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Genetic population structure of Yellowtail Kingfish (*Seriola lalandi*) in temperate Australasian waters inferred from microsatellite markers and mitochondrial DNA

Penny A. Miller [a,1], Alison J. Fitch [a], Michael Gardner [a,b], Kate S. Hutson [b,2], Graham Mair [a,*]

[a] School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, South Australia, 5001, Australia
[b] Australian Centre of Evolutionary Biology and Biodiversity, School of Earth and Environmental Sciences, The University of Adelaide, DX 650 418, Adelaide, South Australia, 5005, Australia

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ABSTRACT

Expansion of finfish aquaculture will see increased incentive for translocation of stocks across geopolitical boundaries. The potential for genetic contamination of stocks arising from translocation and subsequent release or escape of translocated and/or genetically mixed stocks may be a significant risk to wild populations. Assessment of risk requires knowledge of the local population structure for the species across the range that translocation might occur and it is in this context we undertook a population genetic survey of stocks of Yellowtail Kingfish across temperate Australia and New Zealand. Seven polymorphic microsatellite loci were used to analyse a total of 272 individuals sampled from New Zealand, New South Wales, Victoria, South Australia and Western Australia. A subset of individuals from each sample locality was also assessed for variation at the mitochondrial gene ND4. Pairwise analysis of sample location and Bayesian analysis showed that Western Australia *S. lalandi* were genetically distinct from *S. lalandi* sampled from the other localities. No difference was found between New Zealand *S. lalandi* and eastern (New South Wales) or central (South Australia and Victoria) Australian fish. The mitochondrial analysis supported the microsatellite data with western samples possessing unique haplotypes compared with all other sites which shared haplotypes. With the expansion of the Yellowtail Kingfish aquaculture industry and likely translocation of stocks, there is a need for a review of translocation policies that consider genetic diversity as a factor in the development of Yellowtail Kingfish aquaculture in the region.

1. Introduction

The Yellowtail Kingfish, *Seriola lalandi*, (Perciformes: Carangidae) is widespread in the Atlantic and Pacific Oceans and is an important species for both commercial and recreational fishing in Japan, Australia and New Zealand (Nugroho et al., 2001). In Australia the distribution of *S. lalandi* extends from North Island (10°S) in Queensland, across the eastern and southern coasts of the continent, including the east coast of Tasmania, Lord Howe Island (31°S) and Norfolk Island (29°S), to Trigg Island in Western Australia (32°S) (Love and Langenkamp, 2003). In New Zealand, *S. lalandi* occurs from the Kermadec Islands (29°S) to Foveaux Strait (46°S) (Poortenaar et al., 2003). Yellowtail Kingfish can grow to 2.5 m in length and weigh around 70 kg (Gomon et al., 2008). The ideal water temperature for this species is between 18 and 24 °C (Wolvaardt, 2007). Kingfish usually spawn between October and January in the southern hemisphere (Poortenaar et al., 2001). Juvenile Yellowtail Kingfish are found in surface waters offshore and often shelter within flotsam and floating seaweed suggesting passive dispersal of juvenile *S. lalandi* is possible (Diggles, 2002). Currently, there is limited information on the biology and behaviour of the species and it is unknown whether they exhibit active homing or philopatry; moreover, little is known about the location of spawning grounds for the species.

Several *Seriola* spp. (amberjacks) are important aquaculture fishes. Japan currently leads production with 139,000 t per annum (US $1.1 billion per annum; Poortenaar et al., 2003). In Japan, culture of *Seriola* spp. (*S. quinqueradiata*, *S. dumerili* and *S. lalandi*), accounts for approximately 57% of all Japanese marine fish aquaculture (Ohara et al., 2005). Yellowtail Kingfish are currently cultured in South Australia with production expanding considerably since its initiation in 2001. Annual production is currently 3000–4000 t per annum and is expected to increase in the near future to ≥5000 t (ABARE, 2009). There has been some culture of the species in New South Wales (NSW) and Western Australia (WA) but growth of the sector has been constrained to date by limited access to production sites, high mortality rates, disease and growth deformities (Stephens and Savage, 2010; Tachihara et al., 1997). Aquaculture of this species is also under development in New Zealand (NIWA, 2011). Plans have

* Corresponding author. Tel.: +61 8 82015968; fax: +61 8 82017659, +61 8 82017656.
E-mail address: graham.mair@seafoodcrc.com (G. Mair).
[1] Permanent address: School of Plant Science, University of Tasmania, Private Bag 55, Hobart Tasmania, 7001, Australia.
[2] Permanent address: School of Marine and Tropical Biology, James Cook University, Townsville QLD 4811, Australia.

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been made to initiate a breeding programme for the genetic improvement of Yellowtail Kingfish in Australia which could involve the translocation of germplasm between different locations in the formation of a genetically diverse base population.

The South Australian Yellowtail Kingfish aquaculture industry currently relies on hatchery production derived from wild fish captured in the northern Spencer Gulf (34°S). With the majority of temperate Australasian culture of Yellowtail Kingfish taking place in Spencer Gulf using locally derived broodstock, escapees from aquaculture currently pose no significant risk to the genetic structure of this species. However, with the likelihood of expansion of the industry throughout southern Australia and New Zealand and the prospect of genetically composite and eventually genetically improved stocks of mixed origin being used in aquaculture across the region, it is becoming increasingly important to conduct a comprehensive assessment of the risks associated with translocations of stocks of the species. Whilst a major component of this risk will relate to biosecurity and the threat of transmission of pathogens, the potential for genetic contamination of stocks arising from translocation and subsequent release or escape of translocated and/or genetically mixed stocks may also be a significant component of the risk. Risk assessment requires knowledge of the local population structure for the species across the range over which translocation might occur. Escapees from aquaculture or even deliberate releases of hatchery reared stock, if not sourced from the same location, have the potential to interbreed with wild fish, thus possibly modifying the genetic structure of wild populations. This may result in the contamination of potentially important reservoirs of genetic diversity and break up co-adapted gene complexes in locally adapted stocks. Interbreeding of fish from genetically distinct stocks can potentially reduce the fitness and viability of a species and makes it more vulnerable to extinction over time (Chauhan et al., 2007).

Whilst these issues relate to conservation of indigenous genetic diversity, knowledge of genetic structure is also important to the optimal exploitation of this diversity. Maximising genetic diversity in the formation of a base population for a long term breeding programme is an important consideration. The desire to maximise genetic diversity has to be balanced against the risks of future genetic contamination arising from aquaculture (McClelland and Naish, 2007).

Population genetic surveys can reveal genetic resources available as a base for breeding programmes and determine the potential genetic risk of translocation (Johnson, 2000). It is thus evident that understanding the genetic diversity of an economically important species, such as the Yellowtail Kingfish, is vital to optimising its management and ensuring the long term sustainability of both wild and captive stocks (Jeong et al., 2003).

Little is known about the population structure of Yellowtail Kingfish in Australia and New Zealand. Tag and recapture studies in Australia suggest that most Yellowtail Kingfish are relatively sedentary; with some (both juvenile and adults) moving large distances (Gillanders et al., 2001; Hutson et al., 2007a, b). Recaptures show that wild Yellowtail Kingfish migrate naturally between Australia and New Zealand (Holdsworth and Saul, 1998; Gillanders et al., 2001) which is consistent with evidence from microsatellite and mitochondrial DNA (mtDNA) markers that show no significant difference amongst fish sampled from New South Wales and New Zealand (Nugroho et al., 2001). However, Japanese and New South Wales/New Zealand populations show significant genetic divergence (Nugroho et al., 2001) and there are no records of Yellowtail Kingfish movements between Australian and Asian waters.

The aim of this research was to determine if there are genetically distinct populations of *S. lalandi* in temperate Australasia. The present study builds on previous research by Nugroho et al. (2001) through sampling a wide geographical range across southern Australian waters and utilising several microsatellite markers and mtDNA gene sequences to discriminate populations of *S. lalandi*. MtDNA is known to evolve at a much slower rate compared to microsatellite markers, thus this analysis can provide information on historical changes within this species (Anderson et al., 2010). Population structure is an important factor that should be taken into account by the appropriate regulatory authorities when considering applications for translocation of fish.

2. Materials and methods

2.1. Sample collection and DNA extraction

A total of 272 wild *S. lalandi* were sampled from 17 sites and grouped into the five regions, New South Wales ($n=111$) and New Zealand ($n=91$; eastern), South Australia ($n=37$) and Victoria ($n=18$; central), and Western Australia ($n=15$; western) (Figs. 1 and 2). Fin clips (approx 0.5 cm^2) were taken from each individual and stored in 70% molecular grade ethanol. Total DNA was extracted from tissue samples using the Gentra Puregene® DNA Extraction Kit (Qiagen) following the manufacturer's instructions.

2.2. Microsatellite analysis

Nine previously published microsatellite markers *Sdn03* (Nugroho and Taniguchi, 1999), *Sdu 01*, *Sdu10*, *Sdu16*, *Sdu27* (Renshaw et al., 2006), *Sdu 29*, *Sdu32*, *Sdu37* and *Sdu46* (Renshaw et al., 2007) were successfully amplified by polymerase chain reaction (PCR) in preliminary trials and genotyped for all individuals. Primers were synthesised by Geneworks (Adelaide, Australia) and Applied Biosystems (Foster City, USA) with the reverse primer 5′-labelled with a florescent tag: FAM, PET, VIC or NED. Polymerase chain reactions were carried out in 15 μl volumes comprising 10x Hotmaster *Taq* buffer with magnesium, 200 μM dNTPs, 0.4 μM of each forward and labelled reverse primer, 0.03 U Hotmaster *Taq* DNA polymerase (5Prime) and 2–15 μg genomic DNA. Thermal cycling conditions for each locus were: 2 min at 94 °C, followed by 34 cycles of 94 °C for 30 s, annealing (as previously published) for 45 s and extension at 68 °C for 1 min, with a final extension temperature of 68 °C for 10 min.

Samples were analysed on an ABI 3770 (Applied Biosystems) sequencer using LIZ 500 (−250) size standard by the Australian Genome Research Facility (AGRF, Adelaide). Alleles were scored using Genemapper v. 3.7 software (Applied Biosystems). A minimum 5%, blindly scored, sample repeats were performed within and across plates to reduce genotyping errors.

2.3. MtDNA analysis

Twenty six samples were selected for mtDNA analysis from across the 17 sites: New South Wales ($n=6$), New Zealand ($n=6$), South Australia ($n=4$), Victoria ($n=3$) and Western Australia ($n=7$). An approximately 700 bp fragment of the mitochondrial genome, including the 3′ end of the *NADH dehydrogenase* subunit *4* (*ND4*) gene, the tRNA genes $tRNA^{His}$, $tRNA^{Ser}$, $tRNA^{Leu}$ and the 5′ end of *ND5*, was amplified and sequenced using the forward primer ND4 5′ CACCTATGACTAC-CAAAAGCTCATGTAGAAGC 3′ (Arevalo et al., 1994) with the reverse primer H12293-Leu 5′ TTGCACCAAGAGTTTTTGGTTCCTAAGACC 3′ (Inoue et al., 2001).

PCR amplifications were performed in a total volume of 25 μl containing, 0.02 U Hotmaster polymerase (5 Prime), 1x Hotmaster Taq Buffer with 2.5 mM magnesium (5 Prime), 200 μM of each dNTP and 0.2 μM of each primer. PCR conditions were: 2 min at 94 °C, followed by 34 cycles of 94 °C for 45 s, 52 °C for 45 s, and 70 °C for 1 min; with a final extension of 6 min at 70 °C. PCR products were purified using $MultiScreen_{384}$ PCR Filter Plates (Millipore) following the manufacturer's instructions.