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Endocrine Changes Associated with Spawning Behavior and Social Stimuli in a Wild Population of Rainbow Trout (*Salmo gairdneri*)

I. Males

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Rainbow trout (*Salmo gairdneri*) were collected from a natural spawning population at Pennask Lake, B.C. Blood samples were taken from male trout at different stages of spawning and assayed by radioimmunoassay for gonadotropin (GtH), testosterone (T), 11-ketotestosterone (11-KT), and 17 α -hydroxy-20 β -dihydroprogesterone (17,20-P). Testosterone and 11-KT were detected in the plasma of males in spawning condition: 11-KT was at a higher concentration than T and declined to undetectable levels in postspawning fish. There were striking increases in plasma GtH and 17,20-P in males actively courting nest building females. The increase in 17,20-P was evidently not dependent upon an elevation of the pituitary hormone: 17,20-P remained low in males placed with sexually inactive, unovulated females even though GtH levels rose above levels measured in males isolated from females. The significance of the social control of hormone levels is not clear. There is some evidence that 17,20-P and/or GtH may cause an increase in quantity of the milt, and thus the endocrine response to a sexually active female may serve to synchronize maximum male fertility with oviposition readiness in the female. © 1986 Academic Press, Inc.

There has been considerable progress in recent years in describing the annual or seasonal endocrine changes associated with reproduction in teleost fish (recent reviews by Fostier *et al.*, 1983; Peter, 1983). However, much less is known of the acute "short-term" endocrine changes occurring during spawning (Stacey *et al.*, 1984).

The functional significance of many of the observed endocrine events is still far from clear, particularly as they relate to the behavioral processes involved in spawning (Liley and Stacey, 1983). A major reason for the lack of understanding of the behavioral role of gonadal and other hormones is that most studies have concerned captive fish not allowed to participate in a normal spawning process. This is particularly true of investigations involving domesticated strains of rainbow trout, many generations removed from their wild progenitors. It is

possible that the levels and changes in hormones observed in laboratory situations may not be an accurate guide to naturally occurring events (e.g., Licht *et al.*, 1983, found that in the bullfrog, *Rana catesbeiana*, plasma gonadotropins and steroids are highly sensitive to the effects of captivity).

A major factor potentially responsible for differences between restrained laboratory and naturally spawning fish is the two-way relationship between the endocrine system and the biological and physical environment: not only does the endocrine system regulate the behavioral responses necessary for successful reproduction, but it is also responsive to social and other exogenous stimuli (Harding, 1981). Studies with birds in particular (review by Silver and Cooper, 1983) have demonstrated that the smooth progression through the repro-

ductive cycle depends upon the continual interplay between the endocrine system and the environment.

The role of major physical features of the environment—photoperiod, temperature, lunar and tidal rhythms—have been examined in many species of teleost (Lam, 1983; Peter, 1983). Less is known of the role of more immediate physical and social stimuli. A number of studies, reviewed by Liley (1980) and Lam (1983), indicate the role of chemical and behavioral signals in accelerating or synchronizing breeding in fish. Presumably, effects of this nature are mediated by the endocrine system. Direct evidence of behaviorally induced endocrine changes is provided by Kyle *et al.* (1982, 1985) who found a rapid increase in plasma gonadotropin in male goldfish exposed to a spawning pair.

For a better understanding of the relationship between endocrine and reproductive events, it is necessary to assay hormone levels in fish undergoing a natural spawning. Schmidt and Idler (1962), Crim *et al.* (1975), Stuart-Kregor *et al.* (1981), Hunt *et al.* (1982), and Ueda *et al.* (1983b, 1984) have provided an overview of the endocrine events associated with the migration from the sea to the spawning areas in Pacific and Atlantic salmon. Stacey *et al.* (1984) and Scott *et al.* (1984) have provided a detailed account of the endocrine events in white suckers *Catostomus commersoni* through the spawning cycle. None of these investigations involved detailed behavioral observations of the individuals sampled for hormone assay.

In the present study blood hormone levels in wild-caught male rainbow trout were assayed at different stages of the natural spawning cycle. The objectives of this approach were: (a) to compare the plasma hormone profiles of wild fish with those obtained in hatchery stocks, (b) to obtain information on acute, short-term hormonal changes associated with particular events during spawning, perhaps providing information on possible causal relationships be-

tween endocrine factors and behavior, and (c) to evaluate the influence of certain social and physical stimuli on the endocrine condition of the fish.

MATERIALS AND METHODS

(a) *Fish stocks.* Fish were taken from a population spawning in the inlet to Pennask Lake (approx 940 ha) 50 km east of Merritt, B.C. (50° 0' N; 120° 8' W; altitude 1402 m). Most females spawn at 3 years; a few 4-year-old spawners occur. The majority of spawning males are 3 years old, but precocious (2 year) and older (4 year) males also spawn. 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. Fish in the outlet stream spawn at the end of May and early June. In the inlet, spawning commences in early June, peaks in the 3rd or 4th week of June, and persists into early July.

The British Columbia Ministry of Environment Fisheries Branch operates traps on the inlet and outlet streams. Once the required number of eggs is collected the traps are opened and the fish allowed to move on to the natural spawning areas. Fish used in this study were collected at the trap on the inlet stream.

(b) *Holding and experimental facilities.* In 1982, blood samples were taken immediately after fish were collected from the trap, or at various times after fish were placed in wooden or fiberglass troughs approximately 200 × 45 × 45 cm high, enclosed at both ends and above with wire netting. The troughs were placed in the stream and held down by gravel and rocks at a depth of 20–25 cm. Over the main sampling period, June 14–30, water temperature ranged from 10 to 14°. After heavy rain and snow water temperature dropped to 6° on July 4.

In 1983, fish were transported to the laboratory in Vancouver where they were placed, within 10 hr of collection, in flowing dechlorinated tap water in a series of holding tanks (800–1600 liter). Observation facilities consisted of three oval fiberglass spawning channels, each with an independent water supply. The straight portions of each channel (190 × 45 × 45 cm and fitted with clear-glass panels on both sides) were isolated from the semicircular ends by wire mesh partitions; each straight section could be subdivided into two 95-cm long sections.

Gravel and sand (<2 cm maximum diameter) 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°. A constant flow (0.12 m/sec) of water was maintained by two submersible pumps resting on the bottom of the channel at each end.

(c) *Collection of blood samples.* Fish were anesthe-

ized with MS222 and weighed and measured. Blood was collected by caudal puncture using a heparinized 3-ml syringe fitted with 22-gauge needle. Fish sampled once, mainly the 1982 series, provided 2–3 ml blood; 1–1.5 ml was taken from fish sampled serially (1983). Blood was held over ice in a cool box until centrifugation (5 min at 2000g) usually less than 5 min after collection. Plasma was stored on dry ice until transfer to a deep freeze at -80° . Fish to be observed or resampled were given a numbered tag of plastic tape attached by a loop of monofilament line through the muscle just below the anterior rays of the dorsal fin.

Blood samples were collected between 1000 and 1600 hr in 1982. In 1983 most samples were taken between 1000 and 1200 hr; a small number were collected up to 1600 hr. There is no reason to suspect that blood samples from different experimental groups were consistently collected at different stages of the daily fluctuations in plasma hormones described by a number of investigators (review by Zohar and Billard, 1984).

(d) *Hormone assays.* Hormone levels have been measured by means of specific radioimmunoassays.

(i) 11-Ketotestosterone (11-KT) assay was performed according to Fostier *et al.* (1982). The main cross-reactivities, estimated by the ratio of mass of reference to mass of steroid required to decrease the bound label to half its initial value, were 1.1% for testosterone and 1% for 11β -hydroxytestosterone. In the present assay the coefficient of variation was 9% (30.9 ng/ml, $n = 5$).

(ii) Testosterone (T) assay followed the same procedure as for 11-ketotestosterone, but using an anti-testosterone-3-carboxy methyloxime-bovine serum albumin. Cross reactivities were 41% with 5α -dihydrotestosterone, 31% with 11-ketotestosterone, 14% with androstenedione, 6% with 5α androstane- 3α , 17 β diol, 2% with 5α androstane- 3β , 17 β diol, 0.2% with adrenosterone and androsterone, less than 0.1% with dehydroepiandrosterone.

(iii) 17α -Hydroxy-20 β -dihydroprogesterone (17,20-P) was measured according to the procedure described by Fostier *et al.* (1981). The main cross-reactivity was detected with 20 β -dihydroprogesterone (2%). In the present assay coefficient of variation was estimated to be 11% (8.8 ng/ml, $n = 15$).

(iv) Gonadotropin was assayed by the procedure described by Breton *et al.* (1978).

(e) *Spawning behavior.* Tautz and Groot (1975) provide a detailed qualitative and quantitative description of spawning behavior in rainbow trout. The following general account provides a basis for the subdivision of the spawning cycle.

Shortly after ovulation a female initiates spawning activity by selecting a nest site and beginning to dig a nest. *Digging*, which consists of a series of strong body flexures while turned on one side, creates a nest depression 10–15 cm deep by displacing gravel down

stream of the nest site. Bouts of digging are interspersed between periods of *holding* or *probing*. A holding female hovers over the nest for a few seconds to several minutes. In probing the female drops slowly into the center of the nest and pushes her extended anal fin between the larger stones at the bottom of the nest.

A nest building female attracts courtship attention from a male. An *attending* male spends much time hovering just behind or to the side and behind the female, head level with her caudal fin. From the attending position the male may quickly move forward level with the female's dorsal fin and *quiver*. Quiver is a high-frequency low-amplitude undulation of the body performed close to a holding female or, more often, a probing female.

Spawning usually occurs after a period of frequent and intense probing and quivering; the female drops into the nest, as in probing, accompanied by the male. Both male and female *gape*, open their mouths wide, vibrate the caudal peduncle and anal fin, and release eggs and a white cloud of milt. The eggs drop into the interstices of the gravel and within 15 sec the female begins *covering*. Covering digs are relatively gentle digging movements in which the female moves forward several body lengths displacing small gravel to cover the pocket of eggs. Covering continues for 10 min or more and results in a mound of gravel over the egg pocket and a partially excavated new nest 30–40 cm in front of the covered nest. The female may remain quiescent for several hours, holding at the back of the new nest, or she may begin to excavate the new nest and proceed to a second spawning. A female may dig and oviposit into as many as five nests over a period of 3–5 days (Liley *et al.*, in preparation).

(f) *Behavioral recording.* Fish were established, usually as pairs, in spawning channels set on the stream bed (1982) or in the laboratory (1983). In 1982 channels were checked for 5 min at hourly intervals between 0800 and 1700 hr and a note made of, (a) any behavioral activity, and (b) presence, position, and size of a nest. In 1983 channels were checked at 2-hr intervals from 0900 to 1700 hr. Even if fish were apparently inactive at the time of the "check" the presence of a nest or a change in the nest was a highly reliable guide to female activity in the preceding hour.

Depending upon their assigned experimental group, fish were removed and blood samples taken before, during, or after spawning activity. Immediately prior to sampling, a pair was observed for 20 min and the occurrence of digging, covering, probing, and quivering acts recorded on a check sheet. Any evidence of spawning, such as a change in the position of the nest or visible eggs, was also noted. If spawning was suspected this could be checked by searching for a "pocket" of eggs in the gravel in the region of the newly covered nest.

(g) *Statistical procedures.* Data presented in the

figures are the means and standard error. Independent samples were subjected to analysis of variance and Scheffe's test for the comparison of pairs of samples. In cases in which the variances were not homogeneous data were transformed ($\log X + 1$) before statistical testing. A *t* test was applied in the comparison of paired data from serially sampled fish. Unless otherwise indicated the significance level was set at $\alpha = 0.05$.

PLASMA HORMONE LEVELS IN FISH SAMPLED IN THE FIELD (1982)

The following categories of males were sampled:

(a) *Green males*. Males were taken from the trap early in the spawning run (June 14) and immediately assessed for reproductive condition by exerting pressure on the abdomen. Males from which only small amounts of milt could be expressed under firm pressure were termed "green," $n = 10$, standard length (SL) = $30.5 \text{ cm} \pm 0.6 \text{ SE}$, weight = $411.7 \text{ g} \pm 20.9 \text{ SE}$.

(b) *Ripe males*. Males with freely running milt, sampled immediately after stripping by hatchery personnel at the trap on June 26, $n = 10$.

(c) *Post-spawning males*. Males taken from above the trap on July 1 and 2. These males had evidently completed spawning and were moving downstream to the lake, $n = 10$, SL = $29.6 \text{ cm} \pm 0.6 \text{ SE}$. These fish appeared to be in poor condition: thin, pale-colored, and often with fungal growths over damaged areas of skin. In most cases it was difficult to express any milt and the sex of the fish was determined by dissection.

(d) *Courting males*. Males actively courting digging and probing females were removed and immediately sampled June 16–31; $n = 4$. Sampling in this group was curtailed by the change in weather conditions, see (e).

(e) *Inactive males*. Heavy rainfall and snow in early July caused a sudden increase in water level and turbidity, and a lowering of the water temperature from 12 to 6°. Males which had previously been ac-

tively courting females in the spawning channels became inactive, even though there was no reason to consider them to be in a postspawning condition, July 5; $n = 5$. None of these fish had had the opportunity to spawn and, unlike the postspawning males, were in good condition.

Results

Gonadotropin levels were similar across the groups of green, ripe, postspawning, and inactive males (Fig. 1). The four actively courting males had levels of gonadotropin higher than all but one of the 35 males in the other four groups. The sample from one individual in the postspawning fish contained 23.76 ng/ml compared with a maximum of 9.5 ng/ml in all other groups. If the apparently anomalous individual is omitted from the statistical analysis only the courting males differ significantly from all other groups. There were no significant differences in the levels of 11-ketotestosterone in green, ripe, and courting males (Fig. 1). Mean androgen level had declined in inactive fish and was significantly lower than in the green and courting males. 11-Ketotestosterone was not detectable in 8 of 10 postspawning fish.

The progestin, 17,20-P, was detected at similar low levels in green, ripe, postspawning, and inactive males. Courtship activity was associated with a striking and significant increase in this hormone: the lowest level measured in a sexually active fish was 26.61 ng/ml, compared with the highest, 7.43 ng/ml, measured in one of the inactive males.

PLASMA HORMONES IN FISH SPAWNING IN THE LABORATORY (1983)

The most striking feature of trout sampled in the field was the finding that there were marked increases in GtH and 17,20-P in males allowed to court actively nest building females. This finding suggested that it would be of interest to examine fur-

FIG. 1. Plasma levels (mean $\pm$ SE) of gonadotropin, (GtH) 11-ketotestosterone (11-KT), and 17α -hydroxy- 20β -dihydroprogesterone (17,20-P) in male rainbow trout sampled at different stages of spawning at a natural spawning area. G = "green" males collected early in spawning season, small amount of milt released under pressure; R = "ripe" males with freely running milt; PSp = postspawning; C = male collected while actively courting a nest building female; I = males which had not spawned but became sexually inactive because of environmental conditions. Sample size shown at base of each column of upper histogram; X = undetectable.

ther the short-term changes in hormone levels associated with different stages in the spawning process, and to identify the stimuli responsible for the changes that might occur.

Fish trapped in the inlet to Pennask Lake were transported to Vancouver where they were weighed, measured, tested for reproductive condition, and given a numbered

plastic tag. All males proved to be in ripe condition (milt easily expressed) and thus were considered to be in similar condition when they were assigned to one of the following experimental treatments:

(a) *Group A.* Males were held as a group in a bare holding tank isolated from females ($n = 15$; mean SL = $33.13 \text{ cm} \pm 0.68 \text{ SE}$; mean weight $328 \text{ g} \pm 22.19 \text{ SE}$). Blood samples (A1) were taken after 4–6 days, at a time when other males placed with females (Group C) were actively courting.

After the initial blood sample males were either kept in isolation for a further 2–4 days ($\bar{x} = 3.1$) before a second sample was taken (A2, $n = 7$), or were paired with females in a spawning channel and allowed to begin courtship before being resampled (A3, $n = 8$). In some cases the A3 samples were taken as little as 5 hr after the first (3 fish at 5 hr; 1 fish at 24 hr; 1 at 2 days; 1 fish at 3 days; 2 fish at 4 days). Temperature and flow conditions were similar in the holding tanks and spawning channels.

(b) *Group B.* Males were paired with green, unovulated females in the spawning channels 1 or 2 days after arrival in the lab ($n = 16$; SL = 32.88 ± 0.55 ; weight 325.63 ± 15.23). The first blood sample was taken (B1) within the next 48 hr, prior to any nestbuilding activity by the females. A second sample (B2) was taken after the female had begun digging and the male commenced active courtship. The interval between the two samples ranged from 24 to 72 hr and was determined by the time at which females commenced reproductive activity.

(c) *Group C.* Males of the third group ($n = 9$; SL 31.81 ± 0.58 ; weight, 288.13 ± 17.72) were paired with ovulated females within 48 hr of arrival in the lab. In most cases females began nest building within 24 hr: males were sampled (C) after they had been observed in active courtship.

The procedure outlined above allowed direct comparison of first samples from males isolated from females and substrate

(A1) with those exposed to green females and substrate (B1) or actively digging females (C). The role of stimuli provided by females and/or substrate in governing plasma hormone levels could be further examined by comparing fish sampled serially within groups A and B.

Results

GtH levels remained low and similar in the isolated fish (A1) in both first and second (A2) samples (Fig. 2), but increased significantly in the males paired with females (A3). All females were actively nest digging and probing (only 5 were recorded): males attended the females and quivered frequently (Table 1). The magnitude of the increase in plasma hormone was not obviously related to the length of time between first and second samples (Table 2).

There was no significant difference between first and second samples from males paired with green females (B1) which became sexually active (B2), (Fig. 2). Males paired with green females (B1) or active females (C) had significantly higher GtH levels than the isolated males (A1); B1 and C did not differ.

Plasma levels of testosterone increased in the previously isolated males paired with active females (Fig. 2) (A1 < A3). There appears to have been a slight decrease in males maintained in isolation (A2) but the difference between A1 and A2 is not significant, whereas the difference between A2 and A3 is significant.

There was no difference in plasma 11-KT when isolated males (A1), and those with green females (B1) or active females (C) are compared (Fig. 2). There appeared to be an increase in 11-KT levels in previously isolated males placed with active females but the difference between A3 and A1 was not significant, although the difference between A2 and A3 was significant. However, there was a significant decrease in

FIG. 2. Plasma levels (mean $\pm$ SE) of gonadotropin (GtH), 11-ketotestosterone (11-KT), testosterone (T), and 17 α -hydroxy-20 β -dihydroprogesterone (17,20-P) in male rainbow trout sampled in laboratory spawning channels. Group A: A1—isolated from females; A2—2nd sample from males maintained in isolation from females; A3—resampled during courtship of nest building female. Group B: B1—males placed with unovulated, sexually inactive females; B2—2nd sample taken after female commenced nest building and male began courtship. Group C: males courting ovulated nest building females. Sample size shown at base of each column of upper histogram.

11-KT in the males paired with green females which later became active (compare B1 with B2).

TABLE 1
MALE AND FEMALE PRESPAWNING BEHAVIOR IN 20 MIN OBSERVATION PRIOR TO TAKING BLOOD SAMPLE

Male group	n ^a	Female condition	Bouts of quiver by male/20 min	Bouts of female activity per 20 min	
				Dig	Probe
A3	5	Ovulated	14.8 ± 3.8	14.8 ± 2.9	21.2 ± 6.5
B1	16	Unovulated	2.0 ± 0.7	0.1	0
B2	14	Ovulated	15.4 ± 3.1	9.8 ± 1.7	29.6 ± 6.4
C	8	Ovulated	23.2 ± 2.7	14.0 ± 3.2	22.2 ± 4.0

^a Number of pairs for which 20-min observations were recorded.

Plasma 17,20-P showed the most striking changes. There was a marked, significant difference between isolated males (A1) and males paired with active females (C). Males paired with green females (B1) did not differ from isolated males (A1). In males of group A, 17,20-P levels increased after exposure to active females (A3) but remained low in males retained in isolation (A2). In group B, 17,20-P increased significantly as the females became active (compare B1 and B2) to a level not different from other males paired with nest building females (A3 and C).

One of the objectives of this study was to determine whether endocrine changes could be correlated with particular behavioral events such as courtship and

spawning. The majority of samples from A3, B2, and C were taken from fish which were actively courting nest building females, but had not spawned by the time the blood sample was taken (Table 1). Males in these groups quivered alongside females at mean rates ranging from 14 to 23/20 min. Seven of 16 males paired with inactive females (B1) performed small numbers of quivers. The results presented above indicate that active courtship of nest building females is accompanied by an increase in GtH, 17,20-P and, to a lesser extent, T and 11-KT. In a few cases it was possible to identify males which had spawned less than 2 hr before the blood sample was taken ($n = 7$) or within the previous 24 hr ($n = 5$). There was no indication that a recent spawning was associated with a change in GtH, 17,20-P, or androgen over and above those typical of an actively courting male.

TABLE 2
INTERVAL BETWEEN SUCCESSIVE SAMPLES, AND PLASMA GtH AND 17,20-P IN ISOLATED MALES (A1) AND AFTER PLACING WITH NEST BUILDING FEMALES (A3)

Male	Interval between samples (hr)	Gonadotropin (ng/ml)		17, 20-P (ng/ml)	
		A1	A3	A1	A3
111	5	0.84	2.70	2.25	2.64
113	48	2.93	14.91	4.42	31.03
114	5	1.51	4.86	2.9	25.6
118	5	3.33	13.34	9.18	46.24
140	72	1.49	1.77	2.21	4.6
144	24	2.46	6.26	59.66	55.45
147	96	3.52	4.28	1.61	9.81
148	96	0.29	3.43	4.42	36.43

DISCUSSION

Scott *et al.* (1980), Schulz (1984), and Baynes and Scott (1985) have described the annual cycles of plasma testosterone and 11-ketotestosterone in male rainbow trout. In spite of some interstrain differences the general pattern that emerges is that starting 12 weeks or more before the normal spawning time T increases gradually to a peak which occurs several weeks before spawning. Changes in T are followed, with

a delay of 2–4 weeks, by similar changes in 11-KT which reaches a higher concentration than T. The peak in 11-KT occurs shortly before or during spawning. (Note: in none of these and other laboratory studies were males allowed to go through a natural spawning process: spawning period is identified on the basis of the presence of expressible milt.) Plasma T and, to a lesser extent, 11-KT have already begun to decline at the time of spawning, but do not reach their lowest levels for another 12 weeks or more. The same general pattern, an increase in T followed by 11-KT, is found in other salmonids (*Salvelinus fontinalis*, Sangalang and Freeman, 1974; *Salmo salar*, Hunt *et al.*, 1982, Stuart-Kregor *et al.*, 1981; *Salmo trutta*, Kime and Manning, 1982; *Oncorhynchus rhodurus*, Ueda *et al.*, 1983b; *O. keta*, Ueda *et al.*, 1984). The occurrence of high plasma levels of 11-KT just prior to, or during, spawning and maximum development of second sexual characteristics has led to suggestions that this is the primary active androgen in male salmonids and perhaps other species.

The progestin, 17,20-P, has been measured in males of several species and appears to be associated particularly with the spawning period (*Oncorhynchus nerka*, Schmidt and Idler, 1962; *Salmo gairdneri*, Scott and Baynes, 1982, Baynes and Scott, 1985; *O. rhodurus*, Ueda *et al.*, 1983a; *O. keta*, Ueda *et al.*, 1984).

Because in this investigation fish were sampled over a short span of time surrounding spawning it is not possible to make a detailed comparison with the seasonally changing hormone profiles recorded elsewhere. Nevertheless, the present findings are consistent with previous reports. Testosterone, 11-KT, and 17,20-P were all detected in the plasma of males in spawning condition. Testosterone, measured in one group only, was at a lower concentration than 11-KT, a finding in

keeping with reports that maximum T levels are lower than 11-KT, and that the decline in T precedes that of 11-KT.

In contrast with several of the reports referred to above, 11-KT became undetectable in most male rainbow trout less than 2 weeks after the peak of the spawning season. This may be a reflection of the fact that, in comparison with hatchery stocks, postspawning Pennask Lake males are in poor condition and most do not survive long after spawning. Unlike males sampled in hatcheries the spawned-out males were presumably exhausted after several days spent competing vigorously with other males for the opportunity to spawn with nest building females.

Plasma GtH levels peak around the time of spawning in rainbow trout (Billard *et al.*, 1978; Sanchez-Rodriguez *et al.*, 1978); *Oncorhynchus rhodurus*, (Ueda *et al.*, 1983b), and other salmonids (Crim *et al.*, 1975; Stuart Kregor *et al.*, 1981). GtH was present at moderately high levels in all groups examined here. GtH was significantly elevated in males courting nest building females and declined in post-spawning fish to levels found in ripe but unpaired males.

A finding of major interest in this study is the demonstration of a social control of hormone levels. There were striking increases in both GtH and 17,20-P in males placed with nest building females. There are parallel but less marked changes in T and 11-KT in males isolated and then paired with active females (group A). However, in males paired with green females (B1) there was a significant decrease in 11-KT as the females became sexually active (B2).

Which components of the spawning situation provide the effective stimuli is not clear. Green inactive females over a spawning substrate were as effective as nest building ovulated females in causing an elevation of GtH in males. But, in

marked contrast, 17,20-P did not increase unless the stimulus female was actively nest building. Males performed very little courtship of green females, but were highly active and persistent in the presence of ovulated nest building females. Thus, contrary to the expectation that an increase in 17,20-P follows and is the result of a rise in GtH, the lack of a positive correlation between the changes in GtH and 17,20-P suggest that levels of the steroid are not completely dependent upon GtH. The rapid increase in 17,20-P in one group (A3), within 5 hr of exposure to a nest building female, makes it unlikely that the low level of progesterone in males paired with green females is simply a result of a delay in secretion or release of 17,20-P, and raises the possibility that steroid secretion might be in part under neural control as has been reported in mammals (Kawakami *et al.*, 1981). Van den Hurk *et al.* (1982) have reported nerve terminals ending on interstitial cells of the testis of rainbow trout.

There are few other demonstrations of a social control of hormone levels in fish. Kyle *et al.* (1982, 1985) found that GtH measured in male goldfish rose within 2 hr of a male being placed with a spawning pair. Contact with the spawning fish was a necessary component of the stimulus causing the endocrine change. In male *Haplochromis burtoni* and *Xiphophorus helleri* social isolation leads to a reduction in blood androgen and corticosteroid (Hannes and Franck, 1983), and it is proposed that social stimuli cause an increase in androgens and that this leads to higher levels of aggression.

Other less direct evidence points to the role of social stimuli in modulating endocrine levels. Stacey *et al.* (1984) noted that in white suckers *Catostomus commersoni* the onset of spawning was generally associated with a dramatic increase in GtH. GtH levels were low in groups of males from a site at which no females were

present, and in the prolonged spawning season of 1982 GtH levels declined after the first few days of spawning and all males had similar GtH levels regardless of reproductive state. Stacey *et al.* suggest that this low GtH may have resulted from the small number of ovulated females present at the time.

The functional roles of testosterone and 11-KT in male fish have been reviewed by Fostier *et al.* (1983). Androgens have been implicated in sexual differentiation, spermatogenesis, and spermiation, and the development of secondary sexual characteristics. Of particular interest here is the possible role of androgens in spermiation and the production of milt—processes characteristic of the spawning male.

Baynes and Scott (1985) have emphasized the distinction between spermiation (the escape of spermatozoa from cysts formed by Sertoli cells into the testicular tubules) and milt production. The latter depends upon the migration of spermatozoa into the vas deferens where seminal fluid is formed. Baynes and Scott (1985) suggest that spermiation is already complete shortly after the start of milt production.

The onset of milt production in rainbow trout appears to be associated with high androgen levels (Fostier *et al.*, 1982; Sanchez-Rodriguez *et al.*, 1978; Scott *et al.*, 1980; Baynes and Scott, 1985). Fostier *et al.* (1984) report that the volume of stripable milt decreased in parallel with a decrease in 11-KT. Other reports indicate that although the onset of milt formation occurs when androgen levels are high, milt production is maintained or even increased as androgen levels decline (Baynes and Scott, 1985). Ueda *et al.* (1983b) and Ueda *et al.* (1984) found a marked drop in total androgens in two species of Pacific salmon from the prespawning to spawning period. These observations suggest that, even though androgens play a key role in the final stages of spermatogenesis and sper-

miation, it is unlikely that high levels of androgen are essential to the maintenance of milt production.

In contrast to the declining androgen levels in spawning fish, 17,20-P increases dramatically at the time of spawning in trout (Baynes and Scott, 1985) and Pacific salmon (Ueda *et al.*, 1983a; Ueda *et al.*, 1984). Baynes and Scott (1985) noted a close correspondence between the mean number of spermatozoa that could be stripped and the plasma levels of 17,20-P.

The role of 17,20-P in male fish remains poorly understood (Fostier *et al.*, 1983). Scott and Baynes (1982) found that injections of 17,20-P had no effect on milt volume or number of spermatozoa, but did cause an increase in the ratio, $K^+ : Na^+$. In two of three stocks examined, Baynes and Scott (1985) found a close association between 17,20-P levels and the K^+ concentration of seminal fluid. Baynes *et al.* (1981) had earlier suggested that a major effect of a high K^+ is to inhibit activation of spermatozoa prior to release. Baynes and Scott (1985) propose that a major function of 17,20-P in the male is the control of sperm motility mediated by changes in K^+ composition of the seminal fluids.

There is some evidence that milt volume and characteristics may be affected by social stimuli. Kyle *et al.* (1982) measured an increase in expressible milt volumes in male goldfish exposed to spawning pairs. Working with rainbow trout, Büyükhatipoglu and Holtz (1984) examined the effect of the presence of mature females upon milt production. In one season males placed with females showed a significant increase in milt volume (23%) and sperm number (18%). No significant effect was observed in the second season. Sperm density and motility were not affected in any group. In spite of their results and earlier reports referred to in their paper, Büyükhatipoglu and Holtz (1984) conclude that the effect of the females on the reproductive function of the males "was not very evident." It should be noted that in their ex-

periments the sexes were prevented from coming into physical contact by a screen partition, thereby reducing the level of stimulation of the male. Any effect of the female on the male must have been mediated by chemical or limited visual cues.

Milt volumes and characteristics were not measured in this study. However, it was clear that the so-called green males released only small volumes of milt under firm pressure, whereas a few days later the same individuals, actively courting females, would release larger volumes of freely running milt. Our own observations and those of Büyükhatipoglu and Holtz (1984) strongly suggest that at least one of the effects of the endocrine response to a nest building female is an increase in the volume of milt. This results in a synchronization of maximum male fertility with oviposition readiness in the female.

This change in milt production may be a direct effect of the increase in GtH, or it may reflect the increase in 17,20-P. The elevation in GtH may also be responsible for the slight but significant increase in T and 11-KT recorded in previously isolated fish.

At the present time there is no evidence to implicate the hormones measured in this study in the control of spawning behavior. Although androgens have been shown to regulate reproductive behavior in a wide variety of species of teleost (Liley and Stacey, 1983) virtually nothing is known of their role in the causation of spawning behavior in salmonids. The preponderance of 11-KT late in the cycle and close to the time of spawning suggests that 11-KT rather than testosterone (which is also present in large quantities in the female) may be more directly involved in the maintenance of sexual behavior. However, the decline in 11-KT which may precede or coincide with the spawning period in some populations examined implies that high 11-KT levels are not necessary for the maintenance of sexual responsiveness.

There is no evidence that 17,20-P plays a

causal role in the onset and maintenance of spawning activity. The clear association between high 17,20-P levels and the spawning period suggest that this possibility should be examined further.

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