Re-estimation of the mixing proportion of the 'J' and 'O' stocks using alternative stratification

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ABSTRACT

Following a recommendation from the Workshop to review JARPN, we examined the sensitivity of a previous estimate of mixing proportion between 'O' and 'J' stocks to the omission of the samples from sub-area 9 taken in 1995. As suggested in the Workshop, such sample could contain some animals different from the 'O' stock. We re-estimated the proportion of the 'J' stock in sub-area 7 under two stratification for the baseline sample of the 'O' stock (sub-area 9): i) total sample from sub-area 9 (same as in the study presented to the Workshop) and ii) excluding the 1995 samples from this sub-area. For the three genetic markers used, mtDNA control region RFLP, control region sequencing and nuclear DNA (microsatellite), we found no substantial differences in the estimation of stock mixing in sub-area 7 between the two stratification.

KEYWORDS: NORTH PACIFIC MINKE WHALE, STOCK IDENTITY, MIXING, MANAGEMENT

INTRODUCTION

The mixing proportion of 'J' and 'O' stocks in sub-area 11 has been estimated previously using RFLP haplotype frequency data. Butterworth *et al.* (1996) estimated the mixing proportion of the 'J' stock in 0.42 with associated 95% confidence interval (0.27, 0.57) in sub-area 11 in April. Subsequently Pastene *et al.* (1998) estimated the proportion of 'J' stock female animals in the April sample in sub-area 11 in 0.4075 (SE=0.0806) and that of male animals in August in 0.3147 (SE=0.1160).

Recently Goto *et al.* (2000a) used, in addition to mtDNA RFLP data, control region sequencing and nuclear DNA (microsatellite) for estimating the mixing proportion between 'J' and 'O' stocks in sub-area 11, by month and sex. These results were presented to the Workshop to review JARPN. Estimations obtained using RFLP and sequencing data were similar. In general mtDNA and microsatellite yielded the same results, both showing a significant mixing rate of 'J' stock female animals in April. The mixing rate obtained by the mtDNA analyses, however, was higher. On the other hand, a relatively high mixing rate of male 'J' stock individuals was found by the mtDNA analyses in August. Although the microsatellite analysis showed a relatively high rate, the standard error of such estimation was high (Goto *et al.*, 2000a).

In a study on stock identity in the Pacific side of Japan presented to the Workshop, Goto *et al.* (2000b) found some degree of mtDNA heterogeneity in sub-area 9 attributed to samples taken in the western side of this sub-area in 1995. The Workshop to review JARPN noted that some of the mixing rate estimate reported by Goto *et al.* (2000a) were based on the assumption that all samples taken from sub-area 9 were from the 'O' stock and recommended that the sensitivity of these results to omission of the samples for the west of sub-area 9 (i.e. west of 162°E) in 1995 be checked as this area may contain some 'W' stock animals. This paper presents the results of the additional analysis on stock mixing that considered two alternative stratification for the baseline 'O' stock sample (sub-area 9): i) total data and ii) excluding data from 1995.

DATA AND METHODS

Genetic markers, data and mathematical approaches used for estimating mixing proportion were given in the previous report (Goto *et al.*, 2000a). MtDNA control region RFLP haplotype frequencies were derived

from the analysis with eight restriction enzymes (Goto and Pastene, 1997). Sequencing haplotypes frequencies were derived from the analysis of a segment of 487bp of the mtDNA control region. Microsatellite allele frequency data were obtained from the analysis with five loci (GATA417, GT23, GATA98, GATA28 and EV37).

In the case of mtDNA, the composition in the mixed assemblage was estimated by the maximum likelihood procedure. Variances of these estimates consist of the variance from the sampling from mixed stock and the sampling from the baseline stocks ('O' and 'J' stocks) (Kishino *et al.*, 1994). This method had already been applied by Pastene *et al.* (1998) for estimation of mixing proportion in sub-area 11 using mtDNA RFLP haplotype frequency data.

The mathematical model for estimating the mixing proportion using microsatellite data was developed by Dr. A. E. Punt (CSIRO, Marine Research, Australia), who kindly made the calculations for this paper (see Goto *et al.*, 2000a for details of this method).

In Goto *et al.* (2000a), the mixing proportion in sub-area 11 was estimated considering sub-area 6 (28 samples taken during a past commercial operation) as the representative sample of the 'J' stock and sub-area 7 (277 samples taken during past commercial operations and during JARPN) as the representative sample of the 'O' stock. In order to ensure that no whales from the 'J' stock are present in sub-area 7, these authors conducted an estimation of the mixing proportion in that sub-area having as baseline stock of the 'J' stock sub-area 6 (as above) and as baseline stock of the 'O' stock sub-area 9. In this case, the total samples available from sub-area 9 (188) were used. Here we repeat these estimations for sub-area 7 omitting the samples from this sub-area taken in 1995, which could contain individuals different from the 'O' stock.

The mtDNA RFLP and sequencing haplotype and microsatellite allele frequencies of the baseline stocks and of the mixed assemblage of sub-area 7 are shown in Tables 1, 2 and 3, respectively. Data for the baseline stocks of sub-area 9 are given for both the total sample and excluding data from 1995.

RESULTS AND DISCUSSION

Mixing proportions in sub-area 7

Table 4 shows the estimates (and their standard errors) of the mixing proportions for the 'J' stock in sub-area 7 by month and sex. These estimations are shown for mtDNA RFLP, mtDNA sequencing and microsatellite. Results are shown for both options, including and excluding the 1995 samples from sub-area 9.

Females

For each of the approaches used (RFLP, sequencing and microsatellite) there is no substantial differences in the estimation of the 'J' stock proportion for both stratification (including or excluding 1995 data for sub-area 9). There is some apparent differences in the RFLP estimates for May and June. However, the sample sizes are small and the standard errors are large so we can disregard such apparent differences.

Males

In the case of males, there is also agreement between the estimates that included 1995 data in sub-area 9 and those that excluded such data. The only case where these estimations are somewhat different is for the case of RFLP for the month of May. The estimation considering the total samples in sub-area 9 was 0.058 (SE=0.037) and that obtained excluding the 1995 samples was 0.101 (SE=0.040). This result should be seen with caution as such difference in May was not observed for sequencing and microsatellite, which are considered more sensitive genetic markers than RFLP.

On the basis of these results we concluded that the estimates of 'J' stock proportion in sub-area 7 is not sensitive to the use of different stratification for the baseline sample of the 'O' stock. Then we confirm the conclusions of Goto *et al.* (2000a).

One aspect that should be considered in the future is the conduction of sensitivity test for change in the composition in the baseline sample for the 'J' stock. Until now all the estimates of stock mixing between

'O' and 'J' stocks have used a single sample as the baseline for the 'J' stock. Such sample was available from a commercial whaling operation in sub-area 6 in September-October 1982. If new samples become available from the Sea of Japan, estimation of stock mixing should be re-estimated using the new sample set.

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Table 1: mtDNA RFLP haplotype frequency data used in this study. The data in the columns marked by asterisks are omitted from the analyses due to small sample size.

Hap.	Sub-area 6	Sub-area 7												Sub-area 9 All	Sub-area 9 excluding 1995
		April		May		June		July		August		September			
		F	M	F	M	F	M	F	M	F*	M	F	M		
1	0	19	18	15	58	13	51	10	13	3	18	7	28	177	84
2	1	0	0	1	3	1	1	0	0	0	0	0	0	2	0
3	7	0	1	0	1	0	1	2	0	0	0	0	1	5	2
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	20	0	1	0	4	1	2	0	0	0	0	0	1	3	2
6	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	28	19	20	16	66	15	55	12	14	3	19	7	31	188	88

Table 2: mtDNA sequencing haplotype frequency data used in this study. The data in the columns marked by asterisks are omitted from the analyses due to small sample size.

Hap.	Sub-area 6	Sub-area 7												Sub-area 9 All	Sub-area 9 excluding 1995
		April		May		June		July		August		September			
		F	M	F	M	F	M	F	M	F*	M	F	M		
1	18	0	1	0	4	1	2	0	0	0	0	0	1	2	2
2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	2	0	4	0	4	1	0	0	0	0	1	9	5
7	0	1	0	4	3	1	7	0	3	0	3	0	0	15	6
8	0	0	0	0	1	1	2	1	1	0	1	0	2	3	0
9	0	8	7	2	5	0	6	4	0	0	5	2	1	30	12
10	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
11	0	0	0	0	1	0	4	1	0	0	1	0	1	10	2
12	0	0	1	0	2	1	2	1	1	0	1	0	1	7	3
13	0	0	1	1	4	2	5	0	1	0	2	0	3	4	2
14	0	2	1	0	2	2	1	0	2	0	1	0	3	11	6
15	0	1	1	0	4	0	1	0	1	0	1	1	1	6	4
16	0	1	1	1	4	2	2	1	0	1	1	1	2	11	6
17	0	2	1	0	5	0	3	0	0	1	1	0	1	16	8
18	0	0	0	0	1	0	0	0	0	0	0	0	1	3	1
19	0	1	0	0	0	0	1	0	0	0	0	1	1	1	1
20	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0
21	0	0	0	0	2	0	0	0	0	0	0	0	1	3	2
22	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
24	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
25	0	0	1	0	0	0	0	0	1	0	0	0	1	4	2
26	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
27	0	0	1	0	0	0	1	0	0	0	0	0	1	1	1
28	0	0	0	2	1	0	0	0	0	0	1	0	0	0	0
29	0	0	0	2	2	1	1	0	0	0	0	0	0	5	5
30	0	1	0	1	4	0	4	0	2	1	0	1	0	9	6
31	0	0	1	0	3	1	0	1	0	0	0	0	0	2	0
32	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
33	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
34	0	1	0	0	2	0	1	0	0	0	0	0	0	3	1
35	0	1	0	0	2	1	0	0	0	0	0	0	0	0	0
36	0	0	0	1	3	1	1	0	0	0	0	0	0	1	0
37	0	0	0	0	1	0	1	0	0	0	0	0	1	2	1
38	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0
39	0	0	0	0	1	0	0	0	1	0	0	0	0	5	2
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
50	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

(Table 2: Continued)

Hap.	Sub-area 6	Sub-area 7												Sub-area 9 All	Sub-area 9 excluding 1995
		April		May		June		July		August		September			
		F	M	F	M	F	M	F	M	F*	M	F	M		
51	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1
52	0	0	0	0	0	0	0	0	0	0	0	0	0	5	2
53	0	0	0	0	0	0	0	2	0	0	0	0	0	4	1
54	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
55	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
56	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
57	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
58	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
59	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1
60	0	0	0	0	1	1	1	0	0	0	0	0	0	2	2
61	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
62	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
76	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
77	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	28	19	20	16	66	15	55	12	14	3	19	7	31	188	88

Table 3: Microsatellite DNA allele frequency data used in this study. The data in the columns marked by asterisks are omitted from the analyses due to small sample size.

(a) GATA417

Allele	Sub-area 6	Sub-area 7												Sub-area 9 All	Sub-area 9 excluding 1995
		April		May		June		July		August		September			
		F	M	F	M	F	M	F	M	F*	M	F	M		
196	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
200	0	1	0	0	4	0	1	0	0	.0	0	0	2	8	2
204	0	3	2	3	4	1	7	1	3	0	2	0	3	16	4
208	2	3	3	3	14	4	13	3	4	0	2	1	5	36	22
210	1	0	2	0	4	1	3	1	1	0	2	0	0	11	5
212	26	15	10	8	48	18	39	6	9	2	14	7	22	140	62
214	0	0	1	0	0	0	0	2	1	0	0	0	2	2	1
216	28	5	13	11	40	6	28	5	8	2	15	3	22	101	45
218	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
220	1	9	9	4	16	2	13	4	4	0	3	3	7	51	24
224	0	1	0	0	0	0	0	0	0	0	0	0	1	2	2
228	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	58	38	40	30	130	32	104	22	30	4	38	14	64	368	168

(b) GT23

Allele	Sub-area 6	Sub-area 7												Sub-area 9 All	Sub-area 9 excluding 1995
		April		May		June		July		August		September			
		F	M	F	M	F	M	F	M	F*	M	F	M		
88	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
94	6	1	0	0	1	0	0	0	0	0	0	0	0	3	0
96	5	0	1	0	0	0	0	0	0	0	0	1	0	2	1
98	0	4	2	3	14	4	12	1	5	0	3	2	6	32	15
100	1	0	2	0	4	1	2	3	0	0	0	0	1	7	2
102	0	1	3	1	9	0	5	0	0	0	0	0	2	11	3
104	4	4	6	2	18	7	15	1	4	1	6	2	8	50	22
106	9	5	2	3	11	4	8	1	1	1	5	3	4	37	19
108	2	1	4	3	5	2	6	1	2	0	3	1	4	30	16
110	3	5	3	5	18	4	15	4	4	1	5	3	9	50	28
112	22	8	8	4	21	2	21	9	5	2	7	1	15	75	35
114	0	6	1	5	12	4	13	2	1	2	4	1	5	48	21
116	0	3	4	6	15	2	11	0	7	1	5	0	7	28	13
118	0	0	0	0	1	0	0	0	1	0	0	0	1	1	1
120	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Total	52	38	36	32	130	30	108	22	30	8	38	14	62	376	176

(c)GATA98

Allele	Sub-area 6	Sub-area 7												Sub-area 9 All	Sub-area 9 excluding 1995
		April		May		June		July		August		September			
		F	M	F	M	F	M	F	M	F*	M	F	M		
100	0	0	0	0	2	0	1	0	0	0	0	0	0	3	2
104	3	2	2	1	11	2	9	1	1	1	5	0	6	34	19
108	41	19	21	21	74	18	55	16	14	4	20	11	44	188	90
112	12	10	9	6	27	7	24	2	6	3	7	1	10	104	45
116	0	3	5	4	14	3	16	4	6	0	5	2	4	45	19
120	0	0	1	0	2	0	1	1	1	0	1	0	0	2	1
Total	56	34	38	32	130	30	106	24	28	8	38	14	64	376	176

(Table 3 Continued)

(d) GATA28

Allele	Sub-area 6	Sub-area 7												Sub-area 9 All	Sub-area 9 excluding 1995
		April		May		June		July		August		September			
		F	M	F	M	F	M	F	M	F*	M	F	M		
148	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
193	0	0	0	0	0	1	1	0	0	0	0	0	0	2	1
194	0	0	0	0	2	0	0	0	1	0	0	1	0	11	10
198	0	1	5	3	4	0	11	0	2	0	2	0	3	1	0
201	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
202	15	6	5	6	19	2	21	6	6	2	7	2	10	54	24
205	0	2	1	0	1	1	0	0	0	0	0	0	1	5	1
206	0	4	4	6	24	3	17	5	3	1	3	0	9	52	24
209	0	0	1	0	3	1	5	1	1	1	1	0	2	12	7
210	16	5	7	4	17	7	9	5	3	1	5	3	9	63	24
213	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
214	25	12	11	8	36	11	29	5	10	2	14	3	21	102	49
218	0	4	2	2	6	1	6	0	2	0	3	2	5	46	22
222	0	0	3	1	7	1	7	1	2	0	1	2	1	17	8
226	0	0	1	0	2	2	2	1	0	1	0	0	1	7	4
230	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Total	56	34	40	30	121	30	108	24	30	8	38	14	62	372	174

(e) EV37

Allele	Sub-area 6	Sub-area 7												Sub-area 9 All	Sub-area 9 excluding 1995
		April		May		June		July		August		September			
		F	M	F	M	F	M	F	M	F*	M	F	M		
179	17	10	14	10	47	14	38	2	11	3	8	7	17	121	56
181	0	1	0	0	0	1	2	0	0	0	0	0	0	5	3
193	0	1	4	2	12	1	4	5	1	0	4	0	1	23	9
195	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
197	0	0	0	0	0	1	1	0	0	0	0	1	1	8	2
199	18	18	13	14	47	9	37	10	10	2	14	5	26	147	67
201	2	1	0	0	7	0	12	4	2	1	3	0	7	18	11
203	0	0	2	1	1	1	0	1	0	0	0	0	1	4	2
205	17	3	3	2	15	2	13	1	4	2	4	1	7	30	13
207	0	1	1	0	1	1	1	0	0	0	0	0	0	1	0
209	0	1	1	1	0	0	0	1	0	0	1	0	0	5	1
Total	54	36	38	30	130	30	108	24	28	8	34	14	60	362	164

Table 4: Contribution of the 'J' stock to sub-area 7 according estimates that used mtDNA RFLP haplotype frequency, mtDNA sequencing haplotype frequency and microsatellite allele frequency data. The baseline stock for the 'J' stock was a sample from sub-area 6 taken in September-October 1982; the baseline stock for the 'O' stock is presented for two stratification: i) all JARPN samples from sub-area 9 and ii) excluding 1995 samples in that sub-area (P=proportion; SE=standard error).

(a) Females

Month	RFLP All data		RFLP excluding 1995		Sequence All data		Sequence excluding 1995		Microsatellite All data		Microsatellite excluding 1995	
	P	SE	P	SE	P	SE	P	SE	P	SE	P	SE
April(19)	0.000	(0.229)	0.000	(0.229)	0.000	(0.223)	0.000	(0.223)	0.034	(0.103)	0.075	(0.088)
May(16)	0.000	(0.221)	0.063	(0.061)	0.000	(0.217)	0.000	(0.207)	0.000	(0.000)	0.000	(0.000)
June(15)	0.062	(0.079)	0.121	(0.088)	0.052	(0.065)	0.042	(0.066)	0.000	(0.000)	0.000	(0.000)
July(12)	0.067	(0.120)	0.083	(0.118)	0.000	(0.289)	0.000	(0.264)	0.000	(0.000)	0.000	(0.000)
September(7)	0.000	(0.378)	0.000	(0.378)	0.000	(0.350)	0.000	(0.350)	0.267	(0.185)	0.271	(0.184)

(b) Males

Month	RFLP All data		RFLP excluding 1995		Sequence All data		Sequence excluding 1995		Microsatellite All data		Microsatellite excluding 1995	
	P	SE	P	SE	P	SE	P	SE	P	SE	P	SE
April(20)	0.053	(0.066)	0.049	(0.068)	0.035	(0.049)	0.019	(0.050)	0.033	(0.081)	0.044	(0.084)
May(66)	0.058	(0.037)	0.101	(0.040)	0.046	(0.030)	0.034	(0.030)	0.000	(0.000)	0.034	(0.043)
June(55)	0.024	(0.031)	0.047	(0.034)	0.021	(0.026)	0.006	(0.026)	0.000	(0.000)	0.000	(0.000)
July(14)	0.000	(0.267)	0.000	(0.258)	0.000	(0.267)	0.000	(0.258)	0.000	(0.000)	0.000	(0.000)
August(19)	0.000	(0.229)	0.000	(0.223)	0.000	(0.217)	0.000	(0.211)	0.194	(0.119)	0.197	(0.119)
September(31)	0.022	(0.041)	0.017	(0.043)	0.020	(0.032)	0.009	(0.033)	0.082	(0.124)	0.086	(0.124)