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Plasma levels of gonadotropin and 17 α ,20 β -dihydroxy-4-pregnen-3-one in relation to spawning behaviour of rainbow trout, *Oncorhynchus mykiss* (Walbaum)

N. R. LILEY* AND Y. ROUGER†

*Department of Zoology, University of British Columbia, Vancouver, B.C., Canada, V6T 2A9 and †Laboratoire de Physiologie des Poissons, I.N.R.A., Campus de Beaulieu, Rennes, France

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Manipulation of the opportunity to spawn was used to investigate the relationship between endocrine events, egg viability and spawning behaviour in female rainbow trout. Females were prevented from spawning by isolating them from males and gravel for up to 21 days after ovulation. Blood samples were taken before pairing with a male, at the onset of nesting activity, and at the completion of spawning. Plasma hormone levels of gonadotropin (GtH) and 17 α ,20 β -dihydroxy-4-pregnen-3-one (17,20P) were measured by specific radioimmunoassays. There were no qualitative or quantitative differences in the spawning behaviour of females paired on the day of ovulation or 7, 14, or 21 days after ovulation. There was a general decrease in the viability of eggs with increasing retention times. In females paired on the day of ovulation, or after 7 or 14 days, GtH levels increased with the onset of nesting behaviour and declined as fish reached the post-spawning condition. By day 21, GtH levels before pairing were significantly higher than pre-pairing levels in the other three treatment groups, and did not increase at the onset of nesting, or decrease in post-spawning fish. Plasma 17,20P remained high in pre-pairing and nesting samples of all four groups and declined to low levels in fish in post-spawning condition. In females paired on the day of ovulation there was a significant increase in 17,20P from the pre-pairing to the nesting stage. These results suggest that 17,20P plays a key role in the synchronization of behavioural and maturational events at the time of spawning.

Key words: hormones; reproductive behaviour; rainbow trout; egg retention; egg viability.

I. INTRODUCTION

In spite of considerable progress in describing endocrine events associated with gonadal growth and maturation in fish (reviewed by Fostier *et al.*, 1983) little is known of the factors directly responsible for the onset and maintenance of reproductive behaviour in female fish. In particular, the role of the gonadal steroid hormones is poorly understood. Only in one species, the ovoviparous guppy, *Poecilia reticulata*, is there evidence that sexual responsiveness is governed by ovarian oestrogen (Liley, 1972). Stacey (summarized in Liley & Stacey, 1983) demonstrated that stimuli arising from the presence of ovulated eggs in the ovary play a critical role in the onset of spawning in the goldfish, *Carassius auratus*. The stimulus provided by the eggs appears to be mediated by prostaglandin released at the time of ovulation. Experimental evidence suggests that gonadal steroids and the pituitary play a tonic, permissive role, and maintain responsiveness to the stimulus provided by the eggs.

Liley *et al.* (1986) examined the relationship between behavioural and endocrine events during spawning in female rainbow trout, *Oncorhynchus mykiss*

(Walbaum). That study confirmed earlier findings that plasma levels of both oestrogen and androgen decline prior to ovulation, and that plasma concentrations of gonadotropin (GtH) and 17 α ,20 β -dihydroxy-4-pregnen-3-one (17,20P) rose in ovulated fish. Of special interest was the demonstration of a social control of hormone concentrations: levels of gonadotropin and 17,20P increased in response to the stimuli provided by the opportunity to spawn in the presence of a sexually active male. It is not clear from the observed correlations between spawning activity and increased GtH and 17,20P whether these hormones play a causal role in the regulation of female spawning activity and the increase in attractiveness to the males that accompanies nesting behaviour.

In this study we examine further the relationship between behaviour and endocrine events in female rainbow trout. The opportunity for females to spawn was manipulated by controlling the availability of gravel and the presence or absence of sexually active males. It was anticipated that assays of blood samples taken during the spawning cycle of females subject to enforced delays in spawning might allow us to identify the endocrine conditions specifically associated with the onset and maintenance of 'spawning readiness'.

Manipulation of the opportunity to spawn also allowed us to examine the relationship between ovulation and the onset of prespawning and spawning activity. Does the maintenance of behaviour associated with spawning depend upon the presence of a mass of ovulated eggs in the abdominal cavity? If the female is prevented from spawning, for how long will the female remain ready to spawn when given the opportunity? Finally, is there a correlation between the duration or extent of 'spawning readiness' and the viability of the ovulated eggs retained in the abdominal cavity?

II. MATERIALS AND METHODS

FISH STOCKS

Fish were taken from a population spawning in the inlet to Pennask Lake 50 km east of Merrit, B.C. Most females spawn at 3 years; a few 4-year-old spawners occur. The majority, if not all, die after one spawning season.

Shortly after the ice disappears from the lake in early May fish move into outlet and inlet streams. Spawning in the inlet stream commences in early June and persists into early July. Fish used in this study were collected (7 and 20 June and 6 July 1985) from a trap on the inlet stream and transported immediately to the laboratory in Vancouver.

HOLDING AND EXPERIMENTAL FACILITIES

Fish were placed within 10 h of collection in flowing, dechlorinated tap water in a series of holding tanks (800–1600 l). Observation facilities consisted of three oval fibreglass spawning channels, each with an independent water supply. The straight portions of each channel (190 × 45 × 45 cm, fitted with glass panels) were isolated from the semicircular ends by wire mesh partitions; each straight portion of the channel was subdivided by a wire partition into two 95 cm long sections.

Gravel (<2 cm dia.) was placed in each straight section to a depth of 5–8 cm. Water depth was 30 cm and temperature was maintained at 10 ± 0.5° C. A constant flow was maintained by submersible pumps in the semi-circular ends of the channel.

COLLECTION OF BLOOD SAMPLES

Fish were anaesthetized with MS 222 and weighed and measured. Each fish was numbered with a tag of plastic tape attached by a loop of monofilament line through the

musculature just below the posterior rays of the dorsal fin. Blood (1–1.5 ml) was collected by caudal puncture using a heparinized 3-ml syringe fitted with a 22-gauge needle. Blood samples were collected between 09.00 and 12.00 hours in an effort to avoid the possible confounding effects of any daily fluctuations in plasma hormones as described by a number of investigators (review by Zohar & Billard, 1984). Blood was held over ice until centrifugation (5 min at 2000 g) usually less than 5 min after collection. Plasma was stored at -80°C until assay.

HORMONE ASSAYS

Plasma hormone levels of gonadotropin (GtH) and $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (17,20P) were measured by specific radioimmunoassays following the procedures described in Liley *et al.* (1986).

ASSESSMENT OF EGG VIABILITY

Fifty to sixty eggs were 'stripped' into a dry 10 cm watch glass. Milt from two males was immediately added to the eggs. The eggs and milt were stirred gently before water was added and allowed to stand for 1 min. After rinsing with fresh water the eggs were poured into a small incubation tray held in a flow of aerated water. After 14 days the eggs were checked for evidence of development. By 14 days most developing eggs were clearly eyed.

EXPERIMENTAL TREATMENT OF OVULATED FEMALES

Females were maintained in holding tanks without gravel or males, and were checked daily for ovulation. Fish from which loose eggs could be obtained by moderate pressure on the abdomen were assigned to one of the following experimental groups.

Group A

Females permitted to spawn immediately after ovulation ($n=11$; mean weight $259.5\text{ g} \pm 14.8\text{ S.E.}$).

The first 'pre-pairing' blood sample was taken on the day ovulation was detected. After sampling a female was placed immediately into a spawning channel with a male. A second 'onset of nesting' sample was taken after the female had established a nest and had been observed probing—usually 1 day after introduction to the channel. Immediately after taking the blood sample a small batch of eggs was removed, fertilized, and set aside for incubation. A third 'post-spawning' blood sample was taken 24 h after the cessation of digging activity by the female.

Group B

Spawning delayed 7 days after ovulation ($n=9$; weight $286.7\text{ g} \pm 18.9\text{ S.E.}$).

Freshly ovulated fish were placed with other ovulated females in a holding tank without gravel. Seven days after ovulation the first blood sample was taken and the female placed in a spawning channel with a male. Thereafter the procedures were the same as for group A.

Group C

Spawning delayed 14 days after ovulation ($n=7$; weight $289.6\text{ g} \pm 17.6\text{ S.E.}$).

The procedure was the same as for group B except for a delay of 14 days between ovulation and the first blood sample.

Group D

Spawning delayed 21 days after ovulation ($n=7$; weight $257.9\text{ g} \pm 12.4\text{ S.E.}$).

The procedure was the same as for group B except for a delay of 21 days between ovulation and the first sample.

BEHAVIOURAL OBSERVATION AND RECORDING

After fish were established in the spawning channels, each pair was observed daily for five 5-min observation periods between 08.30 and 17.50 hours with 2 h between each observation. Observations were continued until there had been no nesting activity for 24 h. A

check sheet was used to record bouts of the following (see Liley *et al.*, 1986, for a more detailed description).

Female behaviour

Digging, a series of lateral flexures which result in the downstream displacement of gravel and the formation of a circular nest depression.

Covering, shallow digging movements used by the female to cover the eggs and fill in the nest.

Holding, the female hovers over the nest depression.

Probing, the female crouches in the nest depression and 'probes' the interstices of the gravel with the extended anal fin. Note, digging and probing are referred to collectively as 'nesting activities'.

Male behaviour

Attending, the male hovers close behind and to the side of the holding or probing female.

Quiver, the male moves close alongside the female and performs a low amplitude, high frequency undulation of the body.

In addition, evidence of nesting and spawning taking place overnight or between observation periods was also noted as follows.

Nesting. The position and size of a nest was noted at each observation period. The size of the nest was ranked on a four-point scale: 1, a scarcely detectable depression; 2, a deep depression 5–8 cm deep, just exposing a small area of fibreglass; 3, a large steep-sided nest exposing an area of fibreglass 15–20 cm across; 4, a large nest with gently sloping sides, exposing a large area of the bottom of the tank more than 20 cm across.

After the first 2 days of nesting activity the gravel was levelled after the last observation of the day. This allowed us to determine whether digging activity had continued overnight.

Spawning. Spawning rarely occurred during an observation period. Nevertheless, the occurrence of spawning could usually be inferred from a change in nest position: the nest in the previously recorded position was filled and replaced by a new nest 15–30 cm upstream of the 'old' nest. Occasionally eggs were visible in the surface gravel; in other cases spawning was confirmed by excavating the gravel in the position of the old nest.

STATISTICAL PROCEDURES

The Wilcoxon test was used to compare paired, serially sampled data. Because of marked individual variation in plasma measures the test was applied to the increase, or decrease, as a proportion of the initial measure. The Mann-Whitney test was used to compare independent samples. Unless otherwise stated the significance level was set at $\alpha = 0.05$.

III. RESULTS

NESTING AND SPAWNING BEHAVIOUR

All females in each experimental group dug nests and performed probing behaviour. There were no obvious qualitative differences in the nesting behaviours observed (digging and probing). Most females began nesting within 24 h of pairing with a male (Table I, interval between pre-pairing and onset of nesting). Two females paired on the day of ovulation did not establish nests until 7 days after pairing. Although eggs had been obtained from these females, it is likely that these females were not fully ovulated when first paired.

Once established, nests were actively maintained for several days (Table I, interval between onset of nesting and post-spawning). There was a tendency for fish subjected to a delay before pairing to complete nesting activity more rapidly (mean: approximately 4 days) than fish paired immediately after ovulation (mean = 6 days). The difference is not statistically significant.

TABLE I. Collection of blood samples relative to ovulation

Female group	<i>n</i>	Days after ovulation: mean (range)		
		Sample 1 Pre-pairing	Sample 2 Onset of nesting	Sample 3 Post-spawning
A	11	0	2.4(1-7)	8.4(5-15)
B	9	7	8.6(8-10)	12.7(11-15)
C	7	14	15.3(15-16)	18.9(17-21)
D	7	21	22.1(22-23)	26(25-28)

TABLE II. Nesting and spawning of females paired on the day of ovulation (A), or 7 (B), 14 (C) or 21 days (D) after ovulation

Female group	<i>n</i>	Nesting behaviours maximum daily total per 25 min mean (s.e.)		Spawning number of females				
		Digging	Probing	Spawnings per female				
				0	1	2	3	4
A	11	11.5(3.1)	31.6(6.3)	2	3	3	1	2
B	9	9.0(2.6)	32.4(6.7)	1	2	4	2	0
C	7	11.7(4.0)	24.1(4.7)	0	4	2	1	0
D	7	10.9(2.3)	27.6(6.0)	1	2	2	2	0

The frequency and vigour of nesting activities reached a maximum early in the nesting period, around the time of spawning. The maximum daily frequencies of probing and digging were similar in the four groups of females (Table II). Most of the females spawned at least once (Table II). Several females in each group spawned twice, and in some cases there was evidence of three or four spawnings.

EGG VIABILITY

All egg samples taken from groups A, B, and C were undergoing development 14 days after fertilization; samples from two females in group D failed to develop (Table III). There was considerable variability within each group. Eggs of females paired on the day of ovulation showed consistently high levels of viability: in six females over 90% of the eggs developed, and in the female with the lowest survival 63% were alive at 14 days. In contrast, less than 10% of eggs from three B females developed, although eggs from five other females of that group showed over 90% survival. Only one sample of eggs from the C fish showed over 90% survival, and the overall rate (68%) was lower, than that observed in the A fish (difference not significant). Twenty-one days after ovulation there was considerable variability in egg viability: in batches of eggs from two D fish no development occurred; in one female 92% of eggs developed.

TABLE III. Proportion of eggs undergoing development 14 days after fertilization

Female group	<i>n</i>	No. with viable eggs	Mean % of eggs developing (S.E.)	Range (%)
A	11	11	86.6(3.8)	63-100
B	9	9	63.1(14.7)	2-100
C	7	7	68.0(8.12)	30-97
D	7	5	42.4(15.0)	0-92

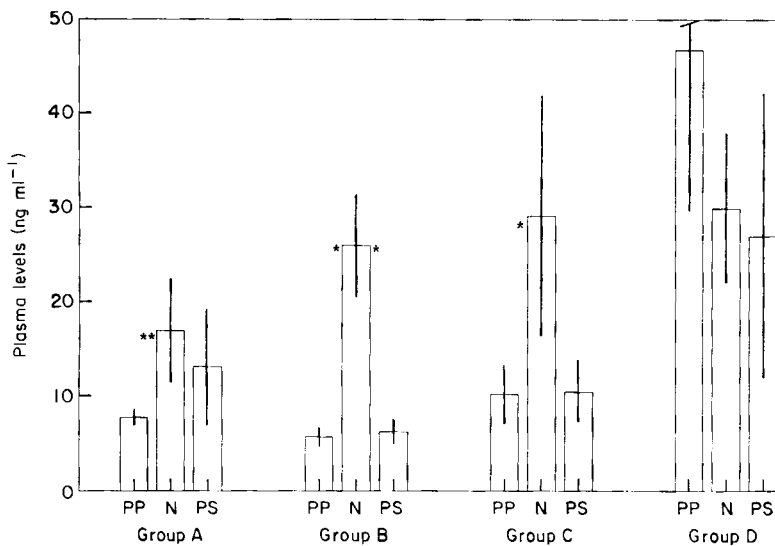


FIG. 1. Plasma levels (mean \pm S.E.M.) of gonadotropin before pairing (PP), at the onset of nesting (N), and following the completion of spawning (PS) in female rainbow trout paired on the day of ovulation (A), or 7 (B), 14 (C) or 21 days (D) after ovulation. The times of the N and PS samples relative to the day of ovulation are listed in Table I. *Significant difference between consecutive samples: $P < 0.05$ two-tailed Wilcoxon matched-pairs signed-ranks test; ** $P < 0.05$ one-tailed test.

PLASMA HORMONE LEVELS

The collection times of blood samples in relation to ovulation are summarized in Table I. Three females in group A, two in group B and 1 in group D died shortly after completion of spawning, before a third sample was taken.

Gonadotropin (GtH)

Females paired with males on the day of ovulation or after 7 or 14 days delay displayed similar patterns of change in plasma GtH concentration (Fig. 1). GtH levels increased significantly from the first sample, taken immediately before pairing, to the second sample taken after the onset of spawning activity. GtH levels decreased by the end of spawning. The decline in GtH from the second sample (female nesting) to the third (post-spawning) sample was significant in females with

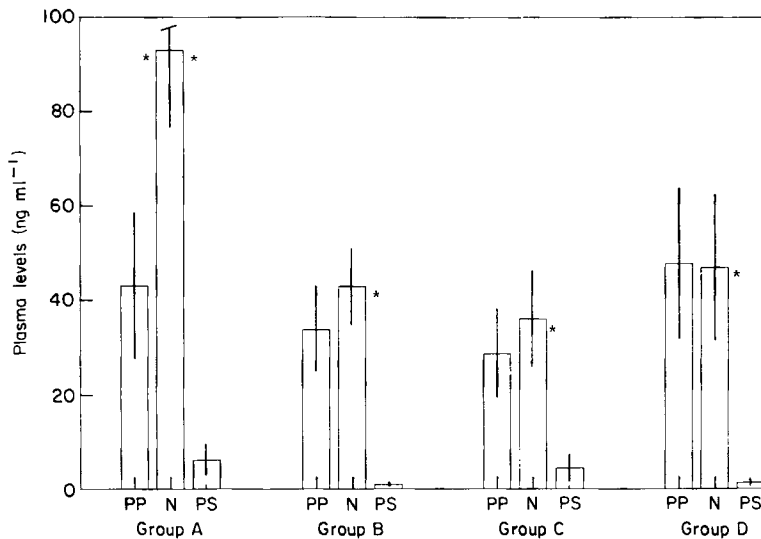


FIG. 2. Plasma levels (mean \pm S.E.M.) of $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (17,20P) before pairing (PP), at the onset of nesting (N), and following the completion of spawning (PS) in female rainbow trout paired on the day of ovulation (A), or 7 (B), 14 (C) or 21 days (D) after ovulation. The times of the N and PS samples relative to the day of ovulation are listed in Table I. *Significant difference between consecutive samples; $P < 0.05$ two-tailed Wilcoxon matched-pairs signed-ranks test.

7 days delay. Six of seven females in group C showed a decrease in GtH from nesting to post-spawning: $P = 0.062$, one-tailed sign test.

Females paired 21 days after ovulation showed a different pattern: GtH levels before pairing were the highest of any group and significantly greater than the first samples from the other three groups. Hormone levels declined by the second and third samples but the differences were not significant. Plasma concentrations of GtH at the onset of nesting and post-spawning did not differ significantly from those recorded in the corresponding samples of the other three groups.

17 $\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (17,20P)

In females paired immediately after ovulation, plasma 17,20P increased significantly at the onset of nesting activity, and decreased by the completion of spawning (Fig. 2). When pairing was delayed 7, 14 or 21 days, there were no marked changes between the pre-pairing and nesting hormone levels. In all three groups 17,20P decreased significantly to low levels by the end of spawning.

Plasma concentrations of 17,20P were similar at the first sampling of the four groups (before pairing). Post-spawning levels were also similar among the four treatment groups. However, 17,20P levels at the onset of nesting were significantly higher in the females paired at ovulation (Group A) than in females subject to a pairing delay.

A striking feature of the results is the demonstration that the retention of the eggs and the delay of spawning are accompanied by high levels of 17,20P. By the time fish paired at ovulation had completed spawning, hormone concentrations had dropped to levels significantly lower than those recorded in the first samples of females paired on day 7. Similarly, the post-spawning levels of females paired on

days 7 and 14 were significantly less than pre-pairing levels 14 and 21 days after ovulation respectively.

IV. DISCUSSION

This study confirms that the occurrence of ovulation and the presence of a mass of freshly ovulated eggs is correlated with the onset and maintenance of spawning readiness in female rainbow trout. The persistence of spawning readiness in fish with retained eggs strongly suggests that stimuli associated with the egg mass play a causal role in the regulation of spawning activity, as has been suggested for the goldfish (Stacey, 1977).

Although the presence of ovulated eggs appears to maintain spawning readiness, there are indications that once initiated nesting may continue in the absence of eggs. In a pilot study in 1983, digging females were stripped of their eggs on two consecutive days. Although over 95% of eggs were removed, females continued to dig nests for up to 5 days following the stripping ($n = 5$; mean days of nesting = 5.2, range 2-7). In a similar experiment with hatchery-raised rainbow trout (N. R. Liley, unpublished) digging persisted for up to 6 days following stripping. On the other hand, spawning readiness does not persist indefinitely: several hatchery females with retained eggs would no longer spawn 20 or more days after ovulation (N. R. Liley, in prep.).

There are few other studies of the relationship between the retention of eggs and readiness to spawn. Spawning of 'ripe' Pacific herring, *Clupea pallasii*, may be delayed 6 weeks or more (Brett & Solmi, 1982; Hay, 1986). In contrast, in *Puntius gonionotus*, a Malaysian cyprinid, spawning readiness disappeared 60 to 90 min after ovulation (Liley & Tan, 1985). Spawning readiness may persist 24 h in the goldfish, *Carassius auratus*, (Stacey, 1977) and between 4 and 24 h following HCG-induced ovulation, in the blue gourami, *Trichogaster trichopterus*, (Cardwell, 1984).

There was a general decrease in egg viability as assessed by survival to the eyed stage with increasing delay after ovulation. However, examination of individual records reveals considerable individual variation: by 21 days two batches of eggs showed no survival; in one batch over 90% developed. This observation implies that, although eggs may retain high viability for some time, once the decrease in viability commences deterioration proceeds rapidly. Within the time span examined, there was no correlation between egg viability and the persistence of spawning readiness.

These findings are consistent with previous studies of the viability of retained eggs in rainbow trout (Nomura *et al.*, 1974; Sakai *et al.*, 1975; Bry, 1981; Escaffre & Billard, 1979; Craik & Harvey, 1984; Springate *et al.*, 1984). All of the above reveal a decline in egg viability with increasing retention. However, it should be noted that assessment at an early stage of development may not provide a reliable guide to the viability of post-hatching stages. Although, Sakai *et al.* (1975) and Springate *et al.* (1984) found that the survival of eggs to eyeing, hatching and swimming followed a similar pattern, there was an increase in the proportion of abnormal alevins as over-ripening progressed (Sakai *et al.*, 1975). Hay (1986) noted that in Pacific herring after delays of more than 2 weeks there is a progressive loss of eggs and larvae, even though the initial fertilization rate remains high.

The persistence of egg viability in trout and herring is in contrast with findings in some other species. In the catfish *Clarias macrocephalus* there is a significant decrease in egg viability 12 h after ovulation (Mollah & Tan, 1983), while in *Puntius gonionotus* eggs are no longer viable 100 min after ovulation (Begum, 1982; Liley & Tan, 1985). Fortuny *et al.* (1988) report a marked reduction in viability of eggs of *Prochilodus platensis* retained more than 2 h after ovulation. Stevens (1966) suggests a maximum 'grace' period of about 60 min between ovulation and the time when eggs must be stripped for successful fertilization in the culture of striped bass, *Roccus saxatilis*.

The contrast between persistence of egg viability and spawning readiness in trout and herring, and the rapid loss of viability and spawning in *Puntius gonionotus* (Liley & Tan, 1985) and the gourami (Cardwell, 1984) with retained eggs, suggests differences in the mechanisms underlying the onset and maintenance of spawning. These differences may reflect adaptations to different environmental demands. *Puntius* and the gourami occupy relatively stable warm water environments. Both are iteroparous, and therefore a failure to spawn does not result in the loss of lifetime reproductive effort. Rainbow trout and herring are coldwater species in which few individuals spawn more than once. In both species reproduction is vulnerable to environmental disturbance. In 1982 we observed that after a short period of heavy rain, low temperatures, and high turbidity, nesting trout abandoned their nests (Liley *et al.*, 1986). Presumably these fish were able to resume nesting when conditions improved. Spawning in Pacific herring may be delayed by natural events such as storms. Furthermore, spawning in herring is synchronized in a series of 'waves'. Both the synchronized spawning and the ability to compensate for naturally induced delays are almost certainly facilitated by the capacity of females to delay oviposition (Hay, 1986).

The measurement of plasma hormones confirm an earlier finding (Liley *et al.*, 1986) that exposure to stimuli associated with the opportunity to spawn results in a marked increase in GtH. This response occurred in the groups with delays of 0, 7 or 14 days between ovulation and the opportunity to spawn. In contrast, by day 21 pre-pairing levels of GtH were already higher than the first two samples of the other three groups. There was no further increase in response to the opportunity to spawn, and GtH remained high compared with the post-spawning levels in the other three groups.

These findings suggest that there are two partially independent processes governing GtH levels. First, plasma GtH levels are maintained or increase following ovulation. This finding is consistent with other reports that in salmonids GtH levels increase prior to ovulation and continue to increase for several days thereafter (references in Goetz *et al.*, 1987). Second, for the first 14 days after ovulation a socially induced increase in GtH may be superimposed upon the general post-ovulation increase. By day 21 the fish is no longer responsive to the social stimulus, or alternatively GtH is already at maximum levels and cannot increase further. Gielen *et al.* (1981) also proposed the existence of two mechanisms controlling plasma GtH in rainbow trout: lesions in the telencephalon abolished the post-ovulatory surge in GtH but did not affect the preovulatory increase.

A number of workers have proposed that the prolonged post-ovulatory rise in GtH may reflect a reduction in steroid negative feedback (discussion in Scott *et al.*, 1983). In addition, the retention of eggs has been implicated as a factor affecting

GtH levels. Jalabert & Breton (1980) found that post-ovulation levels of fish with retained eggs were considerably higher than those of fish stripped immediately after ovulation. Scott *et al.* (1983) found no effect of stripping on GtH levels. In the present study GtH levels were depressed after spawning, but only in the group retaining eggs for 7 days was the decrease statistically significant. Thus although there is some evidence that the presence or absence of eggs may influence GtH levels, this effect was relatively minor and did not obscure the general post-ovulatory increase in GtH.

Maintenance of high levels of 17,20P appear to be associated with the retention of eggs and the capacity to spawn for up to 21 days after ovulation. In addition, there was a marked increase in 17,20P in response to pairing and the opportunity to spawn in females sampled immediately after ovulation. In contrast, there was no increase in 17,20P in response to the opportunity to spawn at 7, 14 and 21 days after ovulation. On completion of spawning 17,20P dropped to low levels in females of all four treatment groups. These results suggest that the capacity of the ovary to synthesize 17,20P and respond to stimuli is at a maximum shortly before and after ovulation, and declines thereafter. The high levels of 17,20P in females with retained eggs may result from storage of 17,20P in the ovary, egg mass and fluids retained in the body cavity. At spawning the stored 17,20P is lost with the eggs and body fluids and is not replaced by the ovary which no longer retains the capacity to synthesize the maturational steroid. Springate *et al.* (1984) found high levels of 17,20P and 17 α -hydroxyprogesterone for up to 12 days after ovulation in rainbow trout with retained eggs. As batches of eggs were removed on alternate days following ovulation, the sharp decline in serum hormones 14 days after ovulation coincided with the cumulative loss of a substantial portion of the original egg mass in each female.

The proposal that there is a post-ovulatory increase in 17,20P in response to behavioural stimuli is consistent with current information regarding the synthesis of 17,20P in salmonids. The general pattern that has emerged from several investigations is one in which levels of circulating levels of 17,20P are low prior to final maturation and begin to increase just before germinal vesicle breakdown (GVBD). They generally increase dramatically during GVBD and may increase even further following ovulation (Goetz *et al.*, 1987). *In vitro* studies with Pacific salmon indicate that the follicles retain the capacity to synthesize 17,20P in response to GtH stimulation for several days following ovulation (Young *et al.*, 1983; van der Kraak & Donaldson, 1986). Furthermore, Nagahama & Kagawa (1982) observed hypertrophy of the granulosa layers of the ovary in amago salmon, *Oncorhynchus rhodurus*, up to 2 days after ovulation. By 7 days the granulosa cells were undergoing degeneration. There is evidence that the granulosa cells play an essential role in the synthesis of 17,20P in the fish ovary (Wright & Wei-xin Zhao, 1988).

The present investigation suggests that spawning readiness in trout is induced by a combination of hormone conditions associated with ovulation, and the presence of freshly ovulated eggs in the abdominal cavity. In goldfish the mass of ovulated eggs alone is sufficient to induce spawning, and this effect appears to be mediated by prostaglandins. Pituitary and gonadal hormones appear to play a permissive role (Liley & Stacey, 1983). It is less likely that the prolonged spawning of trout (as compared with cyprinids) will be found to depend entirely upon stimuli associated with the presence of the eggs. In pilot studies, referred to earlier, nesting activity

persisted for several days after eggs had been removed by stripping, suggesting that at least in the immediate post-ovulation period spawning may persist in the absence of the direct effect of the egg mass and is dependent upon the presence of a tonic mechanism, perhaps mediated by hormones.

As oestrogen and testosterone levels both decline prior to ovulation, it is unlikely that either plays a direct role in the regulation of spawning. On the other hand, increases in GtH and 17,20P are directly associated with ovulation and spawning. Crim *et al.* (1975) proposed that the high levels of GtH present in spawning fish are involved in regulating behaviour. However, although GtH levels do respond to the opportunity to spawn, the persistence of high levels of GtH in post-spawning fish, and the continuing increase to high levels several weeks after ovulation, indicate that GtH does not play a direct role, although an indirect or permissive involvement cannot be ruled out.

It is generally agreed that 17,20P is the major oocyte maturation-inducing hormone in salmonids (Goetz *et al.*, 1987). High levels of 17,20P are associated with the onset and persistence of spawning, implicating this hormone in the control of spawning behaviour. A post-ovulatory role of 17,20P is further indicated by the persistence of the capacity to synthesize 17,20P. Fish stripped immediately after ovulation continue to nest at a time when the ovary retains the capacity to synthesize 17,20P. It will be of interest to determine whether nesting will occur in fish stripped several days after the ovary has lost its capacity to secrete 17,20P.

Young *et al.* (1983) speculated that 17,20P may influence spawning by an effect on the synthesis of prostaglandin, which in addition to a role in ovulation, has been shown to mediate spawning behaviour in goldfish. However, pilot experiments with prostaglandin therapies have so far provided no evidence that prostaglandin is involved in the control of spawning in trout (N. R. Liley, unpublished).

In addition, evidence that 17,20P or its metabolites function as a pheromone in goldfish and perhaps in other species (Stacey *et al.*, 1987) raises the possibility that the high level of 17,20P in fish with retained eggs represents a store of pheromonally active material. Newcombe & Hartman (1973), Honda (1980, 1982), Emanuel & Dodson (1979), Liley *et al.* (1986) and Liley *et al.* (1987) provide evidence that ovulation in salmonids is accompanied by the release of a pheromone that attracts and excites male sexual activity.

In summary, the results of this investigation suggest that 17,20P has a role in the synchronization of maturational and behavioural events surrounding spawning. In addition to its role in oocyte maturation, 17,20P, or possibly its metabolites, may induce and maintain reproductive behaviour in the female, and by its release to the water attract and excite male sexual activity.

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