REVIEW.
2001 CHASE ENCIRCLEMENT STRESS STUDIES ON DOLPHINS TARGETTED IN EASTERN TROPICAL PACIFIC OCEAN PURSE SEINE OPERATIONS.

FINAL REPORT

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EXECUTIVE SUMMARY

An ensemble of studies was conducted by the National Marine Fisheries Service (NMFS) mandated by the International Dolphin Conservation Program Act of 1997 (IDCPA) to characterize the effects of yellowfin tuna fisheries on pantropical spotted dolphins (*Stenella attenuata*) and eastern spinner dolphins (*Stenella longirostris*) populations in the Eastern Tropical Pacific (ETP). Some of these studies focused on determining whether tuna fisheries have patho-physiological effects on these cetacean populations. This report, authored by an external reviewer with expertise in cetacean pathology, reviews the latter studies.

This subset of studies provided data suggesting, but not demonstrating, that the chase and encirclement associated with the fishery have negative effects on the dolphin populations. The findings of St Aubin, 2002 (LJ-02-37C) indicate the presence of muscle damage after the chase and encirclement (see Comments on LJ-02-37C). These findings and those of Cowan and Curry, 2002 (LJ-02-24C) are consistent with - but do not demonstrate - that exertional myopathy (EM) affects some dolphins following the chase. Exertional myopathy is a metabolic disease that induces high rates of delayed mortality. It was first recognized in Africa in free-ranging ungulates chased by air and ground motor vehicles, when they are forced to strenuous exercise *and* submitted to extreme stress (37). We note that lesions often seen in acute stress in animals, such as adrenal hemorrhages, were not observed (Cowan and Curry, 2002 (LJ-02-24C)). Previous work (6) along with the observations of Santurtún 2002 (LJ-02-36C) has documented passive dolphin behavior that is consistent with the clinical signs of EM. This disease has been reported in virtually all vertebrates including pinnipeds and cetaceans since its first description in African wild ungulates. Together these observations warrant the examination of the skeletal muscles of a large number of dolphins to determine whether a definite diagnosis of EM is supported by the morphological manifestations of skeletal muscle damage. In addition, the availability of baseline data in captive *S. attenuata and S. longirostris* would definitely consolidate the interpretation of the hematological parameters study (LJ-02-37C) regarding the diagnosis of EM.

Dolphins severely affected by EM (or by any other disease) are more likely to be killed and their carcass destroyed by predators. Thus they are likely to be underrepresented or not represented at all in samples of live animals. Alternatively or concurrently, a strong negative selection against animals susceptible to EM over 40 years may have resulted in a population composed mostly of dolphins that are resistant to EM. Consequently, any future study addressing the health status of ETP dolphins should target a larger number of dolphins, at least several hundreds, because diseased dolphins are likely to be rapidly destroyed by predators. In this regard, the dolphins currently killed in the tuna fisheries constitute a large and (relatively) cheap source of material that should be urgently exploited for post-mortem examination to detect pathological changes such as EM.

The thermal studies (Pabst et al LJ-02-34C) did not show evidence of hyperthermia, an important clinical sign of EM. This suggests that heat-dissipating mechanisms are highly efficient in ETP dolphins, and that dolphins are efficiently protected against hyperthermia. In LJ-02-30, Mesnick et al suggest that dolphins may have been trained to avoid tuna fishing

operations. Alternatively, ETP dolphins susceptible to hyperthermia and/or EM may have been massively and negatively selected over 40 years by tuna fisheries.

The Skin Molecular Stress Response study (LJ-02-27) addressed an interesting and promising concept. The interpretation of the results however was hampered by several troublesome technical problems. The studies that evaluated the immune functions (LJ-02-25C and LJ-02-35C) did not reveal any immune dysfunction with the important exception of an imbalance between B and T cells. This imbalance has been related to extensive exercise in other species. This abnormality should be further investigated.

Edwards (LJ-02-28 and LJ-02-29) reports mathematical and behavioral data that together strongly suggest a negative impact of the chase on the mother-calf pair. According to studies on free-ranging cetaceans, an increasing proportion of calves is expected over the years in a recovering population. On the contrary, Cramer and Perryman (LJ-02-31) report a significant decline in proportion to calves over a ten-year period.

Considered together, these studies support that tuna fisheries impose a high stress to ETP dolphins and possibly disrupt the mother-calf pairs. In addition, they provide preliminary evidence that exertional myopathy (EM) triggered by the chase and encirclement could affect an unknown percentage of animals.

Recommendations

- **To determine the baseline biochemical parameters of** *S. attenuata* **and** *S. longirostris.* It is important to determine the baseline levels of these parameters in normal, resting domesticated or semi domesticated *S. attenuata* and *S. longirostris.* This would immediately allow the interpretation of the biochemical parameters measured during the CHESS study, particularly with regards to muscle enzymes and HCO_3 levels.

- **To carry out post mortem examinations of a (representative) large number of dolphins killed in seine nets or during chase with an emphasis on the examination of skeletal muscles.** See Wobeser (1994) for an extensive discussion of the number of animals to be examined.

- **Detection of viral infections.** Screen the sera collected during the CHESS study for the presence of antibodies specific against known cetacean viruses, especially cetacean morbilliviruses.

- **To determine whether tuna fishing causes post-release mortality in ETP dolphins (Capture - tagging - release - monitoring experiments).**
  To achieve this goal, it is necessary to tag a large number of ETP dolphins captured in simulated fishery with radio transmitters. Tagged animals will be tracked for three weeks to evaluate mortality and if possible, carcasses will be recovered and examined.

- **Reproductive studies**
  Dolphin carcasses recovered from the tuna fishery should be examined with regards to reproductive success/physiology.

BACKGROUND

Eastern Tropical Pacific (ETP) dolphin populations have sustained heavy losses due to the tuna fishing industry. A particular method used by the industry since the late 1940s is based on the spatial aggregation of dolphins and tuna, and consists of entrapping both dolphins and tuna with a net, the purse seine (22).

From 1959 to 1972, this method, termed "dolphin fishing", caused the death of at least 100,000 dolphins a year with a cumulative loss of 4.9 million dolphins from 1959 to 1972 (National Research Council 1992; Wade 1995 in (6)). In 1972, the Marine Mammal Protection Act, along with the continuing improvement of techniques and equipment and the increased attention of fisherman, led to a decrease in the total observed mortality to 25,000 dolphins in 1991 (22).

In 1995, the Declaration of Panama was signed between the USA and eleven other tuna fishing countries to decrease dolphin mortality to a total of 5,000 a year with the ultimate goal of eliminating all dolphin mortality. In 1996, the total observed mortality was 2,547 dolphins (Lennert and Hall 1997 in (6).

In 1997, the Marine Mammal Protection Act was amended by the International Dolphin Conservation Program Act (IDCPA) to implement the Declaration of Panama. It allowed the importation of yellowfin tuna in the USA from other fishing countries under the label "dolphin safe" if dolphin injury or mortality are not observed during fishing operations. Because of the concern that purse seine "dolphin fishing" may still be detrimental to the dolphin stock, the IDCPA required that research be conducted by the National Marine Fisheries Services (NMFS) to determine if the above fishing method has negative effects on the ETP dolphins (6).

From August to October 2001, a 2-month research cruise, termed Chase Encirclement Stress Studies (CHESS), was carried out in the Eastern Tropical Pacific Ocean. Its objective was to determine whether repeated chase and encirclement, as practiced in the dolphin tuna fishery, have a negative impact by inducing stress on pantropical spotted dolphins (*Stenella attenuata*) and eastern spinner dolphins (*Stenella longirostris*).

The present work is the second review of the studies conducted during that cruise and the first review of two additional reports: one by D.J. St. Aubin (LJ-02-23C), and the IDCPA Report of the Scientific Research Program. In addition to the studies on dolphin stress, the IDCPA report summarizes other concurrent studies carried out on the abundance of ETP dolphin stocks, on the ETP ecosystems, on the quantitative stock assessment of ETP dolphins, and on coastal spotted dolphins genetics. The present report was requested to the author by the "University of Miami Independent System for Peer Reviews" under a consulting agreement.

DESCRIPTION OF REVIEW ACTIVITIES

Fifteen scientific reports from the CHESS research cruise and from demographic studies of ETP dolphin were sent to this reviewer along with one report by D.J. St. Aubin and the IDCPA report of the scientific research program. All documents were examined carefully. A draft report was prepared and submitted prior to a phone call conference arranged on August 16, 2002 to discuss the IDCPA report with relevant NMFS staff from the NMFS La Jolla

Laboratory. The discussion was held with Dr. Karin A. Forney (NMFS), Dr. Stephen B. Reilly (NMFS), and other NMFS members. Dr. Rudy Ortiz, a second reviewer of the CHESS study, contributed to the discussion. A second report (the current report) was written to account for the discussion. The current report fulfills the requirements itemized in the Statement of Work - Consulting Agreement with the University of Miami.

The literature pertaining to diseases and physiology of cetaceans, particularly with regards to stress and capture, was examined. The scope of the literature review was extended to diseases and stress affecting free-ranging ungulates and more generally free-ranging mammals. The summary of findings, individual review of each paper, conclusions, recommendations and references are listed below.

SUMMARY OF COMMENTS

On "Report of the scientific research program under the International Dolphin Conservation Program Act. DRAFT August 2002."

Stress has been suspected as a possible cause for the failure of the ETP dolphin population to recover. To this reviewer's knowledge however, stress *alone* has never been documented to cause the decrease or failure to recover of any population of wild or domestic animals. At the individual animal level, stress alone has been shown to cause severe damage but only in experimental conditions. For instance, fatal heart muscle necrosis alone, without damage to skeletal muscles, is seen in laboratory animals submitted to highly aggressive challenges (see the extensive review by Van Vleet and Ferrans 1986 (35)). Stress however has been involved in the etiology of a well-documented and frequent cause of mortality in wild mammals, especially in free-ranging ungulates submitted to chase by mechanical vehicles. This condition is termed exertional myopathy (EM).

Paleontologists have determined that dolphins and ungulates have a common ancestor, *Pakicetus*, a small ruminant that lived 50 millions years ago (1). This evolutionary relationship has been strongly supported morphologically and molecularly, at the nucleic acid and protein levels (7, 9, 20, 21, 23, 27, 29, 30, 33, 34). This relationship may help explain why infectious agents such as cetacean Morbilliviruses resemble most closely the infectious agents that affect ungulates. Cetacean Morbilliviruses are phylogenically similar to those causing "Peste des petits ruminants", a deadly disease of ungulates, while in contrast phocine (seal) Morbilliviruses (or Phocine Distemper Virus) are closer to canine distemper virus, a deadly virus of carnivores (32). This relationship may also explain why cetacean morbilliviruses can infect bovine cells *in vitro* (36). The same relationships are being defined regarding Herpesviruses (14). The phylogenic relation between ungulates and odontocetes is also manifested by the similarities of their digestive tract. Odontocetes and ungulates both have multiple gastric compartments. The first gastric compartment of both groups is macroscopically and microscopically similar. It is lined by a stratified heavily keratinized epithelium. It is followed by the glandular stomach (although not immediately in ungulates).

The phylogenic relations between ungulates and cetaceans probably suggest that both taxonomic groups are susceptible to the same metabolic diseases, including exertional myopathy (EM). EM is a metabolic disease first recognized in Africa in free-ranging ungulates

chased by air and ground motor vehicles i.e. when these animals are forced to strenuous exercise *and* submitted to extreme stress (37).

EM has been variously termed capture myopathy, exertional rhabdomyolysis, overstraining diseases and stress myopathy. The severe form of EM leads to delayed mortality in 4 to 36 % of wild ungulates and is manifested by damages to skeletal and cardiac muscles, elevation of serum levels of enzymes normally present in intact muscle fibers, metabolic acidosis and hyperthermia. In people and *some* ungulate species suffering EM, damaged muscle fibers release myoglobin, which is eliminated in the urine (myoglobinuria). In these less frequent cases, myoglobinuria may trigger renal damage, particularly in proximal tubules (12, 37).

Over the last two decades, EM has also been described in cetaceans, pinnipeds, birds, canids, marsupials, and even fish. It is now thought that all vertebrates may suffer the disease when they are submitted to strenuous exercise accompanied by excitation (28, 37). Considering the universal presence of EM in animals, because EM has been described in pinnipeds and cetaceans, and because of the ontogenic relationships between wild ungulates and dolphins, it is likely that a certain percentage of ETP dolphins chased during tuna fisheries operations also suffer EM.

Importantly, in 1979, EM was already suspected in the ETP dolphin populations. Sixty-five carcasses killed during the fishing operations were dissected and carefully examined, including skeletal muscles. After careful gross and microscopic examination of the skeletal muscles, no evidence for EM was found (5). For this reviewer, there are several possible explanations for the absence of EM lesions in these earlier studies. Free-ranging ungulates with severe EM are predisposed to predation and accidents (37). Consequently, most explanations listed below are based on the assumption that ETP dolphins with the severe form of EM cannot survive in the wild because they become easy prey to predators such as sharks:

1) **An insufficient number of animals may have been sampled.** The number of animals that must be examined in order to detect a disease with a given prevalence in a population of free-ranging animals has been discussed in minute details elsewhere (8, 38). For instance, with an animal population size over one million animals, and a suspected disease prevalence of 0.5 %, 600 animals should be examined, that is, **about 10 times more than the number of ETP dolphins examined in Cowan and Curry LJ-02-24C** (5). The detection of a 2 %-prevalence requires the examination of 150 animals, or about three times more than the number of ETP dolphins examined here and in 1979. The detection of a 10 %-prevalence requires the examination of 30 animals.
   Wobeser also mentions a useful simple guide, the "rule of three". It is based on the observation that the prevalence is unlikely (95 % confidence) to be higher than 3/*n*, where *n* is the number of animals examined. **Cowan and Walker did not see any evidence of EM in the 65 ETP dolphins in 1979 (5). According to the "rule of three", the prevalence of EM was not higher than 3/65 or 4.6 % (95 % confidence) in ETP dolphins in 1979 (13)**. This statement should be qualified. If the animals most severely affected by EM die after their release because they are vulnerable to predators, -namely sharks- live animals suffering severe EM may never be found. **In fact live animals affected with EM and**

likely to be found are those that are not affected severely, i.e. those that can escape predators.

2) **The "non response" problem** (biased selection) may affect the random sampling method that was used for sampling. The "non response" problem is the equivalent of people who do not respond to questionnaires in epidemiological studies. similarly, in wildlife epidemiological studies, older, more experienced animals may avoid capture, which results in a sample composed of younger animals showing an entirely different pattern of disease (if they show any disease at all) (38).

3) **EM may affect muscles other than those that have been sampled.**

4) **Small herds have been sampled** in the CHESS program compared to the size of herds pursued in the usual operations (Forney et al. LJ-02-32). Because chasing large herds of ungulates predisposes them to EM, the chase of small dolphin schools may lower the prevalence of EM in captured dolphins (17).

5) Dolphins may be progressively, or may have been, trained to avoid the capture (and thus EM) associated with tuna fishing. Mesnick et al (LJ-02-32) provide a fascinating review supporting that dolphins avoid and escape tuna fishing in the most extensive fishing areas. Consequently, as some (or most?) dolphins get trained over the years, they would avoid EM, which would lower the incidence of the disease or even eliminate it in the population.

6) All the above explanations may coexist.

7) No ETP dolphins suffer EM. That would make these animals unique among free-ranging mammals and even vertebrates. This uniqueness is not impossible (but is unlikely) because hyperthermia, believed to play an important role in the development of EM, is probably rare in healthy marine mammals kept in water - water conducts heat more easily than air, and the specific heat of water is high (see also Pabst et al LJ-02-34C).

Except Cornell and Simpson (28) who reported cases of EM in cetaceans, there is not an abundance of cases of EM in cetaceans reported in the literature. There are several potential causes for this:

- In tuna fishery, millions of dolphins have been submitted to high levels of exertion, frequently and over decades, using an extensive deployment of fast noisy motor vehicles, both in the air and water. To this reviewer's knowledge, no other odontocetes populations have been submitted to comparable conditions.
- The causes of death of offshore cetacean species are not well known in general, because of the inaccessibility of the carcasses.
- Oceanaria do not have strong incentive to publish systematically the number or causes of death of marine mammals occurring within their enclosure, especially soon after capture (There is a single report in the literature).

- A dolphin handicapped by myopathy, even to only a moderate degree, is probably the first to be selected from a herd of dolphins (admitting that the affected dolphin is still part of a herd) by predators such as sharks. Other experts are required to assess this risk. Biologists familiar with predator pressure on ETP dolphins should be consulted, particularly with regards with dolphin-shark interactions, and the abundance and identity of shark populations in ETP. Other possible predators of dolphins and their abundance should also be considered.

Over the last two decades, there have been an increased number of instances where diseases have been suspected to play an important role in the decrease of populations of terrestrial (3) and marine mammals (16, 18). These diseases have been detected by examining carcasses of animals stranded on the shoreline, which is unlikely to happen with pelagic species such as the ETP dolphins. **Thus, most likely observers would have missed even large virus-induced mortalities occurring in the pelagic ETP dolphins.** Consequently, it would be important to screen the sera that have been collected during the CHESS study for the presence of specific antibodies against known cetacean viruses, especially cetacean morbilliviruses and/or to look for these viruses by molecular techniques (RT-PCR, ELISA).

Specific changes to be carried out on the IDCPA Draft report

1. This reviewer agrees with the statement that potential muscle injury leading to delayed death warrants future study (p. 7). **It should be clearly stated in the IDCPA Report, however, and as stated on p. 12 of LJ-02-32 and in the abstract of LJ-02-37C, that "hematological parameters (or the data or the results or the observations) indicated that <u>muscle damage</u> followed the exertion of the chase in some dolphins".**
2. **p. 23: for an ungulate to be affected by fatal exertional myopathy, it is not necessary to be submitted to "catastrophic" conditions such as "being trapped under a canopy in the net". It is simply necessary to be submitted to a short but extensive chase, particularly with motor vehicles, terrestrial or airborne. Thus the sentence containing the words "catastrophic aspects of fishery operations" (1st paragraph) should be reworded.**
3. **p. 23. Necropsy sampling should be carried out on at least 600 animals, not 300 animals.**

I totally agree with the importance of the fishery impact on the separation of the mother-calf pair.

Comments on the reviewed CHESS reports (stress and fishery effects)

Further assessment of the potential for fishery-induced stress on dolphins in the eastern tropical Pacific.

Administrative report LJ-02-23C.
D.J. St. Aubin.

This is an excellent review of recent stress studies and of studies documenting other possible fishery-related effects on ETP dolphins.

Histopathological assessment of dolphins necropsied onboard vessels in the Eastern Tropical Pacific Tuna fishery.

Administrative report LJ-02-24C (CIE-S11).
D.F. Cowan, B.E. Curry

The manuscript was appropriately modified except that there was no comment on the absence of skeletal muscles among the samples examined. The examination of skeletal muscles is an important part of the post mortem examination of human and animals. ETP dolphins are wild mammals phylogenetically related to ungulates, and are placed by the tuna fisheries in the same conditions that trigger severe skeletal muscle lesions in ungulates. Therefore, EM should be strongly suspected, and the target organ of EM is skeletal muscle. Thus, the examination of skeletal muscles in this case is particularly important.

Adrenals showed no lesions of acute stress such as hemorrhages.

Investigation of the morphology and autonomic innervation of the lymphoid organs in the Pantropical Spotted, Spinner, and Common Dolphins incidentally entangled and drowned in the tuna purse-seine fishery in the eastern tropical Pacific.

Administrative report LJ-02-25C (CIE-S10)
T. Romano, K. Abella, D. Cowan, B. Curry.

This reviewer had not suggested changes to the manuscript.

Molecular signature of physiological stress based on protein expression profiling of skin.

Administrative report LJ-02-27 (CIE-SO8).
Sarka Southern, Anne Allen, Nick Kellar, Andrew Dizon.

The manuscript was modified but important issues remain to be resolved. I fully agree with the authors in their response to the reviewer comment concerning the choice of proteins as being an appropriate endpoint to measure gene expression in response to stress. However the

quantification of gene expression at the protein level using antibodies is troubled by several technical problems of which the most important are addressed below.

The use of a "cocktail" composed of 40 antibodies is puzzling. The concentration of each antibody is individually optimized, and then this concentration is decreased 40 times for the "cocktail". In the response to the reviewer comments, the authors state that they optimized the concentration of each antibody used singly and then used a concentration 30 - 60 times lower in the antibody cocktail for the spotted dolphins. There is no clear explanation of how the optimization of the antibody concentration was carried out for the antibody "cocktail". Most likely, because of this dilution, only several of the 40 antibodies are solely responsible for the "cocktail" positive reactions (those with the highest affinity and/or the antibodies whose corresponding antigen is most abundant and/or concentrated). It would be important to determine how many and which antibodies are responsible for the positive reactions. This would save expensive reagents, and it would identify the specific proteins whose expression levels are altered, thus providing some information on the patho-physiological mechanisms at play.

In response to the reviewer comments, the authors explain that the validation of the antibody specificity for cetacean SRP antigens could not be carried out because of the scope of the study. This is a fair assessment, but it also limits the impact of the study. The author does not rule out satisfactorily the possibility that the banding pattern is an artifactual change. Argument (1) "the changes are non-randomly distributed" is not clear. Artifacts do not necessarily occur randomly. For instance, a dull microtome blade produces highly repeatable artifacts. Could it be for instance that the stressed samples were cut with the same blade? Argument (2) "the same four types of the banded SRP profiles were observed in two different groups of specimens, regarding processing/preservation" does not rule out that the specimen were inadvertently left drying before being frozen or formalin-fixed. Argument (3) "banded profiles were consistently observed with different markers, and at distant sections" does not rule out that an artifact is possible.

Behavioral contributions to separation and subsequent mortality of dolphin calves chased by tuna purse-seiners in the eastern tropical Pacific Ocean.

Administrative Report LJ-02-28 (CIE-S12).

And

Energetics consequences of chase by tuna purse-seiners for spotted dolphins (*S. attenuata*) in the Eastern Tropical Pacific Ocean.

Administrative report LJ-02-29 (CIE-S13).
EF Edwards.

The changes requested by this reviewer have been made.

Estimation of reproductive and demographic parameters of the eastern spinner dolphin (*S. longisrostris orientalis*) using aerial photogrammetry.

Administrative report LJ-02-31 (CIE-S15).
K. Cramer, WL Perryman

This reviewer had not requested changes.

Chase encirclement stress studies on dolphins involved in Eastern Tropical Pacific Ocean Purse Seine Operations during 2001.

Administrative Report LJ-02-32 (CIE-SO1).
KA Forney, DJ St Aubin, SJ Chivers.

The abstract does not mention the findings concerning muscle damage caused by the chase. Yet in both the manuscript (p. 12) and the abstract of LJ-02-37C, it is mentioned that hematological parameters indicated that muscle damage followed the exertion of the chase. In the same line of thought and for the sake of consistency, on p. 19 (first paragraph) "... in constituents indicative of muscular exertion.." should be changed for "... in constituents indicative of muscle damage... ".

I agree with the authors that exertional myopathy (and any other condition with a low prevalence) would require a larger sample size to be detected (p. 12).

As in LJ-02-37C, articles pertaining to human medicine, sport medicine or human physiology are used for interpreting the blood parameters. Yet, dolphins are phylogenically closer to ungulates than to humans, and exertional myopathy is a pathological entity (although not completely understood). It is not a normal physiological reaction to exercise. Hence, a veterinary textbook in clinical pathology would be insightful for interpreting the hematological parameters (Duncan et al, 3rd edition is nice).

On p.19, 1st paragraph: the authors mention "... and other abnormalities in..., skeletal muscle and kidney" . Yet, this reviewer did not see any description of skeletal muscles in Cowan and Curry 2002 (LJ-02-24C) or elsewhere in the CHESS results. The examination of skeletal muscles from a large number of carcasses will need to be carried out, possibly from the fishery-induced mortality, if EM is to be detected.

Tagging and tracking of *Stenella* spp. during the 2001 Chase Encirclement stress studies cruise.

Administrative report LJ-02-33 (CIE-SO5).
SJ Chivers, MD Scott

The authors addressed all except one of this reviewer's comments. In my comments, I asked whether it was possible that dolphins which dived (rarely) more than 5 meters might have access to cold water during these deeper dives (which would explain that they do not show detectable hyperthermia). The authors address this question by mentioning that human divers did not detect a decrease in water temperature within the net enclosure. Was there any

other data, such as the measurement of water temperature within the net enclosure at various depths? The authors also mention that it is difficult to conceive for them a mechanism by which cooler temperature from below the thermocline depth could be brought near the surface by pursing the net. I was probably not clear when I mentioned some possible explanations: Is it possible that the huge purse seine apparatus, along with the rapid and ample maneuvers of the ship, disturbs the thermocline by mixing the different layers of water?

Measuring temperatures and heat flux from dolphins in the Eastern Tropical Pacific: is thermal stress associated with chase and capture in the ETP-Tuna Purse Seine Fishery?

Administrative Report LJ-02-34C (CIE-SO4).
D. Ann Pabst, WA McLellan, EM Meagher, AJ Westgate.

This reviewer had not suggested any changes to the manuscript.

Investigation of the effects of repeated chase and Encirclement on the immune system of Dolphins (*S. attenuata and longirostris*) in the Eastern Tropical Pacific.

Administrative Report LJ-02-36C (CIE-S03).
T. Romano, M. Keogh, K. Danil

This reviewer had not suggested any changes to the manuscript.

Coping behaviors of spotted dolphins during fishing sets.

Administrative Report LJ-02-36C (CIE-SO6).
E. Santurtún and F. Galindo

The authors have made the adequate changes to the manuscript.

Hematological and serum chemical constituents in Eastern Spotted Dolphins (*S. attenuata*) following chase and encirclement.

Administrative report LJ-02-37C (CIE-S02).
DJ St. Aubin

The manuscript was appropriately modified. In particular, the abstract now mentions that moderately elevated levels of enzymes indicated muscle damage after the exertion of the chase and some dolphins showed alterations suggestive of metabolic acidosis.

The following are some minor comments. On p. 15, The author states that "in other species of dolphins such changes in muscle enzymes are considered subclinical forms of myopathy, which typically resolves in a few days (G. Bossart, pers. comm.)". The dolphins that are referred to are probably captive. Captive dolphins are not exposed to predators or to other

severe stress encountered in the wild. In addition, they do not have to chase prey. The amount of muscular work required in captivity is also limited. Thus, captive animals are effectively in a "convalescence" ward. Consequently, muscle enzyme levels that, in captivity, correspond to "subclinical forms of myopathy" could reflect in the wild a condition that severely impairs the ability to escape predators and to chase prey, and which represents a condition that does not resolve itself, but rather worsens.

To evaluate the significance of the increase in muscle enzyme levels, the author refers to two articles in human sport medicine (19, 26). As stated above, dolphins are phylogenically close to ungulates, not to humans. Thus the veterinary literature (especially that related to ungulates) would be insightful.

On p. 9, the author reports that AST was positively correlated with LDH. Importantly, AST and LDH levels are used as indicators of muscle necrosis in animals (25). Table 4 shows that 3 dolphins of 53 (5.6 %) presumably captured for the first time had elevated CK, the enzyme most sensitive to muscle damage in animals (25).

Response to reviewer comments:

Dr St Aubin mentions that it is "ambitious" to suggest that no dolphins with exertional myopathy (EM) would have survived to be observed with gross evidence of EM. I suggest that this depends mostly on the density and behavior of predators. Here again, the ETP dolphin situation might be compared with that of wild ungulates chased with motor vehicles (airborne and terrestrial). What would be the probability of capturing a wild ungulate with evidence of EM secondary to that chase after it has been released in the wild in Africa? The probability would probably be very remote, considering that predators have a tendency to select preys that seem/are vulnerable. The affected ungulate would not be recaptured; instead, it would be eaten. After all, EM has been detected in wild ungulates only because these animals are accessible and protected from predators after the chase (that is they are kept in an enclosure).

I agree with Dr St Aubin that the single case of suspected EM reported by Colgrove (4) may have been a case of muscle injury due to transportation. Dr St Aubin also raises an interesting related point. Why have many cases of EM not been reported in odontocetes captured in the wild over the years? I suggest that other dolphins have not been submitted to the same exertion, with the same frequency, over such a long time, using such an extensive deployment of fast, noisy motor vehicles, both in the air and water.

In my opinion, a dolphin handicapped by myopathy, even of a moderate degree, will be the first target selected by a predator such as a shark, among a herd of dolphins (admitting that the affected dolphin is still part of a herd). But other experts (within NMFS) are required to better assess this risk. Biologists familiar with predator pressure on ETP dolphins should be consulted, particularly with regards with dolphin-shark interactions, and the abundance and types of shark populations in ETP. Other possible predators of dolphins and their abundance should be also considered.

The author mentions that the phylogenic relation of odontocetes with ungulates should be qualified because 50 million years is a long time. (This issue is important because exertional myopathy (EM) has been described first in ungulates, and ungulates are known to be particularly susceptible to EM). Odontocetes are taxonomically related to ungulates and that relation has been solidly established. Paleontologists have determined that dolphins and

ungulates share a common ancestor, *Pakicetus*, a small ruminant that lived 50 millions years ago (1). This evolutionary relationship has been strongly supported morphologically, molecularly at the nucleic acid and protein levels (7, 9, 20, 21, 23, 27, 29, 30, 33, 34). This relationship may help explaining why infectious agents such as cetacean Morbilliviruses resemble most closely those that affect ungulates. Cetacean Morbilliviruses are phylogenetically similar to those causing "Peste des petits ruminants", a deadly disease of ungulates, while in contrast phocine (seal) Morbilliviruses (or Phocine Distemper Virus) are closer to canine distemper virus, a deadly virus of carnivores (32), and why cetacean morbilliviruses can also infect bovine cells *in vitro* (36). In addition, the same phylogenic relationships are being defined regarding Herpesviruses (14). The phylogenic relation between ungulates and odontocetes is also manifested by the similarities of the digestive tract. Odontocetes and ungulates both have multiple gastric compartments. The first gastric compartment of both groups is macroscopically and microscopically similar. It is lined by a stratified heavily keratinized epithelium and is followed by the glandular stomach (although not immediately in ungulates).

RECOMMENDATIONS AND CONCLUSIONS

The hematological (LJ-02-37C) and necropsy findings (LJ-02-24C) are consistent with the hypothesis that some ETP dolphins suffer exertional myopathy caused by strenuous exercise due to the chase. In turn, EM could cause post-release mortality. To demonstrate this hypothesis, I suggest the following experiments in decreasing order of feasibility and increasing order of cost.

- **To determine baseline biochemical parameters**

To allow the interpretation of any biochemical parameters measured during the CHESS study, particularly with regards to muscle enzymes and HCO_3 levels, it is important to determine the baseline levels of these parameters in normal, resting domesticated, or semi domesticated spotted dolphins. The only way to achieve this goal is to capture animals and maintain them in captivity until they become acclimated to captivity. Scott reports that one female pantropical spotted dolphin is kept captive at Gulfarium, Florida (in (6)). I strongly suggest that the clinical parameters of this animal - and of all other spotted dolphins that have been kept in captivity - be measured.

St Aubin et al (1996) (31) have estimated the differences between circulating levels of adrenal hormones of wild and semidomesticated dolphins (*Tursiops truncatus*). A "correction factor" could be estimated and used to reinterpret the ACTH levels measured in the present study of wild dolphins.

- **Post mortem examination of dolphins dying in seine with an emphasis on the examination of skeletal muscles**

If skeletal muscles have been sampled over the course of the 2001 study, it is imperative that these samples be examined by a trained pathologist to determine whether EM is present.

Based on the prevalence of EM in chased free-ranging ungulates, EM would affect between 4 and 36 % of ETP dolphins, at least the first time they are chased. Each dolphin is captured about 8 times a year on the average (24). Thus, theoretically, any given affected dolphin should show muscle damage at various stages of degeneration and necrosis (acute, versus chronic), assuming that affected dolphins do not die of the disease or are not easy prey for predators, which is unlikely. In fishing areas where fishing is more intense (and where dolphins are likely to be captured more often), the extent, severity and nature (time wise) of the muscle lesions (acute versus chronic) should be higher than in the areas of lower fishing activity.

Wobeser (1994) (38) discussed the number of animals that must be examined in order to detect a disease in a population of free-ranging animals. For instance, with an animal population size over 1,000,000 animals, and a suspected disease prevalence of 0.5 %, 600 animals should be examined. A 2 %-prevalence requires that 150 animals be examined. A 10 %-prevalence requires that 30 animals be examined (The low and high total estimates of abundance (in numbers of animals) are 494,268 to 953,547 for northeastern offshore spotted dolphins; 271,322 to 741,867 for eastern spinner dolphins; and 96,738 to 228,038 for coastal spotted dolphins (11)).

Scott suggested that 2,500 to 3,000 animals a year are killed by the fishery operations (6). High priority should be given to the exploitation of this huge, and for the time being, wasted amount of material since it may permit to answer the major question raised by the CHESS study (for this reviewer): do ETP dolphins suffer EM?

Extensive efforts should be invested to make these carcasses available for standard postmortem examination. If intact carcasses are recovered, complete post mortem examinations should be carried out, including skeletal muscle. Major groups of skeletal muscles should be examined grossly and histopathologically in order to detect muscle damage. Note that skeletal muscles remain reasonably well preserved for a longer time than most organs, which would allow a delay of several hours between death and the sampling of skeletal muscles. (This time frame may vary with water temperature and exposure of carcasses to solar radiation).

Aqueous humor of cadavers and carcasses has been used in humans and in domestic animal species to measure biochemical parameters such as glucose, sodium and chloride urea nitrogen and creatinine concentrations (2). Thus this biological fluid could be used to provide information on these parameters in dead ETP dolphins.

- **Detection of potential viral infections**

Screen the sera that have been collected during the CHESS study for the presence of antibodies against known cetacean viruses, especially cetacean morbilliviruses.

- **Capture-tagging-release-monitoring experiment**

Dolphins severely affected by EM (or any other lesions) following capture are most likely easier prey for predators. Sampling of these dolphins is improbable unless they are tagged before release and then monitored, followed, and if they die, recovered and examined.

Thus we recommend capture-release experiments, similar to those characterizing the nature and frequency of EM in terrestrial mammalsWe suggest the following experimental design. Spotted dolphins could be captured in a simulated fishery, equipped with radio

transmitter, released, monitored and tracked for approximately three weeks in order to determine mortality rates after release.

To determine whether there is a causal relationship between the chase and dolphin mortality due to EM, a "dose-response" type of experiment may be carried out. Different levels of intensity may be applied to the chase. Harthoorn and van der Walt (1974) (15) observed that metabolic acidosis was most pronounced in animals that were forced to run at high speed over short distances than in animals that were forced to a lower speed over a longer distance. We expect that the same relationship will be observed in spotted dolphins. This type of experiment would permit to quantitate the impact of the chase and would allow drawing guidelines for the fishery.

If electronically tagged and monitored dolphins do die after release, carcasses should be recovered and examined by a trained pathologist. Based on the frequency of EM in wild ungulates (4 % to 36 %), the number of spotted dolphins that should be electronically tagged and monitored would be between 30 and 600 (38).

No doubt this type of experiment is expensive. However, considering the cumulative costs of research cruises and other types of work carried out over the last two decades and the high degree of uncertainty still in the air, capture-tagging-release-monitoring experiments may offer a relatively low cost / benefit ratio.

- **Reproductive studies**

Reproductive parameters were collected in the course of the IDCPA Necropsy program for the female pantropical spotted and eastern spinner dolphins. The proportion of lactating and pregnant animals was low. Data collected between 1974 and 1992 showed a wide variation depending of the location where sampling occurred and the year during which sampling occurred.

The carcasses should be examined with regards to reproductive success/physiology. In particular, the ovaries should be examined for the presence of lesions such as follicular cysts, and the number of physiological structures such as *corpora lutea* and follicles should be counted. Mammary glands should be examined microscopically for the potential presence of mastitis and/or nematodes that would impair nursing (6, 10).

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APPENDIX 2. Statement of Work.

STATEMENT OF WORK

Consulting Agreement Between The University of Miami and Dr. Daniel Martineau

Background

The tuna purse seine fishery has used the association between tuna and dolphins to fish in the eastern tropical Pacific Ocean (ETP) 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 (MMPA) in 1972 and the increased use of fishing equipment and procedures designed to prevent dolphin deaths, mortality decreased during the late 1970s, 1980s, and 1990s to levels that are generally considered biologically insignificant.

While changes in the fishery have dramatically reduced the observed mortality of dolphins, the MMPA, as amended by the International Dolphin Conservation Program Act (IDCPA), requires that the National Marine Fisheries Service (NMFS) conduct research consisting of three years of population abundance surveys and stress studies to form the basis of a determination by the Secretary of Commerce regarding 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 Secretary must make a final finding in this regard by December 31, 2002. It should be noted that this issue is controversial and particularly relevant to persons involved with NMFS, the US and non-US tuna industry, and environmental groups.

The topic of this review is the IDCPA Science Report that will be presented to the Secretary of Commerce, along with information obtained under the IDCP, and other relevant information to form the basis of the Secretary's final finding. The IDCPA Science Report is comprised of the results of all research activities required under section 304(a) of the MMPA, as amended by the IDCPA. Each major component of this report has been separately considered in a series of independent peer reviews conducted by the Center for Independent Experts (CIE). These consist of: the Abundance Review (October 15-17, 2001) the Stress Review (February 4-6, 2002), the Ecosystem Review (March 6-8, 2002), and the Assessment Model Review (April 3-5, 2002).

Abundance Review

The topic of this review was the abundance of several species of tropical pelagic dolphins that associate with tuna and are killed in the ETP purse seine tuna fishery. Estimates of dolphin abundance based on cruises carried out in 1998-2000 form a central part of these studies. The main task of the consultant was to review the methods used to estimate abundance from line-transect data, including covariate detection models. The fact that these dolphins occur in a wide range of school sizes presents unique problems for the estimation of expected group size, so considerable effort has been devoted to this analysis. Documents supplied to the reviewers included draft manuscripts describing the covariate analysis, simulations to test the performance of several estimators, calibration of school size estimates, and assignment of partially identified sightings. Background papers included previous relevant publications and reports. The raw data and software used in the analysis were also made available.

Stress Review

The stress studies mandated in the IDCPA include: 1) a review of relevant stress-related research and a three-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; and 3) an experiment involving the repeated chasing and capturing of dolphins by means of intentional encirclement. This review included a suite of studies subsumed under this general topic, and a brief description of these studies follows.

The necropsy program analyzed samples from about 50 dolphins killed incidentally during fishing operations. Historical biological samples and data were analyzed 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. Chase Encirclement Stress Studies were conducted during a two-month research cruise aboard the NOAA ship McArthur in the ETP. During this project, the team worked in cooperation with a chartered tuna purse seine vessel 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.

Ecosystem Review

To complement the three-year abundance studies, population assessments were made for the following years: 1986, 1987, 1988, 1989, 1990, 1998, 1999, and 2000 with a primary goal being to determine if populations that were historically reduced in size are increasing over time. Should the assessments indicate no increase (lack of recovery), three broad categories of factors could be the cause: a) effects from the fishery; b) effects from the ecosystem; c) an interaction between the proceeding two factors. This need to attribute causality for a potential lack of recovery serves as the primary justification for ecosystem studies. By investigating the physical and biological variability of the ecosystem of which the dolphin stocks are a part, we establish a context which can be used to better interpret trends in dolphin abundance. A lack of recovery that is not mirrored by some other change in the ecosystem would largely eliminate an ecosystem hypothesis, leaving fishery effects as the most likely cause.

This review included a suite of studies subsumed under the general topic of ecosystem research in the ETP. The basic approach was to compare ecosystem parameters over time with a primary goal being to look for indications of a potential ecosystem shift. The power of these ecosystem studies increased with the number of environmental variables, taxa, and trophic levels included, and with the time period spanned (although most ecosystem data available for these investigations were collected concurrently with dolphin assessment data aboard NOAA research vessels and are restricted to the late 1980s and late 1990s).

The general components of the ecosystem research included: 1) physical and biological oceanography: sea surface temperature, thermocline characteristics, phytoplankton and zooplankton distribution and relative abundance; 2) larval fishes: distribution and relative abundance; 3) flying fishes: distribution, relative abundance, and habitat relationships; 4) seabirds: distribution, absolute abundance, and habitat relationships; and 5) cetaceans: distribution, absolute abundance, and habitat relationships.

Assessment Model Review

As indicated above, NMFS was charged with essentially determining whether or not the depleted dolphin stocks are recovering, and if so, at what rate and at what level of certainty. The topic of this review was the overall framework that will be to estimate the growth rate of two dolphin populations of interest, the northeastern offshore spotted dolphin and the eastern spinner dolphin, using growth rates estimated by fitting a population model to the three-year and other available estimates of abundance. For this review, estimates from research vessel surveys using line transect methods are available for three periods: 1979-83 (four estimates), 1986-90 (five estimates), and 1998-2000 (three estimates), for a total of twelve estimates over twenty-one years. Reviewers were also asked to evaluate the inclusion or exclusion of a set of fishery-dependent indices of abundance, resulting from data collected by tuna vessel observers. Two types of population growth rate will be estimated: (1) exponential rate of change from 1979-2000 and (2) intrinsic rate of increase under the assumption of a density-dependent model where pre-exploitation population size in 1958 is considered carrying-capacity. Both an aggregated population model and an age-structured model will be used. Bayesian statistics, using a numerical integration method, were used to estimate a probability distribution for the population growth rate.

Specific Reviewer Responsibilities

For the final IDCPA Science Program Review, expertise is needed to review all components of the research described above, specifically with respect to NMFS' incorporation of comments previously received from the topical reviews also described above. Reviewers will be provided with the draft IDCPA Science Report, as well as comments received as a result of the CIE reviews and explanations of how/why such comments were or were not incorporated into the report.

The reviewer's duties shall not exceed a maximum total of 11 days, including:

- 2-3 days to read the draft IDCPA Science Report (to be provided to the reviewers by no later than August 2, 2002);
- 2-3 days to produce a written report of the reviewer's comments and recommendations on the draft report;
- 1-2 days to discuss via telephone, on August 15-16, 2002, with relevant NMFS staff from the NMFS La Jolla Laboratory, the incorporation of comments and any related questions; and
- 2-3 days to revise the written report based on those discussions.

It is expected that each reviewer will have participated in the earlier CIE reviews of IDCPA research described above and will not require general presentations of research results, but will focus on addressing comments and recommendations included in the reviewers' reports in his/her topic area. Reviewers should particularly consider whether the responses to the original review comments are sufficient and acceptable, in a manner similar to the role filled by a journal editor when considering manuscripts revised in response to referees' comments.

Each reviewer's 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 draft IDCPA Science Report provided in advance;

2. No later than August 13, 200, submit a written report of findings, analysis, and conclusion in the individual reviewer's topic area to NMFS;

3. Discuss relevant documents with scientists from the NMFS La Jolla Laboratory via telephone on August 15-16, 2002, to facilitate proper incorporation of reviewers' comments;

4. No later than August 23, 2002, submit a revised written report of findings, analysis, and conclusions based on discussions held with relevant NMFS staff from the NMFS La Jolla Laboratory. The written report[1] (see Annex I) should be addressed to the "University of Miami Independent System for Peer Review," and sent to Dr. David Die, via email to ddie@rsmas.miami.edu, and to Mr. Manoj Shivlani, via email to mshivlani@rsmas.miami.edu.

Signed_____ Date_____

[1] The written report will undergo an internal CIE review before it is considered final. After completion, the CIE will create a PDF version of the written report that will be submitted to NMFS and the consultant.

ANNEX I: REPORT GENERATION AND PROCEDURAL ITEMS

1. The report should be prefaced with an executive summary of comments and/or recommendations.

2. The main body of the report should consist of a background, description of review activities, summary of comments, and conclusions/recommendations.

3. The report should also include as separate appendices the bibliography of materials provided by the Center for Independent Experts and a copy of the statement of work.

4. Individuals shall be provided with an electronic version of a bibliography of background materials sent to all reviewers. Other material provided directly by the center must be added to the bibliography that can be returned as an appendix to the final report.