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REPORT ON CAPTURE STRESS WORKSHOP

BY

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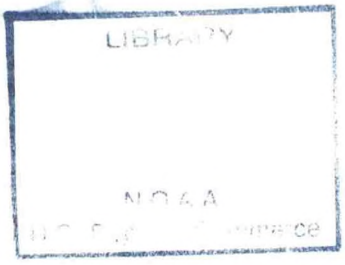

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INTRODUCTION

A workshop on the physiological effects and possibly pathological effects of chasing and capturing dolphins during tuna purse seining was held on 1-3 May 1979 at the Southwest Fisheries Center in La Jolla, California. The workshop was convened to discuss whether or not unobserved mortality was likely among dolphins captured during purse-seining for yellowfin tuna *(Thunnus albacares)* and released apparently unharmed.

Purse seining for yellowfin tuna involves dolphins primarily of the genera *Stenella and Delphinus*. There is an, as yet poorly understood, association between large yellowfin tuna and the dolphins. The dolphin most commonly involved is the spotted dolphin, *Stenella attenuata*. Other dolphins involved are the spinner dolphin, *Stenella longirostris*, and the common dolphin, *Delphinus delphis* but to a much smaller extent than is the spotted dolphin.

In fishing for yellowfin tuna associated with dolphins, fishermen search with 20X binoculars for bird flocks or other evidence of tuna. When a bird flock is located, the ship approaches, and if the birds are feeding over a tuna-dolphin association, speedboats are launched in an attempt to herd the dolphins (the tuna will remain with the dolphins) into a tight group that can be encircled by the purse seine.

The speedboats used are 16-18 feet long, powered with 85 hp or larger engines. The speedboats (directed by the captain via radio) are sent out ahead of the purse seiner and herd the dolphin school in an ever decreasing spiral until the net can be set to encircle the school. Chases last for up to 1.5 hours but average approximately 25 minutes. The distance traveled and the actual speed of the chase have not been measured. Based on behavioral observations and hydrodynamic considerations, there are periods in the chase when swimming speeds probably exceed 10 knots (Dr. Daniel Weihs, personal communication).

Behavioral observations of dolphins confined to the purse seine are relatively easy to make and have led to observations of "rafting" and "passive behavior". Rafting is observed throughout the set. Rafting animals remain motionless in the water often with the flukes hanging down. Some rafting dolphins are observed to sink slowly for a short distance before returning to the surface to breathe. Rafting may be related to passive behavior. Passive behavior is most frequently

observed shortly before the animals are released from the seine. Animals displaying passive behavior are observed to lie against the webbing often with their dorsal fins down, on their sides or in other unusual positions. Some individuals display a posture in which the body is arched with the head and flukes dorsad. When a "passive" animal comes to the surface to breathe, it does so awkwardly with little of the gracefulness normally associated with a swimming dolphin.

Attempts to derive hypotheses concerning the causes or functions of passive behavior led to a consideration of possible physiological reasons for such behavior and to the literature on what has been called "capture myopathy". Descriptions of behavior in animals suffering from capture myopathy are similar in many ways to what has been observed in the "passive" dolphins. Review of the capture myopathy literature raised the concern that some dolphins may be dying after leaving the purse seine. Capture myopathy is a syndrome that has been observed extensively in terrestrial animals that have been chased, captured, and handled. In some percentage of animals captured, death occurs from within a few hours to as much as several weeks, after the capture episode.

Based on this information, it was decided to convene a workshop to discuss the potential effects of chase and capture on the physiology of dolphins. To this end, scientists were selected to participate based on their experience with capture problems in terrestrial animals or with the physiology, pathology, and behavior of marine mammals. A list of the participants in the workshop is appended.

The workshop format was basically informal. Each participant gave a short talk about the work he or she had been doing, and questions and discussions were continuous. Since the problem area could be relatively easily circumscribed, this format was very productive and allowed a group with widely differing backgrounds to communicate successfully. A copy of the agenda is appended.

SYNOPSIS OF RESULTS

Three major conclusions resulted from the workshop. The primary conclusion was that there is probably some mortality that occurs following release of apparently unharmed animals from the purse seine. This mortality results from the stresses of chase and capture. The second conclusion was that the presently available data are insufficient to quantify the rate of delayed mortality. The final conclusion was that more research should be done to resolve the extent of stress and potential mortality.

The remainder of this report will attempt to describe the considerations that went into these conclusions. A short bibliography on literature that covers many of the subjects discussed in the workshop is appended.

TERRESTRIAL MAMMAL DATA

Over the last 10-15 years, a considerable amount of data has accumulated relative to the effects of chasing, capturing, and handling ungulates. A major concern developed when it was found that a large percentage of animals captured died for one reason or another. The classical picture at necropsy showed various muscle lesions and an examination of the serum showed rises in muscle enzymes. This picture gave rise to the term "capture myopathy". As more data have accumulated on this subject over a variety of species, the terminology has tended to become more general, and the term "capture stress" is tending to replace the myopathy term, which of course has the very specific connotation of muscle damage.

A large number of species have been observed to be affected by capture stress. The best known are grazing animals, zebra, various African antelopes, rhinoceros, pronghorn antelope, bighorn sheep, white-tailed deer, moose, elk, and others. Birds have also been found to be susceptible; geese and flamingos are examples. The capture stress syndrome has been reported in fishes and even in one dolphin (*Tursiops truncatus*, Colgrove, 1978). Unfortunately, Colgrove's paper does not exclude the possibility that hyperthermia was the cause of the observed charges. It was discussed at the workshop that the only animals known to be affected by the capture stress syndrome are grazing forms. It should be noted, however, that a similar syndrome is known in domestic animals, e.g., horses and pigs. A condition called azoturia has been observed in racing greyhounds (Davis and Paris, 1974) (Azoturia is named after the dark colored urine that is passed due to the large amounts of myoglobin released from damaged muscle.) which has symptoms very similar to capture stress in other species. It may be that predators in general are less susceptible or that the methods used in capturing them are unlikely to result in a problem.

There are considerable differences among species in their susceptibility to capture stress with animals of some species less subject to the stresses than others. In any species, the effects range from fatal to unmeasurable. Furthermore, the conditions of a chase, capture, etc., are important in determining the severity of the capture stress syndrome. For example, in South Africa Harthoorn found that it was best to capture animals, carry out all necessary procedures, and then release them as soon as possible. This procedure resulted in far fewer mortalities than did techniques where animals were allowed to "rest and recover" for a week to 10 days between handling periods. He found that enzyme levels tended to peak after about 10 days and that during the period when serum enzyme levels were at their highest, the animals were extremely vulnerable to additional stress.

Harthoorn also emphasized that in the normal situation of capturing animals, we do not simply apply one stress. Instead, we normally do several things in a series and each phase of the operation causes more stress. The additional stresses compound the problem. For example, in the South African situation an animal is chased, then grabbed and pulled down, then perhaps loaded into a crate to be transported, and then the diet changed. The additional stresses from each of these operations increases the mortality rate for the animals involved.

Harthoorn and others at the workshop described the physiological and pathological picture of the capture stress syndrome. Unfortunately, most workers in this field have had to depend on collecting their data on capture stress incidentally. Harthoorn has fortunately been able to treat the subject experimentally. Thus, for a description of the capture stress syndrome, it is necessary to rely mainly on Harthoorn's data.

Harthoorn depicts a highly variable physiological picture depending on the conditions of the chase and capture. For example, a short, very fast chase leads to acidemia. A zebra chased for about 5 minutes at maximum speed (2 km) is certain to die. The animal becomes comatose and doesn't respond to the presence of people. The breathing rate slows and the heart rate increases greatly. There is a reduction in the circulating blood volume, a reduction in cardiac output, a fall in systemic pressure, a rise in pulmonary arterial pressure and peripheral stagnation. These symptoms are apparently due to the high degree of anaerobic metabolism during the chase, which causes a large build-up in lactate levels and a consequent severe drop in pH to levels far below any observed in man.

Without treatment an animal in this state is doomed. Treatment with sodium bicarbonate, however, will reverse the pH situation and the animal has a good chance of surviving if then released. Sodium bicarbonate, however, is only effective in this type of chase situation, and although the animal will probably survive, it is susceptible to any additional stresses and will be more vulnerable to predation during the recovery period.

If the chase procedure chosen is a long, low-speed chase which is continued until the animal can be captured without a struggle, the physiological picture is quite different. Such a chase results in alkalosis, and animals die less quickly under these circumstances. After the long chase, vascular hemolysis is a common finding, the cause for which is not known, but which may be due to the pounding of hooves on dry hard ground. The major problem found with a long chase is ruptured muscle fibers. This results in the subsequent release of myoglobin into the blood. The potassium levels are elevated and eventually the myoglobin is precipitated in the kidney resulting in myoglobinemic nephrosis leading to death. Myoglobinuria may be observed.

Chasing animals, although potentially fatal as described above, is not the only area of concern. Animals captured without a chase are also subject to severe and equally fatal stress levels. Dr. Harthoorn gave the example of the sable antelope. These animals were captured with a net and then physically restrained. The results were severe hyperkalemia, acidemia, and a tremendous release of adrenalin. The effects of hyperkalemia on the heart were exacerbated by acidemia, and the addition of adrenalin secretions resulted in a bizzare electrocardiogram. In some instances, the heart went into myocardial fibrillation and the animal died. Even if fibrillation (death) did not occur, the fact that myoglobin was released into the blood resulted in myoglobin precipitating in the kidneys and a high mortality rate.

Harthoorn mentioned that in experimental studies, myoglobin has been found to crystallize in kidney tubules. Stress results in a much more rapid crystallization. Several things are happening that may contribute to this. First, the perfusion pressure is reduced in the kidneys. Acidemia and vaso-spasm caused by adrenalin compound the effects. Thus, even animals that apparently survive without damage may eventually die from myoglobinemic nephrosis. In these situations, death usually occurs in 10 days to 2 weeks. An additional factor in kidney failure may be dehydration. Harthoorn suggested that the capture stress syndrome is brought about when animals are subjected to completely unnatural situations for which there has never been a need to evolve responses.

In the South African situation, a technique has been found to greatly reduce mortality levels due to capture stress. The method of choice is to herd the animals into a large corral and leave them alone for about one month. During this month, they are exposed to the presence of man and regular runs through a capture funnel. After a month of habituation and conditioning through exercise, the animal can be captured and handled without inducing a great enzyme rise and few of the animals die from the experience. The present rate of mortality under the best situations in South Africa has been reduced to 1%, over nearly 1000 animals captured in 1978 (down from a mortality exceeding 50% less than a decade ago) probably the lowest level known from capturing any terrestrial mammals.

Barrett and Chalmers described their studies of capture myopathy in pronghorns, bison, elk, moose and, in one case, a goose. The symptoms and characteristics of the capture stress syndrome are basically as Harthoorn described, with some variation among species. For example, on gross necropsy, the muscle damage and the specific muscles involved in myopathies varies among species. In some species, the muscle becomes very pale, while in others no color change is obvious. Spraker described similar situations.

Barrett and Chalmers reported a short-term mortality rate of 4% in pronghorns that had been captured, weighed, measured, and bled. After release, an additional 3-5% mortality occurred, mostly within 2-5 days but in some up to 2 weeks later. Handling was the basic cause of most of the deaths, with a much lower rate of mortality in those animals not handled. If transportation was added to the procedure, the mortality rate increased to 20%.

Much of the discussion that followed the presentations by various speakers had to do with the effectiveness of various measures of an animal's condition. Cowan for example, mentioned that exercise can induce a rise in some of the enzymes. CPK (creatinine phosphokinase) is one that is likely to be affected by exercise because it is a small molecule. The rises due to exercise as shown by Haigh with moose are not likely to be very large (10-fold or less), whereas the rises due to known cases of captive myopathy are very great (many orders of magnitude). Typical results are that glucose, cortisol, CPK, LDH (lactic dehydrogenase), GOT (glutamic oxaloacetic transaminase), GPT (glutamic pyruvic transaminase), and possibly other enzymes may increase in the serum. The reliability of some of these enzymes as indicators of muscle damage is questionable, depending on the species involved and the conditions under which the blood has been taken, stored, etc. Another area of concern with serum enzyme measurements is that no animal, other than man, has been studied enough so that the physiology is known to the point where enzyme changes can be interpreted with certainty. Most analyses currently being made were developed for human studies. It is possible that in other species other molecules interfere with the tests, making the results unreliable. Another source of problems is that of variations among laboratories. Tests run at separate labs are not comparable in the absolute sense, and in some situations labs change over time. This means that controls must be a constant part of any such work.

Another area of discussion concerned possible predisposing factors in capture stress. Griner discussed the potential for problems in cases of a selenium/vitamin E defficiency. Haigh mentioned that moose captured in early spring while still in poor condition after a winter seemed to have more problems with capture stress. Harthoorn described a similar situation in which poor nutrition during the dry season apparently played a role. The exact significance is not known, but, in general animals that are poorly fed, pregnant, or lactating may be more susceptible.

The capture stress syndrome in general is extremely variable. Spraker suggested that the capture process be looked at in a broad way for any given situation. There may be physical injuries to an animal, ranging from broken bones and abrasions to lacerations. The environmental conditions may cause problems, especially with very high or low temperatures leading to hyperthermia or hypothermia. Probably the most important factor involved, however, is the stress due to fear in animals that have never been in contact with man. An examination of the literature

caused Spraker to suggest that many of the symptoms of the capture stress syndrome could be directly related to shock induced by psychogenic stress. Shock is mostly due to the massive release of epinephrine and norepinephrine from the adrenal gland. These substances are a normal part of the body's defensive system and their release prepares the body for escape, etc. In excess, however, in a terror-producing situation, the combined effects of epinephrine and norepinephrine on the circulatory system may be lethal.

In summary, the capture stress syndrome as seen in terrestrial mammals is a serious problem. Even under the most favorable conditions some mortality occurs when wild animals must be captured. The syndrome is shown to be highly variable in its manifestations, making it difficult to deal with. The psychogenic stresses of the capture situation are apparently very harmful. Added to the stress of capture itself, it militates against survival, and captured animals that are not handled survive better than those that are handled. Also, those that are released soon after capture have a better chance of survival than those kept under captive conditions. The question at this point in the workshop became one of how readily transferable the observations made on terrestrial animals are to dolphins.

MARINE MAMMAL DATA

The discussions of marine mammal data suggested that marine mammals are in fact quite similar to other mammals. Davis, for example, showed that free ranging Weddell seals rely primarily upon aerobic energy production during diving and suggested that porpoises probably behave similarly. This is not to suggest that these animals are incapable of operating anaerobically, however, as the Weddell seal can remain submerged for up to 60 minutes and incur a large lactic acid load. But such long, stressful dives are very unusual in free ranging Weddell seals and require a long recovery period to reestablish a normal metabolic and physiological steady state. Unfortunately, little is known of the diving behavior and physiology of free ranging porpoises. There is no indication of any physiological adaptation in porpoises which would provide special protection against capture myopathy syndrome as compared with terrestrial mammals. Indeed, there is some indication of a small aerobic metabolic scope in porpoises which may limit their ability to greatly increase aerobic energy production over normal levels and require a heavy reliance upon anaerobic energy production if severely frightened or chased. A large anaerobic energy contribution under these conditions would cause rapid lactate production, acid-base disequilibrium, and fatigue.

One source of data on the potential effects of chase and capture on marine mammals is the capture-for-display industry. Prescott described the standard capture techniques and responses of pelagic dolphins, such as *Delphinus delphis*, to this process. One of the problems

he mentioned was acute death. Dolphins often die within minutes after capture, but the reasons for this are unknown. Typically an animal is brought on deck in good shape. Tremors begin and the animal arches its body. Regurgitation occurs and in some instances, is so powerful as to displace the bronchial connection with the blowhole. In such cases, vomitus may be inhaled, asphyxiating the animal. In other sudden death cases, death occurs without an apparent specific cause.

Animals that survive to be returned to tanks on land often show behavioral patterns that are very similar to those described in the purse seine environment. Rafting is common and rafting animals sink to the bottom of the tank and lie there in unusual postures. Often they exhale at this time. Eventually, the animals return to the surface to breathe. The dolphins often appear unable to swim and if they do swim, they bump into the tank walls.

During the discussion of this information, the data published by Walker (1975) on the live-capture fishery were discussed. Those data show a mortality rate for *Delphinus delphis* in captivity of 75% within 60 days after capture. Only 15% of these animals survived for one year in captivity. Walker attributed the high mortality rate to stress, inanition and inappetence which leads to lowered resistance and death or disease.

During the workshop, neither Walker nor Prescott could recall any evidence of capture myopathy-like lesions in animals necropsied after capture.

Possible alternative explanations for passive behavior also came in for some discussion. Geraci suggested that passive behavior could be related to the cataleptic behavior observed in harp seals. Cowan suggested that passive behavior may be learned as a way of avoiding the chaotic situation at the surface of the backdown area. In response to this, Perrin stated that for most of the time that fishing has been occurring passive behavior would have been maladaptive, because most often the passive animals were believed to be dead and were usually "sacked up" with the tuna. Being sacked up with the tuna resulted in approximately 50% mortality for the dolphins involved.

Geraci presented ideas on stress in marine mammals, drawing both from the literature and from his studies of dolphins and seals. Stress affects the output of the adrenal gland. The secretions of the adrenal medulla are mainly catecholamines. Nothing is known about the dynamics of catecholamines in dolphins. The adrenal cortex secretes a variety of substances and is involved via these secretions in such things as water and electrolyte balance, glucose metabolism, etc. Stimulation of the adrenal cortex and the subsequent secretion of steroids results in some measurable occurrences in the physiology of a dolphin. Examples are that lymphocytes and eosinophils decrease in abundance, the other

white blood cells increase in numbers and in severe situations, hemorrhages may occur. Under prolonged stimulation, the cortex may become "exhausted," i.e., no longer responds by appropriate releases of hormone, resulting in hypoglycemia, electrolyte disturbances, and shock. In extreme situations, adrenal cortical hemorrhages may occur.

Geraci went on to describe the metabolic aspects of capture stress. The process of gluconeogenesis provides a source of energy for the muscles. Glucose is broken down to pyruvic acid and to lactic acid. The pyruvic acid goes into the TCA cycle, where GOT and GPT are important enzymes. These reactions, of course, are the source of energy for cellular work involving muscular contraction. Damage to muscle results in cellular enzymes being released into the circulatory system. A background level of these enzymes is always present as cells are constantly being replaced. In some organisms, e.g. man and some laboratory mammals, we have a pretty good idea about how much muscle damage has occurred when a given amount of enzyme is found in the serum. This is not known for most other organisms. In cetaceans, the blood volume is approximately twice that of terrestrial animals, so enzyme levels in terrestrial animals cannot be directly compared to cetaceans. Furthermore, we have no data on excretion rates for the various enzymes in cetaceans. These factors make it very difficult to interpret serum chemistry data from dolphins.

Geraci discussed a syndrome called pinneped hyponatremia. This syndrome occurs in phocid and otariid seals and appears to be related to an inherent deficiency in the adrenal cortical function. The syndrome becomes observable when the animals are stressed. Stresses include molting and captivity, among others, and may lead to death if the stress lasts long enough.

In the dolphin, *Lagenorhynchus acutus*, an adrenal abnormality has been observed in stranded animals. The abnormality is a greatly enlarged adrenal gland. It normally occurs on the left adrenal and is most often found in females (95% of females compared to 15% of males). The causes of this and its impact on the animals is still being investigated by Geraci.

DOLPHINS INVOLVED IN THE TUNA FISHERY

Following Geraci's introduction to dolphin physiology, the data that had been obtained from animals involved in the fishery were presented. Stuntz outlined the methods used in collecting the blood serum data and requested that those persons having experience in using blood serum analyses go over the data for later discussion. A number of the participants examined the data and made comments on them. The consensus seemed to be that without further basic data on the physiology of dolphins and more controls on the laboratory techniques it would be unwise to put much

faith in the values. With this proviso in mind, it was pointed out that certain animals from a sample of 34 animals showed elevated CPK values. The elevated values could be either a result of muscular exertion or of muscle damage. In this regard a possibly very important feature of dolphin physiology was brought up by Geraci. He suggested that dolphins may be able to shut off their kidneys as a way of conserving water during periods of fasting. Ridgway (1972) has obtained data showing that in some species of dolphins kidney function is reduced during periods of fasting and materials may tend to accumulate in the blood during periods when no metabolic water is available for excretion. Interestingly enough, such a state could be responsible for the high variability in some of the data on serum constituents, especially in freshly captured wild animals.

Following presentation of the data on serum constituents, Cowan and Walker presented the data they collected on dolphins killed in the fishery area during the 2nd and 4th cruises of the *M/V Queen Mary* during 1978. The major conclusions of the study were that this population (like most other wild populations) has a considerable degree of parasitism and naturally occurring diseases. It should be remembered that these animals were killed in the purse seine setting and may not be representative of the population as a whole. These are discussed thoroughly in the report and in many cases are in need of further study. No evidence of lesions, scars, or other residue typical of capture myopathy were observed. The cause of death for the animals was more difficult to determine, but it is believed that most of the animals died when they became entangled and suffocated. Some deaths in the purse seine may have been as the result of stress. Some of the lesions found may have been the result of stress, but there is no evidence relating the stress lesions directly to the fishery, since similar lesions have been observed in stranded animals in areas where, so far as is known, the animals have had no involvement with a fishery.

WORKSHOP CONCLUSIONS AND DISCUSSION

The general consensus of the workshop participants was that given the best available information, it is likely that there is some unobserved mortality occurring after the dolphins are released from the purse seine. The data presented from the fishery neither support nor deny this conclusion, since the sample sizes are very small. In the purse seine situation, the animals are not normally handled. This might reduce the impact of captive stress considerably and thus the mortality level could be very low. Given a very low rate, the probability of detecting the situation with the available samples is very small.

Without a very large sample and/or a very detailed experiment, it is impossible to assess the actual amount of any mortality. The question of the amount of mortality we could expect from stress-related causes

was addressed extensively. The main questions by the participants were how the information would be used and why we could still show concern if the mortality levels were very low. The answers to those questions involved the methods used to calculate the original populations (i.e., backcalculate from our best estimate of current abundance) and the fact that a very large number of dolphins (approximately 10,000,000 captures per year) are involved in the fishery. Because of these concerns and considerations, the group felt that it was imperative that a study be designed to deal with the question of the effects of stress on dolphins. Most of the rest of the workshop time was given over to the question of how such an experiment should be designed.

FURTHER RESEARCH

The ideal method of assessing the extent of mortality in affected animals would be by direct observation; for example, capture animals, hold them in a very large area (to minimize the effects of being held captive) and count the animals that die. After death a complete necropsy would be performed to determine the cause of death. Most of the Workshop participants felt that such an approach was impractical due to the very large sample sizes that would be needed to obtain quantifiable results on a problem that may occur less than 1% of the time. They also felt that it would be impossible to do this experiment without the experiment affecting the outcome.

Because of the above considerations, most of the Workshop participants felt that one or more preliminary experiments would be necessary. These experiments would be designed to develop our knowledge and techniques to the point where the appropriate samples could be collected from dolphins captured normally by the fishery. These samples would then be analyzed to determine what percentage of the captured population had been affected (to a lethal degree) with capture stress.

The basic questions to be answered in the preliminary experiments are: (1) What are the effects of stress on dolphins? (2) What is the best way of measuring any effects? and (3) Are there any effects that may be induced by the fishery that would be of sufficient magnitude to result in death or permanent impairment that would harm the reproductive potential of the populations?

Basically, there are two research options open to us and each of them has pros and cons that must be considered. The two options mainly concern where the work is done rather than what is done. The first option is to do the work in the eastern tropical Pacific on dolphins presently involved in the fishery. While this option is attractive, because by using spotted dolphins it would be most directly applicable to the fishery situation, it is logistically very difficult. The second option is to do the work in California waters using *Delphinus delphis* as a model. This option is attractive since the logistics are much

less awesome and because the results will still apply to a fished species albeit a species that forms a minor part of the total number of animals involved in the fishery.

The basic research plan that developed was to purse seine a school of dolphins and hold them for a period of 5 days or so. Data would be collected from the animals upon capture and at intervals throughout the holding period. Capturing and holding a school (50 to 100 animals may be needed) is probably feasible by using the Porpoise School Impoundment System (PSIS) and the holding pens developed for use with that system. The process of monitoring the health of the captured animals entails taking a very large number of physiological measurements from selected animals in the school at regular intervals. Optimally, animals would be sacrificed and a complete workup would be done of each animal.

The measurements to be made at each stage of the experiment are as follows. Upon capture, blood samples would be collected from each individual. Age and sex would be determined as well as possible. Animals would then be placed in holding pens. At this point in the experiment, there are several options to be considered. First is the number of animals per holding pen. Some concern was expressed about having a critical mass of animals to minimize stress while in the pen. It has been the experience of the live capture industry that individuals survive better if they are in a group situation. Discussion on this point suggested that 25-50 animals would be sufficient to minimize any problems in this area. It is important to make certain that a critical mass remains throughout the experiment, so if animals are to be sacrificed on a regular basis, there will have to be a surplus of animals present in the holding pen from the beginning. A second option is to capture several groups of animals and divide them among several holding pens. Some of the groups will only be monitored behaviorally and only animals that die will be removed from the holding pens. This option is basically a good one, but it tends to increase the number of people that will be required for monitoring the animals and thus, complicates the logistics somewhat.

Assuming animals are to be sacrificed, a sample would be taken immediately after capture and bleeding. These animals would provide a baseline upon which to measure stress induced changes that occur later. It is important to remember that, in general, it takes some time for lesions to develop to the stage where they can be readily studied histologically and that the changes in serum enzymes, etc., also take time to appear. Thus, the initial samples will provide baselines for all that follows.

Throughout the holding period, the behavior and acoustic emanations of the animals will be monitored. Focal animals will be selected and followed throughout the period. Thus, we will be able to measure agonistic behavior, respiration rates, activity levels, etc., from

the animals throughout the period and correlate these with acoustic results. These measurements may then be related to the physiological measurements. Such monitoring will also allow us to recover any animals that might die in the holding pens, quickly enough so that the tissues will be in good shape for pathological studies.

The physiological measurements that will be made are intended to be complete enough that a basis will be laid for further studies in the fishery in any attempt to quantify the mortality rate due to stress. The blood samples that have been taken from live dolphins in the fishery were analyzed for the following things: glucose, urea nitrogen, creatine, uric acid, sodium, potassium, chloride, calcium, inorganic phosphates, iron, cholesterol, triglycerides, total bilirubin, GOT, GPT, LDH alkaline phosphatase, total protein, CPK, the LDH isoenzymes, and CPK isoenzymes. These measurements will be made on the blood samples taken from the captive animals. Many of these measurements will be replicated by other methods as a check on the laboratory techniques. Blood samples will be taken and then analyzed serially over a period of weeks as a way of obtaining control data on the effects of freezing dolphin serum. This option is available only if the work is done near San Diego so that fresh samples can be taken immediately to the laboratory for analysis. In addition to the blood serum constituents listed above, the plan is to measure blood gases, lactate, pyruvate, aldolase, thyroid hormones, cortisol, catcholamines, succinate, steroid profiles, blood pH and, with whole blood, to look at RBC sedimentation rate, total eosinophil counts, reticulocyte counts, RBC fragility, O_2 binding rate of hemoglobin and capillary blood gases. Myoglobin levels would be determined both in the serum and in urine, and a complete urinalysis would be performed. Body temperature would be monitored, as would pulse rate.

On the day of capture, as many of these parameters as possible will be measured on as large a number of animals as possible. The sample size will be reduced to 5-10 animals per day on the following days. On each of those days the animals will be sacrificed following bleeding. The animals that are sacrificed will be extensively necropsied and samples will be taken from each organ for histological examination. Any abnormalities will be thoroughly examined visually and if necessary, histochemically. Additional tissue samples will be taken for the analysis of tissue enzymes.

The number and sensitivity of many of the measurements to be made and the necessity for a large technical staff is what makes doing this work locally so attractive. The costs of doing the work locally are also going to be substantially less. A discussion of some of these points follows.

First, two ships will be needed throughout the experiment. In addition, a purse seiner will be needed for the initial capture. The workshop participants envisioned the sequence working as follows. First, the team will be brought together and all of the necessary equipment will be installed aboard a research vessel. At that time, an airplane or helicopter will be used to locate a school of dolphins. Assuming the school was sufficiently large, the tuna seiner would be dispatched, and the set would be made. The initial samples will be taken and the animals placed in holding pens. At that point, the seiner would no longer be needed. The research vessel would now become a working platform and would remain with the holding pens for the next several days. Also needed will be a vessel, or if the research vessel is so equipped, a helicopter for ferrying personnel and samples to and from the beach.

Doing this work on the tuna grounds would mean that only a fraction of the data would be obtained. Many of the kinds of people needed to do this work would probably be unable to make the longer commitment of time away from their normal duties to participate in the research. The specialized analysis equipment that is available here would not be available and often the measurements to be made are seriously affected by freezing or storing the samples. Problems of this sort can be overcome once a baseline of data are available as a standard to compare with, but the initial work needs every possible advantage.

The experiment described above and carried out locally is the ideal. Some options exist which might provide adequate data. For example, the experiment could be carried out without the sacrifice of animals. If this option were chosen, the probability would have to be high that some animals would die, so the pathological data would be obtainable. If no animals were to die of stress related causes during the experiment, the question of the extent of any lesions detected by other methods would remain. The real question here has to do with the extent of changes that are incompatible with the health of an animal. Unless one can relate a rise in selected enzymes (for example) with the extent of damage, it is impossible to say whether or not a given level of change is a problem. If some animals die during the course of the experiment, the pathological questions could probably be answered. Unfortunately, the problems of individual variability tend to minimize the value of such results. Death is a poor indicator of physiological status.

As mentioned in the discussion of dolphin behavior immediately after capture in the live capture industry, sudden deaths occur frequentl in *Delphinus delphis*. They have also been reported in many other species of dolphins. The probability of sudden deaths occurring during the course of the experiment described above is quite high. Given the team of people that would be needed and the kinds of expertise they would have, it might be possible to address some effort to determining

what happens in such cases. Ridgway suggested that such observations would be a very valuable contribution to the knowledge of cetaceans. Another comment was that a study of this sort would be the most extensive taken on any species other than man and a few laboratory animals. Doing such a study on a wild population of animals would be a major contribution to mammalogy and cetology in particular.

A final option for a way to get at the question of the effects of stress in dolphins is to use passive animals in tuna purse seines in a experiment similar to the above. These animals are presumably affected physiologically in some way and given an intensive effort using divers to capture passive animals, blood chemistry data, etc., could be obtained whenever they are observed in a purse seine set. The sacrifice of some of the passive animals for a thorough workup could provide sufficient data to answer the question of stress effects. The objections to this are the same (mainly logistic) as for a full scale study of animals in the fishery and in addition, there is the rather important problem of obtaining baseline data. Finally, there is the possibility that passive behavior is unrelated to stress. Given these drawbacks, the local *Delphinus delphis* experiment remains the best option even though it would not answer directly the question of potential mortality levels from the tuna fishery.

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ATTACHMENT I

WORKSHOP ON THE EFFECTS OF CHASE AND CAPTURE ON DOLPHINS

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ATTACHMENT II

A G E N D A

WORKSHOP ON THE PHYSIOLOGICAL AND PATHOLOGICAL EFFECTS OF CHASE AND CAPTURE OF DOLPHINS

May 1st

0900	Greetings and miscellaneous
0930	Introduction to tuna/porpoise and films
1030	Break
1100	A. M. Harthoorn
1130	M. Barrett
1200	Lunch
1330	G. Chalmers
1400	J. Haigh
1430	M. Anderson
1500	L. Griner
1515	Break
1600	T. Spraker
1630	R. Davis
1700	J. Prescott

Dinner

Drinks, snacks and conversation at Warren Stuntz' house, map attached

May 2nd

0900	S. Ridgway
0930	L. Cornell
1000	Break
1015	J. Geraci
1045	W. Stuntz
1100	W. Walker
1130	D. Cowan
1200	Lunch
1400	Data examination, comparison, etc.

May 3rd

0900	Open discussion