# CHASE ENCIRCLEMENT STRESS STUDIES ON DOLPHINS INVOLVED IN EASTERN TROPICAL PACIFIC OCEAN PURSE-SEINE OPERATIONS DURING 2001.

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**JUNE 2002**

ADMINISTRATIVE REPORT LJ-02-32

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## ABSTRACT

A suite of complementary stress studies was conducted August-October 2001 to investigate potential effects of repeated chase and encirclement on northeastern offshore spotted dolphins (*Stenella attenuata attenuata*) and eastern spinner dolphins (*Stenella longirostris orientalis*). The studies were conducted as part of a larger research program mandated under the 1997 International Dolphin Conservation Program Act (IDCPA) to investigate whether the eastern tropical Pacific tuna fishery is having a significant adverse impact on these dolphin stocks. Known collectively as the Chase Encirclement Stress Studies (CHESS), the investigations included analyses of blood constituents, immune function, thermal condition, behavioral ecology, reproductive parameters, set-associated behavior, and stress-response protein profiles in spotted dolphin skin. Several lines of investigation revealed the expected acute stress-response in chased and captured dolphins. Concurrent histopathological studies in other dolphins sampled in conjunction with the tuna fishery demonstrated focal heart lesions as evidence of prior tissue damage in 36% of the individuals. These lesions are the likely consequence of stress responses that may or may not have been fishery related, but nevertheless are consistent with changes in circulating adrenal hormones observed in the dolphins that were sampled during this study. Considered together and in combination with other IDCPA studies, the investigations that comprised CHESS have provided new and informative baseline data on stress processes in pelagic dolphins involved in tuna fishing operations; however, there is insufficient evidence to establish whether or not repeated chase and encirclement leads to serious injury, negative effects on reproduction, or cryptic post-release mortality.

## INTRODUCTION

The tuna industry has used the association between tuna and dolphins to fish in the eastern tropical Pacific (ETP) for over five decades (National Research Council 1992). Three stocks of dolphins, eastern spinner dolphins (*Stenella longirostris orientalis*), the northeastern offshore spotted dolphin (*Stenella attenuata attenuata*), and coastal spotted dolphins (*Stenella attenuata graffmani*), 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 (Wade 1995). After passage of the Marine Mammal Protection Act in 1972, and the increased use of equipment designed to prevent dolphin deaths, mortality decreased during the late 1970s, 1980s and 1990s. In 1995, the Declaration of Panama was negotiated between the United States and eleven other fishing nations to reduce mortality of dolphins to less than 5,000 per year. The International Dolphin Conservation Program Act (IDCPA), a 1997 Amendment to the Marine Mammal Protection Act, was created to implement the Declaration of Panama. While changes in the fishery during the last few decades have greatly reduced the observed mortality of dolphins, there continues to be concern that the fishing methods currently used are causing stress to the dolphins involved and that such stress may be having a significant adverse impact on population recovery.

Stressful impacts of chase and encirclement by tuna fishing vessels on dolphins can be considered at several different levels. First, an acute fear and flight response, which can be expected based on observations in odontocetes and other mammals (Curry 1999), might be within the adaptive limits of these animals, allowing recovery within a nominal period and little lasting effect on the individual. Second, the stress of chase and encirclement might produce in some dolphins a state of distress, during which excessive physiological responses could compromise short-term survival of individuals. A third outcome relates to the chronic or cumulative effects of repeated chase and capture. Reactivation of stress-response pathways before appropriate physiological equilibriums are restored might transform an adaptive stress response into a distressful one, magnifying the damaging effects of the stressor. Alternatively, repeated exposure to a stressor, even allowing for recovery, might sufficiently insult critical immune and reproductive functions to affect the individual and, ultimately, the population (Curry 1999). Chase and capture by tuna purse-seine vessels, therefore, may affect the recovery of depleted dolphin populations in the ETP if dolphins experience distress or chronic stress as a result of fishing activities.

As a result, the IDCPA required that research consisting of population abundance surveys and stress studies be conducted by the National Marine Fisheries Service to determine whether the "intentional deployment on, or encirclement of, dolphins by purse-seine nets is having a significant adverse impact on any depleted dolphin stock". One of the stress studies outlined in the IDCPA is an "experiment involving the repeated chasing and capturing of dolphins by means of intentional encirclement", also termed the chase-recapture experiment. The planning for the chase-recapture experiment was the subject of several workshops and consultations involving scientific experts (both federal and non-federal), representatives of non-governmental organizations, and representatives of the Marine Mammal Commission and the Inter-American Tropical Tuna Commission (see Curry and Edwards, 1998; Sisson and Edwards, 2000; Donahue et al., 2000). During those meetings, there was extensive discussion about alternative experimental methods and modifications or additions to proposed methods to make the experiment as effective and appropriate as possible at addressing the requirements of the IDCPA.

The resulting design for the Chase Encirclement Stress Studies (CHESS) was a suite of research projects designed to assess whether the health, reproduction and survival of northeastern offshore spotted dolphins and eastern spinner dolphins may be negatively impacted in animals experiencing repeated chase and encirclement during tuna fishing operations, and whether those impacts may affect the populations' ability to recover from their depleted state. The individual research projects were selected to complement each other as much as possible and to be logistically compatible for data collection during a single research cruise. Research techniques for evaluating stress included: analyses of blood samples (St. Aubin, 2002), immunological studies (Romano et al., 2002a), thermal stress studies (Pabst et al., 2002), tagging and tracking (Chivers and Scott, 2002), behavior studies (Santurtún and Galindo, 2002), histopathological studies of stress-activated proteins in skin samples (Dizon et al., 2002, Southern et al., 2002), and investigation of reproductive status and potential cow/calf separation (see Archer et al., 2001). A panel of experts in dolphin physiology and blood analyses was convened by the SWFSC on January 30-31, 2001 to determine the most effective suite of blood parameters for the assessment of stress in ETP dolphins (Curry and Forney, 2001).

The goal of CHESS was to provide data on physiological indicators of stress in chased and captured dolphins, and, if possible, to estimate a range of consequences for individual dolphins' survival and reproduction. To address these issues, CHESS attempted to evaluate the degree of acute physiological stress represented by a single chase and capture event, and undertook multiple recaptures of individual dolphins to establish the potential for chronic and/or cumulative effects on various health indices. Two important concerns regarding the study design were recognized: the absence of baseline data for the species in question, and the critical need to monitor changes in individual dolphins over time. The lack of baseline data for spotted dolphins would limit the ability to recognize the physiological signs of distress in either acutely or chronically stressed individuals, and a deficiency in serial data from individuals would hamper the assessment of chronic effects and the development of prognoses.

Conclusions about the potential for significant adverse impacts of fishery-induced stress will be based on the complete results of all studies mandated in the IDCPA, which include not only CHESS, but also population abundance surveys (Gerrodette 1999, 2000), a review of relevant stress-related research (Curry 1999), necropsy samples from dolphins killed in the commercial fishery (Cowan and Curry, 2002; Romano et al., 2002b), and a review of historical demographic and biological data from the affected dolphin stocks (Archer et al., 2001; Cramer and Perryman, 2002; Mesnick et al., 2002). The results from all research components will be summarized in a final report to Congress evaluating potential significant adverse effects of the fishery on the involved dolphin stocks.

## METHODS

### Field Methods

Research was conducted between August 8 and October 4, 2001 aboard the NOAA ship *McArthur*, and a chartered tuna seiner. The study area identified for CHESS was within the range of the northeastern offshore spotted dolphin and eastern spinner dolphin. All operations were conducted about 300 nmi south of Acapulco, Mexico (Figure 1), where weather conditions, ocean currents and dolphin densities were most likely to be acceptable for conducting the study. During the course of the project, schools of spotted and spinner dolphins were located, chased and encircled by the tuna purse-seine vessel, sampled and tagged by experienced biologists and veterinarians, and subsequently released as a group using the 'backdown' procedure routinely employed during fishing operations (see Coe and Sousa 1972, for a description of the purse-seining process). Radio-tagged focal dolphins were tracked, and attempts were made to recapture the same individual and any associated dolphins over the course of several days. Set operations were largely limited by weather conditions and the availability of identifiable dolphins for recapture. Sampling targeted primarily northeastern offshore spotted dolphins to avoid confounding inter-species effects when interpreting a relatively small number of samples. Spotted dolphins are also less likely to exhibit behavior that increases their risk of injury or death during sets and were therefore preferred for safety considerations. A few eastern spinner dolphins were tagged and sampled when mixed-species schools were captured, but the results presented below refer to northeastern offshore spotted dolphins unless otherwise noted.

### Set operations

To minimize risk to dolphins and field personnel, sets were only conducted when weather and sea conditions were judged acceptable by the tuna vessel captain, set coordinator, and chief scientist. Generally, this required Beaufort sea states of less than 4, low to moderate swells and subsurface currents, and little or no rain nearby. When these conditions were met, the seiner's helicopter crew located a suitable school of dolphins (spotted dolphins or mixed spotted and spinner dolphins), and the seiner set on them using standard procedures. Encountered dolphin schools ranged in size from about 200-3000 animals; however, only a subset of the larger schools was captured to reduce the risk of mortality or injury during set operations. During the chase, the *McArthur* followed the seiner at a distance of a few miles. After the net was 'let go' and the dolphin school was nearly or completely encircled, the biological team was brought to the net aboard the *McArthur*'s rigid hull inflatable boats (RHIBs). The first RHIB carried the set coordinator and the thermal imaging team to the net as soon as possible. The second RHIB followed within 5-20 minutes carrying the sampling team and towing the 4 small rafts used as the two primary dolphin sampling stations. These rafts were secured along the cork line, and the sampling personnel waited in the rafts as the net was retrieved. The third RHIB transported biopsy personnel and the raft used for biopsy sampling.

The first RHIB, carrying the thermal imaging team, entered the net compass as soon as possible and began collecting thermal images within about 20-40 minutes of 'let go'. Thermal imaging continued until about net tie-down, when the RHIB exited the net compass and moved towards the backdown area of the net. As soon as safely possible, a biologist aboard the seiner entered the water and either swam free within the net compass or paddled from a small rubber raft. This biologist's duties included assessing the size and composition of the captured school, locating any previously captured and marked animals, and habituating the dolphins to the presence of swimmers in the water. As directed by the tuna vessel captain, usually after the majority of the net had been retrieved, 1-5 additional swimmers were deployed into the net compass to capture individual dolphins and bring them to the sampling rafts. The swimmers duties also included rescuing any dolphins that became trapped under a net canopy and ensuring that all dolphins were released over the cork line at the end of sampling operations. Behavioral data, including standardized scans of dolphin behavioral states, focal animal sampling, and continuous video records of dolphins in the net, were collected from a platform aboard the tuna vessel by a behaviorist, and an Inter-American Tropical Tuna Commission (IATTC) observer recorded set information on standard IATTC forms.

Throughout the set, the set coordinator monitored all operations from a small inflatable raft and was in radio communication with the seiner crew, the bridge crew of the *McArthur*, the RHIB drivers, and personnel in the dolphin sampling rafts. The set coordinator directed the swimmers to capture individual dolphins and bring them to the sampling rafts. He also monitored the behavior of the dolphins, the net, and environmental conditions, and informed the sampling teams when to terminate operations and prepare for backdown. This decision was generally made after 20-45 minutes of dolphin sampling. If sufficient time was available prior to backdown, one or all of the rafts detached from the cork line, exited the net compass, and were picked up by the nearby RHIBs. Otherwise, the sampling rafts remained attached to the cork line until after backdown was complete. All rafts and scientific personnel were transported back to the *McArthur* aboard the RHIBs as quickly as possible to allow continuous tracking of the radio-tagged focal dolphin following each set. Variations to these procedures and those described below were occasionally necessary to ensure safe operations and appropriate sampling, as determined prior to each set by the chief scientist, or during the set by the seiner captain or set coordinator.

**Dolphin Handling and Sampling**

Our standard setup for dolphin sampling included three sampling stations: two primary stations designated for collecting a complete suite of data from each dolphin, and a third used to collect biopsy samples and/or roto-tag additional dolphins. The primary stations consisted of a pair of rafts that were tied together and attached to the net cork line along one side of the backdown channel (Figure 2). One biologist sat in the partially flooded smaller raft and held the dolphin during sampling. The larger raft contained the sampling gear and three other biologists:

one data recorder, one veterinarian or biologist skilled in collecting blood and attaching tags to cetaceans, and one sampling assistant. After being lifted into the raft, the dolphin was measured (total body length and maximum girth), sexed, and a veterinary thermal probe was inserted 20cm to obtain core body temperatures. Blood was drawn from the flukes or caudal peduncle while a biopsy was obtained, a tag was attached to the dorsal fin, and, for females, pregnancy status was evaluated using a portable Sonograder™ model ultrasound unit and lactation was checked by palpation of the mammary glands.

The third sampling station, a single large raft, was attached to the cork line immediately adjacent to the apex of the backdown channel. During the first few sets, biologists in this raft used biopsy poles to obtain skin samples from as many dolphins as possible. This biopsy method was subsequently replaced with a rapid biopsy and roto-tagging procedure to increase the number of marked dolphins released in association with the focal radio-tagged dolphin. The modified procedure involved swimmers restraining individual dolphins alongside the raft for about 2-3 minutes, while one or two taggers in the raft used a coring device or biopsy punch to create a 6mm hole near the trailing edge of the dorsal fin, and then inserted a roto tag through the hole. The cored tissue sample was saved for laboratory analysis. Another biologist in the raft recorded data, measured total body length, and determined sex of the tagged dolphin.

The ability to select individual dolphins for handling in the net based on age, sex or reproductive state was limited, although two-tone spotted dolphins, which are likely dependent calves, were not brought to the rafts for sampling (for color phase descriptions see Perrin, 1969). However, when possible, females were preferentially sampled because of the priority in assessing stress-related effects on their reproductive status. If a calf was observed associated with a handled female, it was restrained in the water near the sampling raft, and, when possible, a skin sample was obtained to allow future genetic matching of cow/calf pairs within a set.

To meet the objectives of CHESS, we used several different tags that can be described according to their primary purpose: 1) Marked dolphins: roto tags and bullet tags, attached to the trailing edge of the dorsal fin after coring a small hole through the fin, were used to mark previously captured animals; 2) Focal tracked dolphins: saddle packages containing VHF radio tags and either a time depth recorder, time-depth-velocity recorder, or time-depth-velocity-heat-flux recorder, were mounted on the leading edge of the dolphin's dorsal fin using two or three delrin pins, and 3) Other tracked dolphins: satellite tags mounted to the dorsal fin were also deployed to remotely record dolphin movement patterns (for additional detail see Chivers and Scott, 2002). After blood sampling and tagging operations were completed, the dolphins were returned to the water inside the net. All dolphins in the net were released together during backdown.

**Tracking and Recapture**

Following the completion of each set, the focal radio-tagged dolphin was tracked continuously from the bridge of the *McArthur*. Efforts were made to maintain the greatest distance that provided reliable radio signals and direction but minimized potential influences of the *McArthur* on the tracked dolphin's behavior (Chivers and Scott, 2002). After 1-3 days, weather permitting, the seiner's helicopter was directed to the approximate location of the radio signal to visually locate the focal dolphin, and a recapture set targeting the portion of the school with the focal or other tagged dolphin(s) was conducted. Operationally, this procedure was similar to standard fishing operations that target a portion of the school carrying tuna, although the duration of overhead activity by the helicopter may have been greater by an unknown amount.

**Project Summaries**

Among the many approaches that were used, several can be considered together under the broader umbrella of pathophysiological investigations. These investigations include: blood analyses, thermal studies, immunological assessments, and immunohistochemical and histopathological examinations. Additional investigations included dolphin tagging and tracking, behavior, reproductive status, and potential cow/calf separation. Radio-tracking was used primarily as a tool to follow and relocate previously sampled dolphins, but also provided data on school dynamics, movements, and diving patterns. Detailed methodology for each CHESS project can be found in the separate reports (Chivers and Scott, 2002; Dizon et al., 2002; Pabst et al., 2002; Romano et al., 2002a; Santurtún and Galindo, 2002; Southern et al, 2002; St. Aubin, 2002). A brief description of each project follows to provide a general overview.

*Pathophysiological investigations*-- Blood components investigated during CHESS (St. Aubin, 2002) included standard veterinary hematology and chemistry panels, with particular focus on exertion-related enzymes, stress hormones, and immunological indicators. Since stress has been reported to bring about changes in immune function, potentially leading to greater vulnerability to disease, the immune status of dolphins that were chased, captured, and recaptured during CHESS was examined via lymphocyte immunophenotyping, lymphocyte proliferation, and examination of DNA-damage in white blood cells (Romano et al., 2002a). Combined, these blood analyses provide a synoptic evaluation of the animal's health, allowing documentation of potential effects of chase and capture stress. Changes were interpreted relative to the first sample for each animal, the time interval between samples, the time between initiation of chase and sample collection, reference values for these and other species of small odontocetes, established changes in stress and disease, and comparative samples from bottlenose dolphins (*Tursiops truncatus*) recaptured as part of a separate, ongoing investigation in Sarasota, Florida (see St. Aubin 2002).

Hyperthermic stress can cause maladaptive physiological changes or death in mammals (Pabst et al., 2002). In pregnant females, damage can occur to the foetus leading to developmental problems or death. In males, hyperthermia can cause reproductive problems including sperm damage. Thermal stress in chased and recaptured dolphins was examined during CHESS using three approaches 1) measurement of fin and body surface temperatures using infra-red imagery while the dolphins were confined to the net, 2) determination of core body temperatures of dolphins placed into rafts for sampling and concurrent investigations, and 3) attachment of data loggers to the dorsal fins of dolphins to determine heat flux in relation to activity during the time interval until recapture.

Southern et al. (2002) proposed that skin samples can provide an indication of sustained stress periods, because stress-responsive proteins (SRPs) are deposited and maintained through time in the proliferating keratinocytes of the epidermis. A large collection of historical samples was evaluated at the SWFSC as part of other IDCPA stress research. These samples were examined in relation to recent fishing effort in the area where the specimens were collected; samples obtained from bow-riding animals in areas with little or no fishing effort were assumed to be an approximate control. Skin samples were obtained from captured and recaptured dolphins during CHESS to allow direct evaluation of animals with a known capture history during the course of this study. These specimens were compared to those obtained historically during fishery operations and from bow-riding animals in areas of varying fishing intensity (Dizon et al., 2002).

*Tagging and Tracking--* Radio-tags were deployed on captured dolphins to allow recapture of previously sampled individuals. Initially, multiple dolphins were radio-tagged within a single set to allow for simultaneous tracking and alternate recaptures of different individuals if the dolphins remained in a relatively small area. However, after it became apparent that group cohesion was very low among spotted dolphins within a captured school (Chivers and Scott, 2002), single focal dolphins were tagged and tracked during the remainder of the study. Additional animals were marked with roto-tags, which could be deployed much more quickly, to increase the number of identifiable dolphins released from each set. Satellite tagging and tracking studies were intended to provide a longer-term (weeks to months), vessel-independent record of movement of captured dolphins; however, apparent tag failure limited the amount of data obtained from these tags (Chivers and Scott, 2002).

*Behavior studies--* Behavioral data were collected by a behaviorist using scan and focal animal sampling methods after dolphins had been encircled, and from video records of a subset of all sets made (Santurtún and Galindo, 2002). Additional data on dolphin behavior and set characteristics were extracted from standard fishery observer data forms used by the IATTC observer during the project. These data were compared to summary data obtained from the Mexican National Observer Program for 1998-2000 to evaluate the similarity of research sets and actual fishing operations.

*Reproduction and cow/calf separation--* Although the ability to evaluate reproductive condition in the field was limited, a portable ultrasound unit was used to test for pregnancy status of sampled females. Additionally, identifiable cow/calf pairs were noted during each capture and any subsequent recaptures. These data were intended for comparison with previous studies indicating that calves are separated from their mothers during fishing operations (Archer et al., 2001).

## RESULTS & DISCUSSION

### Sampling Operations

The two-month CHESS cruise was divided into two legs (Aug. 4-Sep. 6 and Sep. 10-Oct. 4, 2001) with an in-port in Acapulco, Mexico, Sep. 6-10, 2001 for the *McArthur*. Between the two legs, the tuna seiner continued to make sets on dolphins to collect biopsy samples and to roto-tag additional dolphins. A total of 28 sets, including one that was aborted before completion because of strong currents, were made during the project. Eighteen complete sets were made when both vessels were present and nine when the seiner was working alone during the *McArthur*'s port call (for more information see Table 1 in Chivers and Scott, 2002). Based on the records collected by the seiner's on-board IATTC observer, the average number of dolphins chased was about 700 (range 3-2500), and the average number of dolphins captured was about 59 (range 0-298). Most of the captured animals were spotted dolphins belonging to the northeastern offshore stock, but on several occasions mixed-species schools were pursued. On three occasions, a small proportion of eastern spinner dolphins was captured with the group of spotted dolphins, and in one set a few bottlenose dolphins were also encircled.

The number of dolphins handled and fully sampled during each set ranged from one to nine, and 0-27 additional animals were biopsied and roto-tagged alongside the third sampling station. Dolphins were generally restrained in the water by the swimmers for less than one minute prior to being placed into the raft, and total sampling time for dolphins handled within the primary rafts averaged 9 minutes (range 3-18). Total handling times, including all sampling and tagging operations, were shorter for animals that received roto tags, bullet tags, or had tags removed or replaced, than for dolphins that were radio-tagged or satellite-tagged for the first time (Table 1).

A total of ten dolphins, nine spotted and one spinner dolphin, were outfitted with radio-tag saddle packages. Of these, nine spotted dolphins were tracked as focal animals, and seven were successfully recaptured between one and three times over the course of 1-8 days following initial capture. The total number of each type of tag deployed was: 4 time-depth-recorders (TDRs), 4 time-depth-velocity-recorders (TDVRs), 2 thermal tags, 8 bullet tags, 213 roto tags, and 6 satellite tags. No animals with identifiable natural markings were observed recaptured during the study (Chivers and Scott, 2002). Two animals, one spinner dolphin and one spotted dolphin, died accidentally during the course of set operations and were necropsied in the field according to the protocol described in Cowan and Curry (2002). Results from these dolphins are included with results from other dolphins killed incidentally in the tuna fishery (Cowan and Curry, 2002, Romano et al., 2002b).

Blood was collected from 61 different dolphins, 53 of which were presumed to have been captured for the first time during the course of this study (St. Aubin, 2002). Few marked dolphins were recaptured during subsequent sets, which limited the opportunities for re-sampling dolphins with known chase-recapture histories (St. Aubin 2002). In fact, there were only six occasions when 1-4 non-focal dolphins, initially captured with a focal dolphin 1-3 days previously, were recaptured with the focal animal. One of these was a former focal dolphin whose radio-tag had been removed; two had been previously sampled and tagged in the sampling rafts; and the remaining eight dolphins were only roto-tagged and biopsied raft-side during the previous capture. In addition, a calf associated with one focal female dolphin was recaptured 3 times and two biopsies were obtained.

Skin samples for the stress-responsive protein studies (Southern et al., 2002; Dizon et al., 2002) and for future genetic studies were obtained on 283 occasions, including 14 from individuals known to have been previously captured. Thermal photography provided 623 images of dolphins swimming in the net, 343 of which were of sufficient quality to be analyzed. Body core temperatures were obtained from on 55 occasions from 48 different individuals, and 95 hours of heat flux data were recorded for the two thermal-tagged dolphins (Pabst et al., 2002). Two of the four TDR tags were recovered, yielding tracks of two and six days, respectively, and two of the four deployed TDVR were recovered, each providing a one-day track (Chivers and Scott, 2002).

**Behavior and Ecology**

The dolphin tagging during this study revealed highly fluid school dynamics within the study area (Chivers and Scott, 2002). Cohesion of marked animals following release was very low, apparently due to separation of large schools into small subgroups while foraging at night, and opportunistic regrouping into large schools the following morning (Chivers and Scott, 2002). These patterns of low group cohesion are consistent with previous observations for pelagic dolphins (Perrin et al., 1979; Scott and Cattanach, 1998), and stand in contrast to the strong associations documented for coastal cetacean populations, such as killer whales (*Orcinus orca*) and coastal bottlenose dolphins (Bigg et al. 1990, Wells and Scott 1999). The observed pattern of low group cohesion suggests that separation of schools during chase and capture may not cause splitting of large social groups containing tens or hundreds of individuals within the school; however, a few small groups of 3-5 individuals were captured together during consecutive sets in this study, suggesting that there are small social groups within the larger dolphin schools. The extent to which these smaller groups may be disrupted during fishing operations is not known, although the observation of animals circling outside the purse-seine net during nearly 87% of the sets in this study suggests that some splitting of social groups may occur (Santurtún and Galindo, 2002). Dolphins were also found to be more active when captured in smaller groups, but the causes and implications of this observation are not clear (Santurtún and Galindo, 2002).

Movement patterns observed during CHESS were consistent with those observed during previous tracking studies of spotted dolphins in the ETP (Chivers and Scott, 2002). Furthermore, oceanographic data collected during this study showed that the dolphins were exploiting an area characterized by a thermocline ridge, which is generally associated with high productivity (Chivers and Scott, 2002). Densities of dolphins were not measured directly, but the ease of locating new dolphin schools during our study confirms that we were working in an area where dolphins were abundant. Although the limited scale of movement of the tracked dolphins would suggest a reasonably likelihood of recapturing one of the 237 tagged dolphins by chance, no dolphins other than those immediately associated with the focal animal were ever incidentally recaptured during the 28 sets made. This further suggests that there were large numbers of dolphins in the operating area.

Perkins and Edwards (1999) estimated the frequency with which dolphin schools are encircled during fishing operations in the ETP separately for small (<250 dolphins), medium (250-500), and large schools (>1000). Larger schools had a higher rate of capture than smaller schools, and capture rates for individual dolphins depended on the frequency with which the individuals occurred in groups of varying size. If individuals have a tendency to occur predominantly in larger schools, this would increase their frequency of capture, and vice versa. It has also been documented that spotted dolphin schools increase in size throughout the day and then fragment at night to feed (Scott and Cattanach 1998). The low dolphin group cohesion observed during CHESS suggests that dolphins frequently move from one school to another and that the size of the schools in which they occur may change on a daily basis. The school sizes observed during CHESS, however, do not provide information on the frequency with which individuals occurred in groups of varying size, because the set operations disrupted schooling patterns by capturing only subsets of the entire school. Presumably the frequency of capture will increase primarily as a function of local dolphin densities, because a greater local abundance of dolphins will increase the daily opportunities to form large schools for each individual dolphin. Furthermore, areas with higher dolphin densities may also be more suitable for tuna and therefore may exhibit higher levels of fishing activities. During our study, we made 28 sets during 40 days within a 120 nautical mile radius of the center of operations. A total of about 1500 dolphins were captured and 283 dolphins were marked, but the only marked individuals that were recaptured were those few that remained associated with tracked focal dolphins between successive sets. Thus the likelihood of recapturing animals by chance was very low for the level of 'fishing' effort in our study. Further modeling of school dynamics and fishing behavior may allow assessment of whether the documented school dynamics would act to increase or decrease the frequency of capture for individual dolphins.

**Reproduction**

The original study design for CHESS included targeted sampling of females to document potential disruption of reproduction, either through separation of dependent calves or by causing abortions in pregnant females that were repeatedly chased. The ultrasound unit was determined not to be reliable for assessing pregnancy in spotted dolphins in the field, and the small number of recaptured dolphins limited the amount and types of reproductive data that could be obtained. Blood samples were obtained for 25 of the 31 females handled, and 19 of these 25 were considered sexually mature based on blood hormones, body length, and coloration pattern. Two of the 19 presumably mature females were identified as being pregnant based on blood hormones (St. Aubin, 2002), and neither of these individuals was recaptured. Nine captured females were observed to be with a calf; three of these were recaptured during subsequent sets and in all cases the calf was still present. This included one female and calf who were chased seven times and captured together four times over the course of seven days, and two pairs captured twice over the course of two and three days, respectively. Thus no separation events were documented; however, sample sizes were small and the spotted dolphin calves identified during CHESS were larger calves, not the young calves that are most likely to be separated during chase based on energetics models (Edwards 2002). A temporary separation of some associated individuals was suggested by the observation of dolphins circling outside the purse-seine net during nearly 87% of CHESS sets (Santurtún and Galindo, 2002), but it is unknown whether these individuals may have been separated mother/offspring pairs.

From the biological data collected during CHESS, reproductive parameters were estimated for all 31 female northeaster offshore spotted dolphins handled; 22 of which were considered sexually mature based on length and coloration pattern. Similarly, the data collected during the IDCPA Necropsy Program were used to estimate reproductive parameters for female northeastern offshore spotted and eastern spinner dolphins sampled (see Cowan and Curry, 2002; Romano et al, 2002b). In both studies, the sampled spotted dolphins belonged to the northeastern offshore stock. The proportions of pregnant and lactating females handled during CHESS appeared to be lower than expected, based on biological samples collected from individual dolphins incidentally killed during tuna purse-seine fishing operations between 1974 and 1992. These data were used to estimate the average proportions of pregnant and lactating females for the northeastern offshore spotted dolphin and the eastern spinner dolphin. The estimates were 0.26 pregnant and 0.49 lactating for spotted dolphins (S. Chivers, unpublished data; see also Myrick et al., 1986; Chivers, 1992), and 0.32 pregnant and 0.50 lactating for eastern spinner dolphins (Chivers, 1992). The samples collected by the IDCPA Necropsy Program had equivalent proportions for both the northeastern offshore spotted dolphin and the eastern spinner dolphin. However, the proportion of pregnant and lactating females was lower in the sample collected during CHESS. In fact, only 2 pregnant and 2 lactating females were detected among the 22 sexually mature females tagged and sampled (Table 2).

Although the probabilities of observing only 2 pregnant and 2 lactating female dolphins in a sample of 22 females are low: 0.083 and virtually 0, respectively, the long-term data set shows spatial variation in these measures of reproduction. For example, in 1975, the year with the largest number of northeastern offshore spotted dolphins sampled (n=413 sexually mature females), considerable spatial variation in the proportion pregnant and the proportion lactating can be seen (Figure 3). In the 5$^{o}$ squares from which at least 15 sexually mature females were sampled, the proportion pregnant ranged from 0.117 to 0.625 [pr(pregnant in CHESS)=0.09], and the proportion lactating ranged from 0.25 to 0.75. Additional consideration of the low number of lactating females observed in CHESS may be warranted, because this number appears anomalously low [pr(lactating in CHESS)=0.09]. Without further data, however, it is unknown whether the low proportions of lactating and pregnant females are representative of the overall population, or whether they are an artifact of sampling biases. Potential sampling-related reasons for the low proportions are exclusion of lactating females as a result of selecting only small portions of a school for capture during CHESS, undetected lactation among the females sampled, natural spatial/temporal variation, or biases in the selection of animals within the set.

**Pathophysiological Studies**

There can be little dispute that any form of chase and confinement is stressful to a wild animal, no matter how accustomed it might be to such experiences. By definition, this type of disruption constitutes a stressor. At issue is the nature and degree of the physiological response, the time course to recovery, and the residual effects on the individual. Each of the pathophysiological investigations - blood analyses, thermal studies, immunological assessments, and immunohistochemical and histopathological examinations - addresses one or more of these questions. In many cases, the findings of one line of investigation provided important insights into the conclusions of some of the others. For the most part, however, we can only hint at the time to recovery and any residual or chronic effects due to the paucity of information that was gathered from individuals with a known capture history.

*Thermal Studies*--Hyperthermia can have serious consequences on organ function and reproduction (see Pabst et al., 2002). Dolphins chased by tuna seiners and their auxiliary boats in the warm waters of the ETP might conceivably overheat, and thus would be at risk from the negative effects of thermal stress. This could be an important mechanism through which fishery operations might impact these animals and their populations (Pabst et al., 2002). The thermal status of the dolphins was studied to determine whether dolphins overheat during their attempts to evade capture and while confined to the seine net. Within the three lines of investigation of thermal condition, multiple issues were addressed relative to the effects of chase time, duration of confinement, and sea surface temperatures, along with inherent effects of gender.

The findings showed that chase times longer than 75 minutes resulted in elevated fin and body surface temperatures, but that these differences for the most part did not translate to elevated core body temperatures. It is possible that the small size and thin blubber layer in these animals does not favor the build-up of heat during activity. In the absence of changes of body temperature, Pabst et al. (2002) hypothesized that increased heat production might be detectable as an increase in heat flux across the dorsal fin, a signal that the animals are actively disposing of a heat load to maintain safe core temperatures. In fact, heat flux measurements showed elevations in heat transfer during the chase in one of the two individuals studied. However, there was no statistical correlation with swimming speed, which did not support the hypothesis that faster swimming would result in increased heat production, skin surface temperature and heat loss. Thus, the dolphins appear to be able to manage the thermal burden represented by exertion in the relatively warm waters of the ETP. There was one exception, however - a female dolphin with an elevated core body temperature of 37.9° C (Pabst et al., 2002). The results of blood analyses performed on this individual revealed no abnormalities other than moderately low serum iron, suggesting exertion rather than underlying illness produced the elevation in temperature. Although the relative importance of factors (e.g., chase, encirclement, pursuit by swimmers, dolphin's behavior in the net) potentially contributing to the dolphin's exertion cannot be ascertained, the elevated temperature suggests that hyperthermia and its damaging consequences may be problematic for selected individuals.

*Blood Analyses*--That chase and encirclement constitutes an acute stress for spotted dolphins is supported by changes in hormones typically released following perception of a threat or injury or intense physical activity (St. Aubin, 2002). Hematological signs of neuro-endocrinological stimulation in the spotted dolphins include persistent elevations in norepinephrine, dopamine, ACTH and cortisol, hours after the onset of chase. Changes in these constituents are not unexpected, but their occurrence at such a late stage during the set sequence suggests that the confinement or activities related to the sampling operations were a continuous source of stress. Of these indicators, epinephrine is the most labile in circulation, and the elevated levels could simply be the result of the manipulations necessary to collect the blood sample rather than a reflection of continuous stimulation of the sympathetic nervous system. The scant evidence available for ACTH in cetaceans suggests that, notwithstanding possible species differences and analytical considerations, the pituitary stress response in these animals is especially strong and persistent during confinement in the seine net.

A commonly encountered outcome of chase and capture in wild terrestrial mammals is a condition termed "capture myopathy", which is clinically diagnosed by monitoring changes in circulating levels of enzymes released from damaged muscle (Spraker 1993). Elevations in serum enzymes noted in the dolphins indicate that they sustain some form of muscle damage during the chase (St. Aubin, 2002). Some individuals exhibited particularly large increases in one or two of the indicators considered (CK, AST, and LDH), but there is little basis for predicting whether the extent of the tissue damage increases after the animals are released. In general, it is difficult to directly correlate serum enzyme levels with impaired muscle function (Margaritis et al. 1999); however, the levels attained in dolphins sampled within four hours after being chased do not suggest massive necrosis at that time (Sayers et al. 1999). The release of enzymes from damaged muscle cells is well-documented following exercise in humans and other animals (Suzuki et al. 1999). More severe damage may result if the insult is repeated without allowing recovery (Fielding et al. 2000), although some lines of evidence show either no exacerbation of damage or a blunted response with repeated exertion, signaling protective adaptations in muscle (Suzuki et al. 1999, Chen and Hsieh 2001, Stupka et al. 2001). Serum enzyme concentrations in the small number of recaptured dolphins showed no trend indicating cumulative or persistent effects, even allowing only one day of recovery before being chased again. However, since capture myopathy is a condition that may only affect a vulnerable subset of the population and therefore would require a large sample size to detect, there remains the possibility that some individuals later became compromised as delayed effects developed.

Another indicator of metabolic stress that can occur during exertion is acid-base imbalance. Hypoxia contributes to a build-up of lactic acid, with subsequent changes in constituents such as bicarbonate, and ultimately exacerbates muscle damage. Considerable individual variation was noted in the blood constituents examined that reflect acid-base status in the dolphins, and one individual showed a particularly marked imbalance associated with unusual electrolyte status. However, on recapture the following day, these abnormal findings had resolved. As diving mammals periodically experience episodes of hypoxia, dolphins may have a better capacity to manage lactic acid elevations than do their terrestrial counterparts.

*Histopathological Studies*-- Dolphins that drown in fishing nets show a suite of acute lesions that can be attributed to a 'sympathetic storm', or the overwhelming activation of an alarm reaction (Cowan and Curry, 2002). The lesions include hyper-eosinophilic and wavy fibers, perinuclear vacuolation, and contraction band necrosis in cardiac and skeletal muscle bronchospasm in the lung, and acute necrosis of kidney tubular epithelium. The findings, particularly those in the myocardium, have previously been described in stranded, acutely stressed dolphins of other species (Turnbull and Cowan 1998). The presumed link to excessive release of catecholamines into circulation is supported by the single measurement of blood catecholamine levels taken post mortem from a dolphin that died accidentally in one of the CHESS sets. Concentrations in this animal were an order of magnitude higher than the maximum values observed in live-sampled dolphins. Cardiac arrest is likely the immediate cause of death in these individuals and is consistent with findings in essentially all of the animals sampled for histopathological studies (Cowan and Curry, 2002).

An important question is whether less dramatic increases in catecholamines, such as were documented in the blood analyses in this study (St. Aubin,2002) produce sublethal tissue damage that might eventually contribute to functional impairment, morbidity, and either delayed mortality or heightened sensitivity to subsequent stressful events. The lesions of greatest concern are generally found in heart, kidney and skeletal muscle, and attributable to vascular processes – ischemia, with or without reperfusion – that are mediated by catecholamines. A significant number (36%) of necropsied dolphins had microscopic scars in heart muscle, representing the likely outcome of previous injuries induced by catecholamine surges (Cowan and Curry, 2002). Vascular lesions, such as organizing microthrombi and plaques thought to result from previous endothelial injury, were also noted, as were acute changes in the few samples of skeletal muscle that were examined. These lesions may or may not contribute to functional deficits, and are not necessarily fishery-related. Nevertheless, they demonstrate the potential in spotted dolphins for the development of a form of capture myopathy that may have delayed effects on the individual and could, in some cases, lead to morbidity and mortality.

*Immunohistochemical Studies*-- The hormonal agents involved in the physiological cascade that is the stress response have systemic effects involving virtually all organs and tissues. This premise underlies the application of a novel technique for detecting exposure to stressful events in an animal's recent past (Southern et al., 2002). The approach is based on the dynamics of the rapid epidermal growth in cetacean skin. An epidermal cell's lifetime extends from production in the basal layers to the time when it is shed at the surface (ca. 2 months). Because the basal germinal cells are closely apposed to blood circulation, the initial influence of endocrine signals is likely profound but should diminishes as the cell is pushed to the surface thus producing a time-based record of such influences. The range of cellular responses to such cues is extensive, but a set of 40 stress-responsive proteins have been identified that can be detected immunohistochemically and whose expression seems graded with stress (Southern et al., 2002). The approach has two advantages for studying stress in free-ranging dolphins: skin samples may be obtained in a relatively stress-free manner, and the relative concentrations of proteins may serve as a record of stress events over a much longer time period than can be gained from, for example, analyses of blood, whose responses to the same hormonal events are likely more labile.

Although the dynamics of the accumulation and decay of the stress-responsive proteins detected immunohistochemically are poorly understood, particularly in cetaceans, and the technique presently has no predictive value in terms of the survival of individual animals, initial studies with samples from animals in poor health (and assumed stressed) and animals assumed healthy were very promising (Southern et al., 2002). Recent stressful events (acute) produced no discernable expression pattern differences from patterns observed in healthy animals, but samples from animals in poor health (chronically stressed) showed altered expression patterns.

As part of the IDCPA research program, Southern et al. (2002) analyzed skin samples from nearly 900 dolphins, examining immunohistochemical expression of any of 40 stress-reactive proteins and judging the specimens as displaying normal or altered staining patterns without prior knowledge of the specimen source. The samples were derived from three major groupings: dolphin jaw-sections from the SWFSC fishery mortality tissue archive, biopsies collected from bow-riding dolphins during SWFSC research cruises, and biopsies collected from encircled dolphins during CHESS. Samples from the first and third groupings were thus from "fishery-involved" animals, while samples from the second grouping were thought to have had little experience with purse-seining operations, as judged by their willingness to approach a vessel (Mesnick et al., 2002).

The potential influence of fishery interaction on the probability of detecting altered expression patterns was examined by determining the number of sets within specific spatial and temporal windows (up to 70 days and within 300 nmi) around the place and time where the tissues were collected (Dizon et al., 2002). In the fishery sample, there were significantly more sets preceding the collection of specimens showing an altered expression of stress-reactive proteins in 86% of the time/area windows considered, suggesting a strong correlation with fishing effort. However, in the bow-riding group, significant differences were noted in only 57% of the time/area windows considered, and in all cases the finding of altered skin staining was associated with fewer rather than more numerous sets. This apparent paradox may be a consequence of selective behavioral factors in the bow-riding group (Mesnick et al., 2002). Fishery effort was highly variable within the extensive area from which these samples were drawn. Where fishing activity was higher, only naive, and therefore "unstressed" animals approach the sampling vessel, while where fishing activity was very low, the proportion of normal and stressed animals sampled may be a representative cross-section of the population. This may account for the overall relationship suggesting that normal skin protein patterns in bow-riding dolphins are associated with higher fishing activity.

The frequency of occurrence of an altered expression in samples obtained during the CHESS cruise also showed an inconsistent pattern with respect to fishery effort. For some time and space windows, altered expression patterns were significantly associated with a larger number of sets. However, in the majority of comparisons showing a significant difference (29 of 39 time and space windows), normal expression patterns were observed where fishing activity was greater. Again, sampling biases may play a role in this counter-intuitive result, although the nature of potential biases is less clear than for the bow-riding samples.

Application of this technique for assessing exposure to stressful conditions in a dolphin's recent past is further complicated by the recognition of topographical differences in the expression of stress-activated proteins over the body surface. Skin from the dorsal fin obtained during the application of tags as part of the CHESS study showed a significantly greater percentage of altered patterns than expected from that population based on the historical samples (Southern et al., 2002). When the sample was stratified according to the source of the biopsy (dorsal fin vs. body surface), the occurrence of altered patterns in dorsal fins was disproportionately high. This effect may explain why serial samples from recaptured tagged individuals showed an unexpected and counterintuitive reversion from an altered pattern to a normal pattern in 4 of 11 cases. Two of these animals had been fitted with TDRs, and exhibited hematological and serum chemical changes indicative of moderate systemic inflammatory responses that were deemed significant enough to warrant veterinary treatment if diagnosed in a captive specimen (Townsend and Geertsema, pers. comm.). In all cases, skin samples for examination of stress-activated proteins were first obtained from the dorsal fin at the time site of tag attachment, whereas the follow-up biopsy was collected from the dorsal body wall. Consistency in the sampling site apparently needs to be maintained if this technique is to be used to infer systemic condition in dolphins.

Also still at issue are how quickly a signal might appear in skin and how intense or prolonged the stress needs to be for signal expression. The approach as currently applied evaluates 40 different substances simultaneously to allow rapid profiling; however, greater sensitivity, precision, and understanding of the expressed responses might be obtained through more discriminate application of the technique. Finally, the method does not imply any prognostic information or ability to distinguish whether the provoking stimuli are benign or malignant. Additional research is needed to fully establish the value and application of this methodology for studies seeking to identify the nature and consequences of chronic stress responses, and results pertaining to the question of stress in fishery-involved dolphins are inconclusive.

*Immunological Studies*-- Changes in blood chemical constituents clearly demonstrated the occurrence of a neuro-endocrinological stress response in the dolphins. Activation of the sympathetic nervous system (Madden et al. 1995) and the hypothalamic-pituitary-adrenal axis (Munck et al. 1984) has well-established effects on the immune system, generally leading to suppressed function. For this reason, a comprehensive study of immune function in spotted dolphins was undertaken (Romano et al., 2002a). Three lines of investigation were pursued: quantification of lymphocyte subtypes, assessment of lymphocyte proliferation responses, and evaluation of DNA damage in leucocytes. In the absence of published information on any of these parameters for spotted dolphins, much of the work consisted of establishing apparent baseline data for the population. For the immunophenotyping assays, this baseline had to be determined using reagents developed for other species, and somewhat different results might have emerged with the application of species-specific antibodies. Nevertheless, the tests performed satisfactorily, and yielded data that could be used to identify relative changes in these characteristics.

As for the evaluation of other blood constituents, conclusions regarding the effects of repeated chase and encirclement are limited because of the small number of recaptured dolphins. Important changes in individuals would likely be masked in any attempt to perform statistical comparisons between a few recapture samples and the larger population of nominal first-capture specimens. Still, shifts in lymphocyte subpopulations were noted that are consistent with stress-mediated changes in other species. Glucocorticoids typically reduce lymphocyte counts in circulation because of their toxicity to the B cell, or antibody-producing, subpopulation. This effect was noted in comparisons between the first-capture and recapture datasets, but only when the results were expressed as a proportion of the total lymphocyte cell population. The finding is important because comparisons made on the basis of total lymphocyte counts failed to reveal any trend. The specific effects of stress-activated hormones released during repeated stimulation of the HPA could therefore selectively impact this arm of the immune system.

Despite the apparent reduction in B lymphocytes, proliferation tests using lipopolysaccharide (LPS) revealed no significant changes in this measure of their function in recaptured dolphins. Likewise, the T cell response to the mitogen concanavalin A (ConA) was not affected. Individual variation is extremely high in these measures, and important changes in individuals would likely be missed in comparisons between datasets, particularly when one of the sets is very small, as for the recaptured sample in this study. Changes in these parameters are subtle and often transient in other species after stressful exertion, and logistic constraints on the time of sample collection in the present study may have further obscured any cumulative or residual effects by distorting nominal baseline values. No significant effect on DNA integrity was detected in the samples from recaptured dolphins.

Chronic effects to the immune system could not be explored through tests on circulating lymphocytes, but rather were considered in histological studies on lymphoid organs collected from 57 fishery mortalities (i.e., IDCPA Necropsy Program samples; Romano et al. 2002b). Routine and advanced histological techniques revealed essentially normal, active, functioning lymph nodes. There was no evidence of stress-related changes, such as involution of follicles. Special stains demonstrated patterns of innervation in lymph nodes similar to those observed in other species, including cetaceans, and reinforced the hypothesis that activation of neuro-endocrine pathways can directly influence the function of immune tissue in these animals.

The study plan for the CHESS cruise relied heavily on the ability to recapture dolphins on multiple occasions to monitor trends in blood constituents and other physiological measures and thereby establish whether repeated encirclement produced cumulative changes. The assumption was made that though dolphins appear to tolerate the stress of chase and encirclement, the effects might become chronic if there is a failure to recover between pursuits. This would be manifested by successive elevations or depression of blood constituents established as indicators of stress and organ dysfunction. It was also recognized that individual variation is a major limitation in the use of blood analyses, such that attempts to statistically compare values from a population of recaptured dolphins with those taken to represent baseline levels would likely miss important changes experienced by the recaptured individuals. This was borne out in the limited resampling data that were collected. Only two individuals fitted with the most innocuous tags were recaptured, and the time period between captures was different for each one. Thus, little of statistical significance can be concluded from the trends noted in these animals. Overall, none of the changes that were documented signaled distress or organ dysfunction, but such findings might only be expected in a small subset of the population and require a considerably larger sample size to be detected. Because of the small sample size obtained during CHESS and the rare nature of potential detrimental effects, population-level conclusions cannot be made based on the available data. Further studies that allow larger samples sizes to be obtained and include control groups will be required before population-level inferences can be drawn.

**Sampling biases and caveats**

The highly dynamic nature of dolphin schools, coupled with logistic constraints and environmental limitations, imposed a number of circumstances that could lead to biases in the samples obtained during this study. Sets were made in a relatively small region of the overall stock area for these dolphins (Figure 1), because this area had acceptable weather conditions and sufficiently high dolphin densities. Dolphins captured during CHESS had previously been exposed to moderate-to-high levels of fishing effort in this area, based on IATTC records of set locations (Dizon et al., 2002). If the recent fishery exposure of the captured dolphins was unusual, or if the physiological responses to chase and capture of spotted dolphins in other areas of the eastern tropical Pacific Ocean are different, then the samples obtained during CHESS would not be representative of the population as a whole. At this time, insufficient information is available to address this question specifically; however, most of the physiological measures evaluated are highly conserved across diverse mammalian taxonomic groups. Therefore the responses observed in the subset of dolphins sampled is likely to be representative of other spotted dolphins in the eastern tropical Pacific Ocean.

Too few spinner dolphins were sampled to allow meaningful comparison for this species. Spinner dolphins were not specifically targeted in this study because their more active nature increases the risk of mortality or injury during fishery operations, and their ability to dive deeper and avoid the net as it is being set makes them more difficult to capture repeatedly. In addition, it was recognized that attempting to augment the few opportunities there were to sample spinner dolphins might only further detract from the ability to obtain a robust data set for the spotted dolphins.

Several aspects of the capture operations during CHESS differed from standard fishing procedures, and these could have introduced biases, either increasing or decreasing potential stress experienced by the captured dolphins. Although large schools of up to 2,500 dolphins were chased, smaller subsets were intentionally captured for sampling to reduce the risk of mortality or injury to dolphins during the study, and to allow recapture of the portion of the school containing the tagged target animal(s). This resulted in considerably smaller schools (averaging 59 dolphins) being captured during CHESS than during normal fishing operations. The average school size captured by fishing vessels was 392 and 465 for sets observed by the IATTC in 1998 and 1999, respectively (IATTC, unpublished data), and 350 for the 1998-2000 Mexican National Observer Program (Santurtún and Galindo, 2002). It is not known whether this difference would substantially change the capture experience of the dolphin, for example, by reducing stress due to crowding in the net, or by increasing stress because of separation from the remainder of the school. Small schools were found to be more active than large schools during CHESS (Santurtún and Galindo, 2002), but the reasons for this are not clear.

The chase experience of dolphins during CHESS may have differed in some respects from the fishery standard. Normally, the seiner uses the helicopter to identify and follow the portion of a dolphin school which remains associated with the tuna. During CHESS, the helicopter circled over the school prior to the set to locate tagged animals, and then followed this animal or group of animals for the remainder of the set. The time spent overhead by the helicopter searching for the tagged animal may have increased the dolphin's exposure to helicopter harassment, although the remaining herding operations were qualitatively similar to those normally used to capture the portion of the school with the tuna. Conclusions drawn regarding thermal and blood effects of chase beginning at the time when the helicopter was over the dolphins must therefore be qualified to refer only to the durations of chases encountered during CHESS. Detailed data indicating when the helicopter causes dolphins to begin running prior to a set are not routinely collected by on-board fishery observers, so no data are available for a direct comparison of sets made during CHESS vs. standard fishing operations.

The chase time may also have been increased for some dolphins and prior to some sets by the tracking activities of the research vessel. Tracking of focal dolphins was conducted from the greatest distance that provided a reliable radio signal and minimized disturbance. Preliminary travel speeds calculated for the tracked dolphins in this study were similar, but slightly higher than those reported during a previous tracking study in 1992-93 (Chivers and Scott, 2002). It was also apparent from the track for one of the dolphins with a thermal tag that the dolphin increased its travel speed when the *McArthur* followed within about 1-2 miles. The number of captured and sampled dolphins that were potentially influenced by proximity of the research vessel was small (because of the dynamic school structure) and largely limited to the radio-tagged dolphins. The radio-tagged animals were excluded from the blood analyses presented here because of direct impacts of the tag, and therefore the analyzed sample was not expected to be biased by tagging and tracking activities.

A number of activities took place during the research sets that differed from normal fishing set procedures. After encirclement was complete, a small inflatable boat entered the net compass and slowly approached dolphins to obtain thermal photographs. The boat's engine was kept at slow idle and efforts were made to drift slowly past dolphins to minimize disturbance. The presence of the boat inside the net compass was unusual, although one or more speedboats are generally present outside of the net compass to herd dolphins away from sections of the net and to tow open portions of the net when needed. Underwater noise levels, as assessed by swimmers in the water (M. Scott, IATTC, pers. comm.) were not appreciably increased by the engine noise from the small research boat, but rather were dominated by noise from the purse-seine operations.

The deployment of swimmers and rafts shortly after pursing the net, to assess the dolphin school composition and behavior, also differed from standard fishing procedures, in which a smaller number of swimmers are generally deployed after tie-down (just before backdown). During CHESS, the activities of the swimmers increased after tie-down, when dolphins were captured and guided either to a sampling station, where they were lifted into a small raft, or to the raft-side biopsy/tagging station, where they were restrained in the water for 1-2 minutes. These activities would be expected to elicit a physiological response in the handled dolphins, and influence the behavior and physiological state of other dolphins in the net prior to their capture and sampling. However, there were no significant trends in hormonal stress indices relative to the time interval between tie-down and sample collection (St. Aubin, 2002), suggesting that whatever changes were manifested in the dolphins had already occurred as a result of preceding events and were not exacerbated by the continued presence of swimmers or the handling activities. The only exception might be in the levels of epinephrine, the most labile of the constituents examined, and the one most likely to be acutely influenced during the few minutes of physical restraint prior to sample collection. If late-stage handling procedures necessary for sample collection significantly augmented the stress response in the dolphins, it might only have been revealed some time after release, based on the presumed time course of changes in constituents such as ACTH, cortisol and aldosterone. The residual effects, if any, of this confounding variable could not be determined in the very small sample of recaptured individuals.

The delay in beginning the backdown procedure and presence of the sampling rafts in the backdown area may also have contributed to confusion in the captured dolphins, if they have learned to expect a certain sequence of events during backdown. The behavioral data (Santurtún and Galindo, 2002), and observations of dolphins swimming back towards the seiner after a prolonged sampling period in some of the sets, support the hypothesis that dolphins may have become habituated to the standard sequence of set operations and may have been confused when the procedures were altered during CHESS.

A final source of bias in the sampling during CHESS may have been introduced during the selection of animals for blood sampling and tagging, and by the handling operations themselves. Larger animals were preferentially selected for attachment of saddle packages with radio tags and TDRs, TVDRs or thermal instruments. A mix of smaller and larger animals was generally outfitted with roto-tags or bullet tags.

## CONCLUSIONS

In the aggregate, the pathophysiological studies assessed a broad suite of potential stress responses in spotted dolphins chased and encircled by a purse-seine vessel. Thermal studies determined that chase can increase heat production in spotted dolphins, but that in general the animals can dispose of the heat before experiencing dangerous elevations in core body temperature or manifesting elevated skin temperatures. One female dolphin was significantly hyperthermic, suggesting that selected individuals may be particularly susceptible to overheating. Blood samples revealed elevations in constituents indicative of muscular damage, and pituitary and adrenal stimulation. The degree of elevation of one indicator (ACTH), and the time frame of its occurrence relative to the onset of chase and other stages of fishery operations, suggest that the dolphins continue to exhibit a physiological stress response throughout the time that they are confined in the net, irrespective of biological sampling operations during our study. The changes noted in circulating levels of catecholamines and muscle enzymes appear to correlate with necropsy findings of microscopic damage in heart muscle indicative of earlier sublethal insults to this organ. These types of injuries may contribute to unobserved mortality in some cases. Animals dying in the nets experience cardiac arrest as a result of the massive release of catecholamines, and display characteristic lesions of acute necrosis and other abnormalities in heart, skeletal muscle and kidney. Investigation of immunological characteristics of spotted dolphins established the first baseline values for cell types and functional responses to mitogens in this species, with no unusual results when compared to other cetaceans.

Tissues were examined for evidence of chronic exposure to stress. A study of lymphoid organs gave no indication of involution or hypoactivity commonly associated with chronic stress. Archived skin samples from fishery-killed dolphins were stained with reagents to demonstrate stress-responsive proteins (SRP), and a correlation was identified between the frequency of high SRP levels and the level of fishing effort within various time and distance windows prior to sample collection. The implications are tenuous in the absence of a direct causal link, particularly since biopsy samples from bow-riding dolphins show the opposite pattern and appear to be drawn from a different subset of the population.

The ability to recognize the cumulative effects of repeated chase and capture depended heavily on resampling individuals to establish trends in indicators of stress, organ function, and immune status, because it was recognized that no control animals would be available during this study. Logistic obstacles, principally the lack of strong cohesion within larger groupings of dolphins, confounded attempts to relocate individuals other than those fitted with radio tags for tracking or other lines of investigation. The radio-tagged individuals were excluded from the data set for interpretation of blood indicators, because the attachment of these instruments produced changes in circulating constituents that were unrelated to fishing operations. In the few recaptured animals bearing only small identifying roto-tags, there were statistically and biologically significant decreases in iron and thyroid hormones, consistent with an unresolved stress response. Significant differences were noted on recapture in the muscle specific enzyme CK, however the levels fell rather than increased as might have been expected if the animals were experiencing the delayed onset of capture myopathy. Changes were also noted in the occurrence of stress-responsive proteins in skin, but in a number of cases an abnormal pattern on first capture became normal. Potential sampling biases and a lack of understanding of the short-term development of this signal confound the interpretation of the SRP results. Further biases in the overall CHESS results may have been introduced by a number of required procedural differences in our set operations compared with standard fishing operations. Although some of these biases appear not to have been influential, data are lacking to fully evaluate others.

In conclusion, dolphins are significantly stressed during chase and encirclement, and in some cases this results in sublethal tissue damage, but there is insufficient evidence to establish whether or not repeated chase and encirclement leads to a state of distress, organ dysfunction, immunosuppression, reproductive failure, or insidious mortality. Population-level conclusions about potential chronic stress are, therefore, not possible based on the results of these studies. Future research on chase-recapture stress should focus on the lines of investigation where potential effects were suggested, but not identified conclusively. Furthermore, follow-up studies should include control animals and seek to establish true baseline data for spotted dolphins or closely related species. Future field research must also take into account the great difficulty of recapturing groups of wild spotted dolphins and should employ techniques allowing larger sample sizes to be obtained.

## ACKNOWLEDGMENTS

A study of this complexity could not have been accomplished without the dedication and support of many people. Foremost, we wish to thank the owner/manager, captain, and crew of the tuna vessel for their outstanding efforts and high level of skill, without which we would not have been able to obtain the required samples for these studies. The crew of the NOAA ship *McArthur* provided excellent support, and we greatly appreciated their flexibility to accommodate our ever-changing needs. We are grateful to the Inter-American Tropical Tuna Commission for arranging the tuna vessel charter and providing valuable logistical support and personnel. Special thanks to Michael Scott, David Bratten and Ernesto Bayliss for their support and assistance during this study. We would like to thank the field biologists who participated in this study and contributed greatly to its success: Eric Archer, David Bratten, Kerri Danil, Roger Geertsema, Jim Gilpatrick, John Hyde, Greg O'Corry Crowe, Bill McLellan, Erin Meagher, Jeremy Rusin, Michael Scott, Eduardo Santurtún, Forrest Townsend, Ernesto Vasquez, Andrew Westgate, and Suzanne Yin. We also thank Ann Pabst for her insights and excellent leadership of Team Thermal. Steve Reilly, Andy Dizon, and Frances Gulland provided valuable scientific guidance during the planning of these studies. Laboratory archiving and analyses of biological samples at the Southwest Fisheries Science Center were carried out efficiently at the end of the cruise by Anne Allen, Eric Archer, Nick Kellar, Carrie LeDuc, Ben Maurer, and Kelly Robertson. Nicole Le Boeuf provided valuable assistance and support during all phases of this project. Critical administrative and operational support was received from Barb Decker, Meghan Donahue, Karen Handschuh, and Bobbi Watkins. Research was carried out under Marine Mammal Protection Act Permit No. 774-1634-00; we are grateful to Ruth Johnson and Tammy Adams for their helpful assistance in obtaining this permit. We thank the Center for Independent Experts review panel for their thoughtful reviews of this manuscript and other CHESS reports.

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**Table 1.** Summary of total handling time (in minutes) for dolphins sampled during the Chase Encirclement Stress Studies (CHESS) cruise, by tag type/procedure.

| Dolphin Processing Time (Min) | No Tag Processing | Bullet Tag | Roto Tag | Remove Tag | Swap TDR | Radio Tag | Satellite Tag | Grand Total |
|---|---|---|---|---|---|---|---|---|
| No. of dolphins | 10 | 9 | 33 | 5 | 2 | 10 | 6 | 75 |
| Minimum time | 0:03 | 0:05 | 0:04 | 0:07 | 0:09 | 0:11 | 0:11 | 0:03 |
| Maximum time | 0:11 | 0:12 | 0:14 | 0:13 | 0:10 | 0:17 | 0:18 | 0:18 |
| Average time | 0:06 | 0:07 | 0:08 | 0:10 | 0:09 | 0:13 | 0:14 | 0:09 |

**Table 2**. A summary of the life history data collected by two studies conducted as part of the International Dolphin Conservation Act of 1997 Stress Studies: (1) the Chase Encirclement Stress Study (CHESS) cruise conducted in the fall of 2001, and (2) the Necropsy Program (Cowan and Curry, 2002). "N" is the total sample of males and females collected.

| | | | | | Of Mature Females | |
|---|---|---|---|---|---|---|
| **Sample Source** | **Species** | **N** | **# Females** | **P(Mature)** | **P(Pregnant)** | **P(Lactating)** |
| CHESS 2001 | *Stenella attenuata* | 67 | 31 | 0.71 | 0.09 | 0.09 |
| Necropsy Program | *Stenella attenuata* | 29 | 16 | 0.56 | 0.33 | 0.56 |
| Necropsy Program | *Stenella longirostris* | 26 | 16 | 0.44 | 0.43 | 0.57 |

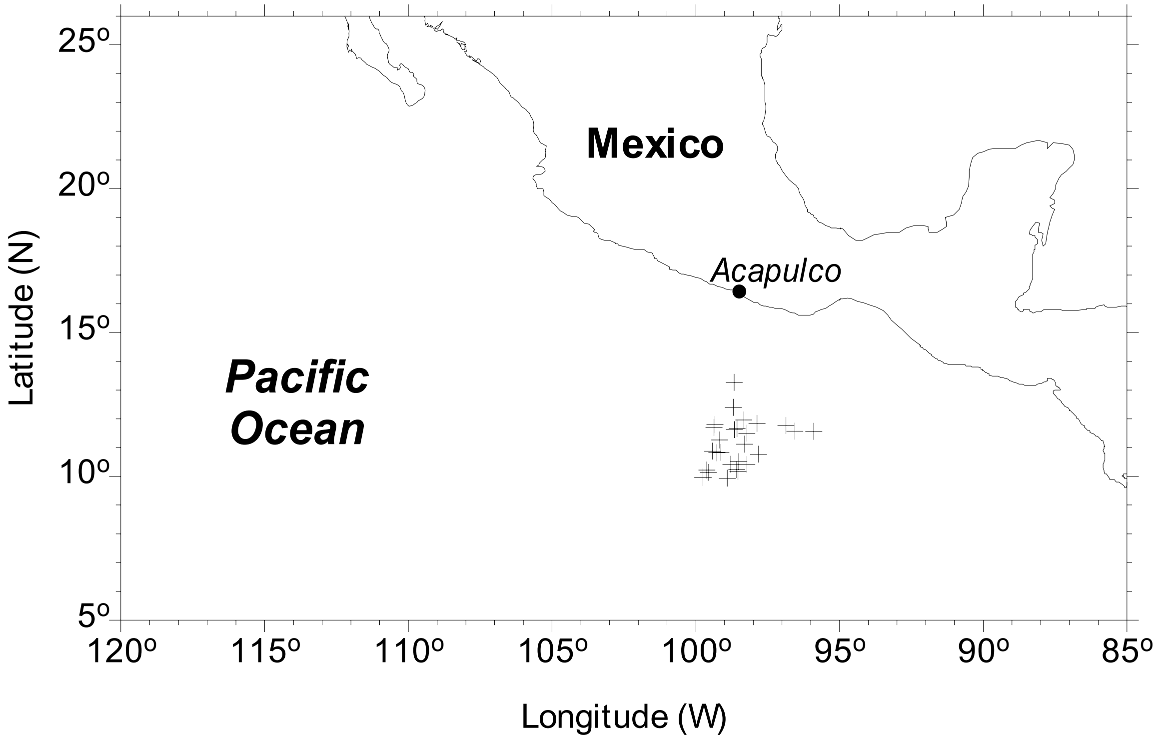


**Figure 1.** Locations of sets (+) on spotted and spinner dolphins during the Chase Encirclement Stress Studies, 2001.


**Figure 2.** Photo illustrating configuration of rafts and personnel for dolphin sampling during the purse seine sets. Raft containing the dolphin (left) is partially flooded.

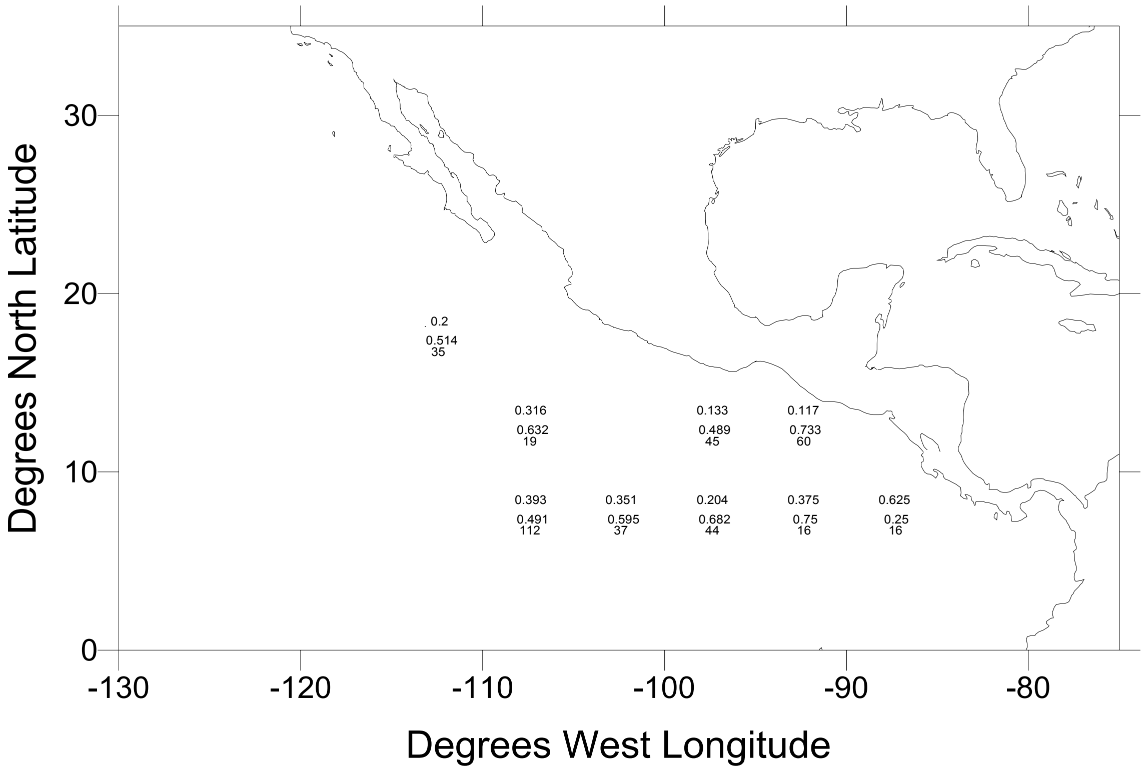


**Figure 3.** For 1975 sample of northeastern offshore spotted dolphins, *Stenella attenuata*, the number of sexually mature females collected (bottom of the cell), the proportion that were lactating (middle) and the proportion that were pregnant (top) are summarized by 5$^{o}$ square. Only the 5$^{o}$ squares with >15 sexually mature females sampled are shown.