Effect of social environment on plasma hormones and availability of milt in spawning male rainbow trout (Oncorhynchus mykiss)

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In this study we investigate the social stimuli responsible for the increases in plasma hormones and in the availability of milt in male rainbow trout allowed to interact with sexually active females. Males were placed in five experimental groups receiving different levels of sensory contact with females: no sensory cues, chemical cues, chemical and visual cues, full sensory and behavioural interaction with a nesting ovulated female, full sensory and behavioural interaction with an inactive postspawning female. Plasma levels of 11-ketotestosterone, 17α,20β-dihydroxy-4-pregnen-3-one and gonadotrophin increased only in males allowed full sensory contact with nesting females. Levels of 11-ketotestosterone did not change in males receiving visual and chemical cues, and decreased in the three other groups of males. The amount of milt that could be stripped from males increased only in those males placed with active females. Spermatocrit did not differ among the groups.

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Dans cette étude, nous avons analysé le rôle des stimuli sociaux responsables de l'accroissement des hormones plasmatiques et de la possibilité de libération de semence chez le mâle de la Truite arc-en-ciel. Les mâles étaient placés dans cinq conditions experimentales caractérisées par différents niveaux de perception sensorielle : aucune perception, perception chimique, perception chimique et visuelle, perception totale et interaction avec des femelles sexuellement actives, perception totale et interaction avec des femelles sexuellement inactives. Les concentrations plasmatiques de céto-11 testostérone, de 17α, 20β-dihydroxy-4-prégnèn-3-one et de gonadotrophine augmentent significativement uniquement chez les mâles ayant une perception totale et des contacts avec des femelles sexuellement actives. Le taux de céto-11 testosterone ne change pas chez les mâles recevant des signaux visuels et chimiques, et il diminue chez les trois autres groupes. La quantité de semence obtenue par pression abdominale n'était significativement importante que chez les mâles ayant des contacts avec des femelles sexuellement actives. Le spermatocrite ne varie pas en fonction de la nature des conditions experimentales.

Introduction

Correlations between gonadal cycles, levels of circulating gonadal steroids, and the appearance of reproductive behaviour have been demonstrated in a variety of salmonids. These correlations suggest that gonadal hormones play a major role in the development and maintenance of male reproductive behaviour (Liley and Stacey 1983). Nevertheless, numerous uncertainties remain. For example, although testosterone (T), 11-ketotestosterone (11-KT) and 17α,20β-dihydroxy-4-pregnen-3-one (17,20-P) appear to be major testicular steroids, there has been no clear demonstration that any of the gonadal steroids or pituitary hormones play a causal role in the regulation of the spawning activities of male salmonids.

As part of an ongoing investigation into the causation of reproductive behaviour in salmonids, radioimmunoassay (RIA) has been used to monitor changes in plasma hormone levels in rainbow trout spawning in laboratory stream tanks (Liley et al. 1986). Both T and 11-KT were present in prespawning fish but declined over the spawning period. In contrast, plasma concentrations of gonadotrophin (GtH) and 17,20-P peaked during final maturation and at the onset of the spawning period. In addition, levels of both hormones increased sharply in males permitted to interact with actively nesting females. It was also noted, but not examined in detail, that the amount of readily 'strippable' milt was greater in males placed with nesting females than in males isolated from females. Büyükhatipoglu and Holtz (1984) made a similar observation, but perhaps because of variation in the effects of their procedures, concluded that the effect of the females on the reproductive function of the males "was not very evident."

In this study we examine further the 'social control' of plasma hormone levels and amounts of strippable milt in rainbow trout. In particular, we attempt to determine the nature of the stimuli responsible for the changes in plasma hormones and milt release. A number of studies have suggested that a chemical signal (pheromone) serves to orientate male trout to a female in spawning condition (Newcombe and Hartman 1973; Emanuel and Dodson 1979; Honda 1980). Does the same or another pheromone elicit the endocrine response and increase in milt volume we observed in males allowed to interact with nesting females? Must the male interact with and court a female, or are visual and (or) chemical cues sufficient to stimulate the male?

In addition to identifying the sensory cues responsible we anticipated that a detailed examination of the endocrine response to social stimuli and the opportunity to spawn may contribute to our understanding of the role of gonadal and pituitary hormones in the synchronization of male reproductive function (behaviour and milt production) with spawning readiness in the female.

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Material and methods

Fish stocks

Fish were taken in June 1985 from a population spawning in the inlet to Pennask Lake (approximately 940 ha, altitude 1402 m), 50 km east of Merritt, B.C., Canada. The majority of spawning males are 3 years old, but precocious (2 year) and older (4 year) males are also spawning. The majority die after one spawning. Spawning commences in early June and persists into early July.

Experimental facilities

The fish were transported to the laboratory in Vancouver where, within 10 h of collection, they were placed in flowing dechlorinated tap water in a series of holding tanks (800−1600 L). After a 2- to 4-day acclimation period the fish were transferred to the experimental tanks. Males paired with females were placed in observation channels. The straight portions of each channel (190 × 45 × 45 cm) were fitted with clear glass panels on both sides. Each straight section was subdivided into two 95 cm long sections and separated from the semicircular ends by wire mesh partitions. Gravel (2 cm maximum diameter) was placed in each straight section to a depth of 5−8 cm. Water depth was 30 cm and the temperature was maintained at 10 ± 0.5°C. A constant flow of water (15−30 cm/s) was maintained by two submersible pumps resting on the bottom of the channel at each end. Two holding tanks were placed near the observation channel; one received water from the spawning channel with active pairs, the other received water directly from the dechlorinated supply.

Experimental procedure

Fifty-eight males (mean wt. = 248 ± 7.1 (SE) g) were taken from holding tanks and, after blood samples were taken and milt was collected, were divided among five experimental groups receiving different levels of sensory contact with females as follows:

Group 1 (n = 12)
No sensory cues from females: the males remained together, isolated from females in a bare holding tank (250 L).

Group 2 (n = 11)
Chemical cues: males were placed together in a holding tank (250 L) receiving water from a stream tank holding 3 or 4 pairs of spawning fish.

Group 3 (n = 12)
Chemical and visual cues: pairs of males were placed downstream of a spawning pair in a spawning channel and separated from the pair by a wire mesh barrier.

Group 4 (n = 12)
Full sensory and behavioural interaction with an active female: males paired with ovulated, actively nesting females.

Group 5 (n = 11)
Full sensory and behavioural interaction with an inactive female: males were placed individually with postspawning females (female had completed spawning more than 7 days previously).

After 4 days, a second blood sample was taken and milt was collected and measured.

Collection of blood samples

Fish were anaesthetized in MS 222, weighed and measured. Blood (1−1.5 mL) was collected by caudal puncture using a heparinized 3-mL syringe fitted with a 22-gauge needle. Blood was held over ice in a Styrofoam box until centrifugation (5 min at 2000 × g), usually 5 min after collection. Plasma was stored on dry ice until transfer to −80°C. Each fish was 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. Most samples were taken between 10:00 and 12:00.

Hormone assays

Hormone levels were measured by means of specific radioimmunoassay. 11-KT was assayed according to Fostier et al. (1982). In the present assay the coefficient of variation was 9.24% (247 ng/mL, n = 15). The procedure described by Fostier et al. (1981) was used to measure 17,20-P. In the present assay the coefficient of variation was estimated to be 4.48% (247 ng/mL, n = 15). Maturational gonadotrophin (GtH II) was assayed by the procedure described by Breton et al. (1978).

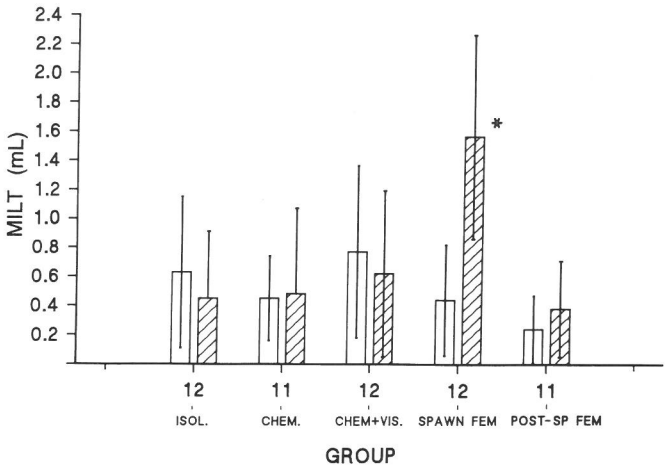

FIG. 1. Milt volume (mean ± SD) obtained before (open bar) and after (hatched bar) males were placed in different social conditions: no sensory cues from females (ISOL), chemical cues (CHEM), chemical and visual cues (CHEM + VIS), full sensory and behavioural interaction with spawning females (SPAWN FEM), full sensory and behavioural interaction with postspawning females (POST-SP FEM). *, Significant difference between consecutive samples: $P < 0.05$ two-tailed paired t-test.

Milt samples

Anaesthetized males were stripped by applying gentle pressure on the abdomen. As milt flowed from the genital papilla it was drawn by suction into a glass measuring tube. Spermatocrit was obtained by centrifugation of a 100 μL sample of milt for 5 min in a microhaematocrit centrifuge.

Spawning behaviour

Fish in groups 4 and 5 were established as pairs in spawning channels. Channels were checked for 5 min at hourly intervals between 08:00 and 17:00. Behavioural activity and the presence, position, and size of nest were noted on check sheets. Liley et al. (1986) provide a description of spawning behaviour in trout.

Statistical procedures

Independent samples were subjected to analysis of variance and Bartlett's test before comparison of pairs of samples. A t-test was used to compare paired data from serially sampled fish. Unless otherwise indicated the significance level was set at P = 0.05.

Results

Similar amounts of milt were collected from males of the different groups on the first day (Fig. 1). Only in males interacting with ovulated females was there a significant increase in strippable milt over the four days of experimental treatment. In the other four groups the volumes of milt stripped from males at the end of the experiment were similar to those obtained 4 days earlier. Spermatocrit values were similar before and after treatment and did not differ among the five groups (Fig. 2).

GtH levels were low and did not differ among the five groups in samples taken on the first day (Fig. 3). There was a marked increase in GtH in males allowed full sensory and behavioural interaction with nesting females for 4 days. GtH was not affected by any of the other treatments.

There was considerable variation among the experimental

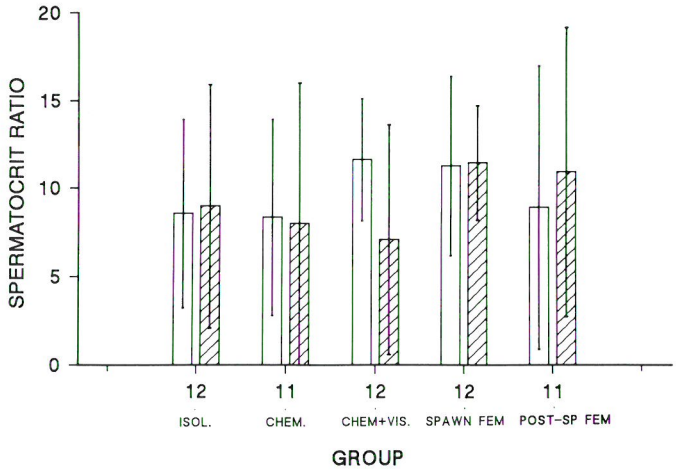

FIG. 2. Spermatocrit (mean ± SD) obtained before pairing (open bar) and after males were placed in different social conditions (hatched bar) (see Fig. 1).

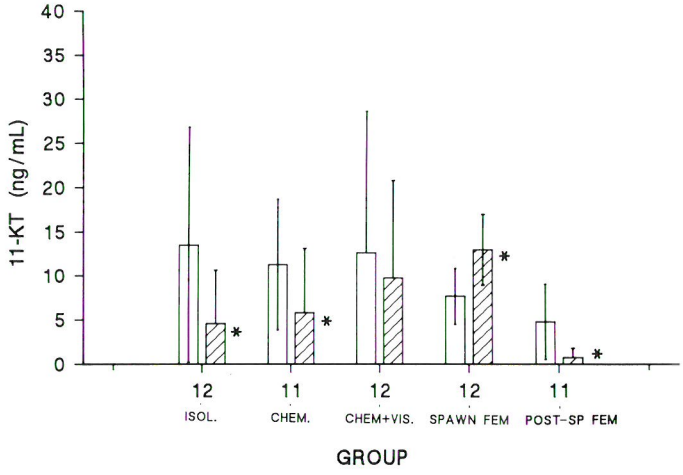

FIG. 4. Plasma levels (mean ± SD) of 11-KT before (open bar) and after (hatched bar) males were placed in different social conditions (see Fig. 1).

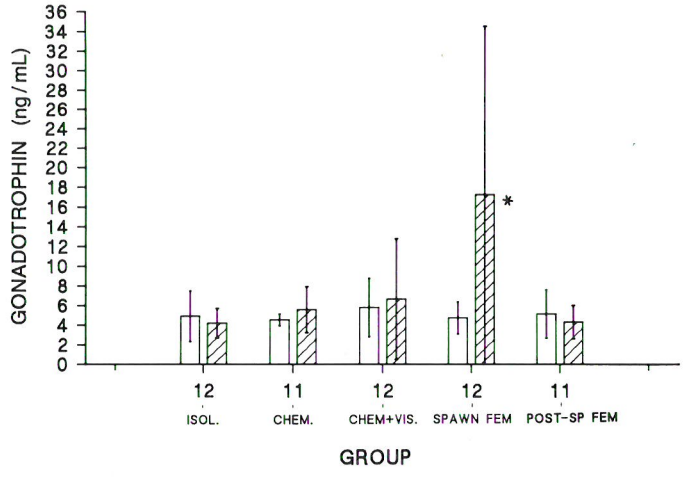

FIG. 3. Plasma levels (mean ± SD) of gonadotrophin (GtH) before (open bar) and after (hatched bar) males were placed in different social conditions (see Fig. 1).

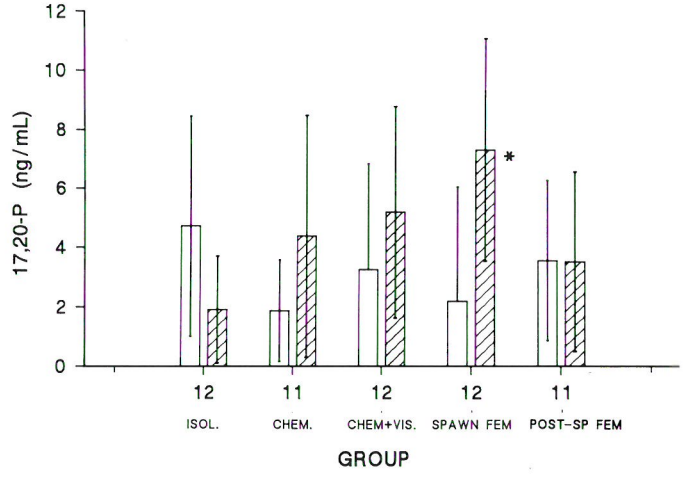

FIG. 5. Plasma levels (mean ± SD) of 17,20-P before (open bar) and after (hatched bar) males were placed in different social conditions (see Fig. 1).

groups in the concentrations of 11-KT and 17,20-P in the samples of plasma taken on the first day (Figs. 4 and 5); differences were not significant (ANOVA). Following four days of treatment, differences between the groups were significant (ANOVA, $P < 0.01$). Serial samples within each group reveal a number of consistent responses. Plasma 11-KT decreased significantly from the first to the second sample in isolated males, males with inactive females, and those receiving chemical cues. Levels of 11-KT rose in males paired with active females and did not change significantly in males exposed to a combination of chemical and visual cues.

Plasma 17,20-P increased over four days in males paired with nesting females (Fig. 5). Differences in other experimental groups were not significant.

Discussion

These results confirm the findings of Liley et al.(1986). Plasma concentrations of GtH, 11-KT and 17,20-P increased in males allowed to interact with nesting, ovulated females. In addition, there was a threefold increase in the amount of strippable milt in those males. There was no increase in milt or plasma hormones in response to chemical cues alone or in combination with visual stimuli, or to pairing with postspawning females. Spermatocrit was not affected by any of the procedures.

At first sight, the present results appear to exclude olfaction from playing a role in mediating the endocrine and milt responses of male trout. Visual, tactile, auditory, or taste signals provided by the nesting female, alone or in combination, may be responsible for the male response. The performance of active courtship by the male may also have a self-stimulatory effect. However, the possibility that the male response also depends upon a chemical signal distributed by diffusion or current cannot be ruled out. Because of rapid dispersion by flowing water, the area within which the concentration of a chemical signal remains above threshold for the stimulation of an endocrine response in the male may be restricted to a small active space close to the source. In such a situation, only when the male is interacting directly with the female is he able to remain within the active space.

Other factors may have affected responses to chemical or

other behavioural cues. There were marked differences in the social environment in the experimental groups: males in Groups 4 and 5 were paired with nesting and inactive females, respectively, whereas males in other groups were maintained in all-male communities (1 and 2) or with one other male (3). The presence of other males may affect the response of some or all males in those groups. In goldfish (*Carassius auratus*), there is evidence that pheromonal androgens released by males may inhibit milt production (Stacey and Sorensen 1991).

The lack of clear evidence of a response to olfactory cues in male trout contrasts with the findings of studies with goldfish. Kyle et al. (1985) measured an increase in expressible milt in male goldfish exposed to spawning pairs, but were unable to demonstrate a response to chemical cues alone. However, Kobayashi et al. (1986) demonstrated a surge in GtH in males in response to an unidentified water-borne chemical released by the female. Dulka et al. (1987) recorded a rapid increase in plasma GtH in males exposed to 17,20-P in the water. This finding and those of Stacey et al. (1989) and Sorensen et al. (1989) led to the suggestion that 17,20-P serves as a preovulatory pheromone, released by the female several hours before ovulation. This pheromone appears to 'prime' the male (Sorensen et al. 1989) and is believed to be an important component in the mechanism that synchronizes the spawning readiness of the male with that of the female.

It is unlikely that the increase in milt quantity recorded here is the direct result of recent spermatogenesis. Baynes and Scott (1985) suggest that sperm formation and the release of sperm from spermatogenetic cysts into the testicular tubules is already complete shortly after the onset of spermiation, i.e., the production of milt (sperm plus seminal fluid). The observed increase in 'available' milt appears to be the result of the migration of mature sperm from the testicular tubules into the vas deferens (Scott and Baynes 1982), where elaboration and 'hydration' of the seminal plasma occur (Clemens et al. 1964; Ueda et al. 1985; Billard et al. 1990). It is milt stored in the vas deferens that may be stripped by applying pressure to the abdomen. Our finding that spermatocrit was not affected by any of the treatments suggests that the increase in milt from males paired with nesting females was not simply due to an increase in seminal plasma alone.

A number of investigations have implicated pituitary hormones in the stimulation of milt production. Clemens et al. (1964) propose that gonadotrophin is responsible for the seminal thinning response in out-of-season carp (*Cyprinus carpio*) and rainbow trout recorded after treatment with pituitary extracts (Clemens and Grant 1965). Injections of salmon gonadotrophin caused an increase in milt in amago salmon (*Oncorhynchus rhodurus*) one month prior to the natural spawning period (Ueda et al. 1985). K. H. Olsen and N. R. Liley (in preparation) demonstrated a marked increase in strippable milt within 4 h of treatment of rainbow trout with pituitary extract, lyophilized GtH, and gonadotrophin-releasing hormone. Hypophysectomy eliminated the increase in milt that occurs in response to water-borne 17,20-P in male goldfish (Dulka et al. 1987).

The role of gonadal steroids in the production of milt is less clear (Billard et al. 1990). Treatment with 17,20-P was less effective than GtH, but more effective than 11-KT or T in causing an increase in milt in amago salmon (Ueda et al. 1985). These investigators conclude that 17,20-P serves as the mediator of gonadotrophin-induced milt production. On the other hand, Scott and Baynes (1982) found that injections of 17,20-P had no effect on milt volume in rainbow trout, but did alter ion balance in the seminal fluids. Scott and Baynes (1982) propose that a major function of 17,20-P is the control of sperm motility through changes in ion balance in the seminal fluid.

Our results are consistent with the proposals of Ueda et al. (1985) and Dulka et al. (1987) that GtH regulation of milt production is mediated by testicular 17,20-P. Both 17,20-P and GtH increased in those males allowed to interact with ovulated females. These were the only males in which there was an increase in strippable milt.

Although androgens play a role in spermatogenesis (Billard et al. 1982, 1990; Fostier et al. 1983), there is little evidence that 11-KT is involved in the final stages of milt formation. Fostier et al. (1984) noted that the volume of milt decreased in parallel with a decrease in 11-KT levels in rainbow trout, whereas Baynes and Scott (1985) found that milt volumes remained high as androgen levels fell. We also found, in those males not allowed to interact with ovulated females, that although androgen levels were falling, milt volumes remained the same as those of the initial sample. Ueda et al. (1985) obtained only a small increase in milt in amago salmon receiving injections of 11-KT.

Plasma concentrations of 11-KT (and to a lesser extent, 17,20-P) were lower and more variable than those in fish of the same population 2 years earlier (Liley et al. 1986). These differences may reflect the fact that, unlike the previous study (fish collected June 6), the present experiments were conducted in the second half of the spawning season (collected June 22). Plasma concentrations of androgens (11-KT and T) are highest prior to the onset of spawning, and decline through the spawning season, whereas 17,20-P and GtH remain low in the prespawning period and rise at the onset of the spawning season (references in Liley et al. 1986). Males in the Pennask population remain in spawning condition after final maturation and the onset of spermiation (milt was readily expressed under gentle pressure) for 3–4 weeks. Because males at the collection site remain trapped between fences on the river for 2–3 weeks, and there is no readily available mechanism for selecting males at precisely the same stage after the onset of spermiation, it is likely that the experimental groups were composed of males tested at different times following final maturation: some males may have moved into the trap at the beginning of the spawning season, other later maturing fish may have entered the trap shortly before collection. Nevertheless, in spite of variability in the condition of the males, and the fact that males in spawning condition would be expected to show declining androgen levels, 11-KT concentrations rose in 10 of 11 males paired with nesting females. In other groups 11-KT remained the same or declined over the four days of the experiment.

Gonadal hormones have been implicated in the control of reproductive behaviour in a wide variety of teleosts (Liley and Stacey 1983); however, little is known of their role in the causation of spawning behaviour in salmonids. The preponderance of 11-KT late in the cycle close to the time of spawning suggests that 11-KT rather than T may be more directly involved in maintaining sexual behaviour (Liley et al. 1986). The decline in 11-KT that may precede or coincide with the spawning period in some populations appears to indicate that high levels of androgen are not necessary for the maintenance of sexual responsiveness. However, in the current investigation plasma 11-KT increased in those males allowed to interact with nesting females. This capacity to respond to the social

situation suggests that 11-KT is involved in mediating an adaptive behavioural response to current 'social demands.' Cardwell and Liley (1991) demonstrated that nonterritorial males of the protogynous stoplight parrotfish (*Sparisoma viride*, Scaridae) have lower levels of T and 11-KT than territory holders. When allowed access to vacant territories nonterritorial males underwent profound increases in T and 11-KT, and maintained territories. Cardwell and Liley (1991) conclude that this endocrine response to the demands of the social environment provides a fine tuning in the control of reproductive behaviour similar to that demonstrated in the other vertebrate groups (see Cardwell and Liley 1991 for references).

The clear association between high 17,20-P levels and the spawning period in salmonids and a number of other teleosts (Fostier et al. 1983) suggests that 17,20-P plays a major role in final gamete maturation in both males and females. The increase in milt in response to injections of 17,20-P (Ueda et al. 1985) and the socially induced changes in plasma 17,20-P concentrations (Liley et al. 1986; this study) indicate that this hormone is a component of a mechanism that ensures an increase in milt synchronized with the availability of a female in spawning condition. Furthermore, as male rainbow trout remain in spawning condition for several weeks (wild) or months (hatchery; Munkittrick and Moccia 1987), the capacity of males to adjust milt production to the opportunity to spawn may be important in conserving gametes over a lengthy spawning period.

The association of the socially induced increase in 17,20-P with the onset of active sexual behaviour by the male also raises the possibility that this hormone is involved in maintaining sexual responsiveness either alone or in conjunction with androgen. There is no evidence that GtH is directly involved in the regulation of spawning behaviour as proposed by Crim et al. (1975). This possibility cannot be ruled out, but it seems more likely that any effect of GtH on behaviour is indirect, through the regulation of the synthesis and release of gonadal steroids from tissues whose capacity to respond alters through the spawning cycle.

Acknowledgements

Preliminary results were presented at the Third International Symposium on the Reproductive Physiology of Fish, St. Johns, Nfld., Canada (Liley et al. 1987). Due to an error, figures presenting data on plasma levels of 11-KT and 17,20-P were transposed in that report.

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