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Additional genetic analyses on the plausibility of the baseline stock scenarios adopted for North Pacific minke whale *ISTs*

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ABSTRACT

For the objective of the *Implementation Simulation Trials* (*ISTs*) of North Pacific minke whale, four baseline stock scenarios were defined during an *ISTs* workshop conducted in January 2002: Baseline A is a two main-stock scenario (J, O) allowing a third stock (W) to occur only in sub-area 9 west, and then only sporadically; Baseline B is a two-stock scenario (J and O) with no W stock; Baseline C is a four-stock scenario overall with three (Ow, Oe and W) to the east of Japan and Baseline D is a three-stock scenario with O and W stocks to the east of Japan mixing across the area 147°-162°E. Some of these scenarios were included under the simple criteria that at least some workshop participants considered the associated underlying hypothesis plausible. Subsequently no detailed discussion had been conducted on the plausibility of these hypotheses. Here we conduct a comprehensive genetic analysis focused to evaluate the plausibility of *ISTs* stock scenario C. Specifically we subjected the underlying hypothesis of this scenario to an *independent test* by conducting a) the mtDNA analysis using a larger data set than used previously in *ISTs* and b) a microsatellite analysis using 17 loci (instead of seven used in previous studies). The analysis incorporates geographical (longitudinal boundaries of putative stocks in scenario C), temporal (early and late periods in the migration season) and biological (sexual components in each putative stock of scenario C) considerations. We used both genetic markers to test the hypothesis that if these stocks are different and separated by hard boundaries (underlying hypothesis of scenario C), then sexual components within a stock are genetically more closely related among them rather than to the same sexual components in other hypothesized stocks. Results of the genetic analysis reject this hypothesis. Genetic diversity at both mitochondrial and nuclear DNA was widely distributed among the sexual components irrespective of any stock division and no significant differences among the three putative stocks were found. Therefore underlying hypothesis of scenario C failed to this independent test. The results are also inconsistent with the underlying hypothesis of scenario D as no evidence of W stock was found in sub-area 9E and no evidence of stock mixing was found for the sector 147°-162°E as postulated by this scenario. Results of this additional genetic analysis are consistent with the single stock scenario to the east of Japan (scenario B), although the sporadic occurrence of the W stocks in part of sub-area 9 can not be discarded (scenario A). Consequently we assign the different scenarios the following weight on plausibility: A=medium, B=high, C= very low and D=low.

INTRODUCTION

The *Implementation Simulation Trials* (*ISTs*) for North Pacific minke whale under the Revised Management Procedure (RMP) started in 1993 (IWC, 1994). The last *ISTs* workshops for this species were conducted in 2002 (IWC, 2003) and 2003.

One of the issues delaying the completion of the trials has been the definition of stocks. The process of proposing and then dropping stock scenarios has not been uncommon during this 10-year period. During the 2001 meeting, the Scientific Committee (SC) established the Terms of References (TORs) for the 2002 workshop (IWC, 2002). The TOR (1) read as 'Review results of further analyses of genetic data for sub-areas 7, 8 and 9 and decide what trial modifications may be necessary in consequence and re-estimation of mixing proportion between 'J' and 'O' stocks in sub-areas 7 and 11' (IWC, 2002 pp 9). The first part of this TOR was related to a collaborative spatial analysis of DNA data in sub-areas 7, 8 and 9 with the aim to investigate the proportions of the 'O' and 'W' stocks in these sub-areas (IWC, 2002 pp 143).

It was understood that after the collaborative analyses the range of plausible hypotheses for the proportions of the 'O' and 'W' stocks in these sub-areas would be developed (IWC 2002 pp143). However, the *ISTs* workshop conducted in 2002 went beyond its own TORs: not only was the 'O' and "W' stock scenario considered but also other hypotheses incorporating additional structure were adopted. This was made despite the strong opposition of some members, who argued on the premature nature of such hypotheses. As a consequence, the *ISTs* process for North Pacific minke whale was delayed once more.

The four baseline stock scenarios adopted in the 2002 *ISTs* workshop are the following:

Baseline A: This scenario proposes two main stocks in the western North Pacific, 'J' and "O' but allows for the occurrence of a third stock ('W') in sub-area 9W, but only sporadically. This scenario is based mainly on the results of genetic (hypothesis testing of mtDNA and microsatellites) and non-genetic analyses discussed during the JARPN review meeting, which overall provide no evidence for additional structure in sub-areas 7, 8 and 9 (IWC, 2001). The 'O' stock distribute in the Okhotsk Sea and in the eastern Japan until at least 170°E. The 'J' stock distributes in the Sea of Japan, Yellow Sea, and East China Sea. O and J stocks mix to each other in sub-area 11 (Goto and Pastene, 1997; Pastene *et al.*, 1998). Evidence for sporadic occurrence of W stock in sub-area 9W derives from an analysis of mtDNA, which showed some degree of heterogeneity in that sector in some years, though microsatellites did not show such substructure (IWC, 2001).

Baseline B: Same as Baseline A but with no 'W' stock animals. This scenario is based mainly on the results of genetic (hypothesis testing of mtDNA and microsatellites) and non-genetic analyses discussed during the JARPN review meeting, which overall provide no evidence for additional structure in sub-areas 7, 8 and 9 (IWC, 2001). It rejects the possibility of W stock because the evidence for its sporadic occurrence in sub-area 9W derives just from the results of a single locus (mtDNA) with no confirmation from other genetic or biological markers.

Baseline C: This scenario proposes the occurrence of four stocks, 'J' 'Ow', 'Oe' and W. The division between 'Ow' and 'Oe' and 'W' stocks occur at 147°E and 157°E, respectively. These boundaries are 'hard' (no mixing across them). This baseline scenario is based on the results of the Boundary Rank (BR) technique. It is noted that the main aim of the BR is to generate a set of stock boundary hypotheses, which should be further evaluated through parameter estimates or by the use of independent data to check these hypotheses. The 2002 Workshop adopted baseline C scenario and its underlying hypothesis without such evaluation.

Baseline D: This scenario propose the occurrence of two stocks to the east of Japan, 'O' and 'W' which mix across the area 147°E to 162°E. The highest densities of 'O' stock animals occur in sub-area 7W and decline to the east, while the highest densities of 'W' stock animals occur in sub-area 9E and decline to the west. This baseline is based in an independent interpretation of the results of genetic analysis (mainly those derived from BR) and on the oceanographic conditions in the sub-areas.

Some of the stock scenarios defined in the 2002 workshop were included in the trials under the simple criterion that at least some workshop participants considered the associated underlying hypothesis plausible. Subsequently no detailed discussion had been conducted on the plausibility of these hypotheses.

Here we conduct a new genetic analysis focused to evaluate the plausibility of *ISTs* stock scenarios and their underlying hypotheses with particular emphasis on scenario C. The analysis is carried out in a comprehensive manner using all the genetic (mtDNA and microsatellite) and biological information (reproductive status of each individual) available to us for western North Pacific minke whale. Specifically we subjected the underlying hypothesis of scenario C to an *independent test* by conducting a) the mtDNA analysis using a larger data set than used previously in *ISTs* and b) a microsatellite analysis using 17 loci (instead of seven used in previous studies).

The analysis was conducted to test the hypothesis that if stocks are different and separated by hard boundaries (underlying hypothesis of scenario C), then sexual components within a stock are genetically more closely related among them rather than to the same sexual components in other hypothesized stocks.

MATERIALS AND METHOD

Samples

Samples used in the present analysis were from two sources: JARPN and JARPN II surveys conducted in sub-areas 7, 8 and 9 between 1994 and 2001 (JARPN) and past coastal commercial whaling samples taken between 1983 and 1987 in sub-area 7 (CS).

Grouping of samples

For conducting the genetic analysis we simulated the geographical boundaries of putative stocks of baseline scenario C: Ow (Japanese coast and 147°E), Oe (147°-157°E) and W (157°-170°E). Samples in each these longitudinal sectors were defined according to sex and maturity status (sexual component): immature male (IM), mature male (MM), immature female (IF) and mature female (MF). Details of the biological analysis and criteria for defining the sexual components were given in Zenitani *et al.* (2000). Stocks moving from low latitudes to higher latitudes in the feeding season could change their distribution longitudinally as the season progresses. For that reason we considered the case of the total samples as well samples grouped by temporal strata: early (April-May-June) and late (July-August-September).

Sample sizes by putative stocks and sexual components are shown in Table 1. Two data sets were used, the first involving those from JARPN 1994-2001 (Table 1-A) and the second involving a combination of CS 1983-1987 and JARPN (Table 1-B). The larger sample size in the second set increases the power of the genetic analysis. The genetic diversity of the sexual components in these longitudinal sectors was examined by mtDNA and microsatellite.

mtDNA analysis

Sequences of the mtDNA control region were used in this analysis. Details of the laboratory work were given in Goto and Pastene (2000).

The differentiation of mtDNA among sexual components within and between putative stocks was quantified using the Analysis of Molecular Variance (AMOVA) (Excoffier *et al.* 1992) as implemented in the computer program AMOVA ver. 1.55. The statistics of primary interest are the haplotype (Fst) and sequence (PHIst), both of which were used. The significance of the observed variance values was tested using a modification of a matrix permutation procedure available in the computer program. All test of statistical significance were based on 2000 random permutation of the original data sets. The level of significance obtained using this procedure is referred to in this paper as the P-value.

We also conducted a homogeneity test for the grouping defined above using the randomized chi-square test.

Microsatellite analysis

Details of the laboratory work were given in previous papers (Abe *et al.* 2000). Except a total of 17 microsatellite loci was used in the present analysis: EV37, EV1, GT310, GATA28, GT575, EV94, GT23, GT509, GATA98, EV104, GATA417, GT211, EV21, DlrFB14, EV14, GT195 and TAA31. The number of loci in this study increased substantially with regard that used in the previous study (Kanda *et al.*, 2002) (17 against 7).

Statistical test for genetic homogeneity were conducted using the computer program GENEPOP (Raymond and Rousset, 1995). The randomized chi-square test was used to compare allele frequencies among sexual classes within and between hypothesized stocks. Decision on statistically significance was made using the chi-square value obtained from summing the negative logarithm of P-values through all loci (Fisher, 1950).

Past commercial whaling samples have not been examined for microsatellite yet. Therefore only the data set shown in Table 1A (JARPN) was used in the microsatellite analysis.

RESULTS

MtDNA Haplotypes

A total of 70 specific sequences (haplotypes) were determined in a total of 558 samples from JARPN. A total of 75 were determined when the commercial samples were added (n=703, Table 1B). To conduct the statistical analysis based on AMOVA, frequencies of each haplotype were obtained for the different groupings shown in Tables 1A and 1B (data not shown).

Results of AMOVA on mtDNA data

Results of AMOVA for the case of JARPN and JARPN+CS data sets were very similar. For clarity we show the results of AMOVA for the latter case only (Table 1B based on larger sample size). These results are shown in Table 2 for both PHIst and Fst statistics. Results obtained by both statistics are similar. In the three cases examined (total sample, early and late periods) the molecular variance is distributed largely within stocks rather than between stocks. No significant P value (at the 5% significant level) was found in the hierarchical analysis. Almost all of the genetic differences detected can be explained by the differences between individuals within as well as between the putative stocks.

Table 3 shows the results of the AMOVA for category Mature Male (MM) in Table1B. This sexual component was chosen for more detailed analysis because it presents the largest sample sizes among the different sexual classes. In this analysis we defined 6 MM strata with two (early and late) in each putative stock. As in the case of Table 2, molecular variance is distributed largely within stocks rather than between stocks. The only significant P values were found for Fst (in bold in Table 3) in the test 'within temporal group'.

Based on the results of a post-stratification analysis conducted by Goto *et al.* (2000), the analysis was repeated excluding animals sampled in sub-area 9W (157°-162°E) in 1995, 2000 and 2001. These animals had been collected almost in the late period in the migration season. When the analysis is made excluding such animals the significant P values found in Tables 3 are not observed (Table 4). This result suggest that mtDNA heterogeneity in putative W stock is attributed to animals in sub-area 9W sampled late in the migration season.

Chi-square test

No significant differences were found among sexual components within putative stocks. Pairwise comparisons among Ow, Oe and W showed just a single significant difference (P=0.049) between Ow (n=319) and W (n=233). When samples from sub-area 9W in 1995, 2000 and 2001 were excluded from the analysis no significant difference was found between Ow (n=319) and W (n=110) (P=0.417).

Microsatellites

Table 5 shows the results of the statistical analysis using microsatellite data. The average number of alleles per locus was 11 ranging from 2 in EV21 to 28 in EV1. To conduct the statistical analysis based on chi-square, allele frequencies in each locus were obtained for the different groupings shown in Table 1A (data not shown).

No significant differences were found among sexual components within putative stock (P values combined for all loci were 0.395, 0.744 and 0.771 for Ow, Oe and W, respectively). Sexual component in each stock were pooled and compared to those in other stocks. No significant differences were found among stocks (P value combined for all loci was 0.207) (Table 5).

As in the case of mtDNA, the statistical analysis was conducted for the sexual component Mature Male (MM) given the largest sample size in this class. No significant difference was found among putative stocks for early, late and both temporal strata combined (Table 5).

DISCUSSION

The aim of the present analysis was to use all the genetic and biological available information on western North Pacific minke whale and examine these data in a comprehensive manner in the context of the stock scenarios being used in the *ISTs*. The analysis conducted here can be considered as an *independent test* for the hypothesis underlying stock scenario C. This scenario derived from hypotheses generated from results of mtDNA using BR. Notwithstanding, the mtDNA data set used in this study is larger than that used previously in *ISTs*, incorporating not only JARPN samples but also the available past commercial samples. In addition we conducted a microsatellite analysis using a larger number of loci than that used in previous studies. Microsatellite analysis has been informative in the case of the North Atlantic minke whale (Andersen *et al.*, 2003). In the case of the North Pacific minke whale they can be used as an independent marker to test hypotheses generated from results of mtDNA analyses. It should be noted that even using a larger number of microsatellite loci in the present study (17 against 7 in previous studies) we failed to find any evidence of additional stock structure in sub-areas 7, 8 and 9.

Below we discuss the implication of the results of our mtDNA and microsatellite analyses in the context of each of the stock scenarios being considered in the *ISTs*.

Scenario C

The underlying hypothesis of baseline stock scenario C establishes that there are three stocks to the east of Japan separated by hard geographical boundaries at 147°E and 157°E, respectively. The concept of 'hard boundary' means no normal overlap of these boundaries by individuals belonging to these putative stocks, with perhaps some very slight long-term interchange only. This idea implies that Ow almost never go east of 147°E, and similarly for Oe. This also implies that Oe animals almost never go close to the coast of Japan. This idea is difficult to explain in the context of varying oceanographic conditions and food distribution. Under such scenario one should expect substantial genetic differences among the stocks and that the different sexual components composing a putative stock will be more closely related among them than they are to the sexual components composing a different stock. Against this expectation, the results of the genetic analysis that simulated scenario C (mtDNA and microsatellites) found that the sexual components in different putative stocks are genetically closely related to each other and there was no genetic support for the boundaries proposed by this scenario.

The statistical analysis on mtDNA data in the present study was based on sequence (PHIst) and haplotype (Fst) statistics. None of these statistics showed significant results in the hierarchical analysis by AMOVA. Only the Fst showed a significant results in the analysis of the male mature group (see below for a discussion of this result in the context of scenario A). Some Committee members believe that haplotype statistics, and particularly the chi-square test, are more sensitive to detect structure. Then we conducted an additional analysis using the randomized chi-square test. Results from the chi-square test are consistent with those from the AMOVA, particularly those derived from the Fst for Male Mature (see below for a discussion of this result in the context of scenario A).

These results constitute a very strong demonstration that the underlying hypothesis of scenario C, e.g. three stocks with hard boundaries at 147°E and 157°E, has no independent support as there are no genetic evidences for multiple stocks as proposed by this scenario. Genetic differentiation among stocks is facilitated by some geographical or ecological 'barrier', which acts restricting the gene flow among them. Minke whales in sub-areas 7, 8 and 9 have been described as opportunistic feeders (Tamura and Fujise, 2002). Distribution of these whales is related to the distribution and dynamics of prey species and the distribution of prey species is defined by oceanographic conditions in the area, which are variable. There is no evidence for ecological barriers at 147°E or 157°E (see Doc. SC/55/IST4 at this meeting by Kawahara *et al.*).

Why then was this unrealistic baseline stock scenario proposed for the *ISTs* trials? To provide an answer to this question it is necessary to review how this scenario emerged in the trials.

Baseline C is based on the results of the BR technique (Martien and Taylor, 2001). Basically this technique involves the following steps:

- Definition of small geographical units in the research area.
- Construction of connectivity matrix according to some assumption on the model of stock structure of the species
- Clustering analysis of geographical units on the basis of their genetic similarities (very similar to the clustering algorithm called UPGMA). The measure of genetic similarity used is the chi-square P value.
- Evaluation of hypothesized population structures using parameter estimation, in particular estimation of dispersal rates across putative boundaries.

The different hypotheses on stock structure derived from this technique will depend on the way as the connectivity matrices are constructed and this in turn will depend on the model of stock structure assumed. For example the models assumed for migratory species should be different from those assumed in the case of non-migratory species. In this regard the authors of BR noted that the technique has only developed for non-migratory species (harbor seals and harbor porpoise) and that minke whales will require a case specific approach because they are not only migratory but also arrive in the different sub-areas at different times according to age and sex (Taylor, 2000). The application of the BR to minke whale was made without consideration of the good advice offered by Taylor (2000).

The BR should be considered just as an exploratory technique and the different stock structure hypotheses derived from this technique should be further evaluated by means of parameters estimates (for example estimates of dispersal rates) or by the use of independent data to check these hypotheses. The use of independent data avoids circular reasoning (using the same data to create and test hypotheses). In the case of western North Pacific minke whale such evaluation was not made during the 2002 workshop, opportunity in which stock scenarios were adopted for *ISTs*.

The only estimations of dispersal rates for western North Pacific minke whale were presented during the 2003 workshop. However these estimations were not presented or discussed in the context of evaluation of the different hypothesized boundaries derived from BR. These were just estimations among Ow, Oe and W once this scenario had been adopted for *ISTs*. Notwithstanding it should be noted that the confidence interval for the estimate for the rate between Ow and Oe included infinity, which correspond to complete mixing.

During the 2002 workshop Martien and Taylor (2002) presented a method to estimate the degree of support for population structure hypotheses generated by BR. In this method the likelihood of an observation is used as a test statistics. However the method does not consider that all assessments are based on different sample sizes, and this could have a large effect on the results. A second and more critical problem is that the method does not provide criteria to assess or evaluate differences among the obtained likelihood estimates. It is essential that the different hypotheses derived from this technique be evaluated through an experimental test before a specific scenario is proposed. Such evaluation was not conducted in the case of the western North Pacific minke whale.

In more general terms, it should be noted that BR was proposed as an alternative to standard hypothesis testing, which has been criticized because in some cases the tests are based on poorly defined *a priori* strata. However the basic procedure of the BR technique can be subjected to the same criticism. What the BR performs is very similar to the procedure used in standard stock structure analysis: following the previously defined connectivity matrix, initial strata are compared statistically and the most similar genetically are pooled and compared to other strata. This process is repeated several times. In the hypothesis testing, previously defined strata are compared statistically and those not differing statistically are pooled and compared to other strata. If we have good previous knowledge on the biology of the species and in particular on the model of stock structure, then we will able to adequately defined *a priori* strata in the case of hypothesis testing or connectivity matrix in the case of BR. If no adequate information is previously available, then both methods can be subjected to the same kind of criticism as the initial strata in the case of hypothesis testing or the connectivity matrix in the case of BR will be poorly defined. It should be noted here that the BR-derived stock structures in western North Pacific minke whale (see Taylor and Martien, 2002) were based largely on a specific assumption of a stock structure model for which the technique had been tested (isolation by distance), however other models are possible for the migratory minke whale.

Hypothesis testing-based studies on western North Pacific minke whale have been based on what originally were arbitrary lines drawn on the map e.g. divisions among sub-areas 7, 8 and 9. However this technique has provided useful information on possible additional structure in these sub-areas. For example, the first evidence for the occurrence of a W stock in sub-area 9 derived from hypothesis testing using mtDNA data (Goto and Pastene, 2000 for Fst statistics; Taylor, 2000 for chi-square). These studies showed near-to-significant P values in the comparisons involving sub-area 9. To investigate the source of these low P values Goto *et al.* (2000) conducted additional hypothesis testing based on a post-stratification of the samples in sub-area 9. They concluded that a possible source of mtDNA heterogeneity was attributed to animals sampled in the western part of sub-area 9 in 1995. Subsequent sampling in sub-area 9W in 2000 and 2001 showed similar results. Therefore results of hypothesis testing on mtDNA data have been informative of the temporal occurrence of W stock in part of sub-area 9.

Scenario D

Results of our genetic analysis are also difficult to reconcile with the underlying hypothesis of scenario D. This hypothesis assumes that O and W stocks mix with each other between 147°E and 162°E. The O stock is predominant to the west and the W stock to the east of this range. Under this scenario only O stock occur between the Japanese coast and 147°E and only W stock from 162° to the east. It should be noted that an AIC analysis based on mtDNA data showed scenario D as much less plausible than A or B (Goto *et al.* 2002). One of the inconsistencies is that the mtDNA haplotype frequency in samples from sub-area 9E (scenario D assume that only W stock is distributed in this sector) is similar to those in sub-areas 7 and 8 (O stock). Furthermore analysis of microsatellite indicated no significant departure from the Hardy-Weinberg equilibrium in the sector 147°-162°E. This is not that we could expect from a situation of mixing between different stocks as propose by scenario D. Scenario D is therefore completely inconsistent with genetic data.

Scenarios B
In general results of the present genetic study are consistent with previous results derived from the analysis of other non-genetic markers and discussed during the JARPN review meeting (IWC, 2001): morphometric (Hakamada and Fujise, 2000), pollutant burden (Fujise *et al.*, 2000), parasite load (Kuramochi *et al.*, 2000) and biological parameters (Zenitani *et al.*, 2000; 2002). Overall no substantial genetic heterogeneity is observed in sub-areas 7, 8 and 9. Therefore these results are consistent with the hypothesis of a single stock in these sub-areas (e.g. the underlying hypothesis of baseline scenario B).

Scenario A
In the AMOVA analysis using Fst statistics we found some mtDNA heterogeneity when only the MM component was analyzed. When samples in sub-area 9W in 1995, 2000 and 2001 were excluded from the analysis these significant differences disappeared (these samples had been taken mainly late in the season). Therefore the heterogeneity in putative W stock is due to animals sampled in sub-area 9W late in the season. These results were similar to that found using chi-square test (see also previous genetic studies by Goto *et al.*, 2002 and Cui *et al.* 2002). These results are consistent with the underlying hypothesis of baseline stock scenario A (the intrusion hypothesis).

The weak point of scenario A is that heterogeneity in sub-area 9W has been shown only by a single loci marker (mtDNA) and, nuclear DNA and all other non-genetic markers have failed to provide evidence for the existence of the W stock. In contrast in the case of the Antarctic minke whale, results from mtDNA (Pastene *et al.*, 1996) have been supported partially from morphometric analysis (Fujise, 1995). Clearly if the W stock exists and has a large population size, the core of its distribution should be to the east of sub-area 9 e.g. sub-area 13. In that case the genetic analysis of samples from sub-area 13 should show sharp difference with any samples taken in sub-areas 7, 8 and 9.

Weighting baseline stock structure scenarios
Based on the considerations discussed above our weighting for the plausibility of the different *ISTs* stock scenarios is as follow:

Scenario A: Medium. This scenario is consistent with mtDNA data. It will make biological sense if both geographical and temporal factors are considered to explain distribution of stocks.

Baseline A specifies that W stock occur in sub-area 9W each year with a 60% probability. In these years 50% of animals in sub-area 9W are W stock. The latter proportion is not confirmed. Furthermore W stock whales have not been detected on the three occasions when sub-area 9E has been surveyed. Thus assuming as many as 50% of the animals in sub-area 9W in some years are from the W stock is erring on the high side. In fact, we consider that this percentage is much larger than is likely to be the case in reality. Accordingly we consider that scenario B should be given more weight than scenario A.

Scenario B: High. This scenario is consistent with genetic and non-genetic data (except for the results of the post-stratification analysis of mtDNA in sub-area 9W).

Scenario C: Very Low. This scenario is inconsistent with both genetic and non-genetic data as detailed above, and its hard boundary assumptions are inconsistent with spatially variable oceanographic conditions and food distributions.

Scenario D: Low. This scenario is inconsistent with both genetic and non-genetic data as detailed above.

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Table 1: Sample sizes used in the present genetic analysis, by putative stock of baseline C scenario and sexual components. Early refers to months April-June and Late to months July-September. IM= Immature male, MM= Mature male, IF= Immature female, MF= Mature female. Table A refers to JARPN/JARPN II samples only; Table B to a combination of past commercial and JARPN/JARPN II samples. MtDNA analysis was conducted on both data sets. Microsatellite analysis only on set A).

A)

Period	Ow				Oe				W				Total
	IM	MM	IF	MF	IM	MM	IF	MF	IM	MM	IF	MF	
Early	27	79	9	4	6	69	5	3	13	56	7	5	283
Late	12	35	1	7	3	63	0	2	3	134	5	10	275
Total	39	114	10	11	9	132	5	5	16	190	12	15	558

B)

Period	Ow				Oe				W				Total
	IM	MM	IF	MF	IM	MM	IF	MF	IM	MM	IF	MF	
Early	63	94	46	6	6	69	5	3	13	56	7	5	373
Late	25	58	11	16	3	63	0	2	3	134	5	10	330
Total	88	152	57	22	9	132	5	5	16	190	12	15	703

Table 2: Results of the mtDNA AMOVA analysis for data set in Table 1B. In the nested analysis the P-value (in parenthesis) is the probability of a more extreme variance component or PHIst than that observed, in comparison to a null distribution of these values on 2,000 random permutations of the data matrix. PHIct and the among stock (AS) variance involve the permutation of whole sexual components (Sco) among stocks; the PHIsc and the among sexual components within stocks (AScoWS) involves the random permutation of individuals among sexual components within stock; the PHIst and the within sexual components (WSco) involves the random permutation of individuals among all sexual components. Early refers to months April-June and Late to months July-September.

Period	Nested analysis					
	AS (%var)	AScoWS (%var)	WSco (%var)	PHIct (P)	PHIsc (P)	PHIst (P)
PHIst						
Early	0.04	-0.52	100.48	0.000 (0.590)	-0.005 (0.694)	-0.005 (0.750)
Late	0.12	0.19	99.69	0.001 (0.448)	0.002 (0.454)	0.003 0.329)
Total	-0.03	0.18	99.86	-0.000 (0.571)	0.002 (0.352)	0.001 (0.271)
Fst						
Early	-0.26	0.16	100.10	-0.003 (0.747)	0.002 (0.329)	-0.001 (0.521)
Late	-0.10	0.61	99.49	-0.001 (0.504)	0.006 (0.213)	0.005 (0.095)
Total	0.07	0.15	99.78	0.001 (0.498)	0.002 (0.303)	0.002 (0.135)

Table 3: Results of the mtDNA AMOVA analysis for geographical and temporal differentiation of MM (Mature male as in Table 1 B). In the nested analysis the P-value is the probability of a more extreme variance component or PHIst than that observed, in comparison to a null distribution of these values on 2,000 random permutations of the data matrix. PHIct and the among stock (AS) variance component involve the permutation of whole temporal groups (Te) among stocks; the PHIsc and the among temporal groups within stocks (ATeWS) involves the random permutation of individuals among temporal groups within a stock; the PHIst and the within temporal groups (WTe) components involves the random permutation of individuals among the all temporal groups. Early refers to months April-June and Late to months July-September.

Period	Nested analysis					
	AS (%var)	ATeWS (%var)	WTe (%var)	PHIct (P)	PHIsc (P)	PHIst (P)
PHIst	0.33	-0.31	99.98	0.003 (0.234)	-0.003 (0.736)	0.000 (0.526)
Fst	0.57	-0.31	99.45	0.006 (0.156)	-0.000 (0.507)	0.005 **(0.024)**

Table 4: Results of the mtDNA AMOVA analysis for geographical and temporal differentiation of MM (Mature male as in Table 1 B but excluding animals from sub-area 9W in 1995, 2000 and 2001).

Period	Nested analysis					
	AS (%var)	ATeWS (%var)	WTe (%var)	PHIct (P)	PHIsc (P)	PHIst (P)
PHIst	0.00	-0.41	100.41	0.000 (0.556)	-0.004 (0.739)	-0.004 (0.833)
Fst	-0.04	0.12	99.93	-0.000 (0.577)	0.001 (0.341)	0.001 (0.354)

Table 5: Results of the chi-square statistical test for genetic homogeneity using microsatellites. Sample sizes are shown in Table 1-A. Figures shown are probability values after 1,000 random permutations. Comparisons are made among sexual components within putative stocks (Ow, Oe and W) and among these putative stocks. Column on the right shows the results of comparison among stocks of Mature Male component (MM).

	No. alleles	Within Ow	Within Oe	Within W	Among Ow-Oe-W	MM Among Ow-Oe-W
EV37	11	0.316	0.469	0.553	0.951	0.918
EV1	28	0.866	0.258	0.821	0.240	0.547
GT310	14	0.423	0.639	0.519	0.421	0.698
GATA28	17	0.090	0.442	0.175	0.063	0.053
GT575	12	0.234	0.732	0.854	0.407	0.746
EV94	6	0.458	0.778	0.439	0.536	0.418
GT23	15	0.478	0.684	0.068	0.065	0.023
GT509	19	0.892	0.113	0.394	0.059	0.265
GATA98	6	0.323	0.189	0.577	0.412	0.409
EV104	5	0.973	0.196	0.814	0.026	0.161
GATA417	14	0.496	0.862	0.546	0.752	0.483
GT211	16	0.457	0.589	0.782	0.983	0.994
EV21	2	0.094	0.524	0.096	0.422	0.124
DlrFB14	5	0.040	0.682	0.395	0.313	0.109
EV14	6	0.601	0.424	0.984	0.853	0.775
GT195	12	0.255	0.217	0.871	0.719	0.531
TAA31	3	0.968	0.779	0.368	0.513	0.251
All loci		0.395	0.744	0.771	0.207	0.201