A note on the collection of associated behavioural data with biopsy samples during cetacean assessment cruises[1]

SARAH L. MESNICK*, PHILLIP CLAPHAM+ AND ANDREW E. DIZON*

Contact e-mail:sarahlyn@caliban.ucsd.edu

ABSTRACT

Understanding the influence of social organisation on the distribution, abundance and genetic structure of cetacean populations is critical in developing better predictive models for management. Field data on cetacean social organisation are far more valuable when collected and analysed together with genetic data from biopsy samples and environmental information (e.g. oceanographic patterns, prey availability). Traditionally, however, studies of cetacean social behaviour and studies of cetacean population dynamics have been conducted independently (Tillman and Donovan, 1986). To integrate these fields, this paper recommends that multi-disciplinary cetacean assessment surveys collect biopsy and associated behavioural data for each sample taken (the minimum data being group size, number of animals biopsied and age class). Examples of sampling forms, outlining the desired information, are provided. Understanding of cetacean stock structure and the processes affecting stock differentiation will best come from a combined genetic, social, ecological and oceanographic approach.

KEYWORDS: BIOPSY SAMPLING; GENETICS; SOCIAL BEHAVIOUR; SURVEY-COMBINED; VOCALISATION; COLOURATION

INTRODUCTION

Understanding the processes influencing stock structure in cetaceans is especially challenging because many species have vast geographic distributions, the marine environment has few geographical boundaries and individuals are highly mobile (Hoelzel, 1994). In addition, many species are long-lived, communicative, and travel in stable groups, suggesting that social behaviour may play an active role in promoting population divergence and the subsequent genetic subdivision of stocks.

The IWC held its first Workshop on the Social Behaviour of Whales in Relation to the Management of Cetacean Stocks in 1978, over two decades ago (IWC, 1979; Tillman and Donovan, 1986). It highlighted species for which social data were critical for effective management, and examined methodological advances in the study of cetacean behaviour. However, little information on social behaviour and social structure has been incorporated into stock identity studies during the intervening years (but see Palsbøll *et al.*, 1995). In part, this is because behavioural data are not routinely collected during cetacean assessment surveys, or are collected in a somewhat *ad hoc* manner (Donovan, 1984). In addition, it is not necessarily a reasonable assumption that the behaviour and social structure of nearshore populations (often more accessible to cetologists studying behaviour) can predict the behaviour of offshore populations (such as those observed during cetacean surveys).

To better understand how cetacean social organisation influences stock structure, it is necessary to determine the tendency of kin to associate in social groups and to obtain data on sex, age and range of dispersal (Hoelzel, 1994). Given the difficulties of obtaining these data in the wild, the use of molecular markers has increased to augment field observations of species-specific behaviour and the results of photo-identification, acoustical, mark-recapture and telemetric studies (e.g. Amos *et al.*, 1993; O'Corry-Crowe *et al.*, 1997). However, biopsy samples for molecular analysis, are often collected opportunistically and information about the behaviour of the animals sampled or their environment is often absent. Such a lack of data makes it difficult to infer anything from molecular analyses about cetacean social structure, or to relate social and genetic structure of cetacean stocks to environmental patterns.

[1] A version of this paper was submitted to the IWC Scientific Committee as SC/49/O25.

WHY ARE DATA ON SOCIAL BEHAVIOUR AND SOCIAL ORGANISATION IMPORTANT IN STUDIES OF CETACEAN STOCK STRUCTURE?

The social behaviour and organisation of cetacean populations have important implications for stock identification and management. A few brief examples are given below. Readers are referred to Tillman and Donovan (1986), Hoelzel (1994) and Hughes (1998) for more detailed treatment of this subject.

What is the tendency of kin to travel together?

Molecular analyses can be used to determine the degree of relatedness among a given set of biopsy samples. Only field observations, however, can indicate what proportion of a group was sampled. Depending on this proportion, different interpretations about group composition are possible. For example, if molecular analyses reveal that all individuals biopsied share identical mitochondrial DNA haplotypes (maternally inherited), but field observations indicate that only a small fraction of the total group was sampled, a conclusion of matrifocal social structure would not be warranted without additional samples. Accurate estimates of relatedness within groups are necessary because the use of multiple samples taken from a group may positively bias estimates of population structure if the group members are more related than average members of a stock (B. Taylor, unpublished data). To correct for this bias, methods of subsampling need to be based on accurate assessment of relatedness within groups.

* *Southwest Fisheries Science Center, National Marine Fisheries Service - NOAA, PO Box 271, La Jolla, CA 92038, USA.*
+ *Northeast Fisheries Science Center, National Marine Fisheries Service - NOAA, 166 Water Street, Woods Hole, MA 02543, USA.*

What is the sex and age composition of groups?
Molecular analyses can be used to determine the gender of a given biopsy sample but the only (non-invasive) method to estimate age class is by field observation. Consider the different possible outcomes when two biopsies are obtained and initial molecular analyses determine that one sample is male and the other female and that the coefficient of relatedness between the two samples is 0.5. Without any field notes on the relative sizes of the animals from which the biopsies were obtained, we would be unable to distinguish among the possibilities that the samples were taken from a mother with a male calf, two juvenile siblings, or an old male accompanied by his adult daughter. With the identification of sex (by molecular analysis) and an estimate of age class (by field observation), biopsy samples can provide a deeper level of understanding of the composition of cetacean groups.

How do social signals influence stock identity and differentiation?
Cetacean social signals, such as acoustic vocalisations and colour patterns, can be useful indicators of stock identity (Payne and Guinee, 1983; Stafford and Fox, 1998), but signal data are often collected independently of tissue samples. Our best understanding of stock identity and of the processes affecting stock differentiation will undoubtedly come from combined analyses. For example, divergence of social signals may rapidly promote population divergence and reproductive isolation with or without molecular, geographical or ecological isolation (West-Eberhard, 1983; Mesnick, 1996). Multidisciplinary studies are our best hope of illuminating such patterns and the underlying processes.

WHAT KIND OF FIELD DATA SHOULD BE COLLECTED?

Cetacean assessment surveys provide a unique opportunity to collect biopsy samples at the same time and place as associated behavioural, ecological and oceanographic data. At a minimum, biopsy records should identify the species sampled and the latitude/longitude of the sampling location. Additionally, the following data should be recorded:

(1) an estimate of total group size (best, high, low);
(2) the number of biopsies taken from the group;
(3) an estimate of the age class of each biopsied animal (e.g. adult, immature, calf);
(4) gender;
(5) species or stock specific behaviours, colour patterns or acoustic signals;
(6) links to other individuals sampled from the same group; and
(7) notes on whether the sampling effort disrupted the whale's behaviour.

These data generally take no more than a few minutes to collect and record and should be made as close to the time that biopsy samples are obtained as possible. Minimally, pen and paper are sufficient. However, computerised event recorders with automated links to GPS can aid in data collection and in linking subsequent sighting records. Links to any photo-identification records and acoustic recordings, as well as any ecological and oceanographic data, should be indicated. An example data sheet is given in Appendix 1.

There are three important caveats to consider when collecting behavioural data with biopsies. First, it is important to assess the potential biases in the collection of the behavioural observations. Sea state, observer effort (time spent observing and the number of observers) and the disruption caused by the biopsy activity itself are three of the most important considerations and should be indicated for each animal sampled. Second, the data forms are presented here only as examples and investigators should tailor the forms, adding and/or omitting particular fields, to suit each individual project. Lastly, although this note has focused on obtaining tissue samples during cetacean assessment surveys, many other sampling opportunities are also available. Incidental sampling on non-cetacean research cruises, during mass strandings and from fishery bycatch are also possibilities that can be pursued.

SIGNIFICANCE AND RECOMMENDATIONS

Enhanced understanding of the social organisation of cetacean populations and the relationship of these populations to their environment is important to the effective management of cetacean species. Together, these data sets can be used to determine aspects of cetacean social structure such as sex-biased dispersal patterns, the tendency of kin to associate, site fidelity and mating systems. Toward this end, we make the two recommendations below.

(1) Cetacean assessment surveys should collect biopsy and behavioural data for each animal (or group) sampled. The most important data to collect are: total group size, the number of animals in each group biopsied and an estimate of the age class of each biopsied animal.
(2) The genetic data collected from biopsied animals should be analysed together with the social, ecological and oceanographic data collected from their environment.

ACKNOWLEDGEMENTS

The design of the data forms was greatly improved by the contributions of G. O'Corry-Crowe and a Working Group established by the International Whaling Commission's Scientific Committee. The Working Group consisted of Philip Clapham, Deborah Thiele, Paul Ensor, Ken Findlay and Steve Swartz. The comments of Per Palsbøll, Fred Serchuk and an anonymous reviewer greatly improved the manuscript.

REFERENCES

Amos, B., Schlotterer, C. and Tauz, D. 1993. Social structure of pilot whales revealed by analytical DNA profiling. *Science* 260:670-72.
Donovan, G.P. 1984. Small cetaceans seen during the IWC/IDCR research cruise in the eastern tropical Pacific, and in particular off Peru. *Rep. int. Whal. Commn* 34:561-7.
Hoelzel, A.R. 1994. Genetics and ecology of whales and dolphins. *Annu. Rev. Ecol. Syst.* 25:377-99.
Hoelzel, A.R. and Dover, G.A. 1991. Mitochondrial D-loop DNA variation within and between populations of the minke whale (*Balaenoptera acutorostrata*). *Rep. int. Whal. Commn* (special issue) 13:171-81.
Hughes, C. 1998. Integrating molecular techniques with field methods in studies of social behavior and management. *Ecology* 79:383-99.
International Whaling Commission. 1979. Report of the sub-committee on cetacean behaviour and management. *Rep. int. Whal. Commn* 29:92-5.
Mesnick, S.L. 1996. Sexual selection and bilogical diversification. Ph.D. Thesis, University of Arizona, Tucson, Arizona. 302pp.
O'Corry-Crowe, G., Suydam, R., Rosenberg, A., Frost, K. and Dizon, A.E. 1997. Phylogeography, population structure and dispersal patterns of the beluga whale, *Delphinapterus leucas*, in the western Nearctic revealed by mitochondrial DNA. *Mol. Ecol.* 6:955-70.
Palsbøll, P.J., Clapham, P.J., Mattila, D.K., Larsen, F., Sears, R., Siegismund, H.R., Sigurjónsson, J., Vasquez, O. and Arctander, P. 1995. Distribution of mtDNA haplotypes in North Atlantic

humpback whales: the influence of behavior on population structure. *Mar. Ecol. Prog. Ser.* 116:1-10.
Payne, R. and Guinee, L.N. 1983. Humpback whale (*Megaptera novaeangliae*) songs as an indicator of 'stocks'. pp. 333-58. *In:* R. Payne (ed.) *Communication and Behavior of Whales.* AAAS Selected Symposium 76. Westview Press, Colorado. xii+643pp.
Stafford, K. and Fox, C.G. 1998. A comparison of the acoustic signals produced by blue whales (*Balaenoptera musculus*) in the North Pacific and their use in stock definition/differentiation. Abstract presented to the World Marine Mammal Science Conference, Monte Carlo, Monaco, January 1998. [Available from *Kate.Stafford@NOAA.gov*]
Tillman, M.F. and Donovan, G.P. 1986. Report of the Workshop on the Behaviour of Whales in Relation to Management, Seattle, Washington, 19-23 April 1982. *Rep. int. Whal. Commn* (special issue) 8:1-56.
West-Eberhard, M.J. 1983. Sexual selection, social competition, and speciation. *Quart. Rev. Biol.* 58:155-83.

Appendix 1

GENETIC SAMPLING DATA SHEET

Instructions and Notes

Purpose
The purpose of this form is to record information about each sampled whale, and the group to which it was associated, to facilitate interpretation of genetic analyses. The form was designed primarily for biopsies, but can also be used for tissue samples collected by other means (e.g. sloughed skin, strandings, tags, etc.) A sample that does not have this information associated with it is of limited use. Data on the individual and the group greatly increases the value of the sample, since it can be used to address a wide variety of questions including social structure, relatedness, paternity, behavioural role and population structure. Given that any research cruise costs a great deal of money to run (and therefore that each sample is expensive), it is vital to maximise the information that is gained from each sample.

Other data
It is very useful to have as much data as possible associated with a biopsy sample. Photographs for individual identification and acoustic recordings for stock identification are particularly useful. Associated ecological and oceanographic data are also important.

What is a group?
The definition of what constitutes a group is not straightforward in all cases. Here, the term 'associated' means two or more whales swimming side by side and generally coordinating their activities (surfacing, diving, direction of movement, etc.). This definition is relatively straightforward when applied to baleen whales, but defining a group in some other species (e.g. dolphins, sperm whales) can be problematic. One of the purposes of this form is to gather information about animals that are closely associated with each other within a group of whales. For example, a group of sperm whales can be spread out over quite a large area but still be associated in the sense of generally moving together in the same direction; but within this aggregation some whales will be more closely associated with each other than with other animals. For this reason, the set of forms includes a third sheet that can be used for free-form notes to record information that may not easily fit into the categories defined elsewhere. The general rule is: the more information the better - it is much easier to discard superfluous data when analysing samples than to try to reconstruct social organisation from inadequate information.

Forms
There are three forms that are designed to be used together.

Master Sheet
This is the first form to use, and one of these should be completed for every biopsied group. The Master Sheet contains fields that need be filled out only once for each group; these fields include the institution, vessel, location of the group, etc. The second part of the Master Sheet provides information on up to two biopsied whales from a group. If more than two whales are biopsied, the additional data should be recorded on one or more Continuation Sheets.

Continuation Sheet
As noted above, this form is used when the Master Sheet is insufficient to record all the required data (i.e. when more than two whales in a group have been biopsied). Each Continuation Sheet has space for data on three whales. As many Continuation Sheets as necessary can be used, but each set *must* be accompanied by one Master Form so that the basic information identifying the research institution and the group is complete.

Notes Sheet
As described above, this form allows for the recording of any additional observations that do not easily fit into the categories provided on the other forms.

Explanation of fields – Master Sheet
Note: All fields MUST be filled out with either data or 'N/A' (Not Applicable)
Page ___ *of* ___ : Fill out the page number, and total number of pages for each group.
Institution: Name (or abbreviation) of the scientific institution conducting the research cruise.
Chief Scientist: Name of the Chief Scientist or Principal Investigator. Please write out at least the last name in full (don't use initials).
Vessel: Name of the research vessel.
Date: Enter this as Day, Month, Year. Month can be either a three-letter abbreviation (JAN) or numeric (01).
Species: The species should preferably be entered as a scientific name (e.g. *M. novaeangliae*) but common names (humpback whale) are also permissible as long as they can be easily interpreted later.
Group size: Enter the estimated minimum (the minimum number of whales the observer was sure was in the group), estimated maximum (possible maximum number present) and best estimate (the best estimate of how many whales were present) of the number of whales present in the group.

How many whales were sampled?: The number of animals in the group from which samples were obtained.
Unique sighting or group identifier number: This number is critical, since it uniquely identifies this group from any other. It must be unique for at least the date concerned; in other words, groups can either be numbered uniquely on each day (no two groups with the same number on that date) or numbered uniquely for the research cruise (no two groups with the same number on that cruise). Assigning this number is up to the Chief Scientist and may be derived from existing data protocols for the research cruise. It doesn't matter what sort of numbering is used, as long as a group can be uniquely identified (with either the number alone, or with the combination of number and date).
Group behaviour: If the general behaviour of the group can be assessed with certainty, circle one of the options given. If the behaviour is unknown or unclear, circle 'undetermined'.
Location: Enter as latitude and longitude. Don't forget to enter N, S, E or W, as appropriate.
Other data available: Indicate whether other data are available that can be associated with this group. Either check the box(es), or enter the number/reference of separate data forms that are available for each category.
Sample number: The number of each sample should obviously be unique. If an individual has been sampled more than once, more than one number can be entered here. Numbers should preferably be sequential.
Whale number: This is an arbitrary number for the individual whale sampled in the group concerned. The easiest method is simply to begin with 1 and continue in sequence. The whale number should be written together with the group ID and the date on the sample so that each sample can be unequivocally linked to a particular animal later on. The whale number may also be used in other notes (e.g. to identify the animal in photographic notes).
Sample type: Circle the type of sample taken (biopsy, sloughed skin or other).
Class of this whale: Circle one option, if known, or 'unknown'.
Approximate size: Although visual estimates of size are not very reliable, it is often useful to give a general relative estimate of the size of the whale relative to the standards (small, midsize, large) for the species. Circle one. (If the animal is a calf, then obviously the approximate size will be small.)
If this whale was closely associated... : Indicate the whale number of any animal(s) in close association with the sampled whale.
Association, behavioural, photo or other notes: Use this space to record any other notes of relevance to this whale, to clarify any uncertainties regarding other fields, or to note possible duplicate sampling of one animal.
Did sampling effort disrupt whale's behaviour?: In your estimation, did the sampling effort significantly disrupt the whales behaviour? Circle one: yes, no, uncertain.
If yes, elapsed time before animal resumes previous behaviour?: Time before the animal returns to its presampling behaviour.

Explanation of fields – Continuation Sheet
The Continuation Sheet is linked to the Master Sheet by two fields: 'Date' and 'Unique sighting or group identifier number'. It is *critical* that these be filled out to avoid confusion if forms get separated later.

All other fields relate to data on biopsied individual whales, and are the same as those on the Master Sheet.

Explanation of fields – Notes Sheet
The Notes Sheet is simply an unstructured form allowing for additional comments or explanations that cannot be accommodated elsewhere.

Additional Notes
Although we recognise that it may not be possible on all cruises, it is very helpful when dealing with species that are individually identifiable (e.g. humpbacks, right whales, blue whales) if each sampled whale is photographed for individual ID. It is particularly useful if the whale is photographed immediately before or after (or during) a biopsy, and that photograph isolated somehow - this makes it very clear which individual was biopsied, which allows for verification in cases where there is confusion when the data are analysed. The easiest method of isolating a photograph is to shoot a spacer or bracket (a photo of something neutral, like a boat, a person, a bird) immediately afterwards, and recording this bracket on the data form (e.g. whale sampled with biopsy 92-14 is dorsal fin photo before shot of boat).

Finally, abbreviations (except for the institution field) should be avoided unless they are likely to be obvious to anyone, or the data forms are accompanied by a sheet which lists abbreviations used.

Questions or comments
This form was designed by a Working Group established by the International Whaling Commission's Scientific Committee. Questions, comments or suggestions for improvement should go to: Phillip Clapham, Northeast Fisheries Science Center, 166 Water Street, Woods Hole, MA 02543, USA (e-mail: *phillip.clapham@noaa.gov*) or Sarah Mesnick, Southwest Fisheries Science Center, P.O. Box 271 La Jolla, CA 92038, USA (e-mail: *sarahlyn@caliban.ucsd.edu*) An electronic version of the form is available upon request from either Phil Clapham or the IWC Secretariat.

GENETIC SAMPLING DATA FORM - MASTER SHEET

Important: use one form (or set of forms) for each group of associated whales

PAGE ____ OF ____ .

Institution _____ Chief Scientist _____ Vessel _____ Date (day, month, year) ____/____/_____

Species _____ Group size: *best* _____ *high* _____ *low* _____ How many whales in the group were sampled? _____

Unique sighting or group identifier number: _____ Group behaviour (circle): *travel feed rest mill courtship aggressive undetermined*

Location: *latitude* _____

longitude _____

Other available data (check √ or include data form numbers if available)					
Photos	*Video*	*Behaviour*	*Effort/vessel track*	*Environmental*	*Other*

SAMPLE NUMBER _____ WHALE NUMBER _____ Sample type (circle): *biopsy sloughed skin other*

Class of this whale (circle): *mother calf adult immature unknown* Approx size (circle): *large midsize small* Sex: *female male unknown*

If this whale was closely associated with another sampled animal from the group, which one(s) - give whale number(s): _____

Association, behavioural, photo or other notes for this whale: _____

_____

Did sampling effort disrupt whale's behaviour (circle): *Yes No Uncertain* If yes, elapsed time before animal resumes previous behaviour:_____

SAMPLE NUMBER _____ WHALE NUMBER _____ Sample type (circle): *biopsy sloughed skin other*

Class of this whale (circle): *mother calf adult immature unknown* Approx size (circle): *large midsize small* Sex: *female male unknown*

If this whale was closely associated with another sampled animal from the group, which one(s) - give whale number(s): _____

Association, behavioural, photo or other notes for this whale: _____

_____

Did sampling effort disrupt whale's behaviour (circle): *Yes No Uncertain* If yes, elapsed time before animal resumes previous behaviour:_____

GENETIC SAMPLING DATA FORM - CONTINUATION SHEET (FOR SAME GROUP)

Date (day, month, year) ____/____/______ Unique sighting or group identifier number: ________________ PAGE _____ OF ____

SAMPLE NUMBER ______________ WHALE NUMBER __________ Sample type (circle): *biopsy* *sloughed skin* *other*

Class of this whale (circle): *mother* *calf* *adult* *immature* *unknown* Approx size (circle): *large* *midsize* *small* Sex: *female* *male* *unknown*

If this whale was closely associated with another sampled animal from the group, which one(s) - give whale number(s): ______________________

Association, behavioural, photo or other notes for this whale: ______________________

____

Did sampling effort disrupt whale's behaviour (circle): *Yes* *No* *Uncertain* If yes, elapsed time before animal resumes previous behaviour:______________

SAMPLE NUMBER ______________ WHALE NUMBER __________ Sample type (circle): *biopsy* *sloughed skin* *other*

Class of this whale (circle): *mother* *calf* *adult* *immature* *unknown* Approx size (circle): *large* *midsize* *small* Sex: *female* *male* *unknown*

If this whale was closely associated with another sampled animal from the group, which one(s) - give whale number(s): ______________________

Association, behavioural, photo or other notes for this whale: ______________________

____

Did sampling effort disrupt whale's behaviour (circle): *Yes* *No* *Uncertain* If yes, elapsed time before animal resumes previous behaviour:______________

SAMPLE NUMBER ______________ WHALE NUMBER __________ Sample type (circle): *biopsy* *sloughed skin* *other*

Class of this whale (circle): *mother* *calf* *adult* *immature* *unknown* Approx size (circle): *large* *midsize* *small* Sex: *female* *male* *unknown*

If this whale was closely associated with another sampled animal from the group, which one(s) - give whale number(s): ______________________

Association, behavioural, photo or other notes for this whale: ______________________

____

Did sampling effort disrupt whale's behaviour (circle): *Yes* *No* *Uncertain* If yes, elapsed time before animal resumes previous behaviour:______________

GENETIC SAMPLING DATA FORM - CONTINUATION SHEET (FOR SAME GROUP)

Date (day, month, year) _ ___/____/_____ Unique sighting or group identifier number: _____ PAGE _____ OF _____

(Use this space for additional notes on the group)