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PHEROMONAL ROLE OF PROSTAGLANDINS ON REPRODUCTION OF GOLDFISH, *CARASSIUS AURATUS*

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Non-induced male goldfish, *Carassius auratus* were exposed to non-ovulated females injected with prostaglandin analogues viz. Prosolvin, Iliren and Lutylase at 1 μg/gm body weight. The volume of milt that could be stripped from male goldfish increased significantly (P<0.05) when fish were exposed to Prosolvin and Iliren treated females for 12 h. Increase in the milt volume was also significant (P<0.05) when males were directly exposed for 12 h to Prosolvin and Iliren in water at concentration of 10 μg/l. Lutylase however, failed to increase the milt volume.

INTRODUCTION

Numerous reports are available on the sex pheromones among insects (Mustaparta and Almaas, 1989). In mammals also sex odours are important. Some of these odours induce an immediate behavioural response via neural pathway (releaser effect) or have physiological effects through neural-neuroendocrine pathway (primer effect) by enhancing plasma concentrations of sex hormones followed by sexual maturity and ovulation (Wilson, 1975; Albone, 1984). However, very little is known about sex odours and their effects in fish. For example, steroid dihydroxyprogesterone (DHP) produced by female goldfish controls not only the onset of ovarian maturation in females but also spermiation in males. Therefore, if added to water, this steroid could be used to control the timing or amount of sperm production. Indeed, preliminary tests have confirmed the feasibility of this procedure to increase male fertility in common carp and goldfish (Zheng *et al*., 1995). A major drawback of multiple hormone injections is that some species are unable to cope up with the stress associated with repeated handling. Handling stress can cause gonadal regression and in some cases even death of the broodstock. One way to avoid excessive handling of the broodstock is to apply non-invasive techniques like using bolus-feeding strategies and also adding reproductive pheromones directly into the water.

It has long been recognized that many animals release specialized chemicals to the environment where they have specific behavioural and/or physiological effects on the

conspecifics. Kittredge *et al*. (1971) were the first to propose that aquatic organisms might commonly use hormones as pheromones. By early 1980s, behavioural studies confirmed that ovulation and pheromone release are closely associated in several species of oviparous fish (Emanuel and Dodson, 1979; Lee and Ingersoll, 1979; Honda, 1980, 1982; Sorensen and Winn, 1984). There is evidence that teleost fish use hormones and their metabolites as reproductive pheromones (Stacey *et al*., 1987).

An intriguing fish pheromone is prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$), which circulates in the blood stream of female goldfish and is associated with follicular rupture and ovulation. When it is released into water together with a metabolite 15-keto-$PGF_{2\alpha}$, it induces increased (but short lived) aggression amongst males and courtship behaviour. Levels of the circulating F-prostaglandins (PGF) increase in goldfish and several other fish at the time of ovulation (Bouffard, 1979; Cetta and Goetz, 1982), presumably reflecting a role modulating follicular rupture (Goetz, 1983). Circulating PGF appears to function as a hormonal signal triggering spawning behaviour through direct action on the brain of goldfish (Stacey and Peter, 1979; Stacey and Goetz, 1982).

In comparison to female teleosts, very little is known regarding involvement of prostaglandins in milt production/spermiation in males. Christ and van Dorp (1972) first identified PGE_1 and PGB_1 in the milt of *Cyprinus carpio*. Nomura *et al*. (1973) recorded PGE_2 in testes of flounder, *Paralichthys olivaceus*; PGE_2 and $PGF_{2\alpha}$ in the testes of bluefin tuna, *Thunnus thynnus* and PGE_1 in the semen of chum salmon, *Oncorhynchus keta*. Nomura and Ogata (1976) later identified PGE_3 in the testes of carp, *Cyprinus carpio* and leopard shark, *Triakis scyllia*. Bouffard (1979) with the help of radioimmunoassay measured PGB_1, PGE_1 and $PGF_{2\alpha}$ in the blood and testes of goldfish. Though direct evidence that PGs help in milt production is not well understood but PGs help indirectly in production of milt in certain teleosts, which is associated with an increase in the level of blood GtH. However, natural prostaglandins have not been commercially used in the field of induced breeding due to their high cost, unstable nature and short shelf-life. In the present study, three synthetic but more stable analogues of $PGF_{2\alpha}$, currently used in veterinary practice, were used to evaluate the pheromonal role of prostaglandin analogues on goldfish reproduction.

MATERIALS AND METHODS

Mature goldfish were collected from broodstock pond and, maintained on artificial fish pellets and live tubifex worms/ mosquito larvae at a water temperature of 25±1 °C. Commercially available prostaglandin analogues used in the present study were: Iliren (contents-Tiaprost-$PGF_{2\alpha}$ analogue, 0.15 mg/ml) (Hoechst Roussel Vet GmbH d-65203 Wiesbaden), Prosolvin (contents-Luprostiol-$PGF_{2\alpha}$ analogue, 7.5 mg/ml) (Intervet,

International B.V. Boxmeer-Holland) and Lutylase (contents-Dinoprost-$PGF_{2\alpha}$ analogue, 5 mg/ml) (Upjhon S.a. Puurs, Belgium).

From the common stock, mature *C. auratus* breeding pairs were selected. Females with reddish swollen vent and bulky abdomen and, active males with presence of white tubercles on the surface of the operculum and secretion of milt were used as the criteria. Selected pairs (20-50 g) were kept overnight in separate spawning aquarium (36"x12"x15") in 50 l dechlorinated water and, checked for ovulation and spawning. Non-ovulated females were used for the present experiments. Two sets of experiments were carried out to study prostaglandin-mediated effects on the milt volume of goldfish.

The first experiment was conducted to study the effects of the three prostaglandin analogues (Prosolvin, Iliren and Lutylase) on the milt volume of male goldfish when they were exposed to prostaglandin-treated females. Treated and control groups contained five pairs each. The females of the experimental groups were given prostaglandin (intramuscular) at a dose rate of 1 μg/g body weight and those of control groups were given an equal volume of fish saline (0.6% NaCl). Males were then exposed to prostaglandin-treated females (1M:1F). The experiment lasted for 12 h. None of the males in the control as well as experimental groups was administered with prostaglandins.

The second experiment was conducted to determine whether direct exposure of male fish to prostaglandins in water would increase the milt volume. Treated and control groups contained five males each. They were placed in 5 l of water containing the three prostaglandin analogues (Prosolvin, Iliren and Lutylase) separately at a concentration of 10 μg/l. The experiment was completed within 12 h. None of the males in the control as well as experimental groups was administered with prostaglandins.

Males were placed belly up on a moist pad and milt was taken out by stripping as described by Kyle *et al*. (1985). Initial pre-treatment stripping was done and males were placed randomly in 50 l of water (separate tanks) and maintained at 25±1 °C as the stock. On the day of experiment males were either exposed to prostaglandin-treated female or to water in which prostaglandin was added directly. Control experiments were run simultaneously. After 12 h of exposure to the treatment, fish were removed from the test aquarium and milt was stripped. Milt was collected in sterilized, pre-weighed glass capillary tubes. Glass capillary tubes were re-weighed and weight of milt was calculated. Milt density as 1 g/ml was used for calculation of milt volume. Data was analyzed by Analysis of Variance (ANOVA) test followed by Tukey's test to determine significant differences ($P<0.05$) between the groups means.

RESULTS AND DISCUSSION

Behavioural changes were observed in males exposed to non-ovulated females injected with prostaglandin analogues. The changes in males were characterized by vigorous chasing of females, pairing and constant nudging of ovipore and side as well. During the series of spawning acts observed, females approached the surface of water in head up position and male followed, both turned on their sides, they broke the water surface and males constantly chased the females. Females gave repeated jerks (spawning acts) however, eggs were not released during these spawning acts. Ovulation and spawning did not take place within 12 h of injection.

There was no pre-treatment difference in milt volume between control and experimental groups. In experiment 1, the males of the experimental groups exposed to prostaglandin analogues such as Prosolvin and Iliren, produced 52.00±10.85 and 45.60±9.41 µl milt respectively, which was significantly higher than the control. Males exposed to Lutylase-treated females however, showed a significant reduction in milt volume (Table 1, Experiment 1). In experiment 2, the non-induced males of the experimental groups when exposed to Prosolvin and Iliren which were added directly into water (10 g/l) produced a significantly higher milt volume of 40.00±10.11 and 16.50±6.61 respectively over control (10.20 ± 2.46). However, in Lutylase treated group there was no significant difference in milt volume to that of control. (Table 1, Experiment 2). Prosolvin and Iliren are veterinary luteolytic prostaglandins, which are 200 times more luteolytic than natural $PGF_{2\alpha}$ (Dukes *et al*., 1974). Results of the present study indicate that Prosolvin and Iliren were very effective in inducing milt volume in male goldfish when exposed to prostaglandin-injected non-ovulated females and also when prostaglandins were directly added into the water. The maximal response was obtained with Prosolvin. Possibly, the different form of prostaglandin analogue in Lutylase failed to increase the milt volume in exposed males.

Table 1. Effect of prostaglandin analogues on milt volume of goldfish, *Carassius auratus*. Experiment 1: Males were exposed to prostaglandin analogue-treated females (1 µg/g body weight). Experiment 2: Males were placed in 5 l of water containing prostaglandin analogues (10 µg/l). Data are presented as Mean ± SE. Means bearing different superscript(s) row-wise are significantly different (P<0.05).

Experiment	Milt volume (µl) after 12 h exposure			
	Control	Prosolvin	Iliren	Lutylase
1	10.20 ± 2.46^{A}	52.00 ± 10.85^{B}	45.60 ± 9.41^{C}	4.20 ± 1.43^{D}
2	10.20 ± 2.46^{A}	40.00 ± 10.11^{C}	16.50 ± 6.61^{B}	12.00 ± 20.00^{AB}

Male fertility during spawning has been positively correlated with milt volume as the depletion in sperm has been suggested to cause decreased fertility (as measured by the proportion of eggs fertilized) in spawning lemon tetra, *Hyphessobrycon pulchripinnis* (Nakatsuru and Kramer, 1982). Because goldfish spawn in large groups in which many males compete for access to ovulated females, it seems likely that the volume of milt that a male release might be an important determinant of its reproductive success. Present study demonstrated that water-borne prostaglandins are effective in increasing the volume of milt that can be stripped from male goldfish. These findings suggest that luteolytic prostaglandin analogues, particularly Prosolvin and Iliren function as reproductive primer pheromones eliciting increased milt volume in male goldfish.

Our results of increase in milt volume when males were exposed to prostaglandin injected non-ovulated females are in agreement with earlier reports of Sorensen *et al*. (1986, 1995). Prostaglandin injection to non-ovulated female goldfish releases an odour that elicits male reproductive behaviour identical to those elicited by odour of ovulated female (Sorensen *et al*., 1986). The tendency of male goldfish to contact the female genital areas (nudging) with snout might expose males to high concentration of such stimulants, which resulted in the significant increase in milt volume in male goldfish. Sorensen *et al*. (1986, 1995) have reported that circulating prostaglandins are cleared to the water to function as pheromone thereby synchronizing male and female sexual behaviour in goldfish. As nothing is known about how goldfish would release injected prostaglandin analogues into water, it is not clear whether natural release of prostaglandin analogues themselves or their metabolites during preovulatory period could result in water concentration sufficient to increase the milt volume in present study. This appears not the direct action of prostaglandins but prostaglandins-mediated pheromonal action. A pheromone-mediated increase in milt volume could affect reproductive success if the increase occurs prior to ovulation and spawning. The observed latency period of 12 h for the prostaglandin-induced milt response suggests primer effect of the pheromone. Intense sex behavioural responses in male goldfish and induced milt volume when exposed to prostaglandin-treated females emphasizes the pheromonal and olfactory potency of circulating prostaglandins/metabolites.

Present results emphasize that injection of prostaglandin analogues to non-ovulated female goldfish not only elicited the spawning acts in females but also induced increase in milt volume in male goldfish. Our findings are in agreement with the findings of Stacey (1976), Stacey and Goetz (1982) and Sorensen *et al*. (1988) related to prostaglandin induced behavioural response in non-ovulated female goldfish. In male goldfish without PG treatment, the blood GtH and milt volume increased due to the presence of gravid female (Kyle *et al*., 1985). Female goldfish, *C. auratus* releases mixture of F prostaglandins (PGFs) in aquarium and this water-borne PGF causes an increase in gonadotropin (GtH) and milt (sperm and seminal fluid) levels in spawning males

(Sorensen *et al*., 1988). In the present study, it was found that prostaglandins in water at a concentration of only 10 μg/l were effective in increasing milt volume. Also, although the threshold water concentration of prostaglandins required to induce the milt response has not been determined, preliminary results indicate that it is less than 10 μg/l.

The clear effects of prostaglandins as a pheromone on goldfish in increasing milt volume warrants field trials under commercial hatchery conditions to determine whether pheromonal milt enhancement can offer practical alternatives to existing invasive techniques. Prostaglandin treatment would reduce labour cost and handling stress by eliminating injections and reduce risk to broodstock.

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