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[Technical Note]

Preliminary Observations of Topical Gill Application of Reproductive Hormones for Induced Spawning of a Tropical Ornamental Fish

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Abstract.—Hormone injection is a common spawning induction technique in aquaculture but is of limited use in small species with low fecundity. An alternative to injection for such species is topical gill application. We conducted an experiment with female rainbow sharks *Epalzeorhynchos erythrurus* (Cyprinidae) in one control group and three treatment groups: (1) topical gill application of dimethyl sulfoxide (DMSO), (2) topical gill application of DMSO and Ovaprim, and (3) an Ovaprim intramuscular (IM) injection. Ovaprim contains a salmonid gonadotropin-releasing hormone analog and a dopamine antagonist. Fish were anesthetized, treated, and placed into recovery tanks. Fish were manually checked for ovulation by exerting abdominal pressure at 8, 10, and 11 h posttreatment and were observed for mortality during a 24-h period. Ovulation varied significantly: we found no ovulation of females in the control or DMSO groups, 78% ovulation in the DMSO plus Ovaprim group, and 100% ovulation in the Ovaprim IM injection group. Only two mortalities occurred in 24 h, both in the injected group. Use of DMSO as a solvent for topical gill application of hormones shows some promise as a spawning induction technique for tropical ornamental fishes.

Hormone injections are used to induce spawning in numerous fish species in aquaculture (Zohar 1989; Mylonas and Zohar 2001). For example, rainbow sharks *Epalzeorhynchos erythrurus* and redtail black sharks *E. bicolor* (freshwater sharks: Cyprinidae) are commonly induced to spawn by use of hormone injections and may be considered as model species for modifying spawning induction techniques (Shireman and Gildea 1989). Broodstock freshwater sharks are relatively large (about 100–120 mm standard length) and fecund (about 10,000 eggs; Shireman and Gildea 1989) relative to other tropical ornamental species, and as such, are ideal candidates for studies of spawning induction. However, injection techniques can be difficult or prohibitively expensive for use in small species of relatively low fecundity, such as many other tropical ornamental fish. For such species, a topical application of spawning hormones might result in less handling stress to the fish and in reduced labor costs for the producer.

An obvious route for topical application of spawning hormones is through the gills. Sherwood and Harvey (1986) reported absorption of mammalian gonadotropin-releasing hormone (mGnRH; luteinizing hormone-releasing hormone) across the gills of goldfish *Carassius auratus* when water or dimethyl sulfoxide (DMSO) was used as the solvent for the mGnRH. In the present study, we report on the gill application of Ovaprim dissolved in DMSO as a potential spawning technique for tropical ornamental species, and we use the rainbow shark as a model species.

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Ovaprim (Syndel Laboratories, Ltd., Vancouver, British Columbia) is a mixture of salmon gonadotropin-releasing hormone analog (sGnRHa [D-Arg^6-Pro^9-NEt sGnRH]) with the dopamine antagonist, domperidone. It is commonly used in the production of freshwater sharks in Florida and many other commercial cyprinids worldwide (Nandeesha et al. 1990; Mohd-Zaini et al. 1994; authors, personal observation). We used DMSO rather than water as a solvent because DMSO is capable of carrying relatively large molecules, such as sGnRHa and domperidone, across membranes (Sherwood and Harvey 1986).

Our objectives were (1) to determine whether Ovaprim induces ovulation in rainbow sharks when dissolved in DMSO and applied to the gills and (2) to determine whether DMSO causes mortality in rainbow sharks. To our knowledge, this is the first report of the use of DMSO as an aid for the gill application of reproductive hormones for the induced spawning of fish.

Methods

This experiment was conducted at the Tropical Aquaculture Laboratory, University of Florida, Ruskin. Broodstock rainbow sharks were obtained from a local breeder. Males (N = 11) and females (N = 39) were placed into separate 750-L concrete tanks and held for 1 week prior to spawning. A representative sample of nine females had a mean weight of 44.4 g (SE = 1.5 g).

Females were randomly assigned to one of four treatments: (1) control (N = 9), (2) DMSO gill application (N = 9), (3) DMSO and Ovaprim gill application (N = 9), and (4) Ovaprim intramuscular (IM) injection (N = 12). All fish were anesthetized in 6 L of aerated water containing a 7-mg/L concentration of metomidate hydrochloride (Aquacalm; Syndel Laboratories, Ltd.). Fish were allowed to remain in the anesthetic for about 5 min. In treatments 2 and 3, fish were removed from the anesthetic, and the treatment solutions (30 μL of DMSO or 30 μL of DMSO plus 20 μL of Ovaprim = 50 μL total) were injected into the mouth with a microsyringe. The opercula were held closed during the application. Afterwards, each fish was placed on a metal tray and covered with a damp paper towel for 4 min. After treatment, fish were placed according to group into one of four 110-L recovery tanks in a recirculating system (i.e., a tank for each treatment and the control). The control fish were anesthetized, placed onto the tray for 4 min, and moved into a recovery tank in the system, but received no gill application. The remaining treatment consisted of an IM injection with 0.5 μL Ovaprim per gram of fish body weight into the dorsal musculature of anesthetized fish. All males also received an Ovaprim IM injection to induce spermiation.

TABLE 1.—Percent ovulation of female rainbow sharks that received gill applications of dimethyl sulfoxide (DMSO), gill applications of DMSO plus Ovaprim, intramuscular (IM) injections of Ovaprim, or no applications (control). Numbers in parentheses indicate the number of females that ovulated and the total number tested. All fish were anesthetized with tricaine methanesulfonate.

Control	DMSO	DMSO + Ovaprim	Ovaprim IM
0% (0/9)	0% (0/9)	78% (7/9)	100% (11/11)

The fish were checked for mortalities and flowing eggs after 8 h. Fish were netted, anesthetized in 6 L of aerated water with a 100-mg/L concentration of MS-222 (tricaine methanesulfonate), and checked by gentle abdominal pressure to induce expression of eggs. Females were classified as having (1) no flowing eggs (i.e., no ovulation) or (2) flowing eggs (i.e., ovulation). Eggs were expressed into dry, clean ceramic bowls and were mixed with sperm from the injected males. Eggs were then placed into two McDonald egg incubation jars (one for DMSO plus Ovaprim and another for Ovaprim IM) until hatching to determine whether viable eggs were obtained. Remaining females were checked again for ovulation at 10 and 11 h posttreatment. We checked for mortalities for 24 h posttreatment.

The hypothesis of equal proportions of females undergoing ovulation in each treatment and control group was tested by use of Fisher's exact test (Uitenbroek 2000). Pearson's χ^2 value was used as the test statistic. The type I error rate (α) was set at 0.05.

Results and Discussion

Ovulation varied significantly by treatment (χ^2 = 14.58, df = 3, P = 0.0022), and no ovulation was observed in either the control fish or the fish given a gill application of DMSO (Table 1). The positive response of females to an Ovaprim IM injection (100% ovulated) compared to no response in the control fish was consistent with previous observations (e.g., Mohd-Zaini et al. 1994). However, the significant percentage of fish (78%) that ovulated following the gill application of DMSO plus Ovaprim demonstrated that this method also effectively delivers GnRH to the fish (see Sherwood and Harvey 1986).

All of the females that ovulated in the DMSO

plus Ovaprim treatment (i.e., seven of seven) and 7 of 11 females that ovulated in the Ovaprim IM treatment yielded eggs that were of low quality. Eggs of low quality were dark and clumped together (Shireman and Gildea 1989). Only a qualitative estimate of egg viability was obtained by observing the hatch of eggs in each jar. Nevertheless, these qualitative observations of hatch agreed with our judgments of egg quality and with the observations made by Shireman and Gildea (1989). The eggs produced by females given a gill application of DMSO plus Ovaprim failed to hatch; the hatch was poor for the eggs produced by the injected females. The poor hatch of eggs from both injected females and gill application females implies that low egg quality, rather than induction technique, was the cause. This may be due to the manual expression of eggs before final maturation of the ova, hence fertilization ability, was reached. Indeed, the fish were not heavily conditioned, and the eggs were not staged prior to the spawning attempt.

One concern over the use of DMSO is its potential toxicity to fish. In a review of organic solvent toxicity, Okumura (1998) reported that the 50% lethal concentration values for various fish species exposed to DMSO in water were in excess of 30,000 mg/L and that safe concentrations were about 300 mg/L. Additionally, DMSO is employed as a solvent for steroid hormones used in experimental sex reversal studies of tilapiine cichlids (*Oreochromis* spp.) (Gissis et al. 1991; Varadaraj and Pandian 1991; Pandian 1993). Nevertheless, preliminary experimental results suggest that DMSO is toxic to the bleeding heart tetra *Hyphessobrycon erythrostigma* (Characidae), a small ornamental species, when used under a protocol similar to that of the present study (J. E. Hill, R. P. E. Yanong, and J. S. Graves, unpublished data).

Despite concerns over DMSO toxicity, mortalities were low in our study (i.e., two mortalities in the injected treatment). Therefore, DMSO is not lethal to freshwater sharks at a gill application dosage rate of about 0.67 μL DMSO/g body weight.

The use of DMSO as a solvent for reproductive hormones applied to the gills shows some potential utility as a spawning induction technique for small fish species. Nevertheless, additional work is needed to address possible shortcomings of the methodology. For example, species-specific toxicity may be an issue. Also, the gill application technique requires handling of individual fish, although the method of handling is potentially less stressful than injection.

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