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Evaluation of Ovaprim and human chorionic gonadotropin doses on spawning induction and egg and larval quality of pinfish, *Lagodon rhomboides*

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ABSTRACT

The use of exogenous hormones to induce final oocyte maturation (FOM), ovulation, and spawning has become commonplace in the reproductive protocols for many species. Dose efficacy can be highly variable among species and factors such as temperature and stress may further obfuscate the hormone's perceived efficacy. The pinfish, *Lagodon rhomboides*, is a marine baitfish species with great potential for commercial development. This species readily undergoes vitellogenesis in captivity, however progression through FOM is unreliable. Therefore, the objectives of this investigation were to assess the effects of various doses of Ovaprim® (sGnRHa + domperidone) and human chorionic gonadotropin (HCG) on ovulation and spawning in pinfish.

Two experiments were conducted to determine efficacious doses of Ovaprim and HCG in pinfish. Ovaprim dosages investigated were 0.25, 0.50, 1.00, and 2.00 mL/kg, injected into the dorsal musculature of female pinfish. Male pinfish received one half the dosage (0.125, 0.25, 0.50, and 1.00 mL/kg) administered to females to ensure spermiation. Experimental HCG doses of 500, 1000, 2000, and 4000 IU/kg were administered to female pinfish with male pinfish once again receiving half the dosage (250, 500, 1000, and 2000 IU/kg). Spawned eggs were collected over a 72 hour period post injection and enumerated and assessed for fertilization, hatching percentage, and survival to first feeding. Additionally, eggs and larvae were photographed and a suite of morphological parameters was evaluated.

Doses of 0.25 and 0.50 mL/kg Ovaprim were efficient at induction of FOM and spawning in pinfish. Superior fecundity, fertilization and 3 day post hatch (DPH) survival rates, as well as larger 3 DPH larvae support lower Ovaprim doses as the preferred choice for use with this species. Conversely, the 4000 IU/kg HCG dose performed the best of all HCG doses evaluated eliciting the greatest spawning frequency from female pinfish.

1. Introduction

Captive propagation of fishes is essential to meet the growing demands placed on finite fisheries resources. To this end, the aquaculture industry must find reliable and cost effective methods to spawn and culture species of interest. Captive spawning of broodstock is an integral component in the commercial production of finfish and significant investments are made in infrastructure to ensure crucial environmental cues can be properly replicated and manipulated. Abiotic factors such as temperature, salinity, and photoperiod play critical roles in the gametogenic cycles of fishes and despite considerable efforts to provide environmental conditions conducive to spawning, many species still fail to undergo final oocyte maturation (FOM) in captivity (Mylonas and Zohar, 2001).

The use of exogenous hormones to induce FOM, ovulation, and spawning has become commonplace in the reproductive protocols for many species including salmonids (Donaldson et al., 1981; Mylonas et al., 1992; Vikingstad et al., 2008), flounders (Berlinsky et al., 1997; Harmin and Crim, 1992), black sea bass, *Centropristis striata*, (Watanabe et al., 2003), European sea bass, *Dicentrarchus labrax*, (Fornies et al., 2001), sea breams (Barbaro et al., 1997; Haddy and Pankhurst, 2000), and ornamental fishes (Hill et al., 2009). Crude pituitary extracts used in the 1930s have been replaced by synthetic gonadotropin releasing hormone agonists (GnRHa) and purified gonadotropins (Zohar and Mylonas, 2001). Advances in induced spawning and its associated biochemistry have resulted in hormone preparations with increased bioactivity across multiple species, decreased risk of disease transmission, and increased options for hormone administration (Patino, 1997).

The pinfish, *Lagodon rhomboides*, is a member of the Sparidae or porgie family, and is distributed from Massachusetts south into the Gulf of Mexico to the Yucatan peninsula (Muncy, 1984). Characterized

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by Oesterling et al. (2004) as marine baitfish species with great potential for commercial development, it is arguably the most popular live bait among anglers in the southeastern United States. Research has shown pinfish to be tolerant of high stocking densities and low salinities with excellent survival in culture conditions (Ohs et al., 2010). Unlike most sparids, pinfish exhibit gonorchoristic development (Cody and Bortone, 1992). They readily undergo vitellogenesis in captivity however progression through FOM resulting in volitional spawning is unreliable (DiMaggio personal communication). Pinfish FOM and ovulation has been successfully induced using human chorionic gonadotropin (HCG), pituitary luteinizing hormone (PLH), and a steroid mixture consisting of estradiol benzoate, testosterone propionate, and progesterone (Cardeilhac, 1976; Schimmel, 1977). Unfortunately, only a single HCG dose (1000 IU/kg) was investigated and all fish were manually stripped resulting in poor egg quality and larval survival in these studies. More recently, DiMaggio et al. (2010) achieved FOM and induced volitional spawning with pinfish using a single intramuscular injection of Ovaprim® (Western Chemical Inc., Ferndale, WA, USA) at the manufacturer's recommended dose (0.5 mL/kg). Results of these experiments illustrate the efficacy of standard induced spawning protocols in captive reproduction of pinfish.

Ovaprim® is a liquid peptide preparation of a salmon gonadotropin releasing hormone analog (sGnRHa, D-Arg^6-Pro^9-Net, 20 μg/mL) and a dopamine antagonist (Domperidone, 10 mg/mL). It is delivered as either an intramuscular or intracoelomic injection and acts directly on the pituitary stimulating the release of gonadotropic hormones while concomitantly preventing dopaminergic inhibition of gonadotropin secretions. Currently, it is legal for use in ornamental finfish broodstock and is indexed by the United States Food and Drug Administration (USFDA) as a Legally Marketed Unapproved New Animal Drug for Minor Species. Human chorionic gonadotropin is legally marketed under the name Chorulon® (Intervet Inc., Summit, NJ, USA) and is currently the only USFDA approved spawning aid for use in finfish. Usually administered intramuscularly, it acts lower in the hypothalamic–pituitary–gonadal axis and resulting eggs and larvae may be inferior in quality when compared to spawns produced with GnRHa. Nonetheless, HCG has proven to be an effective choice for FOM and spawning induction in numerous species.

Species specific dose determination for spawning hormones is still relatively nebulous. General dosage recommendations may be either supplied by the manufacturer, as in the case of Ovaprim® (0.5 mL/kg), or broad ranges may be extrapolated from the scientific literature, as in the case of HCG (100–4000 IU/kg) (Mylonas and Zohar, 2007). Dose efficacy can be highly variable among species and factors such as temperature and stress may further obfuscate the hormones perceived efficacy. Insufficient dosing may prevent the progression of FOM and ovulation whereas overdosing may lead to reduced egg and larval quality and potential death of brood fish in extreme cases. Mylonas and Zohar (2007) recognized that strong, rapid and unnatural stimulation provided by an excessive dose of a hormonal therapy may result in decreased egg quality and viability and thus it is critical to establish appropriate hormone doses unique to the species of interest. Therefore, the objectives of this experiment were to evaluate the efficacy of various doses of Ovaprim® and HCG on ovulation and spawning in pinfish. Numerous qualitative and quantitative variables were analyzed for both eggs and larvae to elucidate the most advantageous dose for each hormone. Results from these experiments will provide valuable information which will help to increase production efficiency and spawning success in this new aquaculture species.

2. Methods

2.1. Broodstock acquisition and conditioning

Pinfish broodstock were collected by commercial fishermen from the Indian River Lagoon near Fort Pierce, Florida, USA and temporarily quarantined in a 2536 L tank with flow-through seawater. Health assessments were conducted and fish were treated for external parasites with 250 mg/L formalin baths (Parasite-S, Western Chemical Inc., Ferndale, WA, USA) and administered a 10 day course of oxytetracycline feed (Terramycin® 200, 2.5 g/lb, Phibro Animal Health, Ridgefield Park, NJ, USA) to reduce the potential of bacterial infection following capture and handling stress. Formalin baths lasted one hour and were conducted on alternate days for a total of five treatments. Following the fifth bath, a subsample of pinfish was reassessed to ensure treatment efficacy and confirm the health status of fish to be used in subsequent experimentation at the University of Florida Indian River Research and Education Center (IRREC) in Fort Pierce, Florida.

Prior to experimentation, pinfish were held in eight 1600 L circular tanks within two recirculating systems inside a greenhouse for a minimum of one year to allow for acclimation to culture conditions. Each system was comprised of four 1600 L culture tanks, a 1600 L sump, bead filter, protein skimmer, two ultraviolet sterilizers, supplemental aeration, and a 1–1/2 hp centrifugal pump. Brood systems contained sterilized natural seawater from the Atlantic Ocean at a salinity of 35 g/L with an ambient temperature and photoperiod in an effort to closely replicate the fish's natural environment and promote natural gametogenesis and reproductive behavior. Furthermore, this acclimation period allowed for uniform nutrition among brood fish, thereby removing the confounding effects of diet on reproductive performance. A maintenance diet which consisted of a 2.0 mm slow sinking pellet (Zeigler Bros. Inc., Gardners, PA, USA, 50% protein, 15% fat, 2% fiber, 12% moisture, and 8% ash) was fed to satiation once daily in the eight months preceding the spawning season. Four months prior to the initiation of spawning experiments the maintenance diet was supplemented with the addition of frozen squid, *Loligo opalescens*, and krill, *Euphausia superba*, and fed twice daily to satiation.

2.2. Ovaprim® dose evaluation

The absence of sexual dimorphism in pinfish required confirmation of gender and reproductive competence via observation of gamete development. Male pinfish selected for experimentation exhibited flowing milt upon gentle palpation of the abdomen anterior to the urogenital opening. Fish which failed to express milt were anesthetized in 125–250 mg/L quinaldine sulfate (Fishman Chemical LLC., Fort Pierce, FL, USA) and a teflon catheter (0.97 mm-inside diameter, 1.27 mm outside diameter) was inserted into the urogenital opening and suction was applied. Collected ovarian samples were placed on a Sedgewick Rafter counting cell to provide scale and photographed with a trinocular dissecting microscope outfitted with a digital camera. As pinfish employ an asynchronous spawning modality, the diameter of vitellogenic oocytes in all stages of development ($n = 100$) within a sample was determined using SigmaScan Pro 5.0 software (Systat Software Inc., Point Richmond, CA, USA). Female pinfish with mean vitellogenic egg diameters >0.450 mm were selected for induced spawning trials. All pinfish were individually weighed and measured for total length (TL) and randomly assigned to one of four Ovaprim® dosage treatment groups. Female pinfish treatment dosages investigated were 0.25 mL/kg (5 μg sGnRHa + 2.5 mg domperidone), 0.50 mL/kg (10 μg sGnRHa + 5 mg domperidone), 1.00 mL/kg (20 μg sGnRHa + 10 mg domperidone), and 2.00 mL/kg (40 μg sGnRHa + 20 mg domperidone) of Ovaprim® injected into the dorsal musculature. Male pinfish received one half the dosage (0.125, 0.25, 0.50, and 1.00 mL/kg) administered to corresponding females to ensure spermiation. Recirculating systems used in spawning trials were identical to the holding systems previously described except each 1600 L tank was outfitted with an external 200 L egg collector engineered to collect both floating and sinking eggs within a 500 μm mesh enclosure. Anesthetized fish were allowed to recover after which one male and one female from the same treatment were stocked in a single 1600 L tank within a recirculating system. Each treatment was replicated once within each of two independent recirculating