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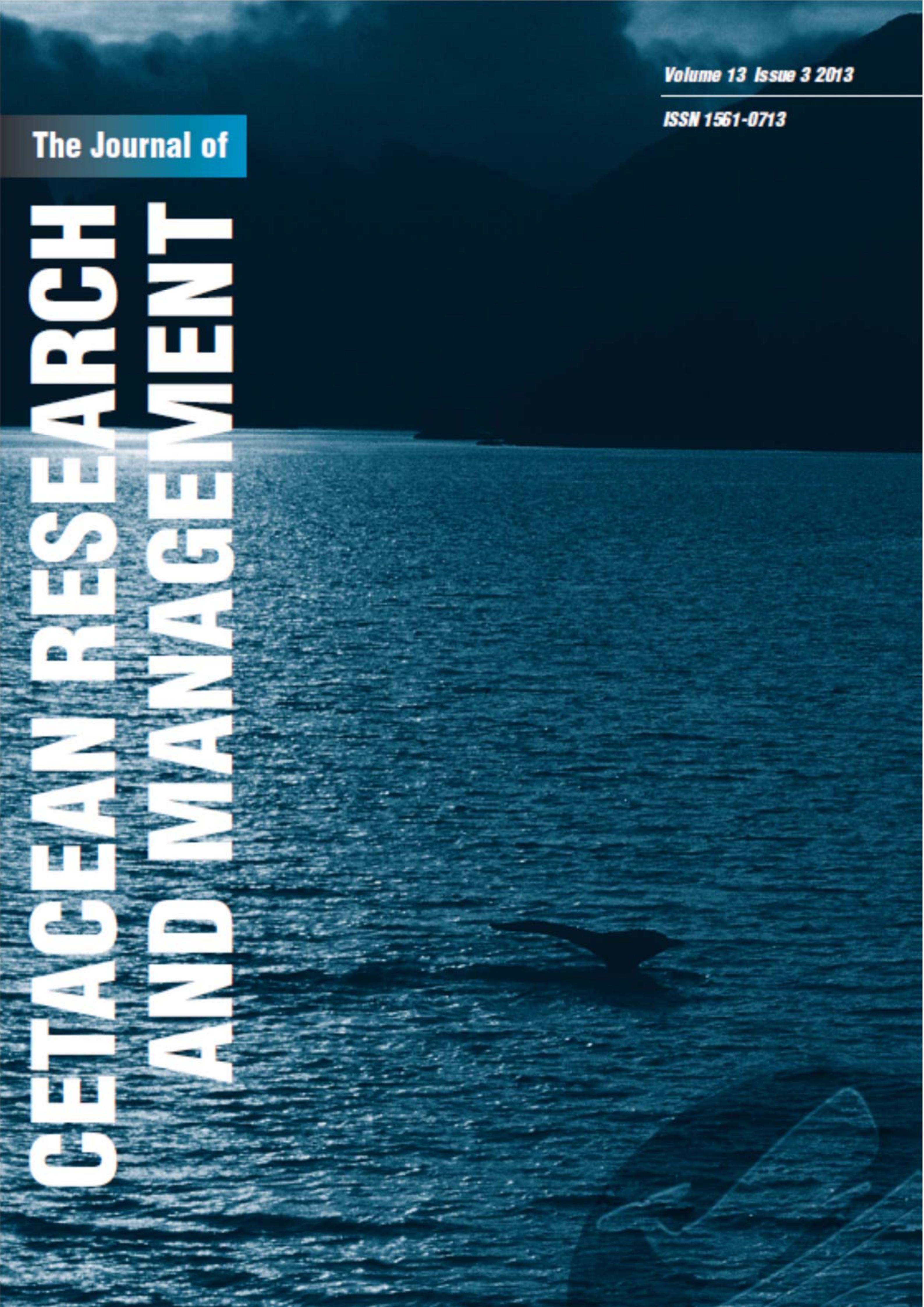

# Contents

# Editorial

Welcome to this the third issue of the thirteenth volume of the *Journal of Cetacean Research and Management*. This volume contains six papers covering a wide range of conservation and management issues.

To submit a manuscript to the Journal, please contact in the first instance Jessica Peers (e-mail: *jessica.peers@iwc.int*). A *Guide for Authors* is published at the end of issue one, and is also available online at the journal address below.

The Journal will be launching online this year. Go to *http://www.iwc.int/jcrm* for details.

G.P. DONOVAN
*Editor*

# A statistical model for quantifying age-reading errors and its application to the Antarctic minke whales

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ABSTRACT

A statistical method for quantifying age-reading error, i.e. the extent of bias and inter-reader variability among readers, is introduced. The method assumes the availability of an independent 'control reader' who produces reference ages for age-reading structures which are also read by additional readers. This control reader is assumed to provide unbiased or consistently biased age estimates so that the additional readers' age-reading outcomes can be standardised. Linear structures in bias and variance are incorporated in a conditional probability matrix representing the stochastic nature of age-determination for each reader. A joint likelihood function for the parameters related to age-reading bias, variance and nuisance parameters is defined based on observed age-reading outcomes from both the control and additional readers. The method is applied to data for Antarctic minke whales taken during Japanese commercial (1971/72–1986/87) and scientific (JARPA: 1987/88–2004/05, JARPA II: 2005/06–2010/11) whaling. A total of 250 earplugs selected according to a predetermined protocol were used in the analyses to estimate the inter-reader variation for four Japanese readers. One of the authors acted as the control reader. The Japanese readers and the control reader differed in terms of both the expected age given the true age, and variance in age-estimates. The expected age and random uncertainty in age-estimates differed among the Japanese readers, although the two readers in charge of age-reading for samples taken during Japanese scientific whaling (JARPA and JARPA II) provided quite similar age-reading outcomes. These results contribute to analyses using catch-at-age data for this species. It should also be noted that the model and approach in this paper can be applied to populations other than the Antarctic minke whales, if a control reader is available, even retrospectively.

KEYWORDS: ANTARCTIC MINKE WHALES; AGE-READING ERROR; EARPLUGS

## INTRODUCTION

The primary source of information on the abundance of many cetacean populations is estimates of abundance from sightings surveys (e.g. Branch, 2011; Matsuoka *et al.*, 2011) and from mark-recapture studies (e.g. Larsen and Hammond, 2006; Paton *et al.*, 2011). This information allows an evaluation of recent (up to the last 20–30 years) trends in abundance when a time-series of comparable estimates is available. However, inferences regarding the status of populations relative to management reference points are more precise if estimates of abundance and information from other data sources are used to fit population dynamics models. Although a variety of population dynamics models has been applied to cetacean populations, most of those presented to the IWC Scientific Committee in recent years have been age- and sex-structured.

Population dynamics models have been proposed as one way to test the hypothesis that the abundance of Antarctic minke whales (*Balaenoptera bonaerensis*) increased in abundance prior to the start of directed harvesting during the early 1970s, perhaps due to the 'krill surplus' which has been postulated to have arisen because of the substantial declines of species such as blue, fin and sei whales (e.g. Laws, 1977; Mori and Butterworth, 2006). The output from these models can also provide the information needed to assess the impact of environmental factors on, for example, reproductive success, as is common in fisheries assessments (e.g. Maunder and Watters, 2003; but also see Haltuch and Punt, 2011).

Age-structured assessment models can be divided into two major 'classes': Virtual Population Analysis (VPA) and 'integrated' methods. VPA methods assume that the catch age-composition is measured with negligible error compared to that associated with the remaining data sources such as estimates of abundance from sightings surveys, while 'integrated' methods allow for sampling and other sources of error with the age-composition data, albeit at the expense of greater complexity. Both of these classes of model have been applied to data for Antarctic minke whales (e.g. Mori *et al.*, 2007; Mori and Butterworth, 2008; Punt and Polacheck, 2005; 2006; 2007; 2008), and both suggest an increase in abundance from ~1930 to ~1970. However, Punt and Polacheck (2005) found that there were substantial differences between growth curves estimated internally to the 'integrated' model based on the length and age data collected from the commercial catches or externally using JARPA[4] data. Several possible explanations for this result have been explored, including time-varying growth and time-varying fishery selectivity. However, one key possible reason for this discrepancy is age-reading error.

Age-reading error can be divided into age-reading bias (i.e. the expected age assigned to age-reading structures, in this case, earplugs, for animals of a given age, differ from the actual age) and random age-reading error (i.e. variation about the expected age for a given age-reading structure). Both of these types of error can be consequential for assessments based on population models. For example, Reeves (2003) found that random age-reading error led to 'smoothing' of recruitment estimates (i.e. large year-classes

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[4] JARPA is the acronym for the Japanese whaling programme under special permit in the Antarctic (e.g. see IWC, 2008).

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'smoothed' to adjacent, less abundant, year-classes). More importantly perhaps, a 'drift' of age-reading methods could have led population models to estimate spurious trends in recruitment for the Antarctic minke whales (Butterworth and Punt, 2009).

The impact of age-reading error can be included in population assessments by specifying a matrix which defines the conditional probability of an animal of true age *a* being aged to be that age or some other age, *a*′, $P(a'|a)$. The model-predictions upon which the likelihood component in the assessment for the age-composition data is based are then a function of the model-estimate for the observed catch of animals of age *a* after accounting for age-reading error. Given $P(a'|a)$, this prediction would be:

$$C_{a'} = \sum_{a} P(a' \mid a) C_a \qquad (1)$$

where $C_a$ is the model-estimate of the catch of animals of age *a*, and $C_{a'}$ is the model-estimate of the catch of animals of (perceived) age *a*′ after accounting for age-reading error. Therefore, it is crucial for the analyses which use catch-at-age data to have information on $P(a'|a)$ for each age reader.

This paper introduces a method for quantifying age-reading errors. It was applied to data from an age-reading experiment conducted for the Antarctic minke whale. The experiment involved one reader (Lockyer) reading 250 earplugs using a protocol designed by the Scientific Committee of the IWC (Butterworth and Punt, 2009) and comparing the resulting age-estimates with values obtained from past and current age readings by Japanese scientists.

## METHODS

### The age-reading experiment

*Selection of samples and experimental protocol*

The experiment involved reading 250 earplugs from female minke whales caught in Antarctic Area IV (Table 1). The plugs were chosen from five groups of years (50 from each group), corresponding to periods near the start and the end of commercial whaling, and the start, middle and end of JARPA sampling (1974/5–1976/7, 1982/3–1984/5, 1989/90–1991/2, 1997/8–1999/2000 and 2003/4–2004/5; referred to as Periods I–V respectively). Random selection was achieved by allocating all the female earplugs for the period a number from 1 to *N*. A random number was then drawn from [1, *N*] and that plug selected, unless it was seen to be damaged or to have deteriorated in quality, in which case another random draw was made. The length of the whale, previous age readings, and the names of the original age readers (Kato, Masaki or Zenitani) were known for the selected whale. In addition to these three readers, a new reader (Bando) who aged all of the samples taken during JARPAII so far (2005/06–2010/11) also read 100 recent samples three times so that his reading outcomes could be standardised against the others.

All of the earplugs were read twice (Lockyer), with randomised sample order both initially and after each complete set of readings. Fifty of the plugs (10 from each of the five periods), again selected at random, were read a third time. Another two readers (Zenitani and Bando) also read each earplug three times (see Table 1). All readings were blind, i.e. the reader had no knowledge of other data pertaining to the whale from which the earplug was taken.

*Detailed procedure of Lockyer's age-readings*

A sample of 100 specially selected earplugs, independent of the experimental sample, was made available to Lockyer, who had expressed a wish to undertake a 'trial' reading of minke whale earplugs in general during 1–2 December, 2009. Lockyer read 50 earplugs from this sample to become re-familiarised with the Growth Layer Group (GLG) counting methods for this species (Lockyer, 1984). Although the specimens had their true ID numbers, they were read 'blind'. The results of this trial, although not part of the experimental design, helped to refine the design of a proposed age recording form.

The first reading began on the afternoon of 2 December 2009, and continued each day until completion, with readings on 3, 4, 6 and 7 December 2009, with approximately 50 earplugs read each day or a maximum of 70 on any one day, with breaks every two hours to rest the eyes. A *Nikon* binocular microscope was used to examine all earplugs with an eye objective 10 × B22 and zoom magnification ×0.8–×8 facility. Even at maximum magnification, it was only just possible to read all GLGs at the earplug base of some of the older animals. Five earplugs were placed in water in separate petri dishes with individual labelling for examination at any one time. These were then replaced in sample jars before the next set of five.

The second readings began after a 2-day break on 10 December, 2009, and continued on 11, 12, and 15 December, 2009. A break was then taken 16 December, 2009, before the third reading of a sub-set of 50 ear plugs took place. These readings were completed on 17 December, 2009.

Table 1

The number of samples employed in the experiment.

| | | Group 1 | Group 2 | | | |
|---|---|---|---|---|---|---|
| | Period | Lockyer* | Masaki | Kato | Zenitani | Bando |
| Period I | 1974/75–76/77 | 50 (10) | 50 | 0 | 0 | 0 |
| Period II | 1982/83–84/85 | 50 (10) | 0 | 50 | 0 | 0 |
| Period III | 1989/90–91/92 | 50# (10) | 0 | 28# | 22# | 0 |
| Period IV | 1997/98–99/2000 | 50 (10) | 0 | 0 | 50 | 50 |
| Period V | 2003/04–04/05 | 50 (10) | 0 | 0 | 50 | 50 |

*The numbers in parenthesis indicate how many plugs were read three times by Lockyer. #The fifty plugs selected for this period were originally read either by Kato or Zenitani.

An *Excel* data book (Appendix 1) was updated regularly (usually after reading 10 earplugs) throughout the experimental readings, compiling all information written on a working form. This also helped to make convenient breaks between each microscope use and avoid monotony. Reading efficiency depends greatly on the degree of alertness and the day's reading session was terminated on two occasions because of the onset of tiredness.

The colour of the earplugs varied from pale ivory through tan to dark brown. The earplugs for young animals were usually pale cream, while most earplugs for older animals appeared dark. However, this was not always consistent. The pale colouration frequently made it difficult to discern any GLG differentiation, and earplugs for apparently very young animals were often very difficult to age. In addition, accessory laminae were sometimes present and led to difficulties with age determination. For this reason, occasionally two possible alternative readings were provided because the reader could not be certain which to choose. Normally, not in this experimental setting, a reader might refer to biological data to help resolve such issues.

An example of an earplug used in this experiment and age-reading outcomes are shown in Fig. 1. This earplug was read by three readers (Lockyer, Zenitani and Bando) whose age-reading outcomes are (1st = 15, 2nd = 16), (1st = 15, 2nd = 15, 3rd = 14) and (1st = 16, 2nd = 16, 3rd = 16), respectively.

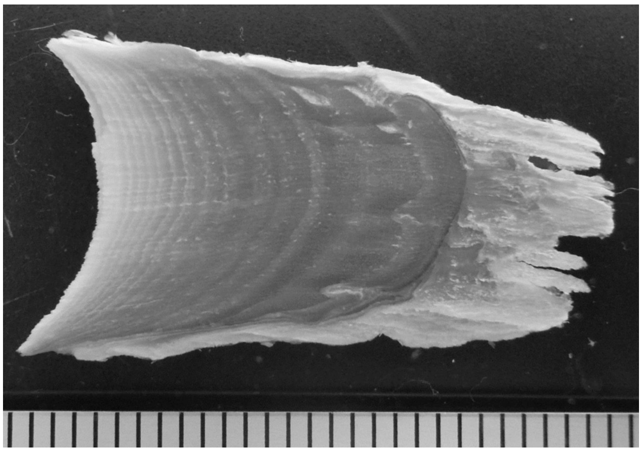

Fig. 1. An earplug used in the experiment. This earplug, which was collected during Period V, was read by Lockyer, Zenitani and Bando, whose age-reading outcomes were (1st = 15, 2nd = 16), (1st = 15, 2nd = 15, 3rd = 14) and (1st = 16, 2nd = 16, 3rd = 16), respectively. The photo was taken and provided by Takeharu Bando.

Lockyer was unable to obtain an age for all of the earplugs (Table 2). Age estimates could be obtained for more than 86% of the plugs for each trial, although the proportion of plugs which could be read decreased between the first and second trials. All the records of ages from the Japanese readers were 'valid' except for 1% of values by Bando, which were recorded as 'minimum'. The bulk of the analysis is based on the 'valid' readings only, although sensitivity tests consider the use of the data from the other categories in Table 2.

### Statistical analysis

*Conditional probability for age-reading errors*

Suppose that two groups of readers independently obtain age-estimates using a common set of $n$ samples (here $n$ = 250). Group 1 consists of only one reader (Lockyer), who conducted age-reading at most three times for all the $n$ samples. Group 2 consists of four readers (Masaki, Kato, Zenitani and Bando). Masaki, Kato and Zenitani read different earplugs, whereas Bando read the 100 samples from Periods IV and V, which Zenitani also read. The sample sizes for Masaki and Kato were respectively $n_M = 50$ and $n_K = 78$, and Zenitani and Bando read earplugs three times for their samples of $n_Z = 122$ and $n_B = 100$, respectively (Table 1).

Let $a_{1jk}$ ($j = 1,2,\ldots,n$; $k = 1,\ldots,r_j$) be the observed ages by Group 1 (assuming that it is a 'valid' count) of the $j$-th sample during the $k$-th of $r_j$ trials ($r_j$ = 2 or 3). Similarly, for Group 2, let $a_{2jk}$ ($j = 1,2,\ldots,n_M$) and $a_{2j}$ ($j = n_M + 1,\ldots,n_M + n_K$) respectively denote the observed ages by Masaki and Kato and $a_{2jk}$ ($j = n_M + n_K + 1,\ldots,n;k = 1,2,3$) denote the observed counts for the $j$-th sample during the $k$-th trial by Zenitani. Also, let $\tilde{a}_{2jk}$ ($j = 151,\ldots,n$; $k = 1,2,3$) be the readings by Bando. As noted above, Lockyer did not assign 'valid' ages to all of the samples during all of the trials (Table 2). In such cases, the notation changes accordingly. For example, the data for the 'either' or 'interval' categories can be denoted $a^{(1)}_{1jk}$ and $a^{(2)}_{2jk}$ respectively (for 'either' the age is either $a^{(1)}_{1jk}$ or $a^{(2)}_{1jk}$ , while for 'interval' the age-estimate is between $a^{(1)}_{1jk}$ and $a^{(2)}_{1jk}$). The 'minimum' counts by Lockyer and Bando were treated in a similar manner.

Now, consider the joint probability distribution of the observations. Let $b_i(a;\phi)$ and, $\sigma_i(a;\phi)$ respectively, denote the expected age and standard deviation for the age-estimates for the $i$-th Group for an animal of true age $a$, where is a

Table 2

Types of data provided by Lockyer.

| Index | Category$ | Data type | 1st trial | 2nd trial | 3rd trial |
|---|---|---|---|---|---|
| 0 | Valid | Age | 228 (91.2%) | 216 (86.4%) | 43 (86%) |
| 1 | Either | Age1 or Age2 | 2 (0.8%) | 4 (1.6%) | 0 |
| 2 | Minimum | Age > = | 11 (4.4%) | 10 (4.0%) | 3 (6.0%) |
| 3 | Interval | (Age1, Age2) | 1 (0.4%) | 0 | 0 |
| 4 | May be missing | Age | 1 (0.4%) | 0 | 0 |
| 10 | Uncertain | Age | 2 (0.8%) | 12 (4.8%) | 3 (6.0%) |
| 100 | Unreadable | N/A | 5 (2.0%) | 8 (3.2%) | 1 (2.0%) |

$'valid': a single age was recorded; 'either': two possible ages were offered; 'minimum': only a minimum age was counted; 'interval': a range of possible ages was given; 'missing': part of the plug was missing; 'uncertain': the reader was not confident in the age estimate. The numbers in parentheses are percentages for each trial.

vector of unknown parameters. The variability in age-reading is expressed as a matrix form $\{P_i(a'|a;\phi)\}_{a,a'=L,\ldots,H}$, where:

$$P_i(a'\mid a;\phi)\propto \exp\left[-\frac{\left(a'-b_i(a;\phi)\right)^2}{2\sigma_i^2(a;\phi)}\right] \qquad (2)$$

is the conditional probability that the *i*-th group draws age-reading outcomes *a′* given that the true age of the animal is *a,* and $\sum_{a'=L}^{H} P_i(a'\mid a;\phi)=1$ for all *a* (Punt *et al*. 2008), where *H* and *L* are respectively maximum and minimum possible ages.

The expected age for Reader 1 is assumed to be proportional to the true age:

$$b_1(a)=(1+x)a\ . \qquad (3)$$

On the other hand, the expected age for the readers in Group 2 is a linear function of true age *a*:

$$b_2(a;\phi)=b_L+(b_H-b_L)\frac{a-L}{H-L}\ . \qquad (4)$$

This is a 2-parameter model from Punt *et al*. (2008). The parameters of equation (4) should relate to each reader when considering hypotheses related to reader effects. The values of *L* and *H* are pre-specified (*L* = 0 and *H* = 70) and are not estimated.

The functional form of the age-reading error standard deviation for the two Groups is also assumed to be a linear function of true age:

$$\sigma_i(a;\phi)=\phi_{iL}+(\sigma_{iH}-\sigma_{iL})\frac{a-L}{H-L} \quad (i=1,2)\ . \qquad (5)$$

As for the expectation, the parameters in the equation (5) are specific to the reader concerned.

*Likelihood function*

Let $\beta=(\beta_L,\ldots,\beta_H)$ be the true age composition of sampled animals, which is unknown. The contribution of *j*-th sample by Reader 1 to the likelihood given the true age (say *a*) is:

$$P_1(a_{1j}\mid a;\phi_1)=\prod_{k=1}^{r_j} P_1(a_{1jk}\mid a;\phi_1) \qquad (6)$$

where $a_{1j}=(a_{1j1},\ldots,a_{1jr_j})$ and $\phi_1$ is the vector of parameters of interest. By considering the distribution for Group 2 in a similar way, the joint probability distribution of age-reading outcomes by the two groups is provided by a mixture form as:

$$\Pr(a_{1j},a_{2j};\phi,\beta)=\sum_{a=L}^{H}\beta_a P_1(a_{1j}\mid a;\phi_1)P_2(a_{2j}\mid a;\phi_2) \qquad (j=1,2,\ldots,150) \quad (7)$$

$$\Pr(a_{1j},a_{2j},\tilde{a}_{2j};\phi,\beta)=\sum_{a=L}^{H}\beta_a P_1(a_{1j}\mid a;\phi_1)P_2(a_{2j}\mid a;\phi_2) \times P_2(\tilde{a}_{2j}\mid a;\phi_2) \quad (j=151,\ldots,250) \quad (8)$$

where $\phi_2$ is the parameter vector for Group 2 and $\phi=(\phi_1,\phi_2)$. Finally, the full likelihood function for the parameters is:

$$Like(\phi,\beta)=\prod_{j=1}^{150}\Pr(a_{1j},a_{2j};\phi,\beta)\prod_{j=151}^{n}\Pr(a_{1j},a_{2j},\tilde{a}_{2j};\phi,\beta)\ . \quad (9)$$

The data that are not in the 'valid' category can be included in the likelihood. For example, when the data type is 'interval' as $[a_{1jk}^{(1)},a_{1jk}^{(2)}]$, the distribution is:

$$P_1\left([a_{1jk}^{(1)},a_{1jk}^{(2)}]\mid a;\phi\right)=\sum_{a'=a_{1jk}^{(1)}}^{a_{1jk}^{(2)}} P_1(a'\mid a;\phi)\ . \qquad (10)$$

The parameters in the expectation and variance structures are of interest in this model, whereas $\beta_L,\ldots,\beta_H$ are nuisance parameters. To make the estimation easier and to reduce the number of nuisance parameters, a functional constraint is incorporated on the parameters for the true age composition of the sample $\beta_a(a\geq A)$ as $\beta_a=\beta_A\exp(-Z(a-A))$, where *A* is the largest number which satisfies:

$$\frac{\#\{j=1,\ldots,n\mid a_{1j1}\geq A\}}{n}>q\ , \qquad (11)$$

and *Z* is a mortality parameter. The threshold value *q* is, of course, *ad hoc*, but the constraint is nevertheless useful in cases such as this experiment. The value *q* = 0.20 is used as a base case assumption, in which case the value of *A* is set at 28.

*Scenarios*

Table 3 lists the scenarios considered in this paper. Lockyer is taken to be the control reader for Cases 1, 2, 3 and 5, and Zenitani for Case 4. Case 5 examines the sensitivity of the results to using all of the data i.e. not only the 'valid' data but also the 'either', 'interval' and 'minimum' data. Several alternative models are considered based on the covariates included in the models for the mean and variance structures for age-reading Group 2 (see Table 4).

## RESULTS

Histograms and scatter plots of the 'valid' age-reading outcomes from Lockyer do not suggest evidence for between-trial bias (Fig. 2). Similarly, there is no evidence for between-trial bias for Zenitani (Fig. 3) and Bando (Fig. 4). Consequently, trial was not considered as a covariate in the analyses. The age-reading outcomes of the two primary

Table 3

The scenarios considered in the analyses.

| | Bias in control reader | Data |
|---|---|---|
| Case 1 (Base) | Lockyer 0% | 'valid' only |
| Case 2 | Lockyer 10% | 'valid' only |
| Case 3 | Lockyer –10% | 'valid' only |
| Case 4 | Zenitani 0% | 'valid' only |
| Case 5 | Lockyer 0% | Index = 0,1,2,3 |

Table 4

Assumptions regarding the covariate effects and parameters. In all the models, constraints $!_{iH}\leq !_{iH}$ for all the readers are imposed.

| Model | Assumption |
|---|---|
| 0 | No reader effects. |
| 1 | Reader effects in Group 2 only in the mean structure. |
| 2 | Reader effects in Group 2 only in the variance structure. |
| 3 | Reader effects in Group 2 both in the mean and variance structures. |
| 4 | Reader effects both in the mean and variance structures, but the expected ages by Zenitani and Bando are same. |

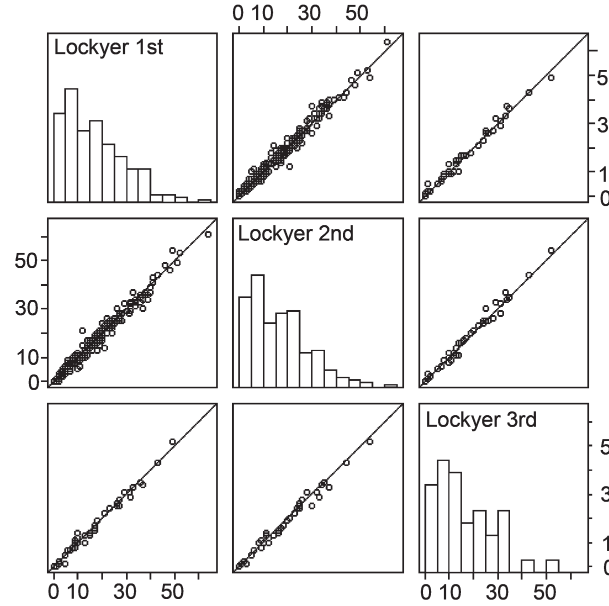

Fig. 2. Scatter plots and histograms for Lockyer's age-reading data for her three trials.

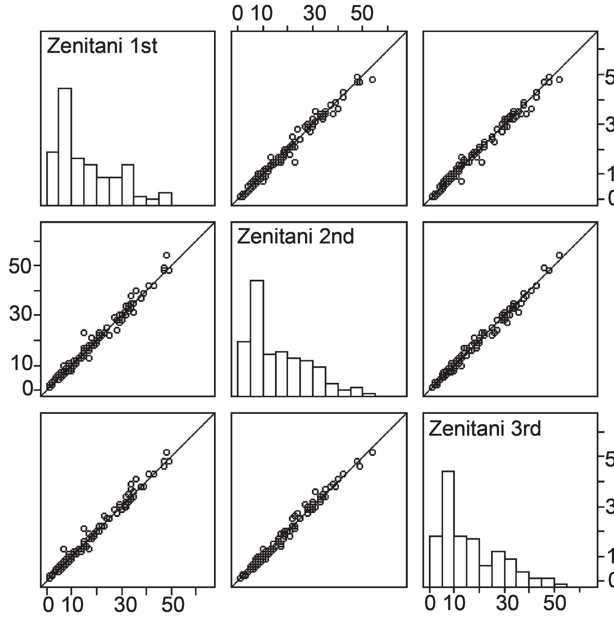

Fig. 3. Scatter plots and histograms for Zenitani's age-reading data for her three trials.

readers for JARPA (Zenitani) and JARPA II (Bando) appeared similar (see Fig. 5).

Fig. 6 plots the age-reading outcomes for each of the Japanese readers (single estimates for Masaki and Kato, and the medians of the three estimates from the three trials for Zenitani and Bando) against the age-estimates by Lockyer. These plots indicate a consistent discrepancy between the age-estimates obtained by Lockyer and those obtained by the Japanese scientists. In fact, under Case 1, where Lockyer's bias is assumed to be zero, the estimated ages by the four Japanese readers appear negatively biased (solid lines in Fig. 6). Fig. 7 shows the difference in absolute and relative biases among the Japanese readers against the control reader. The standard errors and coefficient of variation for the control and Japanese readers also differ (Fig. 8).

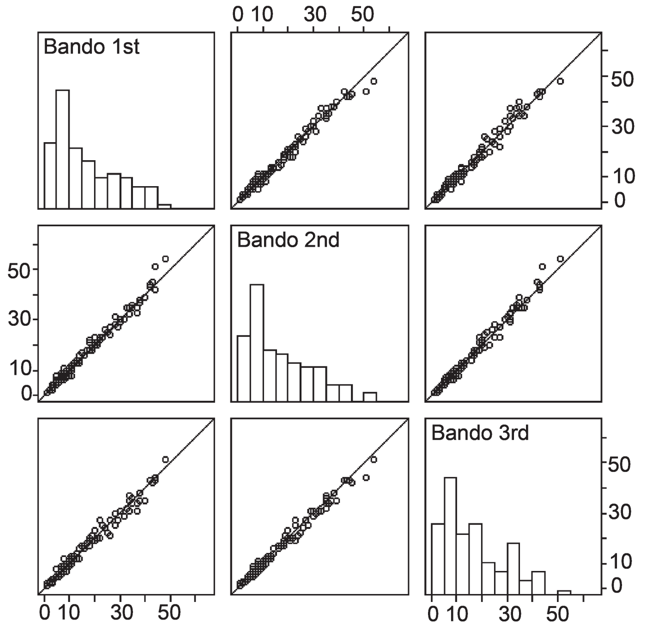

Fig. 4. Scatter plots and histograms for Bando's age-reading data for his three trials.

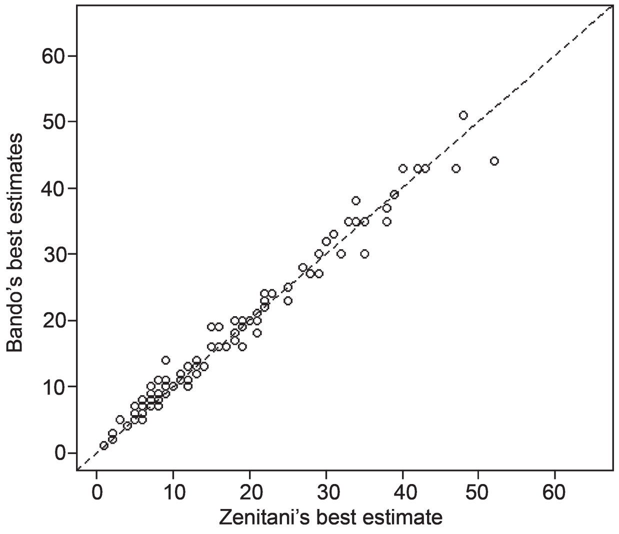

Fig. 5. Scatter plot of the best estimates for the two primary readers in JARPA and JARPA II (Zenitani and Bando).

Table 5 summarises the results of the parameter estimation and model selection for the various models under Case 1. Incorporating a reader effect into the mean component tended to improve the goodness of fit substantially (in terms of model selection criteria) compared to incorporating these effects into the variance structure (i.e. the extent of random age-reading error). Model 3, in which the reader effects were incorporated in both the mean and variance structures, led to the most parsimonious fit to the data. The adequacy of the fits for Model 3 in Case 1 is confirmed by Fig. 6.

Table 6 provides estimates of parameters which could be used to compute age-reading error matrices. It should be noted that the differences in parameter estimates between Cases 1 (base case) and 5 (which uses data for indexes 0–3 in Table 2) are almost negligible.

Age-reading error matrices based on Model 3 could be

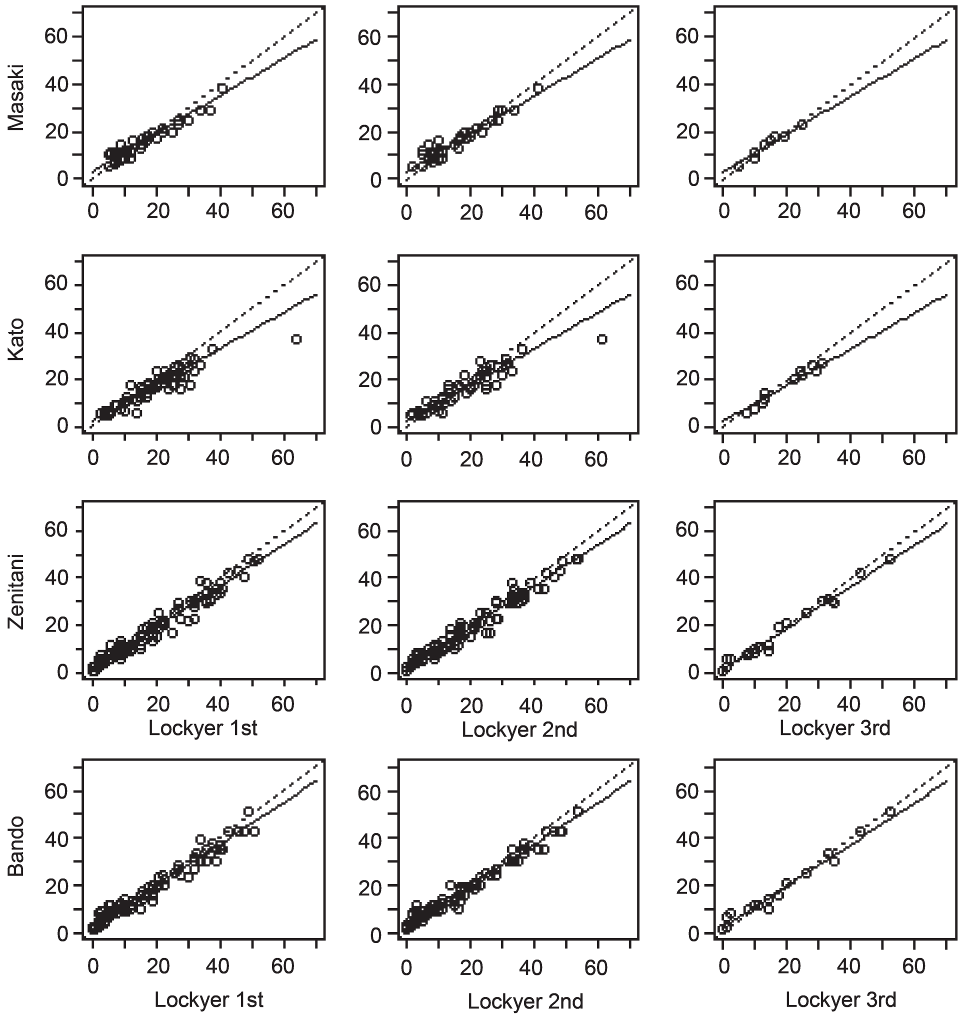

Fig. 6. Scatter plots of the 'best' age-estimates from the four Japanese readers against Lockyer's 1st, 2nd and 3rd trials ('valid' data only). The dashed lines show the 1–1 lines. Lockyer's age-estimation is assumed to be unbiased (Case 1).

incorporated into assessments of the impact of age-determination error on the outputs from age-structured models for Antarctic minke whales (e.g. Punt, 2010; Punt *et al.,* 2013). It should be noted that the analyses on which this paper are based are predicated on Lockyer's age-estimates. It cannot necessarily be assumed that Lockyer provides unbiased estimates of true age. Overall, the results suggest that the age-reading errors for Lockyer and the four Japanese readers differ.

## DISCUSSION

A statistical method was introduced for quantifying age-reading error and the extent of inter-reader variability for the readers who have read Antarctic minke whale earplugs. The

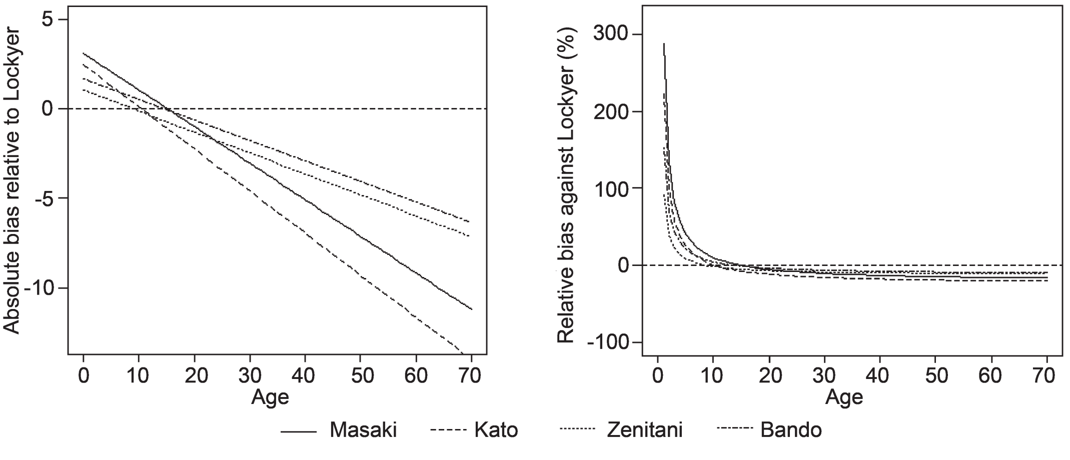

Fig. 7. Absolute (left) and relative (right) biases for the Japanese readers relative to the control reader (Lockyer), who is assumed to be unbiased (Case 1).

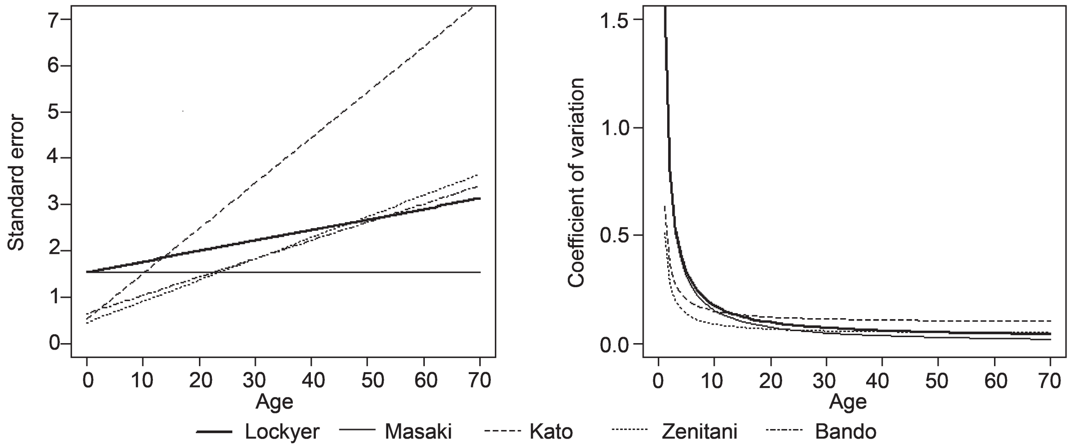

Fig. 8. Standard errors (left) and coefficients of variation (right) for the control and Japanese readers if the control reader (Lockyer) is assumed to be unbiased (Case 1).

method is based on that of Punt *et al.* (2008) but allows for data for estimates that are given as intervals or two values to be used in addition to data where only a single age-estimate is available. The availability of the independent control reader was key to standardising outcomes by the other readers. It is not possible to evaluate possible biases in the control reader, so sensitivity analyses were conducted to determine how the estimates of the parameters of the model change depending on assumptions regarding the extent to which the control reader is biased.

The results suggested that the expected age and random uncertainty in age-estimates differed among the Japanese readers, although the two readers in charge of age-reading for JARPA and JARPA II provided similar age-reading outcomes. This is likely because the new reader Bando had a training period to develop his reading skill using JARPA samples which had previously been aged by Zenitani (these samples were, of course, not chosen from the 250 samples on which this study was based).

The analyses of this paper assumed that the age-composition of the catch was the same over more than 30 years to reduce the number of nuisance parameters. However, the assessment of age-reading errors is subject to confounding if the catch age-composition changes over time. The impact of possible violation of this assumption was examined by assuming different age-compositions for Periods I–III and Periods IV and V. The fit of the model to data was better than for the base-case when the catch age-composition was assumed to differ among periods, but Model 3 remained the best model. The values of the parameters which determine

Table 5

Results of the analysis (upper value = estimate; lower value = SE) for the Case 1, where age-reading by the control reader (Lockyer) is assumed to be unbiased. Note that number of parameters does not include the number of nuisance parameters for the age composition.

| | | | | | Reader 1 (Lockyer) | | Reader 2–1 (Masaki) | | Reader 2–2 (Kato) | | Reader 2–3 (Zenitani) | | Reader 2–4 (Bando) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Model | Loglike | Number of parameters | Δ-AIC | Δ-AICc | $b_{L1}$ | $b_{H1}$ | $b_{L21}$ | $b_{H21}$ | $b_{L22}$ | $b_{H22}$ | $b_{L23}$ | $b_{H23}$ | $b_{L24}$ | $b_{H24}$ |
| 0 | –3004.0 | 6 | 106.18 | 104.54 | 0 | 70 | 1.39 | 61.17 | | | | | | |
| | | | | | | | 0.10 | 0.51 | | | | | | |
| 1 | –2958.6 | 12 | 27.38 | 26.50 | 0 | 70 | 2.65 | 60.51 | 2.33 | 56.21 | 1.03 | 62.92 | 1.58 | 63.93 |
| | | | | | | | 0.44 | 2.06 | 0.38 | 1.27 | 0.10 | 0.55 | 0.10 | 0.57 |
| 2 | –2978.1 | 12 | 66.40 | 65.52 | 0 | 70 | 1.68 | 61.86 | | | | | | |
| | | | | | | | 0.28 | 0.76 | | | | | | |
| 3 | –2938.9 | 18 | 0.00 | 0.00 | 0 | 70 | 3.08 | 58.79 | 2.45 | 56.01 | 1.03 | 62.85 | 1.64 | 63.64 |
| | | | | | | | 0.53 | 1.91 | 1.36 | 4.10 | 0.09 | 0.54 | 0.11 | 0.61 |
| 4 | –2964.7 | 16 | 47.60 | 47.29 | 0 | 70 | 3.10 | 58.74 | 2.63 | 55.49 | 1.24 | 63.07 | | |
| | | | | | | | 0.53 | 1.90 | 0.60 | 2.00 | 0.08 | 0.52 | | |

| | Reader 1 (Lockyer) | | Reader 2–1 (Masaki) | | Reader 2–2 (Kato) | | Reader 2–3 (Zenitani) | | Reader 2–4 (Bando) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Model | $!_{L1}$ | $!_{H1}$ | $!_{L21}$ | $!_{H21}$ | $!_{L22}$ | $!_{H22}$ | $!_{L23}$ | $!_{H23}$ | $!_{L24}$ | $!_{H24}$ |
| 0 | 1.62 | 3.43 | 0.56 | 4.17 | | | | | | |
| | 0.18 | 0.66 | 0.06 | 0.34 | | | | | | |
| 1 | 1.64 | 3.36 | 0.52 | 3.98 | | | | | | |
| | 0.17 | 0.60 | 0.06 | 0.28 | | | | | | |
| 2 | 1.46 | 3.38 | 1.66 | 1.66 | 0.08 | 9.53 | 0.57 | 3.39 | 0.84 | 3.22 |
| | 0.15 | 0.50 | 0.27 | 0.27 | 0.35 | 1.56 | 0.08 | 0.41 | 0.12 | 0.44 |
| 3 | 1.55 | 3.14 | 1.55 | 1.55 | 0.54 | 7.37 | 0.46 | 3.66 | 0.66 | 3.41 |
| | 0.17 | 0.51 | 0.24 | 0.24 | 1.64 | 4.40 | 0.06 | 0.38 | 0.09 | 0.40 |
| 4 | 1.50 | 3.21 | 1.57 | 1.57 | 0.75 | 6.85 | 0.42 | 3.83 | 0.91 | 3.07 |
| | 0.15 | 0.49 | 0.24 | 0.24 | 0.44 | 1.39 | 0.06 | 0.38 | 0.11 | 0.42 |

Table 6

Results of the analysis (upper value = estimate; lower value = SE) for Cases 1–5 under the best model (Model 3). The values in italics are pre-specified.

| | Reader 1 (Lockyer) | | Reader 2–1 (Masaki) | | Reader 2–2 (Kato) | | Reader 2–3 (Zenitani) | | Reader 2–4 (Bando) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Model 3 | $b_{L1}$ | $b_{H1}$ | $b_{L21}$ | $b_{H21}$ | $b_{L22}$ | $b_{H22}$ | $b_{L23}$ | $b_{H23}$ | $b_{L24}$ | $b_{H24}$ |
| Case 1 | *0.00* | *70.00* | 3.08 | 58.79 | 2.45 | 56.01 | 1.03 | 62.85 | 1.64 | 63.64 |
| (Lockyer: unbiased) | | | 0.53 | 1.91 | 1.36 | 4.10 | 0.09 | 0.54 | 0.11 | 0.61 |
| Case 2 | *0.00* | *77.00* | 3.10 | 64.27 | 2.67 | 60.66 | 1.09 | 69.04 | 1.70 | 69.85 |
| (Lockyer: 10% bias) | | | 0.52 | 2.11 | 0.56 | 2.16 | 0.10 | 0.64 | 0.12 | 0.70 |
| Case 3 | *0.00* | *64.00* | 3.08 | 53.22 | 2.62 | 50.27 | 1.14 | 56.35 | 1.74 | 57.15 |
| (Lockyer: 10% bias) | | | 0.53 | 1.66 | 0.65 | 1.85 | 0.09 | 0.49 | 0.11 | 0.54 |
| Case 4 | 0.00 | 75.52 | 3.00 | 63.51 | 2.68 | 59.58 | *0.00* | *70.00* | 0.71 | 70.26 |
| (Zenitani: unbiased) | 0.00 | 0.62 | 0.52 | 2.11 | 0.55 | 2.10 | | | 0.12 | 0.62 |
| Case 5 | *0.00* | *70.00* | 3.01 | 59.03 | 2.36 | 55.99 | 1.02 | 62.65 | 1.63 | 63.41 |
| (Case 1 with index = 0~3) | | | 0.51 | 1.87 | 0.47 | 1.78 | 0.09 | 0.57 | 0.11 | 0.63 |

| | Reader 1 (Lockyer) | | Reader 2–1 (Masaki) | | Reader 2–2 (Kato) | | Reader 2–3 (Zenitani) | | Reader 2–4 (Bando) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Model 3 | $\sigma_{L1}$ | $\sigma_{H1}$ | $\sigma_{L21}$ | $\sigma_{H21}$ | $\sigma_{L22}$ | $\sigma_{H22}$ | $\sigma_{L23}$ | $\sigma_{H23}$ | $\sigma_{L24}$ | $\sigma_{H24}$ |
| Case 1 | 1.55 | 3.14 | 1.55 | 1.55 | 0.54 | 7.37 | 0.46 | 3.66 | 0.66 | 3.41 |
| (Lockyer: unbiased) | 0.17 | 0.51 | 0.24 | 0.24 | 1.64 | 4.40 | 0.06 | 0.38 | 0.09 | 0.40 |
| Case 2 | 1.47 | 3.44 | 1.56 | 1.56 | 0.75 | 7.46 | 0.45 | 4.10 | 0.69 | 3.63 |
| (Lockyer: 10% bias) | 0.16 | 0.58 | 0.24 | 0.24 | 0.40 | 1.50 | 0.06 | 0.43 | 0.09 | 0.46 |
| Case 3 | 1.49 | 3.02 | 1.56 | 1.56 | 0.73 | 6.25 | 0.48 | 3.33 | 0.63 | 3.22 |
| (Lockyer: 10% bias) | 0.15 | 0.45 | 0.24 | 0.24 | 0.51 | 1.35 | 0.06 | 0.36 | 0.09 | 0.35 |
| Case 4 | 1.87 | 2.65 | 1.48 | 1.48 | 0.70 | 7.44 | 0.41 | 3.97 | 0.64 | 3.62 |
| (Zenitani: unbiased) | 0.16 | 0.51 | 0.25 | 0.25 | 0.38 | 1.45 | 0.06 | 0.41 | 0.10 | 0.44 |
| Case 5 | 1.60 | 3.05 | 1.49 | 1.49 | 0.45 | 7.40 | 0.47 | 3.58 | 0.65 | 3.51 |
| (Case 1 with index = 0~3) | 0.15 | 0.48 | 0.24 | 0.24 | 0.41 | 1.47 | 0.06 | 0.37 | 0.09 | 0.42 |

age-reading bias and variability were also quite similar (see Fig. 9) suggesting that how the nuisance parameters are treated only has a small impact on the final results.

The original motivation of the experiment and analysis was to provide quantitative information on age-reading error for use in the statistical catch-at-age analysis. Punt *et al.*

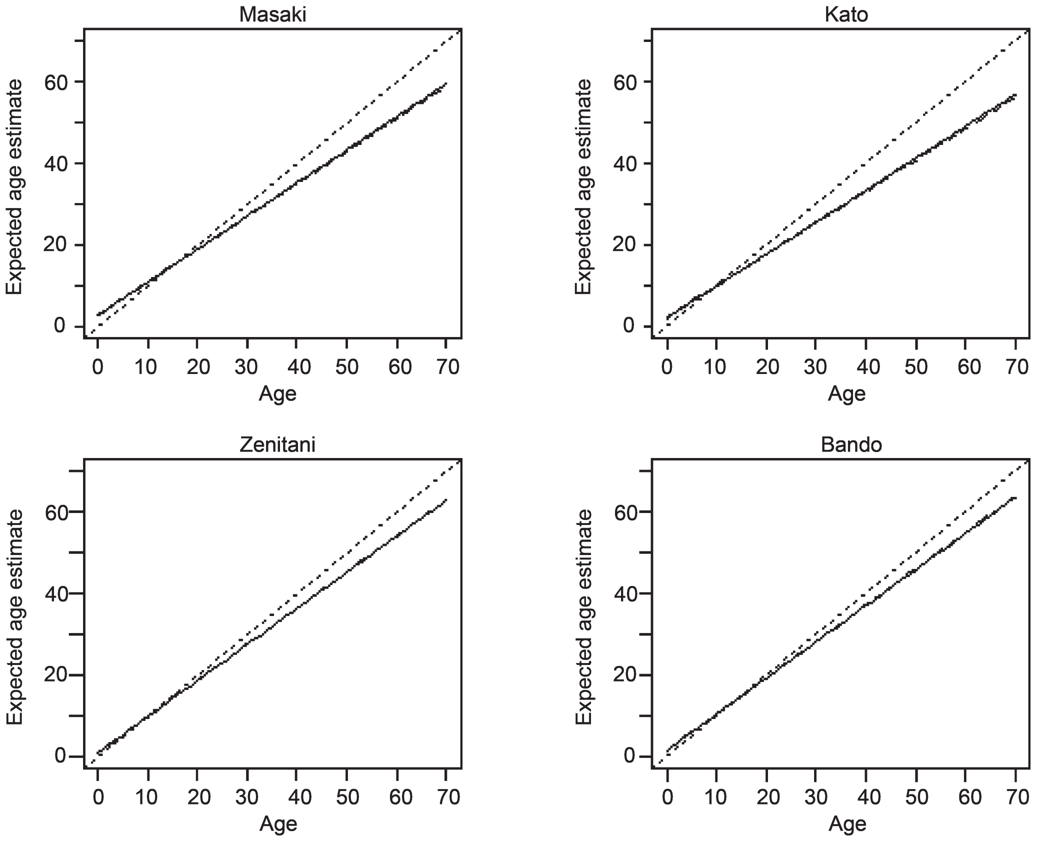

Fig. 9. Sensitivity of the relationship between expected age and true age for the base-case analysis (solid lines) and for the sensitivity test in which the catch age-composition is assumed to change over time (shaded lines). The dashed lines show the 1–1 lines.

(2013) confirm that the results of the statistical catch-at-age analysis for Antarctic minke whales are sensitive to whether age-reading error is ignored or accounted for.

It should also be noted that the model and approach shown in this paper are applicable to populations other than the Antarctic minke whales provided that a control reader is available, even retrospectively as was the case in this study.

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## Appendix 1

## *EXCEL* DATABOOK USED DURING READINGS

Specimen ID number: ____________________

**Age readings**

Trial counts – given in sequence: ____________________

Agreed count from trials based on weighted mean (CHL): ____________________

Best count according to Japanese method of average of counts: ____________________

**Comments**

Plug complete? Yes ______ No ______ Comment: ____________________

Neonatal line present? Yes ______ No ______

Central cut? Yes ______ No ______

General appearance: ____________________

Readability Excellent ______ Good ______ Poor ______ Unreadable ______

Other: ____________________

## NOTES FOR DATABOOK:

### Specimen ID number

This refers to the experimental number provided for this reading stage of the experiment.

### Age readings

In general the following descriptors were used. When there is uncertainty about age, the age is prefixed by ca – e.g. ca N. When part of the plug is missing, + is suffixed on the age. However, + can also be applied in young animals (range up to 6 GLGs) where a new GLG is forming at the edge but maybe incomplete. Other ways of giving this are e.g. N – N + 1 – in other words a range. Sometimes two possible ages are offered because of difficulties in reading. Here the ages will be e.g. N or P. Where only a minimum age is counted in difficult to read plugs, the age will be given as e.g. >N. Sometimes this notation is also used for incomplete plugs.

### Trial counts - given in sequence

This gives the numbers of GLGs counted in sequence. The minimum number of trials is three, but may be many more depending on the confidence of the reader in what is being seen. It should be noted that before recording counts, the ear plug has been scanned several times to get a feel for the GLG patterns with rough counts made. The written counts reflect when the reader is more confident in the counting.

### Agreed count from trials based on weighted mean (CHL)

In cases where there is no consistency of count, the mean may be weighted to the most recent count depending on the relative confidence in the reading.

### Best count according to Japanese method of average of counts

The mean here is a simple mean and treats all readings equally.

### Plug complete? Yes or No; comment

Yes denotes that all parts of the core were found, even if in two or more pieces. A comment will usually describe how many pieces or what is missing.

### Neonatal line present? Yes or No

Yes means that at least part of the neonatal Line has been identified.

### Central cut? Yes or No

Yes means that the core is adequately exposed at the centre line.

### General appearance

Information on colouration, relative size, etc. is given here. However, this has not been consistently provided, but has often been added if there has been a problem with reading. If the plug or part of it is attached to the glove finger, this is noted.

### Readability - Excellent; Good; Poor; Unreadable

E – Excellent means very clear GLGs and little error likely in reading.
G – Good means generally quite readable with mostly clear GLGs. However, there may be some error.
P – Poor means parts of the plug are difficult to read because GLGs are obscure or irregular. A large margin of error is likely in GLGs.
U – Unreadable means that the clarity of GLGs is so poor and/or confusing, that any GLG count provided is likely to be erroneous or incomplete.
Combinations of categories e.g. G/P mean partly good and partly poor – often which part will be specified e.g. P (top)/G (base).

### Other

Here expanded information on readability may be given; also possible transition phase age if determined.

# A note on the northernmost record of the Antarctic minke whale (*Balaenoptera bonaerensis*) in the Eastern Pacific

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ABSTRACT

In this study the first record of the Antarctic minke whale (*Balaenoptera bonaerensis*) in Ecuador (2°7.35'S, 80°45.7'W) is presented. It was a single stranding of a calf of 3.43m in length. The species was identified based on morphological characteristics such as the number and colour of baleen plates, the number and extension of ventral grooves, lack of white flipper patch and the waved greyish light colouration pattern on the flanks, among others. Although only a handful of records exist of this species in the Eastern Pacific, it confirms that the breeding area of this species at least reaches the equator in this region.

KEYWORDS: MINKE WHALE; DISTRIBUTION; SOUTH AMERICA; BREEDING GROUNDS; PACIFIC OCEAN; SOUTHERN HEMISPHERE

## INTRODUCTION

Until around twenty years ago the taxonomic status of minke whales was insufficiently defined. Minke whales from both hemispheres were considered conspecifics and generally referred to as *Balaenoptera acutorostrata* Lacépède, 1804 (e.g. Leatherwood *et al.*, 1983). In 2000, the Scientific Committee of the International Whaling Commission (IWC) recognised the Antarctic minke whale, *B. bonaerensis* Burmeister, 1867 as a different species (IWC, 2001). In the Southern Hemisphere, *B. bonaerensis* coexists partially sympatric with a southern dwarf form that is genetically more related to the northern minke whale *B. acutorostrata* (Arnold *et al.*, 2005; Reilly *et al.*, 2008). There is still uncertainty, however, about the stock structure of the Antarctic minke whale (e.g. Pastene, 2006).

The three minke whale sub-types can be identified based on morphologic and skeletal differences and body colouration. The most evident external differences between the Antarctic and the two forms of *B. acutorostrata* are the lack of a white patch on the flippers and a dark throat patch, which are present only in the dwarf and northern minke sub-types (Arnold *et al.*, 1987; Best, 1985). Differences also include the position of the dorsal fin, the colour and number of baleen plates, an asymmetrical body colouration and several subtle colouration designs in different parts of the body (Arnold *et al.*, 1987; Arnold *et al.*, 2005; Best, 1985; Best, 2007; Bushuev and Ivashin, 1986).

Based on surveys of whaling ships, Kasamatsu *et al.* (1995) suggested that there are two breeding areas for the southern minke whale in the Pacific Ocean; in the eastern and the western Pacific, mainly distributed between 10°S and 20°S. However, the minke whale is considered an uncommon species in the Eastern Pacific (Jefferson *et al.*, 2008). The species does not appear in records of whaling activities along the South American coast during the 20th Century even when catches of more commercially valuable whales such as sperm (*Physeter macrocephalus*), Bryde's (*B. brydei/edeni*) or blue (*B. musculus*) decreased considerably (see Clarke, 1980). Previous records of the species in the Southeast Pacific are from young bycatch specimens in artisanal fishing gillnets in Peru at 8°25'S and 12°29'S (García-Godos *et al.*, 2013; Van Waerebeek and Reyes, 1994). There are few records from the Galapagos Islands, Ecuador (0º30'ºS, 90º30'W), referred to as *B. acutorostrata* (Merlen, 1995; Palacios, 2003) and a westward record (8º03'S, 109º54'W) made on 14 November 1985 (Robert Pittman, pers. comm.). Our specimen from the continental coast would be the first confirmed record of *B. bonaerensis* in Ecuadorian waters and the northernmost one of the species in the Eastern Pacific.

### The specimen

A calf female rorqual of 3.43m in length with a unique central ridge on the head stranded at San Pablo, Ecuador (02°7.35'S, 80°45.7'W) on 31 October 2004 (Fig. 1). The specimen was initially misidentified as a sei whale (*B. borealis*) (Félix *et al.*, 2011), however, a more detailed analysis of the available morphological information showed that the specimen was in fact an Antarctic minke whale (*B. bonaerensis*).

The specimen was relatively fresh when examined. The epidermis was present on around 90% of the back and 30% of the belly. Some bloating was noted, especially of the tongue (Fig. 2). The skin on its right side was in better condition as the animal was lying on its left side and the sun had started to dry it. Thus, colour descriptions below refer to this right side of the animal. On the left side a brownish contusion wound of around 40cm without epidermis extended from behind the eye to the base of the pectoral fin. The absence of bruising suggests the wound could have occurred after death, possibly due to sunburn and abrasion from the beach. The external examination did not help to determine the cause of death. 12 standard body measurements were taken from this specimen to the nearest centimetre (Table 1).

The specimen was dark grey on the back changing to light grey toward the flanks and white on the ventral side. Along

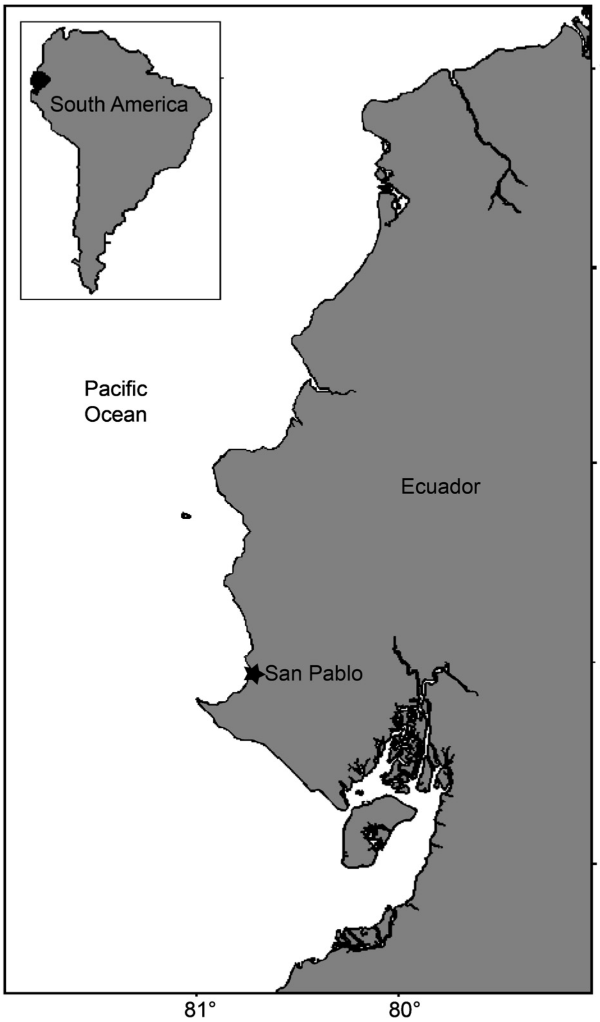

Fig. 1. The site of the stranding at San Pablo, Ecuador.

Fig. 2. Different views of the specimen of *B. bonaerensis* stranded at San Pablo, Ecuador.

the flanks three major light greyish streaks in a waved pattern were observed extending from the lower part up to the mid flank; one located in front of the dorsal fin, another one in thoracic area and a smaller one over the flipper. Another light grey streak was also visible in the caudal area. Two irregular and light grey streaks extended from the blowholes rearward and to left side of the body (Fig. 3). Ventrally, most of the epidermis was lost and the specimen looked whitish and slightly pink in the ventral and genital area. The skin at the throat was in better condition and was white. The flipper's surface was uniformly grey with darker tips, the most common pattern found in Antarctic minke whales flippers (Best, 1985; Bushuev and Ivashin, 1986). Flippers had a white anterior border and dark grey rear border, and were white ventrally. The dorsal fin was tall and falcate, but was still flexible. There were 62 ventral grooves that ended before the navel, which was in the process of healing.

The complete left row of baleen was collected. It was 742mm in length and contained 265 individual baleen plates (Fig. 4). The first 3/4 of the gum, measured from the base, was grey and the remainder was whitish. The gum has a sinuous and asymmetrical form; its external height was 28mm and internally 13.6mm. The gum maximum width was 65.1mm at 320mm from the tip. The row is maintained in formalin (5%) in the Museo de Ballenas at Salinas (Cat N° 115 MBABO 01).

The colour of the plates' surface was not assessed when collected. However, their outer colouration was clearly visible in photographs taken when the specimen was found

Table 1

Body measurements of the Ecuadorian specimen expressed in centimetres and as percentage of total length. The rightmost column contains data from Best (1985) for reference.

| Measure | Length (cm) | % total length | Mean % body length (Best, 1985) |
|---|---|---|---|
| Total length (tip of the upper jaw to deepest part of flukes notch) | 343 | 100 | 100 |
| Tip of upper jaw to centre of the eye | 76 | 22.2 | 20 |
| Tip of the upper jaw to angle of gape | 74 | 21.6 | 18.7 |
| Tip of upper jaw to blowhole along midline | 54 | 15.7 | 14.3 |
| Tip of upper jaw to tip of dorsal fin | 231 | 67.3 | 71.5 |
| Tip of upper jaw to midpoint of umbilicus | 193 | 56.3 | 53.7 |
| Tip of upper jaw to midpoint of genital aperture | 249 | 72.6 | 66.5 |
| Length flipper (anterior insertion to tip) | 54 | 15.7 | 15.8 |
| Length flipper (axilla to tip) | 39 | 11.4 | 11.9 |
| Height dorsal fin (fin tip to base) | 15 | 4.4 | 3.7 |
| Width, flukes (tip to tip) | 83 | 24.2 | 29 |
| Distance from nearest point on anterior border of flukes to notch | 27 | 7.9 | 7.2 |

Fig. 3. Rostrum and upper part of the head showing the central keel and the two blowhole streaks.

(Fig. 3). Most plates had a dark grey external border in the upper part and white tips, particularly along the posterior and central parts of the row. Border plates became lighter towards the tip, changing from dark grey to a red colour, to cream-white. Due to the colour transition on the external border of the row, plates were separated into three colour categories: (1) all white, cream-white plates located in the tip of the row (*n* = 45, 17%); (2) mostly reddish, behind the white part (*n* = 33, 12.4%); and mostly dark grey (the remaining from the original count, *n* = 172, 64.9%). These numbers should be dealt with cautiously as they were qualitatively estimated from photographs. Over time the plates have lost their original colouration, becoming lighter due to the preservation media. This colouration pattern is no longer visible. The largest individual baleen plate was 81mm, measured on the external side from the gum to the beginning of the bristles, and its width was 59mm, measured along the base of the gum, which gives a proportion width/length of 0.73.

## DISCUSSION

Most morphological characteristics that could be evaluated in the Ecuadorian specimen coincided with detailed descriptions of Antarctic minke whales, including the number of ventral grooves, lack of the white flipper patch, blowhole streaks, lack of dark throat patch and the distinctive greyish waved coloured pattern in the flanks (e.g. Arnold *et al.*, 1987; Best, 1985; Best, 2007). Notwithstanding this, other features such as caudal and head chevrons were not seen in our specimen and this could be related to the age of the animal, sunburnt skin and/or post-mortem changes.

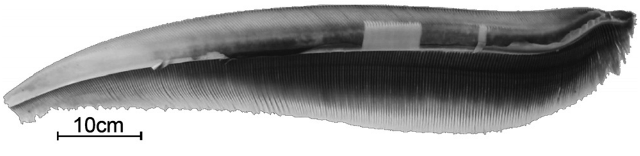

Fig. 4. Left baleen plates row curated at the Museo de Ballenas in Salinas, Ecuador. Colour photos available online (*www.iwc.int/publications*) shows the original colour when the piece was collected.

The number and colour of baleen plates is another essential identification feature. According to Best (1985; 2007), Antarctic minke whales show asymmetrically-coloured baleen, with 20–25% white baleen on the left side and 35–40% white baleen on the right side, with the remaining plates having a black outer border. For the specimen examined in this study, most plates were bicoloured and some had three colours. The specimen had 17% of plates with a white outer border located at the tip and around 33 baleen plates (12.4%) with different proportions of red with white or dark grey after the white section. Reference to the reddish colouration of the plates cannot be found in the literature, which leads to the hypothesis that this is a temporal condition of young specimens and with time this colouration would disappear. The proportion width/length of the largest plate (0.73) is within the wide range given by Best (1985).

Most of the external measurements of the Ecuadorian specimen are outside of the range of Antarctic specimens reported by Best (1985) and Ohsumi *et al.* (1970) (see the data shown in table 5 in Best, 1985). This is not surprising as those data are from larger animals killed during whaling operations and body proportions may change with age. One example of this is the position of the dorsal fin which is known to be located more posterior in the Antarctic form than the dwarf form (Best, 1985; Arnold *et al.*, 1987). For the Ecuadorian specimen it was located in a more anterior position than for the dwarf form.

The size at birth of the Antarctic minke whale is between 2.7 and 2.9m (Ivashin and Mikhalev, 1978; Best, 1982), which indicates that the Ecuadorian specimen would be a calf of a few months old. The presence of this calf in Ecuador during the breeding season of the Southern Hemisphere Mysticeti, as well as other calves from Peru (Van Waerebeek and Reyes, 1994; García-Godos *et al*., 2013), confirms that this species breeds in the Eastern Pacific at least as far north as the equator, just as it occurs in the western and eastern Atlantic Ocean (e.g. Siciliano *et al*., 2011; Segniagbeto *et al.,* 2012). The distribution of Antarctic minke whale in wintering areas is poorly known and no areas of concentration have been reported; their distribution is probably offshore and disperse (Best, 1982; Kasamatsu *et al*., 1995; Jefferson *et al*., 2008). The few reports of the species from the Eastern Pacific appear to be consistent with this hypothesis. Since they have been reported just occasionally, strandings and bycatch victims seem to be a unique source of information in the Eastern Pacific. It is not possible to establish the affinity of this and other specimens from the Southeast Pacific with a particular Antarctic minke whale stock, as genetic studies have not been conducted. Emphasis should be put on obtaining tissue samples for genetic studies to assess stock identity and population structure.

## ACKNOWLEDGEMENTS

The authors are grateful for the advice of Dr. Koen Van Waerebeek who first noticed the original mis-identification of this specimen and suggested that the authors make a more detailed description and respective rectification. Two anonymous reviewers provided valuable comments to improve the manuscript.

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# Preliminary study on genetic differences between two species of finless porpoises, genus *Neophocaena*, with lack of genetic divergence between two subspecies of the narrow-ridged finless porpoise, *N. asiaeorientalis*: cytochrome *b* sequence analyses

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ABSTRACT

Using samples from bycaught finless porpoises, cytochrome *b* sequences were analysed and phylogenetic trees were constructed. The aims were to: (1) determine genetic divergences within the genus *Neophocaena*; (2) examine interspecific divergences between *N. asiaeorientalis* and *N. phocaenoides*; and (3) examine intraspecific divergence between *N.a. asiaeorientalis* and *N.a. sunameri.* For this purpose, complete cytochrome *b* sequences for 12 *N.a. sunameri* specimens, collected from fishery markets at Pohang in southeastern Korea, were obtained, and these sequences were compared to the corresponding partial (402bp) and complete (1,140bp) sequences of *Neophocaena*, obtained from GenBank. From a maximum likelihood tree with the partial sequences of the two *Neophocaena* species, two clades were detected, corresponding to the two species, with average genetic distance of 1.64%, four fixed site differences (1.00%), and a $G^{st}$ value of 0.64, although we did not examine the specimens from Southeast Asia and contiguous South China Sea. Furthermore, from the complete sequences, we recognised a lack of genetic divergence between the two subspecies of *N. asiaeorientalis*, with a $G^{st}$ value of 0.06 and two pairs of identical sequences between them, indicating that our results do not support current subspecies classification. Thus, we newly found that our cytochrome *b* sequencing results are useful for the examination of interspecific and intraspecific divergences in *Neohpocaena*, although further genetic analyses with additional specimens of *Neophocaena* across its distributional range are necessary to confirm the findings in this study.

KEYWORDS: GENETICS; TAXONOMY; BYCATCH; SEA OF JAPAN; ARABIAN SEA; INDIAN SEA; EAST CHINA SEA; NORTHERN HEMISPHERE; FINLESS PORPOISE

## INTRODUCTION

Mead and Brownell (2005) noted that the geographic distribution of the finless porpoise (*Neophocaena phocaenoides* Cuvier, 1829) extends from the Indo-Pacific Ocean to Japan, including coastal waters and some rivers, and they recognised three subspecies (*N.p. phocaenoides*, *N.p. asiaeorientalis*, and *N.p. sunameri*). However, Pilleri and Gihr (1975) had previously reported that the genus *Neophocaena* comprises three distinct species, *N. phocaenoides*, *N. asiaeorientalis*, and *N. sunameri.* Jefferson and Wang (2011) reclassified finless porpoises as two distinct species from the review on the previous studies of finless porpoises with morphological and molecular characters: the Indo-Pacific finless porpoise, *N. phocaenoides*; and the narrow-ridged finless porpoise, *N. asiaeorientalis*, with two subspecies (Yangtze finless porpoise, *N.a. asiaeorientalis*, and East Asian finless porpoise, *N.a. sunameri*).

Molecular genetic studies for taxonomic reconsideration have become widespread during the past decade and mitochondrial DNA (mtDNA) is a sensitive genetic marker suitable for studies of closely related taxa or populations of a variety of species (Sunnucks, 2000). To examine population subdivisions of *Neophocaena*, *F*-statistics were utilised on the basis of the mtDNA control region (Yang *et al.*, 2008; Yoshida *et al.*, 2001), nuclear microsatellites and mtDNA control region (Li *et al.*, 2011; Wang *et al.*, 2008), nuclear microsatellites (Chen *et al.*, 2010), and nuclear introns (Ju *et al.*, 2012). Although Li *et al.* (2011) obtained cytochrome *b* sequences of *N.a. sunameri* from the Yellow Sea, they did not perform any further genetic analyses with these cytochrome *b* sequences. Thus cytochrome *b* sequences and phylogenetic trees have not been used to determine genetic divergences within the genus *Neophocaena*.

Regarding evolutionary rates, the cytochrome *b* gene varies at a slower rate than the control region (Lopez *et al.*, 1997), and the use of DNA barcoding approaches with mtDNA cytochrome *b* sequences was demonstrated in the discrimination between the two mongoose species of the genus *Herpestes* (Bennett, 2011) and among delphinid cetacean species (Amaral *et al.,* 2007a). Thus, it is valuable to examine whether or not population subdivisions between finless porpoises are revealed by examining the 'conservative' cytochrome *b* gene sequences and conventional phylogenetic trees.

In this study, 12 *N.a. sunameri* specimens were used. They were collected from fishery markets at Pohang in southeastern Korea and their complete cytochrome *b* sequences were obtained. These sequences were compared to the corresponding partial (402bp) and complete (1,140bp) sequences of *Neophocaena*, obtained from GenBank, in order to examine interspecific divergence between two species of *Neophocaena* (*N. asiaeorientalis* and *N. phocaenoides*) and intraspecific divergence between two

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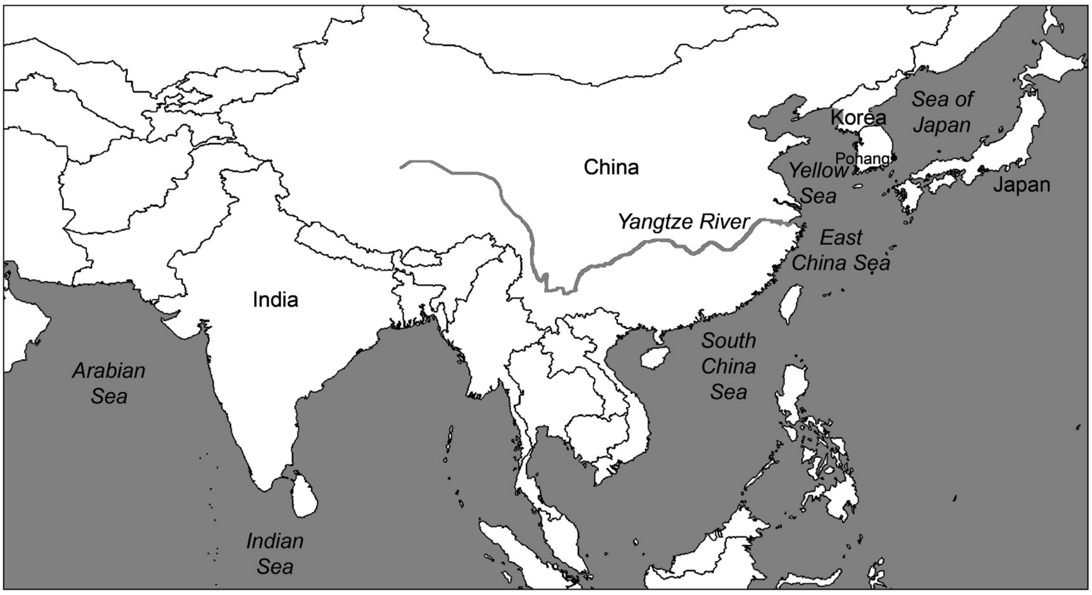

Fig. 1. A map covering the distributional range of two species of genus *Neophocaena*, with collection site of *N. asiaeorientalis sunameri* at Pohang in southeastern Korea. *N. phocaenoides* ranges from the Arabian Sea to the South China Sea, whereas *N.a. asiaeorientalis* is found in the Yangtze River, and *N.a. sunameri* inhabits in East China Sea, Yellow Sea, and the waters of Korea and Japan, including the Sea of Japan (East Sea).

subspecies of *N. asiaeorientalis* (*N.a. asiaeorientalis* and *N.a. sunameri*).

## MATERIALS AND METHODS

For this analysis, 12 specimens (specimen nos. 2507-09, 2511, 2513-15, 2538-40, 2542, and 2548) of *N.a. sunameri* were collected from fishery markets at Pohang in southeastern Korea. They were caught from the Sea of Japan (East Sea) as bycatch in 2013, as given in Table 1. A map detailing the distributional range of the two species of *Neophocaena*, with the collection site of *N. asiaeorientalis sunameri* at Pohang in south-eastern Korea is shown in Fig. 1. Small pieces of muscle tissue were taken and preserved in a deep freezer.

From muscle samples, total cellular DNA was extracted using a Genomic DNA extraction kit (Intron, Daejeon, Korea). The cytochrome *b* gene was PCR-amplified using the primers CB-out1 and CB-out2 (Cassens *et al.*, 2000). PCR thermal cycle for cytochrome *b* sequence was as follows: 94°C for 5 minutes; 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute (32 cycles); and 72°C for 5 minutes. To remove primer and unincorporated nucleotides, the amplified product was purified using a DNA PrepMate kit with a silica-based matrix (Intron Co.). The purified PCR products were analysed with an automated DNA Sequencer (Perkin Elmer 377) at Bioneer Co. (Seoul, Korea).

The complete sequences (1,140 bp) of the cytochrome *b* gene were obtained from 12 *N.a. sunameri* in Korea, and these sequences were compared to the corresponding ten complete sequences of two subspecies in *N. asiaeorientalis*, obtained from GenBank, as given in Table 2. In addition, from the cytochrome *b* complete sequences of *N. asiaeorientalis*, obtained from this study and GenBank, cytochrome *b* partial sequences (402 bp; site nos. 12-413) were obtained and analysed together with the corresponding five partial sequences of *N. phocaenoides*, obtained from GenBank, as listed in Table 2.

Sequence alignment, detection of parsimonious informative sites, model selection, calculation of nucleotide distances, tree constructions with 1,000 bootstrapped replications, and estimation of coefficient of evolutionary differentiation ($G^{st}$) were conducted using MEGA5 (Tamura *et al.*, 2011). The Jukes-Cantor (JC) model, which showed the lowest Bayesian information criterion scores, was selected by the program, and maximum likelihood trees were constructed. Fin whale, *Balaenoptera physalus* (NC001321) and common dolphin, *Delphinus delphis* (AF084084) were used as outgroups.

Table 1

Specimen number and cytochrome *b* complete haplotypes of 12 *Neophocaena asiaeorientalis sunameri* specimens, collected from fishery markets at Pohang in southeastern Korea. Among the 12 sequences nine haplotypes were identified.

| Specimen number | Cytochrome *b* complete haplotype |
|---|---|
| 2507 | CB01Korea |
| 2508, 2513, 1538, and 2548 | CB02Korea |
| 2509 | CB03Korea |
| 2511 | CB04Korea |
| 2514 | CB05Korea |
| 2515 | CB06Korea |
| 2539 | CB07Korea |
| 2540 | CB08Korea |
| 2542 | CB09Korea |

## RESULTS

Nine cytochrome *b* complete haplotypes were obtained from 12 *N.a. sunameri* specimens, as shown in Table 1, and these nine haplotypes were deposited under the accession numbers from KJ472895 to KJ472903. Within 19 haplotypes of *N. asiaeorientalis* (9 haplotypes from this study and 10 haplotypes from GenBank), 31 sites (2.72%) were variable,

Table 2

*GenBank* identification of 15 cytochrome *b* haplotypes in the genus *Neophocaena*, used in this study. The ten haplotypes of *N. asiaeorientalis* were complete[1] (1,140bp) sequences and five haplotypes of *N. phocaenoides* were partial[2] (402bp) sequences.

| Species name | Locality | Accession number (complete[1] or partial[2] cytochrome *b* haplotype) |
|---|---|---|
| *N. a. asiaeorientalis* | Yangzte River | HM137084[1], HM137092[1], HM137098[1], and HM137100[1] |
| *N. a. sunameri* | Yellow Sea | HQ108395[1], HQ108397[1], HQ108415[1], HQ108419[1], and HQ108420[1] |
| | East China Sea | NC021461[1] |
| *N. phocaenoides* | Indian Sea | EF203442[2], EF203444[2], and EF203438[2] |
| | Arabian Sea | DQ364692[2] and DQ364691[2] |

and 16 sites (1.40%) were parsimonious informative. The average JC distance among nine haplotypes of *N.a. sunameri* from Korea was 0.44%.

A maximum likelihood tree with 19 cytochrome *b* complete haplotypes of *N. asiaeorientalis* from four regions in East Asia is shown in Fig. 2, and the 19 haplotypes from East China Sea, Yellow Sea (China), the Yangtze River, and Sea of Japan formed one clade (Gp 1), with within group average JC distance of 0.57% and a $G^{st}$ value of 0.06. In addition, one haplotype (HM137098) of *N.a. asiaeorientalis* from the Yangtze River was identical to one haplotype (CB02Korea) of *N.a. sunameri* from the Sea of Japan, and another haplotype (HM137092) of *N.a. asiaeorientalis* from the Yangtze River was identical to another haplotype (HQ108397) of *N.a. sunameri* from the Yellow Sea. Additionally, HQ108415 from the Yellow Sea was identical to CB04Korea from the Sea of Japan and HQ108420 from the Yellow Sea was identical to CB08Korea from the Sea of Japan.

Another maximum likelihood tree with 24 cytochrome *b* partial haplotypes (402bp) of two *Neohpocaena* species from the Arabian Sea, Indian Sea, East China Sea, Yellow Sea, and Sea of Japan is shown in Fig. 3, and two clades (Gps 1 and 2) were recognised: the 19 haplotypes of *N.*

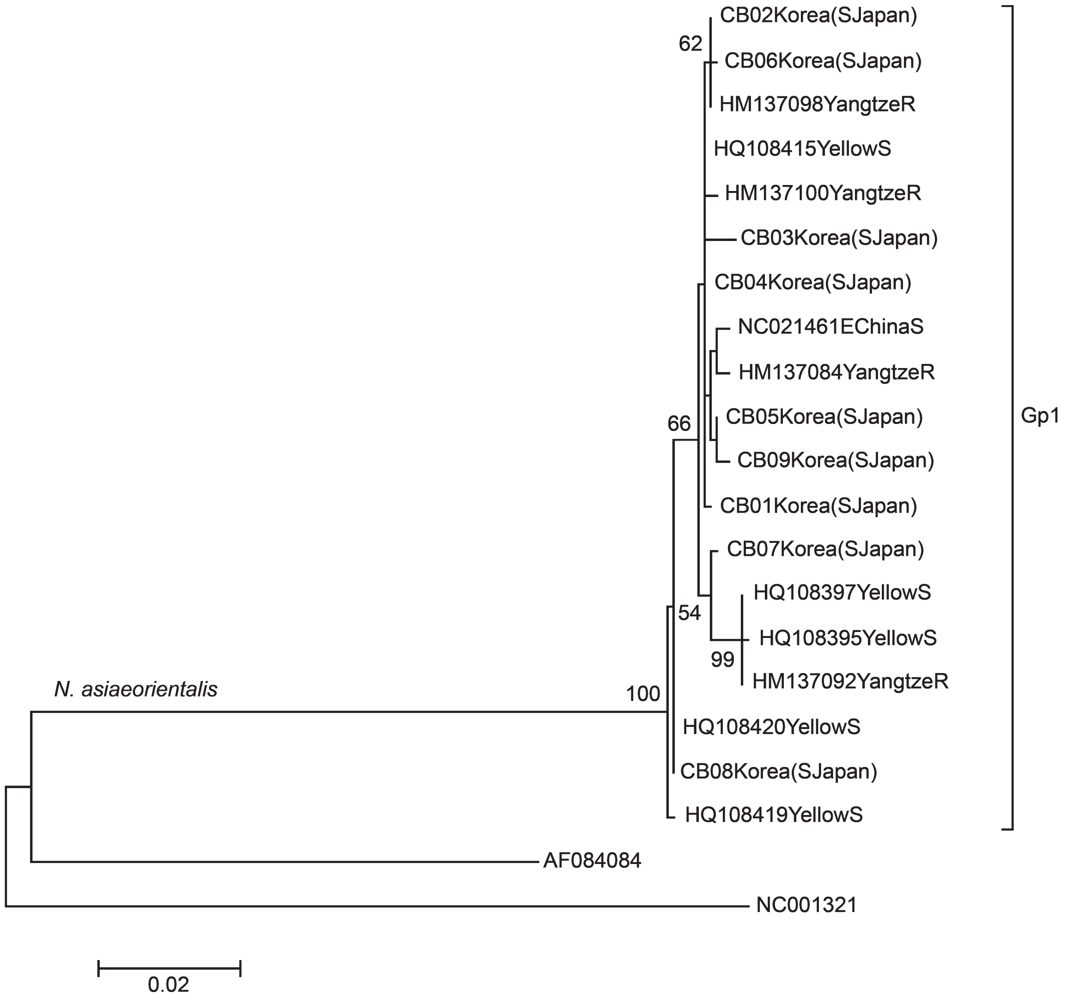

Fig. 2. A maximum likelihood tree with 19 cytochrome *b* complete haplotypes (1,140 bp) of *N.asiaeorientalis*. Nine haplotypes of *N.a. sunameri* from Korea were obtained in this study, as given in Table 1 and 10 haplotypes of *N. asiaeorientalis* were obtained from GenBank, as listed in Table 2. The tree was constructed with 1,000 bootstrapped replications, and the bootstrap values >50% are reported at the internodes. Location name follows haplotype name or accession number in each haplotype, obtained from this study and GenBank. Fin whales, *B. physalus* (NC001321) and common dolphins, *Delphinus delphis* (AF084084) were used as outgroups.

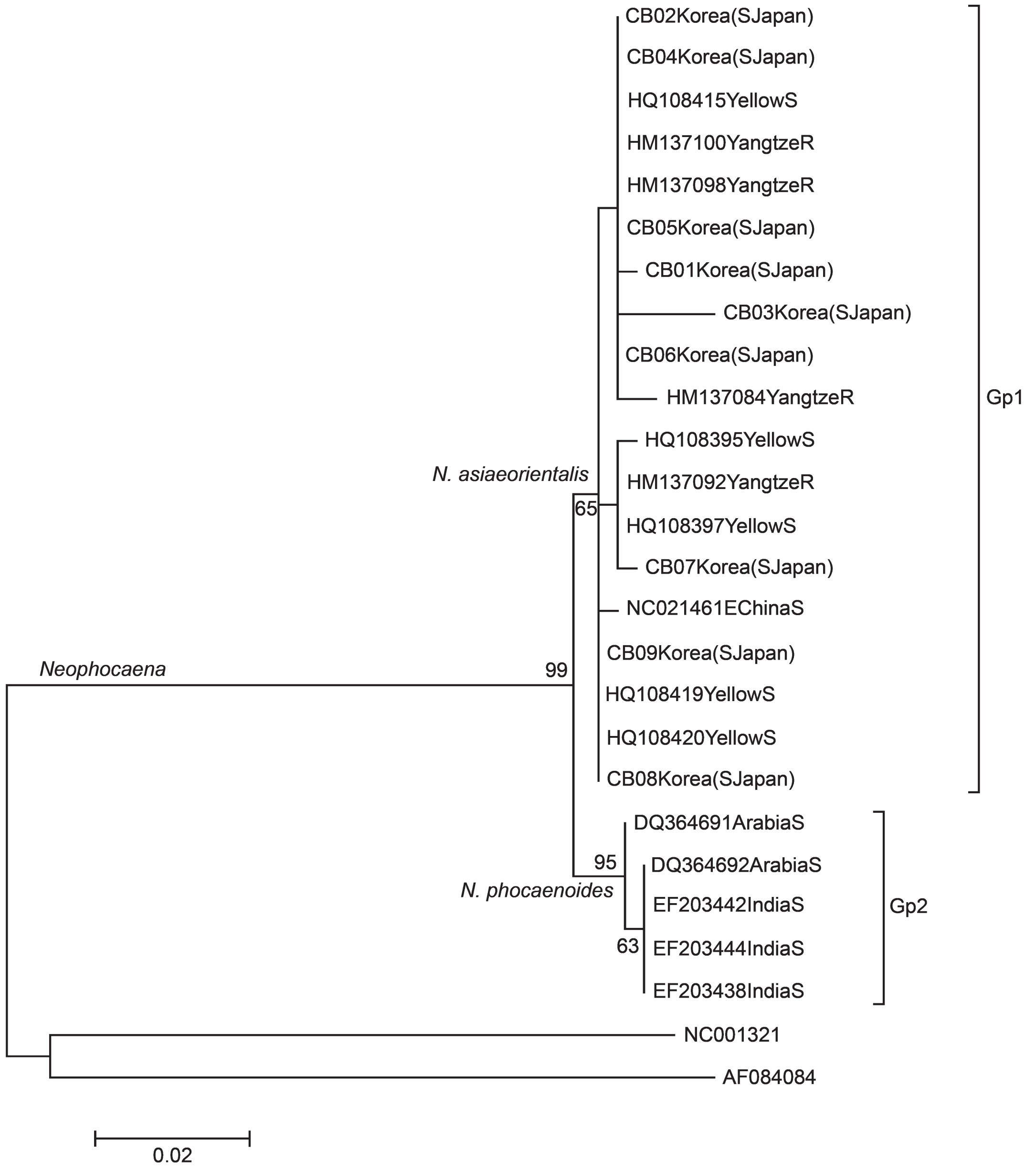

Fig. 3. A maximum likelihood tree with 24 cytochrome *b* partial (402 bp) haplotypes of two species in the genus *Neophocaena*. Nine haplotypes of *N.a. sunameri* from Korea were obtained from this study, as given in Table 1, and ten haplotypes of *N. asiaeorientalis* and five haplotypes of *N. phocaenoides* were obtained from GenBank, as listed in Table 2. Fin whales, *B. physalus* (NC001321) and common dolphins *Delphinus delphis* (AF084084) were used as outgroups.

*asiaeorientalis* from the four regions of East China Sea, Yellow Sea, the Yangtze River, and Sea of Japan (Gp 1) were distinct from the five haplotypes of *N. phocaenoides* from the Indian and Arabian Seas (Gp 2), with average JC distance of 1.64%, and four fixed site differences (1.00%) at site numbers. 60, 145, 261, and 408, and a $G^{st}$ value of 0.64.

## DISCUSSION

Jefferson and Wang (2011) reported that the sharing of mtDNA control region haplotypes and nuclear DNA alleles between the two species of finless porpoises was a common result amongst the previous molecular studies with the genus *Neophocaena*. Li *et al.* (2011) and Ju *et al.* (2012) could not find any obvious groupings in the two species of finless porpoises from conventional phylogenetic trees based on nuclear intron, microsatellite and mtDNA control region sequences, so they distinguished between the two species by using *F*-statistics. Additionally, Wang *et al.* (2008) noted that the shared DNA in *Neophocaena* was due to insufficient time since divergence to allow complete lineage sorting that would result in fixed genetic differences.

From our study based on cytochrome *b* partial sequences (Fig. 3), it was found that *Neophocaena* comprises two clades (Gps 1 and 2), corresponding to the two species of *N. asiaeorientalis* and *N. phocaenoides*, with average JC distance of 1.64%, four fixed site differences (1.00%), and a $G^{st}$ value of 0.64, although the specimens from Southeast Asia and the contiguous South China Sea were not examined.

The nuclear genes vary at a slower rate than mtDNA sequences (Steppan *et al.*, 2005), and the mtDNA cytochrome *b* gene is more conservative than the mtDNA control region (Lopez *et al.*, 1997). The variability of microsatellites is often so high that it is possible to address issues such as discrimination at the individual level (Wan *et al.*, 2004). In addition, the cytochrome *b* gene was used as one of the barcoding genes (Bennett, 2011) and it has several advantages when compared to the control region in phylogenetic analysis of the genus *Delphinus* (Amaral *et. al.*, 2007b). Furthermore, the $G^{st}$ value is equivalent to the $F^{st}$ value (Halliburton, 2004), and the $F^{st}$ value above 0.25 indicates 'very great' genetic differentiation and between them, whereas differentiation is negligible when $F^{st}$ is as small as 0.05 or even less (Wright, 1978).

Cytochrome *b* sequences were used (not analysed by former researchers to discriminate the two species of *Neophocaena*) and two genetically distinct species were found (Fig. 3; Gps 1 and 2), indicating that the cytochrome *b* gene is a useful marker to distinguish the two species. However, further genetic analyses with specimens throughout the distribution range of the two species (especially the specimens from Southeastern Asia and contiguous South China Sea) are necessary to confirm our findings. It was also considered that complete lineage sorting has occurred in the cytochrome *b* gene of the two *Neophocaena* species, because the time after divergence was long enough to result in fixed genetic differences between the two species.

In morphological and molecular studies of the East Asian finless porpoise, *N.a. sunameri*, Jefferson and Wang (2011) noted that the Yangtze River finless porpoise, *N.a. asiaeorientalis,* is distinct from it, although they noted that obvious distinction between the two subspecies was not revealed by previous analyses. Li *et al.* (2011) and Ju *et al.* (2012) used *F*-statistics with microsatellite, mtDNA control region, and nuclear intron markers to distinguish between two subspecies of *N. asiaeorientalis*. However, Yang *et al.* (2002) noted that the differentiation between the Yangtze and Yellow Sea populations was not significant from the control region analysis.

In this study, based on cytochrome *b* complete sequences of *N. asiaeorientalis* (Fig. 2), a lack of genetic divergence was found between *N.a. asiaeorientalis* from the Yangtze River (Gp 1, in part) and *N.a. sunameri* from the East China Sea, Yellow Sea, and Sea of Japan (Gp 1, the rest), with a $G^{st}$ value of 0.06 and two pairs of identical sequences between them. Huelsenbeck *et al.* (1996) reported that a classification should be the product of all available characters distributed as widely and evenly as possible over the organisms studied. Jefferson and Wang (2011) noted that there is still some uncertainty about *N.a. asiaeorientalis'* isolation in the Yangtze River proper, and Pilleri and Gihr (1975) reported that finless porpoises from Japan and China have been considered as the same subspecies in a previous morphometric analysis.

The sequencing results presented here do not support the current classification, recognising two subspecies within *N. asiaeorinetalis.* In future, genetic analyses with more specimens of *Neophocaena* across its distribution range are needed to confirm the findings of this study.

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# An analysis of historical bottlenose dolphin (*Tursiops truncatus*) strandings in the Mississippi Sound, USA using classification and regression trees (CART)

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ABSTRACT

Trends in bottlenose dolphin (*Tursiops truncatus*) strandings can be used to examine several factors associated with mortality and life history and are essential for detecting unusual mortality events (UMEs). This study characterised stranding trends in the Mississippi Sound (MS) region of the northern Gulf of Mexico (GoM) from 1996–2009 using kernel density estimation (KDE) and classification and regression tree (CART) analysis. An annual mean of 26.1 strandings ($n$ = 14), SD = 13.7, 95% CI [18.2, 34.0] and a peak in strandings during spring (March–May) were evident from our analyses. Neonates stranded almost exclusively in spring indicating that this is the dominant breeding and calving season in this area. Spatial distributions revealed that the majority of dolphins stranded along central and western portions of the MS Sound near Gulfport, MS and on Ship Island during the spring and summer months, but were more often found in the eastern MS Sound during winter and autumn. Our CART analyses indicated that 1996, which contained a declared UME, was anomalous from other years as the number of adult, sub-adult and juvenile strandings was relatively high during the autumn and winter. Further, our analyses showed that the location of those strandings on Ship Island in autumn and winter was unique from all other years in the historical record. These results represent historical conditions that can be used as a baseline for future studies of the effects of environmental disturbances, including UMEs, in MS. This research also demonstrates the versatility and usefulness of CART for describing historical trends, detecting departures from the norm and explaining UMEs within the framework of a single analysis. This approach represents an objective assessment tool that could be used to assist governmental agencies with determining the onset of a UME and could help support or refute the cause of these events.

KEYWORDS: COMMON BOTTLENOSE DOLPHIN; STRANDINGS; GULF OF MEXICO; NORTHERN HEMISPHERE; STATISTICS; DISTRIBUTION

## INTRODUCTION

The enactment of the US Marine Mammal Protection Act (MMPA) in 1972 and amendments in 1992 that created the Marine Mammal Stranding Network have generated increased awareness of bottlenose dolphin (*Tursiops truncates*) strandings in the USA (McFee and Hopkins-Murphy, 2001). Since that time, much has been learned about the species as strandings have provided unique opportunities to study factors associated with mortality (Lipscomb *et al*., 1996; Meador *et al*., 1999) and life history (Hubard and Swartz, 2000; Mattson *et al.,* 2006; McFee and Hopkins-Murphy, 2001; McFee *et al*., 2006) that have influenced management and conservation of this protected species. Further, because bottlenose dolphins are considered a sentinel species (Wells *et al*., 2004) periodic assessment of stranding trends is critical for gauging the effects of environmental perturbations on ecosystem health.

A major focus of strandings research is Unusual Mortality Events (UMEs), which can result from viral infection (Lipscomb *et al*., 1996), bacterial infection (McFee and Lipscomb, 2009), biotoxins (MMC, 1996) and many other potential factors. A UME is defined in the MMPA as 'a stranding that is unexpected; involves a significant die-off of any marine mammal population; and demands immediate response.' The Working Group on Marine Mammal UMEs, composed of members from scientific and academic institutions, conservation organisations and federal and state agencies, is charged with examining the nature of a stranding event and determines if the event should be declared a UME[2]. Criteria developed by the working group (and see Gulland, 2006) for determining the onset of a UME are:

(1) unusual magnitude;

(2) marked change in temporal dynamics;

(3) marked change in spatial dynamics;

(4) marked change in species, age, or sex distributions;

(5) unusual pathologic findings, behaviour patterns, clinical signs, or physical condition;

(6) mortality or morbidity among depleted, threatened, or endangered populations; and

(7) stranding of critically endangered species.

These criteria, while necessary, are somewhat subjective as no formal quantitative method for determining the onset of a UME is defined. With respect to the unusual magnitude of a stranding event (Criteria 1), National Marine Fisheries Service (NMFS) has stated 'There is no set formula for determining what magnitude would trigger a response. The NMFS Southeast region has used a formula of the historic mean plus two times the standard deviation to determine a threshold level,' but also states that the magnitude of a stranding event must be weighed against other knowledge (NOAA, 2004). While there is no replacement for expertise of the working group, there is a need for quantitative

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[2] Marine Mammal Working Group Charter available at: *http://www.nmfs.noaa.gov/pr/health/mmume/history.htm.*

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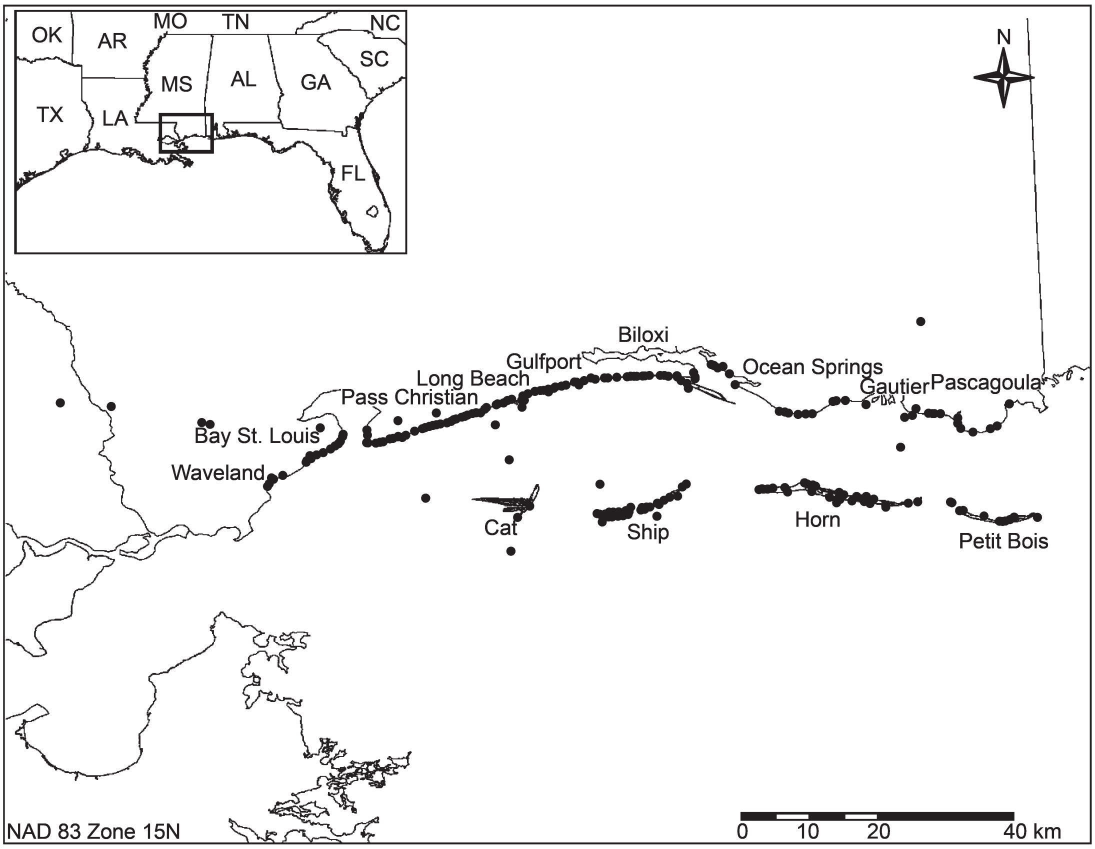

Fig. 1. Study area and location (black circles) of 364 bottlenose dolphin (*Tursiops truncatus*) strandings in the Mississippi Sound from 1996–2009. Strandings occurring inland were found along the Pearl (left) and Escatawpa (right) rivers.

methods that incorporate such varied information into a single analysis. Application of statistical tools that complement the expertise of the Marine Mammal Working Group are essential for assessing the unusual nature of a marine mammal mortality event.

The northern Gulf of Mexico (GoM) is an area with dense populations of bottlenose dolphins (Waring *et al*., 2009) that has been subject to numerous environmental disturbances over the last several years including freshwater flood events (Carmichael *et al*., 2012), hurricanes (Miller *et al*., 2010), and oil spills (Rico-Martinez *et al*., 2013) that have impacted ecosystem health in the region. Coinciding with these events is the longest running UME on record in the northern GoM. The UME began in 2010 and continues to date, and has included 1,271 cetacean strandings over a region extending from the western border of Louisiana to the Florida Panhandle[3]. While the cause remains unknown, several potential factors may have contributed to this UME including a prolonged bout of cold weather, unusually large freshwater floods (Carmichael *et al*., 2012), disease and the Deep Water Horizon (DWH) oil spill. While the duration, geographic spread and magnitude of this event have been unique compared to past UMEs, a thorough understanding of historical stranding trends is needed to provide essential context for assessing spatial, temporal or demographic departures from the norm.

In this paper, a historical assessment of bottlenose dolphin strandings is presented for the Mississippi (MS) Sound from 1996–2009, to describe pre-UME stranding trends in this region. Our analysis approach highlights classification and regression tree analyses (CART), which we believe is well suited for identifying patterns in strandings including anomalous events, and thus could be used as a quantitative assessment tool for identifying UMEs.

## METHODS

### Study Area

The MS Sound encompasses a 2,000km$^2$ area separated from the larger GoM by six barrier islands (i.e. Cat, West Ship, East Ship, Horn, Petit Bios and Dauphin Islands) (Eleuterius, 1978) (Fig. 1). Water depth ranges from 1 to 7m, mean annual water temperature ranges from 9°C in winter to 32°C in summer and salinity ranges from 0 to 33 parts per thousand (ppt) (Christmas, 1973). Population estimates in 2007/08 indicated the total number of dolphins ranges from 2,255 during the summer months to 1,413 during the winter (Miller *et al*., 2013) indicating that this is among the most densely populated areas within the northern GoM.

### Data collection

Data collected for this study were obtained from the NMFS database[4], which archives data received from state marine mammal stranding networks (MMSN). The primary stranding respondent and member of the MMSN in MS is the Institute for Marine Mammal Studies (IMMS), which has been an active member of the national stranding network since 1984, and currently responds to all strandings in MS. Information collected by respondents for this study included

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[3] Data available at: http://www.nmfs.noaa.gov/pr/health/mmume/.

[4] (Available at: http://www.nmfs.noaa.gov/pr/health/networks.htm).

geographic coordinates, sex, body condition and straight body length in accordance with NMFS level A marine mammal stranding reports (NOAA, 2012). Respondents also took photographs and documented visible evidence of human interaction. Age classes were determined using NMFS designations where perinates were <115cm, juveniles were 115–227cm, sub-adults were 227–247cm and adults were >247cm (NMFS, 2011). The period of record for our analyses extended from 1996 to 2009 and included one year with a declared UME (1996). This year was retained in the analyses because this is an important part of the historical record that we believe is essential for a complete understanding of stranding trends in the region. Further, inclusion of this year allowed us to determine how 1996 was different from typical years among spatial, temporal, and demographic factors.

### Data analysis

To describe the spatial distribution of strandings over the historical record, kernel density estimation (KDE) was used within the 'Spatial Analyst' toolbox in the program *ESRI ArcMap 10.2*. This required creation of point shapefiles from geographic coordinates of strandings for selected demographic and temporal categories as an input layer to generate a KDE representing strandings per km$^2$. A search radius of 1km was used for this analysis.

To describe historical trends for the region, means, standard deviations (SD) and 95% confidence intervals were calculated for both yearly totals and for total seasonal strandings among age classes. Seasons were defined as spring (March–May), summer (June–August), autumn (September–November) and winter (December–February). Only strandings of known length were used in age class estimates. To more thoroughly examine 1996, which contained a declared UME, a formula (mean + 2SD) used by NMFS Southeast Region was applied to determine a marked increase in stranding magnitude for yearly and seasonal strandings (NOAA, 2004). To further quantify historical trends, CART was used, which works by repeatedly splitting a dependent variable into homogenous groupings with respect to a set of independent variables. CART is well suited for ecological data because it can handle missing data, non-linear relations and high order interactions (De'ath and Fabricius, 2000), which are common problems with strandings data. CART works by recursively partitioning a continuous or categorical dependent variable into subsets that maximise the homogeneity with respect to single or multiple continuous or categorical explanatory variables while minimising the sums of squares. The output consists of an easily interpretable tree, similar to a decision tree that can be used to explain complex relationships. The strength of this approach for exploring stranding trends lies in its ability to incorporate the magnitude of a stranding event with spatial, temporal and demographic factors into a single analysis. CART is also considered an adequate replacement for traditional statistics such as log-linear models (De'ath and Fabricius, 2000) that are commonly used to analyse stranding data (McFee *et al*., 2006).

CART was conducted using the *mvpart* package within the freely downloadable program R 2.12.1 (R Development Core Team, 2011). Classification and regression trees were constructed to explore spatial, temporal and demographic stranding trends during this period. Regression trees were used to explain the distribution of stranding counts among years, seasons and age classes using 224 observations ($n$ = 224), representing unique combinations of years, seasons and age classes for the historical record. Classification trees were constructed from all stranding records with known stranding location ($n$ = 364) to explain the spatial distribution of strandings among years, seasons and age classes. The dependent variable in classification trees, spatial location, included eight major cities along the MS Gulf Coast (Pascagoula, Ocean Springs, Biloxi, Gulfport, Long Beach, Pass Christian, Bay St. Louis and Waveland) and four MS Barrier Islands (Petit Bois, Horn, Ship and Cat) where strandings were located during the study period (Fig. 1). For each analysis, 100 sets of ten-fold cross-validations were run (De'ath and Fabricius, 2000) and two trees were selected to explain stranding distributions including: (1) the tree within one standard deviation of the smallest cross-validation error (CVE) (Breiman *et al*., 1984; De'ath and Fabricius, 2000); and (2) the smallest tree that explained the most variation in stranding counts (De'ath, 2002). Cross-validation error is a measure of predictive accuracy and is often used for selecting trees to predict unobserved data where a value of zero indicates perfect prediction and values near or above one are indicative of poorer predictive capacity (De'ath, 2002). Classification and regression trees constructed here were not used for prediction; however, relative CVE was used to select the best descriptive trees that contained the most useful information (De'ath and Fabricus, 2000). For regression trees, the proportion of the total sum of squares (SS) explained by the tree was reported. For classification trees, the misclassification rates (MCR) and null model misclassification rates (NMMCR) were reported, which represents a random prediction (De'ath and Fabricius, 2000).

## RESULTS

From 1996 to 2009, National Oceanic and Atmospheric Administration level A stranding reports were filed for 366 bottlenose dolphins in the MS Sound (Fig. 1). The spatial distribution of strandings in the MS Sound determined by KDE over the historical record (1996–2009) indicated the highest stranding densities (6–10) occurred along a portion of mainland MS Coast between Biloxi Bay and Bay St. Louis and in the central portion of Ship Island (Fig. 2). Estimates also showed moderate stranding densities (2–6) concentrated throughout central portions of the MS Coast between Biloxi Bay in the east and Bay St. Louis in the west and isolated clusters west of Bay St. Louis, near Ocean Springs, near Pascagoula and on Ship and Horn Islands.

Mean annual strandings during the study period were 26.1 ($n$ = 14), SD = 13.7, 95% CI [18.2, 34.0] and ranged from 8 in 2007 to 59 in 1996 (Fig. 3). Based on these data, an annual stranding level at or above 53.5 would be considered a marked increase in magnitude (UME Criteria 1; Gulland, 2006). The total number of strandings in 1996 exceeded this level and was declared a UME. Mean number of strandings was highest in the spring for all age classes but showed no clear pattern among other seasons (Fig. 4). The mean for the adult age class ranged from 0.92 ($n$ = 14), SD = 1.63, 95%

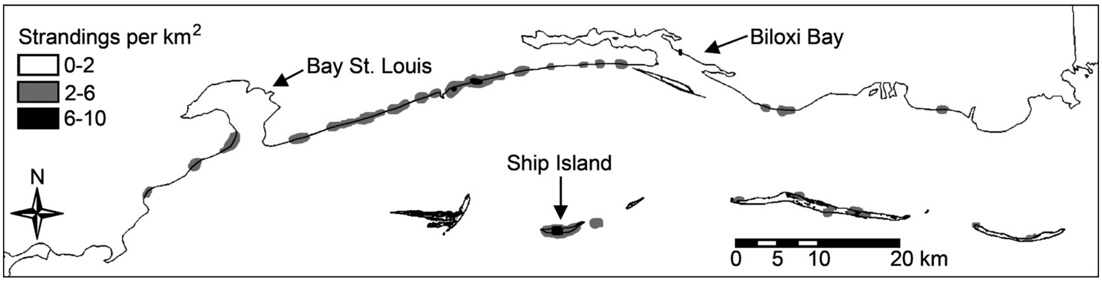

Fig. 2. Kernel density estimations (KDE) of bottlenose dolphin strandings per square km in the Mississippi Sound from 1996–2009.

CI [0, 1.9] in autumn to 1.79 (*n* = 14), SD = 1.31, 95% CI [1, 2.5] in spring (Fig. 4a). Strandings in the perinate age class varied most widely among seasons as they ranged from 0 (*n* = 14) in summer to 4.7 (*n* = 14), SD = 3.1, 95% CI [2.9, 6.5] in spring (Fig. 4d). Adult, sub-adult, and juvenile strandings exceeded the mean +2SD for each respective age class during autumn of 1996 (Figs 4a, b, and c). Adults and juveniles also exceeded this level in winter of 1996.

A regression tree was constructed to explain variation in stranding counts as a function of year, season and age class. The regression tree was pruned based on the relation between relative and CVE and regression tree size and resulted in selection of two trees to explain the distribution of strandings among selected factors (Fig. 5). The first tree contained the lowest CVE (0.87) and had one division among seasons that explained 18% of the total SS (Fig. 6a). This division divided spring strandings from all other seasons. The mean number of spring strandings for each age class within each year was 2.88 (*n* = 56), SD = 2.6, 95% CI [2.2, 3.6]. The second regression tree (CVE = 0.92) contained a total of five divisions and explained 44% of the SS, where the length of each branch was related to the relative amount of the SS explained by each division in the tree (Fig. 6b). The tree divided spring strandings by age class (7% of total SS) where perinates, with a mean of 4.7 (*n* = 14), SD = 3.1, 95% CI [2.9, 6.5] were different than adults, juveniles and sub-adults with a mean of 2.26 (*n* = 42), SD = 2.2, 95% CI [1.6, 2.9] indicating that perinates had a clearly defined stranding peak in spring relative to the other age classes. The left branch of the tree showed that strandings in winter, summer and autumn were divided by year before or after 1997 (9% of total SS). This indicated that 1996 non-spring strandings were unique from all other years of the study period. The next division was among 1996 strandings, which showed that no perinates stranded in autumn, summer or winter (*n* = 3) (5% of total SS). The last division showed that adult, juvenile and sub-adult strandings were concentrated in the autumn and winter and with a mean of 6.17 (*n* = 6), SD = 2.1, 95% CI [3.9, 8.4] compared to a mean of 1.33 (*n* = 3), SD = 1.5, 95% CI [0, 5.1] for summer strandings (5% of total SS).

A classification tree was constructed to explain the spatial distribution of strandings as a function of year, season and age class. For this analysis, the tree with the smallest CVE (0.99) was also the smallest tree that best explained spatial location of strandings (Fig. 7). This tree had an MCR of 71% (NMMCR = 83%). The first division split strandings from 2009 from all other strandings over the period of record. The terminal leaf of the right branch showed that 2008–2009 strandings occurred predominantly along the central MS coast in Gulfport (12) and Pass Christian (9). The left branch of the tree was further divided by year before or after 1997. Strandings that occurred in 1996 occurred predominantly in Gulfport (14) and on Ship Island (14). Strandings from 1997–2007 were further divided by season, where summer strandings were most commonly found along the central MS coast in Gulfport (9) and Long Beach (8). Fall, winter, and spring season strandings from 1997–2007 were further divided by age class where juveniles, which stranded most

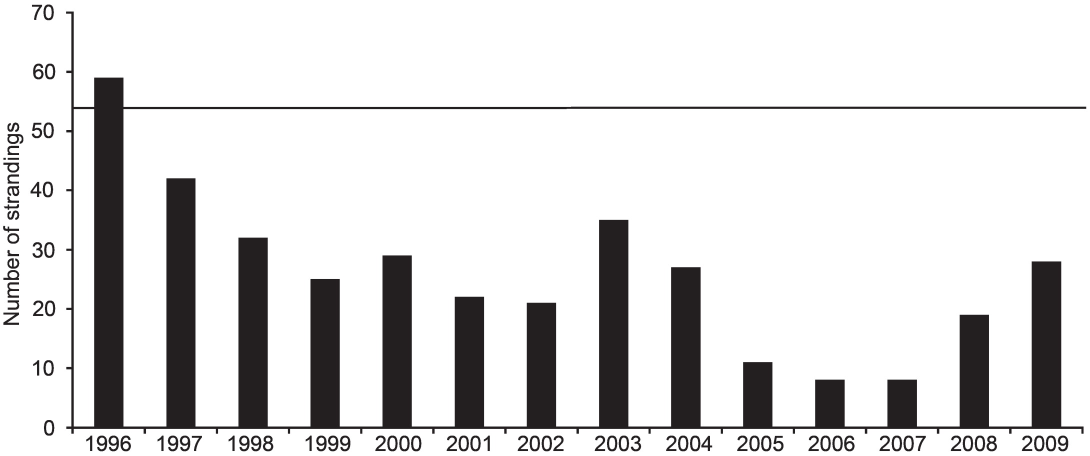

Fig. 3. Stranding totals for each year of the study period (*n* = 14). The horizontal line corresponds to the mean plus two times the standard deviation (53.5) indicating the number of annual strandings that would constitute a marked increase in magnitude as determined by the National Marine Fisheries Service (NMFS) Southeast Region.

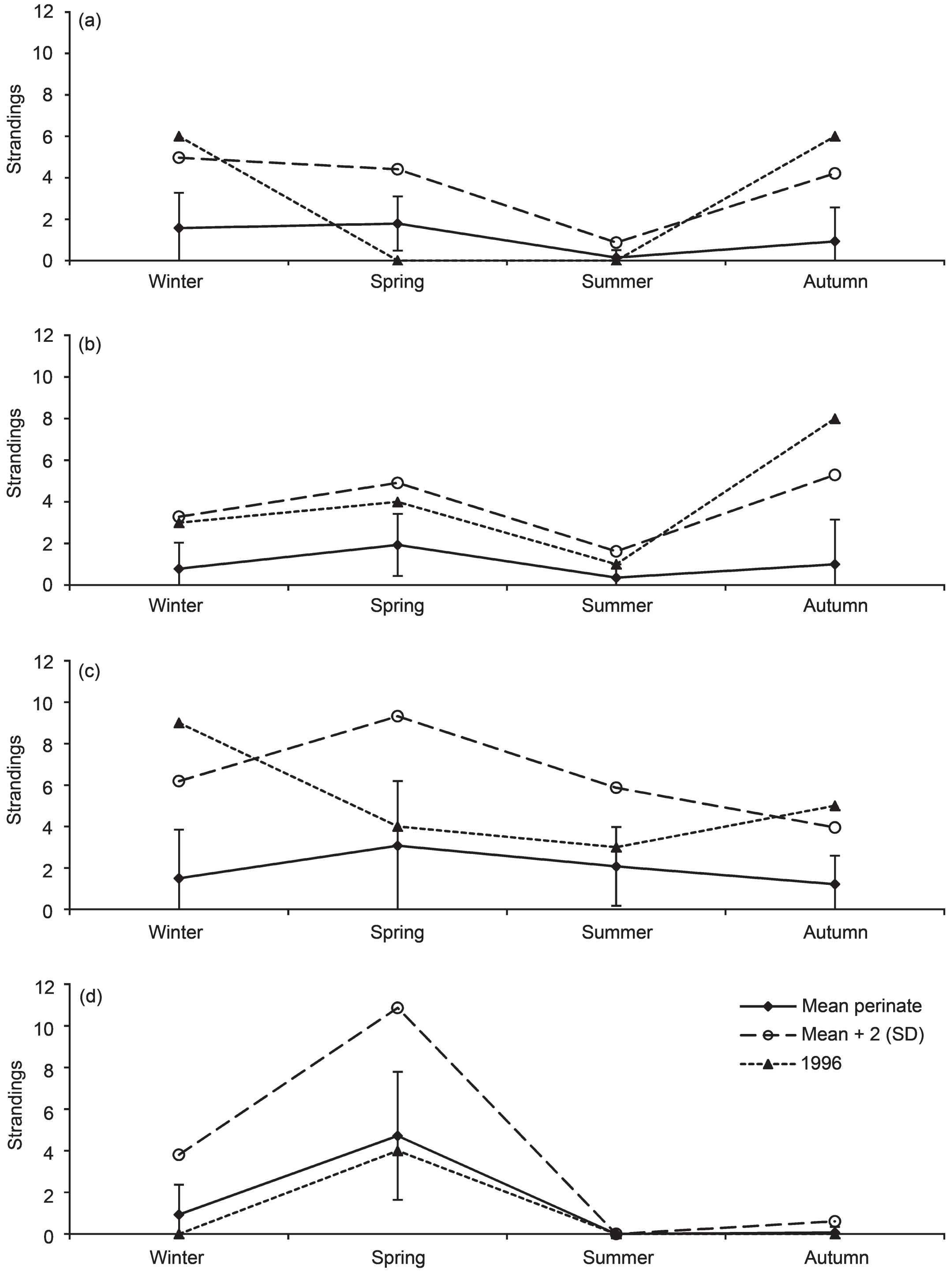

Fig. 4. Mean number of strandings of known length in the Mississippi Sound from 1996–2006 for (a) adults (>247cm), (b) sub-adults (227–247cm), (c) juveniles (115 – 227cm) and (d) perinates <115cm for each season of the year. Seasons were defined as winter (December–February), spring (March – May), summer (June – August), and autumn (September–November). The sample size for each estimate of the mean is 14, which corresponds to the total number of seasons among years that a stranding total was recorded. Error bars correspond to the standard deviation of the mean.

often in Biloxi (13), were different from adult, perinate, and sub-adult strandings, which occurred most often on Horn Island (22) in the eastern MS Sound, and in Gulfport (19) and on Ship Island (18).

## DISCUSSION

### Temporal and demographic trends

The results show that MS has a mean annual bottlenose dolphin stranding rate of 26.1 and highly varied annual strandings over the 14 year period prior to the onset of the current northern GoM UME. It is difficult to determine the effects of surveillance effort on this estimate; however, the occurrence of Hurricane George in 1998 and Katrina in 2005 may have reduced stranding response activity. This seems more evident during the period from 2005–07, which had very low annual totals that may have resulted from reduced surveillance effort following Hurricane Katrina. Other studies have used temporal blocking to try and account for difference in effort (McLellan *et al.*, 2002), but the inability to effectively quantify effort in MS in this study made a

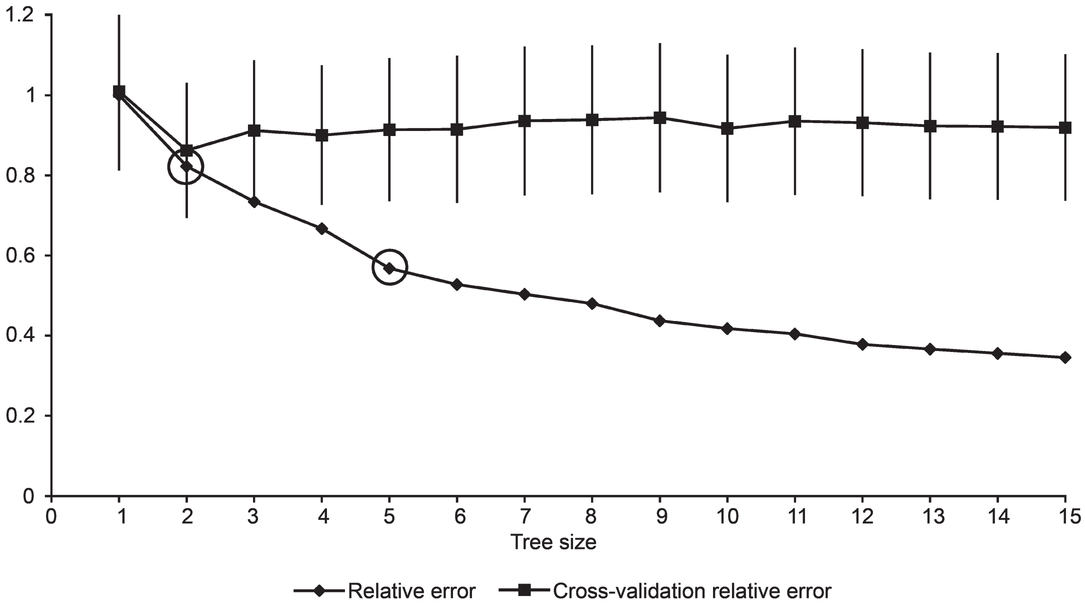

Fig. 5. Relative and cross-validation error (CVE) as a function of regression tree size used to select the tree with the smallest CVE (tree size = 2) and the smallest tree that explained the most variation among stranding counts (tree size = 5), indicated by circles. Error bars correspond to standard error.

temporal blocking scheme inadequate to address this deficiency. Further, the regression tree did not detect any differences in stranding abundance for this time period aside from 1996.

Our results indicated that the spring season (March–May) was the dominant peak stranding season for all age classes, especially perinates. The unimodal distribution of neonate strandings suggests that spring is the dominant breeding and

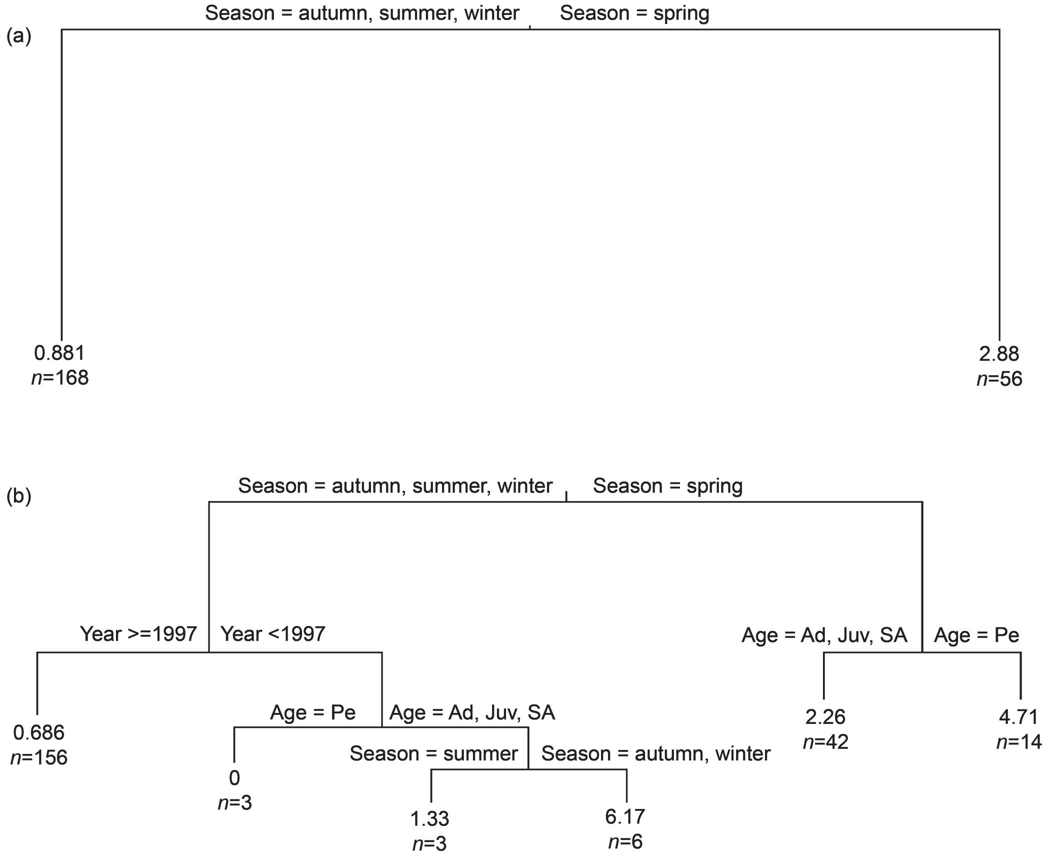

Fig. 6. Regression trees constructed from bottlenose dolphin strandings in the Mississippi Sound from 1996–2009 showing (a) a tree with one division with a cross-validation error (CVE) of 0.82 and a standard error (SE) of 0.17 and (b) a tree with five divisions (CVE = 0.91; SE = 0.18) . The distribution of stranding counts is modeled as a function of year, season, and age class. Seasons include winter, spring, summer, and autumn. Age classes include adult (Ad), sub-adult (SA), juvenile (Juv) and perinate (Pe). The length of each branch corresponds directly to the amount of variation explained by the corresponding factor. The mean number of strandings falling within each bin and the sample size used to derive these estimates is shown below each terminal leaf.

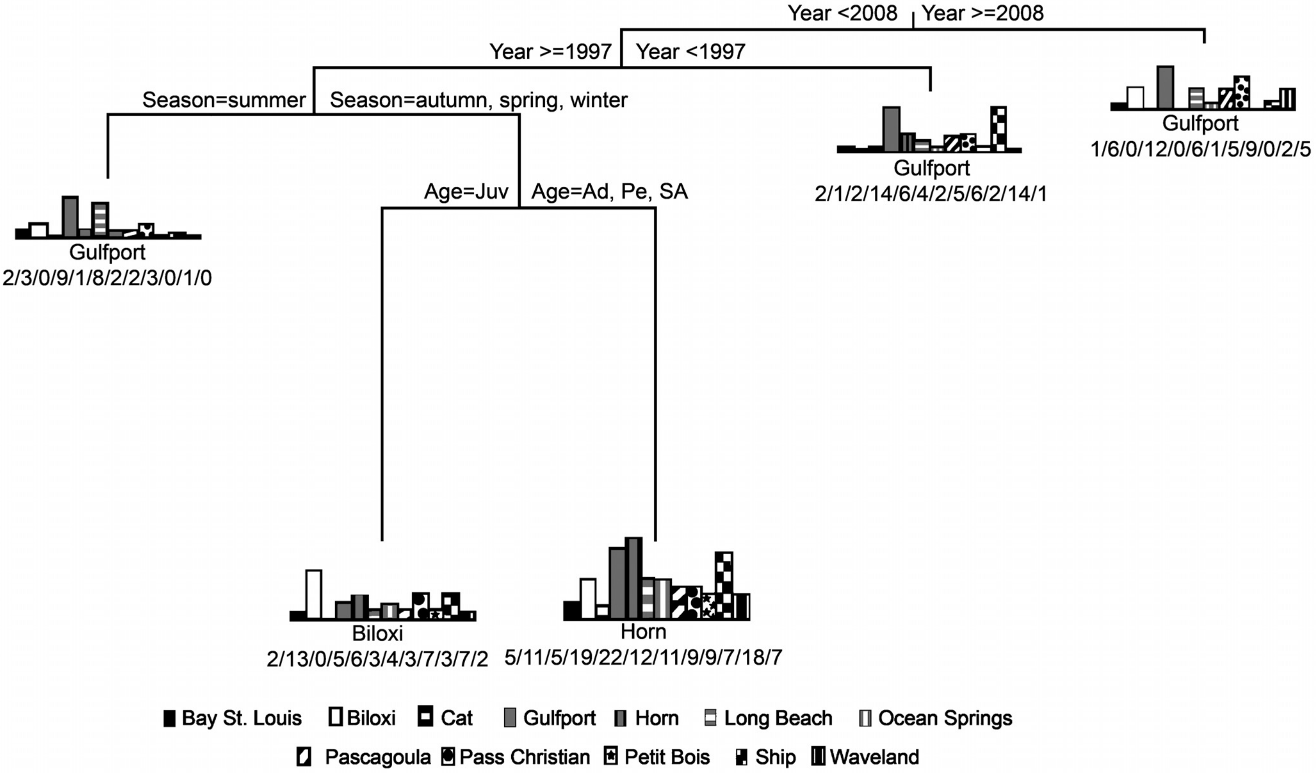

Fig. 7. Classification trees constructed from bottlenose dolphin strandings in the Mississippi Sound from 1996–2009 showing a tree with four divisions (CVE = 1.01; SE = 0.02). The spatial distribution of strandings is modelled as a function of year, season, and age class. Seasons include spring, summer, autumn, and winter. Age classes include adult (Ad), sub-adult (SA) juvenile (Juv) and perinate (Pe). The length of each branch corresponds directly to the amount of variation explained by the corresponding factor. The distribution of strandings is displayed as a bar graph at each terminal leaf showing the frequency of strandings at each location. Written below each terminal leaf is the location with the largest number of strandings along with sequential totals for each corresponding location in the displayed bar graph.

calving season in the MS Sound, which is in agreement with what has been reported for MS (Mattson *et al*., 2006) and other areas within the northern GoM (Fernandez and Hohn, 1998; Wursig *et al*., 2000). Seasonal stranding trends among other age classes were less pronounced, but exhibited peaks in spring as well. Interestingly, this contrasts with bimodal distributions of strandings in portions of the Atlantic Coast that exhibit a spring and autumn peak (McFee and Hopkins-Murphy, 2001; McFee *et al*., 2006). In North and South Carolina, distinct peaks in neonate strandings are seen in autumn and spring (McFee *et al*., 2006), indicating an autumn and spring breeding and calving season in this area.

The year 1996, which contained a declared UME, had the highest number of strandings and was determined to be unique from all other years with regard to the total number of strandings. Regression trees constructed using temporal and demographic explanatory factors revealed that unusual increases in adult, juvenile and sub-adult strandings occurred in autumn and winter of the year, outside of the typical peak stranding season. Historical reports from the area document that this event contained a total of 31 bottlenose dolphins in November and December that were believed to have stranded as a result of a harmful algal bloom (*Karenia brevis*) (MMC, 1996).

## Spatial trends

Spatial trends revealed from KDE indicated that strandings are most often found along the central and western portions of the MS mainland. Classification trees confirmed these findings and showed that juvenile, perinate and sub-adult strandings were most often found along the central MS Coast in cities such as Gulfport during the spring and summer. Such areas have dense human populations and are regularly visited by residents and tourists, thus surveillance effort is naturally higher here than in more isolated areas such as the barrier islands. Ship Island was an exception as KDE also revealed dense clusters of strandings occurred here; however, Ship Island is regularly visited by tourists via ferry from March–October as weather allows. Thus, surveillance effort cannot be ignored when considering these spatial distributions. Since 2010, governmental surveillance has increased throughout all portions of the MS resulting from the UME investigation and the concurrent Natural Resource Damage Assessment (NRDA) investigation. This must be considered in future studies that compare post-oil spill trends with historical trends, and for identifying future UMEs as varied surveillance effort over time and space makes it difficult to determine if stranding trends represent true departures from the historical norm (McLellan *et al*., 2002). Cetacean drift must also be considered as strandings can drift long distances after death depending on tides, winds, decomposition state and the size and buoyancy of the animal (McLellan *et al*., 2002; Peltier *et al*., 2012). The presence of stranding clusters in central and western portions of the MS Sound could partially be the result of a prevalent southeast to northwest current (Morton, 2008). This would indicate that the majority of strandings originated in the central and eastern portions of the MS Sound.

Also important for interpreting spatial trends are the abundance and distribution of live dolphins. The abundance of dolphins in MS Sound fluctuates considerably over the course of a year with higher densities in summer and lower densities in winter (Hubard *et al*., 2004; Miller *et al*., 2013). A large majority of dolphins (73.5%) are considered transients that leave the area during the colder months of the year (Smith *et al*., 2013). No peer-reviewed research has examined spatial distributions of bottlenose dolphins in the entire MS Sound. Previous population research focused in the eastern MS Sound has determined wide distributions within that area throughout the year (Hubard *et al*., 2004; Miller *et al*., 2013). Future studies aimed at examining population dynamics should include greater emphasis on spatial distributions for the entire MS Sound, which will enhance management and protection of dolphins in this region and may provide important clues for understanding stranding trends, including those associated with UMEs.

The cause of death for strandings is impossible to determine with such a large number of factors at play, but theories can be supported or refuted based on the available evidence (Carmicheal *et al*., 2012; McFee and Lipscomb, 2009). Classification trees in this study revealed unique spatial trends in stranding distributions in 1996. A total of 14 strandings were found on Ship Island in 1996 compared to a total of 28 Ship Island strandings for all other years combined. As mentioned previously, this event is believed to be the result of an algal bloom. Repeated testing on Cat Island (just west of Ship Island) showed that marine dinoflagellate cells (*K. brevis*) rose from 13,000 cells per liter on November 14 1996 to 13 million cells per liter on November 20 1996 (MMC, 1996) coinciding with the UME. Such large numbers of strandings on Ship Island and the high concentration of *K. brevis* in that area further support the idea that an algal bloom may have caused this UME and indicate that it may have been most prevalent near the western barrier islands. While the cause of death for these strandings cannot be verified, a clear link between geographic and ecological data is reliable evidence that supports *K. brevis* as a cause of this event. The ability to link geographic and ecological data may be even more critical for detecting emerging diseases in cetaceans (McFee and Lipscomb, 2009).

### Application of CART for exploring stranding trends

This study effectively demonstrated the usefulness of CART for exploring stranding trends. The ability to incorporate different types of data that exhibit varying distributions and missing values into a single analysis (De'ath and Fabricius, 2000; De'ath, 2002) makes CART a versatile tool for exploring stranding data. Historical trends such as peak stranding season and associated demographics were readily defined in our analyses as was the spatial distribution of strandings. More importantly, this approach was able to identify anomalous stranding trends across multiple explanatory factors simultaneously to detect a UME and explain its uniqueness. Factors associated with four of the seven criteria for determining the onset of a UME including those related to magnitude, timing, spatial location and age composition were incorporated. The remaining criteria could also be incorporated if necessary to account for different species of marine mammals, unique pathologies and behaviour patterns. Thus, this approach is a valuable resource to complement the expertise of the Marine Mammal Working Group for determining the onset of a UME within the context of historical data.

CART is an efficient means to explore data and contains easily understandable output that lends itself to explanation (De'ath and Fabricius, 2000; De'ath, 2002). Although strandings in 1996 were more than two SDs above the historical average and were thus part of a declared UME, CART showed that the unusual magnitude was among adult, sub-adult and juvenile age classes out of the typical peak stranding season. Also, CART was able to show that while autumn and winter strandings were typically distributed throughout the MS Coast in autumn and winter, an unusual magnitude of juvenile and sub-adults were stranding on Ship Island in close proximity to an area affected by an algal bloom. Obviously, the declaration of the 1996 UME was made without use of CART; however, it could have helped to efficiently explain the unusual nature of this event and provided greater support for an algal bloom as the cause. Shifting spatial distributions for strandings from 2008–2009 indicate an unusually large number of strandings occurred in Pass Christian, which may not necessarily indicate a UME, but does demonstrate the ability of CART to detect variations in stranding spatial location that could otherwise go unnoticed. Application of a quantitative assessment tool such as CART, that increases objectivity and provides evidence to help to determine the cause of a UME, is important for improved protection of cetaceans worldwide.

The current UME in the northern GoM is an excellent example of an event that has several suspected causes including the DWH oil spill, large freshwater flow events, prolonged bouts of cold weather and the bacterium *Brucella,* among others. Determining the cause and unusual nature of this event relies heavily on well-defined historical trends and adoption of quantitative assessment approaches to assess departures from the norm. Despite the increased awareness and attention cetacean strandings have received in this region, no historical analyses of stranding trends have been published in peer-reviewed literature for MS. Thus, historical stranding trends have not been adequately explored and deserve greater attention as context for understanding the uniqueness of the current UME. Undoubtedly, more work needs to be done to address an incomplete understanding of all aspects of bottlenose dolphin ecology in the northern GoM as this is a critical, yet poorly studied habitat for this sentinel species (Waring *et al*., 2009).

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# Baseline data on abundance, site fidelity and association patterns of common bottlenose dolphins (*Tursiops truncatus*) off the northeastern Tunisian coast (Mediterranean Sea)

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ABSTRACT

The common bottlenose dolphin has been studied intensively in numerous locations around the world but very little is known about this species along the South Mediterranean Basin. In this study, the temporal distribution of dolphins, group dynamics, site fidelity and association patterns of common bottlenose dolphins along the northeastern coastal waters of Tunisia was assessed through mark-recapture photo-identification techniques. Prior to this study, no research has focused on bottlenose dolphins within these waters, despite the potential for human impacts on this species. A total of 718h of boat-based observations, spanning 284 days, were spent at sea between August 2008 and July 2010. During this period, 253h were spent in direct observation of 317 groups of common bottlenose dolphins. Bottlenose dolphins were observed in all seasons, although seasonality was evident, with more encounters during the summer. Photo-identification studies show that 43 individuals used the northeastern coast of Tunisia on a regular basis, while others were present less often. Based on a social structure analysis it was possible to discriminate different communities related with the spatial distribution of the sightings (Zembra island, Hammamet, Kelibia and Galite island).

KEYWORDS: BOTTLENOSE DOLPHIN, SURVEY-VESSEL; ABUNDANCE ESTIMATE; PHOTO-ID; SCHOOL SIZE; SITE FIDELITY; MEDITERRANNEAN SEA; NORTHERN HEMISPHERE; STATISTICS

## INTRODUCTION

*Tursiops truncatus* (Montagu 1821), hereafter referred to as the bottlenose dolphin, has been studied intensively in numerous locations around the world and today is one of the most comprehensively studied cetaceans, primarily due to its coastal proximity, interaction with human activities and adaptability in captivity (Reeves *et al.*, 2002). Ranging from tropical to temperate waters, common bottlenose dolphins show extreme diversity in abundance, distribution, and habitat use (Reynolds *et al.*, 2000). Some bottlenose dolphin communities (e.g. in the Azores (Portugal); Silva *et al.,* 2005) clearly fit a resident pattern, while others (e.g. in the northwestern Sardinian coastal waters (Italy); Díaz López *et al.,* 2013) fit a wide-ranging pattern. Mediterranean bottlenose dolphins occur regularly in a number of coastal areas but empirical data on seasonal abundance, site fidelity and residence patterns of these communities are lacking (Bearzi *et al.*, 2008).

Bottlenose dolphins are affected by man's use of coastal waters, particularly by fisheries activities, aquaculture and habitat modification (Fertl and Leatherwood, 1997; Díaz López *et al.,* 2005; Díaz López, 2006a; Díaz López and Shiray, 2007; Bearzi *et al.,* 2008; Díaz López, 2012). In the Mediterranean, numerous studies have documented bottlenose dolphins interacting with small-scale fisheries: Greece (Casale *et al.*, 1999); Spain (Gazo *et al.*, 2001; Brotons & Grau, 2005; Broton *et al.*, 2008; Gonzalvo *et al.*, 2008); Morocco (Zahri *et al*., 2004); Tunisia (Ben Naceur, 2000); Cyprus (Reeves *et al.*, 2001); Italy (Cannas *et al.*, 1994; Quero *et al.*, 2000; Tringali *et al.*, 2004; Lauriano *et al.*, 2004; Díaz López, 2006b; Díaz López, 2012); Corsica (Rocklin *et al.*, 2009).

The Mediterranean bottlenose dolphin 'subpopulation' is 'Vulnerable' according to the International Union for Conservation of Nature (IUCN) Red List criteria[3]. Thus, it is widely believed that numbers of Mediterranean common bottlenose dolphins have declined in recent decades as a consequence of human activities and habitat degradation (Bearzi *et al.,* 2009) and there is a demand for the development and implementation of conservation management and monitoring programmes (Buscaino *et al.,* 2009; Fortuna *et al.,* 2010; Gaspari *et al.,* 2013; Gonzalvo *et al.,* 2013; Rako *et al.,* 2013).

Although the Tunisian State has develop a national strategy and an Action Plan on Biological Diversity (1998) and is a signatory to most of the international and regional conventions on the protection of the marine environment and its biodiversity: CITES (1974); UNESCO Word Heritage (1974); RAMSAR (1979); the Convention to Combat Desertification (1979); the Bonn Convention (1986); the Convention on Biological Diversity (1993); the Berne Convention (1995); Barcelona Convention (1977/1995); and ACCOBAMS (1996) there have been few studies on the species. Studies that have been undertaken have been mainly focused on strandings (Ben Mustapha, 1986; Bradai, 1991; Kartas and Bradai, 1971, 1991; Karaa, 2005) and on interactions with fisheries (Ben Naceur and Mhenni, 1995; Ben Naceur, 2000; M'kacher, 2004; Benmessaoud, 2008). The lack of information about the presence and distribution of this species makes our ability to assess the impact of human activities on Mediterranean common bottlenose dolphins difficult.

[3] *http://www.iucnredlist.org/details/16369383/0.*

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Data presented here represent the first dedicated effort to assess site fidelity, group dynamics and social structure of bottlenose dolphins along the northeastern coastal waters of Tunisia assessed through mark-recapture photographic-identification techniques. Prior to this study, no research has focused on bottlenose dolphins within these waters, despite the potential for human impacts on this species.

## MATERIALS AND METHODS

### Study area

The study area was located along the northeastern coast of Tunisia (Fig. 1). The area extended 12 n. miles offshore covering an approximate sea surface area of 510km$^2$. This coastal strip is the boundary between the western and eastern Mediterranean basins. The study took place at the intersection point between the Sicilian-Tunisian channel to the north and the south of the Gulf of Hammamet. This zone is characterised by a small shelf and a very rugged and steep slope (Azouz, 1973). The hydrological phenomena are complex and seasonal where two thermal regimes can be considered (Winter–Spring and Summer–Autumn periods) (Gaamour *et al.,* 2004).

### Data collection

Boat-based surveys were conducted *ad libitum* between August 2008 and July 2010 on board a fishing vessel, based at the port of Kelibia (36°50'N, 11°04'E). Although the geographic distribution of effort could vary according to weather conditions, an attempt was made to provide an even coverage of the area (up to 250m depth) with at least three surveys per month.

Surveys were considered satisfactory when the visibility was not reduced by rain or fog, and sea conditions were 3 or below on the Douglas sea state scale (Díaz López, 2006a). Surveys were conducted during daylight at a speed lower than 7 knots with at least two experienced observers scanning the sea surface in search of dolphins from two positions with an eye height of 2m and 5m, respectively. To maintain consistent observation effort two teams of two observers each, organised in consecutive two hours shifts, were scanning the sea surface by naked eye.

In order to analyse the seasonality of bottlenose dolphins in the study area, four seasons were defined: winter (January to march); spring (April to June); summer (July to September); and autumn (October to December).

Upon sighting a group of bottlenose dolphins, searching effort ceased, and the vessel slowly manoeuvred to approach the group in order to minimise disturbance. Position (within approximately 10m of the animals), time, depth, composition and the group size were recorded. A 'dolphin group' was defined as one or more bottlenose dolphins observed in the visual area, usually involved in the same activity, following Díaz López (2006a); and an interaction with a dolphin group was termed an 'encounter'. Searching effort stopped at sighting, and restarted when the encounter was finished. The encounter continued until the group was lost, or weather became adverse.

### Group dynamics

Group size was estimated based on the initial count of individuals observed to surface at one time (Merriman *et al.,* 2009). The group size and age categories were assessed visually *in situ*, and the data were later verified with photographs taken during each sighting. Group composition was determined by counting the minimum number of adults, calves and newborns present. Age class definitions followed those adapted by Mann *et al.* (2000) and Díaz López (2006b).

The Kruskal-Wallis test was performed on data to test the equality of medians of several group size samples. If the test shows significant inequality of the medians, a Tukey's *post-hoc* contrast was performed (Zar, 1998).

### Photo-identification

During each encounter effort was made to photograph all members of the group, using a digital SLR camera (*Canon*

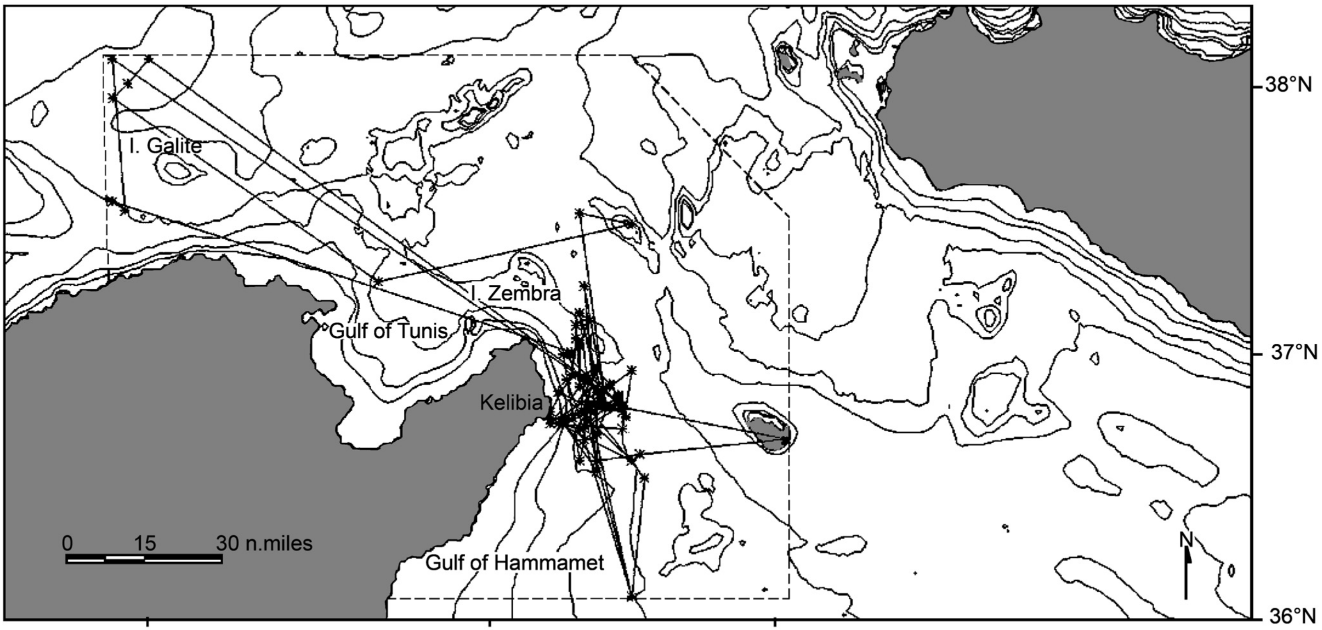

Fig. 1. Study area of the northeast Tunisian survey.

EOS450) equipped with 75–300mm (f: 4–5.6D) zoom lens, in order to determine individual identification based on natural marks on the dorsal fin and surrounding area (Würsig and Jefferson, 1990). To have a representative sampling and to minimise the problem of capture probabilities heterogeneity, attempts were made to photograph the dorsal fins of the majority of dolphins present in a group. At least four pictures were taken for each individual estimated to be in the group (Würsig and Jefferson, 1990).

Only good quality photographs (in focus, with the dorsal fin perpendicular to the plane of the photograph and with the dorsal fin large enough to identify small notches) were used for subsequent analyses (Díaz López and Shirai, 2008). Bad quality photographs or not marked individuals were excluded from the analysis to minimise bias.

Best photographs taken of every dolphin in each encounter were selected and matched with an annual catalogue of identified individuals. If a match was not found, the individual was given a unique identification code (number and letter) and added to the catalogue. Identifications and details relating to group membership, such as sighting location, time, and environmental and anthropogenic conditions were recorded on a database from which individual sighting histories could be reconstructed (Díaz López, 2012).

### Abundance

The two year study period was divided by seasons, resulting in eight sampling periods. Population size was estimated based on all good quality photographs of reliably marked dolphins applying the POPAN model of SOCPROG 2.4 (Whitehead, 2008). ‘Closed’ (Schnabel), ‘mortality’, ‘mortality + trend’, ‘re-immigration’ and ‘re-emmigration’ models were run (Gowans *et al.,* 2000; Whitehead, 1990). The Akaike Information Criterion (AIC; Akaike, 1974) was used to determine the model that best fitted the population for each estimate. Model selection was based on the lowest AIC.

### Site fidelity

To investigate the presence of identified individuals in the study area over time, two temporal sighting rates were calculated; yearly and seasonal. A seasonal occurrence rate was defined as the number of seasons a recognisable dolphin was identified as a proportion of the eight seasons in which at least one bottlenose dolphin was identified. A yearly occurrence rate was defined as the number of calendar years a dolphin was identified as a proportion of the two surveyed years.

Following the methods of Parra *et al.* (2006) and Díaz López (2012) individual dolphins were divided subsequently into three arbitrary categories based on their temporal occurrence rates:

(1) ‘Resident’ category: those bottlenose dolphins seen on the northeastern coast of Tunisia the most often, with both occurrence rates higher (or equal) than 0.5;

(2) ‘Frequent’ category: those bottlenose dolphins with seasonal occurrence rates lower than 0.5 and higher (or equal) than 0.25; and

(3) ‘Sporadic’ category: those bottlenose dolphins seen on the study area seldom, with occurrence rates lower than 0.25.

### Social patterns

Animals photographed in the same group were considered associated. Of those data, only individuals sighted more than three times were considered for the analyses to reduce inaccuracies and biases associated with small sample sizes, and to compare the results with existing studies (Slooten *et al.,* 1993; Quintana-Rizzo and Wells, 2001; Rogers *et al,.* 2004; Gero *et al.,* 2005; Díaz López and Shirai, 2008). Unidentified animals and calves were excluded from the analyses (Díaz López and Shirai, 2008). Calves were excluded because of their unique dependent relationship with their mothers.

The half-weight index (HWI) was used as a measure of association as it accounts best for observer biases inherent in photo-identification techniques (Caims and Schwager, 1987; Bräger *et al*., 1994; Díaz López and Shirai, 2008). This is the most appropriate index as it introduces a bias to correct for missed identifications of one member of a dyad (Caims and Schwager, 1987).

$$\mathrm{HWI} = 2N / (Na + Nb)$$

Where $N$ is the number of encounter that included both dolphins $a$ and $b$, $Na$ is the number of sightings that included dolphin $a$ but not dolphin b, and $Nb$ is the number of sightings that included dolphin $b$ but not dolphin $a$. This index results in values ranging from zero to one, with zero representing two animals never seen together, and one representing two animals never seen apart. The resulting indices were grouped into five association categories: low (<0.20), moderate–low (0.21–0.40), moderate (0.41–0.60), moderate–high (0.61–0.80), and high >0.80 (Wells *et al.,* 1987; Quintana-Rizzo and Wells, 2001).

A hierarchical cluster analysis of associations data was created, which displays the results as a dendrogram. The individuals are arranged on one axis and their degree of association on the other, in order to examine relationships between all dolphins photo-indentified. The cluster was made using complete average linkage (cophenetic correlation coefficient = 0.90).

A permutation test, as in Bejder *et al.* (1998), was used (with modifications as in Whitehead *et al.,* 2005), to test for non-random associations for all data against the null hypotheses that dolphins associate randomly with one another. If some individuals preferentially associate with other individuals (indicating non-random associations), then the Standard Deviation (SD) of the real association indices will be significantly higher than the SD calculated in the random data (Whitehead, 1999; Christal and Whitehead, 2001). Associations were permuted within daily sampling intervals to remove possible demographic effects (i.e. mortality, recruitment or migration to or from the study area; Whitehead, 1999). The calculations of the HWI, clusters analysis and permutation tests were carried out using the compiled version of SOCPROG 2.4 (Whitehead, 2008).

Table 1

Field effort for period study (2008–10).

| Seasons | Winter | Spring | Summer | Autumn | Total |
|---|---|---|---|---|---|
| Days at sea | 66 | 76 | 88 | 54 | 284 |
| Days with sightings | 23 | 13 | 28 | 17 | 81 |
| Sightings per seasons | 35 | 22 | 72 | 35 | 164 |
| No. of individuals photo-id'd | 39 | 31 | 42 | 19 | |

## RESULTS

### Survey effort and sighting rate

In all, 284 days and 718h were spent at sea in satisfactory conditions (Table 1). On average, about 6.5 hours (1.38h per day) were spent conducting observations. During 81 days, 253h were spent in direct observation of 317 groups of common bottlenose dolphins (mean sighting duration = 12.57 ± 0.30min, SD = 5.36min).

### Group dynamics

Group dynamics were examined for 317 independent groups. Group size ranged from 1 to 19 individuals (mean = 5.17 ± 0.16; SD = 2.89; median = 5) (Fig. 2). Group composition revealed 69.77% ($n$ = 221.17) of the individuals encountered in groups were deemed adults, 30.23% ($n$ = 95.83) immature dolphins (calves and/or newborns). Moreover, 12.8% ($n$ = 40.57) were solitary animals, 57.95% ($n$ = 183.70) were groups with immature dolphins (calves and/or newborns) and 30.05% ($n$ = 96.68) groups with only adults. The results revealed no differences in the group size during the two years of research (Kruskal-Wallis test, KW-H (1,317) = 1.77; $p > 0.05$). In contrast, group size showed seasonal fluctuations (Kruskal-Wallis test, KW-H (12,317) = 19.38; $p < 0.05$), with bigger groups during the summer season. Group sizes between groups with immature dolphins and groups formed only by adults were significantly different (5.90 ± 2.88 groups containing immatures vs. 3.46 ± 2.03 groups containing adults only; Kruskal-Wallis test, $p < 0.05$).

### Photo-identification and site fidelity

Only 43 individuals were mark-recaptured using photo-identification techniques from 317 independent dolphin groups encountered during the study period. Eleven of those dolphins (25.58%) were positively identified as females based on strong association and synchronised surfacing with a calf or observation of genital and mammary slits.

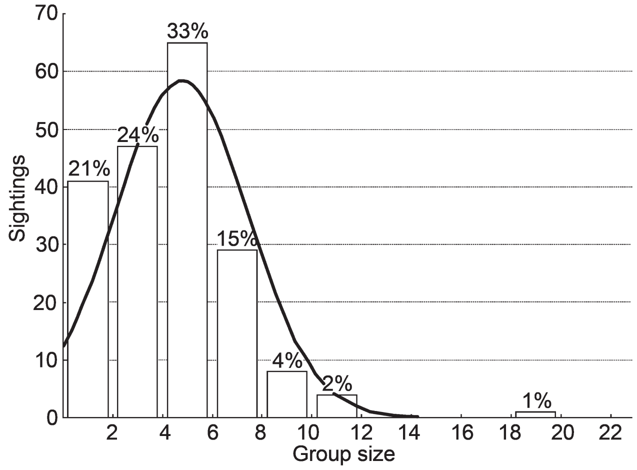

Fig. 2. Histogram of the group size frequency of distribution.

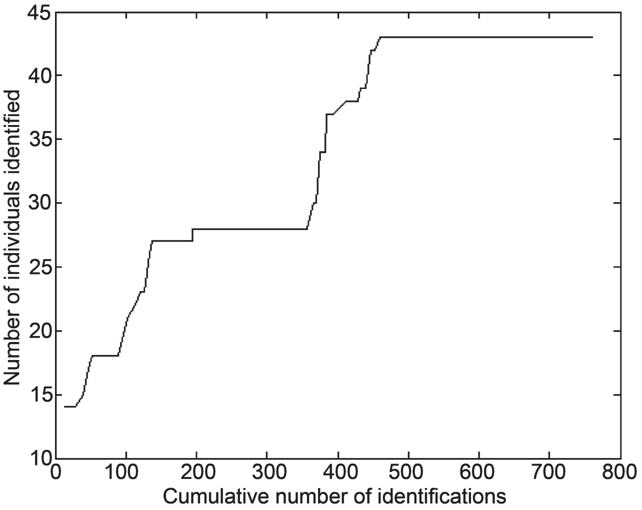

Fig. 3. Discovery curve showing number of mark-recaptured bottlenose dolphins ($n$ = 43) identified between 2008 and 2010.

The cumulative number of identified individuals generally became less steep with time. A flattening of the curve could be interpreted as the catalogue progressively reaching its maximum number of individuals and that probably all individuals present in the study area have been identified (Fig. 3). The average number of photographic recaptures per individual was 31.42 (SD = 11.36).

It is of note that 17 common bottlenose dolphins, accounting for 39.53% of all identified individuals, were identified more than 20 times throughout the study period. However, nine common bottlenose dolphins (20.93%) were identified only once throughout the study period. This shows that some individuals used the northeastern coast of Tunisia on a regular basis, whilst others were present less often (Table 2). Relative to the total number of seasons surveyed, most common bottlenose dolphins identified were sighted occasionally (0.65 ± 0.3 resightings per season) with a peak presence in summer and a minimum in spring ($\chi^2$, $p < 0.05$). Dolphins photo-identified in study area were divided subsequently into three arbitrary categories based on their temporal occurrence rates:

(1) 'Resident' category: this category contained 22 identified adult bottlenose dolphins, accounting for 51.1% for all 43 identified individuals;

(2) 'Frequent' category: this category contained 7 identified bottlenose dolphins, accounting for 16.3% for all 43 identified individuals; and

(3) 'Sporadic' category: this category contained 14 bottlenose dolphins, accounting for 32.6% for all 43 identified individuals.

### Abundance

Table 3 show the abundance estimate for bottlenose dolphins seen in study area. Based on the lowest AIC value (AIC = 214.01), the 'mortality model' seems to be the most adequate model which describes our population. This model describes those associations in which this model assumes a population of constant size, where mortality is balanced by

Table 2

Summary of occurrence pattern of photo-IDed bottlenose dolphins during the research period. The grey coloured cell indicates presence of animals.

| Indiv./ months | Winter 2008–09 | | | Spring 2008–09 | | | Summer 2008–09 | | | Autumn 2008–09 | | | Total sightings 2008–09 | Winter 2009–10 | | | Spring 2009–10 | | | Summer 2009–10 | | | Autumn 2009–10 | | | Total sightings 2009–10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | J | F | M | A | M | J | J | A | S | O | N | D | | J | F | M | A | M | J | J | A | S | O | N | D | |
| RK1 | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 37 | ■ | ■ | | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 28 |
| RK2 | ■ | | ■ | ■ | ■ | ■ | ■ | | ■ | | ■ | ■ | 14 | | ■ | | ■ | | ■ | | | ■ | | | ■ | 6 |
| RK3 | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 41 | ■ | ■ | | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 27 |
| RK4 | ■ | | ■ | ■ | ■ | | ■ | ■ | ■ | | ■ | | 15 | ■ | ■ | | ■ | | ■ | ■ | | ■ | | | ■ | 9 |
| RK5 | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 36 | ■ | ■ | | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | | 26 |
| RK6 | ■ | ■ | ■ | ■ | | ■ | ■ | ■ | ■ | | ■ | | 28 | ■ | ■ | | ■ | | ■ | ■ | ■ | | ■ | ■ | | 17 |
| RK7 | | ■ | ■ | ■ | | ■ | ■ | | ■ | ■ | ■ | ■ | 22 | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | ■ | 22 |
| RK8 | ■ | ■ | ■ | | | ■ | | ■ | ■ | | ■ | ■ | 31 | ■ | | | ■ | | ■ | ■ | ■ | ■ | ■ | | | 17 |
| RK9 | ■ | ■ | ■ | | | | ■ | ■ | ■ | ■ | ■ | ■ | 24 | ■ | | | ■ | | ■ | ■ | ■ | ■ | ■ | | | 19 |
| RK10 | ■ | | ■ | | | | | ■ | ■ | ■ | ■ | ■ | 21 | | | | | | ■ | ■ | | ■ | | | | 9 |
| RK11 | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 38 | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | 31 |
| RK12 | ■ | ■ | ■ | | | | | ■ | ■ | | | | 8 | ■ | | | | ■ | ■ | ■ | ■ | ■ | ■ | | ■ | 21 |
| RK13 | ■ | ■ | ■ | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 35 | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | ■ | 24 |
| RK14 | ■ | ■ | ■ | ■ | | ■ | ■ | | ■ | ■ | ■ | ■ | 15 | ■ | | | ■ | | ■ | ■ | ■ | ■ | ■ | | | 17 |
| RK15 | ■ | ■ | | | | ■ | ■ | | ■ | ■ | ■ | ■ | 12 | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | 17 |
| RK16 | ■ | ■ | | | | | ■ | | ■ | | ■ | ■ | 14 | ■ | ■ | | | | ■ | ■ | ■ | ■ | ■ | | | 11 |
| RK17 | | ■ | ■ | ■ | | | ■ | ■ | ■ | ■ | | ■ | 21 | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | 21 |
| RK18 | | | | ■ | ■ | | | | ■ | ■ | | | 11 | ■ | ■ | | | | | ■ | ■ | | ■ | | | 7 |
| RK19 | | | | | ■ | | | | | | | | 1 | ■ | | | ■ | ■ | ■ | ■ | | | | ■ | | 14 |
| RK20 | | | | | | | | | | | | | 0 | ■ | ■ | | ■ | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | 21 |
| RK21 | | | | | | | | | | | | | 0 | ■ | ■ | | | | ■ | ■ | ■ | ■ | ■ | | | 17 |
| RK22 | | | | | | | | | | | | | 0 | ■ | ■ | | ■ | ■ | ■ | ■ | ■ | | | ■ | ■ | 20 |
| RH1 | | | | | ■ | | ■ | | ■ | ■ | | | 6 | ■ | | | | | | ■ | | ■ | ■ | | ■ | 12 |
| RH2 | | | | | ■ | | ■ | | | | | | 3 | ■ | | | ■ | | | ■ | | | | | | 6 |
| RH3 | | | | | ■ | | ■ | | ■ | | | | 4 | ■ | | | | | ■ | ■ | ■ | ■ | ■ | | | 10 |
| RH4 | | | | | ■ | | ■ | | | ■ | | | 3 | ■ | | | ■ | | ■ | ■ | ■ | ■ | ■ | | | 12 |
| RH5 | | | | | ■ | | | | ■ | | | | 3 | ■ | | | | | ■ | ■ | | ■ | | | | 12 |
| RH6 | | | | | | | ■ | | ■ | ■ | | | 4 | ■ | | | ■ | | ■ | ■ | ■ | ■ | | | | 13 |
| RH7 | | | | | | | | | | | | | 0 | ■ | | | ■ | | ■ | ■ | | ■ | | | | 9 |
| RZ1 | ■ | | | | | ■ | | | | | | | 2 | ■ | | ■ | | ■ | | ■ | | ■ | | | | 8 |
| RZ2 | | | | | | ■ | | | | | | | 1 | ■ | | ■ | | | | ■ | | | | | | 6 |
| RZ3 | | | | | | | ■ | | ■ | | ■ | | 4 | ■ | | ■ | | | | ■ | | ■ | | | | 6 |
| RZ4 | | | | | ■ | | | | | | | | 1 | | | ■ | | | | ■ | | ■ | | | | 7 |
| RZ5 | | | | | | | | | | | | | 0 | | | ■ | | ■ | | ■ | | ■ | | | | 9 |
| RZ6 | | | | | | | | | | | | | 0 | | | | | | | | | | | | | 0 |
| RZ7 | | | | | | | | | | | | | 0 | | | | | | | | | | | | | 0 |
| RG1 | | | | | ■ | | | | | | | | 1 | ■ | | ■ | | | | | | | ■ | | | 3 |
| RG2 | | | | | | | | | | | | | 0 | ■ | | ■ | | | | | | | ■ | | | 3 |
| RG3 | | | | | ■ | | | | | | | | 1 | ■ | | ■ | | | | | | | ■ | | | 3 |
| RG4 | | | | | | | | | | | | | 0 | | | | | | | | | | | | | 0 |
| RG5 | | | | | | | | | | | | | 0 | | | | | | | | | | | | | 0 |
| RG6 | | | | | | | | | | | | | 0 | | | | | | | | | | | | | 0 |
| RG7 | | | | | | | | | | | | | 0 | | | | | | | | | | | | | 0 |

birth. The estimate of population size is 42 individuals (95% CI = 42.0–43.1) with an annual estimated mortality rate of 0.0053.

### Association pattern

The association index for 34 dolphins seen three or more times was examined. The half weight index (HWI) has been selected. Calculated HWIs ranged from 0 to 1. Mean HWIs were found to range from 0.00 to 0.92. All individuals were associated on average 0.28 ± 0.009 times.

The majority of dolphins have low-level associations. For the 820 identifications, the most frequently occurring levels were low level (45%, $n = 316$). However, some identified individuals seemed to form relatively stable groups over the study period with a few dyads that have high and moderate high level associations. For the 820 identifications, only 3% ($n = 22$) have a moderate high-level association and 6% ($n = 43$) have high-level associations (Fig. 4).

The agglomeration plot of hierarchical clustering showed a linear increase in the number of clusters with a slight increase in the rate of agglomeration (Fig. 4). Based on the structure of the dendrogram three different communities related to the spatial distribution of the sightings can be discriminated. The first group of dolphins is present in the north of the study area (Zembra Island). The second in the north-west of the study area (Galite Island) and the third in Kelibia waters (central portion of study area).

Significantly higher associations were identified as the standard deviations of observed association indexes were significant larger than for the randomly permuted data ($p < 0.05$). Association datasets were randomly permuted 10,000 times with 1,000 trials per permutation. The results of the 'permute all groups' test showed that the coefficient of variation of real association was 0.83 which is higher than the coefficient of variation of random association

Table 3

Abundance estimates of marked bottlenose dolphins.

Notations: *N* = estimated population size; CI = 95% confidence interval; N/A = not available; *m* = estimated mortality rate; *t* = estimated trend rate; *e* = estimated emigration rate; *re* = estimated re-immigration rate; *Nc* = number of animals captured; s.p. = number of sampling periods; AIC = Akaike Information Criterion; LogL: log likehood.

| | Estimates | | | | | | Model selection | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Models | *N* | CI | *m* (CI) | *t* (CI) | *e* (CI) | *re* (CI) | *Nc* | s.p. | LogL | AIC |
| Mortality | 42.00 | 42.0–43.1 | 0.00538 (0,003–0,0244) | N/A | N/A | N/A | 43 | 8 | –105.00 | 214.01 |
| Re-immigration | 41.00 | N/A | N/A | N/A | 0.005 | 0.004 | 43 | 8 | –104.54 | 215.08 |
| Mortality trend | 41.85 | 41.9–43.4 | 0.00535 (0.0000–0.0349) | 0.00174 (–0.0089–0.1510) | N/A | N/A | 43 | 8 | –104.70 | 215.41 |
| Closed (Schnabel) | 42.49 | 42.0–44.0 | N/A | N/A | N/A | N/A | 43 | 8 | –106.86 | 215.73 |
| Re-immigration + mortality | 41.32 | N/A | 0.00548 | N/A | 1.05e–009 | 0.020 | 43 | 8 | –104.54 | 217.08 |

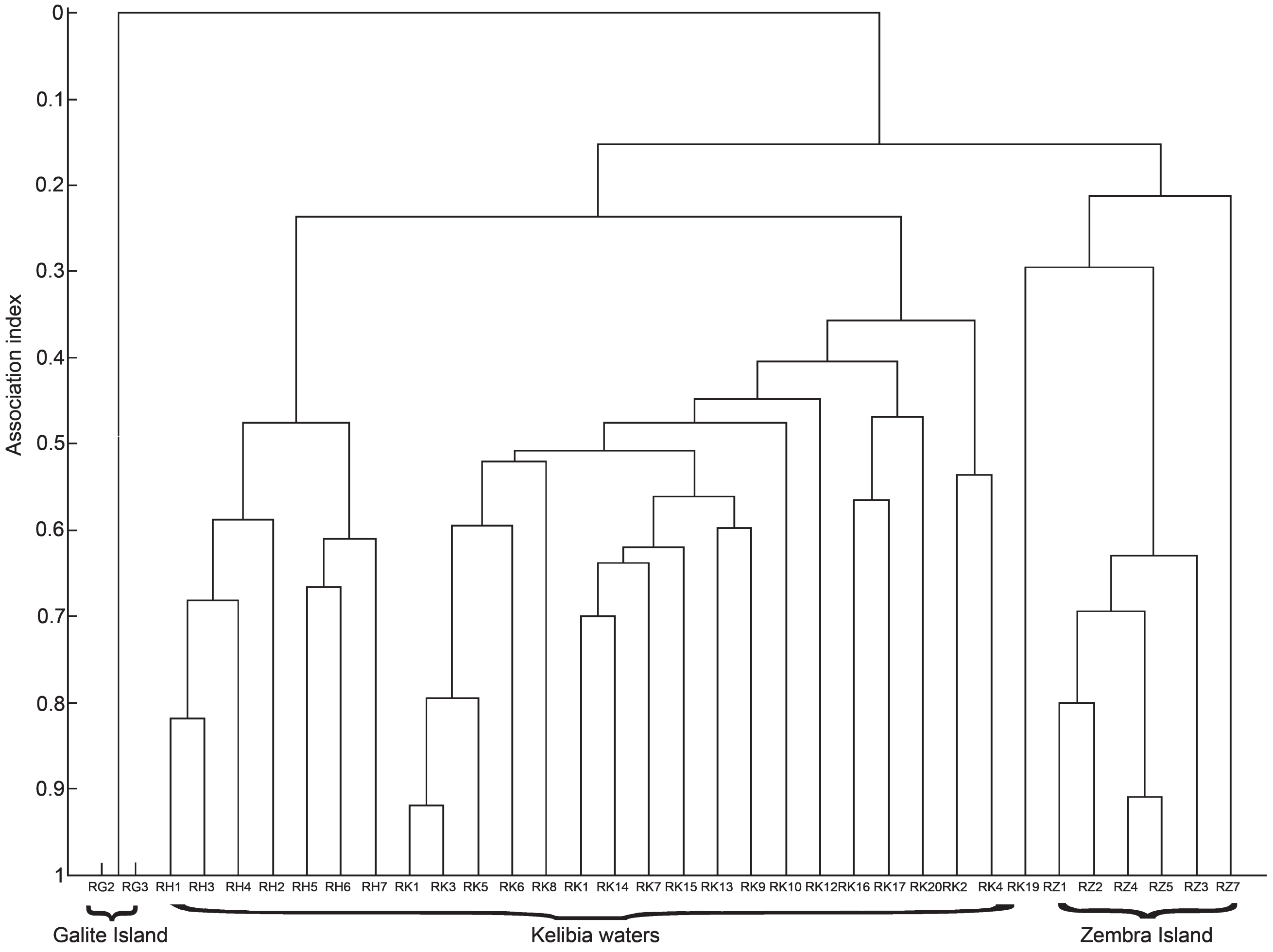

Fig. 4. Dendrogram showing the average-linkage cluster analysis of associations between well-marked individual bottlenose dolphins seen ≥ 3 times in the Kelibia waters, from 2008–10.

(CV = 0.57). Therefore dolphins seen in the same study area in the same sampling period are not likely to group together at random, but show preferred/avoided companionships.

Overall associations between dolphins can be seen in a sociogram (Fig. 5) where points representing the individuals are arranged around the circle and the thickness of lines between the points indicates the strength of their relationship. They are clearly not random, based on the relative absence and asymmetry of linkages through the axes of the sociogram.

## DISCUSSION

This study reveals important information on the occurrence of bottlenose dolphins in northeastern Tunisian waters. Bottlenose dolphins are found year round in this area, which is likely to represent an important part of their habitat rather than simply a corridor between other key areas.

The fact that bottlenose dolphins were the most frequently encountered cetacean species during the surveys was consistent with previous studies conducted in Tunisian waters (Benmessaoud, 2009; Benmessaoud *et al.,* 2013; Ben Naceur, 2004; Karaa *et al.,* 2011; Zanardelli, 2002).

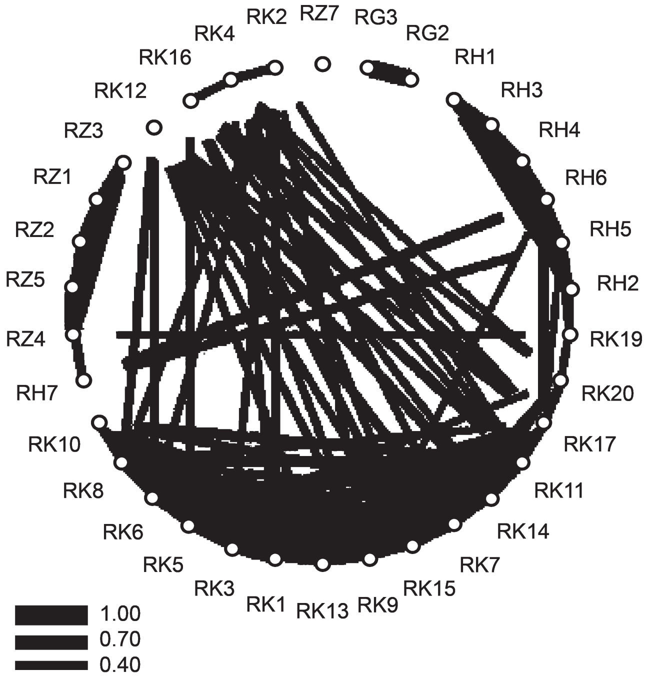

Fig. 5. Sociogram showing associations between all individuals.

Several individuals ($n$ = 29) were resighted on repeated occasions, both within two years, suggesting strong site-fidelity for a significant part of the population. Similar observations were reported in other Mediterranean populations by Pearson (2002), Fortuna (2006), Díaz López and Shirai (2008) and Díaz López (2012). There appear to be both residents and non-residents (both sporadic and occasional visitors) present in community. However, Bearzi *et al.* (2010) documented that bottlenose dolphins in Greek coastal waters that appear to be resident within a given area can temporarily leave 'home' and range over large portions of sea. Likewise, it is clear that the survey area did not encompass the whole range of this regional population. The initial increase in the discovery rate of new individuals during 2008 (Fig. 2) is an attribute of the beginning of the study. However, the alternating increases and plateaus in the discovery curve later in the study could suggest a regular influx of new individuals to the study area and/or unrecognisable animals due to acquired new marks as our study progressed. Nevertheless, other communities of bottlenose dolphins are known to occur in adjacent habitats, and a high number of 'occasional' and 'sporadic' individuals are intermittently observed within the community's core range area. Although bottlenose dolphins were never absent from the study area, site fidelity and residence patterns of identified individuals suggest that there are different degrees of residency among the dolphins using the study area.

Using social structure analysis it was possible to discriminate different communities based on the spatial distribution of the sightings (Fig. 4). Bottlenose dolphin social structure varies drastically, from fission-fusion where individuals make choices to join or leave a group (Díaz López and Shirai, 2008) to being mainly driven by constant companionship (Lusseau *et al.,* 2003). The fission-fusion structure has also been observed along the northeastern coast of Tunisia. Bottlenose dolphins identified in the study area have shown an elasticity of social interaction, where some individuals have a particular affinity for one or more companions, whereas others mutually avoid each other.

Similar group sizes observed here were reported for other areas along the Mediterranean Basin, where groups rarely contained more than ten individuals (Bearzi *et al.,* 1997; Bearzi *et al.,* 2008; Díaz López, 2006a; Díaz López and Shirai, 2007, 2008; Lauriano *et al.,* 2003). Groups with calves were larger than non-calf groups, which according to Norris and Dohl (1980) might be due to females favouring schools larger than those optimal for foraging to provide alloparental care and protection for their young.

The abundance estimate of 42 bottlenose dolphins occurring in the northeastern Tunisian waters represents an approximation of dolphins occurring within these coasts. The results reveal that this population is not closed, and it is affected by changes of density due to the influence of input (immigration and emigration) and/or output (births and deaths). The estimate of the total abundance of bottlenose dolphins in the study area varied among years (Benmessaoud pers. comm.). Based on the lowest AIC value, the 'mortality model' was also selected as the most parsimonious model of bottlenose dolphin population off the northwestern Sardinian coast (Díaz López *et al.,* 2013). The population size there was estimated as 54.8 (95% CI = 44.8–69.5). According to Bearzi *et al.* (2008), the population estimate of bottlenose dolphin inhabiting the semi-closed eutrophic Amvrakikos Gulf, Greece, was 148 individuals (95% CI = 132–180). Several models can be used to describe the size of the bottlenose dolphin population in the same area. Various factors can influence the residence of the species in the same area such as prey availability.

The presence of different communities, with different degrees of site-fidelity, along the northeastern Tunisian coast indicates that the impacts associated with coastal fisheries on bottlenose dolphins could be different between individuals of the same regional population. Consequently, degradation and loss of coastal habitats in a study area can lead to an increase in distance among habitable patches and/or reduction in number of remnant habitats. Likewise bottlenose dolphins present in the study area inhabit an environment greatly affected by human activities including intensive fishing, maritime traffic and tourism. These pressures, particularly fisheries bycatch, may have a strong, adverse impact on population viability and need to be carefully assessed and managed at scales that are consistent with the population structure of bottlenose dolphins (Fortuna *et al.,* 2010; Gaspari *et al.,* 2013).

This study sheds light on how the members of this dolphin community are using the waters of the study area year-round. In order to have an effective conservation of bottlenose dolphin populations, a better understanding of the complexity of ecological, behavioural and social patterns is required in order to facilitate development of management plans. Population management actions should also consider how the impact of human activities differs across geographically distinct areas (Gaspari *et al.,* 2013).

The establishment of protected areas is one important aspect of a coastal zone management plan. These Marine Protected Areas are intended to protect rare, endangered or vulnerable habitats and species and to afford individuals widely differing levels of protection (Wilson *et al.,* 1997). In this context, Tunisia gives priority to environmental protection and sustainable management of natural resources. This includes measures to strengthen the regulation of fisheries, the protection of the marine environment, the prohibition of monk seal, cetacean and sea turtle capture in its territorial waters as well as their trade and captivity. With respect to Marine Protected Areas, we encourage the consideration of a new marine protected area in the Sicilian-Tunisian channel where cetacean diversity is important. We also encourage research on population genetics to assess if habitat diversity plays a significant role in shaping the genetic structure of bottlenose dolphins.

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# Seasonal movements and ecological markers as evidence for migration of common minke whales photo-identified in the eastern North Pacific

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ABSTRACT

In the eastern North Pacific Ocean, common minke whales (*Balaenoptera acutorostrata*) are widespread but encountered relatively infrequently. It is generally believed that they make annual migrations between higher latitudes in the summer and lower latitudes in the winter; however, in some temperate coastal regions where common minke whales have been sighted year-round they have been referred to as resident. To determine movement patterns of common minke whales found in coastal waters of British Columbia and Washington we examined photo-identification data that were collected opportunistically from 2005–12. These data were from four non-overlapping areas between 48°N and 53°N. Despite year-round search efforts, common minke whales were only encountered between April and October. Most of the 44 unique individuals identified in 405 encounters displayed fidelity to areas both within and among years. Five of these whales made relatively large-scale intra-annual movements between areas on six occasions. They were documented to move up to 424km in a northerly direction in spring and up to 398km in a southerly direction in autumn. The seasonal patterns of these movements provide new insights into the foraging ranges and migrations of the individuals. Ecological markers provide evidence that the common minke whales photographed undertake annual long distance migrations. Scars believed to be from cookiecutter shark (*Isistius brasiliensis*) bites were observed on 43 individuals and the majority of whales documented with good quality images each year had acquired new scars since the previous year. Furthermore, the commensal barnacle *Xenobalanus globicipitis* was observed on three individuals. Since these sharks and barnacles are from relatively warm waters, it can be inferred that they interacted with the common minke whales at lower latitudes. These findings may have important implications for the definition and management of common minke whale stocks and/or populations in the eastern North Pacific.

KEYWORDS: MOVEMENTS; MIGRATION; PHOTO-ID; NORTH PACIFIC; ECOLOGICAL MARKERS; FEEDING GROUNDS; SITE FIDELITY; COMMON MINKE WHALE; NORTHERN HEMISPHERE

## INTRODUCTION

The common minke whale (*Balaenoptera acutorostrata*) is found in all the world's oceans (Jefferson *et al.*, 2008). It is the smallest of all rorquals and among baleen whales, is the second smallest species (Stewart and Leatherwood, 1985). Due to its small size and cryptic lifestyle, the common minke whale can be relatively difficult to observe and to study (Martin *et al.*, 2013). Consequently, little is known about the structure of some common minke whale populations.

In the North Pacific, several populations of common minke whale exist. The IWC Scientific Committee has undertaken a thorough review of the stock structure of common minke whales in the western North Pacific (IWC, 2012; 2013; 2014). One population referred to as 'J stock' is exploited in the Sea of Japan, Yellow Sea, South China Sea and coastal waters east of Japan. This population differs both genetically and morphologically from 'O stock' minke whales primarily found in the Sea of Ohkotsk and the western North Pacific (Kato, 1992; Kato *et al.*, 1992; Ohsumi, 1983; Omura and Sakiura, 1956; Park *et al.*, 2010; Wade and Baker, 2010; Wade *et al.*, 2010). There may be other biologically distinct populations in the central and eastern North Pacific where differences in the structure of common minke whale calls have been recorded (Delarue *et al.*, 2012; Rankin and Barlow, 2005). However, common minke whales east of 180°W in the North Pacific have been collectively referred to as the remainder stock because of the lack of commercial exploitation in these waters (Donovan, 1991). For further regional management purposes the United States National Oceanic and Atmospheric Administration (NOAA) has split the remainder stock into the California/Oregon/Washington (CA/OR/WA) stock, the Hawaiian stock and the Alaskan stock.

Ship based line transect cetacean surveys in these regions have shown that common minke whales are relatively common in the central North Pacific around Alaska (Moore *et al.*, 2002; Zerbini *et al.*, 2006), uncommon in the eastern North Pacific (Barlow and Forney, 2007; Carretta *et al.*, 2013; Ford *et al.*, 2010; Williams and Thomas, 2007) and rarely observed around Hawaii (Barlow, 2006). The only research that is specifically focused on this species in these waters has been undertaken either acoustically or in coastal areas where waters favoured by common minke whales are easily accessed. Most of these studies have shown seasonal trends in common minke whale detections. For example, year-round acoustic studies have detected common minke whale calls in deep water off Hawaii from October to May, with peaks in February and March (Oswald *et al.*, 2011; Thompson and Friedl, 1982). These whales have rarely been seen so assessment of behaviour patterns has not been

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possible, but it has been suggested that the calls were related to breeding (Martin *et al.*, 2013). Further north, in the coastal waters of California, Washington and British Columbia, photo-identification studies conducted between April and October found that common minke whales were most abundant from June to September and that individuals showed fidelity to specific feeding areas within and among years (Dorsey, 1983; Dorsey *et al.*, 1990; Hoelzel *et al.*, 1989; Osborne *et al.*, 1988; Stern *et al.*, 1990).

It is generally assumed that common minke whales in the North Pacific migrate to higher latitudes in the spring to feed in cold waters during the summer and to lower latitudes in the autumn to breed in warm waters during the winter (Stewart and Leatherwood, 1985). This is consistent with seasonal trends in common minke whale detections in the eastern North Pacific and with what is known about this species in the western and central North Pacific (Delarue *et al.*, 2012; Gong, 1988; Hatanaka and Miyashita, 1997; Omura and Sakiura, 1956). However, common minke whales have been observed in the eastern North Pacific year-round (COSEWIC, 2006; Dohl *et al.*, 1983; Everitt *et al.*, 1979; Forney *et al.*, 1995; Shelden *et al.*, 2000). Consequently, the putative CA/OR/WA stock is considered to be resident and behaviourally distinct from migratory common minke whales (Allen and Angliss, 2012; Carretta *et al.*, 2013). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has reported that common minke whales in the Pacific waters of Canada are thought to be migratory, but that this region also may include a small resident population that is an extension of the CA/OR/WA stock (COSEWIC, 2006). Stewart and Leatherwood (1985), Leatherwood *et al.* (1988) and Nagorsen (1990) state that common minke whales of the eastern North Pacific move to higher latitudes during summer and lower latitudes during winter and that some populations may also be resident.

Although some common minke whales in the eastern North Pacific may indeed be migratory while others may be resident in certain areas year-round, discrepancies in the literature indicate that further study is required to understand the movement patterns of these whales. Photo-identification can be used to document movements of individual whales (Goley and Straley, 1994; Robbins *et al.*, 2011; Weller *et al.*, 2012). It is also a useful method for documenting ecological markers on their bodies including scars from bites of cookiecutter sharks (*Isistius brasiliensis*) (Bando *et al.*, 2010; Dwyer and Visser, 2011) and barnacles such as *Xenobalanus globicipitis* (Kane *et al.*, 2008). Both of these species are known to be primarily from warm waters (Kane *et al.*, 2008; Nakano and Tabuchi, 1990) and can therefore provide evidence of the movements of whales from such regions when they are documented at higher latitudes (Bushuev, 1990; Mackintosh and Wheeler, 1929; Olafsdóttir and Shinn, 2013; Shevchenko, 1977).

To determine movement patterns of common minke whales from the eastern North Pacific this study examined photo-identification data collected in four temperate coastal areas of British Columbia and Washington between 48˚N and 53˚N. All whales were encountered from spring to autumn and some individuals were documented to make seasonally-based intra-annual movements between areas. These relatively large-scale movements provide insights into seasonally preferred foraging areas and migrations of individuals. Ecological markers observed on almost all whales identified suggest that animals from these waters make annual migrations to lower latitudes. These results may provide useful information for managing stocks and/or populations of common minke whales in the eastern North Pacific.

## METHODS

Common minke whales were photo-identified opportunistically from 2005–12 using vessels primarily focused on the commercial viewing or research of killer whales (*Orcinus orca*), humpback whales (*Megaptera novaeangliae*), gray whales (*Eschrichtius robustus*) and fin whales (*Balaenoptera physalus*). Some cetacean research and commercial whalewatching vessels operated year-round, but the majority of effort occurred from spring to autumn. Whalewatching vessels operated in coastal waters off Vancouver Island, whereas research vessels operated around Vancouver Island and off the central coast of British Columbia. Vessels varied, but most were less than 10m in length with cruising speeds of at least 20 knots. Common minke whales were detected by sight, sound and smell and photo-identified in Beaufort sea-states ≤ 2. When common minke whales were located, they were approached to the minimum allowable distance (20m under research permit or 100m for whalewatching vessels) to obtain identification photographs from both the right and left sides. On occasion, common minke whales were also detected from land and then photo-identified if the animal was close enough to the shoreline. Identification photos were acquired using digital SLR cameras equipped with lenses ranging in focal length from 70–400mm.

All photographic data of common minke whales were managed and analysed using protocols similar to those used for killer whale data in British Columbia as outlined by Towers *et al.* (2012b). An encounter was defined as an event in which identification photographs were obtained from one common minke whale. Data recorded for each encounter included: the date, individual identity, best left and right side photos acquired, photo quality rating and the location. The location of each common minke whale encounter was recorded with a charted geographical place-name, the latitude and longitude and the area in which the encounter took place: NVI (Northern Vancouver Island), SVI (Southern Vancouver Island), CBC (Central British Columbia) or WVI (Western Vancouver Island). All areas were entirely within Canadian waters off the coast of British Columbia with the exception of the SVI area, which also included US waters off the coast of Washington (Fig. 1). The best identification photos from each encounter were assessed for quality and rated as poor, fair or good. Identification photographs were considered to be good when they were in sharp focus, taken from relatively close range, in good lighting conditions and perpendicular to the direction the animal was traveling. Fair quality images were those that fit two or three of these criteria, while poor quality images were those that only fit one or none at all. Positive identifications were based on the distinctiveness of identifying features on an individual and how well those features were photographed. All common minke whales included in the analysis were known from

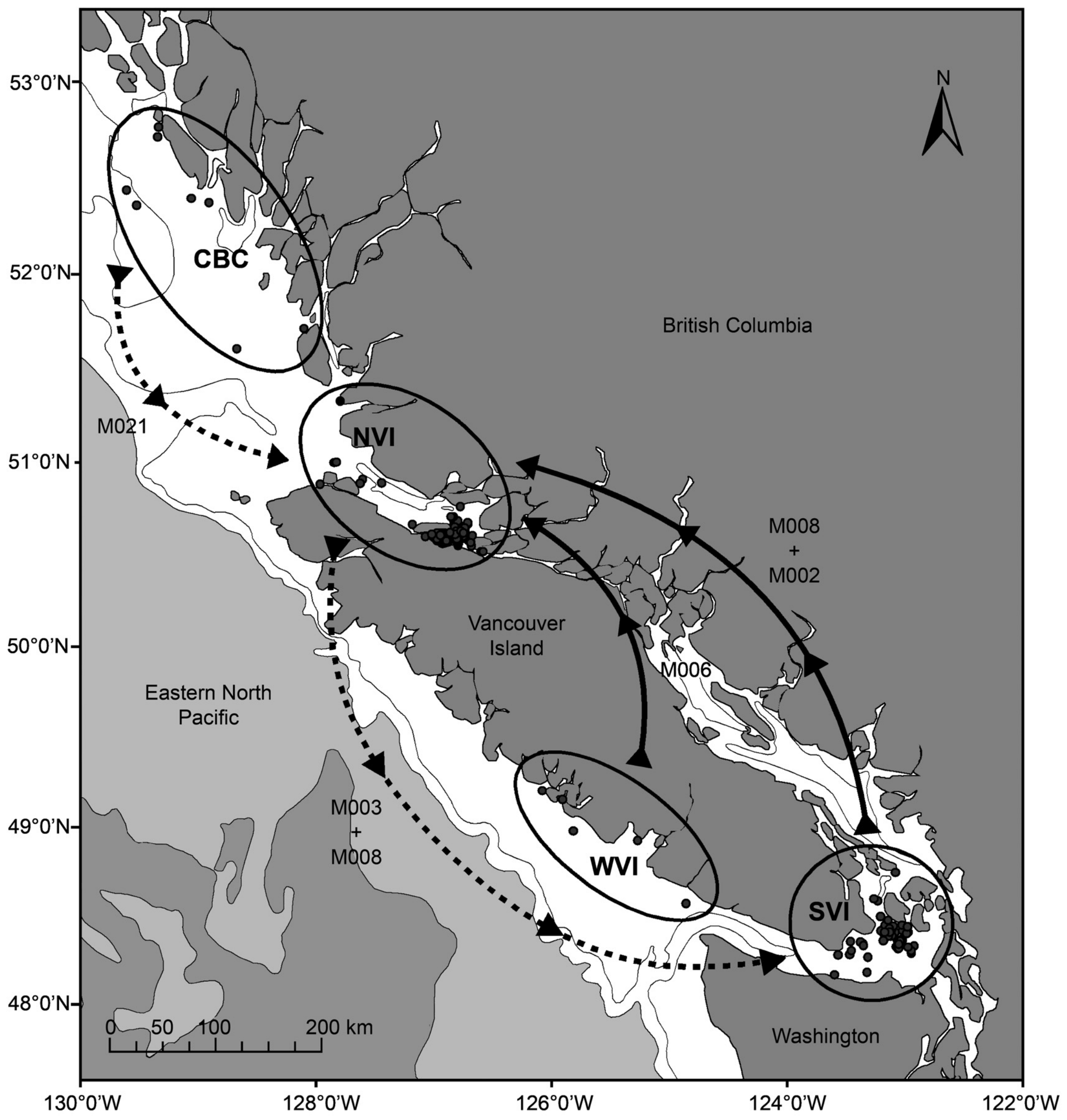

Fig. 1. Locations of common minke whale encounters (solid black circles) in four defined areas and seasonally-based intra-annual movements of five individuals between areas. Solid lines represent northerly movements made between spring and summer. Dotted lines represent southerly movements made between summer and autumn. (Lines and arrows indicate general direction of travel rather than actual travel routes).

good quality identification photos and only encounters of positively identified whales were used for analysis in this paper.

All photographs of the identified common minke whales were scrutinised for ecological markers. Body scars were considered to be from cookiecutter sharks when they were an estimated 3–7cm in size, circular, elliptical or crescent shaped (Jones, 1971) and matched the appearance of scars on other cetaceans attributed to cookiecutter sharks (Bando *et al.*, 2010; Bertulli *et al.*, 2012; Jefferson *et al.*, 2008; Miyashita *et al.*, 2010; Moore *et al.*, 2003; Shevchenko, 1977; Walker and Hanson, 1999). Scars were considered to have occurred within the year if the epidermis was white in colour (Shevchenko, 1977) or if the same individual was seen during the previous year without the scar. As the whale barnacle *Xenobalanus globicipitis* is known to colonise trailing edges of cetacean appendages (Kane *et al.*, 2008), dorsal fins of individual common minke whales were examined for this epizoic crustacean. These ecological markers aided in the identification of individuals, but because they sometimes changed in appearance over the course of a year, they could not always be used as identification features over longer periods of time.

To aid in describing seasonal trends in minke whale distribution, April to June 15 was defined as spring, June 16 to the end of August as summer and September to November 15 as autumn. Intra-annual movements of individual common minke whales between these periods were measured as the minimum by-sea travel distances between two encounter locations. The routes taken by common minke whales were likely to be less direct and/or altogether different.

## RESULTS

In total, 6,990 identification photographs of common minke whales were obtained in 405 encounters. The best images were of good, fair and poor quality in 239, 111 and 55 encounters respectively. Encounters took place in all four of the defined areas (Fig. 1) between April and October. There was a steady increase in the numbers of encounters each

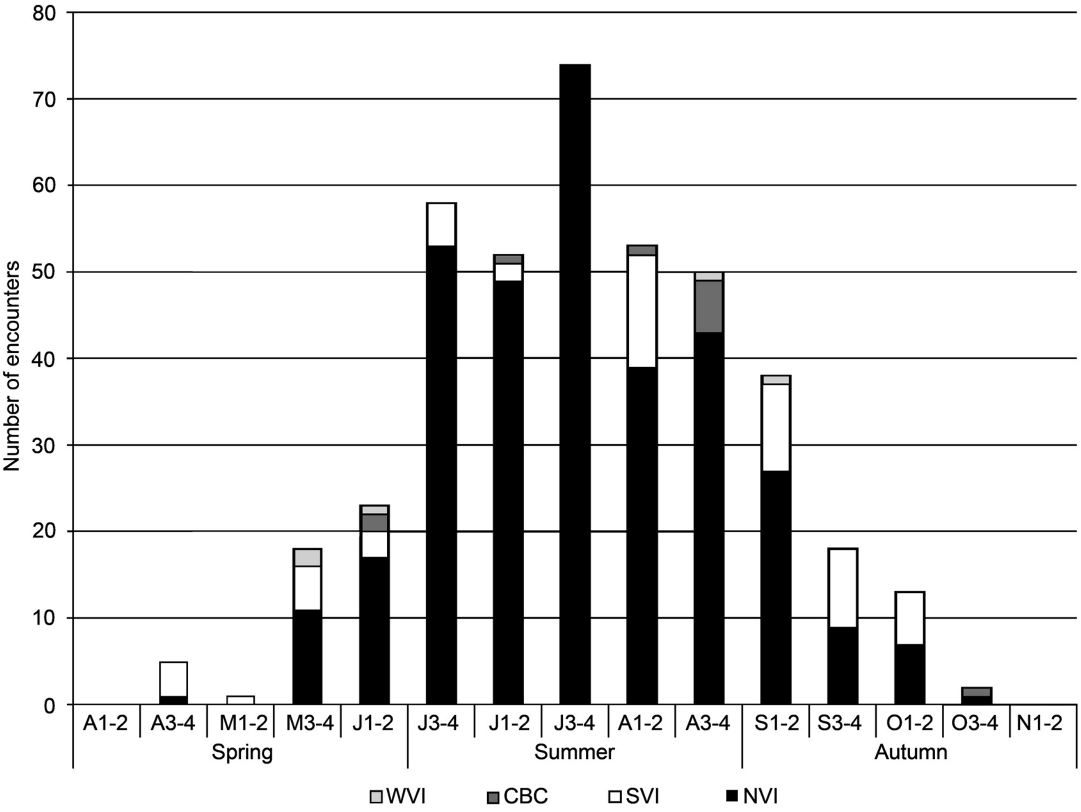

Fig. 2. Seasonal distribution of all encounters with common minke whales from 2005–12, shown for each area by two-week period and season.

month until July, then a steady decline each month thereafter in the NVI area (Fig. 2) where the majority of effort and encounters took place. In the SVI area, the number of encounters fluctuated each month between April and October. The few encounters in the CBC and WVI areas occurred sporadically between May and September (Fig. 2). No common minke whales were encountered from November through March.

A total of 44 unique individual common minke whales were photo-identified. The majority of these whales were documented in more than one encounter. Re-sights of whales within years occurred in both the NVI and SVI areas, while re-sights among years occurred in all four areas. The greatest number of encounters (331) occurred in the NVI area, which led to identifications of 15 individual common minke whales (Table 1). A further 58 encounters occurred in the SVI area where 21 individual common minke whales were identified. There were fewer encounters and identifications in the CBC and WVI areas (Table 1).

Of the 15 individuals identified in the NVI area, eight showed a moderate to high degree of site fidelity to the area as they were encountered there on several occasions during three or more years (Table 2). Three of these whales (M002, M003 and M006) were encountered in another area during years that they were also encountered in the NVI area. Of the seven whales that did not exhibit a high degree of fidelity to the NVI area, two individuals (M008 and M021) were encountered in another area during years that they were also encountered in the NVI area. Two whales known from the NVI area (M003 and M008) were also documented in the SVI area once each during years that they were not encountered in the NVI area (Table 2). In total, five common minke whales were encountered in more than one area intra-annually on six occasions. In all these cases, either the first or last encounter of an individual whale during the course of a year was used to confirm a relatively large-scale movement (Table 2). The distances between encounter locations confirming these movements ranged from 171–424km (Table 3). Temporal intervals between these encounters ranged from 32 to 93 days

Table 1

Total number of minke whale encounters by area and year (a) and number of new whales identified in each area each year, resulting in the total number of unique whales by area (b).

| Year | NVI | SVI | CBC | WVI |
|---|---|---|---|---|
| **(a)** | | | | |
| 2005 | 3 | 4 | 0 | 2 |
| 2006 | 2 | 8 | 0 | 2 |
| 2007 | 12 | 0 | 0 | 0 |
| 2008 | 18 | 6 | 0 | 0 |
| 2009 | 37 | 12 | 1 | 1 |
| 2010 | 99 | 10 | 2 | 0 |
| 2011 | 72 | 9 | 3 | 0 |
| 2012 | 88 | 9 | 5 | 0 |
| **Total** | **331** | **58** | **11** | **5** |
| **(b)** | | | | |
| 2005 | 3 | 4 | 0 | 2 |
| 2006 | 2 | 4 | 0 | 1 |
| 2007 | 2 | 0 | 0 | 0 |
| 2008 | 3 | 3 | 0 | 0 |
| 2009 | 4 | 3 | 1 | 1 |
| 2010 | 0 | 3 | 2 | 0 |
| 2011 | 1 | 3 | 2 | 0 |
| 2012 | 0 | 1 | 4 | 0 |
| **Total*** | **15** | **21** | **9** | **4** |

*Five individuals are known from more than one area.

Table 2

Numbers of encounters for each whale known from the NVI area by year and season. S: spring. S: summer. A: autumn. ***Bold italics*** indicate encounters that were not in the NVI area.

| | 2005 | | | 2006 | | | 2007 | | | 2008 | | | 2009 | | | 2010 | | | 2011 | | | 2012 | | | Encounters | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Whale ID | S | S | A | S | S | A | S | S | A | S | S | A | S | S | A | S | S | A | S | S | A | S | S | A | Total | Areas |
| M001 | | | | | | | | | | | 2 | | | 1 | | 2 | 4 | 4 | | 9 | | | 19 | | 41 | NVI |
| M002 | | | | | | 1 | | 1 | | ***1*** | 2 | | 1 | 5 | | 3 | 3 | | | 10 | | 2 | 11 | 1 | 41 | NVI, SVI |
| M003 | | 1 | ***1*** | | | ***1*** | 1 | 2 | | | 7 | | 1 | 5 | | 1 | 8 | 1 | | 14 | | | 9 | | 52 | NVI, SVI |
| M004 | | 1 | | | | | | 3 | 1 | | 1 | | | 4 | | 1 | 7 | 5 | 1 | 7 | | 4 | 13 | 2 | 50 | NVI |
| M005 | | | | | | | | | | | 1 | | | | | | | | | | | | | | 1 | NVI |
| M006 | | | | | | | | | 3 | | | | ***1*** | 6 | 1 | 2 | 37 | 6 | 5 | 13 | | 2 | 10 | | 86 | NVI, WVI |
| M007 | | | | | 1 | | | | | | | 3 | | | | | | | | 3 | 1 | | | | 8 | NVI |
| M008 | | 1 | ***1*** | | | | | | | | | | | | | | | | ***1*** | 1 | | | | ***1*** | 5 | NVI, SVI |
| M009 | | | | | | | | | | | | | | 4 | 3 | | 2 | 5 | | 1 | | | | | 15 | NVI |
| M012 | | | | | | | | | | | | | | 1 | | | | | | | | | 1 | | 2 | NVI |
| M018 | | | | | | | | | | | | | | 1 | | | | | | | | | | | 1 | NVI |
| M021 | | | | | | | | | | | | | | ****2*** | 3 | | | | | | | | | | 5 | NVI, CBC |
| M022 | | | | | | | | | 1 | | | | | | | 1 | 6 | 1 | | 6 | | | 14 | | 29 | NVI |
| M046 | | | | | | | | | | | | 2 | | | | | | | | | | | | | 2 | NVI |
| M052 | | | | | | | | | | | | | | | | | | | 1 | | | | | | 1 | NVI |

*Only the first of the two encounters was not in the NVI area.

Table 3

Details on the six intra-annual movements between areas documented for five individual minke whales.

| | | | Encounter area details | | | | | Movement details | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Whale ID | Year | Encounters | NVI | SVI | CBC | WVI | Lat/longs (temporally closest between areas) | Distance (km) | Time (days) | Direction |
| M002 | 2008 | 3 | 14/07+16/07 | 25/04 | – | – | 48°44.7'N 123°05.1'W and 50°34.9'N 126°48.0'W | ≥359 | ≤80 | North |
| M003 | 2005 | 2 | 20/06 | 21/09 | – | – | 50°36.4'N 126°48.6'W and 48°23.4'N 123°08.2'W | ≥396 | ≤93 | South |
| M006 | 2009 | 8 | 21/07–14/09 | – | – | 07/06 | 49°09.4'N 125°54.6'W and 50°33.1'N 126°47.6'W | ≥401 | ≤44 | North |
| M008 | 2005 | 2 | 24/07 | 07/09 | – | – | 50°39.0'N 126°44.4'W and 48°23.8'N 123°10.8'W | ≥398 | ≤45 | South |
| M008 | 2011 | 2 | 05/07 | 22/04 | – | – | 48°20.4'N 123°03.7'W and 50°40.0'N 127°11.0'W | ≥424 | ≤74 | North |
| M021 | 2009 | 5 | 16/08–14/09 | – | 15/07 | – | 51°42.7'N 128°06.1'W and 51°00.0'N 127°49.8'W | ≥85* | ≤32 | South |

*Travelled ≥171km between 15/07 and 14/09 encounter locations.

(Table 3). All whales documented making intra-annual movements between areas were found furthest north in summer and furthest south in spring or autumn (Fig. 1, Table 3).

Of the 44 photo-identified common minke whales, 43 had scars believed to be from the bites of cookiecutter sharks. Most scars appeared as grey, oval shaped depressions. These were likely to be older and well-healed bites. No open wounds or fresh bites were observed. The most recently acquired scars were bright white and either circular, oval or crescent shaped (Fig. 3). White scars eventually faded in intensity and were observed as greyish depressions in subsequent years (Fig. 3). Most individuals that were documented with good quality images were found to have some white scars acquired since the previous year (Table 4). These scars were observed on heads, flanks and dorsal fins of common minke whales from all of the four areas.

The commensal barnacle *Xenobalanus globicipitis* occurred on three common minke whales, each identified in one of the three areas around Vancouver Island. Two whales were each host to a single barnacle attached to the trailing edge of the tip of their dorsal fins. The remaining whale had four barnacles attached to the upper trailing edge of its dorsal fin and one at the base (Fig. 4).

## DISCUSSION

The seasonal movements and ecological markers documented in this study together provide new insights into the ranges of eastern North Pacific common minke whales. Almost all individuals had ecological markers on their bodies and the majority of individuals documented with good quality images each year were found to have acquired some of these ecological markers since the previous year. Furthermore, common minke whales were only encountered from spring to autumn despite year-round search effort and five individuals were documented to move up to 424km in a northerly direction in the spring and up to 398km in a southerly direction in the autumn.

Most of the 44 individuals identified during this study, including those documented to make movements between areas, showed fidelity to specific areas within and among years similar to the results of Dorsey *et al.* (1990) and Dorsey (1983). Based on the strong site fidelity exhibited by some of these individuals during summer, it is not surprising that the only encounters confirming large-scale intra-annual movements occurred during spring or autumn when whales would be expected to be migrating. The individuals documented making these movements were animals known from the NVI area, perhaps only because this area had the

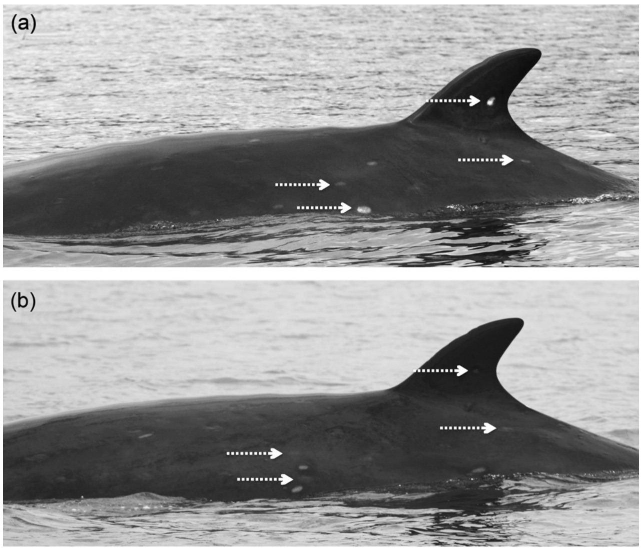

Fig. 3. Identification photographs of common minke whale M001 showing scars believed to be caused by cookiecutter sharks. Photograph (a) was taken on 27 June 2010. Photograph (b) was taken on 8 July 2011. White arrows in both photographs point to the same scars showing how they fade over time. Photograph (b) also shows white scars acquired since the previous year.

most effort, highest number of encounters and is centrally located between other areas where common minke whales were documented. Most of these movements took place between locations in the NVI and SVI areas where common minke whales have previously been observed feeding (Dorsey *et al.*, 1990; Hoelzel *et al.*, 1989; Towers, unpublished data). This suggests that as individuals migrate, they pass through and may stop in other feeding areas along the way. Individuals of other species of baleen whale have also been known to use more than one feeding area while migrating to or from breeding areas (Mate *et al.*, 2010; 1999; Stockin and Burgess, 2005) and in some cases, can be found to move between feeding areas earlier or later than expected (Baker *et al.*, 1985; Barendse *et al.*, 2010; Mizroch *et al.*, 2009; Stern, 2002; Straley, 1990). Although common minke whales in the present study were only encountered from spring to autumn, the few winter sightings of this species reported in the same waters (COSEWIC, 2006; Everitt *et al.*, 1979) could have been individuals feeding or migrating earlier or later than the rest of the population. If a large proportion of the population did not regularly migrate between the eastern North Pacific and warmer waters further south, it would be expected that more of the individuals documented in this study would not have had ecological markers.

Table 4

Total number of encounters from all areas that included good quality identification images each year, the number of unique whales in those encounters and the number of individuals found to have white scars on their bodies believed to have been caused by cookie-cutter sharks since the previous year.

| Year | Encounters with good quality images | Unique individuals | |
|---|---|---|---|
| | | Annual total | With white scars |
| 2005 | 5 | 4 | 4 |
| 2006 | 8 | 8 | 7 |
| 2007 | 1 | 1 | 1 |
| 2008 | 16 | 11 | 10 |
| 2009 | 32 | 15 | 14 |
| 2010 | 63 | 14 | 13 |
| 2011 | 56 | 18 | 17 |
| 2012 | 58 | 15 | 12 |

Fig. 4. Photograph of common minke whale M017 from 1 September 2005 with five *Xenobalanus globicipitis* barnacles attached to the trailing edge of its dorsal fin.

Scars believed to have originated from the bites of cookiecutter sharks were observed on 43 of the 44 common minke whales photo-identified. No open bite wounds were documented and partially healed white scars from bites acquired since the previous year were observed on most individuals. It is possible that the individual without noticeable scars and the few common minke whales that did not appear to have white scars in some years were not photographed extensively enough to see any of the scars they may have possessed. It is also possible that these individuals had not made recent movements into the range of the cookiecutter shark. This shark species primarily occurs within 20° of the equator but has been found to range as far as 38°N in the western North Pacific and 34°N in the eastern North Pacific (Nakano and Tabuchi, 1990). It is primarily known to inhabit oceanic waters (Castro, 1983) with surface temperatures of 18°C or higher (Nakano and Tabuchi, 1990)[4]. Cookiecutter sharks consume a wide variety of prey including tissue from live cetaceans (Jahn and Haedrich, 1987). Open scars from the bites of these sharks have been observed on many species of cetaceans known to be permanent or seasonal inhabitants of warm oceanic waters (Dwyer and Visser, 2011; Gasparini and Sazima, 1996; Jones, 1971; Pérez-Zayas *et al.*, 2002; Shevchenko, 1977; Smultea *et al.*, 2010). Dorsey *et al.* (1990) observed white scars on common minke whales in the eastern North Pacific but despite discussing potential causes and suggesting that the scars were not acquired during the summer, they did not make any conclusions about their origin. It is now widely accepted that scars identical in appearance to those described in the present study and by Dorsey *et al.* (1990) are caused by cookiecutter sharks (Bando *et al.*, 2010; Bertulli *et al.*, 2012; Jefferson *et al.*, 2008; Miyashita *et al.*, 2010; Moore *et al.*, 2003; Pérez-Zayas *et al.*, 2002; Wade *et al.*, 2010; Walker and Hanson, 1999). Other species known to parasitise common minke whales including lampreys (*Petromyzontidae*) (Nichols and Tscherter, 2011; Olafsdóttir

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[4] During the course of this study sea surface temperatures at sampling stations in the NVI and SVI areas ranged from 7°C in winter to 12°C in summer and stations in the WVI and CBC areas reported sea surface temperatures ranging from 6°C in winter to 14°C during summer (*http://www.pac.dfo-mpo.gc.ca/science/oceans/data-donnees/lighthouses-phares/index-eng.html*).

and Shinn, 2013) and the copepod *Pennella balaenopterae* (Bertulli *et al.*, 2012; Dorsey *et al.*, 1990) also cause scars on other cetaceans (Andrews, 1916; Ivashin and Golubovsky, 1978; Pike, 1951; van Utrecht, 1959). However, the scars from these species differ in appearance from those that can be attributed to cookiecutter sharks (Bertulli *et al.*, 2012; Dorsey *et al.*, 1990; Jones, 1971; Mackintosh and Wheeler, 1929; Nemoto, 1955; Samarra *et al.*, 2012; Walker and Hanson, 1999).

The other ecological marker observed on three common minke whales during this study was the commensal barnacle *Xenobalanus globicipitis*. Despite a widespread distribution during its sedentary phase, little is known of this barnacle's larval stage. It has been suggested, however, that this epizoic originates in warm waters and is sometimes carried by its cetacean hosts to higher latitudes during migration (Bushuev, 1990; Olafsdóttir and Shinn, 2013). Fertl (2002) reported that the breeding season of this barnacle is synchronous with the breeding season of its migratory cetacean host. Kane *et al.* (2008) found that *Xenobalanus* is not host-selective and is known to occur on nearly every species of cetacean found in the eastern tropical Pacific. Significantly however, this barnacle species has not been recorded on cetaceans with ranges restricted to cold temperate or polar waters (Kane *et al.*, 2008; Rajaguru and Shantha, 1992). For example, reviews of photographic catalogues of killer whale populations that range within cold temperate coastal waters extending to the north and south of the areas where we encountered common minke whales (Ellis *et al.*, 2011; Ford and Ellis, 1999; Ford *et al.*, 2000; Towers *et al.*, 2012a) showed no occurrence of *Xenobalanus* (Kane *et al*., 2006) or scarring from cookiecutter sharks. This provides further indication that the common minke whales identified during this study move far beyond the areas in which they were encountered.

Although it is not known exactly where the common minke whales encountered during this study migrate to in winter, the seasonal movements and ecological markers documented in this study suggest that their destinations lie somewhere in warm waters to the south of British Columbia and Washington. Considering this, it is clear that common minke whales found in the eastern North Pacific range over a large geographical area. Any non-invasive research focused on determining migration routes and winter destinations of common minke whales in the North Pacific Ocean would be useful for further understanding their population structures.

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