Aquaculture Reports 4 (2016) 1–9

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

The effects of gonadotropin-releasing hormone analog on yellowtail kingfish *Seriola lalandi* (Valenciennes, 1833) spawning and egg quality

A.N. Setiawan[a,*], S. Muncaster[b], S. Pether[a], A. King[a], G.W. Irvine[a], P.M. Lokman[c], J.E. Symonds[a,d]

[a] National Institute of Water and Atmospheric Research, Northland Marine Research Centre, Station Road, Ruakaka 0116, New Zealand
[b] Bay of Plenty Polytechnic, Private Bag 12001, Tauranga 3143, New Zealand
[c] University of Otago, Department of Zoology, 340 Great King Street, Dunedin 9016, New Zealand
[d] current address Cawthron Institute, Private Bag 2, Nelson 7042, New Zealand

ARTICLE INFO

Article history:
Received 12 October 2015
Received in revised form 9 May 2016
Accepted 18 May 2016

Keywords:
GnRHa
Induction
Seriola
Egg quality
Parentage
Reproduction

ABSTRACT

Communal spawning behaviour in marine aquaculture species often results in a few individuals contributing disproportionate amounts of gametes. This can lead to a reduction in genetic variability and increases the risk of inbreeding among successive generations. Therefore, long term sustainability of captive breeding programmes for such species partly depends upon maintaining a sufficiently high proportion of parents contributing high quality gametes during spawning. The current study was conducted to evaluate if the use of slow-release gonadotropin-releasing hormone analog (GnRHa) implants could increase the number of females spawning high quality gametes, and thus increase genetic variation in a captive population of yellowtail kingfish *Seriola lalandi* (Valenciennes, 1833). Broodstock fish received implants with or without 500 μg of GnRHa during the spawning season. GnRHa treatment was associated with a higher proportion of females contributing to spawning. However, compared to eggs from non-GnRHa-treated broodstock, GnRHa significantly decreased the floating rate, fertilisation rate, number of viable eggs and egg oil globule diameter. Overall, the use of slow-release GnRHa implants is a useful tool to increase parental contribution to spawning, but this benefit must be carefully balanced against lower egg quality.

1. Introduction

Genetic selection is an extremely useful tool for improving the productivity and quality traits of farmed organisms (Simm, 2002). One of the key requirements for a successful genetic selection programme is a breeding population with sufficient genetic variation. Such variation allows for individuals with high genetic value to be selected as breeders while minimising the negative effects of inbreeding.

In marine finfish aquaculture, many species spawn communally, often involving displays of dominance/hierarchy in spawning behaviour (Hutchings et al., 1999; Herlin et al., 2008; Trippel et al., 2009). Such behaviour in captive populations can result in a few individuals contributing disproportionate amounts of eggs and sperm (Hutchings et al., 1999; Herlin et al., 2008; Trippel et al., 2009; Symonds et al., 2012) which can lead to a reduction in genetic variability among successive generations of broodstock and result in highly inbred progeny. Therefore, a broodstock development programme for such species should aim to increase the proportion of parents contributing high quality gametes during spawning.

Hormonal manipulation using exogenous gonadotropin-releasing hormone synthetic analog (GnRHa) has the potential to help achieve this goal. The mechanisms of GnRH action on the hypothalamus-pituitary-gonadal axis have been extensively reviewed elsewhere (Mylonas and Zohar, 2001; Zohar and Mylonas, 2001; Nagahama and Yamashita, 2008). In practical terms, the use of GnRHa for inducing spawning and increasing spawning frequency is well-known (Zohar and Mylonas, 2001; Mylonas et al., 2010) and was reported to effectively induce spawning in the greater amberjack *Seriola dumerili* (Risso, 1810) (Mylonas et al., 2004) and in the longfin yellowtail *Seriola rivoliana* (Valenciennes, 1833) (Roo et al., 2014a,b). However, successful aquaculture production also hinges on the quality of gametes produced by broodstock. In a review, spawning induction using GnRHa was reported to have a negative effect on egg quality (measured by floating rate, hatching rate) in some species (Bobe and Labbé,

* Corresponding author.
E-mail address: alvin.setiawan@niwa.co.nz (A.N. Setiawan).

http://dx.doi.org/10.1016/j.aqrep.2016.05.001

2010). However this is not surprising, given that induction success depends to a large degree on it being administered when oocytes are at the right stage of development (e.g., late vitellogenic), and this is likely to vary among female broodstock (Bobe and Labbé, 2010). These studies highlight that careful consideration is needed before the application of GnRH, particularly in new aquaculture species.

The yellowtail kingfish *Seriola lalandi* (Valenciennes, 1833) is a commercially and recreationally fished species in New Zealand and Australia and has been identified as a high value species with great potential for aquaculture (Gillanders et al., 1999; Poortenaar et al., 2001). During the past 14 years, the National Institute of Water and Atmospheric Research (NIWA) has undertaken a captive breeding programme for yellowtail kingfish with the aim of developing broodstock resources capable of sustaining the future industry. Kingfish reared in mixed groups of males and females reliably spawn in captivity (Moran et al., 2007) and are serial batch spawners producing fertilised eggs throughout the spawning season which can last for three to six months each year (November to April). Similar to many other marine species, they spawn communally, resulting in highly imbalanced gamete contributions among individuals (Symonds et al., 2012, 2014). Consequently, our aim was to increase the number of individuals producing high quality gametes that will contribute to the next generation of captive broodstock.

Therefore, the current study was designed primarily to investigate i) if treatment with GnRHa could increase both the proportion of individuals contributing gametes and/or the spawning frequency, and ii) what the implications would be for egg quality of captive yellowtail kingfish during the spawning season.

2. Materials and methods

The study was conducted between 27 January 2012 (administration of implants and placement in treatment tanks) and 29 February 2012 (date of final ovarian biopsy) at NIWA's Bream Bay Aquaculture Park, Ruakaka, New Zealand; this interval coincided with the known spawning time of NIWA's captive first generation (F1) yellowtail kingfish, a period of time selected to ensure that the gonadal development of most fish would be near or at spawning readiness. Indeed, it is possible that some of the females had spawned prior to being moved to their treatment tanks. All animal manipulations and handling were approved by the NIWA Animal Ethics Committee, in accordance with the national guidelines under the Animal Welfare Act 1999 of New Zealand.

2.1. Animal source and husbandry

All fish (14 females, 10 males) used in this study were hatched between 2005 and 2008, and reared in captivity as offspring from wild-sourced broodstock. Fish body mass ranged from 7.3 to 15.6 kg with an average of 10.3 kg and fork lengths from 72.0 cm to 91.0 cm with an average of 80.5 cm (Table 1). Prior to the study, 19 fish were kept in four 12 m^3 (depth 1.2 m) outdoor tanks and five fish in two 20 m^3 (depth 2 m) outdoor tanks (biomass densities of 5.5–8.22 kg/m^3), supplied with flow-through filtered sea water at ambient temperature. Water temperature in the tanks during the study averaged 20.6 °C (range 19.7–21.7 °C). Fish were fed a commercially extruded broodstock diet (Broodmax, E. N. Hutchinson Ltd., Auckland, New Zealand) to satiation three times weekly.

2.2. Experimental design and fish handling

To investigate treatment effects, fish were divided between two groups, i.e., control and GnRHa (Table 1). At the beginning of the study, fish were sedated in 10 ppm Aqui-S® (Aqui-S New Zealand, Ltd., Lower Hutt, New Zealand) prior to anesthesia in 300 ppm 2-phenoxyethanol (Sigma-Aldrich, Australia). Each fish were then given a single intramuscular implant of either a 30 mg custom-made pellet (Castro et al., 2009) of compressed matrix (19:1 cholesterol: cellulose) containing 500 μg GnRHa (D-Ala^6, des-Gly^{10} LHRH ethyl amide, sourced from Ed Donaldson and Associates (British Columbia, Canada)) or a blank pellet with no hormone (control). At this time, gonad biopsy (see Section 2.3.2) and morphometric measurements were also performed. Afterwards, fish were placed in their respective treatment tanks for the remainder of the study. Mean oocyte diameters of the females placed in the control and GnRHa groups were 377.5 ± 195.63 and 334.2 ± 160.91 μm (mean ± SD), respectively, at the start of the study and were not significantly different ($t_{9.75} = 0.42$, $p = 0.675$). Another gonad biopsy was performed at the end of the study.

2.3. Data collection and analyses

2.3.1. Egg collection, quantification and analyses

The methods for egg collection, incubation and quantification in this study were similar to those previously described for yellowtail kingfish (Moran et al., 2007) and hapuku, *Polyprion oxygeneios* (Schneider and Forster, 1801) (Kohn and Symonds, 2012). External surface egg collectors on each tank were monitored several times daily. From 7 February 2012 a fine net (sock) was placed over the outlet at the bottom of each tank to collect the sinking eggs, thus allowing the majority of the eggs spawned from this time until the end of the study to be collected. Collection of sinking eggs from the outlet is not a routine procedure, and was installed only once spawning had started and after poor egg quality was observed in some batches. Thus, information on the total number of eggs spawned was only available from the 7th February 2012 onwards. When eggs were detected, they were collected from the surface egg collectors and the waste sock separately, and the volumes of floating and sinking eggs from both collectors were recorded. All eggs collected at the same time were considered a single batch. The number of eggs produced in a batch was calculated by multiplying the volume of eggs (mL) by the average number of eggs (360) in 1 mL. The numbers of eggs per kg biomass of females in the tank in each batch was also calculated. An assessment of the percentage fertilisation of the floating fraction of each batch of eggs was carried out using a microscope (Moran et al., 2007). The number of viable eggs was calculated by multiplying the number of floating eggs by the percentage fertilisation.

Photos of 30 floating fertilised eggs collected from each batch were obtained using a dissection microscope (Leica MZ7.5) and an attached digital camera (Leica DFC420) as soon after spawning as possible. Images were later analysed (Leica Application Suite 3.6) to obtain egg and oil globule diameters. The stage(s) of embryonic development (Moran et al., 2007) of 100 randomly sampled fertilised eggs were recorded to estimate the number of spawning events in a batch.

2.3.2. Oocyte biopsy, histology and image analyses

Ovarian follicle samples were obtained by applying suction to a catheter (Pipelle Mark II, CCD International (Paris, France); diameter 2.6.mm, length 235 mm) inserted into the genital pore of anesthetised fish. A portion of the sample was placed in Ringer's solution (180 mM NaCl, 4 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 3 mM NaH_2PO_4, 12.5 mM $NaHCO_3$; pH 7.5) and photographs were taken under a dissecting microscope to determine oocyte diameter, in the same manner as above (Section 2.3.1). Measuring fresh samples avoids the underestimation of oocyte diameter due to shrinkage post-fixation and inconsistent positioning of sections through the oocytes. The diameters of the 20 largest well-defined oocytes (judged by eye) in a field of view were measured. If fewer