Elevations in Gonadotrophin Concentrations and Milt Volumes as a Result of Spawning Behavior in the Goldfish

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In many male mammals and birds, exposure to sexual stimuli results in acute elevations of circulating luteinizing hormone (LH) and testosterone (T); a similar phenomenon has now been observed in the male goldfish (*Carassius auratus*). Mature males placed with either a receptive female or stimulus pairs of spawning goldfish had gonadotrophin (GtH) concentrations and expressible milt (sperm) volumes that were significantly greater than those of males kept in all-male groups. This stimulatory effect lasted from 20 min to at least 2 hr for GtH (20°) and from ≤ 1 hr to ≥ 24 hr for milt (14°). When males were separated from the spawning pair by either a solid or perforated clear partition, no elevations of GtH or milt levels occurred. In contrast, these values increased in males placed in contact with a spawning pair, even when that pair contained no female, but a male induced to perform female sexual behavior by treatment with prostaglandin. These results suggest that, in goldfish, access to a spawning situation is necessary for rapid elevations in GtH and milt. Furthermore, it appears that the males must be sexually active in order for these physiological changes to occur, as males that failed to engage in courtship behavior with a spawning pair had GtH and milt values not different from isolated fish. This suggests that male sexual behavior and elevations in milt and GtH are concurrent events that share a common activation pathway in the brain. The increase in milt may be due to both neurally and hormonally mediated events that ensure milt availability for imminent spawning activity. © 1985 Academic Press, Inc.

In many male mammals and birds, exposure to sexual stimuli results in acute elevations of circulating luteinizing hormone (LH) and testosterone (T). These hormonal changes can be induced by the sight and/or smell of a female, and thus probably occur prior to mating in the normal situation (Harding, 1981; O'Connell *et al.*, 1981, for reviews). In fact, anticipatory rises in these hormones or their dependent physiological processes can be evoked even in the absence of a female if the male is presented with a situation where sexual activity has previously occurred (Anonymous, 1970; Kamel *et al.*, 1975; Graham and Desjardins,

1980). Except for preliminary reports of the present work (Kyle *et al.*, 1979; 1982a) similar observations have not been published previously for teleost fishes, although indirect evidence indicates that chronically presented sexual stimuli can activate the pituitary–gonadal axis. For example, several weeks of exposure to the opposite sex facilitated endocrine-controlled events such as maturation of eggs and ovulation in a variety of teleost species (Aronson, 1945; 1965; Chien, 1973; Eaton and Farley, 1974; Chen and Martinich, 1975) and the development of the nuptial coloration in male sticklebacks (*Gasterosteus aculeatus*) (Reisman, 1968). In addition, stimulation of the male endocrine system has been suggested as a mechanism by which exposure to a female or her holding water caused the onset of nest building in several anabantids and cichlids (Liley, 1982).

We present here a series of experiments

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that study the effects of short-term exposure to sexual stimuli on two indices of the reproductive status of the male goldfish (*Carassius auratus*): the concentration of serum "Con A II" gonadotrophin (GtH) and the volume of milt that could be expressed by handstripping. As "Con A II" or "maturational" GtH has an LH-like action on steroidogenesis and spermiation (Idler and Ng, 1979), we hypothesized that circulating GtH levels, as with mammalian LH, may be altered by social conditions. As well, blood GtH levels were substantially higher in spawning male carp (*Cyprinus carpio*; Fish Reproductive Physiology Research Group, 1978) and white suckers (*Catostomus commersoni*; Stacey *et al.*, 1984) than in mature, but prespawning individuals. The volume of expressible milt was measured because milt volumes are reported to vary directly with the dose of exogenous gonadotrophin in hypophysectomized goldfish (Yamazaki and Donaldson, 1968), and thus may be a good indicator of endogenous GtH changes in intact animals, and also because we observed that the female-exposed males in the first experiment appeared to have more expressible milt at the end than at the beginning of the experiment.

The first part of this study (Experiments 1–3) measured the plasma GtH concentrations and milt volumes of male goldfish during exposure to a receptive female or pairs of spawning goldfish. The second part of this study (Experiments 4–6) characterized the sensory information necessary for alteration of GtH and milt levels, by manipulating the type of sensory cues available to the test male.

METHODS

General Protocol

Animals and maintenance. Goldfish were obtained from the Grassforks Fisheries Co., Martinsville, Indiana, and held in 3600- or 1000-liter flowing-water tanks at 14° (Experiments 1–3) or 18° (Experiments 4–6), under a simulated natural spring and summer

photoperiod (Edmonton), for various times prior to each experiment. Animals were fed twice daily with commercial fish food (Ewos pellets). Only mature males, which had expressible milt and tubercles on the pectoral fins and operculum, were used; body weights ranged from 15 to 30 g.

Preparation of stimulus fish. Either a receptive female or pairs of spawning goldfish provided the sexual stimuli for the experimental males; the use of spawning pairs ensured that the test males were exposed to spawning activity, regardless of their own behavioral response (several males may court and spawn with a single female). Normally, females are receptive to male courtship and spawn only when ovulated; however, spawning behavior in unovulated females (Stacey, 1976; Stacey and Peter, 1979) and males (Stacey, 1981) can be induced by intramuscular (im) injection of prostaglandin F₂ α Tris salt (PG; Sigma Chemical Co., St. Louis, Mo.) in physiological saline. PG is thought to be an endogenous signal that coordinates female sexual behavior with the time that ovulated oocytes are in the ovarian lumen (Stacey, 1981; Stacey and Goetz, 1982). In the present study, PG treatment (200 ng/g body wt im) was used exclusively in all experiments, with the exception of Experiment 5, to induce female behavior; Experiment 5 used both PG-treated and ovulated fish. The males of all stimulus (spawning) pairs were fish that had previously demonstrated persistent and vigorous courtship of PG-injected females. All experimental aquaria were illuminated from above by fluorescent lights and screened as much as possible from any outside disturbances; all aquaria contained a spawning substrate of floating, artificial plants. Descriptions of goldfish spawning behavior have been presented elsewhere (Kyle and Peter, 1982; Kyle *et al.*, 1982b).

Milt and blood samples. Fish were anesthetized in solutions of either 0.1% tricaine methanesulfonate or 0.05% 2-phenoxyethanol prior to collection of milt or blood samples. Before stripping the fish of milt, urine was first expelled by applying a light pressure around the urogenital pore and blotting the fluid with tissue paper. Milt expressed during subsequent gentle stroking of the abdomen, in an anterior to posterior direction, was immediately aspirated into a 20- or 50- μ l glass micropipet. The use of excessive force to strip milt was avoided, as this often ruptured the blood vessels around the urogenital ducts. The micropipets were spun for 15 min in a hematocrit centrifuge, and the volumes of milt and packed sperm were measured for calculation of the percentage sperm content or "spermato-crit."

When scheduled, blood was taken from the caudal vasculature while the fish were anesthetized for milt stripping. For experiments that ran over several days, blood sampling was done within an hour of the same time each day to minimize the effects of possible daily

GtH fluctuations (Hontela and Peter, 1978). The blood was kept on ice until clotted, centrifuged at 5°, and the serum stored at -28°. Serum GtH levels were later determined by radioimmunoassay (Crim *et al.*, 1976; Hontela and Peter, 1978; 1980).

Effects of Exposure to Sexual Stimuli

Experiment 1—GtH levels in males exposed to another male or female. A small group of males from a May stock of goldfish were warmed to 20° for 1 week and then placed individually in 65-liter glass aquaria, at 20°. The following morning, either a male or a PG-injected female stimulus fish was added to each aquarium. Experimental males were removed and bled for GtH assay either immediately upon addition of the stimulus fish (0 min) or 20, 40, or 60 min later; this protocol was repeated at the same time of day for several days.

Experiments 2 and 3—Milt and GtH levels in males exposed to spawning goldfish. These experiments were performed in July, using a stock of fish obtained several months earlier. In Experiment 2, 17 mature males were initially (0 hr) stripped of milt and placed into three 225 liter tanks, at 14°. Ten of these males (stimulated) were divided equally between two tanks, each of which contained two pairs of activity spawning goldfish, while the seven remaining fish (controls) were added to the third, empty tank. The males were repeatedly removed, stripped of milt, and returned to the experimental tanks at 1, 3, 6, 12, and 24 hr; blood samples were taken at 1 and 3 hr. This protocol was repeated on the following day with a new group of goldfish, giving a total of 14 control and 20 stimulated males.

Experiment 3 modified the protocol of Experiment 2. Experimental males were moved into the test tanks, at 14°, on the afternoon preceeding the test day. On the test day, a presample of blood and milt was taken, and stimulus pairs of spawning goldfish were added to two of three tanks 1 hr later (0 hr). Blood and milt samples were taken at 2 hr and milt volumes were determined again at 6 and 24 hr.

Effects of Manipulating Sensory Cues

Experiment 4—Effects of contact with vs visual exposure to pairs of spawning goldfish. Male goldfish were stripped of milt (0 hr) and placed individually in 65-liter glass aquaria, at 20°. Each aquarium was arranged in one of the following three ways:

1. *Isolated*—The aquarium was covered on all sides by black plastic.
2. *Contact*—The aquarium contained a stimulus pair of spawning goldfish.
3. *Visual*—The aquarium was as for the "Isolated" treatment, except that the long edge was uncov-

ered and butted against the "Contact" aquarium such that the isolated animal could observe the fish spawning in the adjacent tank.

Two hours later, the experimental males were stripped of milt, bled, and killed for determination of their gonadosomatic index ($GSI = \text{gonad wt} \times 100 / \text{total body wt}$). This protocol was followed for 3 successive days in March, with five replicates of each treatment/day (total of 15 fish/treatment).

Experiment 5—Effects of contact with vs separation from spawning pairs containing either ovulated or PG-treated females. In June, 65-liter glass aquaria, at 20°, were covered on all sides by black plastic and divided in half by crosswise, clear plexiglass partitions, each perforated by six holes, 1.5 cm in diameter. Water was circulated between the tank halves by an air lift system. Stimulus fish were first arranged in the test tanks and allowed to spawn; the experimental males were then stripped of milt (0 hr) and given one of five treatments:

1. *Isolated*—One experimental male was placed on each side of the partition; there were no stimulus fish in this aquarium.
2. *PG female-contact*—One test male was placed with a spawning pair of fish; the female of this pair was made receptive by PG treatment.
3. *PG female-separated*—One test male was placed on the other side of the partition from the spawning pair described above in 2.
4. *Ovulated female-contact*—One test male was placed with a spawning pair that was composed of a male and an ovulated female. Ovulation was induced by selecting mature females from a stock held at 12°, slowly warming them to 20° in the afternoon, and injecting them intraperitoneally (ip) with 4 I.U. human chorionic gonadotropin (hCG; Sigma)/g body wt in the evening prior to the test day. About 80% of the females treated in this manner had ovulated by the morning of the test day and then were transferred into the experimental aquaria.
5. *Ovulated female-separated*—The test male was placed on the other side of the partition from the spawning pair described above in 4.

Blood and milt samples were taken at 1.5 hr, and the males were killed for GSI determinations. There were four replicates of each treatment/day for 3 days, giving a final sample size of 12 fish/treatment.

Experiment 6—Effects of contact with vs separation from either heterosexual or homosexual pairs of spawning goldfish. In April, experimental aquaria were arranged as in Experiment 5. After the stimulus fish were added to the tanks and allowed to spawn, experimental males were stripped of milt (0 hr) and given one of five treatments:

1. *Isolated*—One test male was placed on one side of the partition and the other side was left empty.
2. *Male/female-contact*—One test male was placed

with a heterosexual spawning pair on one side of the partition.

3. *Male/female-separated*—One test male was placed on the other side of the partition in the aquarium described above in 2.
4. *Male/male-contact*—One test male was placed with a homosexual pair of spawning goldfish on one side of the partition. The male performing the female role was previously olfactory tract-sectioned, an operation that reduces male, but not female, behavior (Stacey and Kyle, 1983), and injected with PG on the test day, which induces female behavior in males as well as in females (Stacey, 1981).
5. *Male/male-separated*—One test male was placed on the other side of the partition in the aquarium described above in 4. Courtship and spawning behavior were observed for about 1 min at 15 min intervals during the 2-hr test.

Blood and milt samples were taken at 2 hr and the animals were killed for GSI determinations. The procedure was followed for 3 days with five replicates of each treatment/day giving a total of 15 fish/treatment. During the behavioral tests, we noticed that a few of the test males displayed clamped fins and were less active than normal; as these symptoms usually indicate the presence of disease, the data from these fish were discarded, thus reducing the sample size by 1–3 fish in some treatment groups.

RESULTS

Effects of Exposure to Sexual Stimuli

In Experiment 1, exposure to a sexually receptive female for 40–60 min caused a significant increase in serum GtH concentrations in male goldfish over the initial (0 min) values (Fig. 1). In contrast, the GtH levels of those fish exposed to other males did not change over the duration of the test. Also, the GtH values for the female-exposed fish were higher than those for the male-exposed fish at the 20-, 40-, and 60-min sample times. Similarly, “stimulated” males that were placed in groups with spawning pairs of goldfish had significantly higher serum GtH concentrations than did unstimulated controls at the 1-hr (Experiment 2, Fig. 2) and 2-hr (Experiment 3, Fig. 3) sample times, but not at the presample time (Fig. 3) or at 3 hr (Fig. 2). In Experiment 3, a significant decline was found between the presample and the 2-hr serial

blood samples for the controls, but not the stimulated group; this decline contributed to the significant difference between treatments found at the 2-hr sample time (Fig. 3).

Figures 2 and 3 show that significantly more milt could be stripped from males after 1 to 24 hr of exposure to pairs of spawning goldfish than from males kept in all-male groups. Although the behavior of all individuals in the group could not be determined easily, most males were observed to court the females at some time during the test.

Spermatocrits were not different between treatments at any sample time and averaged (mean $\pm$ SE) $31.7 \pm 1.3\%$ for Experiment 2 and $32.0 \pm 2.1\%$ for Experiment 3.

Effects of Manipulating Sensory Cues

In contrast to the first series of experiments, male goldfish exposed to sexual stimuli in Experiments 4–6 did not always show significantly elevated GtH levels, even when placed in contact with a spawning pair. In Experiment 4, for instance, neither the males permitted only to view sexual activity nor those permitted contact with a spawning pair showed GtH values that were significantly different from the isolated controls (Fig. 4). In agreement with the first series of experiments, males in contact with a PG-treated, receptive female in Experiment 5 had GtH values that were significantly higher than either the separated or isolated groups; however, males in contact with an ovulated female had GtH values that were not significantly different from either the “PG female-contact” group or the other treatments (Fig. 5). We noticed that the “PG female-contact” males courted consistently throughout the test period, while the “ovulated female-contact” males were less sexually active during the latter half of the test, when many females had oviposited all of their eggs and were no longer receptive. The GtH concen-

FIG. 3. Milt volumes and serum GtH concentrations (mean $\pm$ SE) of male goldfish held at 14° and repeatedly sampled at various times while kept either in an all-male group (controls, C; clear bars) or with pairs of spawning goldfish (stimulated, S; shaded bars). *P* values calculated by one-tailed *t* test (for milt and GtH, C vs S) or by paired *t* test, two-tailed (GtH, presample vs 2 hr).

FIG. 4. Milt volumes and serum GtH concentrations after 2 hr of stimulation (mean $\pm$ SE) of male goldfish that were held at 20° and isolated from (I), in contact with (C), or visually exposed to (V) a spawning pair. Values among treatments were compared by ANOVA and Duncan's multiple range test; ★ indicates a significant ($P \leq 0.05$) difference from the isolated group. Milt values between times were compared by paired *t* tests; ▽ indicates a significant decline ($P \leq 0.05$, one-tailed) between the two sample times.

FIG. 5. (a) Milt volumes and (b) serum GtH concentrations after 1.5 hr of stimulation (mean $\pm$ SE) of male goldfish that were held at 20° and isolated from (I), in contact with (C), or separated with a transparent, perforated partition from (S) a spawning pair containing either an ovulated (OV) or PG-treated (PG) female. See legend to Fig. 4 for statistics and significance symbols.

FIG. 6. (a) Milt volumes and (b) serum GtH concentrations after 2 hr of stimulation (mean $\pm$ SE) of male goldfish that were held at 20° and isolated from (I), in contact with (C), or separated with a perforated partition from (S) either a heterosexual (♀) or homosexual (♂) pair of spawning fish. Statistics and significance symbols as in Fig. 4, except that Kruskal-Wallis and nonparametric comparison tests were used to compare GtH values and the data were combined to yield three groups: I, C, and S.

trations measured in Experiment 6 were highly variable and not normally distributed. A nonparametric ANOVA (Kruskal-Wallis test) revealed no significant differences between groups, although the two "contact" groups ("male/female-contact" and "male/male-contact") had the highest means; however, the combined GtH values for these treatments were significantly higher (Kruskal-Wallis and nonparametric *a posteriori* comparison tests) than values for either the combined "male/female-separated" and "male/male-separated" groups or the "isolated" group (Fig. 6).

Milt volume data in Experiments 4–6 were analyzed by comparing both individual pre- and poststimulation values (paired *t* test) and means among treatments (ANOVA and Duncan's multiple range test). In all experiments, isolated males and males prevented from contacting the spawning pair by either a solid (Experiment 4) or perforated (Experiments 5 and 6)

transparent partition yielded significantly less milt on the second stripping (1.5 or 2 hr) as compared to the first (0 hr; Figs. 4–6). These separated males appeared to be aware of the adjacent, but inaccessible, spawning activity and spent much of the test time oriented toward or swimming along the side of the tank or partition next to the spawning fish. However, if contact was allowed between the test male and a spawning pair, regardless of whether that pair was heterosexual (containing either an ovulated [Experiment 5] or PG-treated [Experiments 5 and 6] female) or homosexual (containing a PG-treated male that played the female role [Experiment 6]), then the volume of milt obtained on the second stripping was equal to the first (Figs. 5 and 6). Initial (0-hr) values were not different among treatments for Experiments 4–6. Poststimulation (2-hr) milt levels that were significantly higher than the isolated group occurred only in those males permitted to contact a spawning pair (Figs. 4–6). As in the first series of experiments, spermatozoa were not different between treatments at any sample time for Experiments 4–6 (data not shown); GSI also were not different between treatments and averaged $3.64 \pm 0.15\%$ for Experiment 4, $3.48 \pm 0.19\%$ for Experiment 5, and $4.11 \pm 0.14\%$ for Experiment 6.

In Experiment 6, where the behavior of the test males was routinely observed during the 2-hr experiment, 5 of the 13 males placed in contact with the heterosexual spawning pair and 5 of the 12 males placed in contact with the homosexual spawning pair did not court or spawn. The data for the combined "contact" groups, therefore, were divided into sexually active and inactive categories and compared with the isolated controls (Fig. 7). After 2 hr of exposure to a spawning pair, sexually active males had higher milt and GtH levels than either the inactive or isolated fish. In contrast, sexually inactive males were not different from isolated fish in either the volume of expressible milt or concentration

FIG. 7. Milt volumes and serum GtH concentrations after 2 hr of stimulation (mean $\pm$ SE) of isolated, sexually inactive, and sexually active male goldfish held at 20°. Those values underlined by the same line are not significantly different at $P < 0.05$ (ANOVA and Duncan's multiple range test for milt and GSI, Kruskal-Wallis and nonparametric comparison tests for GtH).

of GtH. The initial (0-hr) milt volumes and GSI were similar between groups.

DISCUSSION

This is the first report in fish of rapid elevations in blood GtH concentrations and expressible milt volumes after exposure to sexual stimuli (either a receptive female or several pairs of spawning goldfish). The first part of the study (Experiments 1–3) found that serum GtH levels in male goldfish at 20° were increased after 20 min of exposure and remained elevated for up to 1 hr as compared to nonstimulated controls. A similar time course occurred at 14°; serum GtH levels of sexually stimulated males were elevated at 1 hr (the earliest sample time) and 2 hr and were no longer significantly different from the control fish at 3 hr. The transitory nature of the gonadotrophin increase in response to sexual stimuli also was noted for LH in mammals, with concentrations peaking in about 15 min and declining to baseline levels in 60 min (Maruniak and Bronson, 1976; Kamel *et al.* 1977; Coquelin and Bronson, 1979). In Experiment 3, the serum GtH concentrations of control fish declined between

the 0- and 2-hr serial blood samples, a phenomenon also noted in goldfish by others (Chang *et al.*, 1982) and in mammals (Harding, 1981). No significant decline between samples occurred in males exposed to spawning fish, suggesting a facilitation of GtH secretion at this time (2 hr, 14°).

Experiments 2 and 3 also found that more milt could be expressed from male goldfish after 1 to 24 hr of contact with a pair of spawning fish than could be expressed from unstimulated controls. The similarity of spermatocrit values between treatments indicates that both sperm and fluid volumes were proportionately increased, suggesting facilitation of milt "loading" into the sperm ducts and/or milt production.

While the sight, sound, or smell of a female can cause rapid, transient elevations of LH and T in some male mammals and birds, the second part of this study (Experiments 4–6) showed that visual and chemical stimuli alone from either a PG-treated or an ovulated spawning female goldfish did not elevate serum GtH concentrations or milt volumes in the male. These elevations only occurred in males allowed to contact the spawning pair, even when a PG-treated

male played the female role in the spawning pair. Furthermore, males that failed to court or spawn when placed in contact with a spawning pair were not different from isolated fish in their GtH and milt levels. In contrast, sexually active individuals had milt and GtH levels of significantly higher than the isolates or inactive fish, suggesting that sexual activity and elevations in milt and GtH are concurrent events which may share a common activation mechanism in the brain. As well, it appears that the male need not spawn in order for the GtH "surge" to occur. The fish in Experiment 1 were courting, but had not spawned by the 20-min sample time, when GtH levels were significantly elevated.

While significant elevations in serum GtH levels were only found in fish allowed contact with a spawning pair, this treatment did not always result in a measurable GtH increase; those males in contact with a PG-treated female in Experiment 4 and ovulated females in Experiment 5 had GtH values not significantly different from isolated fish. Possibly, exposure to a single pair of spawning fish within a relatively small space was not as effective a stimulus as exposure to several spawning pairs within a larger aquarium, as was the case in Experiments 2 and 3, where elevations in GtH were clearly demonstrated. This latter condition would more closely mimic the group spawning behavior of goldfish in natural ponds. As well, the failure to find increased GtH levels may reflect the intensity of ongoing spawning behavior at the time of sampling. For example, the males in contact with PG-treated females in Experiment 5 had elevated GtH levels while, as previously mentioned, blood GtH levels of males in contact with ovulated females in this experiment were not different from those of either the isolated or PG female-exposed groups. Many of the ovulated females (ovulation induced by hCG treatment) had oviposited most of their eggs by 60 min and were no longer receptive, re-

sulting in reduced male courtship behavior at the 90-min sample time, while the PG-treated females were still spawning at the end of the experiment.

The function of the sexually-stimulated surge of GtH in male goldfish is not known. There is yet no evidence clearly indicating a causal role for GtH in the activation of male behavior (see discussion in Liley and Stacey, 1983); however, a GtH increase during courtship may affect ongoing behavior indirectly by altering blood steroid levels. We suggest that a more likely alternative is that the GtH surge acts in a feed-forward manner to facilitate subsequent reproductive processes that are rate-limited by hormone availability. As gonadotrophin treatment is commonly used to stimulate spermiation in intact fish (Billard *et al.*, 1982, for review), the GtH rise in sexually stimulated goldfish may enhance milt production in preparation for future spawnings.

Under natural spawning situations, the GtH surge in males may precede actual spawning. Female goldfish undergo ovulation in response to a GtH surge in the latter part of the scotophase (Stacey *et al.*, 1979), while ovoposition may be delayed until the first part of the photophase when male courtship becomes intense (personal observations). Ovulated females produce a pheromone which both attracts males (Partridge *et al.*, 1976) and stimulates courtship activity (Stacey, unpublished results), and from the present results we can hypothesize that GtH levels in the male would rise within 20 min of the onset of courtship behavior. Injection experiments first detected increased volumes of expressible milt in mature males between 1 and 3 hr after ip treatment with 25 ng carp GtH/g body wt at 14° (Kyle, 1982). This dose was sufficient to cause a serum level of about 35 ng/ml 1 hr after ip injection into mature male goldfish at 12° (Cook and Peter, 1980), a concentration within the range of the endogenous levels measured in this study. Taken

together, this information suggests that an encounter with an ovulated female could result in a GtH-stimulated increase in expressible milt later the same morning.

Exogenous gonadotrophin treatment of intact (Clemens and Grant, 1964) or hypophysectomized (Yamazaki and Donaldson, 1968) goldfish causes gonadal hydration, decreased spermatocrit (seminal thinning), and increased volumes of expressible milt. However, neither the sexually stimulated (present study) nor the hormonally induced (Kyle, 1982) increase in expressible milt were associated with decreased spermatocrit in mature goldfish. Presumably, the sperm ready for release in these mature males had already undergone optimal dilution. Thus, endogenous or exogenous GtH may have accelerated the process of milt production without altering the fluid content of the product.

The mechanism of GtH action on milt production likely involves steroid hormones, as sex steroids (testosterone, methyl testosterone, 11-ketotestosterone, and progesterone) stimulate spermiation in goldfish (Yamazaki and Donaldson, 1969; Billard, 1976). Recently, elevated levels of 17α -hydroxy- 20β -dihydroprogesterone (17α - 20β P) have been found associated with spermiation in rainbow trout (*Salmo gairdneri*; Scott and Baynes, 1982) and with spawning in the white sucker (*C. commersoni*; Scott *et al.*, 1984). In the trout, plasma 17α - 20β P levels are positively correlated with both the volume of expressible milt and the K^+/Na^+ ratio in the milt fluid, suggesting that this steroid may maintain high K^+ concentrations, which inhibit sperm motility (Baynes *et al.*, 1981). Thus, elevated GtH levels may indirectly affect milt quality as well as milt quantity; whether similar changes in 17α - 20β P and K^+/Na^+ ratios occur in the goldfish is not known.

Although the GtH surge seen in sexually-stimulated males may result in elevated expressible milt volumes up to 24 hr after ex-

posure to a spawning situation, the fact that treatment with carp GtH resulted in increased milt volumes at 3 hr, but not 1 hr postinjection (Kyle, 1982), while sexually-stimulated elevations are clearly seen after 1 hr of treatment, suggests that the milt increases at 1 hr may occur via a GtH-independent mechanism. For example, accelerated movement of milt into the posterior portion of the sperm duct would result in elevated expressible milt volumes without necessarily involving a GtH-mediated increase in milt production. Accumulation of milt in readiness for release during spawning is probably accomplished by contractions of the sperm duct (Billard *et al.*, 1982). In fish, hormones of the neurohypophysis, urophysis, and the sympathetic nervous system have been shown to influence sperm duct contractility and sperm "release" as a result of hand-stripping (Berlind, 1972; Billard, 1977) and thus may be potential mediators of sexually stimulated milt volume increases. However, injection of goldfish pituitary homogenate (65.4 μ g wet wt tissue/g body wt, which was slightly less than 2 pituitaries/fish), goldfish urophyseal homogenate (20 mg dry wt tissue/fish), or adrenalin (40 μ g/g body wt) failed to significantly increase milt volumes 1 hr postinjection at 14° (Kyle, 1982).

Demski and coworkers have evoked sperm release in goldfish by electrically stimulating a brain pathway running from the medial olfactory tract to the preoptic area, brain stem, and rostral spinal cord (Demski and Hornby, 1982; Demski and Dulka, 1983), a mechanism that involves contractions of the testes and sperm ducts (Dulka, 1983). This pathway also projects to the supracommisural ventral telencephalon (Demski and Northcutt, 1983), an area critical for the expression of spawning behavior in male goldfish (Kyle and Peter, 1982; Kyle *et al.*, 1982b). Activation of those brain areas involved in sexual behavior may also mediate sperm duct contraction, loading of the posterior ducts with

milt, and, consequently, a rapid increase in expressible milt volume during sexual stimulation.

In summary, this study demonstrates rapid elevations in serum GtH and expressible milt volumes coincident with sexual activity in the male goldfish. This increase in expressible milt volume may be due to both neurally (sperm duct contractions and duct loading) and hormonally (GtH-stimulated milt production) mediated events that are activated during the performance of courtship behavior. This mechanism would ensure that milt is available for release when ovulated oocytes become available for fertilization. In addition to increasing milt quantity, GtH also may alter milt quality (i.e., composition of milt fluid) and other, as yet unidentified, reproductive functions that are rate-limited by hormone availability. This study also suggests that laboratory fish denied sexual interaction may present a misleading view of normal physiological processes that occur during the breeding season. For example, blood GtH levels in males of several teleost species sampled during the spawning season under conditions where spawning does not occur did not show significant GtH elevations (Billard *et al.*, 1982). However, in the white sucker, (Stacey *et al.*, 1984) and rainbow trout (N. R. Liley, personal communication), plasma GtH levels were elevated in animals captured on the spawning beds. The lack of sexual stimulation normally available in the wild also may explain the poor milt yield in laboratory-held rainbow trout and pike (*Esox lucius*); only 5–20% of the spermatozoa present in the testes of mature males could be collected by hand-stripping, with the bulk of the spermatozoa remaining in the testes where they were reabsorbed (Billard *et al.*, 1982).

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