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## Developing broodstock resources for farmed marine fish

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### Abstract

The expansion of New Zealand aquaculture will benefit from diversification into new high value finfish such as Yellowtail kingfish (*Seriola lalandi*) and Hapuku (groper, *Polyprion oxygeneios*). To ensure success, domesticated selected broodstock need to be established along with systems that can reliably produce high quality gametes and juveniles. Collections of wild Kingfish and Hapuku broodstock were completed by the National Institute of Water and Atmospheric Research from 2000 onwards. Over 100 wild broodstock have since been reared to establish the parental stocks. Wild females of both species are very fecund and millions of eggs have been produced each breeding season. One of the main challenges is to ensure that multiple parents contribute during spawning to create genetically variable, selected offspring (F1). Due to the current reliance on communal group spawning, multiplex microsatellite DNA marker panels were developed to determine the parentage of the resulting F1 progeny. Although F1 from multiple parents have been identified, some broodstock produced a disproportionate number of the progeny. This has highlighted the need to manage parental contribution to the F1, which would otherwise exclude many individuals from contributing to the gene pool. As a result we are developing techniques to control mating and to maximise the number of founding wild parents in the programme.

**Keywords:** groper; hapuku; kingfish; aquaculture; breeding; DNA markers; parentage

### Introduction

Marine finfish farming is continuously seeking new species suitable for intensive culture. Two new high value finfish, Yellowtail kingfish (*Seriola lalandi*) and Hapuku (groper, *Polyprion oxygeneios*), have been identified by the National Institute of Water and Atmospheric Research (NIWA) as suitable candidates for New Zealand aquaculture due to their rapid growth up to market size and excellent flesh quality and texture. During the past 10 to 12 years, NIWA has undertaken captive breeding programmes for wild caught Hapuku and Kingfish respectively with the aim of evaluating the performance of these species for aquaculture and developing broodstock resources capable of sustaining the future industry.

Wild Hapuku and Kingfish reared in mixed groups of males and females reliably spawn in captivity (communal spawning) (Moran et al. 2007; Anderson et al. 2012). Both species are serial batch spawners producing large volumes of fertilised eggs throughout the spawning season which can last for three to six months each year. However, little is known about their spawning behaviour, mate choice and the ability of individual broodstock to produce viable gametes and progeny during the spawning season. Studies in other marine species have shown that communal spawning results in a disproportionate contribution of eggs and sperm by a few individuals (Hutchings et al. 1999; Herlin et al. 2008; Trippel et al. 2009). This can lead to a reduction in genetic variability among successive generations of broodstock and result in highly inbred progeny.

Therefore a priority of the broodstock development programme for both species was to develop tools to determine the parentage of the progeny produced by communal spawning and if needed, to establish methods to control breeding such as pair mating.

### Materials and Methods

#### *Broodstock and fertilised egg collection*

Broodstock were caught off the East Coast of Northern New Zealand between 2002 and 2009, and held at NIWA, Bream Bay Aquaculture Park. All broodstock were individually tagged and fin clipped. Rearing conditions were similar to those described by Moran et al. (2007) (Kingfish) and Anderson et al. (2012) (Hapuku). During the reproductive season, broodstock spawned naturally and the positively buoyant eggs were collected from the tanks two to three times per day. A sample of at least 60 fertilised eggs at the blastula (Kingfish) or gastrula (Hapuku) stage or later was collected each day from each broodstock tank and preserved in 100% ethanol for subsequent parentage analysis. Percentage fertilisation was assessed according to the method of Anderson et al. (2012).

#### *DNA extraction and microsatellite analyses*

DNA was extracted from fin clips and eggs preserved in ethanol using Chelex 100 and acid-base boiling procedures respectively. Fin clips (1 mm<sup>2</sup>) were submerged in 200 µL extraction buffer (5% Chelex 100, 0.1% Tween20, 4 µg proteinase K) and incubated at 60°C overnight before boiling for 10 minutes to

**Table 1** Number of fertilised eggs produced by each male (M) and female (F) Hapuku parent in broodstock Tanks 1 and 2. Sorted in ascending order by the total number of eggs produced per parent. Broodstock that did not produce any fertilised eggs are not included.

<b>Tank 1</b>		Female parent						<b>Total</b>
Male parent	F4	F9	F6	F5	F8	F7		
M13		3	2	5		4	<b>14</b>	
M3	16	1	5		4	6	<b>32</b>	
M8	9	7	6	8	1	16	<b>47</b>	
M6	3	19	6	12	4	16	<b>60</b>	
M4	18	15	13	12	5	20	<b>83</b>	
M1	12	6	4	16	24	48	<b>110</b>	
M9	10	18	7	34	34	15	<b>118</b>	
M14	37	43	76	39	54	35	<b>284</b>	
<b>Total</b>	<b>105</b>	<b>112</b>	<b>119</b>	<b>126</b>	<b>126</b>	<b>160</b>	<b>748</b>	

  

<b>Tank 2</b>		Female parent							<b>Total</b>	
Male parent	F1	F2	F10	F14	F12	F15	F11	F3		F13
M15							3			<b>3</b>
M2									9	<b>9</b>
M12			1		2	22	3	6	14	<b>48</b>
M7			2	3	12	11		12	14	<b>54</b>
M5			1		3	6	19	28	59	<b>116</b>
M10	1	1	8	39	18	5	29	20	40	<b>161</b>
M11			29	2	11	35	30	52	10	<b>169</b>
<b>Total</b>	<b>1</b>	<b>1</b>	<b>41</b>	<b>44</b>	<b>46</b>	<b>79</b>	<b>84</b>	<b>118</b>	<b>146</b>	<b>560</b>

inactivate the enzyme. Single eggs were homogenised in 100  $\mu$ L of 0.2 M NaOH, boiled for 20 minutes, then 100  $\mu$ L of an acid solution (0.1 M Tris (pH 8.1), 0.2 M HCl) was added.

A combination of published markers and novel microsatellites isolated by genomic (454) sequencing were used to develop parentage testing panels. Both species were sequenced using single pass, long polymerase chain reaction (PCR) reads. Sequences were repeat masked and filtered for quality before simple sequence repeats (SSRs) of 10 to 14 units were identified and primers designed for those with suitable flanking sequences. The SSRs were screened using 8 to 12 samples and all polymorphic markers were run across a further 48 samples to check for information content, Mendelian inheritance and the presence of null alleles. The most suitable markers were combined into multiplex PCR panels. The Hapuku panel consisted of nine SSR markers, five described by Ball et al. (2000) (Pam010, Pam017, Pam021, Pam025, and Pam035) and four identified by sequencing. All eight markers in the Kingfish panel were discovered by genomic sequencing.

PCR conditions were optimised for each multiplex. For both panels the annealing temperature was 56°C with a MgCl<sub>2</sub> concentration of 2.0 mM. The primer concentrations for Hapuku ranged from 0.1  $\mu$ M to 0.6  $\mu$ M, and for Kingfish from 0.1  $\mu$ M to 1.0  $\mu$ M. Using fluorescently labelled primers, the amplification products were run on an ABI3730 genetic analyser (Applied Biosystems) and allele sizes determined using the GeneScan™-500 LIZ® Size Standard and GeneMapper® Software v.3.7 (both Applied Biosystems).

#### *Parentage assignment*

Pedigrees were assigned using a proprietary pedigree analysis programme developed by AgResearch (KG Dodds, Personal communication). DNA profiles of the progeny were compared against all combinations of parents within the tank. The probabilities and limit of detection were calculated to assist in the parentage analysis. For both species > 95 % of pedigrees were uniquely assigned.

#### *Manipulation of mating groups in Kingfish*

**Rotational mating.** In order to increase the possible parental combinations of wild Kingfish, males and females were swapped among tanks once during the ambient spawning seasons (January/February) of 2009/2010 and 2010/2011. Egg production was monitored prior to handling and anaesthetised broodstock were sexed by gonad biopsy. In 2010, males and females were mixed and placed together into three 20 m<sup>3</sup> tanks. In 2011, 40 males and females from six different 20 m<sup>3</sup> tanks were swapped and mixed among the six tanks. Their ability to spawn and produce fertilised eggs was monitored for 30 and 39 days pre- and post-handling in 2010 and 2011 respectively.

**Pair mating.** Production of 1:1 crosses is required to achieve full control over inbreeding. This was tested in the 2008/2009 spawning season, using two pairs, consisting of one male and one female, placed in two separate 10 m<sup>3</sup> tanks and egg production was monitored for at least four weeks post-handling.

**Table 2:** Number of fertilised eggs produced by each male and female kingfish parent in broodstock tanks 1 and 2 in 2007/2008 and 2008/2009. Sorted in ascending order by the total number of eggs produced per parent. Broodstock that did not produce any fertilised eggs are not included.

2007/2008 spawning season										
Tank 1					Female parent					
Male parent	F5	F9	F10	F15	F6	F17	F16	F11	F12	Total
M9					1	2				3
M11			3	8	3	14	28	14	12	82
M12	1	1	3	2	28	36	28	50	70	219
<b>Total</b>	<b>1</b>	<b>1</b>	<b>6</b>	<b>10</b>	<b>32</b>	<b>52</b>	<b>56</b>	<b>64</b>	<b>82</b>	<b>304</b>
Tank 2					Female parent					
Male parent	F7	F4	F2	F8	F14					Total
M8	1	1	3	4	3					12
M5	4	6	21	14	25					70
M6		13	31	47	42					133
<b>Total</b>	<b>5</b>	<b>20</b>	<b>55</b>	<b>65</b>	<b>70</b>					<b>215</b>
2008/2009 spawning season										
Tank 1					Female parent					
Male parent	F16	F2	F11	F15	F12	F10	F17	F14		Total
M8	2	4		15	5	14	1	11		52
M6	1		5	1	8	7	51	12		85
M11	2	4	13	10	17	5	20	15		86
M12		6	10	18	22	28	16	23		123
M5	1	17	5	14	8	15	14	76		150
<b>Total</b>	<b>6</b>	<b>31</b>	<b>33</b>	<b>58</b>	<b>60</b>	<b>69</b>	<b>102</b>	<b>137</b>		<b>496</b>

### *Predicting the parental contribution to the F1 – Hapuku and Kingfish.*

The potential parentage of F1 Hapuku and Kingfish juveniles produced from communal spawning was predicted through analysis of the actual contribution each parent made to the fertilised eggs genotyped. The feasibility of producing an F1 population with sufficient parents for future selective breeding was then deduced. It was assumed a random sample of 2,000 F1 would be reared, tagged and genotyped. Based on these assumptions the number of parents contributing at least 10 F1 progeny to the breeding programme each season was calculated. The predicted level of inbreeding, the inbreeding coefficient (F), was determined from the effective population size (Ne), according to the formula:

$$\Delta F = 1/2 Ne$$

where  $\Delta F$  is the current coefficient of inbreeding (Falconer & Mackay, 1996). As unequal numbers of male and female parents were predicted, the formula for Ne used was:

$$Ne = 4 Nm Nf / Nm + Nf$$

where Nm and Nf are the number of male and females parents respectively per generation.

### *Results*

#### *Hapuku*

**2010 spawning season.** To study the spawning success of individual wild broodstock in captivity, fertilised eggs were collected from two spawning tanks during the 2010 spawning season (August to December) containing a total of 15 male and 26 female broodstock. There were 117 batches of eggs and an estimated 17.2 million fertilised eggs produced. In order to establish which individual broodstock were spawning, 1,351 individual fertilised eggs from 42 batches were genotyped using the Hapuku nine microsatellite multiplex DNA panel and parentage was successfully assigned for 1,308 eggs (96.8 %).

All 15 males contributed to the pool of fertilised eggs sampled compared to only 15 out of 26 (58 %) of the females. The proportion of fertilised eggs produced by each female and male within each tank also varied (Table 1). In Tank 1, the six females that produced fertilised eggs contributed fairly evenly, with between 14.0% to 21.4% of the eggs produced per female. In Tank 2, four of the nine females produced 75% of the eggs. In both tanks, the number of eggs fertilised per male was highly variable and ranged from 1.9% to 38.0% (Tank 1) and 0.5% to 30.2% (Tank 2) per male (Table 1).

**Table 3** Kingfish rotational mating in 2010 and 2011. Egg production prior to and after exchanging Kingfish broodstock among tanks.

Year	Manipulation	Number of egg batches	Percentage fertilisation	Estimated number of eggs (million)
2010	Prior to handling	49	83.8	26.3
	Total after handling	44	83.2	16.5
2011	Prior to handling	115	94.4	102.9
	Total after handling	74	91.2	47.0

**Table 4** The predicted number of parents contributing to the F1 generation, effective population size and inbreeding coefficient.

Species	Year	Total number of broodstock in tank	Number of predicted males	Number of predicted females	Effective population size	Change in inbreeding coefficient per generation (%)
Kingfish	2007/2008	26	7	12	17.7	2.8%
	2008/2009	13	5	8	12.3	4.1%
Hapuku	2010	41	14	13	27.0	1.9%

### Kingfish

#### 2007/2008 and 2008/2009 spawning seasons.

Fertilised eggs were collected from two spawning tanks during the 2007/2008 spawning season (October to February) (Tank 1 contained: 11 females and three males; Tank 2: nine females and three males) and from one tank in 2008/2009 (Tank 1: eight females and five males). There were 152 batches of eggs and an estimated 76 million fertilised eggs produced in 2007/2008 from the two tanks. There were 80 batches of eggs and an estimated 115 million fertilised eggs produced in 2008/2009. In order to establish which individual broodstock were spawning, parentage was determined for 1,015 individual fertilised eggs from 66 batches (2007/2008:  $n = 45$ ; 2008/2009  $n = 21$ ).

In 2007/2008 all six males contributed to the pool of fertilised eggs sampled compared to 14 out of 20 (70%) of the females; this varied by tank with 82% in Tank 1 and 56% in Tank 2. The number of fertilised eggs contributed by each male and female within each tank also varied (Table 2). In both tanks one of the males dominated the spawning and fertilised over 60% of the eggs genotyped. In 2008/2009 combining the broodstock into one tank resulted in less dominance by a single male (Table 2) with the maximum percentage of eggs fertilised by one male being 30%. Fertilised eggs were produced by 8 out of 11 (73%) of the females in the combined tank.

**Rotational mating.** Handling and exchanging Kingfish males and females among tanks had no detrimental impact on their spawning capabilities. The percentage of fertilised eggs produced was similar before and after handling (Table 3). Broodstock continued to spawn uninterrupted and some tanks spawned within 24 hours of handling. However, total fertilised egg production for the same period before and after swapping

broodstock was reduced, especially in 2011. This may reflect seasonal differences in egg production.

**Pair mating.** Two pairs were established in December 2008. Within 24 hours the males were observed pursuing the females in both tanks. The first fertilised eggs (97% fertilisation) were produced four days later from Tank 1. The first eggs were produced from Tank two 37 days later but were unfertilised. Therefore the female was removed and replaced by a third female. This new pair produced fertilised eggs three days later. Overall, 11 batches of fertilised eggs, approximately 5.5 million, were collected from Tank 1, and four batches from Tank 2, approximately 3.0 million.

#### Parental contribution to the F1

Based on the proportion of eggs produced by each parent in the spawning tanks studied, the number of parents potentially contributing to the F1 population each year was predicted (Table 4). The total number of males ( $N_m$ ) and females ( $N_f$ ) contributing each year was low for each species (13 to 27). Unacceptable levels of inbreeding were predicted in this situation.

### Discussion

NIWA has successfully collected and bred from communally reared wild Hapuku and Kingfish broodstock during multiple breeding seasons. Multiplex microsatellite DNA panels were developed for both species and have proved to be effective for the determination of parentage of individual fertilised eggs collected from spawning tanks. The parentage analysis has shown that like other marine species (Trippel et al. 2009; Na-Nakorn, 2010) not all Hapuku and Kingfish broodstock contributed during communal spawning. There was no evidence of any fertilised eggs from 18 to 44% of the females. Individual male contribution also varied considerably with some males dominating

spawning. This was especially true for Kingfish when only a small number of males were placed in the tanks. As a consequence the predicted effective population size of both species was less than the number of available broodstock. This increases the potential for inbreeding where ideally the change in the coefficient of inbreeding should not exceed 1% per generation (Meuwissen & Woolliams, 1994).

The captive populations of wild broodstock of both species reared at the NIWA, Bream Bay facility have increased over the years. Since this study was undertaken additional parents have spawned. However, there is a balance between the resources required to rear increasing numbers of large (20 to 30 kg) marine finfish broodstock versus developing methods to more efficiently spawn the existing broodstock on site and/or replacing broodstock that do not participate in spawning. The rotational mating and pair mating methods tested with Kingfish were successful and demonstrated that this species is amenable to handling and exchange during spawning. It is therefore possible to combine different males and females, either individually or in groups, to generate new families and increase the chances of specific individuals contributing to the next generation. This is particularly useful if key individuals are mated that would otherwise not be represented in the next generation. The identification of dominant individuals also means they can be removed from spawning tanks and replaced with new broodstock to ensure that the effective population size of the future breeding population increases. It is also important to ensure that the sex ratios are relatively balanced to reduce the chances of a small number of males dominating spawning. Maintaining groups of genetically variable F1 populations derived from different spawning years as the foundation for the future industry, is an important and critical investment for New Zealand aquaculture. Therefore NIWA's on-going broodstock management programmes for both species involve implementation of the spawning methodologies developed, monitoring of spawning contribution, and also parentage assignment of the surviving F1 progeny to ensure that F1 from multiple parents are kept for future breeding.

## Acknowledgements

The authors would like to thank technical staff at the NIWA, Bream Bay Aquaculture Park for their invaluable assistance. This research was supported by funding from the Ministry of Science and Innovation.

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