

## Voyage of the Odyssey – Research Focus

### *Toxicology:*

Today, there are major concerns about the ubiquitous accumulation of persistent organic chemicals in man and animals. Concerns include issues of immunosuppression, neurological function, reproduction and cancer. There are many data that point to possible problems at local and regional levels, but there is a lack of globally integrated data that allow a consistent appraisal of exposure and risk in a manner that covers entire ocean basins. We propose to measure concentrations of polynuclear aromatic and -halogenated aromatic hydrocarbons (PAHs and HAHs) in marine mammals, especially great whales from each of the major ocean basins. We will also look for changes in macromolecular biomarkers of exposure including foreign compounds bound to DNA (adducts) and cytochrome P4501A induction. These data will allow a better understanding of the risks associated with chronic exposure to important toxicants. Burdens of selected organic chemicals will be analyzed by gas chromatograph mass spectroscopy, cytochrome P4501A analysis by immunohistochemistry, and adduct analysis by capillary electrophoresis.

### Toxicology - Whales

- Background
- Aims
- Methods
- Available Resources

### Toxicology - Albatrosses

- The appropriateness of using albatrosses as an assay species
- Status of albatross research and opportunities
- Review of current work on albatrosses
- Estimated Contamination and effects of organohalogens
- Program Elements
- Duration and schedule of study
- Candidate Sites
- Recommended candidates for permanent albatross study sites
- Spills
- Albatross planning committee

### *Genetics:*

Even though the backbone of the Voyage is the toxicology research, we will also be collecting data and doing work in several other areas whenever such work does not interfere with our primary focus. We feel that such additional research will add support to the on-going research and maximize the benefits that can accrue from this three-year study. A prime example of this work is work on DNA fingerprinting that will be carried out in the laboratory of William Amos of Cambridge University. The place where animals are sampled may bear little relation to where they have fed or spent most of their lives. However, genetic analyses help in assigning individuals to populations which assists in defining migratory patterns, which in turn aids in interpreting other analyses.

### Genetic studies:

It is expected that approximately 10-15 samples will be collected from a maximum of six species at a maximum of fifty geographic locations. Given how little we know about the worldwide distribution and movements of most species of whale, such a sample resource will prove invaluable for a wide spectrum of current and future

research. Broadly, the foreseeable benefits can be divided into those which accrue from the Voyage data set alone, and those which would accrue to related and collaborative studies.

### Stand-alone benefits

1) Type specimens and the regulation of whaling Recent studies have highlighted the benefits of being able to take an unknown sample of whale meat and match it to a control sample, thereby establishing the species and oceanic region of origin. The Voyage of the Odyssey should result in a unique reference database of genetic type specimens. Such a resource would provide an invaluable tool for the regulation of possible future commercial whaling operations, as well as for the identification of samples from stranded whales.

2) A temporal transect and global change Current genetic studies are hampered by a lack of information about historical status. Low genetic variability may be interpreted either as the natural characteristic of a species, or as the consequence of severe population depletion. Equally, genetic homogeneity may indicate extensive dispersal patterns or unnatural mixing caused by human disruption of the marine environment. The Voyage of the Odyssey will provide an opportunity to assemble an invaluable reference data set for future studies, describing both the distribution and levels of genetic variability among the target species.

3) Stock identity Stock identity has been the subject of intense debate for many years: what is a stock and how should it be managed. Unfortunately, many species of whale are distributed globally and most individual whales are capable of swimming thousands of miles in their lifetime. Consequently, the relationship between different populations remains for the most part obscure. Photo-identification studies can help to reconstruct general patterns of movement, but they will never be able to elucidate functional relationships between populations: in other words, gene flow.

Most on-going population genetic studies focus on one or at best a few different populations within a species. The only significant exception is that of the humpback whale but, even here, some populations remain unsampled. To construct a realistic population model for any given species, data must be collected from as many sub-populations/geographic regions as possible. The voyage of the Odyssey provides a unique opportunity to do this. In terms of methodology, all samples will be typed for two classes of genetic marker: nuclear and mitochondrial. Mitochondrial genes are inherited only from the mother, and hence tell us only about female lineage. By contrast, nuclear markers are inherited equally from both parents. Both approaches are informative, but one used without the other can be misleading. For example, a common pattern among mammals is for females to remain faithful to the region where they were born and for males to disperse, a system that minimizes inbreeding. In such species, a mitochondrial study would probably indicate a highly fragmented population structure, with each natal area appearing isolated from every other. By contrast, a nuclear genetic study of the same species could indicate a single homogeneous population.

### Genetic variability and population size

It is common knowledge that bottlenecked populations, those that have been reduced to very small numbers, often show reduced genetic variability. Conversely, large populations tend to be highly polymorphic. In other words, levels of genetic variability tend to correlate with population size. The flip side of this relationship is that by measuring genetic diversity, an indirect relative assessment of population size can be obtained. The Voyage sample set should provide an ideal opportunity to calibrate genetic methods for population size estimation with sightings surveys and other methods such as the acoustic surveys that will be going on concurrently.

## Collaborative benefits of genetic studies

1) A context for single population studies Few populations can be considered 'closed', lacking both immigration and emigration, particularly in the marine environment. At the same time, most cetacean studies focus on a single study population. Clearly, many aspects of a single population study would be improved if rates of immigration and emigration could be determined. For example, immigrants might differ considerably in their pollutant loads, thereby artificially conflating inter-individual variability.

The Voyage sample set will provide a context for single population studies. Wherever populations are differentiated genetically, a worldwide reference set will facilitate the identification of immigrant individuals. Similarly, destination populations for emigrants may be identified by the fact that they appear genetically closer to the study population. Those wishing to construct population genetic models will benefit greatly from data concerning the genetic character of neighboring populations.

2) Assistance in Interpreting Toxicology Studies In order to interpret observed levels of toxins in the body tissues of individual cetaceans it is vital to know as much as possible about past behavior of the cetaceans. Clearly, the current position of an animal may not necessarily represent the environment in which it has spent the majority of its life. Genetic analysis allows us to add an extra dimension to the reconstruction of global migration patterns, and hence will aid in the interpretation of other analyses.

3) Stimulation of Future Studies It is a truism to state that one never knows what one will find until one looks. Again, with the possible exception of the humpback whale, no one has yet examined a cetacean species on such a global scale as we are proposing in the Voyage of the Odyssey. Whereas many of the results that the Voyage will yield may well be in line with expectations, there are equally likely to be some surprises. Such leads will form the basis of exciting new lines of research.

## *Bioacoustics*

Marine bioacoustics concentrates on understanding the acoustic behaviors of marine animals with particular focus on marine mammals (whales and dolphins). As such it serves as the focal point for a wide range of disciplines including behavioral ecology, biological oceanography, population biology, psychoacoustics, neuroethology, electrical engineering, statistics, applied mathematics, and applied physics.

## Marine Bioacoustics Program - Cornell University

The research deals with basic descriptions of acoustic behaviors of whales on an ocean scale. The objective is to understand the relationships between these animals and the physical and biological features of the ocean in which they evolved and survive. To accomplish this, Dr. Clark is actively leading several major research efforts in the North Pacific and North Atlantic. This effort requires development and implementation of advanced digital signal processing systems deployed either remotely or linked back to Cornell or to Navy facilities. It also requires the constant integration of the physical and biological sciences. This research has attracted a great deal of attention because of its scope and the fact that it is revolutionizing the way we think about the movements, distributions, populations, and behaviors of whales. As an example of scale, Clark's laboratory is presently acquiring daily data from ocean deployed systems such that the total numbers of whales they detect and track (using passive acoustics) are greater on a quarterly basis than the summed total of all whale survey efforts over the past twenty years. An example of an intriguing insight comes from the work on blue whales, which make extremely loud, low frequency (infrasonic) signals. These signals have an uncanny suite of features superbly designed not only for long-range communication (Clark can detect a blue whale's sounds at a distance of 1000 nautical miles), but also for acoustic navigation and tomography. This is to say that the sounds, and patterns of sounds, made by blue whales are such that we, as scientists, can even use them as rudimentary acoustic signals to probe the physical structures of the ocean. In fact, at this point, humans cannot produce, even with our most

advanced machines, the kinds of sounds that a blue whale routinely makes. However, we have in the last 5 to 10 years, begun to learn how to make loud low frequency sounds for probing the ocean. A deep concern is that unless we are very careful, these human-made acoustic probes could prove to be a threat to the survival of species like the blue whale.

Global warming will eventually affect everyone, and everyone will eventually recognize that the rate at which the earth's average temperature is changing is of great relevance to their lives. The problem at present is that no one knows the rate at which global warming is proceeding. To end this ignorance it is important to devise a way to take the earth's temperature. The ATOC Experiment (Acoustic Thermometry of Ocean Climate) is perhaps the most notable attempt to do this. It takes advantage of the fact that sound travels faster through warm water than through cold water. Thus, by making a loud sound underwater on one side of an ocean and measuring accurately the time the sound takes to reach a hydrophone (underwater microphone) at a known distance on the other side, one can determine the average temperature of the water between source and receiver. When the average water temperature is hotter the trip will be shorter than when the water is colder. (It is the great length of the trip that averages out the different speeds of travel associated with areas of hotter and colder ocean water hence the need, if one is to take advantage of this integrating effect, to make a sound loud enough to cross an entire ocean.) Such measurements made repeatedly, a few times a day over many days make it possible to cancel out confounding variables and after several months or years to determine with great accuracy an average temperature for the entire ocean. The oceans are 71% of the earth's surface and contain almost all of its surface heat capacity, therefore knowing the average ocean temperature over several years enables us to know the rate of global warming. As global warming proceeds, the temperature of the oceans will slowly rise. With ATOC measurements we will finally know how bad the problem is and therefore what steps can be taken to avert disaster. The results of such research are of fundamental importance to humanity. However, because the ATOC sound signal has to be loud enough to be heard across oceans, many people are concerned that these sounds may pose serious problems for whales. But most whale species make very loud sounds themselves and some can even be heard across ocean basins. Whales must have some way to deal with the fact that these very loud sounds are occurring so close to their ears. Which is to say that we do not know whether sounds like the ATOC signals really pose a serious threat to whales. The objections raised to the ATOC experiment have delayed its beginning. But because the results it promises are so important to humanity we need to know as soon as possible whether whales are significantly disturbed by ATOC sounds. Whenever possible during the Voyage, the Ocean Alliance will make its research vessel *Odyssey* available to Dr. Clark to assist in his research on this question. We anticipate that the collaboration between TOA and Clark will produce work of fundamental importance.

A more recently divulged threat involving loud, low-frequency sounds is SURTASS LFA (Surveillance Towed Array Sonar System - Low Frequency Active), a ship-based sonar system under development by the U.S. Navy for detection and tracking of submarines and for supporting fleet and anti-submarine warfare exercises. It will use an array of acoustic transmitters suspended, on average, 100 meters beneath a ship and will transmit at frequencies between 100 and 1,000 Hz (a band in which many whales produce sounds, and where therefore their hearing is thought to be sensitive). The transmitters may be turned on as much as ten percent of the time, during which they will broadcast at classified sound intensities (the National Resources Defense Council has estimated that the intensities may be as much as two hundred times the intensity of ATOC sounds). There is widespread concern that such sounds might seriously damage the hearing of whales located close to the source, or that it could seriously mask communications between whales located at safer distances.

Accumulating data and knowledge about natural ambient noises provides the background for understanding the biological significance and survival value of a rich variety of sounds produced by whales, especially larger whales communicating with low frequency (LF) sounds. The National Marine Fisheries Service consistently monitors, by shipboard and aerial surveys a band of North American coastal waters roughly 300 miles wide, running from southern Mexico to central California. From these surveys estimates for eastern Pacific blue whale populations are derived for some species in California. These are independently corroborated through

photo identification work by John Calambokidis. However, neither of these studies takes into account the fact that throughout their sampling periods other blue whales are being detected acoustically in deep ocean between California and Hawaii. These animals are not included in the estimates. The same is true for humpbacks in the Caribbean. According to Christopher Clark this is the kind of underestimation going on throughout the world's oceans. Acoustic censuses thus indicate that we are consistently undercounting whales to an unknown extent. Such uncertainty accounts for the biases and poor confidence intervals in our estimates of populations and consequently of their rates of recovery. We will combine acoustic survey and visual survey techniques to improve censusing techniques. We will also work in conjunction with Clark when he is monitoring the US Navy's SOSUS arrays. Positions of animals tracked on SOSUS will be relayed to the Odyssey, which will travel to the area and confirm species identification. Odyssey will also provide comparative data on species abundance in the locale indicated.

### Marine mammal responses to low frequency (LF) sound

The uncertainties concerning potential impact of human-made LF sounds on whales are large, and under these conditions the procedure is to err on the side of caution. This has led to decisions being made based on lack of knowledge rather than from experience. Response thresholds and levels of impact are best determined through scientific research, not legal compromise, and any improvement in our understanding of how and under what conditions marine mammals respond to low frequency sounds will be extremely valuable.

The work we plan in conjunction with Voyage will largely involve a limited number of species that are known to produce and/or hear LF sounds. Certain species of whales are known to produce loud (as great as 190 dB re 1 $\mu$ Pa) long, patterned sequences of low frequency sounds. Some, like the songs of male humpbacks definitely appear to be part of breeding activity, while others, like the infrasonic pulses of blue and fin whales, though most probably used for communication, could also function for navigation and as a form of low frequency active sensing. The predominate eastern Pacific blue whale signal consists of a pair of contrasting sounds. The first is a 10 second sequence of amplitude-modulated tones at 17-90 Hz, followed by 30 seconds of silence, followed by a 20-25 second constant frequency (CW) tone at 17.5 Hz that changes into a frequency-modulated (FM) chirp (called so because it resembles a bird 'chirp' when greatly speeded up). Such pairs of signals are repeated every two minutes. Signal bandwidth and variability appear to be associated with different bathymetries (shallow vs. deep). Under optimum ambient conditions, these signals are detectable at ranges out to 1000 miles. We have examples of whale FM chirps exciting the first 4-5 modes in a surface-ducted, shallow water environment. Such patterns of ocean-modified whale signals have even been used to perform rudimentary acoustic tomography. If humans can use whale signals as acoustic probes (and we are now implementing our own probes that have remarkably similar features to blue whale sounds) it seems likely that whales having had 30 million years of lead time, are using their own sounds to investigate the ocean.

As for hearing LF sounds, recent anatomical evidence indicates that, not surprisingly, the ears of LF whales are specialized for hearing LF sounds. In contrast, the ears of dolphins and pinnipeds (except for the elephant seal for which there is evidence that it has good LF hearing) are adapted for high frequency hearing, extending in the case of dolphins well into the ultrasonic range.

The greatest potential risk of acoustic impact from human generated LF noise such as ships traffic noise or the sounds associated with seismic profiling or even signals like those used in acoustic thermography (the ATOC experiment) can be confined to large whales. This potential risk is real, but there is extremely limited evidence by which to estimate the risk. Instead we are reduced to invoking the 120 dB rule of thumb, and inferring impact based on comparisons with levels that produce auditory impairment in terrestrial animals including humans. This is not a very responsible way to define risk and impact. What is needed is more real, empirical scientific data about what constitutes a sound having a deleterious impact on whales, so decisions based on reality can be made.

## Additional relevant use of bioacoustics data

TOA houses the world's largest library of acoustic recordings for two whales species (humpback and right whales). It stems from Dr. Roger Payne and Katharine Payne's work on humpback whale vocalizations. We will extend this collection from the R/V Odyssey. Analysis will be carried out using the analysis program "Canary" (developed by Christopher Clark at Cornell University).

Throughout the course of the Voyage, TOA personnel will use directional hydrophones and acoustic arrays to record and monitor natural acoustic behaviors of cetaceans. Analysis of these recordings will provide deeper understanding of the natural variability in ambient noise from marine mammals and other sources on global, regional and site-specific scales. Data of this kind from the southern oceans will be especially valuable.