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Research paper

Effects of neuropeptides and sex steroids on the pituitary-gonadal axis of pre-pubertal F1 wreckfish (hāpuku) *Polyprion oxygeneios in vivo*: Evidence of inhibitory effects of androgens

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ABSTRACT

The ability to advance puberty in broodstock that have a long generation interval and mature at large size is a highly valuable tool in contemporary aquaculture enterprise. Juvenile male and female wreckfish 'hā puku' *(Polyprion oxygeneios)*, a candidate for commercialization in aquaculture, were subjected to treatment for 8 weeks with two implants, one containing steroid (blank; estradiol-17β, E2; 11-ketotestosterone, KT; 17 α-methyltestosterone, MT), the other peptide (blank; gonadotropin-releasing hormone analog, GnRHa; kisspeptin, Kiss2-12). The expression of target genes (glycoprotein homone α-subunit, *gpa*; follicle stimulating-hormone β-subunit, *fshb*; luteinizing hormone β-subunit, *lhb*; GnRH receptor, *gnrhr*) in the pituitary was assayed by quantitative PCR. KT and MT decreased mRNA levels of all target genes in both male and female hāpuku, suggestive of a strong inhibitory tone by these steroid hormones. E2, GnRHa and Kiss2-12 were largely ineffective, regardless of whether they were administered alone or in combination with steroid implants. Clear differences in release and/or clearance rates between E2 and KT from implants were evident, in part explaining our observations. Advancement of puberty was not achieved, and we pose that different hormone doses and/or administration during more advanced stages of gonadogenesis need to be considered to move this field forward.

1. Introduction

The timing of puberty in farmed fish is a commercially significant biological issue. On the one hand, early or precocious puberty in species such as European sea bass *(Dicentrarchus labrax)* (Carrillo et al., 2015), Atlantic cod *(Gadus morhua)* (Karlsen et al., 2006) and Atlantic salmon *(Salmo salar)* (Bromage et al., 2001; Taranger et al., 2010; Andersson et al., 2013) can affect growth, feed utilization, health and welfare due to investment in gametogenesis, re-allocation of energy at the cost of somatic growth and fillet quality (Bromage et al., 2001; Okuzawa, 2002; Newman et al., 2008). On the other hand, precocious or early puberty reduces generation intervals which allows for more rapid selection for desired traits in broodstock with long generation intervals (Symonds et al., 2012).

Puberty in vertebrates is defined as the developmental period during which the ability to reproduce is acquired for the first time, reflecting a functional brain-pituitary-gonadal axis (BPG axis) (Okuzawa, 2002; Kumakura et al., 2004; Taranger et al., 2010). Pubertal development depends on an array of intrinsic and extrinsic factors (c.f., Taranger et al., 2010) and is governed by pituitary gonadotropins, i.e., follicle-stimulating hormone (Fsh) and luteinizing hormone (Lh) (Dickey and Swanson, 1998; Mateos et al., 2002), glycoprotein hormones that consist of a common α- (glycoprotein hormone-α, Gpα) and a specific β-subunit (Fsh β, Lhβ).

The hypothalamic decapeptide gonadotropin-releasing hormone (GnRH) is a key driver of gonadotropin synthesis and release (Pierce and Parsons, 1981; Mateos et al., 2002; Taranger et al., 2010; Berkovich et al., 2013). Upon release, gonadotropins act on the gonads to regulate gametogenesis and steroidogenesis; in turn, various peptides and gonadal steroids (androgens and estrogens) act on the brain and/or pituitary to regulate gonadotropin synthesis and release, thus generating a feedback loop (Okuzawa, 2002; Swanson et al., 2003; Levavi-Sivan et al., 2010).

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Within the last 20 years, there has been growing recognition that kisspeptins (products of the *kiss* genes) have an important role in pubertal development and other reproductive processes in teleosts (Parhar et al., 2004; Filby et al., 2008; Elizur, 2009; Zmora et al., 2012; Carrillo et al., 2015), as they do in mammals (see review by Clarkson et al., 2010). Indeed, a dual kisspeptin system (*kiss1, kiss2* and their receptors) has been identified in array of fish species, including zebrafish and medaka *(Oryzias latipes)* (Kitahashi et al., 2009), European sea bass (Felip et al., 2009), European eel *(Anguilla anguilla)* (Pasquier et al., 2012) and sablefish *(Anoplopoma fimbria)* (Fairgrieve et al., 2016). In the zebrafish, both kisspeptins appear to have different functions; Kiss2 is thought to be mostly involved in regulating reproductive events (e.g. Lh production) while its paralogue, Kiss1, is more likely to be involved in controlling non-reproductive events such as metabolism (Servili et al., 2011). Kisspeptin neurons in some brain nuclei have shown sensitivity to sex steroids, and thus, are subject to the steroid feedback loop (Kanda and Oka, 2012).

Given their generic ability to modulate gonadotropin synthesis and release, kisspeptins, GnRH (or a synthetic analog, GnRHa) and/or sex steroids have been used experimentally to advance puberty in a variety of fishes, albeit with mixed success. For example, chronic administration of Kiss1-10 and Kiss2-10 to pre-pubertal male kingfish, *Seriola lalandi,* indicated that effects varied with reproductive stage at the time of treatment (Nocillado et al. 2013). Two-year-old previtellogenic (ovaries with perinucleolar oocytes) red sea bream *Pagrus major* could be induced to enter puberty after administration of a single GnRHa implant (Gen et al., 2001), but other species have required additional treatment with sex steroids and/or dopamine agonists (Holland et al., 1998). Accordingly, treatment of immature male yearling rainbow trout (*Oncorhynchus mykiss*) with both GnRHa and the androgen testosterone (T) resulted in precocious puberty by 60 days post-administration (Crim and Evans, 1983). Furthermore, T exerted positive effects on the brain and pituitary of pre-pubertal European sea bass, resulting in acceleration of gonadal development (Zanuy et al., 1999).

The wreckfish 'hāpuku' (*Polyprion oxygeneios*) is a candidate species for aquaculture in New Zealand. Reaching sexual maturity after 10–13 years in the wild (Francis et al., 1999) and after 5 years when bred in captivity (Wylie et al., unpublished data). Late pubertal onset is a notable hindrance to cultivation in species with long generation intervals; puberty advancement can shorten generation times and improve selective breeding outcomes. Furthermore, the ability to collect seed from smaller broodstock has logistical and possible economic benefits as smaller fish are generally easier to handle, and cheaper to medicate and accommodate than larger broodstock.

Hence, the main objective of this study was to advance puberty in juvenile hāpuku using synthetic neuropeptides (kisspeptin; GnRHa) and/or sex steroids (estradiol-17β (E2); 17α-methyltestosterone (MT), 11-ketotestosterone (KT)). The efficacy of these treatments was evaluated by recording changes in organ indices (gonadal and hepatic), in gonadal stage and oocyte diameters, and in pituitary function (reflected in transcript abundances of gonadotropin subunits *fshb*, *lhb*, *gpa* and gonadotropin-releasing hormone receptor (*gnrhr*).

2. Materials and methods

2.1. Animals

Mixed-sex captivity-acclimated hāpuku broodstock (F0) of varying age and size, captured from the wild prior to 2009, were maintained at NIWA's Northland Marine Research Centre, Ruakaka, New Zealand, under simulated natural photoperiod and a controlled temperature regime that cycled between 10 and 19°C. Spontaneously spawned eggs were collected in August-December 2010; following incubation and hatching, larvae were reared on enriched rotifer and *Artemia* before being weaned onto artificial feed (Skretting, Australia) for on-growing (Symonds et al., 2014). At around 6 months of age, F1 juvenile hāpuku were anesthetized with 0.025 mL/L Aqui-S (Aqui-S New Zealand Ltd.) and implanted with a passive integrated transponder (PIT) tag for identification; thereafter, fish were maintained under routine husbandry conditions until use in late summer (February 2013).

During the experimental period, the F1 hāpuku (>24 months old) were maintained under a decreasing water temperature (range 17–16 °C) and a simulated ambient local photoperiod (latitude ~35° South) that was advanced by two weeks. Fish were hand-fed till satiation once daily on dry pelleted food (Nova 9 mm, Skretting, Australia). All animal manipulations and handling were approved by the NIWA Animal Ethics Committee, in keeping with the guidelines of the Australian & New Zealand Council for the Care of Animals in Research and Teaching.

2.2. Experimental design

Two experiments were run concurrently on juvenile (pre-pubertal) hāpuku of the same age class and pedigree over an eight-week period. The first experiment (Trial I) aimed to examine the efficacy of sustained-release delivery systems ('implants') containing synthetic sex steroids in elevating plasma levels of KT or E2 (Section 2.2.1). In the second experiment (Trial II), the ability of neuropeptides and/or sex steroid implants to advance puberty was evaluated (Section 2.2.2). All fish were anesthetized prior to handling and manipulation (Section 2.4).

2.2.1. Trial I: Elevation of plasma sex steroid levels by sustained-release implants

Thirty-six hāpuku of unknown sex were measured (mean body weight (BW) 4.2 ± 0.5 kg; total length (TL) 61.9 ± 1.5 cm) and blood-sampled by syringe from the caudal vein at the start of the trial, T = 0. At the same time, each fish was administered a single intramuscular implant containing 0.5 mg of synthetic sex steroid (either KT or E2) or a blank (Section 2.3) at the base of the dorsal fin with a syringe. Once implanted, fish were divided among six 1.5 m^3 semi-recirculating temperature-controlled indoor tanks (2 tanks/treatment group). Fish were then repeatedly blood-sampled at 48 h (0.3 week), 1 week, 2 weeks and 8 weeks post-implantation before being euthanized and terminally sampled (Section 2.4).

2.2.2. Trial II: Puberty advancement

Hāpuku (N = 120; mean BW 3.5 ± 0.1 kg; TL 56.9 ± 0.5 cm) were subjected to puberty advancement using the same conditions as those described for Trial I. At T = 0, baseline samples were collected from 12 fish (Section 2.4). On the same day, the remaining experimental fish were subjected to administration of one implant containing no steroid or 0.5 mg E2, KT or MT ('steroid treatment': 4 levels; see Section 2.3) and one implant containing no neuropeptide or 0.25 mg of GnRHa or Kiss2-12 ('peptide treatment': 3 levels; see Section 2.3). Implants were injected into the dorsal muscle on either side of the dorsal fin. Thereafter, the fish (N = 9 per treatment group) were assigned to one of twelve 1.5 m^3 semi-recirculating temperature-controlled indoor tanks and maintained for 8 weeks until termination of the Trial for tissue collection (Section 2.4).

2.3. Manufacture of sustained-release implants

Implants (30 mg each) were prepared by compounding of a powder mixture composed of 95% cholesterol and 5% cellulose