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Report on the Findings of the Chase Encirclement Stress Studies (CHESS)

Executive Summary

For over five decades, the tuna industry has used the association between tuna and dolphins to fish in the eastern tropical Pacific (ETP). Three stocks of dolphins, eastern spinner *(Stenella longirostris orientali*s), northeastern offshore spotted *(Stenella attenuata attenuat*a), and coastal spotted *(Stenella attenuata graffman*i), were depleted by high mortality associated with tuna purse-seine nets, with approximately five million dolphins killed between 1959 and 1972 (Wade 1995). After passage of the Marine Mammal Protection Act in 1972, and the increased use of equipment designed to prevent dolphin deaths, mortality has decreased gradually since the late 1970s. In 1995, the Declaration of Panama was negotiated between the United States and eleven other fishing nations to reduce mortality of dolphins to less than 5,000 per year. While changes in the fishery during the last few decades have greatly reduced the observed mortality of dolphins, there continues to be concern that the fishing methods currently used are causing stress to the dolphins 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 studies of population abundance and stress be conducted by the National Marine Fisheries Service (NMFS) 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. As a result, the Chase Encirclement Stress Studies (CHESS) was initiated and conducted between August and October 2001, and consisted of a suite of research projects directed towards the central question of *whether repeated chase and encirclement as part of tuna fishing operations can have a negative impact on the health, reproduction and survival of spotted and spinner dolphins, and consequently affect the ability of this population to recover from excessive fishery-related mortalities in past decades.*

The following primary results were obtained from the various studies. The relatively elevated concentrations of adrenocorticotropin (ACTH), cortisol (F) and catecholamines would suggest an acute neuroendocrine stress response. Studies of the immune system revealed some changes in lymphocyte subsets; however the overall effect was no change in immune function. Post-mortem evaluation of lymphoid organ morphology indicated that the immune system was not compromised. Thermal studies also revealed some statistically significant correlations, but the overall result was that core body temperature was maintained regardless of the chase duration. Necropsies reveal the presence of benign cardiomyopathy with unknown functional consequences. Scarring in cardiac tissue suggests exposure to previous sublethal damage. During the encirclement process, two of the approximately 1,500 animals encircled (0.13 %) died, with the cause of death attributed to “sympathetic storm” characterized by the pronounced release of catecholamines. Tagging data would indicate that following release dolphins stay in a relatively confined area and exhibit high travel speeds. During backdown dolphins begin to congregate at the release end of the net indicating that these animals are familiar with the operation and have been habituated to it. Although the increased alterations in stress related proteins (SRP) suggest the occurrence of stress in spotted dolphins exposed to fishing activity in ETP, implications derived from these data must be evaluated with caution. The idea of using skin-derived SRPs to quantify the effects of chase and encirclement on dolphins is intriguing; however, it is novel and requires further methodological validation for this particular application.

Overall, the likelihood that encircled dolphins exhibit an acute neuroendocrine response typical of exercise- or psychological-induced stress is not surprising, especially in the present series of studies since the animals were chased before being handled for various manipulations. Despite the quality of the researchers participating in these studies, the limitations of working in

the middle of the ocean and the number of logistical constraints associated with this operation make it difficult to derive the required science to properly address the underling question: *does repeated chase and encirclement as part of tuna fishing operations have a negative impact on the health, reproduction and survival of spotted and spinner dolphins, and consequently affect the ability of this population to recover from excessive fishery-related mortalities in past decades?* Also, the lack of *a priori* criteria to determine whether the procedures had *significant adverse impact* on the dolphins was disconcerting. The possibility of maintaining captive spotted and spinner dolphins in order to produce the missing baseline data and obtaining better estimates of abundance via increased tracking and tagging efforts are among the recommendations detailed in the present report.

Background

In the ETP the association between tuna and dolphins by the tuna industry has been used for decades. The historically high levels of dolphin mortality in tuna purse-seine nets have depleted the stocks of three species of dolphins: eastern spinner, northeastern offshore spotted and coastal spotted dolphins. An estimated 4.9 million dolphins were killed between 1959 and 1972. After passage of the Marine Mammal Protection Act in 1972 and the increased use of equipment designed to prevent dolphin deaths, dolphin mortality associated with the tuna industry has decreased gradually since the late 1970s. While changes in the tuna fishery have greatly reduced the observed mortality of dolphins, the concern that the stress induced by the current fishing methods is adversely impacting the recovery of the aforementioned dolphin populations persists. As a result, the International Dolphin Conservation Program Act (IDCPA) required that studies of population abundance and stress 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 by IDCPA include: A) review of relevant stress-related research and a 3-year series of necropsy samples from dolphins obtained by commercial vessels, B) one-year review of relevant historical demographic and biological data related to the dolphins and dolphin stocks, and C) an experiment involving the repeated chase and recapture of dolphins by means of intentional encirclement.

The necropsy program analyzed samples from about 56 dolphins killed incidentally during fishing operations. Stress related proteins (SRP) from the skin of dolphins killed in the fishery and live-sampled via biopsy were analyzed from archived samples at the Southwest Fisheries Science Center (SWFSC). The Chase Encirclement Stress Studies (CHESS) were conducted in the ETP during a 2-month (August - October 2001) research cruise aboard the NOAA ship, McArthur. 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 fishing operations. Groups of dolphins were found to be much more dynamic than previously recognized, making it extremely difficult to recapture dolphins over the course of several days to weeks, as planned. Ultimately, 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. Individually radio-tagged dolphins and 1-4 associated roto-tagged dolphins were recaptured on several occasions spanning 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 recaptured individuals, 283 skin samples, of which 17 were from previously captured and sampled animals, 449 analyzable thermal images, 52 core-body temperatures, and 95 hr 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 recapture. Analyses of samples and data were conducted at SWFSC and at other contracted laboratories.

Review Activities

As reviewers of the CHESS project, our goal was to objectively evaluate the science as presented in written manuscript form and later presented orally in the presence of a review panel at the SWFSC in La Jolla, CA between February 4 and 6, 2002, and not the policies revolving around the tuna fishery. Personally, I evaluated the merits of the science and its ability to address 1) whether or not fishery-related stress may *significantly and adversely impact* the recovery of the aforementioned dolphin populations, and 2) whether or not repeated chase and encirclement as part of tuna fishing operations can *negatively impact* the health, reproduction and survival of dolphins, subsequently affecting the ability of the population to recover from excessive fishery-related mortality incurred from past decades. The oral presentations began with an introduction of the CHESS review (Dr. S. Reilly) and an overview of CHESS (Drs. K. Forney and D. St. Aubin). After the introductions the following topics were presented on the first day (February 4th): tagging and tracking (Dr. S. Chivers), behavior during sets (Mr. E. Santurtún), thermal studies (Dr. A. Pabst), blood analyses (Dr. D. St. Aubin), immunological studies (Dr. T. Romano), and SRP studies (Drs. S. Southern and A. Dizon). The second day (February 5th) of presentations included the results of the necropsy studies (Drs. D. Cowan and T. Romano), a synthesis of the CHESS results (Drs. K. Forney and D. St. Aubin), and the presentation of the non-CHESS studies, which included cow/calf behavior and energetics modeling (Dr. L. Edwards), reaction to research vessels (Dr. S. Mesnick), and reproductive and demographic parameters (Ms. K. Cramer). We were asked by Dr. Reilly to not critically evaluate the non-CHESS presentations as part of our review because these studies fell outside the normal realm of the reviewers' research interests and professional training. Dr. Reilly conveyed to reviewers that he would have a separate set of panelists with research backgrounds in population biology, behavior, and modeling to review the science of these presentations. Nonetheless, the non-CHESS presentations broadened our understanding and perspectives of the dynamics involved with the dolphin-associated tuna fishery. The review panelists were all in agreement with Dr. Reilly that the scope of these presentations are beyond the breadth of our research training and background to appropriately evaluate their potential contribution to addressing the aforementioned questions regarding the impact of the tuna fishery on the recovery of dolphin populations. On the third and final day (February 6th) of the meeting, the reviewers convened to discuss their preliminary impressions of the results and to discuss some pertinent issues that warranted extensive review such as data to indicate the presence or absence of capture myopathy. We were also shown a videotape of an actual fishing set, which helped place some of the logistical constraints and limitations into perspective.

Summary of Findings and Specific Recommendations

The following subsections identify specific strengths and weaknesses in their ability to address whether or not chase and encirclement adversely affect the recovery of the dolphin populations in question. The sections are listed in order of significance and importance to addressing the central issues of negative impact on recovering dolphin populations.

Tracking and Tagging

Unfortunately the lack of a large sample size of satellite tagged dolphins precludes the ability to make any significant inferences regarding the post-release movement and swimming speeds of these dolphins. The average daytime swimming speed was reported as 4.9 knots (kn); however the post-release speeds for the two animals for which data were available were 5.2 and

6.2 kn, or 6 and 26.5 % increases, respectively, in average daytime swim speed. Both of these post-release swim speeds are well within the range of average daytime swim speeds recorded for other spotted dolphins during the course of CHESS. The wide range in increased post-release swim speed suggests that a large degree of individual variability exists. Increasing the tagging efforts may elucidate whether this increase in post-release swim speed is significant. The post-release swim speeds are not the maximum speed reported for these dolphins (S. Chivers, personnel communication). Therefore, given the limited data available it would appear that the animals, upon release, departed the capture area at a velocity consistent with their normal daytime speed. Although the tagging data indicates that these herds of spotted dolphins were more dynamic than anticipated, increasing the tagging effort may help elucidate the population size. Unfortunately, only 90 (6 %) of the total number of animals encircled were roto- or radiotagged, and of these only 11 (14 %) were still associated with the focal dolphins on the next day. The ability to track a satellite tagged dolphin for as much as 20 days was interesting and may lend for advancing this technologies ability to address the issues at hand. If this team of researchers could deploy a larger number (3x to 4x) of satellite tags on spotted dolphins, then they could better elucidate the post-release behavior and activities of these dolphins, which may be more informative than any of the other data sets obtained in regards to the addressing the question at hand. Also, increasing the number of satellite tagged animals may provide information on how frequently the same dolphin gets recaptured over the span of the satellite tag's transmission life. This information could also be delineated by rototagging as many as dolphins as feasible. Despite the methods used to attach the various tags deployed, I believe the greatest amount of information can be obtained from these technologies, especially when it comes to addressing the issues of adverse impact at the population level. Information on individuals over the course of years could be possible using rototags if observers could accurately record tag numbers during sets, thus making the deployment of a large number of tags paramount to addressing the negative impact issues. The ability to discern whether or not an environmental (biotic or abiotic) stimulus can negatively or positively impact a species at the level of the population is contingent on the monitoring of the recruitment of new individuals to that populations and of the growth (or lack thereof) of the population throughout the period of the perturbation. This monitoring effort would be in units of years. Therefore, it would be my recommendation to drastically increase the tagging and tracking efforts on these populations of dolphins to properly address the impact issues since these data can evolve information on individuals and on the population over the course of years, which is required, and obviously lacking at present.

Behavioral Observations

The behavioral observations would suggest that these dolphins are habituated to the encirclement procedures. During the fishing efforts by the Mexican tuna fleet between 1998 and 2000 approximately 96 % of the encircled dolphins were released by backdown, whereas only 4% were actively released suggesting that dolphins recognize backdown and realize their eventual release. Although behavioral differences between small (< 100 animals) and large (> 100 animals) herds were revealed, these differences are likely due to the difference in available area (i.e., m^2) per animal in the net. Determining whether a difference in animals released by backdown was present between the two herd sizes would have been informative. If a difference was observed, this could imply that school size can affect the backdown release of animals and, thus, impact whether or not policy could be instituted to restrict the setting of nets on herds of a particular size. Moreover, if herd size negatively impacts the release of animals, then this variable could potentially reduce survivability of encirclement, which may be significant to addressing the long-term effects of the fishery on population growth. Because behavioral observations are not invasive and do not require the physical handling of animals that blood sampling does, for example, much information can be obtained from these efforts, which may prove more beneficial to addressing the issues of negative impact than invasive procedures. The lack of video taping of animals in the nets from underwater is unfortunate and could prove useful in future studies.

Necropsies

The main objective of the necropsy program was to evaluate the pathophysiological and immunological condition of dolphins killed in the fishery, with an additional goal of assessing overall health and disease status of the animals. Results of the necropsies indicate that parasitism was the major natural disease factor. Essentially all animals sampled died with highly stereotypical pathologies; cardiac injury with implied impairment of cardiac conduction, arrhythmia and muscle contractility and evidence of smooth muscle spasm. The investigators concluded that these injuries resulted from massive shock and stress reactions, "in the setting of the study" suggesting that the interpretation may not have been completely objective and partially biased to conform to the goals of the studies. Capture myopathy-related injury of the heart resulting from acute shock can potentially be fatal. Turnbull and Cowan (1998) hypothesized that dolphins are particularly susceptible to stress cardiomyopathy. The two animals that died from net entanglement in CHESS displayed pathologies indistinguishable either in kind or severity from those collected during fishery operations. A large number of animals examined had lesions of the heart associated with small patchy myocardial scars and abnormalities of small vessels consistent with mural injury due to spasm implying that some animals suffer cardiac injury great enough to result in death in the nets, while most animals may suffer sublethal injury, but survive either to resolve the lesions without scar, or to replace damaged tissue with scar. Also, only two (0.13 %) of approximately 1500 encircled dolphins died during the course of CHESS suggesting that these animals, although potentially susceptible to cardiomyopathy, are not susceptible to the lethal effects of these cardiomyopathies, and thus are resistant to the consequences of tissue damage induced by chase/encirclement procedures. Cowan and Curry (2002) caution that lesions found during CHESS are not specific to the fishery in that they can be found in dolphins in a variety of locations and circumstances. Because of the numerous underlying uncertainties associated with the histories of these particular animals that died during CHESS, stating that these animals died "in the event of chase and encirclement" as opposed to "resulting from chase and encirclement" would be more accurate. Previously, necropsies of dolphins killed incidentally during stock assessment and gear technology research did not reveal substantial evidence of "delayed mortality" related to the fishery (Cowan and Walker 1979). Adrenal hypertrophy is a well-documented consequence of chronic stress (i.e., Ortiz et al. 1999); however the lack of an examination of adrenal glands in the present study was unfortunate. An extensive evaluation of post-mortem adrenal glands may provide a better understanding of the catecholamine-induced cardiomyopathy suggested to have occurred in the present examinations. Also, if fisheries are suspected in negatively impacting the reproduction of these populations, then the reproductive organs of both genders should be examined and evaluated as vigorously as the heart and lymphoid organs were in the present study.

Nonetheless, the present data provide a substantial data set from which future studies can be compared.

Stress may cause the involution of lymphoid organs and decrease cellular compartmentalization (Dominguez-Gerpe and Rey-Mendez 1997; Fukui et al. 1997). However, results of the present examinations did not reveal any indications of tissue involution as has been observed in other stressed mammals. Necropsies of dolphins indicate these animals possess the morphology characteristic of a functioning and intact immune system. Because prior history of chase and encircled individuals was unknown, definitive conclusions regarding the effects of repeated chase/encirclement based on lymphoid organ morphology are not possible. However, based on lymphoid organ morphology in other cetaceans (Romano et al. 1993; Cowan and Smith 1999), there was no indication, based on morphology, of a compromised immune system in dolphins that were necropsied in association with CHESS.

Immunological Studies

It is generally well accepted that repeated and/or excessive stress can negatively affect an animal's health by suppressing its immune system. For example, exercise-induced stress increases lymphocytes with changes returning to normal concentrations as early as 15 minutes following the activity (Robertson et al., 1981; Simon, 1991). However, chronic exertion (i.e., marathon) increased T-cells two days after the activity (Gmunder et al. 1988) indicating that the immune system responds differentially to the intensity and duration of the stimulus. Restraint-induced stress has also been reported to acutely reduce B-cell concentrations to a greater extent than T-cells (Dhabhar et al. 1995). The changes in lymphocytes observed in dolphins during CHESS are similar to those previously reported for exercise- and restraint-induced stress. However, many variables confound the proper interpretation of these results. Exercise and restraint produce different responses in T- and B-cell concentrations, and dolphins in CHESS were exposed to both stimuli within a period of one to four hours. Were the observed differences due to the chase-induced exercise or to the restraint of the animals? To what degree does obtaining a sample one to four hours after cessation of the stimulus affect the interpretation of the result as well as the animal's ability to compensate for the stimulus? Because intensity and duration can co-variably affect the immune response to exercise, measures should be taken to account for these co-variables in the analyses. The authors conclude that some of the significant differences observed were possibly due to species-specific variations and more so related to exposure of the dolphins to infectious agents in their environment. Individual variation due to the animal's habituation to the procedures and its conditioning are other confounding variables that need to be addressed further. The lack of baseline data on immunological measurements in these animals greatly hinders the interpretation of the significance of the observed differences. Overall, changes in lymphocyte subsets were reported, however there was no apparent change in immune function suggesting that the immune system of these dolphins is not compromised in response to chase/encirclement. However, the measurement period following chase and encirclement was relatively short and more dramatic changes in immune function could have potentially surfaced days or weeks following the initial stress necessitating the ability to 1) accurately follow and recapture the same individuals and 2) track the same individuals for days and weeks to monitor its subsequent behavior and position for recapture after a prolonged period.

Blood Analyses

The neuroendocrine response to stress has been well documented in mammals (Axelrod and Reisine 1984) and a substantial literature exists on circulating indicators of stress in cetaceans (for review see St. Aubin and Dierauf 2001). The relatively elevated concentrations of adrenocorticotropin (ACTH), catecholamines, and cortisol (F) suggest that some of these dolphins exhibited a typical neuroendocrine response to stress. The most intriguing result may be the persistently elevated concentrations of ACTH since this peptide has a relatively short biological half-life in circulation (18 min; Liotta et al. 1978) suggesting that stimulation of the hypothalamus was chronic in some of the animals in the present conditions. However, a number of confounding variables makes an accurate interpretation difficult. The hormonal responses to exercise- (Dressendorfer and Wade 1991) and restraint-induced stress (Glavin et al. 1994) are well defined with some obvious overlap between the two in the present study. That is to say, from the results it is virtually impossible to discern what portion of the observed hormonal response was associated to the chase (or exercise) and what portion was attributed to the restraint of the animals during blood sampling. This conundrum necessitates obtaining reliable baseline data, under “non-stressed” conditions. The behavioral data suggests that these animals may be habituated to the chase and encirclement procedures and the necropsy data suggests that these dolphins have been previously exposed to this stress. We have shown previously that monkeys trained and habituated to a stressful stimulus did not continue to exhibit an increase in excreted F (an indicator of stress) suggesting that the neuroendocrine response was diminished after the animals were conditioned to the stimulus (Wade and Ortiz 1997). Because many of the animals have undoubtedly been previously encircled and most likely habituated to these procedures, as suggested by their behavior during backdown, the possibility exists that the large variability in many of these measurements suggests that many of these animals are conditioned to these procedures while others are not. This possibility is consistent with our previous suggestion that in wild bottlenose dolphins most animals were less susceptible to a neuroendocrine stress response (Ortiz and Worthy 2000). In humans, F can acutely (for hours) suppress circulating testosterone (T) (Cumming et al. 1983); therefore, a correlation between F and T would have been interesting because this correlation may have provided some insight on the question of whether stress can negatively impact reproduction. Also, a T:F ratio as a function of time to capture would have been informative. We have shown in stressed rats that the T:B (corticosterone) ratio increases following the peak elevation in excreted B on day one of exposure suggesting that the glucocorticoid-induced suppression of circulating T is gradually released as the animals become habituated to the stimulus (Ortiz et al. 1999). The large variability in serum sex steroids may be reconciled by the fact that these hormones are secreted episodically throughout the day (i.e., Ellis and Desjardins 1982); therefore correlating concentration with time of day may be useful to determine the effects of time of day on sample concentrations. Mean concentrations of aspartate aminotransferase (AST), creatine kinase (CK), and lactate dehydrogenase (LDH), indicators of skeletal muscle necrosis due to capture myopathy, were considered elevated relative to those values reported for several other species of odontocetes (Bossart et al. 2001), but were similar to those values reported for net-impounded harbor porpoises (Koopman et al. 1995). Nonetheless, these values reported for cetaceans likely represent subclinical catabolism of skeletal muscle secondary to capture (Bossart et al. 2001) and pale in comparison to values observed in clinical exertional rhabdomyolysis (capture myopathy), which are typically in the thousands of units per liter ($U\ L^{-1}$) (i.e., Hartup et al. 1999). If anything, a small number of animals exhibited subclinical capture myopathy, but overall, this condition does not appear to be a serious consequence of the capture. Again, the large variability in concentrations suggests that large individual discrepancies exist within these populations of dolphins, and only well controlled time course studies will be able to reconcile these differences. All though the ranges in ACTH, F, catecholamines, glucose, and muscle enzymes suggests that some animals exhibited a neuroendocrine stress response and/or some degree of capture myopathy, data obtained from an appropriately controlled study are required to properly evaluate the significance of the present results.

Thermal Studies

Exercise-induced thermal stress has been well documented in terrestrial mammals (ie, Antognini et al. 1996). Therefore, core body temperature may provide an important index for monitoring stress. Core body temperature was not positively correlated with total chase duration, regardless of gender, and recorded body temperatures were similar to those reported for bottlenose dolphins repeatedly captured in Sarasota, FL (Pabst et al. 2001) suggesting that the chase and encirclement procedures performed in CHESS did not adversely affect the dolphin's ability to properly thermoregulate. The lack of consistent significant differences in surface temperatures and temperature differentials (= $T_B - T_{H20}$) makes this data difficult to interpret in it's applicability to addressing the question of impact on the population. Dolphins regulate body temperature by controlling blood flow to the surfaces of primarily the dorsal fin and flukes (Pabst et al. 1999). Therefore, the observed increases in temperature differential suggest that these dolphins are properly thermoregulating in the presence of a stimulus (exercise). The recording of high core body temperatures in association with elevated skin surface temperatures (or temperature differentials) in the same individuals would have been disturbing and suggestive of this animal's inability to thermoregulate in response to chase and encirclement, but apparently such complementary measurements were not observed. The use of thermal data loggers could potentially reveal more useful information on the long-term effects of chase-encirclement, and could provide a better indication of an animal's response to a thermal challenge because of its ability to record temperatures over a long period. Unfortunately, data from thermal data loggers were obtained from only two animals, and as interesting as some of the associations between changes in heat flux and chase/encirclement procedures are, the lack of sample size makes any conclusions statistically prohibitive. The deployment of more (with the number dependent on power analysis calculations) thermal data loggers is encouraged.

Stress Response Proteins (SRPs)

Although data from skin biopsies indicated a high frequency of altered expression profiles for SRPs from the fishery-involved samples suggesting the occurrence of fishery-induced stress, the lack of methodological validation for this application is disconcerting. The crossreactivity of the antibodies used for each respective ligand (40 proteins) requires validation, especially when all antibodies were combined in a single cocktail. The lack of consistency in sampling sites (jaw, back, or dorsal fin) may have also confounded the results. Also, the effects of solar UV radiation on these proteins need to be elucidated to determine if duration of exposure to sunlight could have further confounded the results. Could the altered SRP profiles have been a consequence of UV damage as opposed to an index of neuroendocrine responsiveness to fishery-induced stress? Although the idea of profiling SRPs as an index of stress is intriguing, the method and technique require rigorous validation to be considered a useful quantifying tool. Some criteria should be developed to insure consistency of its use in future studies.

Conclusions and Cumulative Recommendations

In summary, the objective of the present set of studies was to determine if stress induced by the chase and encirclement of ETP dolphin species, which are associated with the tuna fishery, is significantly impacting the health and reproduction of these dolphins and their subsequent recovery from extensive fishing efforts observed between 1959 and 1972. More simply, the studies tried to address whether acute effects of chase and encirclement could lead to chronic conditions, which may negatively impact the subsequent survival and reproduction. The hormonal data would suggest that some of the animals exhibited a neuroendocrine response; however, the lack of baseline, control data hinders the interpretation of the magnitude of this response and its significance to subsequent survival and reproduction. Also, some of the animals may have exhibited subclinical capture myopathy, but again the significance of this condition can not be properly evaluated given the lack of existing baseline data regarding this condition in "unstressed" dolphins. The health of dolphins that were chase and encircled does not appear to be negatively impacted based on the data which indicates the immune system was not acutely compromised. The small number of recaptures prohibits the ability to address the chronic effects on the immune system. The necropsy data suggests that the two dolphins that were incidentally killed during CHESS were victims of cardiomyopathy and cardiac lesions possibly resulting from a catecholamine "storm". However, the presence of a pre-existing clinical condition making these animals more susceptible to chase and encirclement stress cannot be ruled out. The fact that only two (0.13 %) of approximately 1,500 animals succumbed to the CHESS procedures may be more indicative of the overall impacts and may suggest that these animals are quite resistant to these procedures. Moreover, the congregation of encircled dolphins at the release end of the net at backdown suggests that they are conditioned, if not habituated, to these procedures and probably realize their impending release at that time. The lack of exertion-induced hyperthermia suggests that encircled dolphins are properly thermoregulating. Overall, the lack of baseline, control data for all measured parameters prohibits the formulation of any substantive and definitive conclusions. The small sample sizes especially in the recaptures do not provide sufficient power to statistically resolve the central issues. However, the present data provides some basis from which future studies can be compared.

The following recommendations should help to delineate the central issue of whether chase and encirclement procedures adversely impact the dolphin populations being affecting their health, reproduction and survival as well as provide perspective from which the significance of CHESS can be properly evaluated:

1) It is strongly recommended that efforts be made to obtain 10 to 20 animals for captivity so that controlled studies on the physiology of these animals can be accurately and properly evaluated. From that, the significance of the observed changes reported in the present set of studies can be determined.
2) Interpretation of data from future analyses of necropsy samples could benefit from a separate evaluation of samples from an independent pathology lab, which would obviate any potential bias in the interpretation. The suite of necropsy analyses should also include an examination of reproductive organs and the adrenal glands.

3) Tagging and tracking efforts need to be greatly enhanced in terms of total number of deployed tags and transmitters so that subsequent behaviors and movements of encircled dolphins can be monitored and statistically evaluated. This type of data, in my opinion, will prove to be most useful in trying to address chronic effects of the fishery as well as improve abundance/population data.

4) Prior to the initiation of any future studies a suite of *a priori* criteria should be developed to determine if a hypothesis of prediction can be rejected or accepted.

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Appendix II:

STATEMENT OF WORK

Consulting Agreement Between The University of Miami and Dr. Rudy M. Ortiz

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____________