FURTHER ASSESSMENT OF THE POTENTIAL
FOR FISHERY-INDUCED STRESS ON DOLPHINS
IN THE EASTERN TROPICAL PACIFIC

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BACKGROUND

Uncertainties regarding the effects on dolphins of intentional encirclement during the conduct of tuna-fishing operations in the Eastern Tropical Pacific have been responsible for a series of investigations into the life history, population dynamics, behavior, and pathophysiology of key species, notably the pantropical spotted dolphin, *Stenella attenuata*, and the eastern spinner dolphin, *S. longirostris orientalis*. A number of these investigations were mandated as part of the International Dolphin Conservation Program Act passed by Congress in 1997. Included in the mandate was a review of stress-related research on dolphins and other mammals with the intention of identifying mechanisms through which the physiological and psychological stresses imposed by chase and confinement might adversely affect dolphins. In a comprehensive review, Curry (1999) concluded that “it is plausible that stress resulting from chase and capture in the ETP yellowfin tuna purse seine fishery could have a population level effect on one or more dolphin stocks.”

Curry (1999) described the sequence of events leading up to the encirclement and capture of dolphins targeted in ETP yellowfin tuna purse seining operations. The procedure includes low overflight and pursuit by helicopters, approach and herding by speedboats, encirclement within a net deployed by the seiner, and progressive restriction of space as the net is hauled in prior to backdown. There is no question that such activities constitute a stressor for any animal, and the potential effects of activation of the stress response have been thoroughly documented. Curry (1999) explored multiple pathways of effects, including the acute stimulation of sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis, the short and long-term consequences of their activation on metabolism, immune function and reproduction, and the physiological and pathological outcomes of exertion (e.g., hyperthermia and capture myopathy). In addition, social and psychological factors such as isolation, aggression, and novelty were considered for their direct effects on dolphin stocks and their contribution to the stress response. Direct evidence of these combined effects on the health and productivity of individual cetaceans is sparse, much less on cetacean populations, and thus the conclusion that an effect is plausible rests on extrapolation from findings in other species. Even so, there are few, if any, examples from the wild in which stress alone has been shown to be responsible for the decline of a mammalian population. The extension of the findings of laboratory investigations into specific mechanisms of stress-mediated effects on reproductive and immune systems, for example, to predict outcomes at the population level in the wild is therefore very tenuous.

The purpose of the present report is to expand the review by Curry (1999) with respect to some more recent lines of research and identify any pertinent information that was omitted from the earlier report. Against the backdrop of decades of literature presented in the earlier review, there has been no fundamental discovery during the past three years to substantially alter our understanding of how stress affects homeostasis in mammals, and specifically dolphins (St. Aubin and Dierauf 2001). Nevertheless, some relevant studies reinforce conclusions in the earlier review, while others have explored additional indicators that might bear further examination in future studies.
CHASE AND CAPTURE IN ODONTOCETES

Subsequent to Curry’s review, two studies were published that add to the literature available on the nature of the stress response in odontocetes; two other reports discussed here were not considered in the original analysis. The studies reinforce the conclusion that chase, confinement, and restraint can elicit changes in circulating hormones indicative of a mammalian stress response, albeit one that takes on characteristics that appear to be specific to cetaceans.

Ortiz and Worthy (2000) examined levels of cortisol, aldosterone, and vasopressin (AVP) in bottlenose dolphins, *Tursiops truncatus*, chased and encircled as part of an investigation into health status of the Atlantic coastal stock. Circulating steroid values were higher than those measured in unrestrained captive dolphins voluntarily allowing venipuncture (St. Aubin et al. 1996). Ortiz and Worthy examined the data for temporal effects relative to the start of the capture and also to the actual restraint, but found none and concluded that the dolphins were not mounting a significant adrenocortical response.

The findings highlight the difficulty in using cortisol as an indicator of acute stress in dolphins, particularly within the time frame represented by the study in question. Resting levels are typically quite low compared with other marine mammals (St. Aubin 2001), and changes following controlled stimulation are often modest (St. Aubin and Dierauf 2001). The absence of a significant difference in cortisol levels between dolphins sampled 20-25 minutes after capture and those sampled 20 minutes later likely reflects individual variation in the time course of expression of cortical stimulation, blurring the distinction between the two subsets of data. The authors noted that some of the dolphins had cortisol values above 4 mg/dL, which would be considered elevated for a bottlenose dolphin and therefore indicative of a stress response.

In the same study, the investigators determined concentrations of AVP because of its known influence on adrenocorticotropic hormone (ACTH), and therefore on cortisol secretion. No correlation was found between levels of AVP and cortisol, suggesting a dissociation of this mechanism in dolphins. A directed study (e.g., AVP infusion) has not been undertaken in any cetacean to confirm the presence or absence of a relationship between the two. However, the lack of a clear stress signal from the posterior pituitary represents another obstacle to identifying an unequivocal physiological measure of the activation of higher centers during the stress response in these animals.

One constituent not generally considered as part of the endocrine stress response in mammals is aldosterone. Studies on a variety of marine mammal species, including odontocetes, have shown that aldosterone may play a particularly significant role, as judged by its apparent sensitivity to ACTH stimulation (St. Aubin 2001, St. Aubin and Dierauf 2001). Within the first 45 minutes following capture in bottlenose dolphins, Ortiz and Worthy (2000) observed no temporal pattern suggesting a mounting response of this indicator. Nevertheless, levels during their sampling interval (238 ± 134 pg/mL) were already elevated above those reported for resting captive dolphins (28 ± 38 pg/mL) (St. Aubin et al. 1996), reinforcing the suggestion that aldosterone secretion is enhanced as part of the stress response in these animals.

Ortiz and Worthy concluded that bottlenose dolphins chased, captured in a seine net, and restrained for blood collection within approximately 45 minutes showed little evidence
of a neuroendocrine stress response. This was further supported by the lack of correlation between cortisol and glucose levels, in consideration of the expected hyperglycemia that would result from elevations in glucocorticoids. While the indices examined did not show appreciable changes from recognized baseline values, it would be misleading to suggest that the activities preceding the collection of samples were not stressful to the dolphins. It is likely that the full expression of the stress response occurred some time later, particularly for constituents such as glucose for which such changes might be out of phase with those for cortisol. In other odontocetes, cortisol-mediated changes in other constituents such as thyroid hormones and eosinophils appear hours after stressful events, even when changes in cortisol are relatively small (Thomson and Geraci 1986, St. Aubin and Geraci 1988, 1989, 1992).

St. Aubin et al. (2001) reported on circulating levels of cellular, chemical and biochemical constituents in 183 beluga whales, Delphinapterus leucas, most of which were subjected to variable duration of chase, manual capture and restraint. Most of the samples were collected following relatively short chases (< 15 minutes), but intervals up to 45 minutes, including post-capture handling time, were also encountered. Concentrations of cortisol (3.2 ± 1.6 mg/dL, range 0.5-7.3 mg/dL) clearly included values elevated above those determined in resting, captive beluga whales (1.8 ± 0.75 mg/dL, range 0.66-4.17 mg/dL) (Schmitt and St. Aubin, unpublished). Levels of aldosterone showed a particularly wide range (118 ± 220 pg/mL, range 0-1200 pg/mL), also with concentrations above those for captive belugas (11 ± 4 pg/mL, range 10-33 pg/mL). No attempt was made to further examine the data in relation to the chronology following the onset of the chase. Nevertheless, the findings confirm earlier reports that chase and capture elicit an adrenocortical response in beluga whales (St. Aubin and Geraci 1989). The extreme expression of this response (distress) was documented by Orlov et al. (1991) in belugas captured and immobilized out of water for 44 hours, after which circulating cortisol levels were in the order of 20 mg/dL. They also reported that ACTH stimulation produced gradual elevations in cortisol that peaked 3-6 hours after administration (Orlov et al. 1991).

Koopman et al. (1995) obtained blood samples from 27 harbor porpoises, Phocoena phocoena, trapped in fishing weirs for up to three days, and then captured using seine nets in a process that required 30 minutes to 2 hours. These circumstances mimic certain elements of the conditions experienced by dolphins in the ETP, but also include several important differences that are useful in examining the specific factors that contribute to an observed pattern of diagnostic indicators. The duration of their confinement is considerably longer and, unlike dolphins in the ETP, harbor porpoises are not chased, but rather swim voluntarily and unknowingly into traps set for fish. Ultimately, though, they are captured following a chase of similar duration. While in the weirs, the animals apparently are able to feed, and exhibit behaviors similar to those when unconfined, except that respiration rates are somewhat elevated. Findings in blood samples might therefore be expected primarily to reflect the more recent stress of net capture, since their initial HPA and the SNS responses to confinement would likely have subsided after one to three days.

Blood samples from the porpoises showed levels of cortisol (8.8 ± 3.3 mg/dL, range 4.2-19.9) that are the highest reported “baseline” values for any cetacean (St. Aubin 2001). Harbor porpoises are not commonly held in captivity, and published data on serum cortisol in acclimated animals are not available. Comparable values may only be seen in stranded
cetaceans such as pilot whales experiencing agonal circulatory collapse (Geraci and St. Aubin 1987). The investigators were unable to distinguish which factor(s) (e.g., time out of water, degree of manipulation, tag attachment) contributed to the observed circulating concentrations, but noted that the highest values were found in animals fitted with electronic tags. No details were provided regarding the sequence of procedures or other time factors to help elucidate the expression of the stress response in these animals. Notwithstanding the possibility that much of the observed response could have been due to procedures other than chase and capture within the weir, or that harbor porpoises exhibit unusually high resting cortisol values for a cetacean, the data demonstrate that cortisol can be significantly elevated as part of the stress response in some species of odontocetes. The concurrent elevations in glucose (up to 250 mg/dL) appear to corroborate the effect that elevated glucocorticoids were having on the metabolic state of the animals.

**FACTORS AFFECTING THE ACUTE STRESS RESPONSE**

The potential for marked individual variation in the response elicited by a given stressor has long been recognized. Curry (1999) acknowledged this factor, and it bears further consideration here since it has important ramifications in efforts to understand the effects of fishery-associated stress on dolphin populations. Such effects may only be experienced by a subset of the population, complicating detection in any experimental protocol while still having a significant adverse effect on the population’s ability to increase at the maximum predicted rate.

In humans, a bimodal HPA response to high-intensity exercise stress has been described (Petrides et al. 1997, Negrao et al. 2000). The two groups are identified as high responders (HR) and low responders (LR), and are distinguished by differences in the degree of change in circulating levels of ACTH, cortisol and AVP following exercise (Petrides et al. 1997). Singh et al. (1999) noted that HR and LR groups are consistent across different types of stimulation – both physical (exercise) and psychological (test scores on anxiety traits). The authors postulated that the adrenal cortices of HRs are hypertrophic and/or hypersensitive to ACTH (Singh et al. 1999). HRs also show greater anticipatory stress response, as judged by higher heart rate and arterial blood pressure. However, HRs and LRs cannot be distinguished on the basis of catecholamine responses either to psychological of exercise stress.

**EFFECTS OF GENDER**

Gonadal steroids modulate functioning of the HPA, and are also sensitive to its activity. Consideration should therefore be given to the potential for gender-specific responses, and variable responses as a function of reproductive hormones status, particularly in females. Evidence for such relationships is variable. Deuster et al. (1998) observed that women show significantly greater exercise-induced changes in glucose, AVP and cortisol, but not lactate or ACTH. The experimental conditions included pre-treatment with
dexamethasone to suppress adrenal function, making it difficult to extrapolate the findings to unmedicated subjects. Subsequent investigations by the same investigators did not reinforce these gender differences (Deuster et al. 2000). Although basal levels of cortisol and growth hormone (GH) are higher in women, exercise uniformly affects levels of these constituents in both men and women (Sandoval and Matt 2002). Differences in exercise-induced changes in glucose and lactate were noted, however.

During the mid-luteal phase of the menstrual cycle, when levels of progesterone and estrogen are relatively high, the release of ACTH and AVP, but not cortisol, is enhanced in exercising women (Altemus et al., 2001). Galliven et al. (1997) and Horton et al. (2002) found no significant effects of menstrual cycle phase on different aspects of metabolism during exercise. No comparable data exist for cetaceans, but the possibility remains that female dolphins during critical stages of their reproductive cycle could experience larger hormonal responses to the stress of exercise than in males, or than females at other stages.

EFFECTS OF EXERTION

Exercise by itself stimulates the HPA and SNS, and causes release of muscle enzymes indicating damage to muscle cells. Circulating enzyme levels are reliable indicators of muscle damage (Suzuki et al. 1999, Fielding et al. 2000, Zajac et al. 2001) and the degree of change is broadly proportional to the extent of damage (Sayers et al. 1999). However, Margaritis et al. (1999) found no correlation among enzyme levels, muscle soreness, and muscle function in humans monitored for four days after participation in a triathlon.

Most organisms can endure periodic insults to muscle sustained during even strenuous exercise without compromising health or survival. However, exercise becomes distressful as individual approaches exhaustion, or if the exercise is coupled with fear associated with conditions that it is unable to control. At such times, the excessive and sustained release of catecholamines enhances muscle damage, resulting in a syndrome termed capture myopathy (CM). Several forms of CM are described, some of which are acutely fatal, while others are manifested after days or weeks (Spraker 1993). The threshold between benign, reparable muscular damage and potentially fatal CM is not distinct, and can differ among individuals and species. Ungulates, for which there is the greatest body of literature relating to CM, appear to be particularly vulnerable to its effects, although it has been shown that virtually all vertebrate taxa are potentially susceptible (Williams and Thorne 1996).

Few specific cases of CM have been documented in odontocetes. Colgrove (1978) reported transportation-associated myopathy in dolphin, but the pathogenesis of this condition differs fundamentally from a more restrictive definition of CM as a sequela to exertion coupled with overwhelming fear. During earlier, unsophisticated transports, dolphins suspended for hours in stretchers unsupported in water often experienced poor perfusion of somatic tissues, and hypoxic necrosis of muscle was a likely outcome (Antrim and McBain 2001). These conditions are much like those observed in stranded cetaceans that may have rested onshore for some period of time prior to rescue or euthanasia (Geraci
and St. Aubin 1987). In such cases, trauma (crush injury), hypoxia and vascular collapse (shock) can result in massive necrosis of tissue with subsequent elevations of circulating levels of enzymes. Such individuals observed for several days during attempted rehabilitation typically have difficulty swimming, and sometimes develop spinal curvature as a consequence of unilateral devitalization of epaxial musculature (pers. observation). The development of myopathy in a transported dolphin or a stranded whale is not a good example of CM per se.

Recently published data add to the literature documenting muscle enzyme leakage following exertion in odontocetes (Koopman et al. 1995, St. Aubin et al. 2001). Elevated levels of enzymes (CK, LDH and AST) indicative of muscle damage were noted in harbor porpoises, relative to values published for other cetaceans (Koopman et al. 1995). In the case of AST, some of the increment might also reflect liver damage, since infection of the biliary tree with the trematode Campula oblonga is a relatively common occurrence in this species. However, reported ranges for ALT and GGT, other indicators of damage to liver and bile ducts, were not uniformly high, though it is conceivable that individual animals had liver enzyme profiles that were indicative of hepatic involvement. Thus, the observed enzyme profile for the group suggests that the porpoises sustained some degree of muscle necrosis during the chase and capture efforts. In beluga whales, some of the chases animals displayed modestly elevated levels of CK, AST and LDH, but the full expression of exertional effects on muscle may not have been evident in samples collected for the most part less than 30 minutes after chase (St. Aubin et al. 2001). Whales monitored for days or weeks after such captures showed no sustained enzyme elevations suggestive of a more extensive or enduring necrosis of muscle tissue (St. Aubin and Geraci 1989). All of the sampled harbor porpoises were radio tracked for up to 17 days, suggesting that the degree of capture myopathy experienced by the porpoises was apparently benign (Koopman et al. 1995).

Hypoxic conditions in active muscle contribute to the development of CM through the production of lactic acid (metabolic acidosis). Exhaustive exercise in humans can increase lactic acid concentrations in muscle for up to 24 hours (Zajac et al. 2001). Marine mammals show some apparent resistance to tissue hypoxia as an adaptation to prolonged diving, though their reliance on anaerobic metabolism is less than was suggested in earlier physiological literature. Nevertheless, there is some evidence for acid-base imbalance in chased and restrained odontocetes, as judged by high anion gaps in some animals (Koopman et al. 1995, St. Aubin et al. 2001). Anion gap increases as bicarbonate is consumed by lactic acid. Elevations in sodium and potassium, without concurrent changes in chloride, produce the same effect. Specific factors contributing to high anion gaps in harbor porpoises and beluga whales were not identified (Koopman et al. 1995, St. Aubin et al. 2001). Reported values for CO₂ showed a wide range, but in neither study could the source of the blood sample be unequivocally identified as arterial or venous. This factor, combined with the absence of specific data on lactic acid concentrations, complicates the diagnosis of metabolic acidosis in these animals, and leaves open to speculation whether acid-base imbalance might underlie myopathy.
EFFECTS ON THE IMMUNE SYSTEM

Recent studies have provided further evidence that exertion affects indicators of immune function. In people, 90 minute exertion in untrained subjects 3 days in a row initially induced peripheral neutrophilia and increased interleukin-6, but the response subsided progressively, likely as a result of progressive increases in catecholamine response (Suzuki et al. 1999). Circulating neutrophils are increased immediately following various types of exercise, and remain elevated longer in individuals subjected to additional exertion (Fielding et al. 2000). By contrast, Chen and Hsieh (2001) found no changes in circulating neutrophils after exercise repeated daily for a week. Levels of interleukins were elevated immediately post exercise, but showed no progressive changes over the course of the study. Papanicolaou et al. (1997) noted that catecholamines released during exercise stimulate secretion of interleukin-6, but the effect is blunted by administration of glucocorticoids. Suzuki et al. (2000) highlighted the bimodal response of the immune system to strenuous exercise – suppression of lymphocyte function but enhancement of neutrophil activity. The latter effect is closely tied to the inflammatory response to damaged muscle (Smith 1991, Fielding et al. 2000).

In thoroughbred horses, the exertion associated with a race elevated cortisol in samples drawn 40-60 minutes after running, and 12-16 hours later, reduced lymphocyte proliferation responses and total white blood cell counts (Nesse et al. 2002). The authors acknowledged that the clinical consequences of such changes were unknown but advised handlers to be aware that a horse’s immune system might be temporarily compromised following a race. This caveat underlies the majority of such studies – i.e., what the consequences of the observed changes might be on the individual’s response to immunologic challenge.

Deuster et al. (1999), following on a series of studies examining individual differences in stress responses in humans, examined basal patterns of lymphocyte phenotypes and cytokines in individuals classified as “high” and “low” responders. The two groups showed different baseline values for these constituents, but similar responses to high intensity exercise. It appears that the mechanisms for glucocorticoid-mediated effects on the immune system are unaffected by different “set-points” for HPA activity within a group or population.

EFFECTS ON REPRODUCTION

An extensive literature supports the generally accepted view that stress can have a negative effect on reproductive success (Curry 1999). Lending further support to this tenet is a study by Dobson and Smith (2000), who reported that stressors such as milk fever or lameness increased the calving to conception interval by two weeks in dairy cows, and that an average of 0.5 additional inseminations were required for conception. They postulate, based on studies in ewes, that stress interferes with the amplitude and
frequency of pulsatile secretions of gonadotropin-releasing hormones and luteinizing hormone. Reduced or delayed secretion of these substances results in slower growing follicles which produce less estradiol.

SUMMARY AND CONCLUSIONS

Stress research is a field marked by both useful, unifying concepts and detrimental, contradictory findings (Munck et al. 1984, Sapolsky et al. 2000). Among the more problematic issues surround the role of glucocorticoids as the principal effectors of the stress response. A recent review categorizes these effects as permissive, suppressive, stimulatory, and preparative (Sapolsky et al. 2000). All are considered to be largely beneficial to the survival of the organism, at least in the short term, but damaging if sustained, particularly with regard to the suppressive effects on the immune system and reproduction.

The stress response of dolphins needs to be considered from two perspectives: as a population and as individuals. Numerous studies and manipulations of cetaceans representing a variety of species demonstrate that these animals mount a stress response to chase and capture. On balance, recovery from changes in changes glucocorticoids, muscle enzymes, and immunologic indicators does occur. At the same time, literature and experience with these animals highlights the marked individual variation that exists, making a subset of the population particularly vulnerable to the effects of such stresses as fishery-related activity. Thus, it is plausible that enhanced responsiveness in some individuals could predispose them to chronic effects of repeated stimulation of HPA axis.


St. Aubin, D.J. and Geraci, J.R. 1988. Capture and handling stress suppresses circulating levels of thyroxine and triiodothyronine in beluga whales (Delphinapterus

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