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Fabrication of Silastic Implants for In Vivo Steroid Delivery in Fish

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Abstract.—The use of hormones is a basic tool for managing reproduction in aquaculture. Delivery of a steroid over an extended period via implantation permits the manipulation of phenotypic sex for some fishes for which administration of the steroid in feed is not an option. An implant fabricated from Silastic materials and filled with 17α-methyltestosterone (MT) has been used to sex-reverse several species of fish. The fish must be large enough to accommodate the implant during the period of gonadal differentiation; the diffusion of 5 mg of MT will take place over a period of about 1 year.

The management of fish reproduction frequently involves administering hormones using various delivery systems (Crim 1985). Artificial propagation may require gonadotropins and/or steroids, while induced sex reversal requires androgens or estrogens (Shelton 1989; Hodson and Sullivan 1993; Mylonas et al. 1997). The life history stage and objective will determine which mechanism needs to be used. The attributes of a particular delivery system may be compromised by its disadvantages (Zohar 1996). For example, injection can administer precise doses but frequent handling may induce physiological stress. Incorporating chemicals into feed permits easily adjustable administration, but only if the fish actually eats the diet and the material is not modified during digestion (Pandian and Sheela 1995). The efficacy of a therapeutic given by immersion is affected by its solubility in water as well as its absorption by the fish. The implantation of an agent reduces the frequency of handling, but the carrier or type of capsule and various physical factors that alter in vivo diffusion into a fish's circulatory system may affect the precision of the dosage.

This note describes the fabrication of a delivery system that we have successfully used to direct the gonadal differentiation in fish, commonly known as sex reversal (Shelton 1989). We were encouraged to provide a detailed description by the numerous inquiries we have received about the specifics of making the implant. The implant is relatively simple to construct and can deliver an androgen over an extended period to fish species that cannot be treated with more "conventional" methods (such as by addition to feed or immersion), but it is limited to species that undergo gonadal differentiation at a relatively large size. Implant systems using a cholesterol matrix deliver a hormone as the carrier disintegrates (MacKinnon and Donaldson 1978; Lee et al. 1986; Garcia 1989, 1990). The implant described herein is fabricated from Silastic medical-grade tubing (dimethylpolysiloxane, Dow Corning Corp., Midland, Michigan), with which delivery is via diffusion; the molecular size of the material in the capsule, its solubility, and the relative pore size of the Silastic all influence the rate of movement into the circulatory system. This capsule was initially developed by Moore (1981); we tested it for delivery of 17α-methyltestosterone (MT) in grass carp *Ctenopharyngodon idella* to sex-reverse genetic females into functional males (Jensen et al. 1983; Boney et al. 1984; Shelton 1986). It was subsequently used successfully for the same purpose in silver carp *Hypophthalmichthys molitrix* (Mirza and Shelton 1988) and paddlefish *Polyodon spathula* (Mims et al. 1995; Mims and Shelton 1998). In each of these species, the labile period of gonadal differentiation occurs in relatively large-size juveniles (~12–18, 10–13, and 45–55 cm total length, respectively), and oral delivery cannot be used because of their particular feeding habits.

The materials needed to fabricate the implants are as follows: (1) Silastic tubing with an inner

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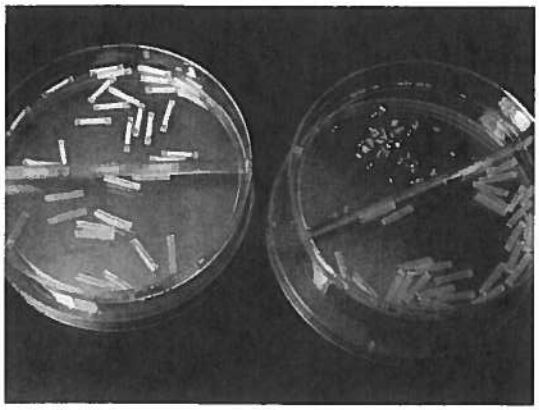

FIGURE 1.—The petri dish on the right contains 12-mm segments of Silastic tubing in one chamber and 1-mm lengths of Silastic rod in the other; the lower chamber of the petri dish on the left contains tube segments with one end plugged and ready for packing, while the upper chamber has finished implants containing 5 mg of 17α-methyltestosterone (MT).

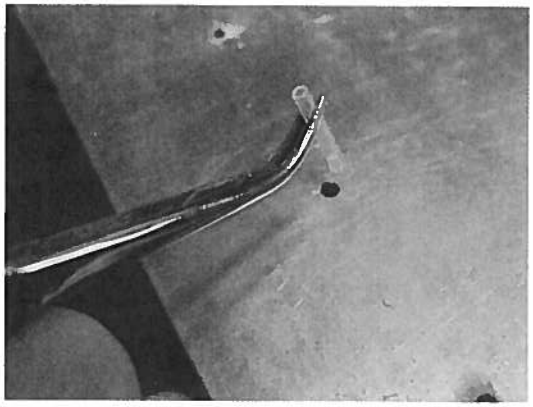

FIGURE 2.—To facilitate MT packing, segments of Silastic tubing are positioned in holes drilled in an aluminum block (the diameter and depth of the holes are determined by the tube dimensions).

diameter of 1.02 mm and an outer diameter of 2.16 mm, (2) Silastic rod (TPI 515–01) with an outer diameter of 1 mm, and (3) silicone adhesive. A source for the Silastic tubing and rods is Technical Products of Georgia, Inc., Medical Division, 2416 Park Central Boulevard, Decatur, Georgia 30035. These materials can be purchased in various lengths and cut for fabrication. The use of MT is regulated by the U.S. Food and Drug Administration, and experimentation must take place under the authority of an Investigational New Animal Drug protocol. Appropriate precautions should be taken during fabrication; we recommend using rubber gloves to avoid skin contact and having an exhaust hood or other respiratory protection.

The following description is for making a single implant, but in practice we prepare a number of the various components ahead of time. During the development and testing of the implants, precise amounts of MT were weighed before packing and the implant was subsequently reweighed to verify the correct net weight; however, precision is not as critical in practical use since delivery extends for about 12 months. The diffusion rate is a function of the hormone structure, surface area of the capsule, and environmental temperature (Boney et al. 1984). The Silastic tubing is first cut into 12-mm lengths and a Silastic rod into 1-mm lengths (Figure 1; see the petri dish on the right), then silicone adhesive is added to one end of the Silastic tube and one of the 1-mm rod segments is inserted to plug that end. When the adhesive is dry the capsule is ready for packing with MT (Figure 1; see the lower half of the petri dish on the left). To facilitate hand packing, several holes 11 mm deep are drilled in an aluminum block to hold the precut lengths of Silastic tubing (Figure 2). The MT is then weighed and added through the open end of the tubing (Figure 3); folding one edge of the weighing paper facilitates directing the hormone into the opening. The powder is periodically tamped with a straightened paper clip; the working volume of the tube (which is 10 mm long with an inner diameter of 1.02 mm) will accommodate about 5 mg of MT, depending on the degree of tamping. If any of the MT spills, it can be brushed onto the weighing paper and inserted during a second or third packing. When the full amount of the hormone has been inserted, more adhesive is added to the open end of the tube and another 1-mm

FIGURE 3.—Packing weighed MT into a Silastic tube; the spilled MT was later collected and added to the tube.

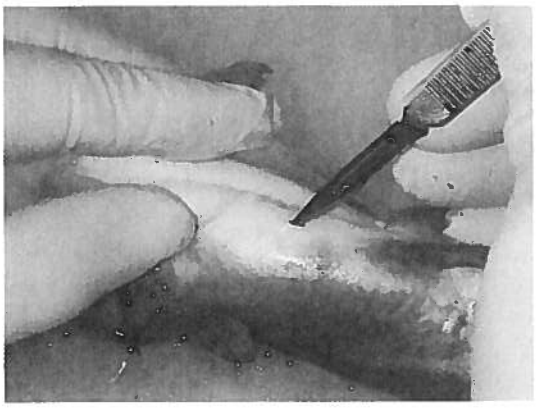

FIGURE 4.—A small incision in a fish's abdominal wall permits insertion of the MT implant; the elasticity of the tissue closes the incision without suturing.

segment of Silastic rod inserted and allowed to dry. The implant is then ready for use. These implants can be stored dry under refrigeration.

Depending on the species and ontogeny of gonadal differentiation, at the appropriate time or age an implant can be inserted into the peritoneal cavity through a small incision (Figure 4). The required dose of anesthesia varies among species. No sutures are required to close the small opening, and antiseptic can be topically applied to the incision. As an example, grass carp are implanted at about 100 mm total length, but fish as small as 75 mm can accommodate and retain the capsule (Rothbard et al. 2000). The MT diffusion rate is initially high, but within 1 month it decreases and stabilizes at a lower level: 5 mg is sufficient to maintain releases over a 1-year period (Shelton 1986). The rates of MT release in an implanted grass carp were estimated to be 10–15 μg/d between 5°C and 25°C, respectively. This system has delivered sufficient levels of MT to induce functional sex reversal in several species of fish when the treatment began before gonadal differentiation and continued until the process was completed.

Acknowledgments

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