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Short Communication

Genetic structure of Chilean populations of *Seriola lalandi* for the diversification of the national aquaculture in the north of Chile

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ABSTRACT. *Seriola lalandi* has been recognized as a potential aquaculture species in Chile, however, little is known about the genetic structure of local populations. This is important, as the current production system is based on an initial wild catching and ill management of these stocks can cause reduced genetic variability. To assess the genetic structure of local *S. lalandi* we evaluated 27 published microsatellite markers developed from genomic libraries of other species of the genera. However only 12 markers could be used to properly assess the populations, most of these markers showed deviations from Hardy-Weinberg equilibrium with moderate inbreeding (F = 0.12). This species tends to show schooling behavior, so in all likelihood mating between relatives within small groups of fish is not unexpected. The population structure was assessed using Structure software, showing the presence of admixture with varying levels of individual ancestry. This was seen in both populations, without significant genetic differentiation. This may be explained by the migratory behavior, with mating between different populations likely to happen in small groups. Management of aquaculture resources is essential to secure a sustainable production system; this study is the first to provide estimates of genetic diversity of Chilean populations of *S. lalandi*.

Keywords: *Seriola lalandi*, genetic structure, aquaculture, Chile.

Estructura genética de poblaciones chilenas de *Seriola lalandi* para la diversificación de la acuicultura nacional en el norte de Chile

RESUMEN. *Seriola lalandi* ha sido reconocida como una especie de gran potencial acuícola en Chile, sin embargo, poco se conoce sobre la estructura genética de las poblaciones locales. Esto es importante, ya que el sistema de producción actual se basa en una captura silvestre inicial y una mala gestión puede causar una reducción en la variabilidad genética. Para evaluar la estructura genética de *S. lalandi*, se utilizaron 27 marcadores de microsatélites desarrollados a partir de bibliotecas genómicas para otras especies del mismo género. Sin embargo, sólo 12 marcadores pudieron ser utilizados para evaluar adecuadamente las poblaciones, la mayoría de estos marcadores presentó desviaciones del equilibrio de Hardy-Weinberg, con endogamia moderada (F = 0,12). Esta especie tiende a mostrar comportamiento de cardumen por lo que con toda probabilidad el apareamiento entre parientes dentro de pequeños grupos de peces no es inesperado. La estructura de la población se evaluó mediante el programa computacional Structure, que muestra la presencia de una mezcla con diferentes niveles de ascendencia individual. Esto fue observado en ambas poblaciones, sin diferenciación genética significativa, y se puede explicar por el comportamiento migratorio con apareamiento entre diferentes poblaciones que es probable que ocurra en grupos pequeños. El manejo de los recursos acuícolas es esencial para asegurar un sistema de producción sostenible y este estudio es el primero en proporcionar estimaciones de la diversidad genética de las poblaciones chilenas de *S. lalandi*.

Palabras clave: *Seriola lalandi*, estructura genética, acuicultura, Chile.

Yellowtail kingfish (*Seriola lalandi*) is a pelagic carnivore with a worldwide distribution covering Australia, New Zealand, Japan (Nugroho *et al.*, 2001), South East China Sea (Randall & Lim, 2000), the Mediterranean Sea (Nakada, 2008) and the Pacific coast of America, from Canada up to Chile (Eschmeyer & Herald, 1999; Dyer & Westneat, 2010). Commercial aquaculture production of this species has been successfully established in Japan (Nakada, 2002), Australia (Hutson *et al.*, 2007) and New Zealand (Moran *et al.*, 2008) as demand of yellowtail fishes continuously grows and fishing quotas have been fulfilled (Nakada, 2008). In Chile, production of *S. lalandi* is increasing with the support of governmental agencies as a part of programs for the diversification of the aquaculture industry (PDACH). However, little has been done to understand the behavioral traits and the population diversity of this species in Chile. This is essential in order to establish long-term management and sustainable policies for breeding stock populations in the aquaculture industry (Gjedrem, 2000). In order to assess the genetic population structure of *S. lalandi* in the north of Chile, a total of 185 individuals were sampled from two geographical sites. The first sample consisted of 32 individuals gathered from wild stocks caught in March 2012 in Bahia Inglesa (27°6'0"S, 70°51'20"W), and a second sample of 153 individuals from a broodstock population rallied in 2011 in Punta Frodden (26°95'00"S, 70°81'67"W).

Samples obtained from the anal fin were kept in 95% ethanol until genomic DNA extraction with Macherey-Nagel NucleoSpin® 96 kit following manufacturer's instructions. Primers for genetic analysis, obtained for 27 microsatellite markers developed from genomic libraries of *Seriola dumeriili* (Renshaw *et al*., 2007) and *S. quinqueradiata* (Ohara *et al.*, 2005) (Table 1), that have been successfully amplified in *S. lalandi* (Ohara *et al.*, 2005; Miller *et al.*, 2011), were used so far. Genotyping was conducted using the M13 tail protocol described in Schuelke (2000). PCR protocols were carried out in a MaxyGene Gradient thermocycler (Axygen) in 10 µL final volume.

Reactions were carried out with 1xPCR buffer, 15 ng genomic DNA template, 3 mM $MgCl_2$, 0,083 µM of each dNTP and 0.525 units of TopTaq DNA Polymerase (Fermentas). Primer concentration was optimized to 0.25 mM forward, 1 mM reverse and 1 mM M13 fluorescent labeled oligos. Four different labels were used, 6-FAM, NED, PET and VIC to optimize fragment analysis. Fragment analysis was carried out in an ABI 3730 DNA Analyzer. A preliminary analysis revealed 15 markers showing evidence of stutter, large allele dropout, null alleles or weak signal. All these markers were discarded, therefore a total of 12 markers were used for the genetic analysis (see below).

Allele fragment size was first assessed for binning using a power function in Tandem v1.09 (Matschiner & Salzburger, 2009). Genotyping was carried out using the auto-binning option in Genemapper v3.2 (Applied Biosystems) with the binning information previously described. To assess the presence of null alleles and scoring errors Microchecker v2.2.3 algorithm was used with 1000 randomizations (Van Oosterhout *et al.*, 2004).

Genetic parameters were calculated in Arlequin v3.11 (Excoffier *et al.*, 2005). Hardy-Weinberg equilibrium (HWE) analysis showed the presence of several markers departing from HWE in both populations. Additionally, fixation index (Fst) demonstrated that there were no significant differences in terms of allele frequencies between populations. Finally, the average inbreeding coefficient (Fis) was significant within populations.

In order, to determine the presence of a subpopulation structure, the software Structure was used (Pritchard *et al.*, 2000; Falush *et al.*, 2003). The program implements a Bayesian model, conditional on the number of clusters (K = 1-7) and on variation of unlinked markers using a Monte Carlo Markov Chain. Analysis was run assuming correlated allele frequencies and the degree of admixture (α) was inferred from the data. Convergence was assumed, when the variability between α runs is at a minimum value (usually 0.2). When this is the case, individuals have been consistently assigned to one cluster and the distribution of the cluster assignment of each individual is very similar between runs (Pritchard *et al*., 2000). In order, to determine the most likely number of clusters (K) that describe the populations sampled, we used the methodology developed by Evanno (Evanno *et al.*, 2005). In our samples, the best number of clusters was equal to 2, meaning that at least two subpopulations best explain the data (Fig. 1). However, the two clusters identified by Structure do not show a skewed distribution between groups, as individuals from both populations could not be assigned, based on the probability of assignment (Fig. 2). This admixture profile could be explained by migration events, which are common in yellowtails (Gillanders *et al.*, 2001).

In accordance with the finding that the two subpopulations show similar patterns of within population variability, we evaluate the relatedness of individuals within and between these sampled groups. We used TrioML and DyadML estimators as these allow for inbreeding to be considered in the analysis using the Coancestry software (Wang, 2011). For TrioML a third individual was incorporated as control

Table 1. *Seriola lalandi* genetic parameters at 12 microsatellite loci. [+]In relation to the total for each group considered. *Significance at $P < 0.05$. Ho: observed heterozygocity, He: expected heterozygocity, Fis: inbreeding coefficient, Fst: fixation index.

	Locus	Sdu_46	Sequ_20	Sequ_29	Sequ_41	Sequ_42	Sequ_47	Sequ_52	Sequ_57	Sequ_114	Sequ_165	Sequ_216	Sequ_233	Overall
Brood-stock	Individuals	142	123	122	119	59	93	125	117	132	42	46	91	101
	% of genotyped individuals[+]	93	80	80	78	39	61	82	76	86	27	30	59	66
	Alelles	8	27	16	12	18	16	10	15	6	34	10	22	16.17
	He	0.68	0.92	0.89	0.76	0.93	0.88	0.78	0.86	0.33	0.95	0.66	0.93	0.80
	Ho	0.70	0.89	0.90	0.82	0.73	0.80	0.79	0.86	0.30	0.50	0.52	0.71	0.71
	Allelic richness	6.07	19.63	12.83	8.82	16.25	12.81	7.55	12.02	4.32	28.90	9.58	18.12	13.08
	HWE test (*P*-value)*	0.01	0.26	0.00	0.12	0.00	0.05	0.03	0.00	0.09	0.00	0.01	0.00	-
	Fis	-0.04	0.03	-0.01	-0.08	0.22	0.10	-0.02	-0.01	0.08	0.48	0.21	0.23	0.11
Wild	Individuals	28	28	24	18	6	15	28	30	31	9	9	7	19
	% of genotyped individuals[+]	88	88	75	56	19	47	88	94	97	28	28	22	61
	Alelles	5	20	14	7	6	10	7	16	4	8	6	8	9.25
	He	0.63	0.92	0.92	0.83	0.86	0.89	0.75	0.90	0.29	0.89	0.83	0.93	0.80
	Ho	0.71	0.79	0.79	1.00	0.17	0.47	0.89	0.93	0.32	0.67	0.56	0.29	0.63
	HWE test (*P* -value)*	0.87	0.01	0.68	0.45	0.00	0.00	0.11	0.24	1.00	0.06	0.01	0.00	-
	Fis	-0.13	0.15	0.14	-0.21	0.82	0.48	-0.19	-0.04	-0.11	0.26	0.34	0.71	0.23
Overall	Individuals	170	151	146	137	65	108	153	147	163	51	55	98	120
	% of genotyped individuals[+]	92	82	79	74	35	58	83	79	88	28	30	53	65
	Alelles	8	27	18	12	21	18	10	17	6	37	10	23	17.25
	He	0.67	0.92	0.90	0.77	0.94	0.89	0.77	0.87	0.32	0.95	0.70	0.93	0.80
	Ho	0.71	0.87	0.88	0.85	0.68	0.75	0.81	0.88	0.31	0.53	0.53	0.68	0.71
	Allelic richness	5.91	19.73	13.16	8.72	18.32	13.79	7.38	12.70	4.33	28.32	9.62	18.58	13.38
	Probability of exclusion	0.62	0.96	0.93	0.77	0.97	0.92	0.74	0.90	0.30	0.98	0.73	0.96	1.00
	Fis	-0.05	0.05	0.01	-0.10	0.27	0.15	-0.05	-0.01	0.05	0.44	0.24	0.27	0.12
	Fst													0.01

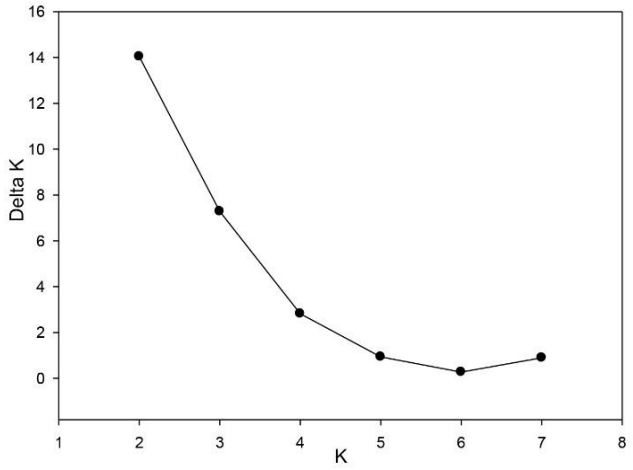

Figure 1. Mean magnitude of delta K using the Evanno Method (Evanno *et al*., 2005), considering 10 chains run per each K evaluated. Higher values indicate the best number of cluster (subpopulations, K), explaining the data.

(outgroup) in order to reduce the likelihood of sampling two identities by state (IBS) alleles and therefore overestimate relatedness. Results showed that individuals within sampled groups are closely related (mean relatedness varying from 0.11 to 0.13), and this was similar both between and within populations and between the TrioML and DyadML estimators (Table 2). These results suggest that mating between relatives is occurring within these populations. Additionally results clearly suggest that schooling behavior of yellowtail king fish and courtship behavior of adult fish involving only a couple or at most 1 female and 2 related males (Moran *et al.*, 2007) could be causing deviations from HWE through closely related mating batches of individuals in the population (Sakakura & Tsukamoto, 1997).

Taken together, the results suggest that the individuals from the two populations belong to a single *S. lalandi* population. Nevertheless, this population is

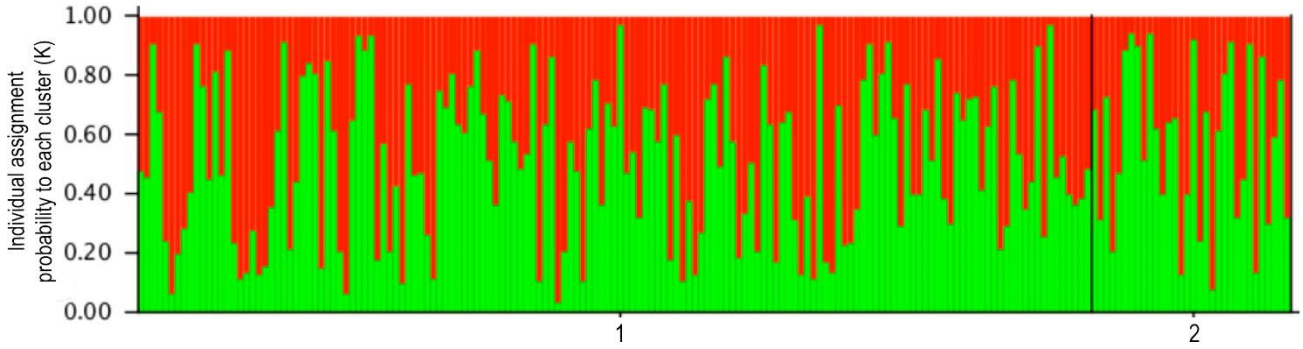

Figure 2. Cluster assignment of for two inferred subpopulations (green and red) in the brood-stock (1) and wild (2) populations.

Table 2. Relatedness within and between the two groups sampled, using TrioML and DyadML estimators. No significant differences were observed between groups with a 95% bootstrapping confidence interval.

		TrioML	DyadML
Brood-stock	Mean	0.11656	0.13267
(11628 pairs)	Variance	0.03294	0.03894
Brood-stock xWild	Mean	0.11463	0.13391
(4896 pairs)	Variance	0.03285	0.04076
Wild	Mean	0.11698	0.14026
(496 pairs)	Variance	0.03042	0.04200
Average	Mean	0.11336	0.13325
(17020 pairs)	Variance	0.03189	0.03956

admixed, probably due to migration of different south Pacific populations, and inter-mating. Long migration patterns are common in *S. lalandi* (up to 3000 km of migration) individuals; this can help display the unique genetic structure pattern of both populations (Gillanders *et al.*, 2001). On the other hand, natal homing conduct (Hutson *et al.*, 2007) and schooling behavior up to sexual maturity age have also been described for yellowtails. The latter supports the considerable mean inbreeding and relatedness coefficients obtained in this work. It is interesting to note that the brood-stock population showed little evidence of a genetic bottleneck, if we consider the similarity of the genetic parameters to its natural population.

From an aquaculture perspective, these results gave important information regarding pedigree management of this species in captivity. *Seriola lalandi* is a pelagic species that spawn spontaneously; therefore, it is not possible to perform contained external fertilization as in other aquaculture species (*e.g.*, salmon). Under these circumstances, markers are essential to keep the pedigree

Table 3. Individual inbreeding for each of the populations analyzed. No significant differences were observed between groups with a 95% bootstrapping confidence interval.

		TrioML	DyadML
Breeding stock	Mean	0.1183	0.1271
n = 153	Variance	0.016	0.018
Wild	Mean	0.139	0.148
n = 32	Variance	0.021	0.023

records, through paternity assignment. This is important in order to maintain the inbreeding rates to acceptable levels, and to select brood stocks based on estimated breeding values using pedigree and marker information. For these to be efficient markers should be relatively easy to be genotyped, and have sufficient variability. The exclusion probability for the set of markers is 1, which is sufficient to accurate pedigree assignment in the brood-stock population. Nevertheless, the use of non species-specific markers is not ideal since many of them may fail to amplify in different populations. We are currently developing a panel of single nucleotide length polymorphisms (SNPs) obtained from massive transcriptome sequencing (Martínez *et al*., 2013; data not shown), that can be used for further pedigree assessment and marker assisted selection giving scope for sustainable breeding programs in this species.

S. lalandi is opening its way as an alternative for diversification of Chilean aquaculture industry, nowadays almost completely dominated by Atlantic salmon. In this context, population studies, which describe the genetic diversity of potentially important aquaculture species present in Chilean coastal waters, such as the yellowtail kingfish, can provide important information for brood-stock aquaculture development. The results of this study provide, for the first time, estimates of genetic diversity in these species, and that there is a scope for properly manage this resource for aquaculture purposes.

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REFERENCES

Dyer, B.S. & M.W. Westneat. 2010. Taxonomy and biogeography of the coastal fishes of Juan Fernández Archipelago and Desventuradas Islands, Chile. Rev. Biol. Mar. Oceanogr., 45: 589-617.

Eschmeyer, W.N. & E.S. Herald. 1999. A field guide to Pacific coast fishes: North America. Houghton, Mifflin, New York, 352 pp.

Evanno, G., S. Regnaut & J. Goudet. 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. Mol. Ecol., 14: 2611-2620.

Excoffier, L., G. Laval & S. Schneider. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinfor., 1: 47.

Falush, D., M. Stephens & J.K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics, 164: 1567-1587.

Gillanders, B.M., D.J. Ferrell & N.L. Andrew. 2001. Estimates of movement and life-history parameters of yellowtail kingfish (*Seriola lalandi*): how useful are data from a cooperative tagging programme? Mar. Fresh. Res., 52: 179-192.

Gjedrem, T. 2000. Genetic improvement of coldwater fish species. Aquacult. Res., 31: 25-33.

Hutson, K.S., I. Ernst & I.D. Whittington. 2007. Risk assessment for metazoan parasites of yellowtail kingfish *Seriola lalandi* (Perciformes: Carangidae) in South Australian sea-cage aquaculture. Aquaculture, 271: 85-99.

Matschiner, M. & W. Salzburger. 2009. TANDEM: integrating automated allele binning into genetics and genomics workflows. Bioinformatics, 25: 1982-1983.

Miller, P.A., A.J. Fitch, M. Gardner, K.S. Hutson & G. Mair. 2011. Genetic population structure of yellowtail kingfish (*Seriola lalandi*) in temperate Australasian waters inferred from microsatellite markers and mitochondrial DNA. Aquaculture, 319: 328-336.

Moran, D., C.K. Smith, B. Gara & C.W. Poortenaar. 2007. Reproductive behaviour and early development in yellowtail kingfish (*Seriola lalandi* Valenciennes, 1833). Aquaculture, 262: 95-104.

Moran, D., R.M. Wells & S.J. Pether. 2008. Low stress response exhibited by juvenile yellowtail kingfish (*Seriola lalandi* Valenciennes) exposed to hypercapnic conditions associated with transportation. Aquacult. Res., 39: 1399-1407.

Nakada, M. 2002. Yellowtail culture development and solutions for the future. Rev. Fish Sci., 10: 559-575.

Nakada, M. 2008. Capture-based aquaculture of yellowtail. In: A. Lovatelli & P.F. Holthus (eds.). Capture-based aquaculture. Global overview. FAO Fish. Tech. Pap., 508: 199-215.

Nugroho, E., D.J. Ferrell, P. Smith & N. Taniguchi. 2001. Genetic divergence of kingfish from Japan, Australia and New Zealand inferred by microsatellite DNA and mitochondrial DNA control region markers. Fish. Sci., 67: 843-850.

Ohara, E., T. Nishimura, Y. Nagakura, T. Sakamoto, K. Mushiake & N. Okamoto. 2005. Genetic linkage maps of two yellowtails (*Seriola quinqueradiata* and *Seriola lalandi*). Aquaculture, 244: 41-48.

Van Oosterhout, C., W.F. Hutchinson, D.P. Wills & P. Shipley. 2004. Microchecker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Resour., 4: 535-538.

Pritchard, J.K., M. Stephens & P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945-959.

Randall, J.E. & K.K.P. Lim. 2000. A checklist of the fishes of the South East China Sea. Raffles Bull. Zool., S8: 569-667.

Renshaw, M.A., J.C. Patton, C.E. Rexroad & J.R. Gold. 2007. Isolation and characterization of dinucleotide microsatellites in greater amberjack, *Seriola dumerili*. Conserv. Genet., 8: 1009-1011.

Sakakura, Y. & K. Tsukamoto. 1997. Effects of water temperature and light intensity on aggressive behavior in the juvenile yellowtails. Fish. Sci., 63: 42-45.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol., 18: 233-234.

Wang, J. 2011. A/b: a program for simula-ting, estimating and analysing relatedness and inbree-ding coefficients. Mol. Ecol. Resour., 11: 141-145.

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