	0.4	0.1	1.0	0.52	0.17	1	0.9	0.3
0010	1.2	0.61	0.18	1.1	0.61	0.18	1.3	0.87	0.25
0336	1.2	0.57	0.17	1.2	0.69	0.20	1.6	1.6	0.47
0700	1.2	0.83	0.24	1.3	0.53	0.15	1.0	0.77	0.22

Table 9. Means, standard deviations and standard errors for plasma T_4 levels, plasma T_3 levels and T_4/T_3 at 8 equidistant sampling times in rainbow trout held at 11.0-13.0°C under a 12L(0700h-1900h):12D photoperiod and fed at 0800h (Experiment 6) (see Figs. 6 and 7).

	T_4 (ng/ml)			T_3 (ng/ml)			T_4/T_3		
Time (h)	X	SD	SE	X	SD	SE	X	SD	SE
0700	3.5	1.3	0.42	3.8	1.5	0.47	0.98	0.38	0.12
1026	5.2	1.7	0.55	5.0	1.2	0.37	1.1	0.43	0.14
1352	4.3	2.1	0.67	5.5	1.3	0.40	0.81	0.40	0.13
1718	3.6	2.6	0.81	4.8	1.3	0.40	0.77	0.56	0.18
2044	2.5	0.94	0.30	5.6	1.8	0.58	0.49	0.16	0.051
0010	2.2	0.92	0.28	4.3	0.83	0.25	0.52	0.24	0.073
0336	4.9	2.1	0.66	4.6	0.90	0.28	1.1	0.43	0.14
0700	5.1	2.2	0.75	4.8	0.95	0.32	1.0	0.41	0.14

Table 10. Means, standard deviations and standard errors for plasma T_4 levels, plasma T_3 levels and T_4/T_3 at 8 equidistant sampling times in rainbow trout held at 11.0-13.0°C under a 12L(2045h-0845h):12D photoperiod and fed at 0800h (Experiment 6) (see Figs. 6 and 7).

	T_4 (ng/ml)			T_3 (ng/ml)			T_4/T_3		
Time (h)	X	SD	SE	X	SD	SE	X	SD	SE
0700	4.2	1.7	0.58	5.7	0.85	0.29	0.77	0.41	0.14
1026	5.9	2.1	0.65	4.3	1.2	0.39	1.5	0.56	0.18
1352	3.6	1.6	0.49	4.1	1.4	0.42	0.99	0.65	0.20
1718	2.5	1.3	0.42	4.4	0.86	0.27	0.58	0.33	0.10
2044	2.2	1.8	0.56	3.8	1.1	0.32	0.59	0.37	0.11
0010	5.4	1.7	0.55	4.5	0.98	0.31	1.2	0.52	0.17
0336	5.3	2.7	0.85	4.9	1.7	0.55	1.1	0.52	0.16
0700	4.1	2.2	0.69	4.8	1.0	0.33	0.85	0.44	0.14

Table 11. Means, standard deviations and standard errors for plasma T_4 levels, plasma T_3 levels and T_4/T_3 at 8 equidistant sampling times in rainbow trout held under complete darkness at 11.0-12.0°C and fed at 1330h (Experiment 7) (see Fig.8).

	T_4 (ng/ml)			T_3 (ng/ml)			T_4/T_3		
Time (h)	X	SD	SE	X	SD	SE	X	SD	SE
0700	0.9	0.4	0.1	2.2	0.83	0.25	0.5	0.2	0.07
1026	1.7	1.2	0.41	2.6	1.0	0.33	0.63	0.34	0.11
1352	2.1	1.3	0.40	3.6	1.4	0.43	0.63	0.39	0.12
1718	1.9	1.8	0.52	3.1	1.0	0.29	0.63	0.48	0.14
2044	1.6	0.92	0.29	2.9	1.1	0.35	0.56	0.26	0.082
0010	0.9	0.3	0.1	3.4	1.1	0.37	0.3	0.1	0.04
0336	0.9	0.3	0.1	3.1	1.3	0.38	0.3	0.2	0.05
0700	1.2	0.90	0.29	2.7	1.5	0.49	0.52	0.27	0.084

Table 12. Means, standard deviations and standard errors for plasma T_4 levels, plasma T_3 levels and T_4/T_3 at 8 equidistant sampling times in starved rainbow trout held at 11.0-12°C under complete darkness (Experiment 7) (see Fig.8).

	T_4 (ng/ml)			T_3 (ng/ml)			T_4/T_3		
Time (h)	X	SD	SE	X	SD	SE	X	SD	SE
0700	0.9	0.3	0.1	1.4	0.49	0.15	0.7	0.2	0.07
1026	1.0	0.44	0.14	1.5	0.88	0.28	0.77	0.32	0.10
1352	1.0	0.74	0.26	2.0	0.81	0.29	0.57	0.46	0.16
1718	1.3	1.6	0.49	1.8	1.4	0.41	0.88	1.2	0.35
2044	1.0	0.45	0.14	1.3	0.57	0.18	0.86	0.26	0.083
0010	0.9	0.7	0.2	1.7	0.60	0.18	0.6	0.3	0.1
0336	0.9	0.4	0.1	1.5	0.73	0.23	0.7	0.5	0.1
0700	1.0	0.55	0.17	1.7	0.80	0.25	0.78	0.67	0.21

Table 13. Coefficients of variation for T_4 and T_3 radioimmunoassays in Experiments 1 through 7.

Experiment	Coefficient of Variation (%)	
	T_4	T_3
1	2.6	3.2
2	15	6.1
3	11	4.8
4	9.5	14
5	26	19
6	23	21
	18@	31@
7	3.5	10

@: From a second set of pooled plasma - used for the last 3 assays.

Coefficient of variation: $\frac{\sigma}{\text{Mean}} \times 100$

= standard error