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REPORT OF THE WORKSHOP ON BLOOD PARAMETERS FOR THE ASSESSMENT OF STRESS IN EASTERN TROPICAL PACIFIC DOLPHINS JANUARY 30 AND 31, 2001 SOUTHWEST FISHERIES SCIENCE CENTER LA JOLLA, CALIFORNIA

By

Barbara E. Curry and Karin A. Forney

ADMINISTRATIVE REPORT LJ-01-07

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EXECUTIVE SUMMARY

The Southwest Fisheries Science Center (SWFSC) is preparing to undertake Chase Encirclement Stress Studies (CHESS) to evaluate the potential effects of fishery-induced stress on dolphins in the eastern tropical Pacific (ETP). The general approach of CHESS is to integrate a suite of complementary research projects that address different ways in which chase and capture stress may manifest itself in the dolphins. One key component of the CHESS research is the analysis of single and repeated blood samples collected from dolphins captured during fisheries operations. To determine the types of blood sampling, analysis and interpretation that should be used for these studies, the SWFSC convened a workshop in La Jolla, California, 30 to 31 January, 2001.

A panel, consisting of three experts in marine mammal veterinary research, was established to provide the SWFSC with formal recommendations. Additional participants shared their expertise in veterinary medicine, immunology, molecular biology, physiology, field biology, and the population biology of dolphins.

The objectives of the workshop were to (1) establish guidelines for blood sampling and analysis in advance of field studies, (2) establish guidelines for the interpretation of blood results from sampled ETP dolphins, (3) determine the types of sample collection, preservation, processing, and analysis that are feasible given field constraints, (4) determine the types of data that may be particularly useful in conjunction with other ongoing investigations of the effects of stress on dolphins in the ETP, and (5) determine the types of controls and reference data that are available for comparison to data that will be collected during CHESS.

The panel discussed blood constituents that could be collected in the field and reliably processed and stored at sea. They identified blood parameters that should be analyzed to best investigate the effects of repeated chase and capture on dolphins. The panel set guidelines for the interpretation of results, and recommended measures for establishing controls for the resulting data set. In addition, the panel recommended that a scientist with expertise and background in marine mammal physiology and stress effects act as the principal investigator for the blood analysis research and experimental design during CHESS.

INTRODUCTION

The International Dolphin Conservation Program Act (IDCPA; U.S. Public Law 105-42), a 1997 amendment to the Marine Mammal Protection Act, mandated that research be conducted by the Southwest Fisheries Science Center (SWFSC) to investigate the potential effects of fishery-induced stress on dolphins in the eastern tropical Pacific Ocean (ETP). The law specifically required that the SWFSC conduct an "experiment involving the repeated chasing and capturing of dolphins by means of intentional encirclement," to determine if the "intentional deployment on, or encirclement of, dolphins by purse-seine nets is having a significant adverse impact on any depleted dolphin stock." In response to this mandate the SWFSC is preparing to

undertake the Chase Encirclement Stress Studies (CHESS) to evaluate the potential effects of fishery-induced stress on dolphins in the ETP.[1]

The SWFSC convened a workshop in La Jolla, California, January 30-31, 2001, to determine the types of blood sampling and analyses that should be used to assess the physiological condition of dolphins that have been chased and captured in the ETP (Appendix 1). In addition, a central focus of the workshop was to identify analyses that would be likely to provide information about the potential effects of fishery-induced stress on survival and reproduction of these dolphins. Such information would make it possible for SWFSC scientists to evaluate a potential range of population-level effects of stress on ETP dolphins. A panel, consisting of three experts in marine mammal veterinary research, was established to provide the SWFSC with formal recommendations. Additional participants shared their expertise in veterinary medicine, immunology, molecular biology, physiology, field research, and the population biology of dolphins, with emphasis on the ETP (Appendix 2).

The objectives of the workshop were to (1) establish guidelines for blood sampling and analysis in advance of field studies, (2) establish guidelines for the interpretation of blood results from sampled ETP dolphins, (3) determine the types of sample collection, preservation, processing, and analysis that are feasible given field constraints, (4) determine the types of data that may be especially useful in conjunction with other ongoing investigations of the effects of stress on dolphins in the ETP (e.g., molecular analyses of skin samples, pathological and immunological analyses of samples from necropsied dolphins, and studies of the potential effects of thermal stress), and (5) determine the types of controls and reference data that are available for comparison to data that will be collected in CHESS.

This report summarizing the proceedings and recommendations of the workshop is based on the agenda found in Appendix 1.

BACKGROUND

Background information was provided by Karin Forney on the concept of a repeated chase and capture study of dolphins in the ETP and the CHESS field research (see also Appendix 3). The initial concept of an experiment involving the repeated chase and capture of dolphins in the ETP, as mandated in the IDCPA, was discussed at a July 1997 research planning meeting (Curry and Edwards 1998). Difficulties inherent to the experiment were discussed by experts and representatives of non-governmental organizations at SWFSC consultations in September 1999 and April 2000 (Sisson and Edwards, 2000; Donahue *et al.*, 2000). These difficulties include the interpretation of effects on individual dolphins relative to population level effects, sample size limitations, obtaining the appropriate controls, and attribution of results. Therefore, the research strategy developed for CHESS includes obtaining repeated measurements of as many different physiological manifestations of stress as is possible (e.g. thermal stress, changes in hormone levels, muscle damage). In addition, the amount of information obtained per capture must be optimized. It is important to identify the range of potential impacts on survival and

[1] The CHESS field research is scheduled to take place in the summer of 2001. A tuna purse seine vessel along with a research vessel will be used for two months at sea to conduct the research.

reproduction so that we can assess these effects at the population level. Thus, investigations and data that can identify problems such as infertility, reduced fertility, or lethal effects that might be attributed to fishery-induced stress, will be especially valuable.

To this end, the general approach of the CHESS study is to integrate a suite of complementary research projects that measure different ways in which chase and capture stress may manifest itself in the dolphins involved in tuna purse seine operations (primarily spinner dolphins, *Stenella longirostris*, and pantropical spotted dolphins, *Stenella attenuata*). The research techniques will include: (a) analyses of single and repeat blood samples, (b) molecular analyses of chronic stress from skin samples, (c) measurement of thermoregulatory processes, (d) satellite tagging and tracking, and (e) documentation of reproductive status and, if observed, cow/calf separation between successive chases. These individual measures, when combined, will provide broad information on the potential for fishery-caused stress in ETP dolphins. Additionally, some of the physiological data may allow estimation of quantitative or qualitative effects on survival and reproduction of individuals, which can be included in a population dynamics model to estimate a range of potential population-level effects. The individual research projects were selected to complement respective weaknesses and strengths wherever possible, while including only projects that are logistically compatible in the course of a single field study.

Blood sampling and analysis will be an important component of the CHESS research. The collection and analysis of blood samples will provide physiological data on stress effects in wild dolphins subject to repeated chase and capture by a tuna purse-seine. Repeated blood sampling from the same individuals will enable researchers to measure blood parameters over the course of days as the animals are repeatedly captured. This will provide information about the individual responses to stress. Analyses of blood samples can provide information about physiological parameters including stress response, muscle damage, immune status, reproductive status, and the sex and relatedness of individuals (Geraci and St. Aubin, 1979; Thomson and Geraci, 1986; St. Aubin and Geraci, 1990; St. Aubin *et al.*, 1990; 1996; Curry and Edwards, 1998).

Some complementary studies that may yield data and information on the physiological condition of dolphins involved in the ETP fishery are underway or planned for CHESS. In particular, studies of stress detected via molecular analysis of skin proteins, studies of the immune competence and pathological condition of these animals, and research on thermoregulatory processes of dolphins are ongoing and may provide results that can be used in conjunction with the results of the blood sample analyses. During the workshop, several participants gave brief presentations summarizing these existing research projects.

EXISTING RESEARCH

Molecular Analysis of Stress Response

Sarka Southern (Appendix 2) described molecular research being conducted by the SWFSC to evaluate the stress response of dolphins in the ETP. New techniques have been developed for molecular analysis of chronic physiological stress, based on detecting changes in

expression levels of proteins and genes in field specimens of skin and blood. Based on the analysis of 90 control cetaceans, this research has identified a group of 32 stress-activated proteins (SAP) whose expression profiles were significantly altered only in clinically diseased or long-term physiologically stressed animals, as compared to the baseline expression profiles identified in normal healthy animals. Previously healthy cetaceans exposed to a brief acute stress, such as rapid drowning or hunting, did not have significantly altered SAP profiles. These SAP can therefore collectively serve as a multi-target marker of chronic stress in cetaceans. The SAPs are structurally and functionally diverse molecules with recognized roles in oxidative stress response, apoptosis, cell growth and differentiation, immunological and neurological signaling and cell adhesion. Cellular stress results in altered oxidative balance, DNA and protein damage, as well as high energy expenditure. Southern has observed that the molecular stress response is a common outcome that is triggered early, spreads rapidly and persists.

In the context of the proposed CHESS project, the SAP expression analysis could be applied for both the skin and blood specimens. Southern suggested that blood samples would be useful for evaluating blood leukocytes, stress activated proteins and stress activated genes. Evaluation of protein patterns in blood samples would be a useful comparison to the measurements that are already being completed for a large number of skin samples from dolphins in the ETP. The results of the proposed analysis of skin samples could provide information on the physiological impact of repeated chase and capture on dolphins during CHESS, for comparison to existing data on normal healthy (biopsied) dolphins. Such control specimens would not be available for the blood analysis. Southern expressed concern whether the physiological perturbation during CHESS would be sufficiently large and rapid to produce detectable changes in the SAP pattern in skin or blood by the time of sampling, since her research has not yet evaluated dose- and time-dependent aspects of the molecular stress response.

Necropsy Studies

Barbara Curry (Appendix 2) provided a brief summary of histopathological examinations being conducted by Daniel F. Cowan M.D. at the University of Texas Medical Branch for tissues collected from dolphins killed during tuna purse-seine operations in the ETP. Results of the pathology analyses are provisional. There is a limited sample size (22 dolphins at the time of the workshop; however, more than half of the tissues have been examined, and the observed pattern indicating an acute stress response is strong and consistent.

Pathological investigations provided data on the status of the major internal organs. For every animal studied microscopically to date, severe myocardial injury of a kind attributable to acute ischemia and/or catecholamine injury was observed. One animal had many myocardial scars of a kind that suggests they resulted from prior injury. In all animals examined thus far, there was also substantial injury to smooth muscle in organs throughout the body, including the intestinal tract and the airway sphincters. These changes are sufficient to account for death. Other findings include non-acute background disease: one vascular tumor of an adrenal gland, frequent minor lungworm (nematode) infestation, and minor stomach and intestinal parasitism. Cardiac and other muscle lesions are all provisionally interpreted to be attributable to an extreme "alarm reaction" or stress response. In this context, these lesions appear to be the result of a stress response attributable to the fishery interaction.

Frances Gulland (Appendix 2) noted that if a handled dolphin did go into capture shock during CHESS, blood analysis might provide information on this type of myocardial injury. Results of histopathology suggest the usefulness of blood analyses that could indicate myocardial and other muscle damage and catecholamine levels, among other constituents.

Immunology Studies

Tracy Romano (Appendix 2) provided a description of the ongoing immunology research being conducted by the U.S. Navy Marine Mammal Program (see Romano, 1993, Romano *et al.*, 1992, 1993, 1994) and the ways in which it can be applied to dolphins captured in the ETP. She discussed the fact that various stressors, as perceived by the nervous system, can compromise the immune system and result in disease or mortality. Incidental capture/entanglement, environmental pollutants (oil, industrial toxins, noise), extreme changes in temperature, housing conditions, social interactions, as well as difficulty in learning a task, have been shown to cause changes in immunocompetence in other mammals (see Curry 1999).

Lymphoid organs (spleen, lymph nodes, thymus) from the ETP necropsied dolphins are being processed and evaluated to determine the "state" of the immune system using histological methods. Lymphoid components are compared with stromal components paying particular attention to lymphoid compartmentation and cell types. Catecholamine histofluorescence is used to determine the degree of postganglionic innervation from the autonomic nervous system, which is activated during stress. Innervation patterns, compartmentation, and nerve targets will be evaluated. Immunohistochemistry for labeling of noradrenergic nerve fibers and lymphoid cell types will be used to determine which cells are targets of autonomic nerves and most likely affected. Overall, the comparison of cellular compartmentation vs. stromal components will give us an idea as to how "active" the organ was. Investigation of cetacean neuro-immune components can be integrated with the study of blood samples collected from dolphins captured in the ETP to assess immunocompetence in these animals. Blood levels of catecholamines, lymphocytes and cytokines can be measured as part of an evaluation of immune status.

Thermal Studies

Michael Scott (Appendix 2) described the studies of the delphinid thermoregulatory system that are currently being conducted by D. A. Pabst, W. A. McLellan, S. A. Rommel, T. K. Rowles, R. S. Wells, T. M. Williams (University of North Carolina, Wilmington). Thermal stress can be associated with chase and capture in terrestrial mammals. Based on observations of terrestrial mammals, it is possible that dolphins undergoing a prolonged, high-speed chase in the warm surface waters of the ETP may be experiencing hyperthermic stress (Curry, 1999). Increased core temperatures in terrestrial mammals can cause maladaptive physiological changes and, in extreme cases, death. Hyperthermia may be most severe in pregnant mammals where blood flow to the uterus is required for adequate thermoregulation of the developing fetus (see Rommel *et al.*, 1993).

The research being developed by Pabst and colleagues has involved extensive pilot field work leading to a study design that can be used as a part of the CHESS research in the ETP. The

researchers use a thermographic camera, which allows observations of thermal stress without handling the dolphins. Because of its efficiency, this technique may also make it possible to obtain a large sample size. Preliminary investigations of bottlenose dolphins, *Tursiops truncatus*, in Sarasota, Florida, have only involved brief chases of less than one minute. Therefore, the researchers have also used the thermographic camera to examine the effects of induced exercise on animals in captive situations. In addition, they have used a thermal probe to measure deep temperature, and have measured heat flux through the dorsal fin using a thermal pack attached to the fin.

Participants agreed that this study may be especially useful for looking at reproductive stress and suggested that it would be useful to identify blood bioassays that could be complimentary. Sam Ridgway (Appendix 2) suggested that portable ultrasound equipment could be used to assess reproductive status under conditions at sea.

LOGISTIC CONSTRAINTS

Because research conditions at sea require special consideration for determining the types of blood sample collection and analysis that are feasible, Scott presented a summary of the logistic constraints involved in developing a study of repeated chase and capture of dolphins in the ETP. Two previous studies that have involved the chase and encirclement of dolphins in the ETP have indicated the types of research conditions that can be anticipated. These studies were tagging and tracking exercises involving tuna and dolphin, that were conducted jointly by the Inter-American Tropical Tuna Commission (IATTC) and SWFSC in 1992 and 1993.

Suitable working conditions could include sea states of up to Beaufort 3 during daylight hours. Once the school is encircled, the pursing of the net will be conducted as during standard fishing operations, until rafts and personnel are deployed into the net for dolphin sampling. Logistic constraints are such that investigators must work within the net. The first small boat to be launched will carry the set coordinator and thermal camera operator. They will take thermal photographs of the dorsal fins of the encircled dolphins as soon after the chase as possible, during the retrieval of the net. Once the net is partially retrieved, up to three pairs of sampling rafts will be deployed and stationed at the cork line near the backdown region of the net, and several swimmers will enter the water to select and handle dolphins for sampling The net will be kept open as long as is possible to increase the working time available, most likely for about 20-30 minutes. Sampling and tagging each animal is expected to take about 7-12 minutes. Photographing and video-taping of the animals can occur during these procedures. A small boat will ferry blood samples to the research vessel for rapid processing. The sampling raft that will contain the dolphins to be sampled will be kept partially flooded, and the safety of the animal will be the primary consideration at all times.

Joseph Geraci (Appendix 2) inquired as to the frequency of capture shock under these conditions. Scott responded that very few dolphins went into shock, and that this was very likely because researchers kept the raft wet to prevent shock. He noted that it appears that handling in the raft may be the primary cause of shock during this type of research, but that dolphins can be

rapidly returned to the water if signs of shock are evident. He defined shock in the dolphins based on breathing pattern and an arching behavior.

TAGGING AND SAMPLING

The panel and participants discussed potential sampling regimes and the technologies available for tagging dolphins that will be chased and captured. Forney emphasized that during the CHESS field research, attempts will be made to repeatedly sample as many individuals as is possible. It will be likely that researchers can sample from one to nine dolphins per set. Based upon logistic considerations, researchers can expect to capture eight to 20 individual dolphins two to four times, and from 30 to 240 dolphins at least once during the two-month field effort. If group cohesion among captured dolphins is strong, additional repeated samples from individuals may be possible. Forney explained the general plan for radio or satellite tags to be attached to several of the sampled dolphins in each set, and to attach other types of tags (such as rototags) to the remaining sampled dolphins. She added that the study requires some type of short-term (days), highly visible tagging method, which could be used to identify as many as possible of the previously unsampled dolphins that may be captured in the set. The radio tags will provide the means of following and relocating previously captured individuals.

In any given set, priority for sampling should be given to those individuals that have previously been sampled, to maximize the chances of detecting cumulative stress effects (i.e., failure to recover between sets) in dolphins with a known recent history. Efforts will be made to sample individuals as many times as possible over the course of days to weeks.

Participants discussed the value of using spaghetti tags for marking large numbers of un-sampled animals in the net. William Perrin (Appendix 2) noted that this technique has been used with some success to identify dolphins in the ETP over periods of one to four days. However, Randal Wells (Appendix 2) and Scott cautioned that studies of bottlenose dolphins have shown that the use of spaghetti tags can cause abscesses and increase the attraction of predators (Irvine *et al.*, 1982; Scott *et al.*, 1990), and recommended against using this technique during CHESS.

Wells suggested that the use of roto-radio tags instead of simple roto tags would greatly enhance the ability to monitor and re-capture the previously sampled animals. These small VHF tags (<20 g in air), when mounted high along the trailing edge of the fin, provide both a visual and a radio cue about the location of an animal of interest, while minimizing size/drag effects from tagging. The transmitters have a battery life of approximately 30 days and a range of several miles (depending on receiving antenna altitude). Wells also noted that if a helicopter were available for tracking, it might be possible to relocate many of the tagged animals over a larger area, providing additional information on group dispersal.

David St. Aubin (Appendix 2) indicated that it is important to note the distinction between animals that are tagged and those that are marked. He cautioned that depending upon the size and style of attachment, satellite, radio or roto tags can cause tissue damage and associated stress. For example, tissue damage associated with tag attachment has been shown to alter lymphocyte and other leucocyte profiles in beluga whales, *Delphinapterus leucas* (St. Aubin *et al.*, 2001). The interpretation of stress-related changes must take the size and style of

attachment for the tags into consideration. Samples from dolphins marked with the less invasive rototags would be more easily interpreted than those from animals with full radio tag packages.

Recommendation 1

The panel recommended that it might be useful to use three types of tags or markings, yielding three categories of animals in the study: (1) short-term marks that can be applied to encircled animals without handling, and can be seen at short distances for a period of days, (2) roto-radio tags that can be detected at moderate distances for animals that have been previously encircled and sampled; these animals would be the primary targets for repeated sampling, and (3) satellite or radio tags (to allow tracking, relocation and repeated sets on the same animals; these individuals are less informative because the tag package itself can cause a stress response, but will be easier to follow and relocate).

Recommendation 2

The panel recommended that priority in selecting animals in a set for blood sampling should be directed to those individuals that have previously been sampled to maximize the chances of detecting cumulative effects (i.e., failure to recover) in dolphins with a known recent history. Furthermore, individuals with the least invasive tags should be targeted whenever possible to minimize potential confounding effects of the tag attachment itself.

BLOOD PARAMETERS, ANALYSIS AND INTERPRETATION

Roundtable discussions were initiated to address the workshop's primary objectives regarding the development of the blood analysis research. These topics included a discussion of the blood parameters that would be both feasible and necessary to evaluate, as well as those that might yield information on the effects on an individual's survival and reproduction. In addition, the types of controls and reference materials that could be used for the study were discussed.

Research Development

Recommendation 3

The panel recommended that it will be necessary to have a scientist with relevant expertise and background experience act as the principal investigator for the blood analysis research and experimental design. Further, the laboratories selected for the various analyses must have prior experience in assaying dolphin specimens to ensure that their equipment can accommodate constituent concentrations that are somewhat different than those for domestic animals or humans. Quality assurance and quality control must be established, using blind duplicates and randomly assigned sample identification numbers. Statisticians should be consulted in advance to determine how the data should be analyzed. Repeated-measures analysis of variance might be employed, but the fact that some individuals will be over-

represented in the time series could be problematic. These issues should be investigated and resolved as soon as possible.

It was agreed that the type of physiological research to be undertaken in CHESS requires the use of an additive approach based on clinical experience. No single blood parameter can be interpreted in isolation and all must be interpreted in the context of other parameters known for that individual. Because of the complexity of potential combinations of blood parameter values, the panel agreed that is was not possible to establish *a priori* a list of parameters that would indicate whether an individual is healthy or stressed, except in extreme cases. For evaluation of stress effects in dolphins involved in purse seine operations, it will be necessary to base interpretation on the sum of integrated data points from all blood analyses for each animal.

Recommendation 4

The panel recommended that the complete blood profile data from a representative subset of CHESS samples should be sent to three independent evaluators with expertise in cetacean clinical pathology for expert interpretation. These evaluators would be tasked with identifying any abnormalities (pre-existing or emerging) and giving a prognosis based on experiences with other cetaceans exhibiting similar profiles.

Recommendation 5

Table 1 provides a list of the blood constituents that the veterinary experts determined could be analyzed to investigate the effects of repeated chase and capture on the dolphins. The blood panel included the following: hematology, chemistry, epinephrine and norepinephrine, reproductive hormones, adrenocorticotropic hormone (ACTH), cortisol and aldosterone, lymphocyte function/cytokines, molecular - skin stress proteins, thyroid hormones, muscle glycogen, and blood gases.

Workshop discussions identified the direction and magnitude of changes expected in blood constituents as a result of chase, encirclement, and modest handling over the course of 1-7 days. Specifically, an uncomplicated stress response would be expected to result in increased levels of white blood cells, neutrophils, epinephrine, norepinephrine, cortisol, aldosterone and changes in levels of glucose. The working assumption is that these changes are not necessarily deleterious, and that recovery should occur with 1-2 days in virtually every instance. Changes of a greater magnitude or lack of recovery would signal a more severe stress response that might compromise the animal. Expert interpretation of such deviations in the context of all other blood parameters would be required for establishing a prognosis for these individuals.

It was determined that the quantity of blood required for these analyses was approximately 75cc (see Table 1) and that all samples, except those for catecholamines could be kept on ice for up to two hours (Geraci and Medway, 1973). Samples to be processed for catecholamines must be iced and returned to the research vessel for further processing within 30 minutes.

Additional Sampling and Analyses

Geraci noted that chronic stress will cause damage that may be quite different from that due to acute stress. In the experimental approach we are discussing here, we are actually attempting to assess the cumulative effects of chase and encirclement. Interpretation of the results of each of an individual's blood parameters in relation to all of the other parameters will be important. In addition, collection of the appropriate samples and interpretation of the results is critical to assessing the differences between acute and chronic stress. For example, a blood sample can indicate that there was muscle damage over a period of hours. A muscle biopsy may show scarring and lesions that have taken place over a longer period of time.

Francisco Galindo (Appendix 2) provided written comments, and stated that it would be useful to take more than one blood sample per individual during each capture so that a profile of changes in levels of stress hormones within each of the captures would be available. The panel noted that this is a valuable suggestion if by chance the net were to stay open for a sufficient period of time. However, the panel also determined that this type of multiple sampling of individuals in a single set would not typically be possible given the logistic constraints of the study. Galindo also suggested that less invasive samples that can reflect the long-term effects should be considered. In particular he noted that the collection of saliva and/or fecal samples might be useful. The panel considered the collection and analysis of cortisol and aldosterone in saliva to be highly experimental, and that collection of both saliva and fecal samples would not be possible given the logistic constraints of the work.

Recommendation 6

The panel recommended that muscle biopsies should be collected from sampled animals whenever possible. These samples can be taken as quickly and easily as blood samples, and can provide information on long-term muscle damage that can complement data from blood analysis.

Recommendation 7

Adrenocorticotropic hormone (ACTH) was identified as a potentially useful indicator of the stress response, however there are as yet no data on this constituent in the plasma of odontocetes. The panel recommended that it may be important to submit in advance a number of samples from other animals to determine whether commercially-available methods are appropriate for these species. Specimens that have been collected from stranded, (i.e., stressed), dolphins would be very instructive.

Controls and Available Reference Data

Participants discussed the types of controls that could be used for evaluating results of blood analyses from the CHESS research. There was general agreement that an inherent part of the study is that some aspects of handling cannot be extracted from end results. Blood samples from dolphins experiencing a range of handling effects would, however, provide a means of investigating handling versus chase/recapture stress for sampled dolphins. St. Aubin suggested that field data sheets would need to note time and the level of handling perhaps by categorizing

handling (e.g., a scale of 1-5). Also, to mitigate the effects of handling, it would be best to always keep the number of people involved in dolphin handling to a minimum.

Galindo suggested that it would be useful to record the behavior of the captured dolphins to obtain data on how the individual differences in behavior relate to the physiological measurements. Wells suggested that a continuous video record (with time stamp) of the overall operation would ensure correct measurement of timing of each of the component activities, and might allow the tracking of the behavior and condition of sampled dolphins prior to their selection for sampling. It would be possible to identify animals exhibiting rafting or sleeping behavior. He noted that it should be possible to videotape the operation from the crow's nest of the purse seiner.

Recommendation 8

The panel agreed that blood samples from animals exhibiting rafting or sleeping behaviors would be valuable, but noted that these individuals should not be selected preferentially because it would skew the results of the study.

The panel also said that if an animal were to exhibit signs of shock during handling, the blood sampled would be valuable. St. Aubin noted that if an animal were to go into shock during handling, it would be because something (e.g., chase) had pushed the animal to its limit prior to handling. Gulland noted that obtaining a core temperature would be very important in this instance.

Several sources of baseline data for "normal" ranges of cetacean blood parameters were discussed, including some potential information from Sealife Park and Sea World, as well as information in the scientific literature (e.g., Asper *et al.*, 1990). Galindo expressed concern over using the generally established blood ranges for cetaceans as a control parameter for hormone values. He suggested that those reference values would only be useful if the diurnal variation of the hormones were known. He commented that the individuals should be used as self-controls over time. The panel agreed with this in general, but noted that we can obtain an understanding of the changes in blood values from known ranges for cetaceans. It was also noted that any blood parameter of concern would exhibit values beyond those caused by diurnal variation.

Recommendation 9

The panel recommended that dolphins sampled during CHESS will serve as their own internal control, with the first sample representing a reference point for repeated samples from the same individual. In addition, studies of other captive, wild, or live-stranded dolphin species should be used for reference. For example, a "reference study" could be conducted using wild bottlenose dolphins that are captured as part of ongoing studies in Sarasota, Florida. Multiple samples from animals captured on more than one occasion could provide valuable insights into the breadth of changes that appear to be tolerable in one cetacean species, since these well-known individuals are the subjects of ongoing monitoring. Submitting these samples to the diagnostic laboratories at the same time as those derived from the dolphins sampled in CHESS studies will provide a reference framework for comparison to larger databases for other cetacean species.

SUMMARY

The panel recommended that it might be useful to use three types of tags or markings, yielding three categories of dolphins in the study: (1) animals that were encircled without handling, (2) animals that were encircled and sampled; these animals would be the primary targets for repeated sampling, and (3) animals that were encircled, sampled and satellite tagged (these individuals would be less informative because the tag itself can cause a stress response, but will be required to ensure relocation of previously caught individuals). Priority in selecting animals in a set for blood sampling should be directed to those individuals that have previously been sampled and marked with the least invasive method to maximize the chances of detecting cumulative effects (i.e., failure to recover) in dolphins with a known recent history.

A scientist with expertise and background experience should act as the principal investigator for the blood analysis research and experimental design, and data sets from blood analyses should be sent to three independent evaluators with expertise in cetacean clinical pathology for expert interpretation.

The panel identified the blood constituents (Table 1) that should be analyzed to best assess the physiological conditions of the dolphins subject to chase and capture: standard hematology and chemistry panels, metabolites, enzymes, proteins, hormones, immunological tests, lactate, blood gas, and possibly troponin. The panel also recommended that muscle biopsies be taken to assess muscle damage over time.

The data set of dolphins sampled during CHESS will serve as its own internal control, with the first sample representing a reference point for repeated samples from the same individual. In addition, studies of other captive, wild, or live-stranded dolphin species should be used for reference. Established normal ranges of cetacean blood constituents will also be useful for interpreting the changes in blood values of chased and captured dolphins.

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Table 1. Summary of blood parameters for evaluation of stress in dolphins (0 = no change, + = increase, - = decrease).

Analysis/Test	Required Blood Volume & Method (Total 75cc)	Estimated Cost	Expected responses during capture on...					Recovery Time (Days)	Interpretation Comments	Background Data Available? (0=no, 1=some, 2=yes)	Quantitative Data Available ?
			Day 0 (Initial capture)	Day 1	Day 2	Day 4	Day 7				
Hematology	5 ml EDTA	$30									
PCV			0	0	0	0	0	0		2	
Hb			0	0	0	0	0	0		2	
RBC			0	0	0	0	0	0	*Depend on other parameters, times & frequency of samples. Could be caused by disease or chase/recapture*	2	
WBC			+	+	++	++	++	1-2		2	Y
Neut			+	+	++	+/++	+/++	1-2		2	Y
Lymphocytes			0	0/-	-	-	-	1-2		2	Y
Eosinophil			0	-	--	--	--	1-2		2	Y
Monocytes			0	0	0	0	0	0		2	
Bands			0	0/+	+	+	+	0		2	
Platelets			0	0	0	0	0	0		2	
Fibrinogen			0	0	+	+	+	0		2	
Sed Rate			0	0	0	0	0	0		2	
Chemistry Panel	20ml whole blood	$30									
Electrolytes											
Na			0	0	0	0	0			2	Y
K			+	+	+	+	+	1	Related to handling, exertion	2	Y
Cl			0	0	0	0	0			2	

Analysis/Test	Required Blood Volume & Method (Total 75cc)	Estimated Cost	Expected responses during capture on...					Recovery Time (Days)	Interpretation Comments	Background Data Available? (0=no, 1=some, 2=yes)	Quantitative Data Available ?
			Day 0 (Initial capture)	Day 1	Day 2	Day 4	Day 7				
Ca			0	0	0	0	0			2	Y
P			0	0	0	0	0			2	Y
Fe			0	-	--	-		1-2	Cumulative effects	2	Y
TIBC			0							2	
Mg			0	0	0	0	0			0	
Metabolites											
Gluc			+	+	+	+	+	1	Should not be cumulative	2	Y
BUN			0							2	
Creatin			0							2	Y
Bilirubin			0							2	Y
TG			0							2	
Chol.			0							2	
Uric Acid			0							2	
Enzymes											
AlAT			0							2	
AsAT			0	+	+	++	++	2	Primarily exertion related	2	
GGT			0							2	
AlkPhos			0							2	
CPK			0	+	++	++	+	2	Primarily exertion related	2	Y

Analysis/Test	Required Blood Volume & Method (Total 75cc)	Estimated Cost	Expected responses during capture on…					Recovery Time (Days)	Interpretation Comments	Background Data Available? (0=no, 1=some, 2=yes)	Quantitative Data Available ?
			Day 0 (Initial capture)	Day 1	Day 2	Day 4	Day 7				
LDH			0	+	+	+	+	2	Primarily exertion related	2	Y
Lipase			0							1	
Amylase			0							1	
Proteins											
Alb										2	
Glob										2	
Profile/ Electrophoresis		+$35								2	
Myoglobin		+$??		+	+	+	0	2	Little background info	0	
Hormones											
Dopamine	10-12 cc							?	Hard to interpret	0	
E	Hep.	$35	++	++	++	++	++	<1		1	
NE	Blood		++	++	++	++	++	<1		1	
Cortisol	*12-15cc*	$10	~+	~+	~+	~+	~+	<1		2	Y
Aldosterone		$15	+	+	+	+	+	<1		2	Y
T4		$5	0	-	-	--	--	2-3		2	Y
T3		$5	0	-	-	-	-	1-2		2	Y
rT3		$15	0	+	+	+	~+	1-2		2	Y
fT4		$5	0	-	-	--	--	2-3		2	Y
ACTH	*~5ml*	$15	+	+/-	+/-	+/-	+	1	No background data	0	

Analysis/Test	Required Blood Volume & Method (Total 75cc)	Estimated Cost	Expected responses during capture on…					Recovery Time (Days)	Interpretation Comments	Background Data Available? (0=no, 1=some, 2=yes)	Quantitative Data Available ?
			Day 0 (Initial capture)	Day 1	Day 2	Day 4	Day 7				
Progesterone	*~5ml*		0						Should not change	2	
Estrogen			0							2	
Testosterone			0							2	
Immunological tests											
phenotyping		Navy lab	0	0/-	-	-	-	1-2	(B cells)	1	
lymphocyte blastogenesis		Navy lab	0	0/-	0/-	-	-	1-2	possibly cumulative	1	
cytokines	10ml EDTA	$100	0	+	+	+	0/-	1		0	
cytokine antibodies	smear or skin	$30		0/+	0/+	0/+	0/+	Unkn.	Very little known about expected responses	0-1	
Tissue Analysis											
Muscle lactate	Muscle biopsy	$ 35	+	+	+	+	+	<1		1	
Muscle glycogen	Muscle biopsy	$ 35	-	-	-	-	-	1-2		1	
Skin proteins cytokines	Skin biopsy	$ 40	+	+	+	+	+	10-20	~60 days, but proteins degrade with time (new method)	0-1	
Others											
lactate	4ml whole blood	$5	+	+	+	+	+	<1		1	
blood gas	(no extra)	$10	+/-	+/-	+/-	+/-	+/-	<1		1	
troponin									Relates to myocardial damage.	0	

APPENDIX 1

AGENDA

NMFS - BLOOD PARAMETER WORKSHOP FOR DOLPHINS IN THE ETP

SOUTHWEST FISHERIES SCIENCE CENTER, LA JOLLA, CALIFORNIA

Tuesday, January 30, 2001 (9:00 AM-5:00 PM)

I. INTRODUCTION (0900-0930)
- Welcome & Introductions
- General Objectives

II. BACKGROUND (0930-1000)
- Brief summary of the overall IDCPA research goals (Reilly)
- CHESS (**CH**ase **E**ncirclement **S**tress **S**tudies; Forney)
  - Background
  - Objectives
  - Logistics

III. EXISTING RESEARCH -- Research Plan Overview – (1000-1030)
- Immunological studies (Romano)
- Molecular studies (Southern)
- Necropsy program (Cowan/Curry)
- Other summaries (Forney)

IV. OUTLINE OF SPECIFIC GOALS & QUESTIONS (1045-1130) – (Forney)
- Ultimate objective
- Critical Questions

V. ROUNDTABLE DISCUSSION (1230-1630)
- Which blood parameters will provide the most informative data?
- Which blood parameters can be assessed given logistic constraints?
- Controls & tie-ins to complementary research.
- WRAP-UP (1630-1700)

Wednesday, January 31, 2001 (9:00 AM-5:00 PM)

V. ROUNDTABLE DISCUSSION (continued)
- Re-cap of previous day's discussion (Forney)
- Anticipated ranges of target blood parameters.
- Interpretation of blood parameters within anticipated ranges.

VI. RECOMMENDATIONS
- Summarize key blood parameters, expected ranges and interpretation.

VII. CONCLUSIONS

APPENDIX 2

LIST OF PARTICIPANTS
BLOOD PARAMETER WORKSHOP FOR THE ASSESSMENT
OF STRESS IN EASTERN TROPICAL PACIFIC DOLPHINS,
JANUARY 30 and 31, 2001
SOUTHWEST FISHERIES SCIENCE CENTER
LA JOLLA, CALIFORNIA

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APPENDIX 3

LIST OF BACKGROUND MATERIALS

Anon. Chase Encirclement Stress Studies (CHESS) 2001 Draft Research Plan. Unpublished .

Curry, B. E., and Edwards, E. F. 1998. Investigation of the Potential Influence of Fishery-Induced Stress on Dolphins in the eastern Tropical Pacific Ocean: Research Planning. NOAA Technical Memorandum NOAA-TM-NMFS-254.

Donahue, M. A., Taylor, B. L., and Reilly, S. B. 2000. IDCPA Research Program Chase-Recapture Experiment Consultation Southwest Fisheries Science Center, La Jolla, CA, 25-26 April 2000. SWFSC Administrative Report LJ-00-15.