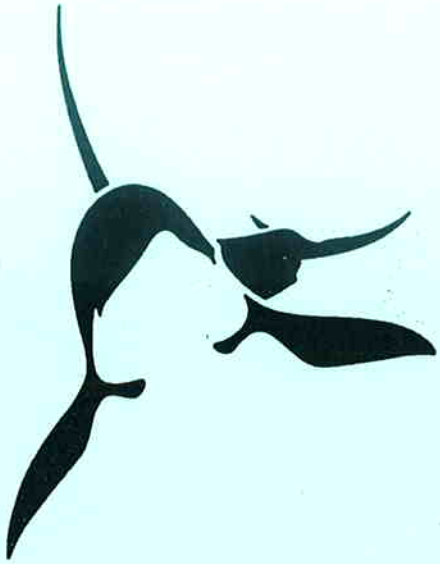

PROCEEDINGS OF THE WORKSHOP

METHODS FOR THE STUDY OF BOTTLENOSE DOLPHINS IN THE WILD

8th Annual Meeting of the European Cetacean Society
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1. Introduction

Giuseppe Notarbartolo di Sciara
Tethys Research Institute, Viale G.B. Gadio 2, 20121 Milano, ITALY

Free-ranging communities of bottlenose dolphins (*Tursiops truncatus*) have provided during the past decades the primary research opportunities in the field of cetacean social ecology in several marine localities around the world, including Argentina, the Gulf of Mexico, Western Australia, California, and Great Britain. Many of the species' traits, including widespread distribution, coastal habits, small group size and contained community size, combine to make the bottlenose dolphin the favourite subject for cetacean social ecology studies. Furthermore, the ecological plasticity of bottlenose dolphins, a species which successfully survives in an extreme range of different conditions - from inland waters to the high seas, from the tropics to cold temperate latitudes, from pristine to severely abused habitats, from "shark-infested" waters to areas where predators are absent - presents unique opportunities for understanding the mechanisms of adaptation of a top predator to its environment, and how the interplay of different factors, such as predation pressure anf food availability, combine together to shape social structure.

From this consideration stems the extreme interest in comparing the social ecology and behaviour of different bottlenose dolphin communities, which have adapted to life in the most diverse marine habitats around the world. Unfortunately, such comparisons toady are practically impossible because the research methods used are so different. As Shane (1990) remarked, "To date, methodology has not been critically assessed by researchers studying bottlenose dolphin behavior, and there has been no attempts to standardise methods".

In organising this workshop, we have not been seized by standardisation frenzy. We fully appreciate the beauty of euristic ingenuity and of the diversity of individual approaches to the solution of the manifold problems which research conditions always present. However, we hope that by improving communication among different research groups, ideas will spread around and circulate, methods will be discussed and compared, and finally, results will become more comparable.

This workshop, which the European Cetacean Society kindly agreed to host, represents only a first small step in the direction indicated by Shane. Its scope was necessarily limited by the time available and by the complexity of many of the subjects that were discussed. Nevertheless, it is the hope of those who organised the workshop that it will have stimulated a process of intellectual exchange among those who are interested in cetacean social ecology.

Thanks are given to Jean-Michel Bompar and Helène Petit, organisers of the European Cetacean Society Annual Meeting in Montpellier, for taking care with competence and enthusiasm of all the logistic aspects of the workshop.

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2. Home range: is this concept applicable to cetacean studies?[1,2]

Daniela Maldini Feinholz
Pacific Cetacean Group, P.O. Box 378, Moss Landing, CA 95039 - USA

Home range is a relatively old concept, very well known to animal behaviourists and ecologists worldwide. One of the many existing definitions of this concept is that of Knight (1965): "...An animal's home range is the portion of a habitat, or several contiguous environmental areas that constitute the region over which this animal moves, while engaged in routine daily activities...".

The tools which have been used to study the movements of individual animals, across their territory, have been first applied to terrestrial species, and subsequently adapted to the aquatic environment, sometimes with major difficulties. For smaller species, like a field mouse, a reef fish, or an invertebrate, the easiest method to determine home range is to observe the home site, either visually, for given periods of time throughout the day, or using video and time lapse photography. Trapping, tagging and releasing (mark-recapture techniques) have been proven useful to monitor long-term movements and seasonal variations in patterns of movement. For example, carefully placed traps, at different distances from a den, can help in determining the radius of an animal's territory (Dice, 1962). An adaptation to this method for the underwater world (which has also been successfully used on land) is photo-identification. This non-invasive technique allows one to follow individual animals through time series photographs taken in different locations. Animals are recognized by individual patterns of scarring, or by notches, or coloration patterns which are unique to that particular individual. This method has been the most widely used for cetacean species, which are otherwise harder to capture and tag.

Radio-tracking, which relies on a continuous signal at a given frequency emitted by a tag placed on the animal, has brought a new perspective to the study of very mobile species. Tags have become smaller, lighter, and more powerful, so that a wider variety of species could be studied. Nonetheless, this method is still relatively expensive, time consuming and hard to use, especially in the aquatic environment. Some of the large baleen whales have been outfitted with radio tags, and followed through the world's ocean using satellite tracking (Hobbs and Goebel, 1982, Mate and Harvey, 1984).

Aerial observations can be useful to monitor ranges encompassing large areas, but are not geared to target a specific individual, unless they are used in combination with radio-tracking and/or photo-ID. An example is the bowhead whale, which presents a pattern of callosities on the rostrum, and can therefore be identified from the air (Howard and Rugh, 1983).

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[1] At the end of the workshop some of us (H. Arnold, P. Evans, D. Goodson, D. Maldini-Feinholz, R. Sponer, N. Tregenza, and J.P. Ulzega) participated in a discussion panel to identify problems associated with the concept of home range in cetacean studies. The results of such discussion are incorporated in this text.

[2] For every study there are limits imposed by species under investigation (such as high mobility, time spent underwater, etc.), habitat, and method used. Since we realize that investigators understand these constraints, and that each study is going to have different requirements, we did not concentrate on such areas, but we dealt directly with the conceptual problems inherent to the definition of home range.

It is relatively easy to see how the concept of home range has been applied to terrestrial or sedentary species, where movements revolve around a nest or a den, generally centrally located within the range. In the aquatic environment, many reef and intertidal species also revolve their lives around their home site, be it a crevice, or rock. When, on the other hand, we try to extend the concept of home range to open ocean species (e.g. pelagic fish, sharks and cetaceans), the reference point, the home site, is missing. The concept of home range loses meaning, and many problems arise from trying to define it.

In the course of the workshop, we identified three main areas of concern when dealing with home range studies: *terminology used*, *temporal scale*, and *size of the study area*. We understand that the list is not exhaustive and we agreed to return to this topic in the future.

Terminology in science is always a complex yet crucial area. We must define our parameters and limit our conclusions to such parameters. We, therefore, propose to substitute the term "home range" with "known range", and not make generalised statements unless we can identify a cycle within the activities of an individual, and demonstrate that this cycle is repeated with statistical significance.

Temporal scale is a major concern in most research projects, and should always be stated and accounted for in our results. For example, if we followed a dolphin for a day and were able to trace its movements, we would have a pretty good idea of what its "home range" was, for that particular day. On the other hand, from this information, we could not infer how significant that particular area is for the individual until some longer-term study had been conducted. The real question becomes the determination of how long a period of time this dolphin should be followed in order to outline its routine territory.

In many cases the amount of time available in the field is going to be set by funding constraints, type of research conducted, and/or weather patterns. Nonetheless, investigators are drawn into making generalised statements about patterns observed and stretch them well beyond the temporal scale within which they are working. In doing so, animals that were thought to be resident in an area as a result of a short term study, may turn out to be transients as a result of a longer term investigation. This situation was experienced in California where a population of coastal bottlenose dolphins (*Tursiops truncatus gilli*) was studied in San Diego first for a 17-month period (Hansen, 1990), then for a nine-year period (Defran *et al.*, 1992). While the short term study found that some individuals were permanent or seasonal residents of that particular area, the nine year study revealed a low number of re-sightings, and little residency (Hansen and Defran, 1990). In Monterey Bay, where the same population was studied for five years, during the first year of the study the re-sight rate of individuals was high and there seemed to be little immigration or emigration (Maldini and Kent, 1992), but the longer-term research is now revealing a much lower fidelity to that particular area than previously thought (Maldini-Feinholz, in progress). In practical terms, it is imprecise to define an animal's home range without stating the period of time that the home spans. This brings us back to the question of whether or not the concept of home range in the case of highly mobile aquatic animals is a biological reality in a temporal sense.

Size of the study area surveyed is also a factor in biasing conclusions about the home range of an individual. A small study area compared to the area used by an animal is not suited to a study of home range, and this term should not be used. For example, the coastal bottlenose dolphin population in California was believed to range from Ensenada, Mexico, up to Point Conception, but it was actually discovered that 38% of the population travelled as far as Monterey Bay (Scott *et al.*, 1993). In many instances we try to talk about an animal's home range when we are in reality talking about the amount of space the animal uses in the area we are sampling. A small study area may, in many cases, lead to fewer sightings, or a lower capture frequency for many individuals. If home range determination is our goal, we should progressively enlarge our study area according to sighting frequency and probability of sighting.

It became clear during the course of the workshop that there are no easy solutions to the conceptual difficulties involved with the definition of home range. In general, the need for longitudinal studies, bigger study areas and a more consistent use of terminology should be stressed.

As a first recommendation, it is useful to state the importance of frequent and close co-operation among researchers working in adjacent areas, when available. A direct comparison of photographs and data is the only way to gain perspective on the temporal, as well as spatial environment of a specific individual.

Combining photo-identification with radio and satellite tracking should add information on individual movements. Developments in acoustic monitoring will allow tracking of animals in this way. It was proposed that a probabilistic approach should be considered when describing use of an area by individual animals, where the distribution map of such an animal is based upon peaks of probability and probability contours.

In general, the concept of home range still presents many challenges to cetacean researchers, and a more standardised approach may become necessary. Our future goal is to increase co-operation amongst researchers dealing with similar species and/or constraints in order to gain some perspective and to compare techniques.

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3. Determination of group size

Giuseppe Notarbartolo di Sciara
Tethys Research Institute - Viale G.B. Gadio 2, 20121 Milano - ITALY

Two main approaches can be considered for the determination of group size in small cetaceans:

- a *stochastic approach,* necessary for large, mostly pelagic groups. In this case you are not interested in the determination of the exact number of individuals - this is often impossible in groups numbering several dozens, hundreds or even thousands of animals (for a discussion of this problem, see Scott and Chivers, 1990). What you really want here is an objective method, as accurate as possible and consistently precise, to measure the size of groups observed for a very short time (often instantaneously during aerial surveys).

- a *deterministic approach,* applicable to small, usually coastal, dolphin groups. You follow the animals for a long time, and want to know exactly how many individuals there are in the group. Furthermore, groups being dynamic units, you want to measure change in group size while you perform the observation.

For the purposes of this workshop, given the coastal nature and the usual small size of *Tursiops* groups (although, as pointed out by V. Cockroft, group size in bottlenose dolphins off South Africa may reach up to several hundred individuals), only the deterministic approach is being discussed.

For a partial review of concepts and definitions related to group size, see Shane *et al.* (1986, p. 39). Würsig's (1978, p. 349) subgroup is somewhat equivalent to what we currently intend as a group. According to Shane (1990, p. 247), a *group* (pod) is defined as "any group of dolphins observed in apparent association, moving in the same direction and often, but not always, engaged in the same activity". For Hansen (1990, p. 404), a group of bottlenose dolphins is simply "any aggregation of one or more dolphins". A useful working definition is given by Wells *et al.* (1980, p. 292), who define a group as "all dolphins sighted within an approximately 100 m radius. This generally accounted for all dolphins visible from the boat at any given time"; they then define primary group (PG) as "the smallest number of dolphins observed to be closely associating and engaging in similar activities", whereas "two or more primary groups often combined to form secondary groups [SG] for periods ranging from minutes to hours".

In general, the instant exact determination of group size in a species like *Tursiops* (coastal ecotype), where groups usually average 5-6 animals, is not the main problem. You follow the animals long enough, often in calm waters, get familiarised with the different individuals, and count them over and over until you are confident that you know how many animals there are. In this task you can often count on the additional support of photo-ID: when you do the matching later on, you reconstruct the composition of the group by listing the presence of different individuals (and often find out that you had underestimated group size in the field!).

The real problem consists in the dynamic character of groups, often described as fusion/fission (Goodall, 1986), showed by many *Tursiops* communities. Given that the size of the group keeps changing while you follow the animals, how do you measure it?

There seems not to be a simple way to solve this problem in *Tursiops*. Although on most occasions we do observe dolphins remaining in groups that are clearly social units showing some temporal stability (the PGs of Wells *et al.*, 1980), the size of which seems like a useful thing to measure when studying their social behaviour, these units too often break up or aggregate together, in such a way as to defy the systematic description and measurement of the phenomenon. The general impression is that the main problem lies in the fact that our general perception of a dolphin "group" is too coarse and simplistic in comparison to the dolphins' own perception of it.

A better knowledge of the bottlenose dolphins' social organisation must then be invoked to make some sense of this. One must understand what are the basic social units, the "molecules" of the dolphin society, those that are unlikely to break up, and this can be done only through an extensive study of the individuals' association patterns within a given community. Such "molecules" may consist, for instance, of adult male duos, or female matrilinear units composed of one to three adults and their offspring. These basic units may coalesce together in "compounds" (groups of 6, 10, or many more, thus becoming SG) for variable periods of time, depending on behavioural state, reproductive condition, food availability, presence of predators, migratory needs, etc, and then split again into the original "molecules" when conditions change. When faced with an SG, with dolphins scattered in small groups over a wide expanse of water, breaking the surface in all directions, the researcher is confronted with a formidable task, in which a stochastic approach must be adopted to obtain the best possible approximation to reality.

To complicate matters further, the basic units/"molecules" can also subdivide at the individual level, with single animals - the "atoms" of the dolphin society - taking off to go on their own, usually for a short time (minutes to hours), but occasionally to join other "molecules" for unknown lengths of time. It should be noted that a separation of an "atom" from the "molecule" may seem such to us; however, this may not be interpreted as a real separation by the animals themselves, who are capable of keeping in acoustic contact with each other over several kilometres (i.e., outside the observer's visual range).

To keep track of the dynamic history of *Tursiops* group size, several methods can be devised. For example:

- you subdivide your sighting in *sets* (Bearzi *et al.*, in prep.), each new set being determined by the joining or leaving of one or more individuals, and then average group sizes from each set to obtain a mean group size for that particular sighting. Disadvantage: this method is unweighted with respect to time, and results can be quite unrealistic (consider the example of a sighting made of two sets: the first of two animals lasting 9 h 55 min, the second of ten animals - with eight joining the initial two - lasting 5 min; you end up with a mean group size of five, although you've been for 99% of the time with two dolphins). Considering the mode instead of the mean does not seem sensible, as most sightings are made up of a very small number of sets.
- you sample group size regularly - e.g., every 3 min - from the beginning through to the end of your sighting. You then average your samples, and the mean will be your weighted group size for that particular sighting (Bearzi *et al.*, in prep.).

The fixed-interval sampling method has the advantage of providing the means for a consistent measure of group size, although its efficiency decreases with the increase in group size and somewhat breaks down in the presence of an SG. This option also allows one to gain a measure of the fluidity of any particular group, by considering the variance of the entire sample of group size values taken every 3 min. Group fluidity is yet another behavioural variable, which can provide useful insight into dolphin social ecology.

It can be concluded that the deterministic approach to the study of group size in a bottlenose dolphin community is subject for the moment to the following major constraints: 1) it makes little sense without considering the phenomenon in a dynamic perspective; and 2) it needs the support of a good knowledge of the individuals' association patterns within the dolphin community under study.

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4. Behavioural states: terminology and definitions

Giovanni Bearzi
Tethys Research Institute - Viale G.B. Gadio 2, 20121 Milano - ITALY

Introduction

If we want to compare behavioural data collected in different areas from different researchers, we should try to standardise the methods of data collection, by adopting general behavioural categories that can be used for any bottlenose dolphin community. Different subcategories can further be adopted for a better description of the many possible activities performed by the dolphins in specific areas.

We consider here only the behavioural data that can be recorded by observing the dolphin behaviour from a small research boat at close range, without the collection of other underwater or acoustic data. We would like to stress the importance of avoiding any personal interpretation of the functional meaning of the behaviour (e.g. "feeding") when the underwater activity is actually unknown, and we suggest the use of objective parameters.

Equipment

We recommend to record the field notes vocally on a portable tape recorder with 90 or 100 minute cassettes, using the tape recorder strictly for the notes relating to behaviour (i.e. not for behaviour *and* photoidentification). A digital alarm timer programmed for emitting an acoustic signal at fixed time intervals (e.g., every 3 min) is a very useful tool. Finally, digital watches for timing the behavioural data, and a digital chronometer for recording the duration of the dives, are also needed.

Procedure

In order to record behavioural data, we propose the adoption of the method defined by Altmann (1974) as *instantaneous sampling*, with the modifications proposed by Shane (1990): the continuous observation of the activity of the dolphin focal group for subsequent periods of 3 minutes, describing the behaviour at the end of each period by using conventional terms.

Other data, such as group formation, group size, speed, dive times, behavioural events, and field notes, can also be referred to the 3-min sample in which the variable was measured.

For the description of the behavioural activities occurring during the 3-min samples we propose the following definitions, some of them previously proposed by Shane (1990) and by Peharda and Bearzi (1993); the 30 seconds cut-off to discriminate between behavioural states was adopted according to dos Santos *et al.* (1990), and Peharda and Bearzi (1993).

Terminology and definition of the proposed behavioural states

Travel (T) - moving steadily in one direction, at the surface or close to it; no dives lasting more than 30 seconds within the 3-min sample; speed = 2.5 - 5 knots:

fast Travel (T+): speed > 5 knots

slow Travel (T-): speed < 2.5 knots (= REST: Shane, 1990)

Dive/Travel (DT) - moving steadily in one direction; at least one dive or portion of a dive lasting more than 30 seconds occurs within the 3-min sample; dolphins, while diving, move in the same general direction kept during the surfacings, also submerging in the direction of the movement, although the route may be not as linear as during *travel*; speed = 2.5 - 5 knots.

fast Dive/Travel (DT+): speed > 5 knots

slow Dive/Travel (DT-): speed < 2.5 knots

Dive (D) - no steady directional movement; at least one dive or portion of a dive lasting more than 30 seconds occurs within the 3-min sample; the pattern is characterised by cycles of long dives (usually lasting 2-4 min) and ventilations.

Surface Feeding (SF) - obvious feeding activities performed close to the water surface, characterized by *feeding splashes, feeding rushes, fish tossing, fish kicking* (Shane, 1990); dolphins are often seen catching fishes by pursuing them in parallel to the water surface with their belly up.

Following of Fishing Boat: active (FBa) - following of a trawling fishing boat, at about 100-300 m from the stern, presumably on top of the net; at least one dive or portion of a dive lasting more than 30 seconds occurs within the 3-min sample; the pattern is characterised by cycles of long dives (usually lasting 2-4 min) and ventilations while travelling in the wake of the boat.

Following of Fishing Boat: passive (FBp) - following of a fishing boat while trawling or navigating; no dives or portion of dives lasting more than 30 seconds occur within the 3-min sample.

Socialize (S) - some or all group members in frequent physical contact with one another, oriented towards one another, and often displaying surface behaviours; no steady directional movement; no dives or portion of dives lasting more than 30 seconds occur within the 3-min sample

Social Travel (ST) - moving steadily in one direction while socialising intermittently; no dives or portion of dives lasting more than 30 seconds occur within the 3-min sample.

Milling (M) - moving in varying directions in one location but showing no surface behaviours and no apparent physical contact between individuals; usually staying close to the surface; no dives or portion of dives lasting more than 30 seconds occur within the 3 min sample.

Topics to be defined or needing further investigation:

- is the 30 sec cut-off to discriminate among behavioural states appropriate?
- are these general behavioural categories appropriate for the description of all the possible behaviours performed by the dolphins?
- how should we consider the behaviours thought to be performed as a response to the research boat or to other boats or "unnatural" sources of disturbance?
- how should we consider 3-min samples where more than one behavioural activity occurs (e.g. 2 min of socialising and 1 min of diving)?
- how to deal with groups of dolphins where one or more individuals perform different behavioural activities (e.g. two dolphins are socialising while five are diving)?
- how to deal with very large groups, where many subgroups of dolphins may be engaged in different behavioural activities?

Summary of the discussion during the working group (Participants: G. Bearzi, S. Berrow, R. Bonaccorsi, S. Harzen, J.R. Heimlich-Boran, D. Herzing, C. Ridet, V. Ridoux, A. Silva, R. Sponer, F. Ugarte, C. Wood). As a result of the short discussion among the participants, the following guidelines were proposed:

Preliminary research

In the preliminary phase of a behavioural investigation only *ad libitum* sampling should be adopted, in order to understand the general behavioural patterns of the population under study. This phase could last for the first 1-2 years. During this time the researchers should try to observe the behaviour, take notes in the field, make preliminary hypotheses and test them.

Definition of methods

After this preliminary research, methods for behavioural data collection should be defined and standardised. Are the behavioural categories and methods used by other researchers adequate to describe the local situation? There are three possibilities: 1) the data collected are not sufficient to answer this question: the possibility to continue the preliminary study should be considered; 2) the general behavioural categories and methods used by most authors satisfactorily describe the situation, and could provide useful comparative insights; and 3) the methods used elsewhere don't fit the particular situation under study, and new methods have to be defined in order to describe properly the behaviour of the community under study.

The possibility of defining behavioural categories which can be considered as subsets of more general ones should be considered (e.g. "surface feeding" or "following of trawling boats" as a subset of "feeding").

Sampling methods

Both *ad libitum* and instantaneous sampling methods (Altmann, 1974; Shane, 1990) can be adopted. No guidelines were given concerning the most appropriate duration of the instantaneous sampling, but the 3-minutes interval first adopted by Shane for wild bottlenose dolphins was considered to be adequate. Four options were discussed: 1) the taking of 3-min instantaneous samples; 2) the taking of *ad libitum* samples; 3) the simultaneous use of both 3-min and *ad libitum* sampling methods, relating *ad libitum* notes to the 3-min sample of occurrence; and 4) the taking of 3-min samples and *ad libitum* notes in separate or alternate sessions, or by different observers, in order to be able to compare the data collected through these two methods. Most participants expressed views in favour of this fourth option.

Definition of behavioural states

A definition of the behavioural states based on dive duration or respiration patterns was proposed by G. Bearzi. The possibility to adopt such a non-subjective method could represent a good option, but has to be tested carefully (J. Heimlich-Boran).

Harzen argued that the 30-sec cut-off for distinguishing among behavioural categories proposed by Bearzi is too short. It can also be different for different bottlenose dolphin communities.

Conclusions

As a half-satisfactory conclusion of the meeting, it was stressed that it is not always necessary to force the standardisation of the methodologies in behavioural studies. Different methodologies can be adopted for obtaining valid results, which can then be compared at a more general level.

This is especially true for long-term studies, capable of affording a description of the situation based on long-term experience and adequate amounts of data.

For short-term investigations, the collection of data using standardised methods (e.g. methods used by experienced researchers working in similar situations) is advised, and can provide quantitative results which can also be used for comparative purposes.

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5. Behavioural sampling

Elena Politi
Tethys Research Institute - Viale G.B. Gadio 2, 20121 Milano - ITALY

Different methods to sample behaviour categories in wild animals have been developed in the past thirty years, and Altmannn (1974) gives an exhaustive review. These methods are:

- *Ad libitum sampling:* Altmann describes it as the "typical field notes", with the observer recording as much as possible, choosing behaviours, individuals and often the times for behaviour recording sessions on an *ad libitum* basis. It is a non-systematic procedure, and therefore it hardly permits comparison among different data sets.
- *Focal animal sampling*: in this case: 1) all occurrences of specified actions of an individual (focal animal), or specified group of individuals (focal subgroup), are recorded during each sample period, that is of predetermined length; 2) a record is made of the length of each sample period and, for each focal individual, the amount of time during the sample that it is actually in view.
- *All occurrences*: the aim of this method is to record all occurrences of certain classes of behaviour in all the members of the group during each observation period.
- *Sociometric matrix completion*: this method is used in those studies where the object is to establish, for each pair of animals, the direction and degree of some relationship. It focuses on individuals and it is recommended as a support to the focal animal sampling method.
- *Sequence sampling*: in sequence sampling of the focus of the observation, instead of the individual, is an interaction sequence. Usually a sampling period begins when an interaction begins, and the sample continues until the sequence terminates.
- *One-zero sampling*: the observer scores whether a behaviour occurs (one), or not (zero) during a short interval of time (sample period).
- *Instantaneous and scan sampling*: it is a technique in which the observer records an individual current activity at preselected moments in time (e.g., every minute on sample session. In the past, behavioural studies on free-ranging bottlenose dolphins were mostly based on a non-systematic *ad libitum* technique; either these were opportunistic descriptions or quantitative approaches (Shane, 1990b).

The first consistent attempt towards developing a systematic and comparable sampling of bottlenose dophin behaviour has been described by Shane (1990a). She adapted the focal animal sampling method described by Altman (1974) to the particular situation of free-ranging dolphins, considering a focal group as a "group of dolphins observed in apparent association, moving in the same direction and often, but not always, engaged in the same activity" (see Chapter 3). To the focal group three different methods were applied, by recording: 1) the ongoing activity during the sample unit, every 3 minutes (defined as instantaneous data); 2) the duration of a certain activity performed by the group during a period of observation (duration data); and 3) all occurrences of surface behaviours during randomly selected 15-minute sampling periods. The comparison of all the data obtained from instantaneous and duration samplings brought the conclusion that although the results were significantly different in a statistical sense, "they did not appear to be significantly different

from a biological point of view". Despite this resulting homogeneity, Shane remarked that the instantaneous data best reflected the dolphins' behaviour.

Shane did not explain the reasons for her choice of 3-min samples; however, this seems to be the best compromise for sampling bottlenose dolphin behaviour: 1) it is short enough to allow, most of the time, the recording of one behavioural state during each sample unit; and 2) there is a high probability (bottlenose dolphin dives usually lasting 1-2 min) of seeing the dolphins at least once during the sample. However, Shane noted two potential problems that affect this sampling method: 1) it can happen that, during the sample period, the activities are not homogeneously performed by all the dolphins; in this case mixed categories (e.g., social/travel) were taken into account; and 2) during the entire sample period the group was submerged; in this case only an arbitrary rule could be applied, such as recording, for the sample in which dolphins were not sighted, the last observed activity. In the same way, another community of bottlenose dophins has been the object of behavioural studies, in which the methodologies described by Shane, partially modified, have been applied (Bearzi and Peharda, 1991; Bearzi *et al.* 1993). In this case, different behavioural categories, performed by the focal group, were recorded on samples of pre-determined length (3 minutes); simultaneously, the instantaneous behaviours (surface behaviours in Shane 1990a) were recorded in real time as all occurrence events, within a 3-minute sample, maintaining their sequence order.

Data gathered using these sampling methods allowed one to describe the dolphins' behavioural budget and frequency of occurrence, in relation to variables such as time of day, season, water depth and temperature, dophins' group structure and composition (Shane 1990a, Bearzi *et al.* 1993). Thus, their advantages consist mostly of a systematic approach, that permits a comparison of data coming from different studies, and a relatively easy way to describe major trends in behaviour of bottlenose dophins.

Still, the nature of the observation in itself limits drastically the knowledge of dolphin behaviour. As mentioned above, the observation of dolphins only when surfacing, does not permit an accurate scan of the activities performed underwater. Thus, the use of different techniques, such as video cameras (both at the surface and underwater) and acoustic devices, may lead us to gather more information on these animals' behaviour. Furthermore, more detailed insights can be obtained if the systematic sampling is performed also on focal individuals (such as mother-calf pairs and identified individuals that are found permanently associated with others), applying different sampling methods. In this case, information on different sex-age classes can be assessed in terms of frequencies, sequences and duration of the behaviour in relation to different variables.

During the discussion among the participants to the workshop, the following points came out:

- The choice of a particular sampling method depends on the nature of questions we want to answer.
- In a preliminary phase of a behavioural study, the observer must understand the general behavioural patterns of the population under study. Secondly, quantitative and standardised sampling methodologies can be applied, with the definition of behavioural categories. Moreover, behaviours cannot be generalised to the five categories that are always mentioned in the literature (travelling, playing, socialising, resting and feeding), since some differences in population behaviours may occur at different locations.
- The importance of using video cameras in order to record dolphin behaviour on long time sessions. The videos with the tape counter give to the observer the opportunity of analysing the behaviour a second time, thus permitting the formulation of new questions. This technique is also rather cheap.
- Acoustic techniques can give more information about the nature of a given behavioral category.

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6. Behavioural Data Analysis

Ben Wilson
University of Aberdeen, Lighthouse Field Station, Cromarty, Ross-Shire, IV11 8YJ - SCOTLAND

Standardisation of behavioural data analysis

An agreed protocol for analysing behavioural data that is used by each research group working on *Tursiops*, sounds like a very desirable goal. However, like the behaviour of the dolphins themselves, the range of analyses that have and can be performed on behavioural data are enormous. Output from different studies, for example, have ranged from simple descriptive work to complex modelling.

In contrast to the general theme of this workshop therefore, behavioural data analysis, *as a whole*, does not appear to be a good candidate for standardisation. This is because (in addition to the size of the subject area alone) the analyses of these data must give us the flexibility and room to test our hypotheses. The depth of this exploration will inevitably vary between studies due to, for example, the amount of information available and the particular questions being asked. Some sort of fixed protocol will obviously leave interesting aspects of each study unexplored.

Having said this, there are undoubtedly several *more specific* areas of research, like the analyses of social structure or the classification of home ranges, where comparable data have been analysed and presented in many arbitrarily different ways in the past. It is these specific areas which require our attention so that the results of our research can be made comparable. Several of these areas were discussed in earlier sessions of this workshop. A further topic came to light during this session.

It concerned the description of group (school or herd) size. At present, even if the definitions of groups which have been used by different researchers were identical, the variety of methods used to describe them, make any formal comparisons impossible. To date, the following (amongst others) have been used: the range; the most common sizes, the mean (along with standard deviation or standard error), and the median (with interquartile range).

Which of these is the most suitable? The first, the *range of values* expression, is of little benefit as it is dependent on the size of the data set being used and does not give any indication of where a central tendency might lie. The expression of the *most common sizes* is also unsuitable as it lacks a definition and therefore cannot be used in comparisons. This leaves the *mean* and *median* expressions along with their appropriate measures of variability.

In the majority of studies, the frequency distributions of group sizes are highly skewed (see Fig. 1); generally there is a greater range of values above the mean than below it. For these kinds of distributions, medians (unlike means) are not over affected by the few, very high value observations on the extreme right of the distribution. It would therefore seem sensible to use medians and inter-quartile ranges as the common descriptors for group size information. Unfortunately, however, mean values have been used in many previous studies, making comparison difficult. To get around these problems, I suggest that authors presenting group size data should show both of these parameters either in a table or by illustrating a frequency distribution of the type shown in Figure 1. The use of an illustrated frequency distribution has the added advantage of allowing other researchers to statistically test the difference in group sizes between studies rather than leaving them to simply compare median or mean values.

If other similarly problematic areas of data analysis come to people's attention, make them known and they too can be discussed via this forum, either through the newsletter or future *Tursiops* workshops.

General points relating to the analysis of behavioural data.

Included here are some general points which arose during the session and are worth consideration when starting a project which involves behavioural data:

1. Consider the data analysis from the very start of a research project:

a) Formulate clear hypotheses or objectives for your research. This will help reduce the number of variables which you will need to record during field work. This approach also encourages the formulation of new research tools and multidisciplinary approaches.

b) Carry out a simple power analysis to estimate the necessary number of samples which will have to be collected to address your research objectives. This will firstly establish whether the project is possible in the time available and secondly will indicate how many resources will be required to carry it out (funding, boat time, field assistants, etc.).

c) Consider how you are going to analyse the data that you are about to collect. If necessary seek statistical advice before you start.

2. At the time of data collection, begin a preliminary data analysis. By doing this you will know when sufficient information has been collected to address your hypothesis. Once this point is reached, you can stop or move on.

3. When carrying out comparisons between different populations, try and quantify the amount of variation which already exists within each. Variation may be temporal; spatial or it can occur between individuals.

4. The analysis of data may take considerably longer to perform than one might at first suspect. Adequate time for these analyses must be allocated if justice is to be done to the data collection effort and to the animals.

Recommended reading (* highly recommended):

Altmann, J. (1974). Observational Study of Behaviour: Sampling Methods. Behaviour, 49:227-267.

Cohen, J. (1977). Statistical Power Analysis for the Behavioural Sciences. Academic Press, New York. ISBN 0-12-179060-6.

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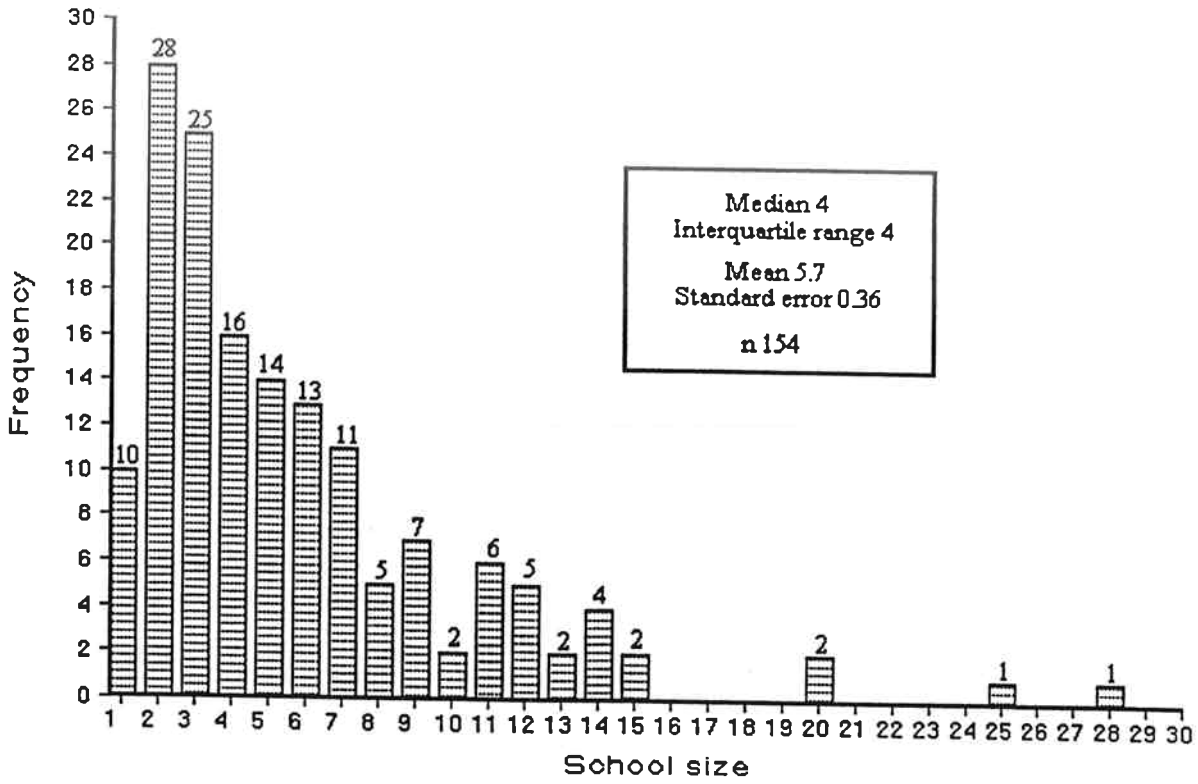

Figure 1. A typical frequency distribution plot of bottlenose dolphin school (or group) size. (fictitious data).

7. Analyses of associations and social structure

James R. Heimlich-Boran and Sara L. Heimlich-Boran
Zoology Dept, Cambridge Univ, Downing St., Cambridge UK CB2 3EJ
DELPHIS, 7 Victoria Street, Cambridge UK CB1 1JP

The duration of samples over which associations are summed depends on the questions of dolphin social organisation being addressed. At one extreme, some killer whale researchers study fine nuances of associations within permanent pods using instantaneous samples of associations recorded in a single frame of film (Bigg *et al.*, 1990). Studies of the fluid associations of dolphins also require short (<15 min) scan samples. In contrast, our pilot whale studies used day-long sample periods to define the broad social group. The choice of the duration of the sample period is also related to the independence of the samples. If the groups are located too closely together in time, the group compositions may be interrelated and dependent. These potential biases must be clearly stated in association studies.

Another critical consideration is whether more than one group of animals will be located within a single sample period. The definition of such groups must be clearly stated in terms of distance or behavioural synchronisation. Sample durations should be short enough to ensure that the groups have not had time to change composition, yet long enough to ensure a complete sampling of the individuals.

Indices of Association

There are three primary association indices currently in use (Cairns & Schwager, 1987):

$$\text{Simple Ratio} = \frac{x}{x+y_{ab}+y_a+y_b}$$

$$\text{Half Weight} = \frac{x}{x+y_{ab}+1/2(y_a+y_b)}$$

$$\text{Twice Weight} = \frac{x}{x+2y_{ab}+y_a+y_b}$$

where: x = times A and B are located in the same group
y = times A and B are in separate groups
y_a = times only A is located
y_b = times only B is located
y_{ab} = times both A and B are located separately

All range from 0 (two dolphins never seen together) to 1 (two dolphins always seen together).

Since it is often not possible to locate all individuals in every sample, the different types of "y" sightings, when the pair is not located together, are isolated. The "y_{ab}" term allows for the situation when multiple groups are identified within a single period.

Ginsburg & Young (1992) considered the Simple Ratio index to be the least biased. The Half Weight Index tends to overestimate associations since it averages the counts of y_a and y_b, thus reducing the denominator. The Twice Weight Index tends to underestimate associations since it double counts the samples in which members of the pair are located separately (y_{ab}). The Simple Ratio Index quantifies an association as a proportion of the number of times a pair was seen together compared to the number of samples in which either member of a pair was sighted. Earlier studies tended to use the Half Weight Index (S.L. Heimlich-Boran 1986; Wells 1986), but recently the Simple Ratio has been employed (J.R. Heimlich-Boran, 1993; Slooten *et al.*, 1993).

Cluster Analysis

Following the tabulation of association indices, it is necessary to conduct a cluster analysis. The most common clustering method is "hierarchical" and "agglomerative", meaning it begins with N individuals as N discrete "clusters" and proceeds to link the highest associated pair. This creates N-1 clusters. This process continues until all individuals are linked into one large cluster. This creates a continuum of possible clusters and there are no clear methods to determine what is a statistically significant level of clusters. We used a method which looks for jumps in the steadily declining association indices used to form more distant clusters, and then uses this to set a level of "significant" associations (Heimlich-Boran, 1993).

A major problem of cluster analysis is "chaining", where, for example, A and B are first linked and then C is linked to this pair because of a high association with only A. Thus, although there may be no association between C and B, they are in the same cluster.

The end result of cluster analysis is a graphical representation of the association data. Dendograms are the most common form, but maximum spanning trees and multi-dimensional scaling diagrams are also used. These all provide a visualisation of the patterns of association which may be used to understand social organisation.

Slooten *et al.* (1993) have recently applied a new technique, termed a "reassociation rate" (after Whitehead *et al.*, 1991), to Hector's dolphin associations. This tests whether the continued association of the same pair occurs differently from random. This technique holds promise.

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8. Photoidentification: field methods

Giovanni Bearzi
Tethys Research Institute - Viale G.B. Gadio 2, 20121 Milano - ITALY

The basic recommendations for conducting photo-identification studies in the field are summarised, and suggestions are given in order to obtain a standard procedure for taking and filing the photographs.

Approaching the dolphins

A small (4-6 m) and manoeuvrable boat reduces disturbance. A noiseless engine (e.g. Honda 4 strokes, 45 HP) is preferable. Sudden and erratic changes of speed and direction should be avoided.

It is best to approach the dolphins by gradually converging on their route from the side which allows best photo opportunities with respect to light.

Never drive through the school or in front of it; do not force the approach if the dolphins show signs of being disturbed. Don't try to get too close to the dolphins; rather, let them approach you. Take your time and don't hurry up if the approach is not easy: let them get used to your boat

Equipment

Autofocus lenses are now definitely fast enough for use on small cetaceans: we suggest the use of 35 mm cameras provided with a very luminous zoom such as 80-200 mm f 2.8 (the ones produced by Minolta, Nikon and Canon are among the best); luminous lenses allow one to photograph under most light conditions and with a faster shutter speed; luminous, high-quality lenses, although expensive, reduce film wastage, and the economic advantage of such an "investment" should be considered; telephoto lenses such as 300 mm f 2.8 are also OK, if you can afford them, but they are pretty heavy and less easy to handle.

A motor drive or power winder is highly recommended; a speed of three frames per second is usually enough to get three photographs of a dolphin during a single surfacing (blowhole-back, dorsal fin, peduncle).

Use a shutter speed of 1/500 or greater; 1/1000 is usually the most appropriate for photographing dolphins in motion with a 200 mm lens.

The use of a data back is recommended; we would suggest printing the date on a marker ("blank") photograph at the beginning of each roll and of each sighting, and the time on all the photographs relating to the same sighting.

Film

We recommend the use of colour slide films of 100 ISO speed; colour and fine grain are necessary to provide adequate information on animals that can often be recognised only by very subtle details.

Kodachrome 64 is among the most used and recommended of colour slide films; Ektachrome EPR 64 or Fujichrome Velvia 50 are cheaper, can be home-rolled and are developed in a shorter time in most European countries. Use a good professional laboratory for the development of the film

Marker photographs (blanks)

To separate field events and allow reconstruction of the sighting history, use blanks for identifying each roll, and for marking the beginning and the end of the sighting. Use a tape recorder to relate every blank to date, time, subject, and field event. Always try to use a different subject for blanks, if you have enough fantasy or subjects available; otherwise shoot a board with written notes.

Photo-identification notes

Use a small portable tape recorder for taking notes in the field; we suggest using it strictly for the notes relating to photo-identification, and that the behavioural notes be taped on a different recorder.

Extensive use of field notes is recommended, especially to identify the blank subjects, to time the field events (e.g. the changing of a roll), to relate the pictures to the focal group, to define particular features that could go undetected during the slide analysis, and to describe mother-calf associations, etc.

All notes taken in the field should be transcribed on appropriate forms (Fig. 1) as soon as possible after the end of the sighting.

Sighting, Set

The size and composition of a group of dolphins is likely to change while it is being followed, and it is necessary to consider a group of dolphins as a dynamic unit (see Chapter 3).

For many research purposes, it is useful to relate the photo-identification data to such units, because the rough knowledge about *all* the dolphins encountered during a photo-identification survey may be insufficient to describe more complex relationships.

The definition of the conventional term *set* is a useful tool: we suggest defining set for the particular focal group we are observing and photographing. A set is composed of a defined number of dolphins potentially photo-identifiable, belonging to definable age classes; the set changes when the number of animals or the composition of the focal group changes (see Chapter 3). A sighting can include one or several sets; successive sets of the same sighting must share at least one individual dolphin.

Field procedure

1. First step: count the dolphins. Since the photographer cannot easily concentrate on both group size determination and photo-identification, especially with large groups, some assistance from another experienced member of the crew is usually needed.
2. The photographer should know and note which animals are likely to have been "captured" and which ones have not; to take pictures at random leads to possible over-representation of the most approachable animals.
3. Take several photographs of each individual to ensure that photographs suitable for photo-identification are obtained; we would recommend shooting at least 5-10 photographs/animal at close range.
4. Take always many pictures of all the animals, even if some of them are well-known and have already been identified during previous surveys. This will help to document changes in patterns over time. Never consider a field identification without photographs to be sufficient, not even for heavily marked and very well known animals.

5. If the set changes, photo-identification work has to run again: the goal is to identify all the dolphins of all the sets of a sighting.
6. When all the animals in the group have been "captured", you can either leave the group and look for other dolphins, or follow the same dolphins waiting for a change of set; the second option is recommended for socio-ecological studies, and in places where the density of dolphins is low.
7. According to our experience, we would not recommend one to assume that a group is entirely identified if every identifiable individual is represented by a minimum of four usable photographs ("complete sighting group": Ballance, 1990; Würsig and Jefferson, 1990). This may be too intercorrelated with the degree of "confidence" that each individual dolphin has with the boat, and biased by fortuitous factors: some dolphins (e.g. mothers with newborns) are sometimes almost impossible to approach, and no useful pictures of them can be obtained, while other members of the same group can be easily approached and identified.

Photo requirements

1. Try to shoot individual animals alone, not in groups: that complicates the slide analysis.
2. Take photographs of mother-calf pairs together.
3. Photos should be taken as perpendicular to the body axes as possible, and the back of the dolphin should not be arched (e.g. before a steep dive).
4. Try to get photographs of the whole body, not solely of the dorsal fin: scars and other marks on the body can be associated with the dorsal fin shape, becoming useful during the slide analysis.
5. Back-lighted, sharp pictures of the dorsal fin are also often useful.
6. Photo requirements change according to the kind of information one wants to get: for the mark-recapture method, for instance, only high quality pictures should be used, for ensuring equal catchability. For other purposes even poor quality pictures can be used to maximise the amount of available data: judge separately photographic quality and recognisability.

Selection and filing of the slides

1. After the rolls are developed, use the marker photograph at the beginning of each roll for ordering the slide containers chronologically, also with the help of the forms. Write a progressive number on each slide container (avoiding flying sheets).
2. Write on each slide: name (initials) of the photographer, date of the sighting, progressive number of the sighting, number of the set (Fig. 2)
3. Make a conservative selection of the photographs (i.e. discarding only the truly useless slides).
4. Be careful to maintain the chronological order for all the photographs.
5. Write a progressive number on *all* the slides, eventually restarting by 1 at the beginning of each year/research season.
6. Use two separate catalogues: a chronological catalogue including all the slides with sufficient quality taken in the field, and a catalogue for the "type specimen" of each identified animal.
7. Catalogues (Hammond *et al.*, 1990) should be: 1) periodically re-assessed; 2) updated to include the best and more recent photographs (also for documenting any changes in patterns over time); and 3) include photographs of all identifying features, not just the shape of the dorsal fin.

Analysis of the photographs within the same sighting: the reason for a conservative selection of the photographs

During the analysis of the pictures taken during the same sighting, many features that are usually not considered elsewhere can provide useful information. For instance, although many

marks and light scratches on the body can be temporary (and therefore useless for the permanent identification of a given individual), they can be extremely useful in making sense of which dolphin is which in any particular sighting.

The field estimate of group size should match the number of dolphins (temporarily) photoidentified. According to our experience, photoidentified animals often turned out to be more numerous than what we had estimated in the field (especially for large groups).

The analysis of the photographs should also confirm the field estimate of the major age classes.

Match the pictures within the sets first, then among successive sets. Pictures showing only temporary marks may match pictures where permanent marks are readily visible, taken in another set of the same sighting.

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Tethys Research Institute

page _____

date / /19 photographer body: ☐ 8000i ☐ 7000i ☐ other _____

ROLL N.	time	frame/s	X if blank	sigh,set	TOPIC	notes

NOTES

Figure 1. An example of photographic form.

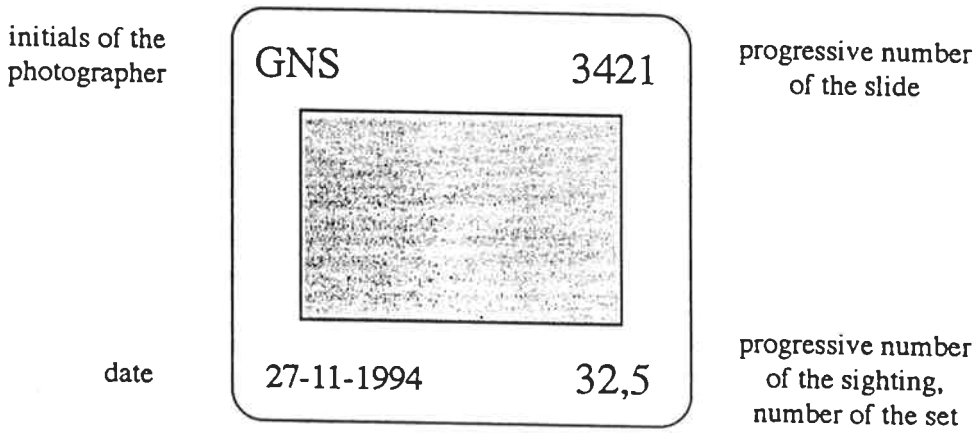

Figure 2. Information on a filed slide.

9. Photo-identification: matching procedures

Giovanni Bearzi
Tethys Research Institute - Viale G.B. Gadio 2, 20121 Milano - ITALY

The following notes were compiled based on a seven year-long (1987-1993) field experience with coastal bottlenose dolphins, totalling 613 hours spent in direct observation and photography of 639 groups of different size and composition. Over 16,000 photographs were taken during this study, 12,000 of which catalogued and matched. Given that our experience relates to a small community (128 animals catalogued to date), some methods discussed here will not apply to larger communities. The method presented here is by no means intended to be definitive. We would like to share our experience with all interested groups, to generate discussion, and help improve procedures.

Equipment: large light table; slide lens; slide projector with zoom lens.

Step-by-step matching procedure suggested:

1. take the first slide of the sighting (in chronological order) and place it on the light table;
2. match the first slide with the second, and check if in them the same animal is represented; if yes, place them on the same row, otherwise begin a new row;
3. match the following slides with the previous ones, one at a time;
4. try to match as many photographs as possible, using all visible body marks;
5. if all individuals in the group have been photographed, you should have a number of rows equal to the number of dolphins composing the group (and the field estimate of the group size, if correct);
6. consider the best slides from each row, and match them with the dolphins already catalogued;
7. a catalogue of drawings of the dorsal fin shapes, classified according to fin type, can help to determine the dolphin's identities. However, always match photographs with photographs, never photographs with drawings;
8. match all the photographs available for each dolphin, considering all body marks, with the photographs in the catalogue;
9. try to match the photographs on the light table with the photographs taken most recently, to use the presence of subtle body marks;
10. before concluding that a match exists, consider as many body marks as possible, and possibly not the shape of the dorsal fin alone: always try to use dorsal fin together with other, independent body marks (scars, spots, nicks on the peduncle etc.);
11. keep in the catalogue a wide collection of good photographs, showing many parts of the dolphin's body and dorsal fin under different light conditions;
12. if you think that you have found a new individual, use the slide projector to project its best slide on a wall or board, zoom until the fin fits a standard size, and trace the dorsal fin's profile on a sheet of paper;
13. match once again the drawing with the catalogue of drawings, considering possible changes in the dorsal fin's features (developing of new nicks, etc.); check (by using the slides) all the animals which look similar;

14. if the new dolphin does not seem to match any of the previously identified individuals, then try to match the new dolphin's slides with those of *all* the dolphins in the catalogue;
15. avoid long sessions at the light table; you will be more efficient with a fresh mind.

Poorly identifiable dolphins

Poorly marked animals can be filed separately and matched among themselves based on temporary marks. The resulting information can be useful for some purposes (i.e. mother-calf associations, confirmation of the group size estimated in the field, etc.).

Data about resightings of these dolphins should not be included in the analysis of the data concerning well identifiable animals carrying permanent marks.

10. Methods for cataloguing identified delphinids

James R. Heimlich-Boran and Sara L. Heimlich-Boran
Zoology Department, Cambridge University, Downing Street, Cambridge CB2 3EJ, UK
DELPHIS, 7 Victoria Street, Cambridge CB1 1JP, UK

The identification of individuals from naturally-occurring marks and scars on the dorsal fin and back has become a commonly-used technique in studies of wild delphinids, providing insight into social behaviour and population dynamics. These results come from long-term censusing, continually comparing newly collected photographs with existing ones to accurately re-identify known individuals and to confidently name new individuals which cannot be matched. Over the long-term, especially if study populations are subject to immigration, a systematic method for cataloguing the accumulating photographs and the identifying characteristics of individuals is essential.

Filing and Storage of Photographic Raw Data

A complete filing and reference system is needed for the storage and retrieval of the raw film data. Each film needs to be uniquely named or numbered. A computer database should be created to store every possible type of information related to the films: date, time, platform, photographer, etc. Each frame of film should also have a separate reference number so that when identifications are made, they can be easily relocated. All materials, whether colour transparencies or black-and-white negatives, should be stored in clear, "archival quality" storage sheets which allow easy viewing of the images without removal from the sheets. These can be stored in ring binders or file drawers for easy access.

Identification catalogue

For efficient review of new films to identify individuals, a system is needed for viewing the raw data. Colour transparencies may be projected. Contact prints may be made of back-and-white negatives, or the negatives may be viewed directly through a dissecting scope. We recommend the use of film/video processor, which can project negatives or positive transparencies onto a TV monitor. Negative images may be converted to positive images and zoom lenses allow details to be examined. It is also possible to record selected images onto video tape (see below).

As unique individuals are identified in the films, it is essential to create a master catalogue of the best image of each individual's identifying characteristics. This allows more than one person to view the images at the same time and can serve as a backup copy of the most important images. We print 5" x 7"(13 x 18 cm or A5) photographs for a print catalogue. Artistic researchers have traced projected dorsal fin images to be used in similar ways.

Categorising identifying characteristics

Identifying characteristics vary from highly distinctive to extremely subtle. The most commonly (and safely) used identifying characters are nicks in the dorsal fin. These have the benefit of being visible from both sides of the animal and do not change over time. General dorsal fin shape

may also be a characteristic, but this can change appearance drastically with different viewing angles. Scratches and scars on the dorsal fin and back are less distinctive since they are usually only visible from one side, can change over time due to healing and can vary appearance in different lighting conditions. Used in combination, these characters can identify a large proportion of individuals, especially given high quality photographs.

A number of techniques have been used to categorise identifying characters to reduce the initial pool of existing individuals which must be examined for matches with new photographs. In our studies of pilot whales (over 500 have been identified: Heimlich-Boran, 1993), we have used 24 categories based primarily on the presence, absence, location and size of dorsal fin nicks, but also including a few unique dorsal fin shapes (e.g. NM = Large Nick in Middle third of fin, HOO = Hooked fin). Defran et al. (1990) presented a "dorsal fin ratio", which can be used on animals with two or more fin notches and is based on the relative location of the top two nicks to the top of the fin. These ratios (or dorsal fin categories) can be sorted in a database and used to locate similarly-marked individuals. Whitehead (1990) used a digitised tracing of the trailing edge of sperm whale flukes which has potential for use with dorsal fins.

Digital image storage

As the number of identified individuals in a catalogue grows, it may be necessary to consider storing digital images on computer or video. Killer and humpback whale catalogues have been recorded onto video disc which allows quick retrieval (KC Balcomb, pers. comm.; Mizroch *et al.*, 1990). We are currently examining the potential of Photo CD format for storage of the pilot whale catalogue. Films can be scanned onto CD by Kodak and then manipulated in software such as Adobe Photoshop. Image databases could be used to store the relevant categorising data. This has the added benefit of allowing desktop publishing of a final catalogue. Unfortunately, all this requires large amounts of expensive computer memory. Eventually, digital cameras will completely replace the need for film, allowing images to be stored directly in digital format.

Computer Automated Matching

The technique of automated computer matching of individuals has been most successfully applied to grey seals, using a 3-D model of a seal head (Hiby & Lovell, 1990). With the appropriate reference points of eye, ear and nostrils, any photograph can be matched to the model. Grey scale intensities are then compared in a numerical matrix to provide matches. The application of a similar technique to dorsal fins is difficult because there are no fixed reference points to standardise photographs before comparison. Additional development of image recognition technology will be needed before dolphin photo-identification studies become fully automated.

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11. Biopsy sampling from free-ranging bottlenose dolphins

V.G. Cockroft
Centre for Dolphin Studies, Port Elizabeth Museum & University of Port Elizabeth
P.O. Box 13147, Humewood 6013, SOUTH AFRICA

Tissue samples for genetic analyses are collected for a number of reasons, and the methods of genetic analysis used provide different types of information. Briefly, biochemical genetic analyses can be used to obtain information at varying levels. It can be used as a taxonomic tool, where in conjunction with morphology and meristics, one obtains information on taxonomic relationships. As an adjunct to this type of research, information on the relationships within species, at the population and/or stock level, can be obtained. Lastly, but often of more immediate importance, genetic analyses can provide information on kinship, sex structures and affiliations within and between groups of animals.

Tissue samples for genetic analyses can take various forms, depending on the material available. Sampling tissue from stranded or incidentally captured animals will depend on the state of the animal/carcass. From live animals, blood samples are least invasive and provide an ideal source of genetic material. From freshly dead carcasses, any tissue can be collected, although dried skin is not recommended; from the carcasses of animals that died several days prior to sampling, it is recommended that skin from inside the blow-hole be collected. Other than fresh blood, which can be frozen, any collected tissue should be stored in alcohol, DMSO or similar preservative.

From free-ranging animals, the preferred collection method is the biopsy. Essentially, this entails removing a sample of skin and sub-cutaneous tissue from an animal or animals. The recommended procedure, and that used most often, is a specially designed biopsy taking tip, which is either attached to the tip of a hand held lance and 'plunged' into the animal, or is attached to a projectile and 'shot' into the animal using either a crossbow (50 to 60 kg pull) or gas operated gun (the sub-cutaneous tissue - blubber - obtained from biopsies of free-ranging cetaceans can also be used for toxicology analyses). Biopsy sampling is cheap and relatively easy in comparison to capturing animals for tissue samples.

Essentially, a biopsy dart consists of a biopsy tip attached to a projectile of some sort. The tip, as described here, is the most important component of this system. The biopsy tip is a 10 mm outside diameter stainless steel tube, with an 8 mm inside diameter (the specifications given here are for what we have found works best and can be changed according to specific needs). The one end is bevelled, to provide a cutting edge and allow penetration into the skin. A barb is placed internally of the cutting end, about 8 mm down from the bevelled tip and retains the sample after penetration. To prevent the tip penetrating too far into the animal, a stainless steel washer is attached (welded or silver soldered) onto the tube, about 33 mm down from the bevelled tip. A section of high pressure, rubber tubing is fitted over the penetrating end, resting against the washer; it extends to about 5 mm from the cutting edge (bevelled end), allowing the tip to 'bite' when it hits the animal. The rubber tubing not only prevents too much penetration, but it also provides back pressure when the tip strikes and penetrates the animal, aiding 'bounce back' or extraction. This assembly is attached to a standard crossbow bolt (or lance). This attachment can be fixed or allow 'quick release'. For easy retrieval of the crossbow bolt after shooting, a standard, egg shaped fishing float is glued to the anterior end of the bolt, just behind the biopsy tip. The float is just big enough to allow the biopsy tip assembly and bolt to float.

The entire cost of this assembly, with crossbow bolt, is approximately US $10!

Prior to shooting, the tip should be washed/cleaned in hydrogen peroxide and a broad spectrum antibiotic. About six of these biopsy tip and bolt assemblies is sufficient for extensive sampling. A team of three to four people is sufficient for sampling. Other than the boat skipper, one or two individuals extract and clean the assemblies and pass cleaned assemblies to the individual shooting. Samples are removed with forceps and stored in the appropriate preservative (See Chapter 12). Should the removal of a sample from the tip prove difficult, the biopsy tip can be removed and replaced with an unused tip.

Obviously, when working with free-ranging animals from a boat, all care should be taken to prevent undue disturbance and harassment of the animals. Our long-term observations suggest that biopsy sampling using this technique and a crossbow has little discernible short- or long-term effect on dolphins.

12. A brief summary of molecular genetic tools and techniques for marine mammal studies

Barbara E. Curry
South West Fisheries Research Center, National Marine Fisheries Service
La Jolla Shores Drive, La Jolla, California 92037, USA

The following summary is intended to serve as an introduction to the types of procedures used for genetic analysis of marine mammal tissue samples.

Tissue preservation

Skin, blood, and organ tissues can all be useful for genetic studies. Tissue can be kept frozen, but, recently, researchers have found that storing skin and organ tissue samples in a saturated salt solution with 20% dimethyl sulfoxide (DMSO) is a convenient way of archiving, and allows samples to be readily preserved in the field.

Once the samples are in the laboratory, a variety of methods may be used to extract DNA from the tissue. Researchers use both mitochondrial DNA, which is maternally inherited, and nuclear DNA, inherited from both parents, to study population structure and relationships between animals.

DNA Structure

The DNA macromolecule is a double helix with the complementary strands linked by hydrogen bonds. These bonds can be readily broken by heating for a few minutes at 95°C to 100°C and will re-form at about 65°C. Nucleotides, the basic units of the molecule, are covalently linked to their complementary base on the other strand, by hydrogen bonds (adenine to thymine, cytosine to guanine).

Restriction Enzymes

Restriction endonucleases cleave DNA molecules at specific recognition base sequences (restriction sites). There are hundreds of known endonucleases that have been isolated from different bacterial species. Lengths of sequences recognised vary from four to six base pairs (bp).

Restriction endonucleases are used as a tool to obtain fragments for DNA fingerprinting and for examination of *Restriction Fragment Length Polymorphisms* (RFLPs), or different DNA fragment lengths amongst individuals. The polymorphisms result from mutations at restriction sites that cause endonucleases not to cut at that site, as well as mutations that create new restriction sites. The technique allows survey of genetic variation across a region of DNA.

Polymerase Chain Reaction

Short sequences (approximately 15 to 36 bp long) known as *primers* are designed to anneal on either side of the target sequence. The target sequence is denatured, the primers are annealed to the

target sequence, and a copy is produced with the use of a DNA polymerase. *DNA polymerases* are enzymes that catalyse the formation of a complementary DNA strand. The cycle of denaturing, annealing, and extension is repeated so that many copies are made from the original DNA and its copy. Repeated cycles allow exponential amplification of the target sequence.

DNA Sequencing

Description of the technique - The "chain termination" method of DNA sequencing, employs the controlled interruption of enzymatic DNA replication. The double-stranded DNA is denatured to produce a single stranded DNA template. A primer is annealed to the template. The preparation is divided into four subsamples; each has all deoxynucleotides (A, C, G, and T), radioactive label, and DNA polymerase. In addition, a dideoxynuceotide (ddA, ddC, ddG, or ddT), lacking the 3'OH group necessary for chain elongation, is added to each of the subsamples. The primer has a free 3'OH group on its deoxyribose, so additional nucleotides can be attached. During the reaction, the DNA sequence is extended in each of the four subsamples. On some fragments, however, a given dideoxynucleotide will terminate polymerisation. The fragments, each varying in length by one bp, are separated by gel electrophoresis and visualised by autoradiography. The fragments in each subsample will terminate with the corresponding dideoxynucleotide (complementary to the deoxynucleotide in the template sequence), and the sequence can be read directly from the autoradiograph.

Use of technique - DNA sequencing is a powerful molecular approach for the inference of phylogenetic history and population structure. It includes the characters (nucleotides) that are the basic units of information encoded in the organism, and provides high resolution for the calculation of genetic distance.

DNA Fingerprinting

The DNA of an animal can be examined using genetic probes as informational tools. A *probe* is a single stranded fragment of DNA containing the complementary code for a specific sequence. The probe is used to identify a small fraction of the total DNA present in a cell and allows that fraction of DNA to be distinguished from the whole.

Each *locus* (region of DNA) consists of many possible *alleles* (different forms of given *genes*, differing in DNA sequence) with frequencies that vary depending upon the population.

Multilocus DNA Fingerprinting

Description of the technique - The technique is based on the presence of hypervariable DNA sequences that can be found in many independently segregating, highly polymorphic loci of the nuclear DNA. The numbers of repeated sequences often vary between individuals.

Individual variation in the pattern of restriction-digested DNA fragments can be visualised with the use of a radioactive probe. The result is a series of bands resembling a product bar-code. Approximately 50% of the bands in the offspring will come from the mother while the remaining bands come from the father. Parental candidates are excluded if bands of the offspring are not found, and a band-sharing coefficient is determined for candidates that share some bands with the offspring.

Use of technique - The technique can be used for paternity testing when the number of comparisons is small. This type of fingerprinting can be prohibitively time-consuming when the number of crosswise comparisons is large (i.e., highly polygynous species).

Single-locus DNA Fingerprinting

Genetic information is obtained from a single chromosomal site, thus resulting in only one (homozygous) or two (heterozygous) bands per individual.

Single-locus minisatellites

Description of the technique - Long strings of highly repetitive minisatellites occur at many chromosomal locations and vary in length because of loss or gain of repeat units. Minisatellites can be cloned, and polymorphism at any one locus may be screened independently.

Use of technique - Minisatellites are highly variable and can be very informative for determining relatedness. However, problems with cloning the minisatellite sequences make the technique difficult to use.

Microsatellites

Description of the technique - Microsatellite DNA sequences (short sequences of di- or trinucleotide repeats) occur abundantly throughout the genomes of higher organisms. Within the microsatellite regions, molecular "slippage", the mislignement of repeat units during replication, generates length variability. These repeat units are useful genetic markers because they are abundant, easy to clone, and can be visualised using polymerase chain reaction procedures and gel electrophoresis to compare fragment lengths.

Use of technique - Microsatellites promise to be a powerful tool with high resolution for the detection of individual variation, population differences, and establishment of pedigrees. These probes also appear to be useful among different species.

Suggested reading

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The International Whaling Commission Special Issue 13, Genetic Ecology of Whales and Dolphins, is a useful compilation of studies that have used molecular genetic techniques for the investigation of population subdivision and social organisation. The book also includes information on sample preservation and biopsy.

Protocol for cetacean tissue sample collection

By collecting tissue samples and storing them in a saturated salt solution and 20% dimethyl sulfoxide (DMSO), we can avoid freezing the tissue.

1) Collect 2-5 grams of skin tissue (this will be 2-3 strips of tissue, about 1.5 cm x 4 cm) from each animal and place it in a vial with all of the tissue submerged. Leave some air space at the top to compensate for changes in air pressure during mailing. Wearing gloves while handling the tissue, and changing gloves in between samples from different individuals, is ideal. *Use a new blade* when collecting from each different individual.

2) Label each tube. Included in the sampling kit are small labels that can be written in pencil and inserted into the vial. If possible, include any other information on a separate piece of paper (a copy of the stranding sheet would be ideal):

- identification number
- sex
- total body length
- date collected
- location of animal when stranded (or biopsed)

3) Wrap parafilm around the cap and the top of the vial by stretching the parafilm as you wrap. This prevents leaking in transport.

Sample preservation

To prepare 3 litres 20% solution of saturated DMSO (Dimethyl Sulfoxide)

1. Need stir plate, stir bar, and 3500 ml beaker.
2. Use 600 ml DMSO to 2400 m distilled H_2O (it is preferable to use sterile water).
3. Place stir bar in beaker. Put beaker on stir plate and saturate with salt (sodium chloride).
4. Store solution in plastic container with lid. Label with contents (20% DMSO) and date.

APPENDIX 1

PROGRAMME OF THE WORKSHOP

9:00	Opening of the Workshop - Introduction (G. Notarbartolo di Sciara)
9:30	Determination of group size (G. Notarbartolo di Sciara)
10:00	Photo-identification: field methods (G. Bearzi)
10:30	Behavioural sampling (E. Politi)
11:00	Behaviours and behavioural states: terminolgy and definitions (G. Bearzi)
11:30	Determination of home range (D. Maldini)
12:00	Collecting biopses from free-ranging bottlenose dolphins (V.G. Cockroft)
12:30	Lunch break
14:00	Genetics as a tool for the study of bottlenose dolphins populations (B. Curry)
14:30	Matching of photo-identification photographs (G. Bearzi)
15:00	Cataloguing identified dolphins (J. Heimlich-Boran)
15:30	Patterns of association (J. Heimlich-Boran)
16:00	Behavioural data analysis (B. Wilson)
16:30	Working groups
18:00	Closure of the Workshop

APPENDIX 2

Workshop Participants

Arnold, Holly
Dept. of Zoology, University of Aberdeen, Lighthouse Field Station, George Street, Cromarty, Ross-shire IV11 8YJ, Scotland, U.K.
ph. (381) 600548 **fax** (381) 600548 **home** (4563) 368

Baines, Mick
Dyfed Wildlife Trust, Cefn Garth, Cilgerran, Cardigan, Dyfed SP43 2PD, Wales, U.K.
ph. (1437) 765462 **home** (1239) 682548

Barros, Dr Nélio
Hubbs Sea World Research Institute, P.O. Box 691602, Orlando FL 3286-1602, USA
ph. (407) 3632664 **fax** (407) 3455397 **home** (407) 8564587

Bearzi, Giovanni
Adriatic Dolphin Project, Tethys Research Institute, Rovenska 28, Veli Losinj 51551 Croatia
ph. (385) (532) 236348 **fax** (385) (532) 236348

Berrow, Dr Simon
Dept. of Zoology, University College Cork , Lee Maltings, Cork, Ireland
ph. (21) 276871 ext 4053 **fax** (21) 277922

Bertram, Dietmar
Institut für Naturchutzforchung (INF), Sr. Thomas Plän, Regensburg, Sömmerringplatz 5, Mainz 55118, Germany
ph. (6131) 673204 **home** (6131) 673204

Birkun, Dr Alexei
Brema Laboratory, 27-2A R. Luxemburg Str., Simferopol 333720, Ukraine
ph. (652) 272570 **fax** (652) 272570 **home** (652) 253503

Bompar, Dr Jean-Michel
GECEM, 243 Rue V. Hugo, La Maison Blanche, La Garde 83130, France
ph. (33) (94) 036767 **fax** (33) (94) 163053 **home** (33) (94) 610691

Bonaccorsi, Rosalba
Ist. Anatomia Comparata, University of Genova, Via A. Noli 19, Bergamo 24126, Italy
ph. (35) 242741 **home** (35) 242741

Bortolotto, Alessandro
Fondazione Cetacea, Via Milano 36, Riccione (FO) 47036, Italy
ph. (39) (541) 601712 **fax** (39) (541) 606590

Bucchini, Luca
Genetics and Microbiology-Tethys Research Institute, University of Pavia, Via Abbiategrasso 404, Pavia 27100 Italy **ph.** (39) (382) 505540 **home** (39) (2) 9793788

Chakroun, Dr Founoun
I. Nat., 43 Ave., Chale Nicolle, Tunis 1082, Tunisia
ph. 280950

Cockroft, Dr Victor
Centre for Dolphin Studies, Port Elizabeth Museum, P.O. Box 13147 Humewood, Port Elizabeth 6013, South Africa **ph.** (27) (41) 561051 **fax** (27) (41) 562175
e-mail PEMVGC@ZOO.UPE.AC.ZA

Creton, Pol
Departement Mammifères Marins, Océanopolis, Port de Moulin Blanc, Brest 29200, France
ph. (98) 344040 **fax** (98) 344069

Curran, Sarah
Dept. of Zoology, Univesity of Aberdeen, Lighthouse Field Station, Cromarty, Ross-shire IV11 8YJ, Scotland, U.K. **ph.** (381) 600557 **fax** (381) 600557 **home** (381) 600548

Curry, Barbara E.
Molecular Genetics Laboratory, Southwest Fisheries Science Center, NMFS - affiliated with Southeast Fisheries 8604 La Jolla Shores Drive, La Jolla CA 92038, USA
ph. (619) 5467094 **fax** (619) 5467003 **home** (619) 2208239

Evans, Dr Peter
Dept. of Zoology, University of Oxford, South Parks Road OX1 3PS, U.K.
ph. (44) (865) 727984 **fax** (44) (865) 727984 **home** (44) (865) 727984

Feinholtz Maldini, Daniela
Monterey Bay Coastal Dolphin Project, Pacific Cetacean Group, P.O. Box 136, Moss Landing CA 95039, USA
ph. (408) 6334438 **fax** (408) 6334438 **e-mail** MALDINI@MLML.CALSTATE.EDU

Fertl, Dagmar
Behavior Laboratory, Marine Mammal Research Program, Texas A&M University, 4700 Avenue W., Galveston TX 77551, USA **ph.** (409) 7404718 **fax** (409) 7404717 **home** (409) 7402687

Gaspar, Raquel
Dept. of Anthropology and Zoology, University of Lisbon, Rua Prof. Queikoz Veloso, Lote-1, 7° esq., Telheiras, Lisboa 1600, Portugal
ph. (1) 7584618 **fax** (44) 721293 **home** (1) 7584618

Goodson, David
Electronic Engineering, Loughborough University, Ashby Road, Loughborough LE11 3TV, U.K.
ph. (44) (509) 222846 **fax** (44) (509) 222854

Guinet, Christophe
Centre d'Etudes Biologiques de Chizé, Centre National de la Recherche Scientifique (CNRS), Villiers en Bois 79360, France **ph.** (33) (49) 096111 **fax** (33) (49) 096526 **home** (33) (49) 075688

Harzen, Stefan
Projecto Delfin, Herdade das Praias, Bairro de Sapec, Setubal 2900, Portugal

Heimlich-Boran, Dr Jim
Dept. of Zoology, Cambridge University, Downing Street, Cambridge CB2 3EJ, U.K.
ph. (44) (223) 313116 **fax** (44) (223) 336676

Heimlich-Boran, Sara
Dept. of Zoology, Cambridge University, Downing Street, Cambridge CB2 3EJ, U.K.
ph. (44) (223) 313116 **fax** (44) (223) 336676

Herzing, Dr Denise
Wild Dolphin Project, P.O. Box 8436, Jupiter FL 33458, USA
ph. (407) 575568 **fax** (407) 5755681 **e-mail** WILDDOLPHIN@ECONET.IGC.ORG

Holcer, Drasko
Fogulinca 27, Sisak 44000, Croatia
ph. (385) (44) 31665 **home** (385) (44) 31665

Hussel, Birgit
Biologische Austalt Helgoland, Wattenmeerstation Sylt, Hafenstrasse 49, List / Sylt 25992, Germany
ph. (4652) 1011

Kiely, Oliver
Dept. of Zoology, U.C.C., Lee Maltings, Prospect Row, Cork, Ireland
ph. (353) (21) 276871 **fax** (353) (21) 270562 **home** (353) (21) 396424

Klinowska, Dr Margaret
Animal Welfare Research Group, Dept. of Clinical Veterinars Medicine, University of Cambridge, Madingley Road, Cambridge CB2 0ES, U.K.
ph. (44) (223) 330845 **fax** (44) (223) 337650 **home** (44) (223) 69026
e-mail MKIO@UKAC.CAM.PHY

Lewis, Dr Tim
30 Bridge St., Aberystwyth SY23 1QB, Wales U.K.
ph. (970) 612449 **home** (970) 612449

Liret, Céline
Dept. Mammals. Marins, Océanopolis, Université de Brest, B.P. 411, Brest 29275, France
ph. (98) 344040 **fax** (98) 344069

Lütkebohle, Thomas
Institute for Marine Science Kiel, Marine Botany, Gutenbergstr. 40, Kiel 24118, Germany
ph. (49) (431) 561358 **fax** (49) (431) 565876 **home** (49) (431) 561358

McDonnell, Peter Joseph
47 La Grande Piece, St. Peter, Jersey JE3 7AE, Channel Islands
ph. (534) 483148 **home** (534) 483148

Miokovic, Danijela
Rebar 145, Zagreb 41000, Croatia

Moreno, Paula
ISPA, Pct. Bentomoura, 2-5° C, Almada 2800, Portugal
ph. (351) (1) 2546456 **fax** (351) (1) 7783208 **home** (351) (1) 2546456

Müller, Monica
Languedoc / Roussillon, Lab. Arago, Observatoire de Biologie Marine de Paris VI, 108 Ave. du Puig del Mas, Banyuls - sur - Mer 66650, France
ph. (68) 887373; **fax** (68) 887394; **home** (68) 885723

Notarbartolo di Sciara, Dr Giuseppe
Tethys Research Institute, V.le G.B. Gadio 2, Milano 20121, Italy
ph. (39) (2) 72001947 **fax** (39) (2) 29401987 **home** (39) (2) 29401927
e-mail GNSTRI@IMIUCCA.CSI.UNIMI.IT

Pierpoint, Chris
Dyfed Wildlife Trust, 7 Market St., Haverfordwest, Dyfed, U.K.

Politi, Elena
Ionian Dolphin Project, Tethys Research Institute, V.le G.B. Gadio 2, Milano , 20121Italy
ph. (39) (2) 72001947 **fax** (39) (2) 29401987 **home** (39) (2) 3314281
e-mail GNSTRI@IMIUCCA.CSI.UNIMI.IT

Pribanic, Sandra
Barcev Trg. 7, Zagreb 41020, Croatia
ph. (385) (41) 674737 **home** (385) (41) 674737

Ridoux, Dr Vincent
Marine Mammals Laboratory, Océanopolis, B.P. 411, Brest 29200, France
ph. (33) (98) 344040 **fax** (33) (98) 344069 **home** (33) (98) 491434

Schmidt, Rolf
Seevogelrrttungs und Naturforschungsstation Sylt e.U., Bahnstrasse 1, Tinnum / Sylt 25980, Germany
ph. (4651) 35311

Silva, André
Instituto Superior de Psicologia Aplicada, P.ta Beira, 2 R/C esq., Oeiras 2780, Portugal
ph. (1) 4435291

Stanenis, Alvidas
Rehabilitation Center, laspi, 25 fl. 9 bd. Botanishin gaden, Yalta, Ukraine
ph. (692) 534393 **fax** (654) 335180 **home** (654) 335180

Strong, Powell
Dyfed Wildlife Trust, Castle House, Newcastle Emlyn, Dyfed SA38 9AF, U.K.
ph. (239) 710187

Tregenza, Dr Nick
5 Beach TCE., Long Rock, Penzance, Cornwall TR20 8JE, U.K.
ph. (736) 753136 **fax** (736) 753463 **home** (736) 711783

Walton, Michael
Sea Mammal Research Unit, High Cross, Madingley Road, Cambridge CB4 3NB U.K.
ph. (223) 311358 **home** (223) 350478 **e-mail** US-MJW@BAS-VAXC.NERC.UCS.UK

Webb, Andy
Seabirds & Cetaceans Branch, Joint Nature Conservation Committee, 17 Rubislaw Terrace, Aberdeen AB1 1XE Scotland, U.K. **ph.** (44) (224) 642863 **fax** (44) (224) 621488 **home** (44) (224) 312484

Wilson, Ben
Dept. of Zoology, University of Aberdeen, Lighthouse Field Station, George Street, Cromarty, Ross-shire IV11 8YJ, Scotland, U.K. **ph.** (381) 600548 **fax** (381) 600548 **home** (381) 600574

Wood, Colin James
School of Environmental Studies, Greenwich University, Creek Road Deptford., London, U.K.
ph. (225) 334511 **fax** (225) 480097 **home** (736) 51747