Hormonal Regulation of Parental Care Behavior in Nesting Male Bluegills: Do the Effects of Bromocriptine Suggest a Role for Prolactin?

Pawel M. Kindler[1,*]
Janice M. Bahr[1]
Mart R. Gross[2]
David P. Philipp[3,†]
[1]Department of Animal Sciences, Animal Genetics Laboratory, University of Illinois, Urbana, Illinois 61801; [2]Department of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 1A1; [3]Center for Aquatic Ecology, Illinois Natural History Survey, Champaign, Illinois 61820

Accepted 4/25/90

Abstract

*The hormonal mechanisms underlying parental care behavior in fish are poorly understood. This study investigates the effects of bromocriptine, a specific dopamine receptor agonist that inhibits secretion of prolactin from the pituitary, on parental care behaviors of nesting male bluegills. Parental male bluegills (*Lepomis macrochirus*) exhibit a complex set of reproductive behaviors including the construction of nests in colonies and courtship of females. After spawning, these males then alone remain to fan and guard the eggs and larvae from predators. Compared with control males, males implanted with bromocriptine showed significantly less defense of their broods and spent significantly more time rim circling their nests, a behavior typical of prespawning males. Because there were no significant differences among male groups in the levels of 11-ketotestosterone (11KT) or testosterone (T), the observed behavioral alterations cannot be ascribed to androgenic effects of bromocriptine. Instead, we suggest that prolactin, mediated through the action of dopamine, is involved in the expression of parental care behavior in this species.*

Introduction

Endocrinology of Fish Parental Care Behavior

Parental care is widespread among fishes (Gross and Sargent 1985). This behavior, typically shown by the male sex, includes nest construction, fan-

* Present address: Department of Zoology, University of British Columbia, Vancouver, B.C., Canada V6T 2A9.
† To whom reprint requests should be addressed.

Physiological Zoology 64(1):310–322. 1991. 0031-935X/91/6401-0901$02.00

ning the eggs to deliver O_2, and guarding the brood from predators. Although the ethology of male parental care has attracted considerable attention (e.g., Keenleyside 1979; Blumer 1982; Pitcher 1986), the endocrine mechanisms underlying this behavior are poorly understood. To study these mechanisms, we test whether bromocriptine, a highly specific dopamine receptor agonist shown to decrease serum prolactin levels (Vance, Evans, and Thorner 1984; Klonoff and Karam 1987), influences parental care behavior in nesting male bluegills (*Lepomis macrochirus*) in their natural habitat.

Several authors suggest that parental care behaviors in male fish are largely mediated by gonadal steroids (Liley and Stacey 1983; Matty 1985; Stacey 1987). However, supporting evidence is limited and comes from only two species, the threespine stickleback, *Gasterosteus aculeatus,* (Smith and Hoar 1967) and the blue gourami, *Trichogaster trichopterus* (Kramer 1972). We have recently demonstrated in the parental male bluegill that serum levels of the two predominant androgens, 11-ketotestosterone (11KT) and testosterone (T), are high during spawning but then rapidly decrease to low or undetectable levels throughout the parental care phase (Kindler et al. 1989). Thus, it is unlikely that either of these steroid hormones is necessary for inducing and/or maintaining parental care behavior in this species.

An alternative mediator of teleost parental behavior, however, may be prolactin. Fiedler (1962) demonstrated that in the wrasse, *Crenilabrus ocellatus,* intramuscular injection with mammalian prolactin induced fanning behavior. Subsequent research showed that prolactin also induced nest construction (Molenda and Fiedler 1971), fanning (Blum 1973), and mucus secretion (Blum and Fiedler 1965) in four other teleosts. Purified fish prolactin gave similar results in the one species tested (Blum 1973). As further evidence, prolactin-sensitive neurons were located in the brains of three species of teleosts that fan their eggs but not in five species that do not (Blum and Fiedler 1973).

Reproductive Biology of Bluegills

The bluegill is a species of teleost native to lakes in east-central North America (Scott and Crossman 1973). At our study site, Lake Opinicon, Ontario, Canada, males of ages 7–10 yr construct nests in colonies, court and spawn with females, and then alone remain in their nests to provide parental care (Gross 1982). Colony formation is usually initiated by an aggregation of parental males at a prospective site in the littoral zone, 0.5–3.5 m in depth. A complex series of social interactions involving aggressive displays and encounters is associated with establishing nest sites within the colony. Nests, saucer-shaped depressions in the substrate, are constructed by the parental males through repeated sweeping actions of their caudal fin. Gravid

females arrive at the colony in a school. On their arrival parental males initiate certain displays, such as rim circling, which involves rapid swimming in tight circles around the perimeter of the nest (Miller 1963; Avila 1976; Colgan et al. 1979). Females typically enter the nests individually and pair and spawn with the males. Each male may spawn with several females before the school leaves the colony area. The males then enter a parental care phase, fanning the eggs and guarding the eggs and larvae from predators until the fry leave the nest. Guarding the brood against predators, primarily nonnesting bluegills (Gross and MacMillan 1981), involves frontal display behavior in which opercular flaps are flared, chasing, and active biting (Colgan and Gross 1977). During this parental care phase males do not spawn again. Once the fry have left the nest the parental males also depart, which ends their parental care activities. This parental care phase lasts 5–13 d, depending on water temperature.

Experimental Approach

The purpose of this study was to investigate the possible involvement of prolactin in mediating the parental care behavior of nesting male bluegills. Our study relied on indirectly manipulating prolactin levels with bromocriptine mesylate via intramuscular implants. Bromocriptine, a semisynthetic ergot alkaloid, is widely regarded as a specific dopamine receptor agonist (Vance et al. 1984; Klonoff and Karam 1987). In turn, dopamine has been shown to be a prolactin inhibitory factor in many vertebrates (Seki and Kikuyama 1982; Ben-Jonathan 1985), including several species of teleosts (reviewed by Peter and Fryer [1983]; see also James and Wigham 1984; Johnston and Wigham 1988; Olivereau, Olivereau, and Lambert 1988). Furthermore, the specificity and apparent lack of side effects of bromocriptine have led to its widespread clinical use for the treatment of hyperprolactinemic conditions in humans (Vance et al. 1984). As a result, throughout this study we have assumed that behavioral changes observed in fish implanted with bromocriptine resulted from the suppression of prolactin secretion. Unfortunately, because of the unavailability of radioimmunoassay (RIA) procedures to measure bluegill prolactin, no direct proof can be offered that prolactin was indeed suppressed. However, it has been recently shown in our laboratories that implants identical to those used in this study can significantly decrease serum prolactin in the rat (Trawick 1989). In any case, an induction of significant behavioral alterations among nesting male bluegills by bromocriptine must be considered as evidence consistent with our proposed role for prolactin.

Material and Methods

Specimens and Treatment

Because the Lake Opinicon bluegill population has been under intensive study for over 12 yr, all components of the reproductive cycle and the location of the spawning grounds could be accurately predicted. This population usually spawns in 4–6 distinct breeding bouts from late May to mid-July, with most individual parental males apparently taking part in only one or two of these (J. E. Claussen, M. R. Gross, and D. P. Philipp, unpublished data). To allow for possible seasonal effects, our experiments were conducted both at the beginning of the spawning season, when males would still have new opportunities to spawn, and at the end of the spawning season, when future spawning opportunities during that year were unlikely.

Both experiments were conducted during the 1987 spawning season. For each experiment, divers observed colonies to determine the onset and duration of spawning. Directly following the cessation of spawning, all nests in a chosen colony were marked with small, numbered tiles, and parental males were randomly assigned to three groups: bromocriptine treatment, placebo treatment, or handling control. Each fish was then caught with a hand net by a diver and brought to a boat anchored near the colony. The fish was weighed and measured, and a small but visible clip was made in the soft dorsal or caudal fin to identify it visually with the treatment group. Each male in the handling-control group was immediately returned to the colony and released directly onto his respective nest. Each male assigned to either the bromocriptine or placebo-implant group, however, was first placed in a small plastic basin lined with clean, wet paper towels. A few scales (2–4) from the anterior dorsal area above the lateral line were removed and a 5-mm-long and 5-mm-deep incision was made with a scalpel. A small pellet (3 mm in diameter; Innovative Research of America, Toledo, Ohio) was inserted into the incision with forceps. Each placebo pellet was made of filler material, including cholesterol, microcrystalline cellulose, α-lactose, di- and tricalcium phosphate, calcium and magnesium stearate, and stearic acid (Osborne, Hobbs, and Clark 1985). Each bromocriptine pellet contained these materials, as well as 5.0 mg of bromocriptine mesylate. To keep the pellet in place, we applied 1–2 drops of Super Glue over the incision. Minimal bleeding resulted from this procedure. Each fish was then immediately returned to the colony and released directly onto its nest. Maximum elapsed time for this procedure (from fish capture to its release) was 2 min. All control and implanted fish remained on their nests and immediately resumed fanning their eggs. All fish survived the experiment, and no fungal or bacterial infections caused by the handling or surgical procedures were detected.

Behavioral Assessments

After implantations, behavioral activities of each male were assessed daily during the remainder of the parental care phase (postspawning days 1–5 and 1–4 for the early-season and late-season experiments, respectively). To determine the relative amount of time individual males allocated to various behavioral activities, we observed each fish for two 2-min sessions during morning hours. The amount of time each individual spent fanning/hovering over the nest, rim circling the nest, and chasing predators was recorded. Intrusions by natural predators were rare during the 4 min of observation. Therefore, to assess individual male aggressiveness, after the completion of behavioral observations, we presented each fish at the nest rim with a lifelike model of a male bluegill. This model, a photograph mounted on Plexiglas and coated with resin, simulates a common-brood predator and readily elicits antipredator aggression from nesting male bluegills (see Coleman, Gross, and Sargent [1985] for details of the presentation technique). Each fish was first presented with the model for a 30-s session, and the order of individual sessions was random. When all fish were tested, a second series of 30-s sessions was conducted. The order of this second series of sessions was identical to that used in the first series, which assured that the two presentations of the model to the same individual were always about 20 min apart. Antipredator aggression was calculated as the total number of bites and opercular flares directed to the model during two 30-s presentations. All presentations were always conducted by the same person (P.M.K.).

Steroid Measurements

In the evening of the last testing day (day 5 and day 4 for the early-season and late-season experiments, respectively), each fish was captured by hand net, weighed, and immediately bled by heart puncture. Blood samples were held overnight at 4°C to allow clotting. Serum was then obtained from each sample by centrifugation at 3,000 *g* for 10 min and frozen at −20°C until assayed. The RIA procedures used in this study to measure 11KT and T concentrations were identical to those reported by Kindler et al. (1989).

Statistical Analysis

Analyses were performed with the Statistical Analysis System (SAS 1985). Repeated-measures and one-way ANOVAs, as well as linear regression procedures, were used to analyze the data, and the statistical rejection level for similarity was $P < 0.05$.

Results

In both the early-season and late-season experiments, males treated with bromocriptine showed less total aggression to the model predator than placebo-implant or handling-control individuals (fig. 1). The differences were statistically significant as early as the second day after implantation and continued throughout the remainder of the parental care phase (days 2–5 and days 2–4; repeated-measures ANOVAs followed by Student-Newman-Kuels [SNK] multiple-range tests, $P < 0.0001$ and $P < 0.05$, respectively). Placebo-implant and handling-control males did not differ in antipredator aggression ($P > 0.05$). Finally, there was no significant difference in the allocation of aggression between bites and opercular flares among the three treatment groups (percentage bites vs. total aggression [bites and flares] for each individual was square root transformed and followed by repeated-measures ANOVAs, $P > 0.05$ and $P > 0.1$ for early-season and late-season experiments, respectively).

Furthermore, compared with placebo-implant and handling-control fish, males treated with bromocriptine spent more time rim circling (fig. 2). The difference was statistically significant less than 24 h after the implantations (days 1–5 and days 1–4; repeated-measures ANOVAs followed by SNK multiple range tests, $P < 0.0001$ and $P < 0.001$, respectively). There was no significant difference in the amount of rim circling exhibited by placebo-implant and handling-control males ($P > 0.05$).

A comparison of the data presented in figures 1 and 2 reveals a significant negative correlation between the time spent rim circling and the level of antipredator aggression among the males treated with bromocriptine for both early-season and late-season experiments ($r = 0.899$, $n = 5$, $P < 0.05$; and $r = 0.931$, $n = 5$, $P < 0.05$, respectively). In contrast, there was no significant correlation between rim circling and aggression among placebo-implant and handling-control fish in either early or late experiments ($P > 0.05$).

Finally, treatment with bromocriptine did not affect serum levels of circulating androgens. In both early-season and late-season experiments the levels of 11KT and T in fish treated with bromocriptine were not statistically different from the levels of those steroids measured in fish from either the placebo-implant or clinical control groups (table 1; one-way ANOVAs, $P > 0.05$ for all four determinations).

Discussion

Bromocriptine implants clearly have a profound effect on the behavior of nesting parental male bluegills. First, implantation with bromocriptine sig-

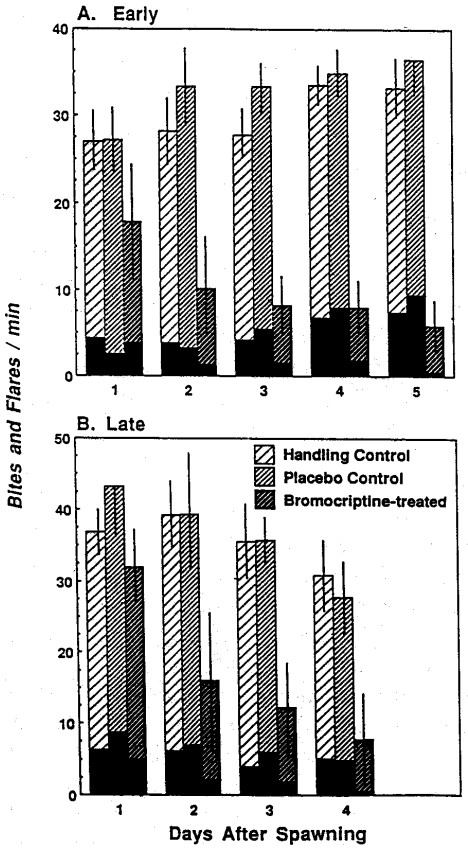

Fig. 1. Aggressive behavior directed to a model brood predator by nesting male bluegills (A) at the beginning of the reproductive season, June 7–11, 1987; and (B) at the end of the reproductive season, July 9–12, 1987. Sample sizes in the early season experiment are n = *9 for the handling-control group,* n = *7 for the placebo-implant group, and* n = *5 for the bromocriptine-treated group. In the late season experiment* n = *5 for all three treatment groups. The black portion of each bar represents the number of bites; the stippled portion of each bar represents the number of opercular flares.*

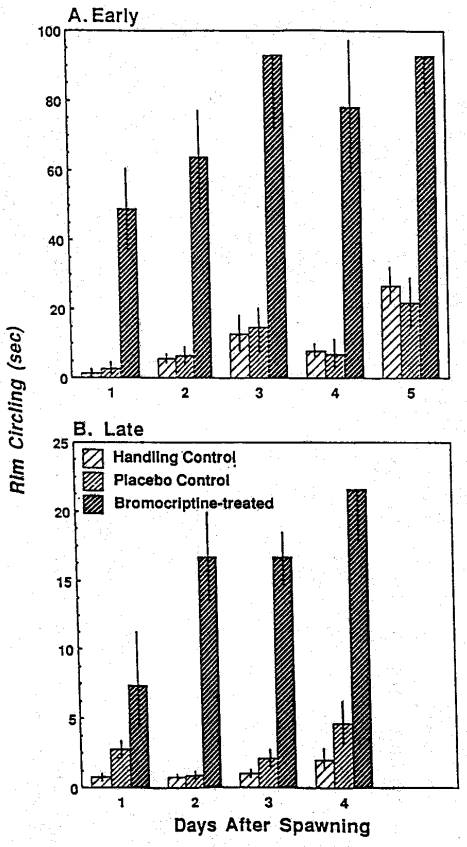

Fig. 2. Rim-circling behavior. These fish were the same individuals as those tested in fig. 1.

nificantly decreases aggression against model predators. Because antipredator brood defense is necessary for survival of offspring (Gross and MacMillan 1981; Bain and Helfrich 1983), this decreased investment in nest defense presumably would decrease their reproductive success.

Second, implantation with bromocriptine significantly increases the amount of time spent rim circling, a behavior characteristic of the prespawning period, and concomitantly decreases the amount of time available for fanning eggs. It is interesting that the developmental success of the offspring was also affected. By day 2, 80% of the early and 100% of the late season's nests of the bromocriptine-treated males were heavily infected with fungus

TABLE 1

Serum 11-ketotestosterone (11KT) and testosterone (T) in parental male bluegills

	Early Experiment			Late Experiment		
Steroid	Handling-Control Males (n = 9)	Placebo-Control Males (n = 7)	Bromocriptine-treated Males (n = 5)	Handling-Control Males (n = 5)	Placebo-Control Males (n = 5)	Bromocriptine-treated Males (n = 5)
11KT . . .	5.3 ± .8	5.6 ± 1.0	4.5 ± 1.0	2.6 ± 1.3	1.6 ± .5	2.0 ± .9
T	2.8 ± .5	2.1 ± .6	1.3 ± 1.0	2.6 ± 1.0	2.0 ± .2	2.0 ± .5

Note. All males were collected on the last testing day of the parental care phase of the early-season and late-season experiments (day 5 and day 4, respectively). Concentrations are expressed in ng/mL ± standard error of the mean.

(*Saprolegnia* sp.). In contrast, 0% of the early and only 20% of the late season's nests of the placebo-implant males, and 11% and 20%, respectively, of the early season's and late season's nests of the handling-control males had obvious fungus. A decreased investment in fanning is the most likely cause for the deterioration in rearing conditions that caused widespread fungal infections and again represents a significant potential reduction in reproductive success.

On the other hand, it has been shown in several species of teleosts that the size of brood influences levels of parental care behavior (Kramer 1973; Pressley 1981; Carlisle 1985; Coleman et al. 1985). For example, in the bluegill, as shown by Coleman et al. (1985), a substantial reduction of the brood size can alone cause a decrease in antipredator aggression by up to 50%. The use of bromocriptine in this study resulted in the decline of these behaviors among treated fish by 83% and 58% when determined on the last day of the early and late experiments, respectively. Therefore, it is likely that the significant decrease in the willingness to defend offspring observed in nesting males implanted with bromocriptine was caused not only by the direct action of the drug. Instead, it may have also resulted, at least to some extent, from a response to the reduction in brood size induced by the increased incidence of fungal infections.

We have previously shown that serum androgen levels among parental males decrease significantly during the parental care phase (Kindler et al. 1989). The results of this study suggest that the changes in behavior induced by bromocriptine are not related to androgen levels. Thus, it appears that androgens are not responsible for the induction and/or maintenance of parental care behavior in male bluegills.

We therefore suggest that an inhibition of prolactin secretion through bromocriptine-induced agonism of the dopamine receptor is responsible for mediating the alteration in parental care behaviors observed in our experiments. We realize that the actions of dopamine in teleosts are not limited to the inhibition of prolactin synthesis. Dopamine may also inhibit the release of gonadotropin (reviewed by Peter et al. [1986]; see also Omeljaniuk, Shih, and Peter 1987; Omeljaniuk, Habibi, and Peter 1989*a*; Omeljaniuk, Tonon, and Peter 1989*b*) and α-melanocyte-stimulating hormone (Olivereau 1978; Olivereau, Olivereau, and Lambert 1987; Omeljaniuk et al. 1989*b*), while stimulating the release of growth hormone from the pituitary (Chang et al. 1985). However, there is no evidence to suggest that one or more of these three hormones is involved in the expression of parental care behavior in fish. The early laboratory experiments by Fiedler (e.g., 1962) and Blum (e.g., 1973) that directly involved the use of prolactin, combined with our indirect but field-conducted experiments, suggest that prolactin may be an

important parental care hormone in fish just as it is in birds (Follet 1984) and mammals (Austin and Short 1984).

Acknowledgments

We thank the management of the Queen's University Biological Station for providing field and laboratory facilities at Lake Opinicon and P. Austin for her invaluable field assistance. Research was supported by funds from the Illinois Natural History Survey to D.P.P., and a Natural Sciences and Engineering Research Council of Canada operating grant to M.R.G.

Literature Cited

AUSTIN, C. R., and R. V. SHORT. 1984. Reproduction in mammals: hormonal control of reproduction. Cambridge University Press, Cambridge.

AVILA, V. L. 1976. A field study of nesting behavior of male bluegill sunfish (*Lepomis macrochirus* Rafinesque). Am. Midland Nat. 96:195–206.

BAIN, M. B., and L. A. HELFRICH. 1983. Role of male parental care in survival of larval bluegills. Trans. Am. Fisheries Soc. 112:47–52.

BEN-JONATHAN, N. 1985. Dopamine—a prolactin-inhibiting hormone. Endocr. Rev. 6:564–589.

BLUM, V. 1973. Die Rolle des Prolactins der Cichlidenbrutpflege. Fortschr. Zool. 22: 310–333.

BLUM, V., and K. FIEDLER. 1965. Hormonal control of reproductive behavior in some cichlid fish. Gen. Comp. Endocrin. 5:186–196.

———. 1973. Prolactinempfindliche Strukturen im Fischgehirn. Fortschr. Zool. 22: 155–166.

BLUMER, L. S. 1982. A bibliography and categorization of bony fishes exhibiting parental care. Zool. J. Linnaean Soc. 76:1–22.

CARLISLE, T. R. 1985. Parental response to brood size in a cichlid fish. Anim. Behav. 33:234–238.

CHANG, J. P., T. A. MARCHANT, A. F. COOK, C. S. NAHORNIAK, and R. E. PETER. 1985. Influences of catecholamines on growth hormone release in female goldfish, *Carassius auratus*. Neuroendocrinology 40:463–470.

COLEMAN, R. M., M. R. GROSS, and R. C. SARGENT. 1985. Parental investment decision rules: a test in bluegill sunfish. Behav. Ecol. Sociobiol. 18:59–66.

COLGAN, P. W., and M. R. GROSS. 1977. Dynamics of aggression in male pumpkinseed sunfish (*Lepomis gibbosus*) over the reproductive phase. Z. Tierpsychol. 43:139–151.

COLGAN, P. W., W. A. NOWELL, M. R. GROSS, and J. W. A. GRANT. 1979. Aggressive habituation and rim-circling in the social organization of bluegill sunfish (*Lepomis macrochirus*). Environ. Biol. Fishes 4:29–36.

FIEDLER, K. 1962. Die Wirkung von Prolactin auf das Verhalten des Lippfisches *Crenilabrus ocellatus* (Forskal). Zool. Jahrb. Abteilung Anat. Zool. Physiol. Tiere 69: 609–620.

FOLLET, B. K. 1984. Birds. Pages 283–350 in G. E. LAMMING, ed. Marshal's physiology of reproduction. Churchill Livingstone, London.

GROSS, M. R. 1982. Sneakers, satellites, and parentals: Polymorphic mating strategies in North American sunfish. Z. Tierpsychol. 60:1–26.

GROSS, M. R., and A. M. MACMILLAN. 1981. Predation and the evolution of colonial nesting in bluegill sunfish (*Lepomis macrochirus*). Behav. Ecol. Sociobiol. 8:163–174.

GROSS, M. R., and R. C. SARGENT. 1985. The evolution of male and female parental care in fishes. Am. Zool. 25:807–822.

JAMES, V. A., and T. WIGHAM. 1984. Evidence for dopaminergic and serotonergic regulation of prolactin cell activity in the trout *Salmo gairdneri*. Gen. Comp. Endocrin. 56:231–239.

JOHNSTON, L. R., and T. WIGHAM. 1988. The intercellular regulation of prolactin cell function in the rainbow trout, *Salmo gairdneri*. Gen. Comp. Endocrin. 71:284–291.

KEENLEYSIDE, M. H. A. 1979. Diversity and adaptation in fish behaviour. Springer, Berlin.

KINDLER, P. M., D. P. PHILIPP, M. R. GROSS, and J. M. BAHR. 1989. Serum 11-ketotestosterone and testosterone concentrations associated with reproduction in male bluegill (*Lepomis macrochirus*: Centrarchidae). Gen. Comp. Endocrin. 75:446–453.

KLONOFF, D. C., and J. H. KARAM. 1987. Hypothalamic and pituitary hormones. Pages 423–435 in B. G. KATZUNG, ed. Basic and clinical pharmacology. Appleton & Lange, Norwalk, Conn.

KRAMER, D. L. 1972. The role of androgens in the parental behavior of the blue gourami, *Trichogaster trichopterus* (Pisces, Belontiidae). Anim. Behav. 20:798–807.

———. 1973. Parental behavior in the blue gourami *Trichogaster trichopterus* (Pisces, Belontiidae) and its induction during exposure to varying numbers of conspecific eggs. Behaviour 47:14–32.

LILEY, N. R., and N. E. STACEY. 1983. Hormones, pheromones, and reproductive behavior in fish. Pages 1–49 in D. HOAR, J. RANDALL, and E. M. DONALDSON, eds. Fish physiology. Vol. 9. Reproduction. Pt. B. Behavior and fertility control. Academic Press, New York.

MATTY, A. J. 1985. Fish endocrinology. Croom Helm, London.

MILLER, H. C. 1963. The behavior of the pumpkinseed sunfish, *Lepomis gibbosus* (Linnaeus), with notes on the behavior of other species of *Lepomis* and the pigmy sunfish, *Elasomma evergladei*. Behaviour 22:88–151.

MOLENDA, W., and K. FIEDLER. 1971. Die Wirkung von Prolactin auf das Verhalten von Stichlings ♂♂ (*Gasterosteus aculeatus* L.). Z. Tierpsychol. 28:463–474.

OLIVEREAU, M. 1978. Effect of pimozide on the cytology of the eel pituitary. I. Prolactin secreting cells. Cell Tissue Res. 189:219–230.

OLIVEREAU, M., J. M. OLIVEREAU, and J. F. LAMBERT. 1987. In vivo effect of dopamine antagonists on melanocyte-stimulating hormone cells of the goldfish (*Carassius auratus* L.) pituitary. Gen. Comp. Endocrin. 68:12–18.

———. 1988. Cytological responses of the pituitary (rostral pars distalis) and immunoreactive corticotropin-releasing factor (CRF) in the goldfish treated with dopamine antagonists. Gen. Comp. Endocrin. 71:506–515.

OMELJANIUK, R. J., H. R. HABIBI, and R. E. PETER. 1989*a*. Alterations in pituitary GnRH and dopamine receptors associated with the seasonal variation and regulation of gonadotropin release in the goldfish (*Carassius auratus*). Gen. Comp. Endocrinol. 74:392–399.

OMELJANIUK, R. J., S. H. SHIH, and R. E. PETER. 1987. In vivo evaluation of dopamine receptor-mediated inhibition gonadotropin secretion from the pituitary gland of the goldfish. J. Endocrinol. 114:449–458.

OMELJANIUK, R. J., M. C. TONON, and R. E. PETER. 1989*b*. Dopamine inhibition of gonadotropin and α-melanocyte-stimulating hormone release in vitro from the pituitary of the goldfish (*Carassius auratus*). Gen. Comp. Endocrinol. 74:451–467.

OSBORNE, C. K., K. HOBBS, and G. M. CLARK. 1985. Effect of estrogens and antiestrogens on growth of human breast cancer cells in athymic nude mice. Cancer Res. 45: 584–590.

PETER, R. E., J. P. CHANG, C. S. NAHORNIAK, R. J. OMELJANIUK, S. H. SHIH, M. SOKOLOWSKA, and R. BILLARD. 1986. Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. Recent Prog. Horm. Res. 42:513–545.

PETER, R. E., and J. N. FRYER. 1983. Endocrine functions of the hypothalamus of actinopterygians: Pages 165–201 in R. E. DAVIS and R. G. NORTHCUTT, eds. Fish neurobiology. Vol. 2. Higher brain areas and functions. University of Michigan Press, Ann Arbor.

PITCHER, T. J. 1986. The behavior of teleost fishes. Croom Helm, London. 553 pp.

PRESSLEY, P. H. 1981. Parental effort and the evolution of nest-guarding tactics in the threespine stickleback, *Gasterosteus aculeatus* L. Evolution 35:282–295.

SAS INSTITUTE. 1985. SAS users guide: statistics. 5th ed. SAS Institute, Cary, N.C.

SCOTT, W. B., and E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Fisheries Res. Board Can. Bull. 184:1–966.

SEKI, T., and S. KIKUYAMA. 1982. In vitro studies on the regulation of prolactin secretion in the bullfrog pituitary gland. Gen. Comp. Endocrinol. 46:473–479.

SMITH, R. J. F., and W. S. HOAR. 1967. The effects of prolactin and testosterone on parental behaviour of the male stickleback *Gasterosteus aculeatus*. Anim. Behav. 15:342–352.

STACEY, N. E. 1987. Roles of hormones and pheromones in fish reproductive behavior. Pages 28–60 in D. CREWS, ed. Psychobiology of reproductive behavior. Prentice-Hall, Englewood Cliffs, N.J.

TRAWICK, D. R. 1989. Modulation of humoral activity in the rat by 17β-estradiol and prolactin. Ph.D. diss. University of Illinois.

VANCE, M. L., W. S. EVANS, and M. O. THORNER. 1984. Bromocriptine. Ann. Intern. Med. 100:78–91.