Tuna fisheries-associated stress in dolphin:

a review of findings from different studies.

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EXECUTIVE SUMMARY

Background

The tuna industry has used the association between tuna and dolphins to fish in the eastern tropical Pacific Ocean for over five decades. An estimated 4.9 million dolphins were killed in tuna purse-seine nets during the fourteen-year period from 1959-1972. While changes in the fishery have greatly reduced the observed mortality of dolphins during the late 1970s, 1980s and 1990s, there continues to be concern that the fishing methods used are causing stress which may be having a significant adverse impact on dolphin populations. The stress studies include findings of a 3-year series of necropsy samples from dolphins obtained by commercial vessels, one-year review of relevant historical demographic and biological data related to the dolphins and dolphin stocks, and an experiment involving the repeated chasing and capturing of dolphins by means of intentional encirclement (details below).

Description of review activities

Upon completion of the research activities, written reports were compiled and submitted to an independent review process. Four reviewers (selected by an independent organization) reviewed written reports and heard presentations on the findings, and submitted independent reports.

Summary of findings

Overview of CHESS (Forney) CIE-S01

The authors provided a comprehensive overview of the Chase Encirclement Stress Studies (CHESS) study, with adequate preamble and discussion of the results from the different studies. In order not to bias the reader in the interpretation of the individual investigations, it might be useful to divide this report in two, the first one as an introduction, and a last one which would be a summary and interpretation.

Tagging and tracking (Chivers) CIE-S05

In this clear report, the authors describe the tagging and tracking efforts on dolphins captured during the CHESS study. In addition to confirming some of the spotted dolphins "natural behaviors" such as diving and feeding patterns as well as herd dynamics, this study clearly demonstrated definitive and quantifiable changes in swimming behavior associated with the chase and capture of spotted dolphins in tuna fisheries-like operations. In two dolphins, swimming speed increased during a chase as well as, and even more significantly so, after release (106 and 90 minutes). This work could have provided a unique opportunity to evaluate the health effects associated with fitting dolphins with different types of transmitters, which could have been done through blood work and histopathology of skin samples at the site of implantation, when the animals were recaptured. Overall, this strategy proved successful and useful, and should be pursued in the future.

Behavior during sets (Santurtun) CIE-S06
This study reported on the behavior of dolphins chased and encircled in nets. Differences were documented relative to the size of the school captured, and interestingly, the animals appeared to be familiar with the backdown procedure, suggesting that they have been captured repeatedly. The major limitation of this study is the lack of "normal" dolphin behavior, which would be modified when animals are chased and captured.

Blood analyses (St. Aubin) CIE-S02
Dolphins were captured after a chase and encirclement, and blood was sampled for hematological and biochemical analyses. Changes compatible with an acute stress response was documented after a chase and capture, while the only two recaptured dolphins showed inconsistent results compatible with a response that would change in time (recapture after 1 vs. 2 days). Once again, a lack of baseline information in "unstressed" animals of the same species, as well as a lack of good understanding of the dynamics of the occurrence of some changes, limited the interpretation of the data. It is possible that some animals experienced a certain level of capture myopathy, a disease entity well characterized in wild ruminants in which it can result in death. The overall difference between a normal and a pathologic stress response could not be determined in this study.

Immunology studies (Romano) CIE-S03
Immune functions were evaluated to assess potential changes related to the stress of fisheries-like chase and capture. It was found that recaptured dolphins had increased proportions of T lymphocytes and decreased proportions of B lymphocytes (except in one dolphin recaptured after two days). The LPS-induced lymphocyte proliferation in the animal recaptured after three days appears reduced, which should be discussed in more details. Although several technical questions arise from this report, and it would benefit from revisions to clarify the presentation and interpretation of the findings, the findings presented are potentially important in the evaluation of possible significant adverse impact of fisheries on dolphins.

Thermal studies (Pabst) CIE-S04
The thermal balance of chased and encircled dolphins was measured to test the hypothesis that these procedures could lead to hyperthermia. It was found that dolphins could increase their ability to dissipate heath upon chase and capture and after release (when they swim fast according to CIE-S05), therefore avoiding changes in deep body temperature. It is interesting that one of the animals had a body temperature deep body higher than all others, and efforts should be made to examine all data available on this animal to determine if it was undergoing an adverse reaction (which would be likely to occur in only a few individuals).

Stress response proteins studies (Southern) CIE-S08
This report suggests the use of cocktails of monoclonal antibodies to detect changes in expression of stress-related proteins in skin biopsies. Several significant problems were

found with the concept and application of the approach. Conceptually, the methods used the same animals to both define and validate the markers, which seemed inappropriate. The specificity of the antibodies used was not validated in cetaceans, and their use in a cocktail defies the purpose of using monoclonal antibodies (specificity for one antigen, allowing the interpretation of changes observed). Lack of knowledge in the dynamics of the changes in expression of stress-related proteins prevents interpretation of the results. The subjectivity of the interpretation ("normal" vs. "abnormal" according to the experience of the investigator) does not ensure reproducibility, and helps little in qualifying and quantifying the stress response. Finally, it appears that the patterns of "normal" vs. "abnormal" expression were affected by the location of the skin sample examined (which differed with studies), and the stress of recapture appeared to reduce the expression of stress-related proteins abnormal patterns. Overall, the usefulness of the (not yet validated) work presented in this report is at best doubtful at this point.

SRP and fishing efforts analysis (Dizon) CIE-S07

This report assesses the relationship between past fishing efforts and the expression of stress-related proteins in skin biopsies. Fishing efforts were estimated in time (up to 70 days) and space (up to 300 nautical miles) with relation to the site of capture. There is a good chance that a set 300 miles away, 70 days ago, when the dolphin in question might have been in a totally different location, resulted in little stress on that dolphin. This report overlaps significantly with the previous report (CIE-S08) and combines the weaknesses of the stress-related protein techniques and applications (see above) and an imprecise quantification of past fishing efforts.

Necropsy results (Cowan) CIE-S11

Necropsies were performed on dolphins that died in fishing activities. While few "natural diseases" were observed in those animals, lesions were described that were compatible, but not necessarily specific for a "sympathetic storm" resulting from an inappropriate acute stress response in the net, resulting in death. Chronic lesions were also compatible with a previous sub-lethal capture-associated stress, but again not specific for such. While this study discusses the apparent cause (and likely mechanisms) of death of animals that actually died in fisheries operations, it does not clearly contribute to our understanding of the fisheries operations on the animals which survived a chase/capture and were released alive.

Necropsy immunology (Romano) CIE-S10

The post-mortem examination of immune organs of dolphins that died in fishing activities revealed no significant abnormalities and showed innervation of immune organs as described in other species. It would be unlikely to detect stress-related abnormalities without quantitative measurements and without tissues from control "unstressed" animals.

Cow/calf behavior and energetics model (Edwards) CIE-S12 and CIE-S13

Those reviews of cow/calf behavior and energetics models, while interesting and relevant to the overall problematic of possible impact of chase and capture on dolphin populations, seemed unrealistic in view of field observations that calves do remain with cows during chase and capture operations.

Reaction to research vessel (Mesnick) CIE-S14

This study beautifully quantified dolphin behavior associated with boat encounter, and a model helped define the relative "meaning" of certain behaviors of different species of dolphins, with differences between targeted species and non-targeted species. While reservations were expressed above (CIE-S07) on the crudeness of the past fishing efforts, it seemed more appropriate to the evaluation of behavior of groups of dolphins upon boat encounter than for the evaluation of past stress in individual animals.

Reproductive and demographic parameters (Cramer) CIE-S15

In this study, attempts were made to identify population demographic parameters by assessing the proportions of calves in different schools, using aerial photography data. The conclusions seemed to be based on scarce data points irregularly spaced in time, and sometimes omitting some data points. The proportions of juveniles might be more demographically relevant than that of calves, since they are more likely to contribute to the future population growth.

Conclusions and recommendations

Several issues were relevant to more than one individual study. The low number of recaptures had a significant impact because several of the study designs relied on the recapture of animals to draw conclusions. The consistent lack of a definition of the stress to be assessed remained a constant problem. The lack of ("unstressed") controls, coupled to the limited knowledge/data in the species studied restricted the ability to interpret results. Given the fluidity of herd dynamics, which allow few recaptures, two strategies would be recommended for future studies. (1) The remote monitoring of animals, using tagging techniques, have shown successful at evaluating what happens to animals after the completion of a capture/release event, as well as the events related to the initiation of a subsequent capture, with a period in between where the animal serves as its own control. (2) The capture of a small number of animals to be used (in captivity?) to determine "normal values" as well as "normal responses" would be extremely useful to interpret potential adverse effects, and does not seem unreasonable given the number of animals that have died and are still dying in fisheries operations.

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 from1959-1972 (Wade 1995). After passage of the Marine Mammal Protection Act in 1972 and the increased use of equipment designed to prevent dolphin deaths, mortality 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:

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

The necropsy program (1) has analyzed samples from about 50 dolphins killed incidentally during fishing operations. Historical biological samples and data (2) 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 (3; 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. Dolphin 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 95 hours 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.

DESCRIPTION OF REVIEW ACTIVITIES

Upon completion of the research activities, written reports were compiled and submitted to an independent review process. The reports were submitted to four reviewers (selected by an independent organization, the Center for Independent Experts). The reviewers' mandate included the review of the written reports, attendance to a three-day meeting at the NMFS laboratory in La Jolla, CA, to hear presentations on the topics addressed in the written reports and to ask questions, and the submission of independent written reports (one per reviewer) on the findings presented. The reports were to review the activities, analyze the findings, draw conclusions and make recommendations relevant to the areas of expertise of the individual reviewers. The process was one of scientific review, and was not expected to address policies.

SUMMARY OF FINDINGS

The following section will review the information presented in individual reports and discuss the strengths, weaknesses and relevance of the data presented and their interpretation. Overall comments are presented in the "Conclusions and Recommendations" section.

Overview of CHESS and summary of results (Forney) CIE-S01

This report gives a comprehensive overview of the CHESS study, which is a good preamble to the following individual reports, and provides a relatively detailed, comprehensive and honest summary and interpretation of the findings of individual investigations as well as of the whole CHESS study. The effort at cross-referencing and relating findings is particularly impressive given the relatively short period of time to integrate the results of several investigations, and the fact that investigators had not previously met to present and discuss findings. In order not to bias the reader in the interpretation of the individual investigations, it might be useful to divide this report in two, the first one as an introduction, and a last one which would be a summary and interpretation.

Tagging and tracking (Chivers) CIE-S05

In this clear and to the point report, the authors describe the tagging and tracking efforts (including pertinent details on tags and methods) on dolphins captured during the CHESS study, in view of their efforts to determine the potential adverse effects of the chase and capture of dolphins during tuna fisheries. In addition to confirming some of the spotted dolphins "natural behaviors" such as diving and feeding patterns as well as herd dynamics, this study significantly contributed to our understanding of modifications of behaviors following a capture. It was clearly demonstrated that, in two dolphins, swimming speed increased during a chase as well as, and even more significantly so, after release. Interestingly, the fast swimming episode (5.2 and 6.2 knots vs 3.1 and 3.6 knots average day swimming speed, respectively) was sustained for a relatively long time (106 and 90 minutes, respectively. Those data were recovered from time-depth-velocity recorders (TDVR), which appeared to be the most useful in providing information on the reactions of the dolphins to the chase/capture activities as they relate to the tuna fisheries.

Of additional interest is the fact that a calf consistently accompanied a female that was repeatedly chased/recaptured. This is of particular relevance to the interpretation of other studies and should be stated more clearly in the report. Also, the recapture of animals fitted with different types of tags provides a unique opportunity to evaluate the health effects (or lack of health effects) associated with fitting dolphins with different types of transmitters. This could be evaluated by monitoring blood parameters (CIE-S02) or histopathology of a biopsy of the skin surrounding the site of implantation upon removal.

In conclusion, although data were available from only two animals, this study demonstrated definitive and quantifiable changes in swimming behavior associated with the chase and capture of spotted dolphins in tuna fisheries-like operations. This strategy proved successful and useful, and should be pursued in the future to evaluate the changes in swimming behavior of dolphins by tuna (-like) fisheries operations which involve or do not involve a capture.

Behavior during sets (Santurtun) CIE-S06

In this study, the behavior of dolphins chased and captured in tuna fishery-like operations was observed to determine if duration of chase/capture or school size affected dolphin behavior, and if behavior in the experimental chase/capture (the CHESS operation) differed from that in "real fisheries". Behavior was classified into active and passive, with active events and passive events and schools size divided as smaller or larger than 100.

The smaller groups tended to exhibit more active behavior, and some underwater behaviors could be interpreted as associated with stress. Interestingly, the data tends to support the assumption that the dolphins anticipate and are familiar with the backdown procedure, suggesting that they have been captured repeatedly, and that they learned the release procedure.

The major limitations of this study include the lack of data on dolphin "normal" behavior. What do dolphins do when not chased and captured? Do they display active behavior? Passive behavior? How often do they display behaviors such as defecating, or mating behavior? This information would be essential for the interpretation of behavior in a net (active behavior may be just normal, and not indicate stress). Similarly, it is difficult to accept an interpretation of "coping behavior" or behaviors associated with "fear" when the "normal" behavior is not defined. Also, behavior was evaluated at the level of the group of dolphins captured. Evaluation of the effects of capture, but most importantly recapture, would benefit from observation at the individual level (for example, does the behavior of dolphins recaptured differ from that in the first capture). Also, the suggestion that the animals are familiar with the backdown procedure suggests that the animals are repeatedly captured, which argues against one of the premises of CHESS that recapture will increase the stress level compared to the "first" capture. Also, if the backdown procedure is a learned behavior, it is possible that the disruption (modification, delay) of this procedure in the CHESS study contributed to confusing the dolphins and creating additional stress. Overall, this study presented potentially useful results to assess the effects of chase and capture on dolphins, but the interpretation of the results would greatly benefit from learning what are the "normal" behaviors of dolphins not chased and captured (could the tagging studies, with swimming speed recordings, be helpful?).

Blood analyses (St. Aubin) CIE-S02

This study was meant to document the possible changes in blood parameters associated with the stress of repeated chase/capture efforts in operations mimicking tuna fisheries. The particular attention paid to the consistent quality of the blood sample processing in order to obtain reliable results is to be commended, especially in view of the difficulties inherent to field conditions. Several caveats were recognized and identified in advance by the investigator, including the lack of "unstressed" controlled dolphins, the fact that handling the dolphins for blood (and other) sampling, which is not done in fisheries operations, may cause additional stress, and the unknown status of the dolphins' previous encounters with fisheries operations at the time of what had to be considered by the investigators the "first" capture of a given dolphin. Those caveats led to the working hypothesis that stress would increase upon repeated recaptures, with the study relying on recaptures for power.

Recaptures in animals for which a blood sample had been collected upon the first capture were the most valuable but included only two animals (captured one and two days after the first capture), and for which results were inconsistent (although some of the inconsistencies could potentially be explained). Animals recaptured without a blood sample at the first capture were compared with animals captured only once to determine the effects of recapture, and changes compatible with stress were documented.

A lack of baseline data in spotted dolphins clearly limited the interpretation of the results obtained in this study. While this study will provide baseline "normal" blood parameters (for chased dolphins), the interpretation of several (present and future) results will depend on a better understanding of the dynamics of changes of some constituents in the spotted dolphin. For example, the dynamics of elevation of ACTH, with the following elevation of cortisol (while ACTH starts to drop) needs to be studies in spotted dolphins to allow the interpretation of results from a single blood sample.

The authors rightfully excluded from the interpretation of the stress effects of capture and recapture the animals that had been fitted with the more invasive types of transmitters. It would be interesting to have those changes discussed to assess the direct effects of those transmitters on the general health of the dolphins.

It is interesting to notice that the cholesterol and triglycerides results from the two recaptured animals suggest that one animal would have not fed within the first day after initial capture, but the other would have fed the next day. It would be interesting to correlate these results with time-depth-velocity recorder data (see CIE-S05) to see if "disturbed" behavior (swimming fast for over 90 minutes after release) returns to normal (including feeding-type diving pattern) within the first day or later.

Some of the data (elevated enzymes, acidosis, etc.) may suggest evidence for capture myopathy, a disease entity well characterized in wild ruminants with potential severe outcome (may include death). Efforts should be made to more extensively address this possibility in the report.

Overall, the results presented in this study were analyzed appropriately, to the extent possible, and contributed to the documentation of a stress response associated with the chase, capture and sampling of spotted dolphins in tuna fisheries-like operations. It is nevertheless difficult to quantify the levels of stress attributed to the recapture. It is also difficult to assess what constitutes a "normal" stress response (a lack of stress response would be abnormal), and what constitutes an "abnormal" or "pathologic" stress response, which could be expected to result in adverse health effects for the animal.

Immunology studies (Romano) CIE-S03

This study investigated the potential effects of the stress of a tuna fisheries-like chase and capture (and recapture) on the immune system of dolphins. An increase in the percentage of T cells and a decrease in the percentage of B cells are reported in recaptured dolphins compared to dolphins captured for the (presumably) first time. These changes are also seen in a dolphin recaptured after one day, but are reversed in a dolphin recaptured after two days (Table 5).

A few technical questions arise from the report. On page 9, for the comet assay, why are only 5-10 nuclei per sector counted, as this seems like a small number. The methods section describes the enumeration of cells upon thawing, with trypan blue to assess viability. The results for viability are never reported in the results section. Are the cells used for phenotyping and proliferation viable cells? Similarly, were control cells used to assess the validity of the tests and account for the daily (inter-experiment) variability? This is especially important for functional assays. While the methods section describes the use of three concentrations for each mitogen, the results report three to four concentrations for each. While the investigators make an effort to assess the possibility for differences in immune functions attributed to the gender of the animals, no effort is made to assess differences attributed to the age of the animals. Two monoclonal antibodies are described in Table 1 to identify CD4 T cells, but the results report only one value; which antibody was used? What were the criteria used to select twenty dolphins to match gender and size with the ten recaptured dolphins? Were they equally matched with the recaptured animals (two first capture for each recaptured one) or were all animals matching a recaptured one used (eleven first capture matching one recaptured dolphin and none matching the other nine recaptured ones). As is well known in clinical pathology, comparing the percentage of lymphocytes over the total number of leucocytes is not a valid practice, since variations of the proportions of other cell types (for example, a large increase in neutrophils) could reduce the percentages of lymphocytes without changing the numbers. Using the total numbers of lymphocytes is a much better practice. What was the rationale/relevance for using an antibody against MHC class II? It is doubtful that (all) T cells expressing MHC class II are in a state of continuous activation. MHC II expression on all lymphocytes has been reported before in bottlenose dolphins (Romano et al. 1992) and beluga whales (De Guise et al. 1997) as well as in swine (Tizard 1992), horses (Barbis et al. 1994, Crepaldi et al. 1986), cats (Rideout et al. 1990) and dogs (Doveren et al. 1985, Doxiadis et al. 1989), making it unlikely to be related to the marine environment. The discussion addresses degree of strand breakage, frequency and type of cells, while the results only report on the degree of cell breakage. Why would DNA damage not be induced in cetaceans that swim with great efficiency? Figures 2-3 and Table 8 are redundant. Figures 4-6 and Table 11 are redundant.

The authors should qualify their conclusions more carefully. For example, the abstract states that "Changes in the lymphocyte percentages in the repeat capture group may increase susceptibility to disease". The results alluded to are indirect (some animals captured twice compared to different animals "presumably" captured only once), and the discussion cites studies with reversible changes. The authors should avoid over interpretation of their findings, or otherwise support those interpretations more strongly.

These authors, as most in this study, struggle with the interpretation of their results and the concept of stress. They compare their results with studies in other species in which exercise is a stressor. In these studies, long-term exercise and cessation of exercise are associated with changes in lymphocyte subpopulations. It is not clear how relevant these studies are in the interpretation of the dolphin study.. Do dolphins ever stop exercising? What does the capture (or recapture) represent?

Two dolphins were recaptured. These animals are likely to best represent the effects of a recapture. The authors should put more emphasis on the changes found in those two animals upon recapture (although there are only two animals). The possible reduction of LPS-induced lymphocyte proliferation in the animal recaptured after three days appears reduced, which should be discussed in more details.

Among the future studies described at the end of the report, several would be difficult to interpret without information on the immunological status (past and present) of the animal sampled. For example, the levels of total immunoglobulins in an animal are difficult to interpret without a detailed knowledge of the past challenges an animal encountered in the past (an animal never exposed to microorganisms would not be expected to raise immunoglobulins).

While the determination of the effects of the stress associated with tuna fishery-like chase and capture of dolphins on their immune system is valuable and potentially important in documenting long-term (and potentially residual) effects, the present report would benefit from a review of the reporting of their findings and their interpretations.

Thermal studies (Pabst) CIE-S04

This report examined the influence of chase and capture on the ability of dolphins to regulate their temperature, by studying deep body temperature, infrared thermography of encircled dolphin backs and dorsal fins, as well as dolphin skin temperature and heat loss, as measured by thermal logger deployments. The hypothesis to be tested was that chase and capture could induce hyperthermy in dolphins.

The data presented and their interpretations were clear. It was demonstrated that deep body temperature did not increase with chase and capture, although the differential between skin and water temperature (increase of skin temperature) increased with longer chases. Thermal data logger deployments were successful for two animals. They demonstrated that heat flux values increased during dolphin escapes (after release) and during the subsequent helicopter chase that initiated the following chase and capture, but sharply decreased as soon as the animals were corralled within the net. It is interesting to note that those episodes match those in which increased swimming speed were recorded (CIE-S05). Overall, it was concluded that dolphins are capable of adjusting their heat dissipation system during a chase and capture operation to avoid changes in deep body temperature.

This study, although the results are based on a relatively small number of animals, was useful to document and quantify the thermal stress in dolphins involved in fisheries-like operations. Interestingly, one animal (out of 48) displayed an unusually high body temperature. Since the effects of chase and capture may be deleterious to some, rather than to all of the dolphins captured, it may be appropriate to emphasize that one animal seemed different from the others, and to make all possible efforts to examine in detail all other data available on this animal.

Stress response proteins studies (Southern) CIE-S08

This report presented a novel approach to determine whether marine mammals experienced stress by evaluating the presence of stress-related proteins in sections of skin biopsies prepared histologically and labeled with individual and a cocktail of monoclonal antibodies to such proteins. While accepting that this approach is novel, significant problems were found with the concept and application of such an approach.

First and foremost, the conceptual development of the approach is puzzling, to say the least. Several proteins with differing functions may be absent or present at different levels in a "normal" animal. A subset of skin samples from marine mammals for which relatively little is known on health status/life history at the time of sampling or before were used to define what is "normal". Then, animals for which, once again, relatively little is known on health status/life history were defined as "stressed". The sections of skin for all animals were then labeled with antibodies to determine if they were stressed or not. In this approach, the same somehow subjectively divided animals were used to both **define** and **validate** the markers to be used later. This does not constitute a valid scientific approach.

Several other problems arise from the development of the method. Forty monoclonal antibodies raised against human, rat, mouse, bovine, equine and bacterial antigens were used in this study. These antibodies were used in cetacean without assessing the specificity of the proteins they recognized, assuming that they recognize in cetacean skin the same proteins as they do in the species against which they were raised and characterized. This assumption is dangerous, as in several occasions cross-reactivity is not specific. As an example, a cross-reacting bovine anti-CD2 labeled virtually all lymphocytes in belugas (De Guise et al. 1997), as anti-CD2 does in mice (Yagita et al. 1989, Sen et al. 1990), while a species-specific anti-CD2 was a T lymphocyte marker (De Guise et al. 2002). Another problem lies in the use of a cocktail of monoclonal antibodies. While the use of some specific monoclonal antibodies might be useful to detect modulation of specific processes (such as oxidative stress of apoptosis), the use of antibodies in a cocktail loses all the specificity of the measurement (which is the basic rationale for the costly production and use of monoclonal antibodies). In addition, it is puzzling to note that the different antibodies are used in the cocktail at concentrations 40 more diluted than when used individually, without reference to the conservation of specificity and sensitivity. Also, the potential for interactions between antibodies when used in a cocktail has not been addressed.

Another fundamentally important deficiency of the study particularly relevant to the interpretation of the results is the lack of knowledge on the dynamics of expression of the proteins of interest in the skin of marine mammals. It was not determined whether those proteins appear within, and stay for, minutes, hours, days, weeks or months in skin cells. The dynamics of appearance and turnover of those proteins in other species was not addressed either. The portion of the epidermis where the cells did or did not produce each of those proteins was not clearly determined either. An additional level of complexity becomes apparent when antibodies are used in a cocktail; do all proteins have the same dynamics of expression and disappearance? It becomes hard to determine if "abnormal" pattern of stress protein expression will "diagnose stress" that occurred five minutes or twenty days ago. Similarly, it is hard to determine when to sample animals in order to detect a "fisheries-related stress".

The interpretation of the results (for validation of the concept) was initially based on a relatively objective computer-assisted quantification of the changes using image analysis to help detect the extent and intensity of a change. It is interesting that this relatively objective and quantitative method of interpretation was later dropped in favor of a more subjective visual interpretation. The qualitative classification ("normal" vs. "abnormal") seems less than ideal, not allowing to quantify a change/response (so stressed that I will die or a bit stressed because I might be late for a meeting). There is no documentation of the "normal response" to a stress, which allows individuals to adjust to situations with different requirements and allow survival.

The quality of the skin sample obtained seems key to the possible use of this method. For example, the variability in the proportion of "normal" samples seemed to vary with the location of the skin sampled, preventing comparisons between studies as well as within a study. The expression of the very proteins measured will also likely be affected by an active inflammatory process or healing after a past injury at that site. Also, the metabolic activity of skin at different locations can be affected by several factors such as vascularization, pigmentation, exposure to ultra-violet light, etc. Where were the skin biopsies from in the animals (from several different species, different geographical locations and collected by different collaborators) used for the validation study?

Finally, the concept of the method developed was that the expression of stress proteins in the skin becomes "abnormal" upon exposure to stress. Those very premises are then negated to explain the detection of "loss of abnormality" (or becoming "more normal") in animals recaptured in the CHESS program. The rapid change from abnormal to normal in recaptured animals (within a few days) also argues against the assumption that the markers chosen would detect "chronic stress".

As a more technical comment, quality control, including accounting for the quality of the samples analyzed (all fresh for the CHESS study, how about the other ones?), as well as description of positive and negative control antibodies for the immunohistochemistry (make sure it worked when it should, and that it did not when it should not), need to be better described. A 13% discrepancy between two analyses of "normal" bow rider dolphins, using only two categories (normal vs. abnormal) seems rather high.

Overall, the usefulness of the (not yet validated) work presented in this report is at best doubtful at this point in time to define if dolphins are "stressed" and/or suffering significant adverse effects from the tuna fisheries.

SRP and fishing efforts analysis (Dizon) CIE-S07

The objective of this study was to interpret the findings of the skin biopsies stress proteins (CIE-S08) in view of the estimated previous fishing efforts for the captured animal based on past records of sets within varying distances from the site of capture and time, assuming that high fishing efforts would result in more stress.

The results presented overlap significantly with the results presented in CIE-S08. In addition, the reservations expressed about the technique and its application (see above) remain a major concern in the interpretation of this report.

Finally, an effort was made to quantify the past fishing effort to which a dolphin was exposed. To do this, the number of sets recorded in a defined radius from the site of capture/sampling of a dolphin and in a defined period of time previous to the capture. Although this may be the best retrospective "estimate" of past exposure to fisheries, it retains a certain level of imprecision. Inherent to the method is the assumption that the dolphin was in the same location in the 10-70 days previous to its capture. Similarly, it is assumed that sets 30-300 nautical miles from that potentially stationary location all had the same stressful effect on a dolphin. In other words, it is assumed that a set that occurred 70 days before a sampling, 300 nautical miles from the site of that sampling, could influence the stress level of a dolphin which might have been in a totally different location at the time of that set. This is imprecise at best. Moreover, animals recaptured within days of a known capture had a modified (decreased) expression of those stress proteins, suggesting that the stress that they may be unlikely to represent a stress that would have occurred several weeks before. In addition, the results presented are inconsistent between studies (likelihood of altered pattern increased with number of sets in fisheries mortality samples while it decreased in bow riding animals). Finally, the results presented may suggest "associations" at best, but certainly not "effects" of fisheries on dolphin stress (as stated on Page 13).

Necropsy results (Cowan) CIE-S11

Necropsies were performed on dolphins which died in fisheries operations in order to determine the background of naturally occurring diseases, defining the cause of death of the animals examined, and identifying the possible effects of stress on those dolphins. It was determined that naturally occurring diseases were rare and mild, and that the individuals that died in fishing operations had lesions compatible with an acute stress leading to death, while other lesions were compatible with outcomes of previous stresses, potentially that of previous chases and encirclement in fisheries operations.

The authors were the only ones to provide an operational definition of stress, which clearly helped in the interpretation of the data they provided. The authors should also be commended for putting the emphasis of their report towards the goal of the study (stress in dolphins), with discussions on the possible mechanisms involved, rather than detailing all the background lesions in the animals examined.

Two major conceptual points need to be made. First, it should be noted that while the lesions found were in fact **compatible** with a "sympathetic storm" (as extensively and clearly explained by the author), they may not be **specific** for the sequence of events described, especially the chronic lesions. The author nevertheless makes a convincing interpretation of the acute lesions to the effect that dolphins which die in tuna seine nets do so acutely, with lesions compatible with a "sympathetic storm", without other disease processes to explain the death of the animals. This, nevertheless, represents the cause of death for the animals that die. It is unclear if the chronic lesions found in the heart contribute to the death of those animals (apparently, the "sympathetic storm" is sufficient to kill an animal), if those lesions result from previous stress related to tuna fisheries, and if the animals chased/captured in tuna fisheries and released alive will undergo a "sympathetic storm" and survive or undergo sub-lethal lesions which may or may not affect their health. In other words, while this study clarifies the apparent cause (and likely mechanisms) of death of animals that actually died in fisheries operations, it does not clearly contribute to our understanding of the fisheries operations on the animals which survived a chase/capture and were released alive.

Necropsy immunology (Romano) CIE-S10

This report examined the histology of immune organs from dolphins that had died in tuna fisheries operations and concluded that (1) no significant abnormalities were detected, and (2) innervation of immune organs previously demonstrated in other cetaceans was conserved in the species examined. A caveat of this study is that, without quantitative measurements and without control "unstressed" tissues, it is unlikely to determine "stress" in dolphins. Also, it is unlikely that histological examination of lymphoid tissues will detect the acute stress related to a capture and death within minutes to few (1-2) hours. The study design is also not meant to address the effects of chronic stress of repeated fishing (capture) efforts.

Cow/calf behavior and energetics model (Edwards) CIE-S12 and CIE-S13

The review of the mother and calf behavior as well as the energetics models presented were very interesting and relevant to the problem studied (stress of chase and capture), but both reports seemed quite pessimistic on the outcome of a chase on a calf, in view of the field observations presented in the CHESS study (see above), where calves were observed with their mother in the net and even upon repeated recaptures. Aerial observations also concluded that cow/calf pairs are usually not separated during chases.

Reaction to research vessel (Mesnick) CIE-S14

This study beautifully quantified dolphin behavior associated with boat encounter, and a model helped define the relative "meaning" of certain behaviors (running opposite of bow riding). Further, the recent fishing effort (in location relative to the sighting site and in time) was correlated with the behavior of different species of dolphins, with differences between targeted species and non-targeted species. Although reservations about the appropriateness of the method to quantify fisheries efforts and its relation to stress in individual dolphins were expressed above (see comments on CIE-S07), the method seems to be more appropriate to evaluate the behavior of groups of dolphins and changes in behavior.

Reproductive and demographic parameters (Cramer) CIE-S15

In this study, attempts were made to identify population demographic parameters by assessing the proportions of calves in different schools, using aerial photography data. The conclusions seemed to be based on scarce data points irregularly spaced in time, and sometimes without taking into account some data points that do not fit the conclusions reached (in particular, with reference to Figure 6). Spatial distribution differences between years should also be carefully examined. Finally, the proportions of juveniles might be more demographically relevant than the proportion of calves for such an effort, since they represent calves that survived through the more "fragile" calf stage, and are more likely to contribute to the future population growth.

CONCLUSIONS AND RECOMMENDATIONS

The main objective of the work presented was to assess the potential significant adverse impact on dolphins of tuna fisheries using repeated chase and encirclement of dolphins. A series of studies to evaluate the effects of fisheries and fisheries-like operations were conducted and a review panel was convened to review the results obtained and their interpretations. While specific comments relative to the individual studies appear above, this section highlights the overall strengths and weaknesses of the work as a whole and issues relevant to several studies.

The reports submitted were generally of relatively good quality, despite the fact that they were assembled in a short period of time. They addressed the potential impact of capture on two important aspects, the ecology and the pathophysiology of dolphins. It should be commended that both types of disciplines were considered, rather than be exclusive of one another. The short time period between the completion of the study and the presentations of the results allowed for limited interactions between investigators, and relatively little cross-reference of results between studies.

Several limitations were recognized and addressed throughout the reports (although not always within a given report). The major problem was probably the fact that herd dynamics were much more fluid than expected, which resulted in relatively few recaptures. Since the design of several of the studies depended on a large number of recapture for the statistical power that would strongly support their conclusions, the low number of recaptures was significant. Also, one needs to remember that some relatively rare events at the population level may not have been represented in the sampling.

Another significant problem throughout the reports (except for one report) is the consistent lack of a working definition of stress. This is of particular significance since the concept of stress was central to all studies. Also, it was not clear whether the overall goal of the studies was to "diagnose stress" (vs. "lack of stress"?), to determine an acute vs chronic stress response, determine a normal vs. pathological stress response, or to determine whether stress had a significant adverse impact on the animals captured (or recaptured). It would be difficult to evaluate of long-term impact of capture in a study of only a few weeks duration.

An issue relevant to all studies is the lack of controls. What are "normal" blood parameters in a dolphin not chased and captured before blood sampling? What is normal dolphin behavior when there are no boats around? What is the "normal" stress response in a dolphin? How rapidly and to what extent do blood components or skin proteins increase after a stress and when do they start to decrease? The lack of knowledge and understanding of normal physiology and behavior clearly affected the ability to interpret the results of most studies.

The different studies presented resulted in different levels of confidence in the conclusions reached, towards the overall goal of assessing the potential adverse impact of fisheries on dolphins. The categories are listed below in decreasing order of importance.

- The most reliable conclusions would have been obtained from controlled studies with animals that would never have been chased previously and/or would be used to voluntary, stress-free sampling (for blood and other samples). This was not possible in this study.
- A study with control animals to document pathophysiological "normal" values in non-stressed animals would have benefited in the interpretation of the data of several individual studies. This was not possible in this study.
- The study of recaptured animals is the next most valuable tool in the evaluation of the stress of recapture, although one has to assume that the "recaptured" animals had not been captured recently before their "first documented capture" during the CHESS study. Unfortunately, as discussed above, the number of animals recaptured was relatively low.

- Tha pathophysiological response of animals captured and sampled once is interesting but the interpretation of the data is limited by the lack of "normal" baseline data in the species of interest.
- Statistical associations (such as that between past fishing effort in time and space and expression of stress related proteins in the skin) have to be interpreted in the context of what they mean (a given animal might have been in a different location than that of capture/sampling and not influenced by previous fishing effort that took place relative to that location), and not interpreted as a cause and effect relationship.
- Other studies use methods that are not validated and generate data that do not have significant relevance. Such studies are unlikely to provide helpful information, whatever their results are.

Given the fluidity of herd dynamics, which led to low recapture rates in the CHESS study, the evaluation of potential significant adverse impact on dolphins of fisheries or fisheries-like operations may be best evaluated in a study that would not count on recaptures for collection of data. Two scenarios could be envisioned: remote monitoring and examination of control animals.

1. Remote monitoring

Some of the tagging studies took advantage of the post-release behavior and physiology to monitor what happens **after** the release following a chase and capture event, which is probably most significant to detect potential adverse effects. Interestingly, the events at the initiation of a subsequent capture effort have also been monitored using tags equipped with different recording devices. The animals also act as their own control between capture efforts, allowing to assess the timing and extent of deviations from and return to "normal".

Different technologies exist to collect different types of data, but long-term follow up of large numbers of animals would appear desirable to assess long-term effects of repeated recaptures and allow for the documentation of events that might be rare in the overall population. While initial handling is required to equip the animals with the tags, the subsequent captures could be done (and documented) randomly by "real" and "normal" fishing operations, given that enough animals are tagged. The data recovered from time-depth-velocity recorders (TDVR) appeared to be most useful (compared to time-depth recorders (TDR) or location (satellite) data a lone in providing information on the reactions of the dolphins to the chase/capture activities as they relate to the tuna fisheries. Maybe a heat flux data recorder could be coupled to a TDVR and data could be recovered through a satellite transmitter.

2. Examination of "control" animals

The examination of "normality" appears to be a constant issue in the studies described. It appears that normal physiology and behavior need to be better determined to allow the qualification and quantification of deviations from normal. The timing and magnitude of normal responses also needs to be determined in order to detect abnormal or pathological responses.

It is estimated that 4.9 million dolphins were killed during the fourteen-year period 1959-1972 (Wade 1995). Although 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, the observed mortality for 1996 was 2,547 dolphins (Lennert and Hall, 1997) and the estimated mortality for 1997 was 3,000 dolphins (Inter-Americal Tropical Tuna Association, IATTC, 1997).

Given the importance of understanding normality in order to assess deviations from it, and given the number of animals that died (and continue to die) in fisheries operations, it does not appear unreasonable to contemplate the capture of a relatively small number of animals to be kept in captivity and used for controlled situations/experiments. The results of such an effort would permit to further the scientific knowledge (normal values, normal responses) that would in turn allow the adequate evaluation of the potential adverse impact on dolphins of tuna fisheries operations.

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APPENDIX I: BIBLIOGRAPHY OF MATERIAL PROVIDED

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APPENDIX II: STATEMENT OF WORK

Consulting Agreement Between The University of Miami and Dr. Sylvain De Guise

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:

1. A review of relevant stress-related research and a 3-year series of necropsy samples from dolphins obtained by commercial vessels.
2. A one-year review of relevant historical demographic and biological data related to the dolphins and dolphin stocks.
3. 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:

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

1. 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.

1. 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.

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

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

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

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

1. 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____________