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ABSTRACT

During the IWC SC JARPNII review workshop conducted in February 2016, information and analyses on stock structure-related issues were presented in 25 scientific papers. The review workshop noted the substantial amount of field, laboratory and analytical work undertaken under the JARPNII program, and the contribution of the work for the assessment and management of sei, Bryde's and common minke whales in the North Pacific. At the same time the workshop provided four medium-long term recommendations, and three short-term recommendations. The latter should be responded by JARPNII scientists 'ideally by the 2016 Annual Meeting but certainly by the 2017 Annual Meeting' of the IWC SC. This paper responds to one of the three short-term recommendations from the JARPNII review workshop that the presence of multiple stocks within sample partitions should be assessed employing STRUCTURE and DAPC for Bryde's and sei whales. Results of additional STRUCTURE analyses supported the hypothesis of a single stock of Bryde's whale in sub-areas 1 and 2, which contradicts the results of the hypothesis testing of significant mtDNA and microsatellite differences between sub-areas 1 and 2. This reflects the well-documented difficulty that STRUCTURE has in detecting weakly differentiated populations. In the case of sei whales, results of the STRUCTURE and hypothesis testing were similar, and they support the existence of a single stock in the pelagic region of the North Pacific.

INTRODUCTION

The International Whaling Commission Scientific Committee (IWC SC) carried out a specialist workshop under the guidelines of Annex P (IWC, 2016) to review final data and results of the Second Phase of the Japanese Whale Research Program under Special Permits in the North Pacific (JARPNII) (JARPNII review workshop). Many of the analyses conducted under the three main objectives of JARPNII, including those on stock structure, were based on a combined data set of JARPNII and the previous JARPN program.

On the research objective on stock structure of large whales in the North Pacific, a total of 25 scientific papers were available for the review workshop. The workshop noted the substantial amount of field, laboratory and analytical work undertaken under the JARPNII program, and the important contribution of the studies for the management of common minke and Bryde's whales under the RMP, and for the *in-depth assessment* of sei whales (SC/66b/Rep 6).

At the same time the review workshop provided four medium-long term recommendations, and three short-term analytical recommendations. The latter should be responded by JARPNII scientists 'ideally by the 2016 Annual Meeting but certainly by the 2017 Annual Meeting' of the IWC SC (SC/66b/Rep 6).

The three short-term recommendations were the following (from SC/66b/Rep 6 p.18):

i) All references regarding randomness of observations (e.g. unassigned common minke whales) should be substantiated by a statistical assessment of the presumed randomness.
ii) The presence of multiple stocks within sample partitions should be assessed (employing, e.g. STRUCTURE and DAPC) for Bryde's and sei whales.
iii) More explicit information on quality checks be provided in each study as well as study-

specific estimates of genotyping and DNA sequencing error rates.

Respond to the third recommendation above can be found in Goto *et al.* (2016). This paper provides the background and the response to the second recommendation above by conducting additional STRUCURE running in the case of Bryde's and sei whales. Because results from STRUCTURE and DAPC were very similar in the case of the North Pacific common minke whale (Pastene *et al.*, 2016a), the authors considered that the running of only one of those two computer programs is fair enough to respond the recommendation from the JARPNII review workshop.

Response to the first recommendation above will be provided to the next meeting of the IWC SC.

BACKGROUND

Bryde's whale
During the final JARPNII review workshop, results of the analyses of hypothesis testing for both mtDNA and microsatellites, were presented. Table 1 shows the number and sources of the genetic samples of Bryde's whales used in the analyses while Figure 1 shows the geographical distribution of the genetic samples. In summary both markers showed no significant genetic heterogeneity between whales in sub-areas 1W and 1E, and the statistical power of the analysis was considered high (see details in Pastene *et al.*, 2016b). This result suggested no stock structure within sub-area 1, and it was consistent with the information on movement from mark-recapture and satellite tracking analyses.

In contrast significant differences were found between whales in sub-areas 1 and 2 for both mtDNA and microsatellites (Pastene *et al.*, 2016b), suggesting the possibility that different stocks occur in these sub-areas.

After examining these results, the JARPNII review workshop recommended that 'the presence of multiple stocks within sample partitions should be assessed employing, e.g. STRUCTURE and DAPC)' (SC/66b/Rep 6).

Sei whale
During the final JARPNII review workshop, results of the analyses of hypothesis testing for both mtDNA and microsatellites, were presented. Table 2 shows the number and sources of the genetic samples of sei whales used in the analyses while Figure 2 shows the geographical distribution of the genetic samples. In summary both markers showed no significant genetic heterogeneity between whales in the western and eastern sectors of the North Pacific (see details in Pastene *et al.*, 2016c). This result suggested no stock structure in the pelagic region of the North Pacific which is consistent with other lines of evidences (e.g. Kanda *et al.*, 2015a).

After examining these results, the JARPNII review workshop recommended that 'the presence of multiple stocks within sample partitions should be assessed employing, e.g. STRUCTURE and DAPC' (SC/66b/Rep 6).

MATERIALS AND METHODS

Samples
The number of samples, sub-area or sector and sources are shown in Tables 1 and 2 for Bryde's and sei whales, respectively. These are the same used in the hypothesis testing presented to the JARPNII review workshop. The geographical distribution of the samples of the two species is shown in Figures 1 and 2, respectively.

Microsatellite analysis
In the case of the two species, genetic variation at microsatellite DNA was analyzed using 17 loci, none of which was designed specifically from Bryde's whales: EV1, EV14, EV21, EV94, EV104 (Valsecchi and Amos, 1996), GT011 (Bérubé *et al.*, 1998), GT23, GT211, GT271, GT310, GT575 (Bérubé *et al.*, 2000), GATA28, GATA53, GATA98, GATA417, GGAA520 (Palsbøll *et al.*, 1997), and DlrFCB17 (Buchanan *et al.*, 1996).

Details of the laboratory procedures were given in Pastene *et al.* (2016b;c).

Analytical procedure
The Bayesian clustering approach was implemented with the microsatellite data in the STRUCTURE version 2.0 (Pritchard *et al.*, 2000) to determine the most likely number of genetically distinct stocks present in the samples. The program is a model-based clustering method for inferring stock structure (K, the number of stocks in the model) using multilocus genotype data with and without information on sampling locations. STRUCTURE allowed for the analyses of the samples without choosing sample units that did not necessarily correspond to real biological stock boundaries. Posterior probabilities for K were estimated from ten independent runs for each value of K=1-5 for Bryde's whale and K=1-3 for sei whale with only genetic information. All runs were performed with a burn-in period of 10,000 iterations and 100,000 MCMC iterations. The ancestry model used for the simulation was the admixture model, which assumes individuals may have mixed ancestry. The allele frequency model used was the correlated allele frequencies model, which assumes frequencies in the different stocks are likely to be similar due to migration or shared ancestry.

Following the recommendation from the JARPNII review workshop, in the case of Bryde's whale STRUCTURE was running for each sampling partition (sub-area 1E, sub-area 1W, sub-area 2 and entire sub-area). In the case of sei whales STRUCTURE was running for the entire research area.

RESULTS AND DISCUSSION

Bryde's whale
Bayesian clustering analyses conducted for different sampling partitions without information on their geographic origins presented the highest likelihood probability at K=1 (Tables 3-6). Figures 3-6 shows estimated stock structure at K=2-5. As evident from the figures, additional stocks were indistinguishable. These results indicated that the samples came from a single stock of Bryde's whale in all sampling partitions examined.

However these results are different from those from the hypothesis testing presented at the JARPNII review workshop (Pastene *et al.* 2016b). This reflects the well-documented difficulty that STRUCTURE has in detecting weakly differentiated populations.

Based on the results of the hypothesis testing, it is suggested that the plausibility of the stock structure hypotheses for western North Pacific Bryde's whale used in the 2007 *Implementation* whale should be re-examined. The results of this study suggest that the two-stock hypotheses (Hypotheses 2 and 3) could be more plausible than the one-stock hypothesis (Hypothesis 1) and the three-stock hypothesis (Hypothesis 4).

Sei whale
Bayesian clustering analyses conducted for all samples in the entire research area without information on their geographic origins presented the highest likelihood probability at K=1 (Tables 7). Figure 7 shows estimated stock structure at K=2-3. As evident from the figures, additional stocks were indistinguishable. These results indicated that the samples came from a single stock of sei whale.

In this case, results of the STRUCTURE and those from hypothesis testing (Pastene *et al.*, 2016c), were similar, and they support the existence of a single stock in the pelagic region of the North Pacific.

The *in-depth assessment* of North Pacific sei whale started at the 2015 IWC SC with two initial alternative stock structure hypotheses: i) a single stock in the entire North Pacific as proposed by Kanda *et al.* (2015a;b), based on several pieces of evidences included the genetics; and ii) a five-stock hypothesis proposed in Mizroch (2015), based mainly on the interpretation of mark-recapture data: Japan coastal; North Pacific pelagic; Aleutian Islands and Gulf of Alaska; eastern North Pacific migratory; and Southern North American coastal stock (coastal California) (IWC, 2015). As noted earlier the IWC SC agreed that discriminating between these two hypotheses is difficult in the absence of genetic data from the potentially extirpated stocks, and thus both hypotheses are plausible (IWC, 2015).

The main purpose of this study was to examine genetically all the available genetic samples of the sei whale in the North Pacific in order to confirm the hypothesis of a single stock in the oceanic regions of the North Pacific (the 'North Pacific pelagic' in the second stock structure hypothesis indicated above). As noted by the IWC SC it is not possible to check the plausibility of other putative stocks under the second hypothesis using genetics analyses because no genetic samples are available from the regions where these putative stocks are proposed.

Results of the present analysis are in agreement with previous genetic analyses based on smaller sample sizes (Kanda *et al*., 2009; 2015a). Kanda *et al*. (2015b) analyzed all non-genetic evidences that were consistent with the single stock in oceanic regions. In fact, the genetic and non-genetic evidences summarized by Kanda *et al*. (2015b) were more consistent with the first hypothesis above, a single stock of sei whales in the whole North Pacific.

It is considered that the evidences for proposing the second stock structure hypothesis (five stocks) are weak and that the IWC SC should evaluate objectively the plausibility of the additional putative stocks other than the 'North Pacific pelagic'.

ACKNOWLEDGEMENTS

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Table 1. Sample sizes of Bryde's whales used in the present study in the microsatellite analyses, by sub-area, sex, and source of samples. Only the samples genotyped for the complete microsatellite loci set were included in the analysis.

Sub-area		Sex	JARPNII	Commerc.	POWER	Jap. dedic.	Total
1	West	Male	274	105		20	399
		Female	346	66		36	448
	East	Male	14	16	10	1	41
		Female	45	12	20	1	78
2		Male				29	29
		Female		1		23	24
Total			679	200	82	58	1019

Table 2. Sample sizes of sei whales used in the present study for the microsatellite analyses, by longitudinal sector, sex, and source of samples. Only the samples genotyped for the complete microsatellite loci set were included in the analysis. Western and Eastern sectors were divided at 180°.

Data source	Sex	Sector		Total
		Western	Eastern	
JARPNII	Male	551		551
	Female	623		623
POWER	Male	1	36	37
	Female	1	40	41
Commercial	Male	88	67	155
	Female	92	55	147
Total		1356	198	1554

Table 3. Results of Bayesian clustering method for samples of Bryde's whales in sub-area 1E.

K	Log P(k/x)*	Variance*	Pr(k/x)**
1	-5745.21	52.09	~1.00
2	-5876.05	357.89	~0.00
3	-6004.32	629.35	~0.00
4	-6061.36	751.07	~0.00
5	-6000.82	609.39	~0.00

* log likelihood of the data for different values of K and the variance.
** Probability for each K.

Table 4. Results of Bayesian clustering method for samples of Bryde's whales in sub-area 1W.

K	Log P(k/x)*	Variance*	Pr(k/x)**
1	-40201	62.23	~1.00
2	-40655	1218.9	~0.00
3	-40975	2019	~0.00
4	-41786	3726	~0.00
5	-42944	6130.4	~0.00

* log likelihood of the data for different values of K and the variance.
** Probability for each K.

Table 5. Results of Bayesian clustering method for samples of Bryde's whales in sub-area 2.

K	Log P(k/x)*	Variance*	Pr(k/x)**
1	-2534.9	44.24	0.91
2	-2537.2	51.82	0.09
3	-2544.8	71	~0.00
4	-2548	79.19	~0.00
5	-2555.5	96.37	~0.00

* log likelihood of the data for different values of K and the variance.
** Probability for each K.

Table 6. Results of Bayesian clustering method for samples of Bryde's whales in the entire research area.

K	Log P(k/x)*	Variance*	Pr(k/x)**
1	-48510	62.71	~1.00
2	-49012	1353.1	~0.00
3	-49368	2223.9	~0.00
4	-50134	3867.4	~0.00
5	-51775	7225.4	~0.00

* log likelihood of the data for different values of K and the variance.
** Probability for each K.

Table 7. Results of Bayesian clustering method for samples of sei whales in the entire research area.

K	Log P(k/x)	Variance	Pr(k/x)
1	-68842.9	69.75	~1.00
2	-69340.5	1653.6	~0.00
3	-71366.9	5968.8	~0.00

* log likelihood of the data for different values of K and the variance.
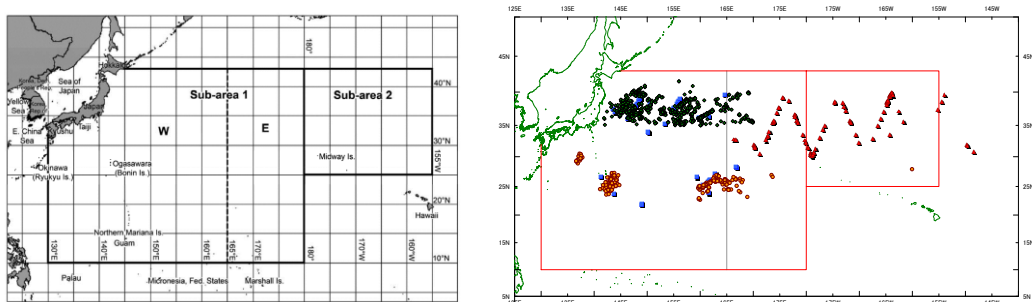** Probability for each K.

Figure 1. Sub-areas used during the RMP *Implementation* of Bryde's whale (left) and geographical distribution of genetic samples from different sources (right): Green: JARPNII samples; Blue: Japanese dedicated sighting survey samples; Orange: past commercial whaling samples; Red: IWC/POWER survey samples (from Pastene *et al*., 2016b).
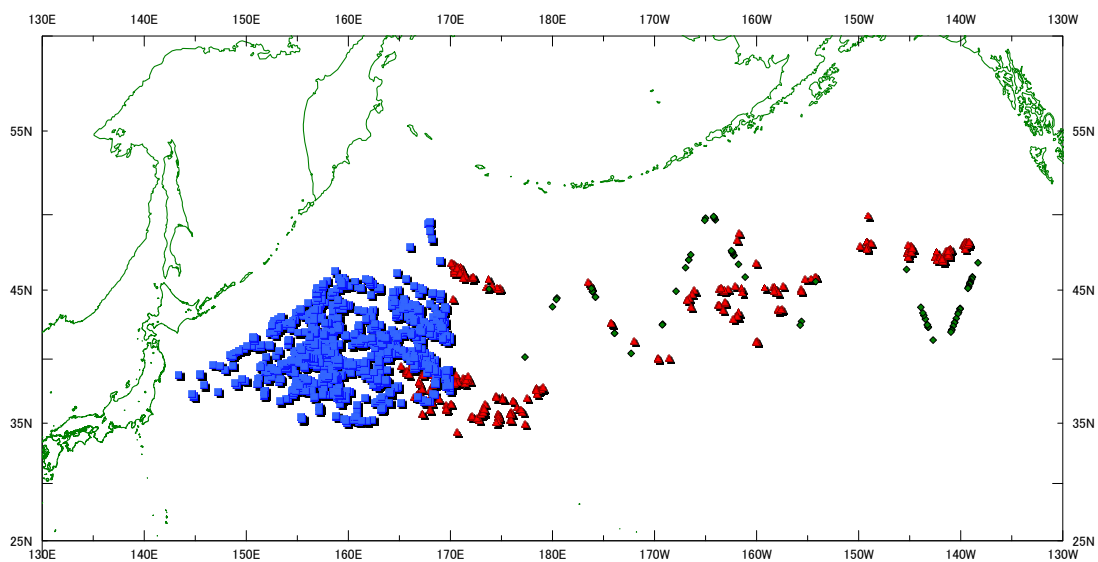

Figure 2. Sampling locations of sei whales used in the present genetic study. Blue: JARPNII samples; Red: past commercial whaling samples; Green: IWC/POWER samples. For the statistical analyses samples were arbitrarily divided into two sectors: Western and Eastern at 180° (from Pastene *et al*., 2016c).
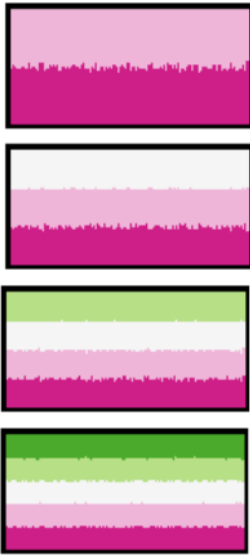
Figure 3. Plot of membership probabilities at K=2-5 for Bryde's whales in sub-area 1E. Each individuals is characterized by a thin vertical line, which is divided into K colored segments on the basis of the individual's membership fractions in K clusters.
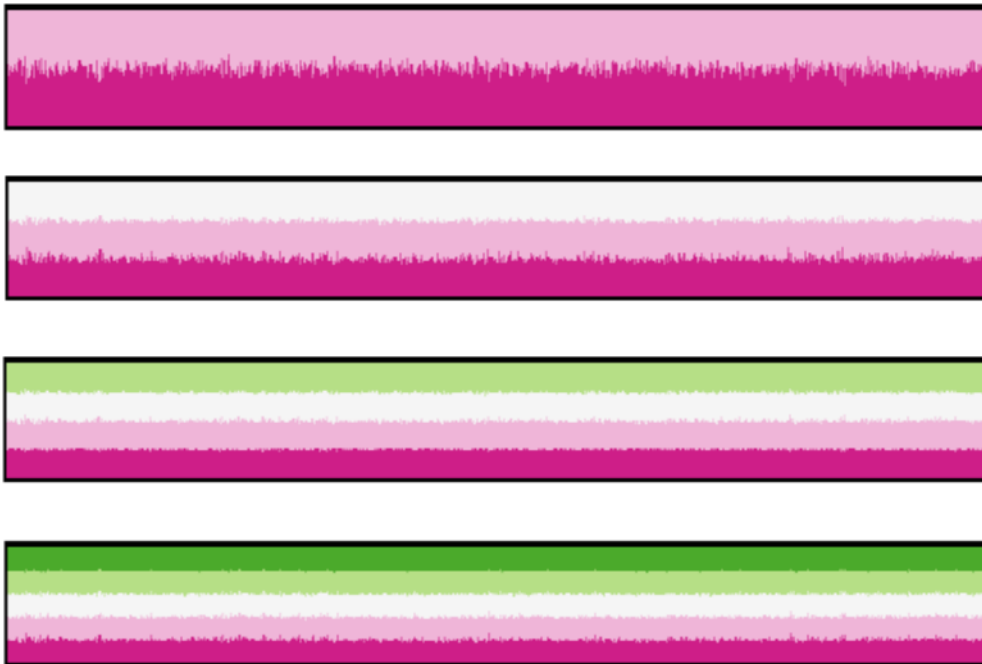

Figure 4. Plot of membership probabilities at K=2-5 for Bryde's whales in sub-area 1W. Each individuals is characterized by a thin vertical line, which is divided into K colored segments on the basis of the individual's membership fractions in K clusters.

Figure 5. Plot of membership probabilities at K=2-5 for Bryde's whales in sub-area 2. Each individuals is characterized by a thin vertical line, which is divided into K colored segments on the basis of the individual's membership fractions in K clusters.
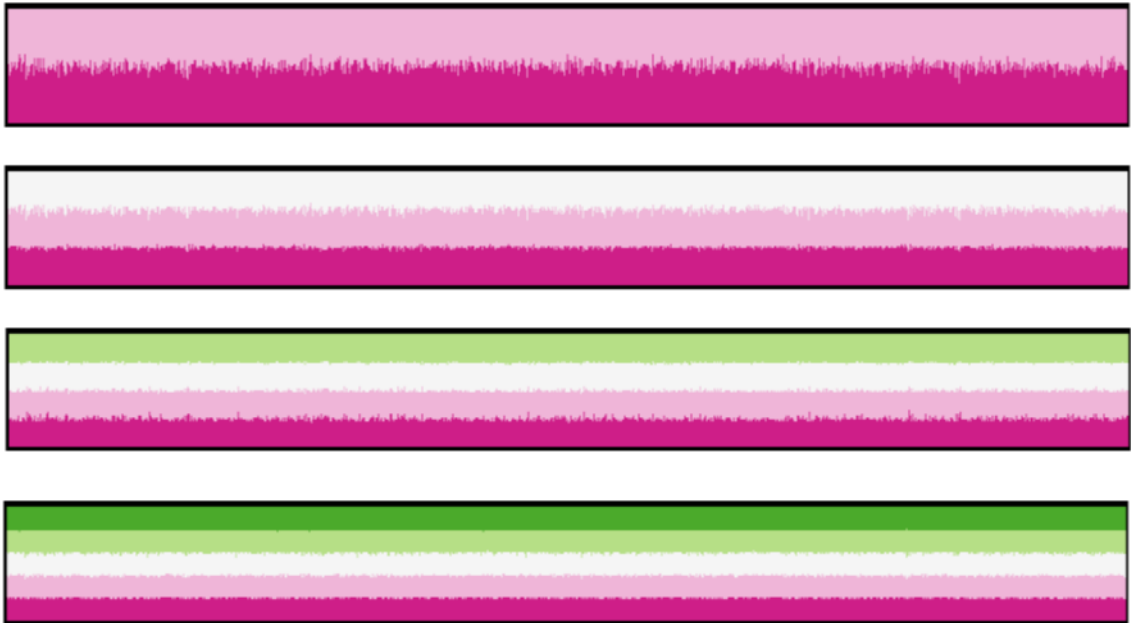

Figure 6. Plot of membership probabilities at K=2-5 for Bryde's whales for the entire research area. Each individuals is characterized by a thin vertical line, which is divided into K colored segments on the basis of the individual's membership fractions in K clusters.

Figure 7. Plot of membership probabilities at K=2-3 for sei whales in the entire research area. Each individuals is characterized by a thin vertical line, which is divided into K colored segments on the basis of the individual's membership fractions in K clusters.