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# Chronic exogenous kisspeptin administration accelerates gonadal development in basses of the genus *Morone*

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ABSTRACT

The present study assesses the effects of chronic administration of peptides to fish, termed kisspeptins, which are the products of the KISS1 and KISS2 genes, and have been shown to control the development of puberty in animals. Using ecologically and commercially important species (white bass, *Morone chrysops*, striped bass, *Morone saxatilis*, and their hybrid) as comparative models, we determined that repeated bi-weekly injections (over 7 weeks) differentially accelerate puberty, as evidenced by increases in the prevalence of spermatozoa in the testes of juvenile fish. Moreover, in sexually mature fish, kisspeptin treatment led to increased gonad weight, gonadosomatic index, and spermatocrit in some white and striped bass. Additionally, mature white bass treated with kisspeptins showed an advancement in oocyte development as determined by histological examination. These gonadal changes occurred in the absence of any photothermal manipulation or hormone injections. To date, this is the first description of kisspeptin-mediated pubertal initiation in fish, and the first evidence that kisspeptins could modulate gonad maturation. Although it remains to be determined how kisspeptins may best be utilized in practice, our findings are a basis for future studies to characterize the molecular underpinnings of the KISS system in various fish species.

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## 1. Introduction

The KISS1 gene was first discovered in the context of cancer, specifically melanoma, where it was demonstrated to be a suppressor of metastasis (Lee et al., 1996; Lee and Welch, 1997a, 1997b; Nash and Welch, 2006; Nash et al., 2007; Beck and Welch, 2010; McNally et al., 2010). Since that time, the KISS1 gene and the KISS1 receptor (KISS1R or GPR54) have emerged as the principal system mediating pubertal development in vertebrate animals. This perspective is supported by studies demonstrating that both KISS1R-knockout mice and mutations in KISS1R in patients cause reproductive/pubertal failure, notably autosomal recessive idiopathic hypogonadotropic hypogonadism (de Roux et al., 2003; Seminara et al., 2003; Mitani et al., 2010). The products of the KISS1 gene are termed kisspeptins, which bind to KISS1R (Kotani et al., 2001; Muir et al., 2001; Ohtaki et al., 2001). The full-length KISS1 protein is 54 amino acids and is termed KP54 (alternatively named metastin) which can be proteolytically cleaved into shorter fragments (e.g., KP10, KP13, and KP14) representing the C-terminus of KP54, and which signal through KISS1R with presumably equal activity (Kotani et al., 2001). Exogenous kisspeptins have been administered to numerous vertebrate animals including humans and rodents, and have been shown to stimulate gonadotropin release including luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone (Gottsch et al., 2004; Dhillo et al., 2005, 2007; Cho et al., 2009; Beck and Welch, 2010; George et al., 2011).

More recently, the KISS1 gene and a paralog termed KISS2 have been identified in teleost fishes (Filby et al., 2008; Kanda et al., 2008; van Aerle et al., 2008; Felip et al., 2009; Kitahashi et al., 2009; Li et al., 2009; Mitani et al., 2010; Shi et al., 2010; Yang et al., 2010; Servili et al., 2011). According to synteny analysis, the KISS gene was duplicated before the divergence of sarcopterygians and actinopterygians, but it seems that the KISS2 gene was lost in placental mammals (Zohar et al., 2010). KISS1 and KISS2 gene sequences are dissimilar; however, they have some sequence similarity at the amino acid level (60–80%) of the smallest known kisspeptin, the decapeptide KP-10 (Mitani et al., 2010). This disparity in amino acid sequences could result in different efficacies on the KISS receptor(s) (Zohar et al., 2010). Indeed, this notion is supported by a study on transfected cell lines expressing either of the kisspeptin-receptors identified in fish (GPR54-1 and GPR54-2) where KISS1 exhibited higher sensitivity to GPR54-1, while GPR54-2 responded to KISS1 and KISS2 with similar sensitivity (Lee et al., 2009). Studies examining the in vivo effects of kisspeptin administration in fish have primarily focused on the hormonal (mainly luteinizing hormone (LH) and follicle-stimulating hormone (FSH) profiles of fish at timepoints soon (e.g., hours) after exogenous administration, or have involved the temporal and spatial characterization of KISS or KISS

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receptor mRNA expression during seasonal variation. More specifically, in goldfish *Carassius auratus*, intraperitoneal administration of KISS1 resulted in an increase in serum LH, while KISS2 treatment showed little effect (Li et al., 2009). Alternatively, in European sea bass, *Dicentrarchus labrax*, intramuscular injections of KISS2 exerted superior effects in terms of LH secretion over KISS1 (Felip et al., 2009). In orange-spotted grouper, *Epinephelus coioides*, intraperitoneal injection of KISS2 decapeptide significantly increased gnrh1 transcript levels in the hypothalamus and follicle-stimulating hormone beta (fshb) transcript levels in the pituitary at 6 and 12 h post-injection (Shi et al., 2010).

Although considerable progress has been made, the precise neuroendocrine mechanisms underpinning reproductive success and failure have remained largely hidden, frustrating efforts to improve fish breeding practices (Zohar et al., 2010). The recent discovery of the KISS system as a central regulator of GnRH and integrator of environmental cues reinforces the fact that our knowledge of the molecular actors and their roles in puberty and fertility is still deficient (Zohar et al., 2010). For our purposes, exploiting the KISS/kisspeptin system, in the setting of aquaculture, abounds with theoretical promise. Maintaining stocks of fish until sexual maturation can be an economic burden to commercial producers due to feed and space considerations, as well as the increased risk of stress and/or disease-related losses (Taranger et al., 2010). This is of particular importance in species where onset of puberty takes substantial time such as groupers (*Serranidae*), tunas (*Scombridae*), and sturgeons (*Acipenseridae*) (Taranger et al., 2010). It is also important in interspecific hybrids, where maintenance of pure-line broodstock of more than one species is necessary to produce the F1 intercross progeny, such as hybrid catfish (*Ictalurus punctatus* × *Ictalurus furcatus*), hybrid tilapia (*Oreochromis* spp.), and hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). Thus, accelerating puberty for earlier reproduction, exerting more control over reproduction to extend breeding seasons, or better synchronizing the timing of spawning to overlap between different species for hybrid production, are all scenarios that could improve the cost-efficiency of many industries (Taranger et al., 2010). Furthermore, propagation of endangered or imperiled species may benefit from improvements in reproductive efficiencies and outputs.

However, aside from such commercial application, understanding the role of kisspeptins and their associated receptors at a fundamental level is equally important in the quest to better understand and exert more control over fish reproduction. To date, no study has demonstrated that delivery of exogenous kisspeptins (either KISS1 or KISS2) could affect gonadal condition or quality. Previous studies have instead focused on either identifying the presence of a KISS1/KISS2/KISS1R axis in different fishes or by characterizing the early molecular signaling events after administration. In the present study, we sought to characterize the physiological consequences of repeated exogenous kisspeptin administration to sexually immature and sexually mature fish with a specific emphasis on documenting the size and histological development level of the gonad. Here, using two model species of closely related fish within the family *Moronidae*, and their hybrid, we demonstrate that KISS1 and KISS2 decapeptides can differentially accelerate puberty onset, increase gonad mass, oocyte size, and sperm density within milt.

## 2. Materials and methods

### 2.1. Fish

The effects of kisspeptin treatment on two different age groups of mixed sex white bass *M. chrysops* that were 8 months (juvenile) and 19 months of age (sexually mature); one 8 month group of juvenile mixed sex striped bass, *M. saxatilis*, one 5 ½ year group of female striped bass (sexually mature), and one 8 month group of hybrid striped bass (juvenile) (Table 1). Fish were either bred on-site at the Stuttgart National Aquaculture Research Laboratory in Stuttgart, AR, USA or obtained from the North Carolina State Pamlico Field Lab, Aurora, NC, USA. Fish were fed a commercial diet daily to satiation. Fish were maintained in common garden fashion; to monitor each individual fish throughout the course of the study, all fish were tagged with alcohol sterilized PIT tags (Biomark, Boise, ID, USA; measuring 8.5 mm × 2.12 mm and 0.067 g) using a standard 12 gauge tagging syringe in the dorsal musculature (DM). Before PIT tagging, and before each peptide treatment, fish were anesthetized in well water containing clove oil (Sigma-Aldrich, St. Louis, MO, USA) to initial loss of equilibrium.

### 2.2. Water quality

Fish were randomly distributed by species (approximately 30 fish per tank, except for adult striped bass which were stocked at a density of six fish per tank) among three 600 L tanks with forced aeration and flow-through well water at 21.8 ± 1.1 °C, pH 7.8, and dissolved oxygen of 8.0 mg/L. No manipulations were made in water temperature or photoperiod.

### 2.3. Peptides

Peptides were designed based on the previously reported (Felip et al., 2009) amino acid sequences of the kisspeptin-10 region in the European sea bass (*D. labrax*); a closely related species with a high homology in GnRH to fish of the genus *Morone* (Zmora et al., 2002). Two amidated peptides termed KISS1 (YNLNSFGLRY-NH2) and KISS2 (FNFNPFGLRF-NH2) were synthesized by CPC Scientific (Sunnyvale, CA, USA). Peptides were diluted in Dulbecco's phosphate-buffered saline (PBS; Cellgro by Mediatech, Manassas, VA, USA) and were injected in the DM twice weekly at a dose of 250 ng/g body weight for 8 weeks ranging from January to May 2010 (Table 1). Control fish were injected with PBS alone. Dosing was selected based on previously reported dosages that elicit gonadotropin release in European sea bass (Felip et al., 2009). Doses were adjusted to account for individual weight gain during the study by weighing fish at the beginning of the study, on week 4, and near the termination of the study (week 7). There

**Table 1**
Species and characteristics of fish used to examine gonad-level responses to chronic exogenous kisspeptin administration.

| Species | Age at start (months) and classification | Starting weight (mean ± SEM g) | Ending weight (mean ± SEM g) | Number of individuals | Number of injections |
|---|---|---|---|---|---|
| White bass *Morone chrysops* | 19 Mature | 289.8 ± 7.7 | 302.1 ± 8.9 | 43 (22 male; 21 female) | 15 |
| White bass *M. chrysops* | 8 Juvenile | 79.6 ± 1.6 | 126.7 ± 2.8 | 90 (46 male; 44 female) | 14 |
| Striped bass *M. saxatilis* | 68 Mature | 1771.6 ± 52.9 | 2116.2 ± 67.7 | 18 (all female) | 14 |
| Striped bass *M. saxatilis* | 8 Juvenile | 111.7 ± 1.8 | 207.7 ± 3.8 | 88 (51 male; 37 female) | 14 |
| Hybrid striped bass *M. chrysops* × *M. saxatilis* | 8 Juvenile | 134.5 ± 3.2 | 208.7 ± 5.1 | 80 (32 male; 48 female) | 15 |