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Genetic parameters for economically important traits in yellowtail kingfish *Seriola lalandi*

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ABSTRACT

The aim of the present study was to estimate genetic parameters for body and carcass traits, visual condition score, and deformity in yellowtail kingfish *Seriola lalandi*, an emerging aquaculture species in Australia. These novel data and genetic parameters are required to solve the problem of how to conduct efficient selection in this and related species. Analyses were performed on a total of 400 data records collected from a yellowtail kingfish breeding population at Cleanseas Tuna Ltd. farm. They were progeny of 22 full- and half-sib families (eight sires and six dams). Six newly developed and four published microsatellite markers were used to construct the pedigree. Genetic parameters were estimated using average information algorithm in ASReml with a multiple trait model. Fixed effects included sex, seal bite and deformity status. Random effects were the additive genetics of individual animal, and maternal and common environmental effects (i.e., dam-tank effect arising from a short period of separate rearing of offspring that came from two different broodstock tanks). The estimates of heritability for body and carcass traits were moderate ($h^2 = 0.15$ to 0.30, s.e. ranging from 0.09 to 0.19). Fillet fat content showed an unusually high heritability (0.94 ± 0.21) with a standard animal model, but was only moderate (0.41 ± 0.26) when tank and dam were included as random effects. The estimate for condition score was 0.15 ± 0.11, whereas the heritability for deformity was close to zero ($h^2 = 0.02$). The genetic correlations between body and carcass (fillet weight and fillet yield) traits were high and positive (0.57 to 0.94, s.e. 0.05 to 0.46). Genetic correlations between body traits and condition score were moderate to high and positive (i.e. favourable). These results suggest that selection for high growth would result in concomitant increase in fillet weight, a carcass trait of paramount importance. It is concluded that there is substantial potential for genetic improvement of economically important traits especially growth performance and fillet weight in the current population of yellowtail kingfish.

1. Introduction

The yellowtail kingfish, *Seriola lalandi*, is an emerging aquaculture finfish species in Australia, with a current production approaching four thousand tons at a value of 20 million dollars/year (Booth et al., 2010) although aquaculture production on *Seriola* species in Japan is very large, accounting for most of Japan's finfish aquaculture (Statistics Department, Ministry of Agriculture, Forestry and Fisheries, Japan 2002). In South Australia, where the majority of production occurs, it is the second most commercially valuable aquaculture industry (Fernandes and Tanner, 2008). There is increasing demand for the species in both domestic and export markets with culture technologies being well developed. Yellowtail kingfish is a premium quality product and is marketed as whole live fish or fresh and frozen fillets, cutlets and loins. In the Japanese sashimi market yellowtail kingfish is an extremely valuable product after tuna. However, with steadily reducing catches and quotas for bluefin tuna, the value and demand for yellowtail kingfish will continue to grow (Love and Langenkamp, 2003). Up to the present, quantitative genetic basis of these traits is not known in yellowtail kingfish and there are no reports on genetic associations between body and carcass traits and flesh quality in this species. Genetic associations between performance and fitness-related traits (condition score and deformity) were also not available in the literature for this species (*S. lalandi*). As yellowtail kingfish are group spawners and produce small larvae (about 4 mm at hatching), physical tagging of offspring is unfeasible. DNA markers function as naturally occurring biological tags that can be used in lieu of physical tags, with the added benefit of enabling other biological observations to be made, such as management of genetic diversity and inbreeding of stocks (Frost et al., 2006). The application of genetic markers for parentage

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allocation and pedigree reconstruction have been practiced in other aquaculture species (e.g., Ferguson and Danzmann, 1998; Hara and Sekino, 2003; Jerry et al., 2004; Ninh et al., 2011; Sekino et al., 2005; Vandeputte et al., 2004). The technology has long been recognised as an effective tool to reconstruct the pedigrees of group spawned and communally reared aquaculture populations, allowing high selection intensity in genetic improvement programmes (Doyle and Herbinger, 1995), to account for environmental effects common to full-sibs (Rodzen et al., 2004) and to achieve a high selection responses (Ninh et al., 2011).

In the present study, we used a total of 10 microsatellites to construct the pedigrees for the breeding population of yellowtail kingfish from which genetic parameters were estimated. Specifically we report: i) heritability for body and carcass (fillet weight and fillet yield) traits including fat content, condition score and deformity, and ii) genetic relationships among traits studied. The main aim of this study was to understand the quantitative genetic basis of traits in yellowtail kingfish such as whole and fillet weight, traits for which the market pays for, to start a formal breeding programme for this species.

2. Materials and methods

2.1. Experimental location

The reproduction, larval rearing, fingerling production and grow-out were conducted at Clean Seas Tuna Ltd., Arno Bay (33° 56.202' S 136° 34.500' E), South Australia. Cages were towed from Arno Bay to Port Lincoln, South Australia (latitude 34° 73' S, longitude 135° 86' E) for harvest which is a distance of about 115 km.

2.2. Animal materials

The original broodstock were eight wild males and six wild females, presumed to be 6–10 years old and estimated to be >15 kg each, caught in the Spencer Gulf (i.e., are presumed to come from the same population) and were held into two broodstock tanks at Arno Bay and all broodstock contributed to the offspring. The two tanks spawned a daily average of 1,290,000 and 974,000 eggs. Broodstock were kept at a density of 3–5 kg/m^3, fed commercial diets, and mass spawned on several consecutive nights between 19th and 30th October 2011. The larvae were stocked in hatchery rearing tanks (8 – 14 m^3) at a density of 90/l, and fed rotifers (Artemia) from day 2 until day 15, Artemia was introduced at day 11 and fed until day 25, fish were weaned onto pellet diet around day 22–25. During larval rearing, water temperature was maintained at about 24.5 °C and 12 h photoperiod. The survival rate from hatching to fingerlings varied from 6 to 14%. Fish continued to be reared in the hatchery until around day 70–80 when they reached 6–8 g when they were transferred to a sea cage, where they were on-grown to harvest (average body weight of 3.1 kg ± 0.35; 427 days post spawning) using standard industry practices. At harvest, 400 fish were randomly sampled from the sea cage with approximately 40,000 fish and transported to the processing factory in Port Lincoln in large, ice-filled containers. They were the G1 progeny of 22 full-sibs and half-sibs families. As there were eight sires and six dams, typically each sire or dam had three to four half-sib groups, ranging from two to 50 offspring per group.

2.2.1. Phenotyping

Measurements and tissue sampling were completed at the processing factory in Port Lincoln and at the laboratory at the Lincoln Marine Science Centre. Four hundred animals were taken for measuring but some data were not available because of seal bites and availability from the factory. Measurements included weights, lengths (mouth to caudal fork), and condition score (an arbitrary measure to grade the general appearance of the fish on a 1 to 5 scale, with 1 being the poorest condition and 5 the best condition; a fish that scored 5 had long, wide and deep body shape, appeared to have a heavy body weight for its length and no deformity, by contrast, a fish that scored 1 had deformity, and short and thin body shape).

Deformity was visually recorded and categorised as lower jaw, nasal erosion or operculum, which were the prevalent deformities found in these fish. In the present study, deformity was defined as a binary trait (coded as 1 for fish which had either low jaw, nasal erosion or operculum, and coded as 0 if these clinical signs were absent).

Both sides of skinless fillets were weighed and used to calculate total fillet weight and percent fillet yield (fillet weight/slaughter weight). Gonads were bagged and placed on ice for later visual identification of sex. Approximately 20 g of muscle tissue were bagged and placed on ice for flesh quality assessment (analysis of fat content).

2.3. Chemical analysis of fat

The crude fat content of the flesh was determined with an ethyl acetate extraction method based on the Norwegian Standard method (NS 9402 E) (NSA, 1994). We sampled tissue from approximately the same region in each animal; approximately 30 mm inferior and anterior from the dorsal fin. This region showed the least seasonal variation and corresponds with the 'Mowi cut', which is a standard cut used in examining fat content in salmonids (Bremner, 2010).

2.4. Marker development

2.4.1. Primer design

For this study we used four published microsatellite primer pairs (references following) and developed primers for six additional microsatellite loci from *S. lalandi* transcriptome sequences. Primers for 25 published microsatellites from two other *Seriola* species (*Seriola quinqueradiata* and *Seriola dumerili*) were tested on *S. lalandi* broodstock and four candidates were selected based on polymorphism and scoring accuracy. These four microsatellites were Sequ38 (Ohara et al., 2003), Sdu21 (Renshaw et al. 2006), Sdu32 and Sdu46 (Renshaw et al., 2007). These were tested and validated using the protocols outlined by Renshaw et al. (2007).

Transcriptome sequences from *S. lalandi* liver and digestive systems were generated using the Roche 454 FLX next generation sequencing platform at the Australian Genome Research Facility. Microsatellite sequences were identified using the QDD pipeline (Meglécz et al., 2010) which includes primer design using Primer3 (Rozen and Skaletsky, 2000). Of the 41,765 reads generated, 250 contigs containing microsatellites were identified, from which 70 were found to have a suitable number of repeat motifs (over 20) and suitable flanking regions on which to design primers. Of these 70 microsatellite sequences, 31 candidate primer pairs with similar T_m (around 60 °C) and minimal dimer interaction were selected for further testing. Forward primers for these 31 microsatellites were tagged with the M13(21) 'universal' sequence 5'-TGT AAA ACG ACG GCC AGT -3' to enable two-stage labelling with the use of an additional fluorescent dye labelled M13(21) primer. Primers were optimised by testing under a range of thermal and reaction parameters and a final six of the most specific and polymorphic microsatellites were selected for production. These loci in addition to the four published microsatellites are Sel001, Sel002, Sel008, Sel011, Sel017 and Sel019. A total of six loci developed from our laboratory in addition to four published loci made up a total of 10 microsatellites for parentage assignments. The thermal parameters for primers with M13 sequences were: 94 °C for 3 min, 20 cycles of 94 °C for 30 s, 60 °C for 30 s (decreasing by 0.5 °C for each cycle), 72 °C for 45 s, 20 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 45 s, 72 °C for 10 min. For the primers without M13 sequences the parameters were: 94 °C for 3 min, 40 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 45 s, 72 °C for 10 min. Considering all 10 loci, the average number of alleles detected was 10 ± 2.10, the average observed heterozygosity was 0.70 ± .05 and the polymorphic information content average was 0.66 ± .06.