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An Assessment of Bigeye (Thunnus obesus) Population Structure in the Pacific
Ocean, based on Mitochondrial DNA and DNA Microsatellites Analysis

by

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Introduction

Knowledge regarding population subdivision is central to sustainable fishery management. Uncertainty regarding bigeye stock structure seriously restricts the confidence that scientists and fisheries managers can place in the regional assessments that have been carried out to date. At a national or sub-regional level, fisheries managers need to have a better idea of the broader surrounding population of bigeye from which the fish in their fisheries are drawn.

Examination of mitochondrial DNA markers is now an established technique for elucidating population genetic structure. While there is little differentiation among yellowfin tuna populations for mtDNA variants (Ward *et al.*, 1994; Ward *et al.*, 1997), there are specific mtDNA polymorphisms that are known to differentiate bigeye tuna from the Atlantic and Indian Oceans (Grewe, unpublished data). mtDNA variation can be used for both population structure analysis and confirmation of species identification. Usually around 3-5% (but sometimes as high as 30%) of tuna samples we receive have been mis-identified by collectors, but all can be unequivocally identified by mtDNA examination (Chow and Inoue, 1993).

Until recently, variation in the much larger nuclear genome has been chiefly assessed through allozyme electrophoresis, but more powerful methods are now available. Pre-eminent among these is the detection and analysis of microsatellite variation. Microsatellites show high levels of genetic variation and high mutation rates, meaning that populations are likely to diverge not only by genetic drift but by mutation as well. Microsatellite markers also have the important advantage over allozyme markers in being able to be screened in alcohol-stored tissue or even fin-clip samples. This greatly simplifies sampling logistics.

Microsatellite analysis is a new technique, and while there have as yet been few studies on fish populations, microsatellite screening in cod has revealed substantially more about population structure than either allozyme or mtDNA analysis (Wright and Bentzen, 1994). Initial results from examination of DNA microsatellites in yellowfin tuna indicate more population subdivision is present in the western Pacific than is apparent from either allozyme or mtDNA analysis (Grewe and Ward, unpublished data.). DNA microsatellite data were examined among yellowfin tuna samples from five locations in the Pacific Ocean (Philippines, Coral Sea, Solomon Islands, Fiji, and California). Analysis of data from four DNA microsatellite loci indicated significant heterogeneity, on one locus between samples collected in the western and eastern Pacific, and on a second locus between samples collected in the Philippines and Solomon Islands and those collected in Fiji and the Coral Sea. Although preliminary, these data are the first indication of genetic structure within what has been assumed previously to be a single Western Pacific yellowfin tuna stock. The data collected thus far are most encouraging, and suggest a finer-scale resolution of yellowfin tuna population structure within the Pacific Ocean than has hitherto been achieved with allozyme and mtDNA markers. These same microsatellite polymorphisms from yellowfin tuna can be used to examine bigeye tuna, substantially reducing the time and cost of the development phase associated with microsatellite analysis.

The present study examines variation of mitochondrial DNA and DNA microsatellite markers among bigeye tuna sampled from various regions throughout the

Pacific ocean. Comparison of these marker frequencies among sampling sites provides an assessment of bigeye population structure in the Pacific Ocean.

Methods

Sampling logistics.

Samples of bigeye tuna were collected from several locations within the Pacific Ocean (Figure 1). Between 69 to 96 fish were examined from each location (Table 1). Approximately 0.5 grams of tissue sampled from individual fish was preserved in ethanol and transported to the CSIRO Marine labs for analysis. DNA from these tissues was extracted using a modified CTAB extraction protocol described in Ward et. al (1994).

DNA Analysis:

Genetic analysis involved the assessment of mitochondrial DNA (mtDNA) and nuclear DNA microsatellite variation. mtDNA variation was examined through restriction digestion of a selected fragment of the mitochondrial DNA genome. The fragment (ATCO) used contains the flanking region between ATPase-6 and cytochrome oxidase subunit III genes and was amplified via the Polymerase Chain Reaction (PCR) using primers described by Chow and Inoue (1993). The restriction enzyme *MseI* was used to produce diagnostic patterns to confirm species identity.

DNA microsatellite loci examined in this study were previously isolated from yellowfin tuna (Grewe, unpublished data). Locus designations were derived from the clonal isolate number from which each locus was sequenced. Primers used to assay fish in the current study were synthesised for ten microsatellite loci. One primer from each locus pair was end labelled with the fluorescent dye 6-FAM. PCR amplifications were carried out using standard conditions in a PE-Applied Biosystems 9600 thermocycler. Microsatellite fragment products for each locus were separated on a PE-Applied Biosystems ABI-377 automated DNA sequencer and sized using GENESCAN collection software. Additional genotyping was then completed using ABI GENOTYPER software.

Statistical Analysis

Variation in microsatellite and mtDNA allele frequencies among sub-populations was assessed using standard monte-carlo chi-square approaches. Significant differences in frequencies would indicate that collection localities represent areas that contain genetically distinct groups. The extent of such differentiation allows an estimate to be made of the number of migrants exchanged between such groups.

Results

Examination of the ATCO fragment revealed three restriction patterns. One of these patterns was diagnostic for yellowfin tuna while the others were diagnostic for

bigeye. One of the two bigeye patterns was rare and present in only two of the 96 individuals from Hawaii and one of 96 Philippine fish.

Two of the 96 Coral Sea and 10 of the 96 East Pacific-1 fish were identified as yellowfin tuna. Interestingly, fish which had the mtDNA pattern diagnostic for yellowfin tuna also contained DNA microsatellite genotypes which were common (freq. > 0.50) in yellowfin but rare in bigeye (freq. < 0.002).

The 10 microsatellite loci used were a mixture of perfect (CA) and imperfect or mixed repeat motifs. With the exception of locus 135.a, an imperfect tri-nucleotide repeat, between four and 35 alleles were resolved per locus (Table 2). Locus 135.a, was monomorphic with an identical fragment of 207 base pairs amplified from both bigeye and yellowfin individuals. Four to 35 alleles were observed at the remaining loci. Identification of allelic state for locus 135.b was unreliable with alleles separated by single base pair units. This locus was therefor dropped from further comparison until new primers can be designed to eliminate ambiguous allele calls.

The remaining eight loci were used to examine a sub-sample of 36 fish from each of two sites (Philippines and Ecuador) that represented the extreme ends of the sampling locations of this study. With rare exception, most alleles at each locus were found in both populations (Table 3). Frequencies of alleles present at locus 102, 113, 117, and 121 were no greater than 0.17 with more than half of the alleles present at frequencies less than 0.07. Statistical comparisons indicated that allele frequencies at each loci were not significantly different between these two populations.

Locus 144, 125.a, and 208.a had allele frequencies which were greater than 0.20. These loci and locus 161, which has shown significant variation in yellowfin tuna, were chosen to examine larger numbers of individuals from additional sample locations. Additional fish from Philippines (n=95), Coral Sea (n=94), Hawaii(n=95), and E.Pacific-1 (n=84) have been examined for locus 208.a (Table 3). Allele frequencies among these four populations were also not significantly different. Work is currently in progress to increase numbers of individuals assayed from the extended sample locations listed in Table 1.

Discussion

The analysis of ATCO provided limited variation from which to compare mtDNA haplotype frequency differences among sample locations. Another fragment, with higher levels of variation will be required to further assess population structure of bigeye using mtDNA. Two regions of the mitochondrial genome that have shown promise include the d-loop and ND-4. Primers for these regions have been synthesised and are currently being used to assay the populations.

Analysis of ATCO provided valuable information by identifying individuals which were not bigeye. Initially, microsatellite analysis indicated a high degree of population subdivision. However, this was strictly due to the presence of yellowfin tuna in the sample. The presence of yellowfin among the bigeye collected for this project indicates the necessity of genetic identification of each individual. Mis-identified fish were between 40-60cm in length. Interestingly, yellowfin collected in

this size range for a previous study have been mis-identified in the field as bigeye which may suggest identification of these two species becomes problematic at these lengths.

Analysis of microsatellite allele variation could not provide diagnostic characters which could be used for species due to overlap of allelic characters. However, they did offer some characters which were of rare frequency in one species but common in the other. These characters did permit some degree of recognition that alternative species (ie. yellowfin) were present in a sample of bigeye.

Six of the nine loci examined had greater than 20 alleles segregating in each population. The number of alleles present at each locus did not appear to be related to the type of repeat motif but rather seemed closely linked to the number of repeat units in the original clone isolated from yellowfin tuna. This perhaps reflects the likelihood that the most common allele will be cloned during construction of the microsatellite library. Thus, when a small repeat was found it usually corresponded to a locus for which there was limited size variation and only a few alleles were present in the population. Correspondingly, if a large allele was cloned and sequenced, this usually meant that a large number of alleles would be found segregating at that locus.

Large numbers of alleles ($n > 25$) observed at some loci will require sample sizes greater than 36 fish to achieve desired precision on estimates of allele frequency. This degree of precision will in turn affect the statistical power of assessments on the degree of differentiation among populations if any is present. Thus, there is limited use for loci such as 113 and 117 with 35 and 27 alleles respectively. This is especially true for these loci where allele frequencies are partitioned evenly across size categories and are at frequencies less than 0.10.

The large numbers of alleles observed for the majority of loci examined indicates that increased numbers of individuals are required to more fully assess variation among the Pacific wide bigeye sampling locations. With the exception of 125.a and 144, there were greater than 20 alleles segregating each locus. Thus the lack of significant differentiation between the Philippine and Ecuador samples was hardly surprising given a sample size of 36 fish per population and a minimum of 20 alleles present. Analysis of 72 to 100 fish should help address the issue of sample size and large numbers of alleles. Comparison of results between two less variable loci (125 and 144) versus two with higher allele numbers (161 and 208) should also help to guide which loci will be most useful in future examinations of population structure.

References :

- Chow, S. and S. Inoue. 1993. Intra- and interspecific restriction fragment length polymorphism in mitochondrial genes of *Thunnus* tuna species. National Research Institute of Far Seas Fisheries. 30:207-225
- Ward, R.D., Elliott, N. G., Grewe, P. M. and Smolenski, A. J. (1994). Allozyme and mitochondrial DNA variation in yellowfin tuna (*Thunnus albacares*) from the Pacific Ocean. Marine Biology. 118:531-539.

Ward, R.D., Elliott, N. G., Innes, B. H., Smolenski, A. J., and Grewe, P. M. (1997).
Global population structure of yellowfin tuna (*Thunnus albacares*) inferred from
allozyme and mitochondrial DNA variation. In press.

Figure 1. Approximate sampling locations where fish were collected from for the current project. Corresponding longitude and latitudes for each sample location are listed in table 1.

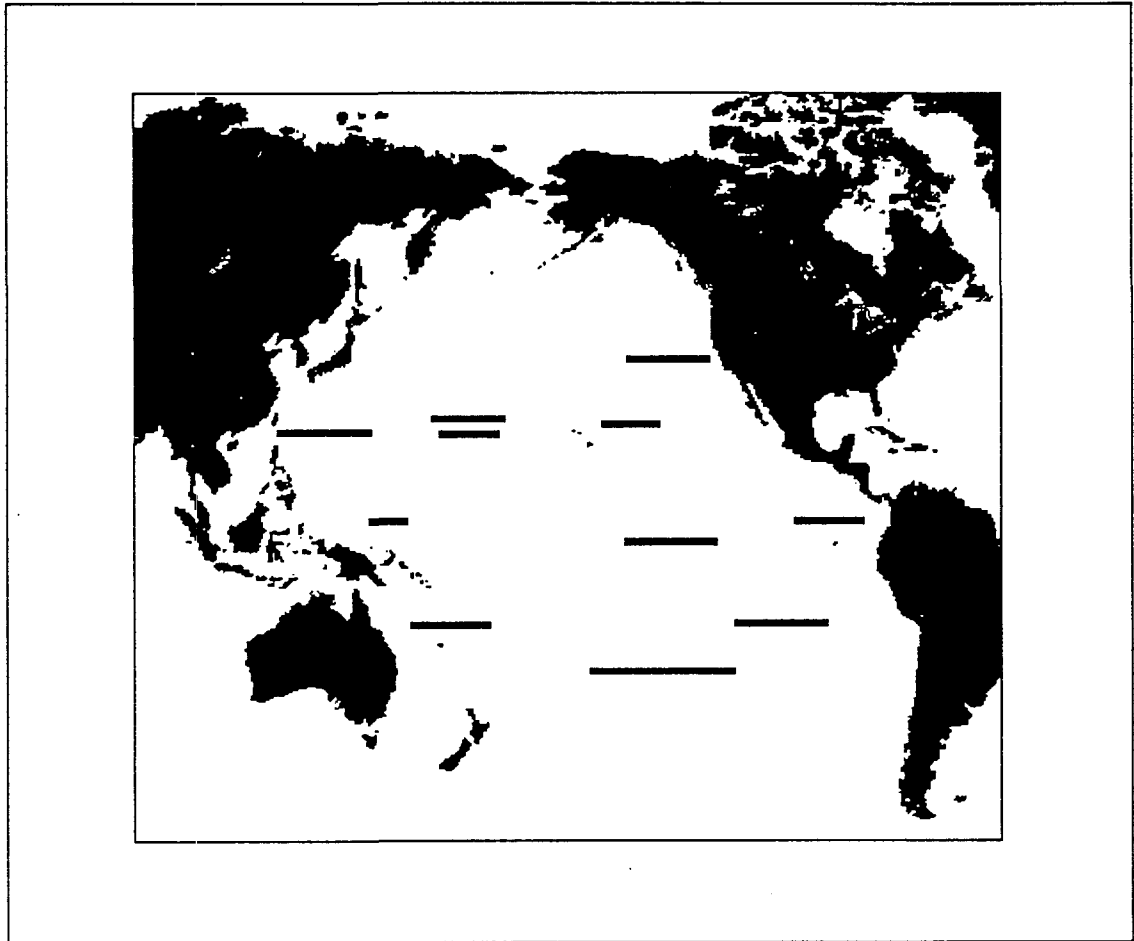


Table 1. Location and approximate number of individuals (n) if fish collected from each of the sample sites examined by the current study. Approximate longitude and latitude are give for each location. Size range of fish comprising the sample is given as either fork length in centimeters or as weight in kilograms.

location	n	Lat. ; Long.	size
Philippines	96	10N ; 122E	20-30cm
FSM	96	3-5N ; 137-141E	27-57cm
Coral Sea	96	16S ; 147E	100-150cm
Marshall Islands	86	10N ; 166E	130-150cm
Hawaii	96	20N ; 155W	10-60kg
East Pacific-1	96	5S ; 115W	40-50cm
East Pacific-2	50	1N ; 130W	38-52cm
East Pacific-3	69	(see Fig.1)	50-150cm
French Polynesia	96	6-21S ; 142-150W	60-130cm
Ecuador	96	0° ; 85W	100-220cm

Table 2. Variation in numbers of alleles observed at ten DNA microsatellite loci examined among 400 yellowfin and 72 bigeye tuna individuals.

Locus	Motif	Yft (n=400)	Bet (n=72)
102	(GA) ₂ (CA) ₃₂	-	24
113	(CA) ₁₂	25	35
117	(CA) ₁₂	17	27
121	(CA) ₄ (TA)(CA) ₇	-	21
125.a	(CA) ₁₀	-	4
144	(CA) ₆	-	5
135.a	(CCA)(CCG)(CCA) ₄	1	1
135.b	(CA) ₁₀ (TA)(CA) ₉	22	27
161	(CA) ₁₉	29	24
208a	(CA) ₁₀	8	25

Table 3. Allele frequencies observed at nine DNA microsatellite loci among populations sampled for the current study. A dash indicates that an allele was not observed. The asterisk denotes that a random sub-sample of 36 fish was chosen from the total sample collected from that location.

Locus 102

Allele	Ecuador* (n=35)	Philippines* (n=34)
134	0.01	-
138	0.13	0.07
142	0.01	0.01
144	0.11	0.15
146	0.04	0.03
148	0.16	0.07
150	0.06	0.06
152	0.01	0.04
154	0.04	0.07
156	0.09	0.09
158	0.11	0.04
160	0.06	0.06
162	0.01	0.01
164	-	0.04
166	-	0.04
168	0.04	0.06
170	0.01	0.04
172	0.01	0.01
174	0.01	-
176	0.03	0.01
182	0.01	0.03
186	-	0.01
192	-	0.01
202	0.01	-

Table 3. (continued)

Locus 113		
Allele	Ecuador* (n=34)	Philippines* (n=36)
105	-	0.01
113	-	0.01
115	-	0.01
117	0.03	0.04
119	0.04	0.07
121	0.01	0.03
123	0.07	-
125	0.04	0.06
127	0.03	0.03
129	0.04	0.08
131	0.07	0.04
133	0.06	0.03
135	0.07	0.04
137	0.01	0.03
141	-	0.06
143	0.07	0.06
145	0.06	0.10
147	0.06	0.03
149	0.03	0.01
151	0.01	0.03
153	0.04	0.03
154	-	0.01
155	0.07	0.03
157	-	0.06
159	0.04	0.03
161	0.01	0.01
163	-	0.01
165	0.01	-
167	-	0.01
169	0.03	-
171	-	0.01
173	0.03	-
177	-	0.01
179	0.01	-
183	-	0.01

Table 3. (continued)

Locus 117		
Allele	Ecuador* (n=34)	Philippines* (n=36)
164	0.01	0.03
174	0.03	-
176	0.01	0.01
178	0.07	0.01
180	0.07	0.10
182	0.12	0.08
184	0.13	0.11
186	0.03	0.04
188	0.01	-
190	-	0.01
192	0.03	0.01
194	-	0.06
196	0.06	0.06
198	0.06	0.13
200	0.06	0.06
202	0.04	0.04
204	0.06	0.04
206	0.01	0.03
208	0.04	0.03
210	0.01	0.03
213	0.03	0.03
215	0.03	0.03
217	0.04	-
219	0.01	-
221	-	0.01
225	-	0.03
229	-	0.03

Table 3. (continued)

Locus 121		Ecuador* (n=35)		Philippines* (n=36)	
Allele					
248		0.01	-	-	-
264		0.01	0.01	0.01	-
266		0.04	0.04	0.04	-
268		0.13	0.13	0.07	-
270		0.01	0.01	-	-
272		0.06	0.06	0.06	-
274		0.16	0.16	0.11	-
276		0.03	0.03	0.11	-
278		0.09	0.09	0.08	-
280		0.09	0.09	0.14	-
282		0.13	0.13	0.07	-
284		0.04	0.04	0.11	-
286		-	-	0.03	-
288		0.10	0.10	0.08	-
290		0.01	0.01	0.01	-
292		0.04	0.04	0.01	-
294		0.01	0.01	-	-
296		0.01	0.01	0.03	-
298		-	-	0.01	-
300		0.01	0.01	-	-
304		-	-	0.01	-

Locus 125.a

Allele	Philipp.	FSM	Marshall	Coral Sea	Hawaii	F. Poly	E.Pac-1	E.Pac 3	Ecuador
	(n=92)	(n=93)	(n=73)	(n=95)	(n=96)	(n=96)	(n=84)	(n=64)	(n=103)
149	-	-	0.007	-	-	-	-	-	-
151	-	-	0.007	0.005	0.005	-	-	0.016	-
153	-	0.005	-	-	-	-	-	-	-
156	-	-	0.007	-	0.005	-	-	-	-
158	0.902	0.876	0.870	0.868	0.875	0.904	0.946	0.898	0.893
160	0.082	0.108	0.110	0.111	0.104	0.091	0.048	0.070	0.087
162	0.011	-	-	0.016	-	0.005	-	0.008	0.010
164	0.005	0.011	-	-	0.010	-	0.006	0.008	0.010

Table 3. (continued)

Locus 144										
Allele	Philipp.	FSM	Marshall	Coral Sea	Hawaii	F. Poly	E. Pac-1	E. Pac 3	Ecuador	
	(n=94)	(n=92)	(n=80)	(n=93)	(n=96)	(n=98)	(n=84)	(n=64)	(n=105)	
162	-	-	-	-	0.005	0.005	0.006	0.008	-	-
164	-	0.005	-	-	-	0.010	-	-	-	-
166	0.005	-	-	-	-	-	-	-	0.010	-
168	0.165	0.130	0.188	0.108	0.125	0.128	0.167	0.125	0.138	-
170	0.005	0.005	0.019	0.022	0.010	0.020	0.018	0.023	0.029	-
172	0.628	0.636	0.563	0.624	0.583	0.566	0.536	0.523	0.514	-
174	0.197	0.223	0.225	0.242	0.276	0.265	0.274	0.320	0.310	-
176	-	-	0.006	0.005	-	0.005	-	-	-	-
Locus 135.a										
Allele	Philippines* (n=36)			Ecuador* (n=36)						
207	1.00			1.00						
Locus 161										
Allele	Philipp.	FSM	Marshall	Coral Sea	Hawaii	F. Poly	E. Pac-1	E. Pac 3	Ecuador	
	(n=56)	(n=92)	(n=80)	(n=93)	(n=96)	(n=96)	(n=84)	(n=64)	(n=103)	
172	-	0.005	-	-	0.005	0.005	-	-	-	-
176	-	-	0.006	-	-	-	-	-	-	-
180	0.054	0.027	0.044	0.054	0.021	0.030	0.054	0.023	0.009	-
182	0.027	-	0.019	0.005	0.016	-	0.018	0.016	-	-
184	0.018	0.022	0.019	0.032	0.016	0.020	0.024	0.016	0.009	-
186	0.027	0.016	-	0.005	0.016	0.040	0.012	0.008	-	-
188	0.054	0.065	0.075	0.048	0.089	0.056	0.065	0.063	0.065	-
190	0.063	0.076	0.075	0.059	0.057	0.025	0.048	0.055	0.037	-
192	0.027	0.027	0.031	0.027	0.016	0.020	0.006	0.031	0.046	-
194	0.080	0.049	0.050	0.065	0.089	0.051	0.089	0.078	0.037	-
196	0.054	0.049	0.088	0.048	0.036	0.071	0.071	0.063	0.028	-
198	0.027	0.043	0.056	0.054	0.083	0.051	0.060	0.055	0.065	-
200	0.071	0.130	0.056	0.070	0.078	0.096	0.071	0.078	0.083	-
202	0.036	0.060	0.050	0.054	0.052	0.030	0.048	0.055	0.037	-
204	0.054	0.043	0.063	0.038	0.036	0.061	0.065	0.016	0.046	-
206	0.080	0.071	0.044	0.059	0.057	0.076	0.083	0.086	0.046	-
208	0.036	0.054	0.038	0.097	0.078	0.056	0.060	0.070	0.102	-
210	0.134	0.130	0.081	0.118	0.125	0.152	0.125	0.094	0.148	-
212	0.009	0.011	0.044	0.038	0.047	0.030	0.018	0.039	0.019	-
214	0.045	0.038	0.031	0.048	0.031	0.025	0.012	0.047	0.093	-
216	0.027	0.027	0.038	0.032	0.010	0.040	0.042	0.031	0.019	-
218	0.045	0.016	0.056	0.032	0.016	0.030	0.012	0.023	0.037	-
220	0.027	0.038	0.013	0.005	0.026	0.010	0.012	0.031	0.046	-
222	0.009	-	0.019	0.011	-	0.015	0.006	0.008	-	-
224	-	-	-	-	-	0.010	-	0.016	0.019	-
226	-	-	0.006	-	-	-	-	-	0.009	-

Table 3. (continued)

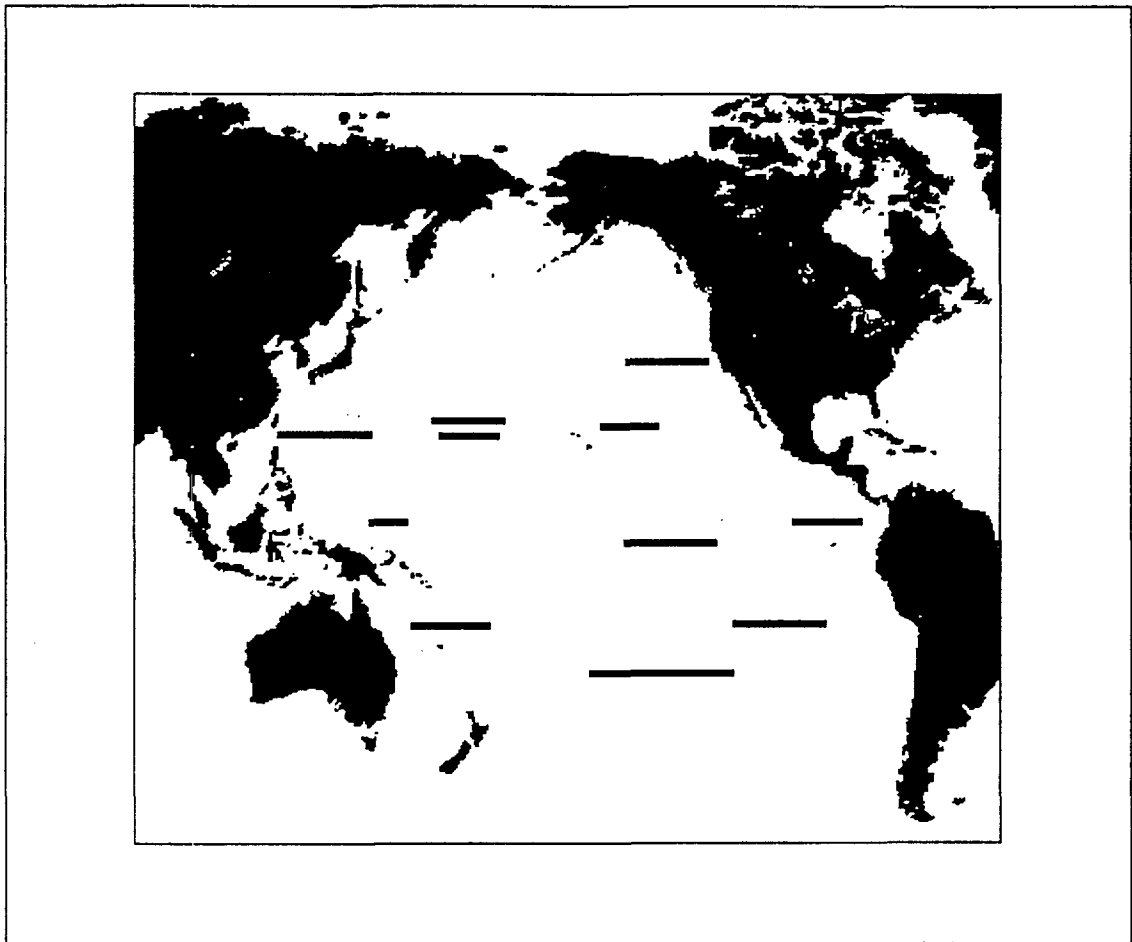
Locus 208.a									
Allele	Philipp.	FSM	Marshall	Coral Sea	Hawaii	F. Poly	E. Pac-1	Ecuador	
	(n=57)	(n=92)	(n=83)	(n=96)	(n=95)	(n=98)	(n=84)	(n=59)	
94	-	0.005	0.012	0.021	0.005	-	-	0.008	
96	0.009	0.033	0.024	0.010	0.016	0.010	0.036	0.059	
98	-	0.016	-	-	0.005	0.010	-	-	
100	0.009	0.016	-	0.021	0.021	0.010	0.012	0.008	
102	0.316	0.255	0.235	0.219	0.147	0.209	0.238	0.212	
104	0.237	0.326	0.259	0.271	0.363	0.321	0.333	0.331	
106	0.228	0.109	0.223	0.156	0.158	0.184	0.155	0.169	
108	0.026	0.049	0.054	0.052	0.074	0.056	0.054	0.042	
110	0.009	0.011	0.018	0.036	0.016	0.031	0.012	0.025	
113	0.026	0.033	0.006	0.021	0.016	0.010	0.018	0.008	
115	-	-	-	0.016	0.026	0.005	0.018	0.008	
117	0.009	-	0.006	-	-	-	0.006	0.008	
119	-	-	0.006	-	0.005	0.010	0.006	-	
121	0.009	0.033	0.036	0.021	0.011	0.020	0.018	0.017	
123	0.018	-	-	0.021	0.021	0.020	0.006	0.017	
125	0.009	0.011	0.018	0.005	0.005	0.026	0.006	0.017	
127	-	0.033	0.030	0.026	0.016	0.036	0.024	-	
129	0.026	0.033	0.036	0.036	0.037	0.015	0.036	0.025	
131	0.026	0.022	-	0.016	0.021	-	0.018	0.008	
133	0.009	0.011	0.024	0.021	0.005	0.010	0.006	-	
135	0.009	0.005	-	0.016	0.021	0.010	-	0.008	
137	-	-	0.012	0.005	0.005	-	-	0.017	
139	0.026	-	-	0.005	-	-	-	-	
141	-	-	-	0.005	-	0.005	-	-	
148	-	-	-	-	0.005	-	-	0.008	

Table 3. (continued)

mtDNA (DL19-12s)

Allele	Philipp. (n=85)	FSM (n=89)	Marshall (n=88)	Coral Sea (n=93)	Hawaii (n=93)	F. Poly (n=96)	E.Pac-1 (n=84)	E.Pac 3 (n=62)	Ecuador (n=101)
AS	0.259	0.258	0.159	0.290	0.312	0.281	0.250	0.290	0.228
AP	0.200	0.225	0.341	0.323	0.194	0.260	0.262	0.194	0.287
BP	0.188	0.124	0.182	0.151	0.215	0.219	0.167	0.226	0.139
BR	0.082	0.079	0.023	0.043	0.054	0.042	0.071	0.032	0.099
AR	0.071	0.056	0.068	0.032	0.075	0.042	0.060	0.097	0.020
AQ	0.059	0.135	0.091	0.043	0.097	0.063	0.107	0.065	0.079
DP	0.047	0.011	-	-	-	-	-	-	-
ES	0.024	-	-	-	-	-	-	0.016	-
FQ	0.024	-	-	-	-	-	0.012	-	0.010
AT	0.012	-	-	-	-	-	-	-	-
BQ	0.012	0.011	-	0.011	-	-	0.012	-	0.010
BY	0.012	-	-	-	-	-	-	-	-
BZ	0.012	0.011	0.011	0.011	-	-	0.012	-	0.020
AA ₁	-	-	-	0.011	0.011	0.010	-	-	-
AC ₁	-	-	-	0.011	-	-	-	-	-
AD ₁	-	-	-	0.011	-	0.021	-	-	-
AE ₁	-	-	-	-	-	-	-	-	0.030
AY	-	0.011	-	-	-	-	-	-	-
AZ	-	0.011	-	0.011	-	-	-	-	-
BA ₁	-	-	-	0.011	-	-	-	-	-
BD ₁	-	-	-	-	-	0.010	-	-	-
BS	-	0.045	0.080	0.022	0.022	0.031	0.024	0.016	0.030
CP	-	0.011	-	-	-	-	-	0.016	0.010
CW	-	-	-	0.011	-	-	-	-	-
DR	-	-	0.011	-	-	-	-	-	-
DS	-	-	-	-	-	0.010	-	-	-
EA ₁	-	-	-	-	-	-	0.012	-	-
EP	-	0.011	0.034	-	0.011	-	-	0.016	0.020
EW	-	-	-	-	-	-	-	0.016	0.010
FS	-	-	-	-	-	-	0.012	-	-
GP	-	-	-	-	-	-	-	-	0.010
HP	-	-	-	-	0.011	0.010	-	0.016	-
IS	-	-	-	0.011	-	-	-	-	-

Figure 1. Approximate sampling locations where fish were collected from for the current project. Corresponding longitude and latitudes for each sample location are listed in table 1.



Summary of Zaychin and Purdovkin Analysis of 4 Loci.

Dmitrii Zaykin and Alexander Pudovkin
Institute of Marine Biology, Vladivostok 690041, Russia.
Zaykin, D.V. and Pudovkin, A.I. Two programs to estimate Chi-square values using pseudo-probability test. -J.Hered., (in press)

Locus 208.a

name of input file: "all-208.txt"
row x col = 8 x 25
df = 168, X² = 199.771, chi-sq₀₅ = 198.862
Monte Carlo testing: 5000 runs made
estimated probability of homogeneity (P) is 0.0384
95% confidence interval for P: 0.0332516 - 0.043903

Locus 161

name of input file: "all-161.txt"
row x col = 9 x 26
df = 200, X² = 202.789, chi-sq₀₅ = 233.604
Monte Carlo testing: 5000 runs made
estimated probability of homogeneity (P) is 0.425
95% confidence interval for P: 0.411328 - 0.43873

Locus 144

name of input file: "all-144.txt"
row x col = 9 x 8
df = 56, X² = 59.3571, chi-sq₀₅ = 74.468
Monte Carlo testing: 5000 runs made
estimated probability of homogeneity (P) is 0.325
95% confidence interval for P: 0.312086 - 0.338048

Compare Philippines to Ecuador for Locus-144

name of input file: "loc144pe.txt"
df = 5, X² = 8.68297, chi-sq₀₅ = 11.070
Monte Carlo testing: 5000 runs made
estimated probability of homogeneity (P) is 0.112
95% confidence interval for P: 0.103409 - 0.120889

Locus 125

name of input file: "all-125.txt"
row x col = 9 x 8
df = 56, X² = 57.579, chi-sq₀₅ = 74.468
Monte Carlo testing: 5000 runs made
estimated probability of homogeneity (P) is 0.3876
95% confidence interval for P: 0.37414 - 0.401146

mtDNA HApotypes

name of input file: "spc-haps.txt"
row x col = 9 x 33
df = 256, X² = 285.335, chi-sq₀₅ = 293.918
Monte Carlo testing: 5000 runs made
estimated probability of homogeneity (P) is 0.0456
95% confidence interval for P: 0.0399928 - 0.0515564