Report

Center for Independent Experts
Dolphin Stress Review

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I. Executive Summary

The tuna industry has used the association between tuna and dolphins to fish the eastern tropical Pacific (ETP) for over 5 decades. Dolphin mortality initially associated with tuna purse-seine fisheries was high, however, with improved fishery methodologies, observed dolphin mortality has markedly decreased in the last few decades. Nevertheless, stocks of dolphins in the ETP are not recovering as projected and concern exists that present fishing activities are causing "stressors" that are adversely impacting population recovery. As a result, the International Dolphin Conservation Program Act required that research consisting of "stress" studies be conducted by the National Marine Fisheries Service to determine if intentional deployment on, or encirclement of dolphins by purse-seine nets is having a significant adverse impact on any depleted dolphin stocks. A suite of multidisciplinary and multi-institutional pathophysiologic studies was conducted by an impressive array of researchers in 2001. These studies included investigations of various blood parameters, immune function, thermal condition, set-associated behavior, and pathology. The results of these studies were reviewed in February 2002 at the Southwest NMFS Laboratory in La Jolla, California.

In general, the scientific research presented at the review was well designed, synergistic and implemented to maximize results. In a few cases, new reference data were produced that likely will prove useful for future dolphin health assessment evaluations. Additionally, in many instances, the findings of one investigation provided complementary data for other studies. As a whole, these studies broaden our scientific understanding of these dolphin species.

Pathophysiologic effects consistent with an acute "stress" response were demonstrated in chased and captured dolphins but no clear evidence was demonstrated that these effects were sufficient to cause morbidity or mortality. Concurrent histopathologic studies demonstrated heart lesions consistent with acute "stress" which probably reflected excessive catecholamine release. Chronic lesions likely secondary to previous tissue injury also were reported. The latter lesions may or may not be related to chase and encirclement. Thus, these studies have provided new and informative baseline data on acute "stress" responses in dolphins involved in tuna purse-seine fishing operations. However, sufficient evidence was not presented to conclude that chase and encirclement are having a significant adverse impact on depleted dolphin stocks. Logistic difficulties and lack of dolphin herd cohesion resulted in minimal dolphin recaptures, therefore no valid conclusions could be made to address the critical issue of the effects of recurring or chronic "stress" which may be impacting dolphin population recovery. The research plan with suggested modifications should be repeated in future studies with additional preliminary population modeling to help increase the likelihood of recaptures. Consideration of the cost/benefits of modifying the reported sample acquisition protocol to a large-scale individual dolphin tagging, tracking, and recapture plan to address the problem of low group cohesion also is recommended. Additionally, the feasibility of using a captive population of spotted dolphins to provide critical baseline pathophysiologic data should be investigated.

II. Background

The yellow fin tuna industry has used the association of tuna and dolphins to fish in the ETP for over 50 years. Historically high levels of dolphin mortality in tuna purse-seine nets seriously depleted three stocks of dolphin. These stocks included eastern spinner dolphins (*Stenella longirostris orientalis*); northeastern offshore spotted dolphins (*Stenella attenuata attenuata*) and coastal spotted dolphins (*Stenella attenuata graffmani*). From the review materials provided, it is estimated that 4.9 million dolphins were incidentally killed in this fishery between 1959-1972 depleting the northeastern stock of spotted dolphins to less than 50% of its original abundance. The use of new equipment and new techniques in the tuna purse-seine fishery gradually decreased direct fishery-associated dolphin mortality between the late 1970's until the present. While incidental dolphin mortalities are currently low (in 2001 less than 1000 spotted dolphins were killed in the fishery from an estimated stock of about 500,000 animals [S. Reilly, personal communication]), stocks of pantropical spotted dolphins are not recovering as projected and concern exists that present fishing methodologies are causing chronic "stressors" (i.e., those "stressors" that produce pathophysiologic states not immediately fatal and/or detrimental to reproductive success) that may be having a "significant adverse impact" on population recovery. As a result the International Dolphin Conservation Program Act (IDCPA) required that research consisting of abundance surveys, ecosystem studies, and clinicopathologic determinations of "stress" be conducted by the National Marine Fisheries Service. The purpose of this research was to determine if the "intentional deployment on, or encirclement of, dolphins by purse-seine nets is having significant adverse impact on any depleted dolphin stocks." The "stress" studies outlined by the IDCPA included a review of relevant "stress"-related research and a 3-year necropsy review from dolphin tissue samples obtained on commercial tuna vessels, a 1-year review of relevant demographic and clinicopathologic data from dolphins from the ETP and a multifaceted field research experiment intentionally designed to repeatedly chase and capture dolphins. The latter studies were defined as Chase Encirclement Stress Studies (CHESS) and, according to the background material provided, were planned as the result of several workshops and consultations involving scientific experts, representatives of non-governmental organizations and staff of the Marine Mammal Commission and Inter-American Tropical Tuna Commission. CHESS consisted of a suite of projects designed to determine if repeated tuna fishery-related chase and encirclement could have deleterious effects on the health, survival and/or reproduction of spotted and spinner dolphins in the ETP. And, if such effects were demonstrated, if they could impact the ability of the dolphin population to recover from the fishery-related mortalities of the past. CHESS was one project specifically mandated by Congress to address questions of whether the tuna fishery causes sufficient "stress" to dolphins during chase and encirclement to hinder recovery of the population. The designed CHESS were conducted during a 60-day research cruise aboard the NOAA ship *McArthur* in the ETP from August – October 2001. CHESS also involved the concurrent use of a chartered tuna purse-seiner that performed actual dolphin associated tuna purse-seine operations involving standard dolphin chase and encirclement techniques.

III. <u>Review Activities</u>

The "stress" studies mandated by the IDCPA and reviewed February 4-6, 2002 at Southwest NMFS Laboratory in La Jolla, California included a wide range of multi-institutional and multidisciplinary studies. The studies reviewed were categorized and presented as follows: dolphin tracking and tagging associated with the fishery; dolphin behavior during purse-seine operations; thermal studies; a "stress" responsive skin protein study and clinicopathologic studies (including hematologic, serum analyte, and immunologic parameters, and gross and microscopic anatomic pathology). Additionally, studies outside the purview of the reviewers were presented that provided important ancillary information. The latter included studies of dolphin cow/calf behavior, dolphin energetics, dolphin reactions to research vessels, and dolphin reproductive and demographic parameters.

The principal investigators from each of the above sections provided oral presentations of variable length followed by a time period for reviewers' comments, questions and a general discussion. Lengthy discussions of scientific methodology, research results and conclusions often were made by the reviewers. Presentations were made primarily on February 4-5. On February 6 the reviewers were shown multiple videos of actual dolphin-associated tuna purse-seine operations in the ETP and had time to privately discuss the review process, data evaluation and recommendations. The Statement of Work (see Section VI, B) requests that a written report based on the reviewer's area of expertise be provided by March 15, 2002. This document will serve as the requested report.

<u>General Methodology Review</u>

The CHESS area was within the range of the northeastern stock of pantropical spotted dolphin and eastern spinner dolphin approximately 300 nmi south of Acapulco, Mexico. During CHESS, spotted and spinner dolphin herds were located, chased, encircled by the tuna purse-seine boat, experimentally evaluated (see below), and eventually released by a standard tuna fishery backdown procedure. Individual “focal” dolphins were tracked with radio transmitters and recaptures of the same “focal” dolphin and associated herd animals were attempted over the next several days. Set operations were limited by weather conditions and the presence of tagged identifiable dolphins for recapture. Sampling targeted primarily spotted dolphins to limit species-specific variation and for animal health considerations.

Dolphin herd sizes encountered ranged from 200-3000 animals. A subset of the herd was captured to reduce dolphin mortality and injury during the set procedure. After encirclement of the herd was complete, scientific teams were sequentially deployed in rigid hull inflatable boats (RHIBs) from the *McArthur*. The initial RHIB carried the set coordinator and thermal imaging personnel; the second RHIB followed 5-20 minutes later with the biologic sampling personnel and tow rafts used as 2 primary sampling locations. The third RHIB transported biopsy team personnel and equipment.

After a majority of the set had been retrieved, swimmers captured individual dolphins and guided them to sampling rafts. Behavioral data were collected by a behaviorist and an Inter-American Tropical Tuna Commission observer recorded standard set information aboard the tuna vessel.

From 1 to 9 dolphins were examined, sampled and tagged during each set (see CIE-S01 for details). Dolphins were restrained by swimmers in the water for generally less than 1 minute. Within the sampling raft, dolphins were restrained for an average of 9 minutes (range 3-18 minutes). Skin biopsies were taken in the backdown region of the net. It was difficult to select individual dolphins based on age, sex or reproductive status. When possible, priority was placed in sampling adult females to help determine possible effects of “stress” on reproduction. From the data collected and presented, it appeared that all dolphins were handled in a safe and humane manner. Two dolphins died accidentally in capture (not sampling) operations.

IV. Summary of Studies with Conclusions and Recommendations

A. Tagging and Tracking Studies (CIE-S05)

Briefly, 15 pantropical spotted dolphins (*Stenella attenuata*) (9 fitted with VHF radio transmitters and 6 fitted with satellite transmitters) were tagged and tracked. The dolphins tracked with VHF radio tags also carried time-depth recorders (TDRs) (n=3), time-depth-velocity recorders (TDVRs) (n=4) or time-depth-velocity-heat flux recorders (thermal) (n=2). Dolphins were tracked for varying time periods; 1 to 6 days for VHF tagged animals and 2 to 20 days for satellite tagged animals. Additionally, 213 visual tags were attached to spotted dolphins and 8 short-range radio tags (7 spotted and 1 spinner) were attached to obtain data of dolphin group captive associations. Dolphin movement patterns, dive characteristics and herd dynamic observations were reported from the tagged and tracked animals.

Seven of the 9 focal dolphins tracked were recaptured and the tags removed after 1-6 days. One dolphin was recaptured 3 times, another dolphin was recaptured twice and the remaining 5 were recaptured only once.

Nine focal dolphins were tracked for up to 6 days and 6 dolphins were satellite tracked for up to 20 days. The focal dolphins traveled from 71 to 134 nm per day with a median swimming speed of 4.2 kn.

Four dolphins exhibited diurnal differences in diving patterns similar to those previously described.

During the day, dolphins generally dove to depths of 15-20 m in a U-shaped pattern with little change of depth at the deepest depth. These dives were usually above the thermocline. However, at night the dolphins dove deeper usually well below the thermocline. Median dive depths at night were 12-18m.

Dolphins were observed to react first to the presence of the helicopter associated with the purse-seine operation. Therefore, for the purposes of measuring the effects of stress, the start of the chase was associated with the time the helicopter was overhead.

The TDVRs deployed provided limited data indicating that during chase and often release from the net during backdown, dolphin swimming speed exceeded speeds for normal traveling or feeding.

Conclusions and Recommendations

This was a well-designed study that produced informative data on movements of dolphins tracked and the associated diving patterns. Highly fluid herd dynamics within the study area resulting in low group cohesion were demonstrated. Low group cohesion has been reported for pelagic dolphins (Perrin et al., 1979). Low herd cohesion was apparently due to separation while foraging at night and regrouping opportunistically the following morning. This is a plausible hypothesis but additional tracking data is indicated for verification of the cause of this observation. A distinct diurnal/nocturnal trend was reported that indicated (along with oceanographic data) that the dolphins were exploiting a productive area with a shallow thermocline. Additionally, the authors of this study suggested that based on the data, small social groups likely exist within a large herd and that chase and encirclement may therefore not involve separation of large social groups. These suggestions also require more in-depth tagging and tracking studies.

It was postulated that by setting on small portions of large dolphin herds that the chances of tagging multiple dolphins from a stable subgroup would be enhanced. This was not the case. Questions that might be asked are: 1) could the methodology of sampling a smaller subgroup of a large dolphin herd cause the introduction of an experimental variable that caused the apparent highly fluid dynamic population?, and 2) could sampling a smaller subgroup actually contribute to the observed clinicopathologic parameters of acute “stress”? These questions need to be addressed in future studies. Further population modeling of fishing behavior and herd dynamics is recommended to possibly help in increasing the frequency for capture and repeated recapture of individual dolphins. Additionally, attempts for tagging and tracking including the chase and encirclement of large herds of dolphins as typically done in routine purse-seine operations are encouraged for future research. The feasibility of modifying the sample acquisition protocol to a large scale individual tagging, tracking and recapture plan also may prove useful. The repeated recapture of individual dolphins is critical and an absolute necessity to answer the question if fishing methodologies are having a “significant adverse impact” on dolphin population recovery.

The apparent increased swimming speeds of dolphins during chase and post release are interesting data that should be further investigated as possible “stressors” in the chase and encirclement fishing procedures. However, the sampling size from this study is too small to produce conclusive results as to a cause and effect relationship.

In addition to experimental sampling limitations, sampling biases may also be present in the experimental model. Sampling biases may include that only spotted dolphins were sampled and that dolphins in the net were exposed to new and increased human activity (e.g., swimmers, rafts, boats, handling, specimen collection techniques, and delay in backdown). These factors must be taken into account as possible experimental causes of “stress” influencing data collected in other CHESS studies and/or the ability to recapture non-focal animals. These may be largely unavoidable but important limitations for such a study.

Perhaps one of the more interesting but worrisome findings was that the proportions of pregnant and lactating females handled during CHESS appeared lower that expected based on previous studies. This may represent sampling bias. However, this observation warrants further consideration in future tracking, tagging and necropsy (see Section G, Conclusions and Recommendations) studies as it has obvious implications for a population’s recovery.

In conclusion, because the dolphin herds studied proved to be more dynamic than anticipated, this unexpected variable prevented an adequate sample size of repeat captures that were not focal animals. Therefore, adequate data providing information on the repeated effects of chase and encirclement over the course of several days to weeks including patterns of diving and movement were not obtained. This is the great limiting factor of the CHESS studies.

B. Coping Behavior During Sets (CIE-S06)

A behaviorist collected data using scan and focal methods after dolphins were encircled, and from video records of a subset of all sets. Data on dolphin behavior were also obtained from fishery observer data forms used by the Inter-American Tropical Tuna Commission (IATTC) observers. These data were compared to data obtained from the Mexican National Observer Program from 1998-2000. The objectives included: 1) to determine whether differences in duration of the set affect dolphin behavior in the net; 2) to determine whether dolphin behavior changes with the size of the herd captured; and 3) to examine aspects of set operations during CHESS compared to actual Mexican tuna fleet operations.

Briefly, it was reported that, in general, dolphin activity was increased in small herds compared to large herds and that no significant differences in duration were found between commercial and CHESS sets. Additionally, the proportion of dolphins engaged in active behaviors during the backdown was significantly greater in small herds than in large herds. The remaining data can be found in CIE-S06.

Conclusions and Recommendations

Behavioral investigations of dolphins are not in this reviewer's field of expertise. However, a few comments on the presentation and written report are warranted. This particular review briefly addressed (as did CIE-S11) the basic theoretical pathophysiologic aspects of acute and chronic "stress", provided a useful definition of "stress", and applied these concepts into a problem associated model for CHESS. This is critical for an understanding of the purpose of these studies. This presentation clearly focused that when the duration or intensity of a "stress" factor are transitory, the physiological effects are beneficial and no pathologic changes are produced (i.e., acute "stress" is an adaptive physiologic mechanism). However, when "stress" factors are chronic these same physiologic mechanisms can cause a suite of pathologic changes involving cardiovascular, digestive and metabolic disease with concurrent inhibition of immunity, reproduction and growth and behavioral changes that negatively influence all of the above in various ways. This is the primary question CHESS addresses, namely does chase, encirclement and backdown represent physical and psychological "stressors" that produce over time, chronic "stress" which affects dolphin health and/or reproductive success.

Specifically, the investigators appear to have addressed their objectives with the inherent limitations of most dolphin behavioral studies of this type (e.g., determining procedures for estimating numbers of dolphins captured and released, ability to characterize behaviors from a distance and underwater, etc.) and the fact that it was not possible to obtain adequate data from recaptured dolphins. The latter is a significant recurring problem for the each CHESS study section.

A few comments on the behavioral findings can be made. First, it was suggested that the high percentage of dolphins released by backdown indicated that the dolphins were familiar with the backdown procedure. Does this suggestion infer that “desensitization” or familiarization with backdown resulted in decreased “stress”? This may be a question to address in future studies.

An interesting finding was the observation of behaviors (e.g., defecation, aggression and vocalizations) often associated with psychological “stress” in net set dolphins and the resultant speculation that this could indicate short-term welfare problems for the captured animals. Mating behavior was noted to increase in the result section but not mentioned in the discussion of this paper. Over the years, I have commonly observed increased mating/sexual behavior in bottlenose dolphins (*Tursiops truncatus*) with various social or disease “stressors”.

Second, as discussed previously, the smaller number of dolphins captured per set during CHESS was expected because protocols were designed to limit the number of dolphins for dolphin and research personnel safety reasons. Did purposefully limiting the number of dolphins per set introduce an experimental bias to behavioral tracking and other pathophysiologic data? As indicated above this should be addressed in future studies.

Third, it was noted that smaller captured groups of dolphins were more likely to include separation of social subgroups (i.e., mother/calf pairs) that could increase the likelihood of active or agitated behavior. This was supported by the observation that dolphins were observed circling the outside of the net in almost 80% of the sets, and in some cases these dolphins tried to swim back over the cork line. Further research is recommended to evaluate these separation events and if cow/calf pairs were involved. This could shed light on the potential effects of this observation as a chronic “stressor”.

C. Thermal Studies (CIE-S04)

Chase and capture can be associated with thermal “stress” in terrestrial mammals and the resultant increased body core temperatures can cause pathophysiologic changes and death (Antognini et al., 1996; Beringer et al., 1996). Hyperthermia also can cause fetal changes resulting in low birth weight, retarded growth, skeletal and neurologic development abnormalities and acute fetal “stress” leading to death (Rommel et al., 1993). Additionally, in terrestrial mammals, high testicular temperature can interfere with spermatogenesis and sperm maturation.

It is possible that dolphins associated with tuna fishing operations in the warm ETP that undergo prolonged chase operations may be experiencing hyperthermic “stress”. The resultant “stress” may have a deleterious impact on dolphin health and reproductive success despite the presence unique reproductive countercurrent heat exchanger mechanisms (Pabst et al., 1998; Rommel et al., 1998).

The effects of prolonged chase and capture on thermoregulation in dolphins are unknown, however, comparative studies indicate that severe hyperthermia can cause health related conditions and reproductive problems.

This CHESS component was developed to test the hypothesis that ETP dolphins that are chased and captured in the tuna fishery suffer thermal “stress”. Deep body temperatures of captured dolphins, dorsal fin and body surface temperatures of swimming net-corralled dolphins, and dorsal fin heat flux values and skin surface temperatures of free-swimming dolphins were used to investigate thermal responses of dolphins to chase and capture.

Conclusions and Recommendations

This was a well-designed and interesting study that resulted in useful and informative data. It utilized three complementary and synergistic data sets to investigate the thermal biology of ETP dolphins. In this manner, multiple thermal issues were addressed in relationship to chase time effects, capture time confinement, area surface temperature and potential gender effects.

In general, the findings supported by the data suggested that longer chase times resulted in elevated fin and body surface temperatures but homeostatic mechanisms appeared to maintain normal core body temperatures in both male and female dolphins. The elevated surface temperatures were likely a result of vascular heat delivery to the surface of the dolphin. Additionally, data indicated that a positive relationship did not exist between deep body temperature and either the length of the chase or the amount of time spent within the net corral. Heat flux data indicated that dolphins appeared to be able to manage the thermal burden represented by exertion in warm ETP waters.

Some minor concerns exist regarding experimental design, methodology and data interpretation when the three data sets are examined individually. First, core body temperature results may have been biased from the experimental procedure of handling the dolphins. In my clinical experience, this is not a factor in other captive dolphin species but the possibility of developing experimental controls should be investigated for future studies.   Additionally, the deep body temperature measurements were measured at an insertion depth of 20 cm. The utilization of different probe depths may provide important thermal data in future controlled studies. Second, the statistical evaluation of infrared thermal image was complicated because images could not be correlated to any individual (i.e., multiple images of a single individual could exist within the data set). Finally, only two individuals were sampled in the heat flux studies; therefore, the results may not be representative of the reaction in ETP spotted dolphins. The experimental set should be expanded, again with control animals, to determine the thermal/vascular effects of the data collecting attachment in spotted dolphins.

The thermal researchers in the paper and oral presentation acknowledged many of these limitations. However, when combined, the results of the three approaches were unique and did provide valid insights into the thermal biology of chased, encircled and captured ETP spotted dolphins.

D. <u>Hematologic and Serum Analyte Studies (CIE-S02)</u>

This study had three objectives which were to: 1) expand the meager hematologic and serum analyte data sets for spotted dolphins in both number of specimens and evaluated parameters; 2) examine these data for clinicopathologic indications of acute “stress” responses; and 3) evaluate similar data in repeatedly captured dolphins for indications of chronic additive changes that could cause morbidity, eventual mortality, and/or reproductive failure from the “stress” represented by each capture.

The suite of blood analyses included routine hematologic and serum chemical analytes including a standard complete blood count with leukocyte differential counts, fibrinogen, and 28 serum constituents consisting of electrolytes, metabolites and enzymes (see CIE-S02). Hormones analyzed included adrenocorticotrophic hormone (ACTH), aldosterone, cortisol, total thyroxine, total triiodothyronine, free thyroxine, reverse triiodothyronine, testosterone, progesterone and estradiol. Epinephrine, norepinephrine and dopamine were determined using high-pressure liquid chromatography. Statistical analyses were performed using a software system and included: ANOVA, ANCOVA and Pearson correlates, where appropriate. Baseline data were examined for the effects of age and sex, and for the influence of time between specific events and blood collection.

Blood for at least some analyses was taken from 61 different dolphins, 53 of which were found to have been captured for the first time during the study. Ten dolphins fitted with tags were blood sampled at the time of known second capture; 6 on the day after tagging; 1 dolphin after 2 days; and 3 dolphins after 3 days. However, only 2 of the 10 captured with tags were blood sampled when first caught.

Conclusions and Recommendations

This was an informative, in-depth study that used laboratory methodologies with good quality control and quality assurance. The absence of the latter has been a serious flaw in other similar studies. The data collected offers, for the first time, a degree of baseline clinicopathologic data from spotted dolphins from the ETP. Additionally, the data indicated clinicopathologic evidence of acute “stress” responses to chase, encirclement and capture. Some dolphins sampled had hemograms suggestive of a “stress leukogram” (i.e., leukocytosis with absolute neutrophilia, lymphopenia and eosinopenia [Bossart, 2001]). Hyperglycemia, hypoferremia, decreased thyroid hormone levels, and elevated blood levels of cortisol also were consistent with an acute “stress” response. Apparent significant elevations in circulating catecholamines and ACTH after chase, encirclement and capture help, for the first time in this species, to define the degree of acute “stress” although, as indicated, the correlation between severity of the “stress” and deviation of blood results are tenuous. The results suggested that spotted dolphins exhibit a classical acute “stress” response as seen in other odontocetes and terrestrial mammals following capture “stress” or after exogenous administration of ACTH (Thomson and Geraci, 1986; St. Aubin and Geraci, 1989b, 1990). Thus, the study fulfilled its first two objectives. However, the critical third objective (i.e., to evaluate findings in repeatedly captured dolphins for evidence of additive changes that would signal an inability to recover from the “stress” encountered by each capture) was not realized. While the experimental design was to obtain serial blood samples from repeatedly captured dolphins, logistic difficulties and lack of spotted dolphin herd cohesion resulted in only 10 recaptures but only 2 of these dolphins were blood sampled when first handled. Therefore, no valid conclusions can be made to address the critical issue of the pathophysiologic effects of recurring or chronic “stress” which may be impacting the population’s recovery.

Two limitations of a study of this type complicate data evaluation. The first limitation involves the recognition of individual variation that occurs compared to a population mean of the same blood analyte. In captive marine mammal medicine, it is often impossible to recognize a clinically significant blood analyte value when compared to a mean value obtained from a heterogeneous population of the same species representing various states of health as well as sex, reproductive status and age. Because of this, health monitoring in captive marine mammals references normal values established over time for each individual animal (Bossart, 2001). Therefore, the possibility remains that the individual spotted dolphins of this study were demonstrating other clinicopathologic changes but that the analyses were unable to detect such changes due to wide variation in the initial values within the population. A captive study population of these dolphin species would help address these issues (see Section E, Conclusions and Recommendations).

The second limitation of this type of "stress" study is that the methodologies involve actions that themselves can induce a "stress" response especially in wild animals. This can be the result of the action of blood collection and prolonged confinement. This phenomenon can confound result interpretations and is another reason to obtain multiple samples from the same individual to assess trends in relation to an animal's baseline clinicopathologic values. Thus, this was a limiting feature of this study since repeated sampling of the same dolphins could not be made in large enough numbers.

Another interesting finding was the elevation of mean blood levels of CK, AST and LDH compared to other species of odontocetes (Bossart, 2001), but similar to those reported for net-captured harbor porpoise (Koopman, 1995). These enzymes in other species are generally found in striated muscle and may markedly increase in the blood following severe muscle exertion with subsequent rhabdomyolysis, profound acidosis and sometimes myoglobinuria, renal failure and death (Hulland, 1993; Kock et al., 1987; Colgrove, 1978; Chalmers et al., 1977; Lewis et al., 1977). Thus, this observation as discussed briefly in CIE-S01 may represent a "benign" or subclinical form of exertional rhabdomyolysis or the beginning of a clinical form of the same that could result in subsequent morbidity or mortality. Subclinical forms of exertional rhabdomyolysis are not uncommon in other marine mammals and the clinicopathologic changes usually resolve within a few days (Bossart, 2001). It must be cautioned that in this study more data is necessary to draw any conclusions as the presence or absence of an exertional rhabdomyopathy (capture myopathy).

One minor comment involves the etiology of the possible globular fraction changes observed. Serum protein electrophoresis of banked serum may help characterize the nature of the presumed globular fraction changes.

E. Immunologic Studies (CIE-S03)

In this study, various measurements of the immune system were carried out (on blood samples collected in CIE-S02) to determine the effects of repeated chase and encirclement in dolphins from the ETP. Immune system measurements characterized aspects of immune system structure and function. Lymphocytes were isolated from dolphin peripheral blood and labeled with monoclonal antibodies to cell surface proteins to determine specific lymphocyte subsets. Additionally, lymphocytes were cultured and incubated with T and B cell dependent mitogens to measure proliferation. DNA damage also was assessed using a Comet Assay to visualize DNA strand breakage.

Briefly, lymphocyte percentages and absolute lymphocyte numbers were determined for T cells, B cells, T helper lymphocyte an class II+ cells in the first time capture group (n=51) and the repeat capture group (n=10). Significant increases were documented in T cell percentages and decreases in B cell percentages in the repeat capture group compared to the first time capture group. Lymphocyte percentages from these subsets remained significant after matching 20 dolphins in the first time capture group with dolphins in the repeat capture group, with the addition of a significant decrease in lymphocyte percentages and an increase in the T/B ratio. First time captured males had significantly higher absolute class II+, T cells and T helper cells than first time captured females. No differences were noted in lymphocyte proliferation or DNA damage between either group. It was speculated that changes in lymphocyte percentages in the repeat capture group might increase a dolphin's susceptibility to disease.

Conclusions and Recommendations

This study characterized for the first time portions of the structure and function of the immune system for this dolphin species. Specifically, lymphocyte subsets and the effects of chase and encirclement were described in a concise detailed study. The data indicated that some changes in lymphocyte subset percentages occur as a result of repeated chase and capture with no apparent change in immune function. The changes observed are similar to other studies that have examined "stress" effects on the immune system (Dhabhar et al., 1995; De Guise et al., 1996).

While the applied science is exceptional in this study, conclusions on the effects of chase and encirclement on the immune system need to be made with extreme caution. The measured immune system parameters of this study give a very brief glimpse of an extraordinarily complex, dynamic, and multifaceted system. Due to the complex nature of the immune response, individual variation with many of these parameters would not be considered unusual. Additional individual variables that confound immunologic data interpretation include variation to the type of "stressor", intensity and duration of "stressor" and desensitization to the "stressor", as well as species-specific variation, flux of health and physiologic factors including age, sex and reproductive status. The authors acknowledged some of these limitations. These factors make comparisons of data from different animals within a population very difficult. A suite of a wide range of immunologic measurements may be necessary to determine immune system structure and function and the potential effects of disease and environmental "stress", including capture and encirclement. In addition to the measurements made in this study, the measurement of various cytokines, quantification of immunoglobulin classes, further lymphocyte phenotyping and genomic analyses of cellular components may provide more information on these effects on the immune system for future studies. As the authors correctly state, one of the major obstacles in characterizing the immune system of marine mammals is the lack of species-specific reagents and assays. Hopefully, this obstacle will be able to be overcome with additional applied research since meaningful studies depend on it. This cannot be overemphasized for a study of this type.

Sampling biases are a problem in this study as discussed previously. Dolphins in the repeat capture group were not represented in the first time group, hence comparisons were not made from the same individual dolphin. This is a significant limiting factor when attempting to determine immunologic effects due to great individual variations in measured parameters. The fact that dolphins were captured and handled also is likely an experimentally induced “stressor”. This is an inherent limitation to this type of study.

Another problematic and limiting feature of all of the clinicopathologic parameters measured from CHESS hematologic, serum analyte, immunologic and SRP studies is that baseline values in normal healthy dolphins of these species are not available. As the authors indicated, data obtained for the first time in chase and encircled dolphins cannot be considered truly baseline and so comparisons are difficult if not impossible to make. The methodologies used in this study are recent and still being characterized to establish baseline immunologic parameters for cetacean species maintained in captivity. In captive situations, internal and external variables that influence immune parameters can be controlled. Ideally, critical baseline data for comparative studies including hematologic, serum analyte and immunologic studies should be obtained from a captive healthy group of this species. Minimally, first time capture samples from the same repeat chased and captured dolphins are needed before any meaningful conclusions can be made on clinicopathologic data. This statement holds true for all future CHESS clinicopathologic studies that are highly recommended to address these issues.

F. Stress-Activated Protein Studies (CIE-S07 and CIE-S08)

A new technique was used in CIE-S07 and CIE-S08; therefore, this section will serve as a summary for both studies. A new immunohistochemical technique was designed to detect the molecular signature of pathophysiologic “stress” based on the expression profile analysis of multiple molecular “stress” response proteins (SRPs) in skin. The authors postulated that skin samples provide an indication of sustained “stress” periods in a dolphin’s recent past because SRPs are deposited in keratinocytes of the epidermis. “Stress”-associated changes in SRP expression profiles were reported using 90 reference specimens from normal and “stressed” mammals obtained by opportunistic sampling from eight species. Forty SRPs were identified and reported to have altered expression profiles due to “stress”. Skin samples were examined in relation to fishery effort at the time and in the area of collection with skin samples from bow-riding dolphins in areas of little fishing effort used as approximate controls. Skin samples also were collected from captured and recaptured dolphins during CHESS to permit direct evaluation of dolphins with a known capture history. In turn, these specimens provided a comparison to those obtained historically during fishery operations and from bow-riding dolphins from areas of varying fishing intensity (CIE-S07).

The potential influence of fishery interaction on the probability of detecting altered expression patterns was examined by determining the number of sets within specific spatial and temporal times. High frequencies of altered expression patterns characterized the fishery-involved samples relative to the non-fishery involved samples; however, the authors speculated that potential sampling biases regarding sampling location confounded comparisons. It was concluded that within the fishery mortalities, more sets, occurring prior to sampling increased the likelihood of an altered expression pattern. However, within the bow-riding biopsies, more sets decreased the likelihood of an altered expression pattern. The authors suggested from the SRP results that in fishing mortality cases, fishing sets in the recent past caused "stress". However, in the bow-riding biopsies fishing sets in the recent past altered behavior.

The primary conclusion made in CIE-S08 was that the SRP immunohistochemical technique represented a "new, effective and practical biomarker of stress" with wide potential application in medicine and conservation biology.

Conclusions and Recommendations

The basic concept of immunohistochemical testing for SRPs is intriguing and may be useful in this type of "stress" research. However, serious concerns exist about the design and scientific methodology used in this section. Considerable technical and analytical variability exists in the design of the study that does not permit a meaningful comparison of the two groups of "stressed" and "non-stressed" dolphins. These variables are applicable to basic concepts and limitations of the immunohistochemical technique and greatly influence the validity of immunohistochemical data.

The first variable involves tissue fixation and includes elapsed time between death and tissue sampling, elapsed time between tissue sampling and fixation and duration of fixation before processing. These fixation issues seem to vary widely or are not indicated and need attention since they greatly influence the accuracy and validity of the immunohistochemical technique.

Another more concerning variable is the great diversity and number of markers used in the methodology. Differences in antigen cellular location (nuclear, intracytoplasmic, cytoplasmic membrane) and the influence on image analysis would be anticipated but is not addressed. Additionally, in a "cocktail" of antibodies as used in this study, non-specific or cross binding within the "cocktail" itself could occur. This, in turn, would affect the immunohistochemical data. Greater sensitivity and precision would be produced by limiting the technique to a single antibody per tissue tested. These variables should be addressed in a controlled validation study.

Concern also exists that the staining technique, including antigen retrieval, may not be applicable to all of the antibodies tested and the possibility that some of the antibodies tested may not react at all in fixed and processed tissues.

These issues need to be addressed before the technique can be considered an effective and practical biomarker of “stress” with wide application in medicine and conservation biology. Development of a laboratory animal model for the technique is suggested. This would permit experimental manipulation with adequate controls for technique validation.

Once the immunohistochemical technique is validated, interpretative difficulties with the methodology remain to be addressed. One difficulty is that the pathophysiologic mechanisms of SRP antigen deposition are unknown in cetaceans. Another difficulty involves the apparent topographic differences in the expression of SRPs over the body surface. Finally, the prognostic significance of immunostaining is not implied and needs further characterization. As indicated previously, controlled studies of the immunohistochemical technique in a laboratory animal model would be useful to address these issues. Additionally, an experimental captive population of spotted dolphins would help address the issues of the SRP pathophysiologic mechanisms in cetacean skin. The latter may be a necessary prerequisite for interpreting future SRP immunohistochemical data. The scientific benefit of a captive experimental population of spotted dolphins for generating critical baseline pathophysiologic data is also discussed in Sections D and E – Conclusions and Recommendations.

G. <u>Histopathologic Studies (CIE-S11)</u>

Tissues from 56 dolphins (26 *Stenella longirostris*, 28 *S. attenuata*, 2 *Delphinus spp.*) that were collected during tuna vessel shipboard necropsies were examined microscopically by standard histopathologic techniques. The objectives of this study were to: 1) define a background of naturally occurring disease in the ETP dolphin population; 2) define, if possible, the cause of death of individual dolphins; and 3) identify lesions of disease or injury that might be attributed to “stress” related to prior chase and encirclement.

For objective1, the authors indicated that the major natural disease factor in the dolphins evaluated was parasitism, primarily pulmonary nematodiasis. Additionally, mild chronic interstitial nephritis also was commonly reported. The authors concluded that the background of disease was “almost trivial” when compared to parasitic disease found in coastal odontocetes and that the population sampled appeared generally healthy with adequate functioning immune systems.

For objective 2, the authors reported that all dolphins examined had nearly universal myocardial microscopic changes including hyalinized and wavy fibers, perinuclear cardiomyocyte vacuolation and contraction band necrosis. These changes were widespread and frequently observed in the conduction system. Contraction banding also was reported in the smooth muscle of intramural coronary arteries and occasionally small artery eccentric intimal plaque formation was present. Hyalinization of myocytes and architectural disturbance were reported in the intramural coronary arteries. Additionally, contraction banding of smooth muscle of the intestine and urinary bladder and in the media of vessels of many organs was reported. Bronchospasm and pulmonary air trapping were almost universal. Acute tubular necrosis of segments of the renal tubules with intraglomerular reflux also was very common. These acute changes were postulated to be related to the immediate circumstances of encirclement and indicated to the authors that the cause of death in these cases was acute endogenously generated catecholamine-induced myocardial injury leading to cardiac arrhythmia and sudden death. The microscopic data were interpreted to be the result of a “sympathetic storm” indicating the pronounced release of catecholamines (i.e., an overwhelming activation of an alarm reaction).

For objective 3, the authors reported microscopic evidence consistent with the healing of acute injury related to the fishery or other “stressors”. The microscopic findings found to validate this assumption were small patchy fibrous myocardial scars in about 71% of the cases. These scars were located in similar regions of the heart as the acute lesions reported above. Additionally, intramural coronary artery intimal fibrous plaques were reported. It was hypothesized that these lesions were sequelae to the acute lesions observed with the implication that acute mortality was related to the degree of cardiac injury (i.e., a high degree of cardiac injury results in acute mortality while dolphins that suffer similar but less severe cardiac injury resolve the lesions with or without scar formation).

Within the sample set, no apparent difference was noted in microscopic findings among species or geographic origin of location. Two animals that died from net entanglement in the CHESS study had lesions indistinguishable in type or severity from dolphins collected during fishery operations.

Conclusions and Recommendations

This study applied classical anatomic pathology to microscopically evaluate tissues from dolphins that died primarily in previous tuna fishing operations. It also provided an exceptional operational definition of “stress” and theoretical considerations of the pathophysiologic mechanisms of “stress” in dolphins. This is critical information for an interpretation of the entire CHESS program.

For the second time in these dolphin species, the data provided a suite of likely interrelated tissue changes including some fascinating myocardial and vascular lesions (first report was by Cowan and Walker, 1979). It was postulated that the etiopathogenesis of the majority of the lesions was related to the acute lethal or sublethal effects related to a “sympathetic storm” or the overwhelming maladaptive activation of an alarm reaction.

The study addressed each of the objectives. It was determined in the first objective that parasitism was the primary natural disease factor but was not considered to be a significant factor in mortality. This would be an expected finding in most healthy terrestrial or aquatic wildlife species. An important although understated point is that the dolphin population sampled, from a histologic standpoint, appeared to be generally healthy with an adequately functioning immune system. This is important information from a population perspective as it implies from the limited data set that dolphin morbidity or mortality due to natural disease is not having a “significant adverse impact” on population recovery. The necropsy study should be expanded to include more individual animals to help confirm this implication as well as for other reasons detailed below.

The statement regarding an adequate functioning immune system has to be made with a degree of caution. Immune system function was inferred by microscopic structure of immune system tissues. Immune system function is usually, but not always, inferred by microscopic structure. Also, in this study, not all immunologic tissues (all lymph nodes, bone marrow) appear to have been evaluated. Albeit important, this is a minor point.

Additionally, it would be interesting to attempt to document the reproductive status of the dolphins necropsied. Microscopic evaluation of testicular, ovarian and uterine tissues as to state of reproductive activity and past reproductive events (e.g., presence of spermatogenesis, folliculogenesis, corpora lutea, corpora albicans, corpora atretica and new, potentially useful, microscopic techniques for determining past pregnancies) could help to characterize reproductive parameters in the ETP dolphin population. For example, our laboratory is presently investigating the evaluation of the degenerative changes of uterine blood vessels in bottlenose dolphins (*Tursiops truncatus)* to determine past female reproductive history. In horses, studies of the degenerative changes of endometrial blood vessels correlated the degree of degeneration with parity and age (Gruninger et al., 1998; Oikawa et al., 1993). We are employing this technique with studies of captive dolphin reproductive tracts with known age and parity, with the intent of determining potential application for estimating parity in free-ranging dolphins.

The second objective was to define, if possible, the cause of death in the dolphins examined. This objective was realized with the presentation of a hypothetical pathophysiologic mechanism for the observed microscopic lesions. The results suggested the cause of death of dolphins dying in purse-seines was a catecholamine cardiomyopathy leading to myocardial arrhythmia and sudden death. The changes observed are consistent with an insult caused by a sudden and massive release of catecholamines or by vasospasm particularly of intramural coronary arteries with resultant ischemia and reperfusion. This theoretical pathophysiologic mechanism was supported by the single blood catecholamine measurements taken from a dolphin that died accidentally in a CHESS set. Catecholamine levels in this dolphin were an order of magnitude higher than the maximum values in live-sampled dolphins. The histopathologic evidence provided is quite detailed and distinctive, and the pathologic mechanisms leading to these lesions are well documented in the clinical and experimental literature as indicated by the authors (Lunt and Rose, 1987; Cebelin and Hirsch, 1980; Mukherjee et al., 1982; Muntz et al., 1984; Hinkle and Thaler, 1982). However, it appears that a strong association is made between fishery operations and the observed lesions. The authors did indicate briefly that the lesions observed in dolphins from non-fishery related mortality studies (Turnbull and Cowan, 1998; Cowan et al., 1986) also were consistent with injury caused by massive release of endogenous catecholamines or by vasospasm. Additionally, similar myocardial lesions have been described in other cetacean species that are not associated with fisheries (Bossart et al., 1985). Therefore, it must be stressed that the lesions found in the ETP dolphins are non-specific to the tuna fishery (also see below).

The possible association the authors make with capture myopathy and the renal lesions (and some blood analyte values discussed previously) cannot be discounted (reviewed by Curry, 1999) and this association needs further investigation (see Section D, Conclusions and Recommendations). However, acute tubular necrosis of renal tubules can be a result of a long list of etiologies that cause prolonged renal ischemia. Therefore, this association should be made with caution.

The third objective was addressed with the observation that a significant number of dolphins examined had lesions of the heart and small blood vessels that were consistent in size, location and type with being the result of the acute lesions described above. These lesions included multifocal myocardial fibrosis and changes of intramural coronary arteries consistent with chronic spasm. It was speculated that these types of lesions could cause delayed mortality and they might be attributed to “stress” related to prior chase and encirclement observations. These are not implausible speculations. However, this suite of lesions is not uncommon in other terrestrial wildlife and non-fishery associated marine mammal species. As the authors indicated in the last sentence of the abstract “it is possible that residua in these animals could have been produced by non-fisheries related stress in the normal environment”. I suspect this is probably the case in this study. Additional research is required to confirm the pathologic mechanisms involved.

H. Morphology and Autonomic Innervation of Lymphoid Organs (CIE-S10)

Lymphoid organs including spleen, lymph nodes, thymus, and gutassociated lymphoid tissue were collected and examined from ETP pantropical spotted dolphins, spinner and common dolphins incidentally entangled and drowned in the tuna fishery to help gain an understanding of the effects of repeated chase and encirclement. The general gross and microscopic anatomy and autonomic innervation of these tissues were characterized to help demonstrate the possible effects of repeated chase and encirclement. Similar studies in laboratory animals have demonstrated that “stress” can cause lymphoid tissue involution with decrease in various cell types (Dominguez-Gerpe and Rey-Mendez, 1997 and 1998; Fukui et al., 1997).

Lymphoid organs were collected from 57 dolphins incidentally killed in ETP purse-seine fisheries. Lymphoid tissue functional morphology was examined by traditional microscopic methods. Autonomic innervation of lymphoid organs was determined using catecholamine fluorescence histochemistry and immunocytochemistry.

Briefly, it was concluded that the lymphoid organs examined displayed characteristic mammalian morphology that was consistent with an intact and functioning immune system. The authors reported no evidence of involution or lymphoid depletion as seen after “stress” in terrestrial mammals.

Catecholamine-containing and tyrosine hydroxylase positive nerve fibers were demonstrated in close association with lymphoid cells of the spleen and mesenteric lymph nodes demonstrating an anatomic link between the nervous and immune systems. It was suggested that the latter finding needs further investigation to characterize the functional implications.

Conclusions and Recommendations

This was a well-structured investigation that provided useful and important scientific data. It is important to note that the lymphoid tissues of these dolphins had a microscopic appearance consistent with a functioning and intact immune system. Therefore, not only are pathologic changes associated with “stress” absent but changes associated with other disease states also are absent. As indicated in Section G, this may provide important information for population recovery analyses.

Additionally, the study provided conclusive data of an anatomic link between the nervous and immune systems in these species of dolphins. This link has been demonstrated in other terrestrial mammals. This has important implications for future “stress” studies as it implies that neurotransmitters associated with “stress” may affect the immune response and lymphocyte distribution. Studies as detailed by the authors in the Discussion Section of CIE-S10 should be conducted in future free-ranging experiments.

The one significant limitation of this study was that no data were obtained on the effects of repeated chase and encirclement on the immune system. The prior history of chase and encirclement for each necropsy case in this study is unknown. Hence, no information was provided to help determine the effects of repeated chase and encirclement, which is a stated objective of the study. This is critical missing data as the functional morphologic effects of "stress" on lymphoid organs in dolphins, are likely time dependent and dependent on the duration and intensity of the "stressor".

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APPENDIX A

All files in Adobe Acrobat PDF format (click here to get latest free Acrobat Reader)

CIE-S01	Karin A. Forney, David J. St. Aubin, and Susan J. Chivers	Chase encirclement stress studies on dolphins involved in eastern tropical Pacific Ocean purse seine operations during 2001
CIE-S02	David J. St. Aubin	Hematological and serum chemical constituents in eastern spotted dolphins (*Stenella attenuata*) following chase and encirclement
CIE-S03	Tracy Romano, Mandy Keogh, and Kerri Danil	Investigation of the effects of repeated chase and encirclement on the immune system of dolphins (*Stenella attenuata* and *Stenella longirostris*) in the eastern tropical Pacific
CIE-S04	D. Ann Pabst, William A. McLellan, Erin M. Meagher, and Andrew J. Westgate	Measuring temperatures and heat flux from dolphins in the eastern tropical Pacific: Is thermal stress associated with chase and capture in the ETP-tuna purse seine fishery?
CIE-S05	S. J. Chivers and M. D. Scott	Tagging and tracking of *Stenella* spp. during the 2001 Chase Encirclement Stress Studies Cruise
CIE-S06	Eduardo Santurtún and Francisco Galindo	Coping behaviors of spotted dolphins during fishing sets
CIE-S07	Andrew Dizon, Anne Allen, Nick Kellar, and Sárka Southern	Stress in spotted dolphins (*Stenella attenuata*) associated with purse-seine tuna fishing in the eastern tropical Pacific
CIE-S08	Sarka Southern, Anne Allen, Nick Kellar and Andrew Dizon	Molecular signature of physiological stress based on protein expression profiling of skin
CIE-S10	Tracy Romano, Karen Abella, Daniel Cowan, and Barbara Curry	Investigation of the morphology and autonomic innervation of the lymphoid organs in the pantropical spotted, spinner, and common dolphins (*Stenella attenuata*, *Stenella longirostris* and *Delphinus delphis*)

		incidentally entangled and drowned in the tuna purse-seine fishery in the eastern tropical Pacific
CIE-S11	Daniel F. Cowan, Barbara E. Curry	Histopathological assessment of dolphins necropsied onboard vessels in the eastern tropical Pacific tuna fishery (images added 2002-01-29)
CIE-S12	Elizabeth F. Edwards, Ph. D.	Potential effects of chase and encirclement by purse-seiners on behavior and energetics of spotted dolphin (*Stenella attenuata*) mother-calf pairs in the ETP
CIE-S13	Elizabeth F. Edwards, Ph. D.	Energetics consequences of chase by tuna purse-seiners for spotted dolphins (*Stenella attenuata*) in the eastern tropical Pacific Ocean
CIE-S14	Sarah L. Mesnick, Frederick I. Archer, Anne C. Allen, and Andrew E. Dizon	Evasive behavior of eastern tropical Pacific dolphins relative to effort by the tuna purse-seine fishery
CIE-S15	Katie Cramer, Wayne L. Perryman	Estimation of reproductive and demographic parameters of the eastern spinner dolphin (*Stenella longirostris orientalis*) using aerial photogrammetry

APPENDIX B

STATEMENT OF WORK

Consulting Agreement Between The University of Miami and Dr. Gregory D. Bossart

Background

The tuna industry has used the association between tuna and dolphins to fish in the eastern tropical Pacific Ocean for over five decades. Three stocks of dolphins were depleted by high historical levels of dolphin mortality in tuna purse-seine nets, with an estimated 4.9 million dolphins killed during the fourteen-year period 1959-1972. After passage of the Marine Mammal Protection Act in 1972 and the increased use of equipment designed to prevent dolphin deaths, mortality decreased gradually during the late 1970s, 1980s and 1990s. While changes in the fishery have greatly reduced the observed mortality of dolphins, there continues to be concern that the fishing methods used are causing stress to the dolphins involved and that such stress may be having a significant adverse impact on population recovery. As a result, the International Dolphin Conservation Program Act (IDCPA) required that research consisting of population abundance surveys and stress studies be conducted by the National Marine Fisheries Service to determine whether the "intentional deployment on, or encirclement of, dolphins by purse-seine nets is having a significant adverse impact on any depleted dolphin stock". The stress studies mandated in the IDCPA include:

A. A review of relevant stress-related research and a 3-year series of necropsy samples from dolphins obtained by commercial vessels.
B. A one-year review of relevant historical demographic and biological data related to the dolphins and dolphin stocks.
C. An experiment involving the repeated chasing and capturing of dolphins by means of intentional encirclement.

The necropsy program (A) has analyzed samples from about 50 dolphins killed incidentally during fishing operations. Historical biological samples and data (B) have been analyzed at the Southwest Fisheries Science Center (SWFSC) to investigate stress-activated- proteins (SAPs) in the skin in dolphins killed in the fishery and live-sampled via biopsy. Historical data were also examined to assess separation of cows and calves during fishing operations. The Chase Encirclement Stress Studies (C; CHESS) were conducted during a 2-month research cruise aboard the NOAA ship McArthur in the eastern tropical Pacific Ocean from August - October 2001. During this project, the team worked in cooperation with a chartered tuna purse seiner to study potential effects of chase and encirclement on dolphins involved in tuna purse seine operations. Dolphins groups were found to be much more dynamic than previously recognized, making it extremely difficult to recapture groups of dolphins over the course of several days to weeks, as planned. In the end, nine different dolphins were tracked for 1-5 days during the course of the study, including two animals outfitted with a thermal tag that recorded heat flux, temperature, and dive data. Individual radio-tagged dolphins and 1-4 associated roto-tagged dolphins were recaptured on several occasions spanning shorter periods of 1-3 days. Six satellite tags were deployed to record movement and dive data on dolphins that were not recaptured.

Biological data and samples were collected from as many captured dolphins as possible, and include: 70 blood samples, of which 18 were from repeat captures of marked individuals; 283 skin samples, of which 17 were from previously captured and sampled animals; 449 analyzable thermal images; 52 core temperatures; and 95hrs of heat flux data. Females with calves were noted on several recapture occasions, and one known calf was skin sampled during an initial and subsequent capture. All samples and data are being analyzed at SWFSC and other contracted laboratories.

General Topics for Review

This review includes a suite of studies subsumed under the general topic of "Stress Studies". Up to 17 separate papers will be provided covering the studies described below. The general components are as follows:

- Necropsy samples: Analysis of tissues from dolphins incidentally killed in the fishery.

- Blood samples: Analysis of blood samples collected from wild dolphins captured using purse seine methods to assess A) general health, B) immune function, and C) stress response to capture.

- Stress-activated protein studies: Analysis of skin samples to assess levels of stress-activated proteins in dolphins that were A) killed in the fishery B) captured once C) captured repeatedly and D) bow-riding research vessels.

- Thermal studies: Analysis of thermal images, deep core temperatures, and heat flux data derived from thermal tag deployments on wild dolphins.

- Fishery-related behavior: Analysis of behavioral data from dolphins captured using purse seine methods.

- Behavioral ecology: Analysis of tracking data for dolphins captured, tagged, tracked and recaptured during field studies, to investigate school dynamics and movement patterns.

- Cow/calf separation: Analysis of composition of dolphin schools to investigate separation of lactating females and their calves.

- Dolphin swimming energetics: Analysis of the energetic costs of being chased, particularly for lactating females and associated calves.

Documents supplied to reviewers will include draft manuscripts on topics listed above, and a number of background papers (relevant publications and reports).

Specific Reviewer Responsibilities

The reviewer's duties shall not exceed a maximum total of two weeks, including several days to read all relevant documents, three days to attend a meeting with scientists at the NMFS La Jolla Laboratory, in San Diego, California, and several days to produce a written report of the reviewer's comments and recommendations. It is expected that this report shall reflect the reviewer's area of expertise; therefore, no consensus opinion (or report) will be required. Specific tasks and timings are itemized below:

1. Read and become familiar with the relevant documents provided in advance;

2. Discuss relevant documents with scientists at the NMFS La Jolla Laboratory, in San Diego, CA, for 3 days, from 4-6 February 2002;

3. No later than March 15, 2002, submit a written report of findings, analysis, and conclusions. The report should be addressed to the "UM Independent System for Peer Reviews, " and sent to David Die, UM/RSMAS, 4600 Rickenbacker Causeway, Miami, FL 33149 (or via email to ddie@rsmas.miami.edu).

Signed________________________Date____________