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Estimating sex ratio in North Pacific Albacore (*Thunnus alalunga*) Using Genetic Methods

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Abstract

Albacore (*Thunnus alalunga*) is a pelagic tuna species that supports a lucrative fishery worldwide. Like all tuna species, Albacore are not sexually dimorphic. This means that accurate identification of sex in Albacore is only possible through direct observation of gonads. This process is costly, time consuming, and lethal, and often necessitates histological confirmation of sex due to the large numbers of immature animals captured in some fisheries. Genetic methods have shown to be successful in facilitating sex determination via PCR and DNA sequencing. We used a PCR-based method to genetically determine sex in 1255 Albacore and show that larger size classes are dominated by male fish.

Introduction

Albacore (*Thunnus alalunga*) is a pelagic tuna species that supports a lucrative fishery worldwide. Currently, Albacore in the Pacific is managed as two separate stocks, one in the North Pacific Ocean and one in the South Pacific Ocean. Albacore are highly migratory and, as such, management requires an international effort. The Albacore Working Group (ALBWG) of the International Scientific Committee for Tuna and Tuna-like Species in the North Pacific Ocean (ISC) is tasked with conducting regular stock assessments of North Pacific Albacore to estimate population parameters, summarize stock status, and develop scientific advice on conservation needs for fisheries managers. These assessments are provided to the Regional Fishery Management Organizations (RFMOs) tasked with managing this stock. The most recent stock assessment for North Pacific Albacore was completed in 2023 (ALBWG 2023).

In the North Pacific Albacore assessment, based on the estimated sex-specific growth, natural mortality and limited anecdotal information, it is assumed that sex ratio is approximately 1:1 until albacore reach age 3, after which males become more common due to a higher estimated mortality in females beyond this age (2017 ISC assessment). In some fisheries, the sex ratio is heavily male biased for albacore >100 cm. Whether these phenomena are related to selectivity of the fishery or reflective of natural processes is unclear. The 2023 North Pacific Albacore stock assessment identified a lack of sex-specific size data as a source of uncertainty and suggested the collection of sex-specific age-length samples and the collection of sex ratio data by fleet.

Like all tuna species, Albacore are not sexually dimorphic. This means that accurate identification of sex in Albacore is only possible through direct observation of gonads. This process is costly, time consuming, and lethal, and often necessitates histological confirmation of sex due to the large numbers of immature animals captured in some fisheries. As a result, the sex ratio and sex-specific size information for Albacore fisheries which operate throughout the North Pacific Ocean are difficult to obtain. Given the identified uncertainty of sex-specific size data in the 2023 assessment, tools that can improve the efficiency of determining the sex of an individual are highly desirable.

Over the past decade, and with the advantages of high-throughput, next-generation DNA sequencing technology, genetic methods for the determination of sex in fishes have been developed for several fishes. In 2019, Suda et al. published an improved genome for the Pacific Bluefin Tuna (PBF; *T. orientalis*) and identified regions of the genome that contained multiple single nucleotide polymorphisms (SNPs) that corresponded with the sex of the individual. Suda

et al. (2019) developed three simple PCR assays that were able to identify male PBF individuals based on these sex-linked SNPs.

Given the close relationship and genetic similarity between PBF and Albacore, Chiba et al. (2019) tested the efficacy of these assays in Albacore from port samples in Japan, finding good concordance between phenotypic and genetic sex (n=105; 94.3%, 100%, and 97.1% for Suda et al.'s 2019 Pair I, II, and III, respectively). We used a modified version of primer set II of Suda et al. (2019) to facilitate medium-throughput, PCR-based analysis.

Methods

Tissue samples (fins clips and muscle) were collected by various groups either on-board fishing vessels or shortly after landing. DNA was extracted by combining ~5mg tissue (fin clip or muscle) with 150µL 10% Chelex resin beads suspended in deionized water and heated to 60˚C for 20 minutes, followed by 103˚C for 25 minutes. PCR was performed using fluorescently labeled male-specific primer pair II of Suda et al. (2019) and a fluorescently labeled primer pair that amplified a portion of the 12S ribosomal RNA as an internal positive control (Table 1). The fluorescently labeled primer allowed for PCR produced to be visualized on an ABI 3730 genetic analyzer allowing for 96 samples to be visualized in an automated manner in a single run which is more efficient than simple agarose gel electrophoresis methods for large sample numbers. 10ul PCR reactions were prepared using 5ul Qiagen Multiplex Mastermix Plus, 1ul of each male-specific primer at 10uM, 0.2ul of each 12S primer at 10uM, 0.7ul diH2O, and 1ul DNA (~10-100ng). The male-specific primer set is very similar to the female sequence and initial PCR trials following Chiba et al. (2021) produced instances of non-specific binding of the male-specific primer pair in histologically confirmed female samples. Therefore, the annealing temperature was raised and the number of cycles reduced until this non-specific binding was undetectable. The optimized thermal cycling protocol was a two-step protocol as follows: 95C for 5 minutes, followed by 29 cycles of 95C for 30sec and 65C for 45sec. Genetic sex determination was compared with histological sex determination for 87 individuals for which both data types were available (SWFSC unpublished data).

Results

Overall, we assigned sex using genetic methods to 1,255 individuals. Among these, histological data were available for 87 samples, of which only one sample was a mismatch with the genetically assigned sex resulting in 98% accuracy of the genetic method. In total, there were 831 males and 424 females yielding an overall sex ratio of the sampled individuals of 1.96:1 male:female (M:F). This ratio, however, was not consistent across size classes. Sex ratio was relatively consistent across size bins up to the 90cm bin and ranged from 1.2 to 1.6:1 M:F (Table 2, Fig. 1). In size bins greater than 90cm, the sex ratio increasingly shifted to male dominance with each subsequent size class. The M:F ratio was 2.9:1, 18.6:1, and 23.0:1 for size bins of 100cm, 110cm, and 120cm, respectively. This pattern appeared consistent across fisheries when excluding size bins with fewer than ten individuals (Table 2, Fig. 1).

Discussion

Sex ratio is an important component when evaluating population dynamics, especially if unequal contributions by males and females are suspected. Given the fact that Albacore are not sexually dimorphic, and that there is an indication that sex ratio of a cohort may change with

age, it is important to develop alternative, non-invasive, and cost-effective methods to determine the sex of an individual. Our results corroborate the use of primer pair II of Chiba et al. (2021) coupled with an internal positive control as a method capable of determining sex in North Pacific Albacore with high accuracy.

It is important to remember that this assay only examines a small region of the Albacore genome that appears to be associated with sex. This genetic "marker" should not be presumed to be a causal factor in the phenotypic display of male or female characters which is almost certainly determined by a host of genetic, epigenetic, and environmental factors during development.

These results corroborate earlier studies which indicated that larger size classes taken by the fishery are dominated by male fish. This highlights the importance of including samples from a wide range of size classes rather than from a specific fishery as fishery behavior (e.g., encounter rate or intentional selection of specific sizes) can influence the observed sex ratio. There are several factors that may explain the dominance of males in larger size classes of fishery samples including differential mortality and growth, and spatiotemporal variation in the distribution of males and females and we suggest these as future courses of study.

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Table 1. Primers used to genetically determine sex in albacore in this study.

Primer Name	Sequence (5' to 3')	Size
sca64_3726411_F	GCAGACAAAAAGCCATTCG	~145
sca64_3726411_R	HEX/CTGATGWCCTCTGTAACACAATCAT	~145
12saL	AAACTGGGATTAGATACCCCACTAT	~470
12sbH	HEX/GAGGGTGACGGGCCGTGTGT	~470

Table 2. Number of males and females and their ratios as determined genetically for the five fisheries from which samples were examined.

Size Bin	HI H&L			HI LL			Japan LL			Taiwan LL			USTROLL		
(cm)	Female	Male	M:F	Female	Male	M:F	Female	Male	M:F	Female	Male	M:F	Female	Male	M:F
0	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
10	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
20	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
30	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
40	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
50	0	0	-	3	3	1.00	0	0	-	0	2	-	34	50	1.47
60	0	0	-	13	31	2.38	6	12	2.00	0	1	-	47	60	1.28
70	0	0	-	27	39	1.44	19	22	1.16	1	4	4.00	23	30	1.30
80	0	0	-	54	66	1.22	4	2	0.50	30	36	1.20	4	17	4.25
90	0	2	-	46	50	1.09	15	14	0.93	30	42	1.40	0	1	-
100	1	5	5.00	37	119	3.22	0	4	-	20	46	2.30	1	0	0.00
110	2	0	-	1	124	124.00	0	0	-	5	25	5.00	0	0	-
120	0	0	-	1	22	22	0	0	-	0	1	-	0	0	-
130	0	0	-	0	0	-	0	0	-	0	1	-	0	0	-
Total	**3**	**7**	**2.33**	**182**	**454**	**2.49**	**44**	**54**	**1.23**	**86**	**158**	**1.84**	**109**	**158**	**1.45**

Figure 1. Proportion of male (blue) to female (red) albacore in 10cm size bins for all samples analyzed and by individual fishery. See Table 2 for sample sizes by fishery and size class. "*" indicate size bins comprised of fewer than 10 individuals.