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Article *in* Aquaculture Reports · November 2016

DOI: 10.1016/j.aqrep.2016.05.001

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

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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,

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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

Table 1
Experimental groupings of broodstock and their properties.

Experimental groups	Implant	No. of fish		Weight and length ± SD		Density (kg/m^3)
		Male	Female	Male	Female	
Control	Sham	5	7	9.9 ± 1.66 kg 78.0 ± 5.00 cm	11.3 ± 2.64 kg 83.1 ± 4.78 cm	11
GnRHa	GnRHa	5	7	9.0 ± 0.98 kg 78.0 ± 5.00 cm	9.9 ± 1.84 kg 81.7 ± 3.73 cm	9.7

Table 2
The number of spawning events, batch collection and genotyping, and genetic contribution for each group during the experimental period. Gamete contribution was determined through genotyping of broodstock and eggs.

Experimental group	No. of spawning events	No of egg batches collected	No of egg batches genotyped	Mean no. eggs genotyped per batch (range)	No. of fish contributing gametes/total no. fish		Mean no. fish per batch (range)	
					Male	Female	Male	Female
Control	22	16	6	25.3 (10–33)	5/5	3/7	3.8 (3–5)	1.5 (1–2)
GnRHa	23	15	3	14.7 (7–27)	5/5	5/7	4 (3–5)	2.3 (1–4)

than 20 clearly defined oocytes were available, additional follicles from the same fish were analysed. Oocyte diameter measurements were made using Leica Application Suite 3.6 (Leica Microsystems Ltd., Wetzlar, Germany).

To determine the oocyte development stage, one portion of each ovarian biopsy sample was fixed in 10% neutral buffered formalin and processed using routine histological practices. Following serial dehydration with ethanol and xylene, the tissue was embedded in paraffin and sectioned at 4 μm. The sections were then mounted on glass slides and stained with haematoxylin and eosin for light microscope analyses. Images were captured and micrographs analysed to determine the developmental stage of the samples. The software ImageJ 1.4.6r (Rasband, E.; National Institute of Health, USA) was used to view the micrographs, and to measure oocyte diameter and evaluate developmental stage (determined using the criteria in Table 3). The development stages of the 20% most advanced or atretic oocytes on the slide were scored (Lokman et al., 2007) based on a 5-point scoring system (see Table 3). To obtain a representative sample size per fish, a minimum of 30 oocytes were scored. When this was not possible from one slide, additional non-adjacent slides from the same fish were scored until the minimum number of oocytes was scored. The scoring system, partly based on a previous description (Shiraishi et al., 2010) is described in Table 3 and Fig. 1.

2.3.3. Parentage assignment of eggs

Genotyping of yellowtail kingfish broodstock and eggs has been previously described (Symonds et al., 2012). Finclip samples were taken from individually tagged broodstock and individual embryos (blastula stage or later) were homogenised for genetic sampling. The parentage testing panel comprised of eight polymorphic microsatellite DNA markers (Symonds et al., 2012). Pedigree assignment was conducted by GenomNZ (AgResearch Ltd., Invermay, New Zealand) using a proprietary analysis programme. We sampled a total of 270 eggs from 9 batches of eggs, representing contribution of 24 potential parents, giving a ratio of 11.3 progeny/potential parent. This ratio is comparable to similar studies on broadcast spawning farmed species e.g., gilthead seabream *Sparus aurata* (Linnaeus, 1758), 6.8 (Castro et al., 2007); abalone *Haliotis asinina* (Linnaeus, 1758), 11.3 (Selvamani et al., 2001); and Japanese flounder *Paralichthys olivaceus* (Temminck and Schlegel, 1846), 12.1 (Hara and Sekino, 2003).

2.4. Statistical analyses

Most statistical analyses were conducted using SYSTAT 13 (SYSTAT Software Inc., San Jose, CA, USA). Egg quantity and quality parameters were analysed using two-sample *t*-tests (Control vs GnRHa) using each batch as a datum. To determine the effects of implant type on egg and oil globule diameters, linear mixed effects models (implant type as a fixed factor, spawning batch as random factor) were employed using each egg as a datum. Linear mixed effects models (implant type and sample time (start or end) as fixed factors, individual females as a random factor) were also employed to determine the effects of implant type on oocyte diameter change over the study period. Goodness of fit test was used to analyse differences between the two groups in the proportion of parents contributing gametes. To analyse the effects of GnRHa on oocyte development stages, we employed a mixed model binomial logistic regression in R 3.13. software (R Core Team, 2015) using the package *glmm* (Knudson, 2015). Each stage was analysed separately, with implant type and sample time (start or end) as fixed factors, and individual females as a random factor. Differences were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Number and timing of spawning events

A total of 31 batches of eggs were collected and analysed during the study which represented 45 spawning events (Table 2). Control and GnRHa-treated groups had 22 and 23 of spawning events, respectively. Fish in the control group spawned between 31January and 28 February; fish in the GnRHa group spawned between 2nd and 26th February (Fig. 2). The average period between spawning events was 1.34 and 1.10 days for the control and GnRHa groups ($t_{41} = 0.688$, $p = 0.495$), respectively.

3.2. Parentage analysis

In total, 196 out of 270 eggs from 9 batches were genotyped, representing 24 families. All 10 males contributed progeny and at least 8 of the 14 females did, representing 43 and 71% of the females in the control and GnRHa groups, respectively (Table 2). At least 60% of the males in each tank contributed to each batch. The genotyping data showed that the GnRHa implants significantly increased the proportion of females contributing gametes. Three out of seven females contributed eggs to six genotyped batches in the control

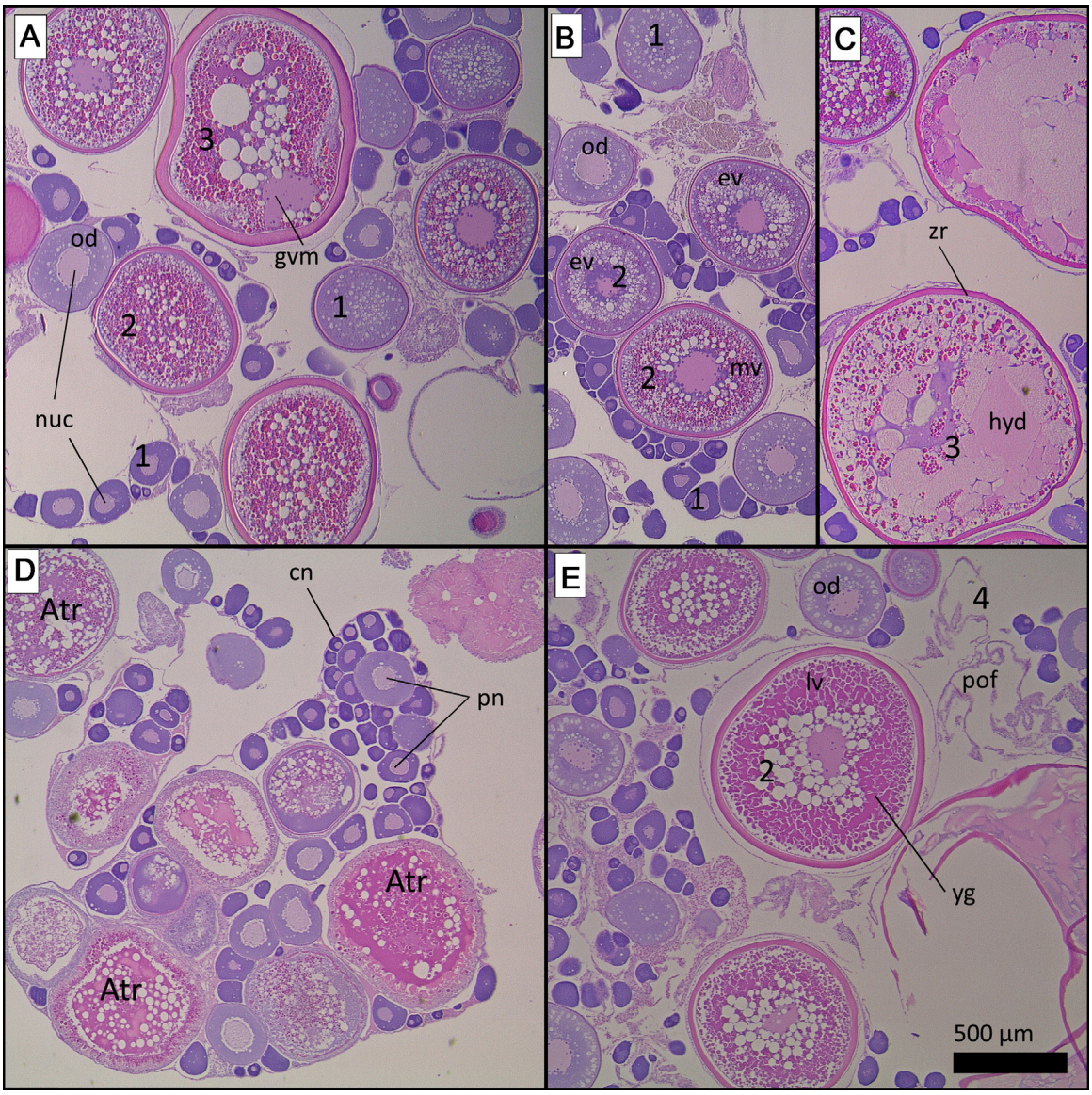

Fig 1. Representative micrographs of histological sections of yellowtail kingfish ovarian follicles at different stages of oogenesis. Refer to Table 3 for descriptions of each stage. Numbers denote the oocyte score (stage). Oocyte stages and structures are denoted by the following: cn = chromatin nucleolus oocyte; pn = perinucleolus oocyte, od = oil droplet stage (oil droplets are white) oocyte; ev = early vitellogenic oocyte; mv = mid-vitellogenic oocyte; lv = late vitellogenic oocyte; hyd = hydrated oocyte; pov = post-ovulatory follicle; atr = atretic oocyte; nuc = nucleus, yg = yolk globules; zr = zona radiata; gv = germinal vesicle; gvm = migrating germinal vesicle. Magnification is 50×.

Table 3
Oocyte scoring criteria. Description of oocyte score based on the developmental stages as determined from histological observations. Histological sections were stained with hematoxylin and eosin.

Oocyte score	Developmental stage	Histological description
1.	Pre-vitellogenic	Ooplasm mainly basophilic, stained dark purple with no or very few oil droplets visible. Oil droplet stage: oil droplets (white dots or globules) are clearly visible around the nucleus and the ooplasm stained light purple; at later stages, the oil droplets are very prominent in the ooplasm.
2.	Vitellogenic	Early vitellogenic stage: yolk granules (stained pink) are visible at the periphery of the oocyte. Mid-late vitellogenic: The ooplasm was composed mainly of yolk granules which may have started to coalesce; the oocyte is surrounded by a thick zona radiata.
3.	Post-vitellogenic	Germinal vesicle migration or breakdown has occurred, yolk granules heavily coalescing to form large globules that dominate the oocyte, or oocyte is hydrated.
4.	Post-ovulatory	Post-ovulatory follicle: empty/'deflated' follicular layers which may appear stringy.
Atr.	Atretic	Atretic follicle: visible breakdown of zona radiata, follicular layers and/or ooplasm content.

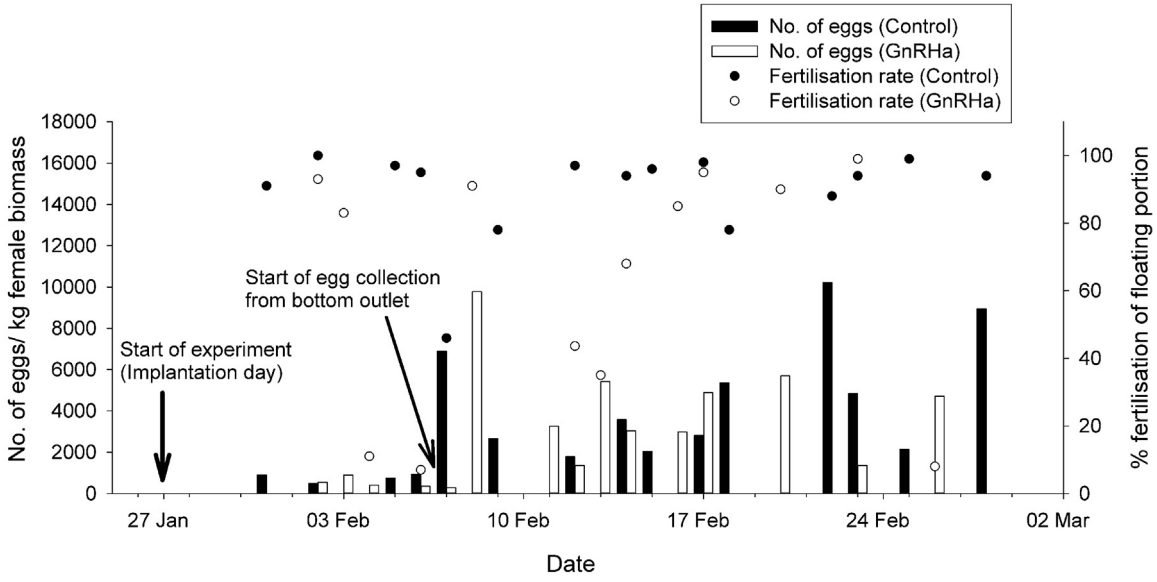

Fig. 2. The numbers of eggs per kg female and fertilisation rates of the control and GnRHa groups. The total number of eggs spawned including sinking eggs collected from the tank outlet were only available after 7 February 2012 (indicated by arrow; see text for detail). Prior to this, only eggs that floated to the egg collectors were quantified. Fertilisation rates were calculated only from the floating portion.

group, whereas five out of seven contributed eggs in three genotyped batches in the GnRHa group (Goodness of fit test: $\chi^2_1 = 23.06$, $p < 0.001$).

3.3. Egg quality

Fecundity (the number of eggs produced per batch or per female) was not statistically different between control and GnRHa-treated fish. However, GnRHa implants were significantly associated with reductions in the percentage of floating eggs, the percentage of fertilised eggs and the estimated number of viable eggs per batch (Table 4).

Treatment with GnRHa also significantly decreased oil globule diameters, but not egg diameters (Table 4). This finding led us to further investigate if GnRHa implants altered the relationship between oil globule size and egg size (Fig. 3). We employed a linear mixed model with oil globule diameter as a dependent variable, egg diameter as a covariate, implant type as a categorical fixed factor and spawning batch as a random factor. Using this model we did find a significant effect of egg diameter ($F_{1,548} = 137.97$, $p < 0.001$) and implant type ($F_{1,548} = 4.04$, $p = 0.045$), consistent with the previous result that GnRHa reduced oil globule diameter. We also found a tendency for the relationship (slope) between egg and oil globule diameters to be more pronounced in eggs of GnRHa-treated fish (interaction effect: $F_{1,548} = 2.70$, $p = 0.101$; Fig. 3).

3.4. Oocyte development

The linear mixed model revealed a significant interaction effect ($F_{1,501} = 12.81$, $p < 0.001$) of implant type and oocyte diameter at the start and end of the experiment. The average oocyte diameter of fish in the GnRHa group was noticeably greater at the end than at the start of the study period, an observation different from the control group (Fig. 4). Parentage analyses showed that four out of the five fish that were known to spawn in the GnRHa group had maximum oocyte diameters of <500 μm at the start of the study, whereas the corresponding oocyte diameters for all three fish that spawned in the control group were >800 μm (Fig. 5).

Histological analyses showed that most oocytes at the start and end of the study were at stage 1 (previtellogenic) (Fig. 6) and that atretic oocytes were present in 25 out of 29 biopsy samples. Binomial mixed logistic regression models were fitted for each developmental stage separately, with sampling time and treatment as fixed effects, and individual female as random factor. Each egg was treated as a binary (1 or 0) datum for being at a particular developmental stage. There was a significant interaction effect between treatment and sampling time for the occurrence of vitellogenic oocytes (stage 2) ($z = 3.184$, $p < 0.002$; Fig. 6); vitellogenic oocytes were virtually absent at the end of the study in ovaries of GnRHa-treated females. Similarly, between the start and end of the study, the occurrence of post-ovulatory follicles (stage 4) increased among fish in the control group, but decreased among GnRHa-treated fish (interaction effect: $z = 3.983$, $p < 0.001$; Fig. 6). Similar conclusions were arrived at when the data were analysed using linear mixed model with sampling time (start or end of study) and treatment (control and GnRHa) as fixed effects, and individual fish as random factor with the percentage of oocytes at each developmental stage as the dependent variable.

4. Discussion

This study has shown that slow-release GnRHa implantation significantly increased the proportion of females contributing eggs in captive yellowtail kingfish. This finding is consistent with that reported for a congener, the greater amberjack (Mylonas et al., 2004), which showed that GnRHa implantation induced spawning. Accordingly, exogenous GnRHa can be a useful tool in mitigating the problem of disproportionate genetic contribution and inbreeding in an aquaculture species. However, GnRHa implantation was shown to have a detrimental effect on egg quality; it was associated with decreased floating rate, lower fertilisation rates, decreased numbers of viable eggs and smaller oil globule diameters. Despite a greater number of females spawning, no significant increase in the number or frequency of spawning events was observed.

The fecundity (eggs per kg of body mass) and fertilisation rates of the control and GnRHa-treated F1 females in this study were considerably lower than reported for other *Seriola* species. In greater amberjack (Fernández-Palacios et al., 2013) and longfin yellowtail (Fernández-Palacios et al., 2015) GnRH induction was not associated with low fecundities or fertilization rates. Induc-

Table 4
Values for egg quality parameters measured in the experimental groups by batch. All values are presented as the mean for each group ± SEM, except for total numbers eggs and viable eggs. Numbers in parentheses denote the sample size for each group. Statistically significant differences between the treatments are denoted by ** ($p < 0.010$).

Egg quality parameters	Control	GnRHa	Statistical test and p-values
Total number of eggs produced (×1000)	3614.40	2836.80	N/A
Total number of viable eggs (×1000)	1588.39	486.80	N/A
Number of eggs (×1000)	301.20 ± 48.27 (12)	257.89 ± 52.82 (11)	$t_{21} = 0.61$, $p = 0.551$
Number of eggs (×1000)/kg female biomass	4.27 ± 0.68 (12)	3.88 ± 0.80 (11)	$t_{21} = 0.37$, $p = 0.716$
Floating eggs (%)**	54.2 ± 6.5 (12)	23.9 ± 7.1 (11)	$t_{21} = 3.17$, $p = 0.005$
Eggs fertilised (%)**	89.7 ± 3.5 (16)	62.2 ± 10.0 (13)	$t_{27} = 2.81$, $p = 0.009$
No. of viable eggs (×1000)**	132.37 ± 23.76 (12)	44.25 ± 13.47 (11)	$t_{21} = 3.15$, $p = 0.005$
Egg diameter (mm)	1.349 ± 0.002 (360)	1.325 ± 0.003 (210)	$F_{1,551} = 2.31$. $p = 0.129$
Oil globule diameter (mm)**	0.312 ± 0.001 (360)	0.301 ± 0.001 (209)	$F_{1,550} = 8.09$, $p = 0.005$

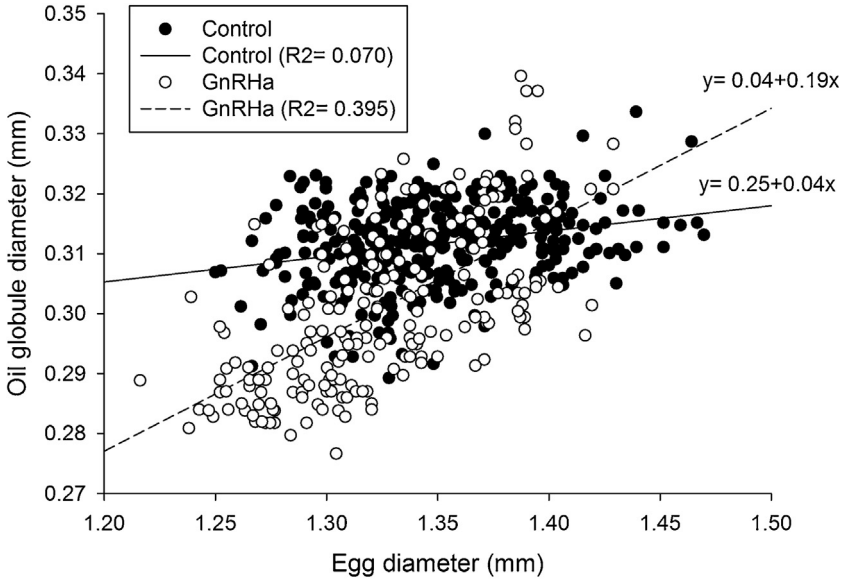

Fig. 3. Relationship between egg diameter and egg oil globule diameter. Filled (●) and empty circles (○) denote eggs from the control and GnRHa groups, respectively. Solid (—) and dotted (– –) lines denote the regression lines for control and GnRHa groups, respectively. The equation for each slope is displayed above the corresponding lines. Refer to the text for the statistical analyses.

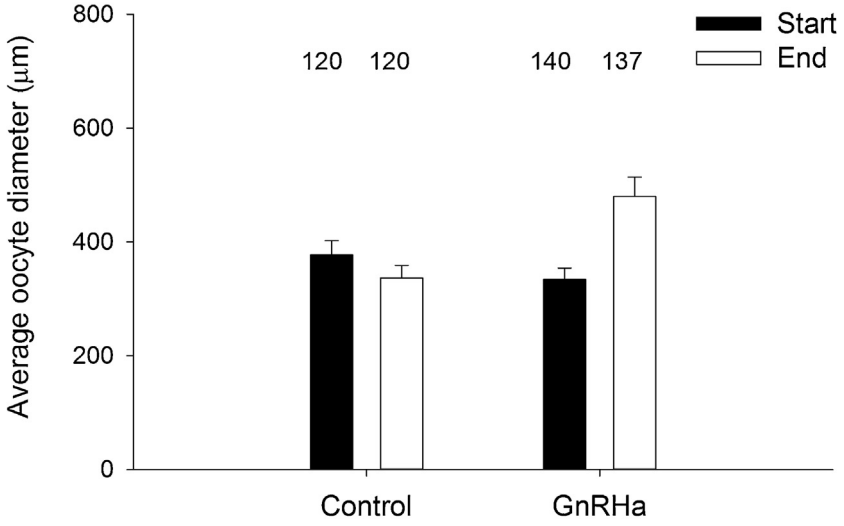

Fig. 4. The average of the mean diameters of the largest 20 biopsied oocytes from each of control and GnRHa-treated females at the start and end of the study. Error bars denote 1 SEM. Numbers above bars denote sample size (number of eggs). Please refer to the text for the statistical analyses.

tion with GnRHa produced season-long fecundities of 300,000 and 900,000 eggs/kg body mass, with fertilisation rates of over 90% (Fernández-Palacios et al., 2013, 2015; Roo et al., 2014a,b). However, wild-caught broodstock were used in these studies, and GnRHa was administered via serial intramuscular injections of 20 μg/kg body mass. In 2015, wild-caught broodstock at NIWA produced over 1 million fertilised egg/kg body mass (unpubl. data), with fertilisation rates of over 90% without the use of exogenous

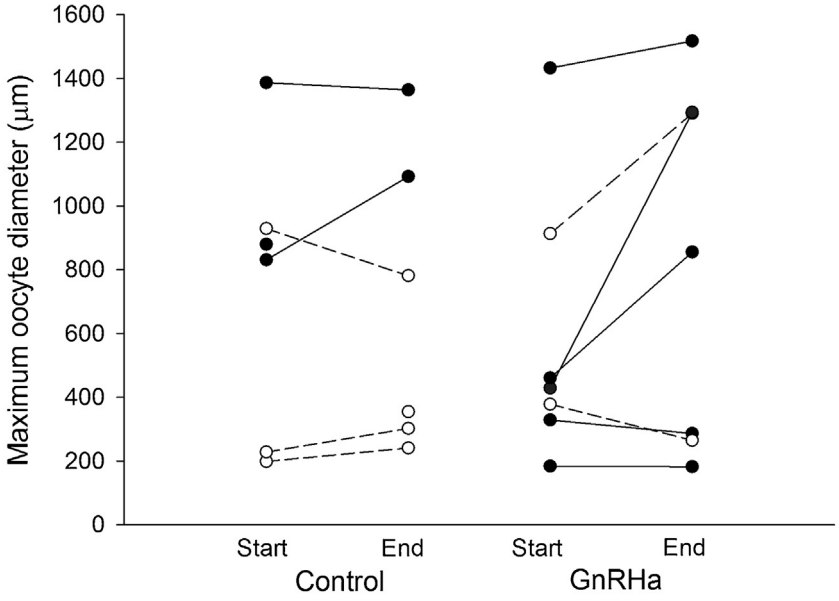

Fig. 5. The maximum oocyte diameters of individual fish from the control and GnRHa-treated groups at the start and end of the experimental period (connected by a line). Two fish in the control group were only sampled at either the start or end of the experiment. Fish that were confirmed to have spawned are denoted by filled circles (●) and solid lines (—).

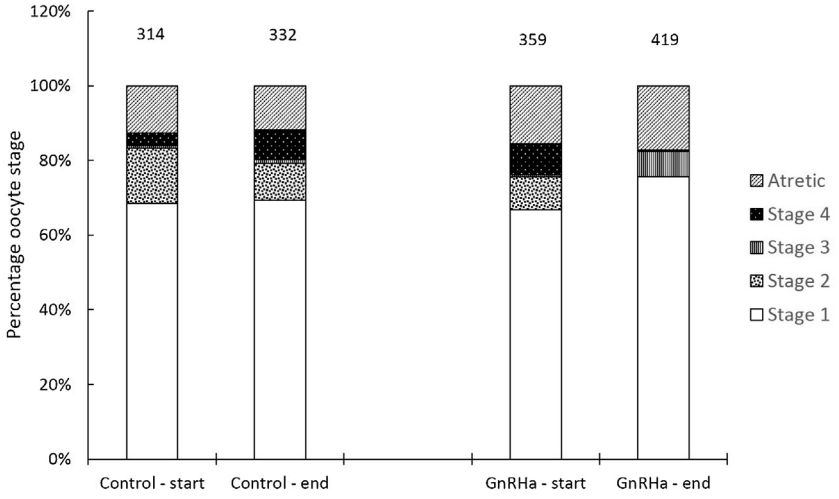

Fig. 6. The developmental stage distribution (percentages) of the 20% most advanced biopsied oocytes of fish in the control and GnRHa groups at the start and end of the study. Values above bars denote the numbers of oocytes scored. The developmental stages are as follows: Stage 1 = pre-vitellogenic, Stage 2 = vitellogenic, Stage 3 = post-vitellogenic, Stage 4 = post-ovulatory, Atretic = atretic follicles. Please refer to Table 3 and Fig. 2 for descriptions of the developmental stages. Refer to the text for the statistical analyses.

hormones. In contrast, the current study was conducted on captive-bred fish and GnRHa was administered via slow-release implant. Fertilisation rates after GnRHa implantation in greater amberjack and in the present study were 22% (Mylonas et al., 2004) and 62%, respectively. These results suggest that serial GnRHa injections may be a more effective means of induction in this genus.

One of the constraints of our study was the limited number of available fish for the experiment, not uncommon when working with larger marine broodstock. This constraint necessitated including females whose vitellogenic oocytes were smaller than would be considered optimal for GnRHa induction. Indeed, 5 out of the 7 females in the GnRHa group had maximum oocyte diameters less than 500 μm. In studies on greater amberjack and longfin yellowtail, GnRHa treatment was only administered to females with oocyte diameters greater than 500 μm to ensure successful induction (Fernández-Palacios et al., 2013, 2015). Nevertheless, four fish in the GnRHa group containing oocytes with maximum diameters of less than 500 μm at the start of the experiment subsequently spawned, whereas the two females in the control group with similar oocyte diameters did not. This suggests that the GnRHa treatment may have advanced vitellogenesis and ovulation. Indeed, the GnRHa group had increased oocyte diameters at the end of the experiment, in contrast to the control group in which oocyte diameters decreased (Figs. 3 and 4). However, it should be noted that as not all egg batches were genotyped, it is possible that the three females in the control group with oocytes of similar sizes also spawned but their eggs were not detected. Furthermore, this ovulation advancement was associated with poorer egg quality, which is likely related to the sub-optimal timing of the implantation.

Histological analyses showed that the ovaries of most females contained oocytes at all stages of development, including atretic oocytes, consistent with the fact that the study was conducted in the middle of the spawning season. The analyses also showed a decrease in the proportion of vitellogenic oocytes as the season pro-

gressed. This decrease, from 8.8% to 0%, was most apparent among GnRHa-treated females (Fig. 6). The increase, from 0.5 to 6.7%, of oocytes in the post-vitellogenic stage in GnRHa-treated fish was mainly driven by one female, and thus, not indicative of an overall increase. The proportion of post-ovulatory follicles increased in the control group, whereas it decreased dramatically in the GnRHa group throughout the study period. Exogenous GnRHa in aquaculture is generally used to induce final oocyte maturation, or in a few cases advance vitellogenesis, but only for oocytes that are sufficiently advanced, e.g., early vitellogenic (Mylonas and Zohar, 2001). In dusky grouper *Epinephelus marginatus* (Lowe, 1834), GnRHa promoted completion of vitellogenesis only for oocytes greater than 350 μm diameter (Marino et al., 2003). Therefore, it is possible that in the present study, the absence of vitellogenic oocytes by 33 days after implantation was due to GnRHa advancing vitellogenesis faster than the 'natural' rate of their recruitment from earlier developmental stages.

The advantages of using GnRHa to advance final oocyte maturation in species that do not readily spawn in captivity (Larsson et al., 1997; Marino et al., 2003; Mylonas et al., 2010) or as a breeding management tool (Mugnier et al., 2000; Garber et al., 2009) are well documented. However, similar to our findings, some studies have reported decreases in egg quality following GnRHa spawning induction (Bobe and Labbé, 2010). Compared to controls, GnRHa-treated fish had lower fertilization rates in turbot *Scophthalmus maximus* (Linneaus, 1758) (Mugnier et al., 2000) and Atlantic cod *Gadus morhua* (Linneaus, 1758) (Garber et al., 2009), reduced egg floating rates in Senegal sole *Solea senegalensis* (Kaup, 1858) (Agulleiro et al., 2006), compromised survival to hatch in European sea bass *Dicentrarchus labrax* (Linneaus, 1758) (Forniés et al., 2001) and Atlantic cod (Garber et al., 2009), and reduced larval survival rates in rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) (Bonnet et al., 2007a). Increased alevin abnormalities in rainbow trout were also associated with GnRHa treatment (Bonnet et al., 2007a,b). These negative observations reinforce the importance of optimising GnRHa induction for each species and carefully selecting females to be induced based on oocyte development.

Functional genetics approaches have shown that translation of maternally-inherited mRNAs are vital for the development of the early embryo (e.g., embryonic cleavage, development of body axes), before zygotic transcription takes place (Traverso et al., 2012). Spawning induction using GnRHa has been shown to affect, presumably indirectly, the maternally-inherited mRNA levels of genes involved in various functions (e.g., cell proliferation, apoptosis, enzyme activity, RNA binding, molecular transport, etc) in the eggs (Bonnet et al., 2007b). In the case of rainbow trout, it was shown that the egg mRNA levels of apolipoprotein C1 (APOC1) were markedly increased due to GnRHa treatment (Bonnet et al., 2007b). Apolipoprotein C1 is involved in the down-regulation of lipoprotein lipase (LPL) (Bonnet et al., 2007b), which is thought to be important in lipid incorporation into the oocyte of European sea bass (Ibáñez et al., 2008) and eel *Anguilla australis* (Richardson, 1841) (Divers et al., 2010). Sufficient lipid reserves in the egg are vital as fuel for the developing embryo and larvae (Ronnestad et al., 1994, 1998; Silversand et al., 1996; Wiegand, 1996).

There was little relationship between oil globule diameter and egg diameter in naturally spawned eggs (Fig. 3), whereas in the GnRHa treatment the smaller eggs often also had smaller oil globules. The observation that oil globule size remained relatively unchanged regardless of egg size under natural spawning further reinforces its likely importance as post-hatching energy reserve for the developing larvae, as has been reported for various marine species (Chambers et al., 1989; Ronnestad et al., 1994, 1998 Silversand et al., 1996).

In capelin *Mallotus villosus* (Müller, 1776), oil globule size was highly correlated with post-hatching lifespan (Chambers et al., 1989). Interestingly, yolk size was also highly correlated with oil globule size in capelin (Chambers et al., 1989), the former being the main energy source for the developing embryo and at early larval stages in various species (Silversand et al., 1996; Wiegand 1996; Ronnestad et al., 1998). If such a correlation also occurs in yellowtail kingfish, it would suggest that GnRHa treatment incurs a nutritional cost in the embryos and larvae produced from smaller eggs. Indeed, this cost may partly explain the lower hatch rate and higher incidence of larval deformity/mortality associated with GnRHa-treatment in many species (Forniés et al., 2001; Bonnet et al., 2007a,b; Garber et al., 2009). It is possible that GnRHa treatment caused eggs to be ovulated prematurely, before the process of oocyte lipid accumulation had been fully completed.

5. Conclusions

Gonadotropin-releasing hormone analog slow-release implants increase the number of spawning females in yellowtail kingfish. However, clear reductions in various measures of egg quality were observed following GnRHa treatment, which is likely related to suboptimal timing of GnRHa administration in terms of gonadal development. It is recommended that hormonal induction only be performed if there is a clear purpose or benefit, especially in species that readily spawn in captivity. However, although its usefulness for production purposes in yellowtail kingfish is still under question, the study demonstrate the effectiveness of GnRHa treatment in breeding programs as a tool for increasing genetic contribution.

Acknowledgements

We are grateful for the support and assistance of the staff and students at the National Institute of Water and Atmospheric Research (NIWA) Bream Bay Aquaculture Park, Bay of Plenty Polytechnic and University of Otago. We would also like to thank Sheri Johnson and Patrice Rosengrave for their constructive comments on this manuscript, and Judy Hewitt, Bruno Buzatto and Carly Wilson for statistics advice. Funding for this study was received through the National institute of Water and Atmospheric Research Aquaculture and Biotechnology Programme 3 (2012/13 SCI). The funding body did not have a role in the study design, data collection, analyses and reporting of this study.

References

Agulleiro, M.J., Anguis, V., Cañavate, J.P., Martínez-Rodríguez, G., Mylonas, C.C., Cerdà, J., 2006. Induction of spawning of captive-reared Senegal sole (*Solea senegalensis*) using different administration methods for gonadotropin-releasing hormone agonist. Aquaculture 257, 511–524.

Bobe, J., Labbé, C., 2010. Egg and sperm quality in fish. Gen. Comp. Endocrinol. 165, 535–548.

Bonnet, E., Fostier, A., Bobe, J., 2007a. Characterization of rainbow trout egg quality: a case study using four different breeding protocols, with emphasis on the incidence of embryonic malformations. Theriogenology 67, 786–794.

Bonnet, E., Fostier, A., Bobe, J., 2007b. Microarray-based analysis of fish egg quality after natural or controlled ovulation. BMC Genomics 8, 55.

Castro, J., Pino, A., Hermida, M., Bouza, C., Chavarrías, D., Merino, P., Sánchez, L., Martínez, P., 2007. A microsatellite marker tool for parentage assessment in gilthead seabream (*Sparus aurata*). Aquaculture 272 (Suppl. 1), S210–S216.

Castro, L.R., Claramunt, G., Krautz, M.C., Llanos-Rivera, A., Moreno, P., 2009. Egg trait variation in anchoveta *Engraulis ringens*: a maternal response to changing environmental conditions in contrasting spawning habitats. Marine Ecol. Prog. Ser. 381, 237–248.

Chambers, R.C., Leggett, W.C., Brown, J.A., 1989. Egg size, female effects, and the correlations between early life-history traits of capelin, *Mallotus villosus*—an appraisal at the individual level. Fishery Bull. 87, 515–523.

Divers, S.L., McQuillan, H.J., Matsubara, H., Todo, T., Lokman, P.M., 2010. Effects of reproductive stage and 11-ketotestosterone on LPL mRNA levels in the ovary of the shortfinned eel. J. Lipid Res. 51, 3250–3258.

Fernández-Palacios, H., Schuchardt, D., Roo, J., Hernández-Cruz, C.M., Izquierdo, M., 2013. Multiple GnRHa injections to induce successful spawning of wild caught greater amberjack (*Seriola dumerili*) matured in captivity. Aquacult. Res., http://dx.doi.org/10.1111/are.12330.

Fernández-Palacios, H., Schuchardt, D., Roo, J., Hernández-Cruz, C., Izquierdo, M., 2015. Spawn quality and GnRHa induction efficiency in longfin yellowtail (*Seriola rivoliana*) broodstock kept in captivity. Aquaculture 435, 167–172.
Forniés, M.A., Mañanós, E., Carrillo, M., Rocha, A., Laureau, S., Mylonas, C.C., Zohar, Y., Zanuy, S., 2001. Spawning induction of individual European sea bass females (*Dicentrarchus labrax*) using different GnRHa-delivery systems. Aquaculture 202, 221–234.
Garber, A.F., Fordham, S.E., Symonds, J.E., Trippel, E.A., Berlinsky, D.L., 2009. Hormonal induction of ovulation and spermiation in Atlantic cod (*Gadus morhua*). Aquaculture 296, 179–183.
Gillanders, B.M., Ferrell, D.J., Andrew, N.L., 1999. Size at maturity and seasonal changes in gonad activity in yellowtail kingfish (*Seriola lalandi*; Carangidae) in New South Wales, Australia. N. Z. J. Mar. Freshwater Res. 33, 457–468.
Hara, M., Sekino, M., 2003. Efficient detection of parentage in a cultured Japanese flounder *Paralichthys olivaceus* using microsatellite DNA marker. Aquaculture 217, 107–114.
Herlin, M., Delghandi, M., Wesmajervi, M., Taggart, J.B., McAndrew, B., Penman, D.J., 2008. Analysis of the parental contribution to a group of fry from a single day of spawning from a commercial Atlantic cod (*Gadus morhua*) breeding tank. Aquaculture 274, 218–224.
Hutchings, J.A., Bishop, T.D., McGregos-Shaw, C.R., 1999. Spawning behaviour of Atlantic cod, *Gadus morhua*: evidence of mate competition and mate choice in a broadcast spawner. Can. J. Fish. Aquat. Sci. 56, 92–104.
Ibáñez, A.J., Peinado-Onsurbe, J., Sánchez, E., Cerdá-Reverter, J.M., Prat, F., 2008. Lipoprotein lipase (LPL) is highly expressed and active in the ovary of European sea bass (*Dicentrarchus labrax* L.), during gonadal development. Comp. Biochem. Physiol. A 150, 347–354.
Knudson, C. (2015). Generalized Linear Mixed Models via Monte Carlo Likelihood Approximation v.1.04. CRAN, <tp:e-path>https://cran.r-project.org/web/packages/glmm/index.html.</doc_ref</tp:e-path».
Kohn, Y.Y., Symonds, J.E., 2012. Evaluation of egg quality parameters as predictors of hatching success and early larval survival in hapuku (*Polyprion oxygeneios*). Aquaculture 342 (343), 42–47.
Larsson, D.G.J., Mylonas, C.C., Zohar, Y., Crim, L.W., 1997. Gonadotropin-releasing hormone analogue (GnRH-A) induces multiple ovulations of high-quality eggs in a cold-water, batch-spawning teleost, the yellowtail flounder (*Pleuronectes ferrugineus*). Can. J. Fish. Aquat. Sci. 54, 1957–1964.
Lokman, P.M., George, K.A.N., Divers, S.L., Algie, M., Young, G., 2007. 11-Ketotestosterone and IGF-I increase the size of previtellogenic oocytes from shortfinned eel *Anguilla australis*, in vitro. Reproduction 133, 955–967.
Marino, G., Panini, E., Longobardi, A., Mandich, A., Finoia, M.G., Zohar, Y., Mylonas, C.C., 2003. Induction of ovulation in captive-reared dusky grouper Epinephelus marginatus (Lowe, 1834), with a sustained-release GnRHa implant. Aquaculture 219, 841–858.
Moran, D., Smith, C.K., Gara, B., Poortenaar, C.W., 2007. Reproductive behaviour and early development in yellowtail kingfish (*Seriola lalandi* Valenciennes 1833). Aquaculture 262, 95–104.
Mugnier, C., Guennoc, M., Lebegue, E., Fostier, A., Breton, B., 2000. Induction and synchronisation of spawning in cultivated turbot (*Scophthalmus maximus* L.) broodstock by implantation of a sustained-release GnRH-a pellet. Aquaculture 181, 241–255.
Mylonas, C.C., Zohar, Y., 2001. Use of GnRHa-delivery systems for the control of reproduction in fish. Rev. Fish Biol. Fish. 10, 463–491.
Mylonas, C.C., Papandroulakis, N., Smboukis, A., Papadaki, M., Divanach, P., 2004. Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using GnRHa implants. Aquaculture 237, 141–154.
Mylonas, C.C., Fostier, A., Zanuy, S., 2010. Broodstock management and hormonal manipulations of fish reproduction. Gen. Comp. Endocrinol. 165, 516–534.
Nagahama, Y., Yamashita, M., 2008. Regulation of oocyte maturation in fish. Dev. Growth Differ. 50, S195–S219.
Poortenaar, C.W., Hooker, S.H., Sharp, N., 2001. Assessment of yellowtail kingfish *Seriola lalandi lalandi* reproductive physiology, as a basis for aquaculture development. Aquaculture 201, 271–286.
Core Team, R., 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Ronnestad, I., Koven, W.M., Tandler, A., Harel, M., Fyhn, H.J., 1994. Energy metabolism during development of eggs and larvae of gilthead sea bream (*Sparus aurata*). Mar. Biol. (Berlin) 120, 187–196.
Ronnestad, I., Koven, W., Tandler, A., Harel, M., Fyhn, H.J., 1998. Utilisation of yolk fuels in developing eggs and larvae of European sea bass (*Dicentrarchus labrax*). Aquaculture 162, 157–170.
Roo, J., Fernandez-Palacios, H., Hernández-Cruz, C.M., Mesa-Rodriguez, A., Schuchardt, D., Izquierdo, M., 2014a. First results of spawning and larval rearing of longfin yellowtail *Seriola rivoliana* as a fast-growing candidate for European marine finfish aquaculture diversification. Aquacult. Res. 45, 689–700.
Roo, J., Fernandez-Palacios, H., Schuchardt, D., Hernández-Cruz, C.M., Izquierdo, M.S., 2014b. Influence of hormonal induction and broodstock feeding on longfin yellowtail *Seriola rivoliana* maturation, spawning quality and egg biochemical composition. Aquacult. Nutr., http://dx.doi.org/10.1111/anu.12188.
Selvamani, M.J.P., Degnan, S.M., Degnan, B.M., 2001. Microsatellite genotyping of individual abalone larvae: parentage assignment in aquaculture. Mar. Biotechnol. 3, 478–485.
Shiraishi, T., Ohshimo, S., Yukami, R., 2010. Age, growth and reproductive characteristics of gold striped amberjack *Seiola lalandi* in the wates off western Kyushu, Japan. N. Z. J. Mar. Freshwater Res. 44, 117–127.
Silversand, C., Norberg, B., Haux, C., 1996. Fatty-acid composition of ovulated eggs from wild and cultured turbot (*Scophthalmus maximus*) in relation to yolk and oil globule lipids. Mar. Biol. (Berlin) 125, 269–278.
Simm, G., 2002. Genetic Improvement of Cattle and Sheep. Farming Press.
Symonds, J.E., Walker, S.P., van de Ven, I., Marchant, A., Irvine, G., Pether, S., Gublin, Y., Bruce, M., Anderson, R.M., McEwan, K.M., 2012. Developing broodstock resources for farmed marine fish. Proc. N. Z. Soc. Anim. Prod. 72, 222–226.
Symonds, J.E., Walker, S.P., Pether, S., Gublin, Y., McQueen, D., King, A., Irvine, G.W., Setiawan, A.N., Forsythe, J.A., Bruce, M., 2014. Developing yellowtail kingfish (*Seriola lalandi*) and hāpuku (*Polyprion oxygeneios*) for New Zealand aquaculture. N. Z. J. Mar. Freshwater Res. 48, 371–384.
Traverso, J.M., Fostier, A., Bobe, J., 2012. Egg transcriptome, the maternal legacy to the embryo. In: Fletcher, G.L., Rise, M.L. (Eds.), Aquaculture Biotechnology. John Wiley and Sons Ltd., Chichester.
Trippel, E.A., Rideout, R.M., O'Reilly, P.T., Herbinger, C.M., Neil, S.R.E., Hamilton, L., 2009. Communal spawning leads to high potential for inbreeding in gadoid culture. Aquaculture 296, 27–35.
Wiegand, M.D., 1996. Composition, accumulation and utilization of yolk lipids in teleosts. Rev. Fish Biol. Fish. 6, 259–286.
Zohar, Y., Mylonas, C.C., 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. Aquaculture 197, 99–136.