The Use of Animal-Borne Imaging to Assess Post-Release Behavior as it Relates to Capture Stress in Grey Reef Sharks, *Carcharhinus amblyrhynchos*

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Introduction

harks, like many species of fish, are exploited by extensive recreational and commercial fisheries throughout the world. In an effort to control fishing mortality and restore stocks to sustainable levels, fisheries management agencies have implemented measures that result in the release of sharks by recreational and commercial fisheries (NMFS, 2007). Such measures range from minimum sizes and bag limits to the complete prohibition on retention of various species (NMFS, 2007). To date, little is known of the post-release behavior and potential mortality associated with the catch and release of sharks (Skomal, 2006).

Regardless of fishing gear, captured fish are exposed to varying degrees of stress, which includes the cumulative impacts of physical trauma and physiological stress (Skomal, 2007). The magnitude of either stressor is dependent on capture method and handling. Physical trauma, which is characterized by external and internal tissue damage associated with the capture method, can be quantified through physical and histopathological examination of fishes after capture (Skomal, 2007). Physiological stress refers to homeostatic disruptions of the internal milieu of fish associated with high anaerobic activity, muscular fatigue, and time out of water (Skomal, 2006). Typically, changes in blood biochemistry, par-

ABSTRACT

Sharks are subjected to extensive commercial and recreational fisheries worldwide. Current management, which imposes bag limits, minimum sizes, and quotas, mandates the release of large numbers of sharks each year, but little is known of post-release behavior and survivorship. Using animal-borne imaging technology, we examined the effects of handline capture on post-release behavior of six grey reef sharks, Carcharhinus amblyrhynchos, at Johnston Atoll (Central Pacific) as it relates to physical trauma and physiological stress induced by capture. To quantify the extent of physical trauma, 25 grey reef sharks (including these six), ranging from 56-135 cm fork length, were examined for evidence of external tissue damage after 2.0-12.8 minutes of handline capture. In addition, these fish were blood sampled to quantify relative changes in acid-base biochemistry. Although blood lactate increased and blood bicarbonate decreased significantly relative to the duration of the capture event, blood pH did not drop significantly and there was no evidence of respiratory or metabolic acidoses. Post-release behavior, as evidenced by animal-borne imaging, included group (n=3) and solitary (n=2) activities that had been previously described in this species. A single shark exhibited aberrant behavior, which included a two-minute period of disorientation, lack of movement, and loss of equilibrium; this behavior was attributed to extensive physical trauma associated with hook damage. When coupled with quantified information relative to the capture event, we found that animal-borne imaging is a useful tool for collecting direct observations of post-release behavior in sharks so that fishery managers and researchers can better assess the impacts of various capture techniques.

ticularly acid-base status, relative to the capture event are used to provide quantitative information about the magnitude of physiological stress (Wells et al., 1986; Skomal, 2006). However, to develop meaningful measures to reduce the lethal and sub-lethal effects of catch and release, indicators of physiological stress and physical trauma must be directly linked to observations of post-release behavior and survivorship.

Standard methods for assessing post-release behavior in fishes, which typically include natural or artificial confinement (Muoneke and Childress, 1994), are simply not applicable to large fishes like sharks. To date, methods for assessing survivorship and behavior in sharks have included conventional tagging, acoustic telemetry, and high technology satellite tagging (reviewed by Skomal, 2007). While these methods provide indirect observations that can be used to characterize behavior (e.g., depth, rate of movement), direct observations of shark interactions with the environment, conspecifics, and other animals are lacking.

Animal-borne imaging systems have been used on numerous animals, including sharks, to provide direct observations of behavior and how it relates to ecology and life history (e.g., Heithaus et al., 2001). However, no study to date has deployed an animal-borne imaging system to assess post-release behavior as it relates to capture stress. This application not only provides direct observations to fishery managers, but also allows researchers to determine if behavioral data are related to the natural ecology of the species or induced by stress. The objective of this study was to deploy an animal-borne imaging system, CRITTERCAM (National Geographic Society, Washington, DC),

to observe the post-release behavior of grey reef sharks, *Carcharhinus amblyrhynchos*, captured on handlines at Johnston Atoll in the Central Pacific. These observations were compared to levels of physical trauma and physiological stress quantified through direct observation and blood sampling, respectively.

Methods

This work was conducted at Johnston Atoll (Central Pacific, 16.735°N, 169.528°W) in 2001-2003 as part of a larger comprehensive study on the ecology of grey reef sharks in this area (P. Lobel, pers. comm). In April 2001 and 2002 and June 2003, 25 (15 males and 10 females) grey reef sharks ranging from 56-135cm fork length were caught on handline and brought immediately to the vessel for blood sampling and, in some cases, tagging before release. The handline comprised a single barbless shark hook crimped to stainless steel wire (1 m) attached to braided nylon line (8 m). To quantify the capture event, the time from first hook-up to physical handling of the shark was defined as fight time, handling time was defined as the duration of time initiated when the shark is first secured to time of release, and total fight time was the cumulative period of fight time and handling time. All sharks were quickly inspected for signs of physical injury, hook placement, and tissue damage. All hooks were cut with bolt cutters and removed from the shark prior to release.

To observe post-release behavior, six of the blood sampled grey reef sharks were randomly chosen and fitted with the CRITTERCAM animal-borne imaging system (Gen 4, National Geographic Society; see Marshall, 1998 for system details). This particular model of the CRITTERCAM differed from that described by Marshall (1998) in that it was smaller (7.6 cm diameter, 32 cm length) and contained a mini DV tape-based image recording system with 2 hr 5 min video capacity. The system was placed over the dorsal fin of each shark using a V-style clamp secured together with a programmable electronic burn-wire system and a back-up galvanic magnesium link. For maximum duration deployments, the burn-wire was programmed to release after two hours.

Blood Analysis

Once close to the vessel, each shark was secured by the tail and inverted to induce tonic immobility. Blood was drawn as quickly as possible by caudal venipuncture in volumes of 2-3 ml and processed immediately for blood gases $(pO_3 \text{ and } pCO_3)$ and pH with a portable blood gas analysis system (IRMA, International Technidyne Corporation, Edison, NJ, USA); blood bicarbonate (HCO₃-) was derived using standard equations (Tietz, 1987). All blood gas and pH measurements were standardized to 25°C. After blood gas analysis, 5 µl of the remaining blood was used to measure whole blood lactate with a portable blood lactate analyzer (Lactate Pro, Fact Canada Consulting Performance Ltd., North Quesnel, BC, Canada); blood lactate levels were not determined in the three sharks sampled in 2002 due to the lack of an analyzer. Changes in blood biochemistry relative to total fight time were modeled using regression analysis (Statgraphics Plus 4.0, Manugistics, Inc., Rockville, MD).

Results

Using a handline, grey reef sharks were quickly brought to the vessel and secured for blood sampling and Critteram deployment; average fight, handling, and total fight times were 4.3 min (1.0-10.0), 1.9 min (0.3-7.3), and 6.0 min (2.0-12.8), respectively (Table 1). All 25 sharks were hooked in the jaw and, with two exceptions, showed no signs of extensive tissue damage and only minor bleeding from the hook wound. In two sharks, GR0203 and GR0303, the hook point pierced the upper palette and exited the orbit of the eye causing tissue damage and bleeding from this region.

Based on regression analysis, we found that blood pH and gases (pO_2 and pCO_2) were not significantly influenced by total fight time (Figure 1A, B). However, blood lactate and bicarbonate increased and decreased significantly with total fight time, respectively, although the relationships based upon the values of the correlation coefficient 'r' were weak (Figure 1C, D).

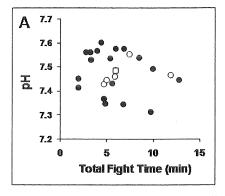
TABLE 1

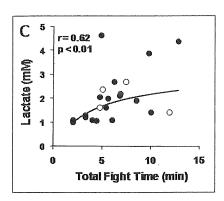
Grey reef sharks blood sampled and observed with Crittercam (indicated by track and behavior).

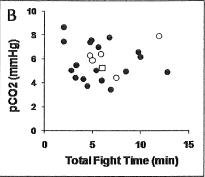
Comple	le Date FL (cm) Sex Time (min)							Behavior
Sample	Date	FL (GIII)	SEX	Fight		Total Fight	Track	Dellavior
GR0101	4/3/2001	91	F	4.7	0.7	5.4	HOUN	
		107	F	3.1	2.8	5.9	42	Group
GR0201	4/19/2002				0.3	2.8	44	Group
GR0202	4/21/2002	114	F	2.5			0.0	Abarrant
GR0203	4/21/2002	124	M	5.0	1.0	6.0	20	Aberrant
GR0301	6/21/2003	91	M	2.0	3.0	5.0	58	Group
GR0302	6/22/2003	112	F	5.0	1.0	6.0		
GR0303	6/25/2003	102	M	4.0	0.4	4.4		
GR0304	6/25/2003	76	M	5.4	1.5	6.9		
GR0305	6/26/2003	71	М	1.0	1.0	2.0		
GR0306	6/26/2003	56	М	2.5	0.8	3.3		
GR0307	6/26/2003	91	F	5.2	1.6	6.8		
GR0308	6/27/2003	107	F	3.4	1.5	4.9		
GR0309	6/27/2003	117	M	4.6	7.3	11.9	71	Group/Solitary
GR0310	6/28/2003	66	F	4.0	0.8	4.8		
GR0311	6/28/2003	135	М	4.1	3.4	7.5	126	Solitary
GR0312	6/28/2003	72	F	3.0	0.3	3.3		
GR0313	6/30/2003	135	М	5.5	4.3	9.8		
GR0314	6/30/2003	124	М	2.9	2.7	5.6		
GR0315	6/30/2003	112	F	3.0	1.0	4.0		
GR0316	6/30/2003	124	М	5.0	1.3	6.3		
GR0317	6/30/2003	132	М	7.0	1.5	8.5		
GR0318	6/30/2003	122	F	10.0	2.8	12.8		
GR0319	6/30/2003	109	М	9.0	1.0	10.0		
GR0320	6/30/2003	102	М	1.0	1.0	2.0		
GR0321	7/1/2003	124	М	4.0	0.8	4.8	121	Solitary
Mean				4.3	1.9	6.0		

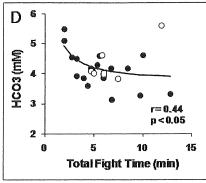
FIGURE 1

Linear relationships of total fight time to blood (A) pH, (B) pCO_2 , (C) lactate, and (D) HCO_3 in grey reef sharks; Crittercam-monitored sharks are indicated with open circles and square (GR0203).









CRITTERCAM deployments ranged from 20-126 minutes (Table 1); tracks shorter than the maximum of two hours were associated with the V-clamp slipping off the relatively

small dorsal fin of this species. Grey reef sharks exhibited three behaviors (Table 1). Upon release, three sharks (GR0201, GR0301, GR0309) immediately joined a school of six

or more sharks and moved synchronously as a group through multiple depths with little horizontal movement. These sharks frequently displayed "nose to tail" following behavior during these tracks (Figure 2). One of these sharks (GR0309) remained with the group for only nine minutes and exhibited solitary behavior thereafter. Two sharks (GR0311, GR0321) remained solitary for the entire deployment, moving frequently from the surface to the bottom while covering large horizontal distances. Although these sharks encountered solitary conspecifics, neither shark initiated group behavior. A single shark, GR0203, exhibited what appeared to be aberrant behavior. Upon release, the shark moved along a nearby bulkhead, abruptly collided with the bottom rubble, and stopped, thereafter settling on the bottom and rolling on its left side as it lost equilibrium (Figure 3). The shark remained on the bottom for two minutes before resuming solitary swimming behavior. Unfortunately, the CRITTERCAM remained on this shark for only nine additional minutes before slipping off. Although this fish did not exhibit blood biochemistry that would be indicative of severe physiological stress (Figure 1), it was one of the sharks that suffered internal tissue damage associated with its right eye, which was pierced by the point of the hook (Figure 4).

FIGURE 3

Still image captured from Crittercam video footage showing grey reef shark GR0203 settled on bottom with equilibrium loss, rostrum of shark is resting against coral rubble; note orientation of nearby fish in upper-right corner.



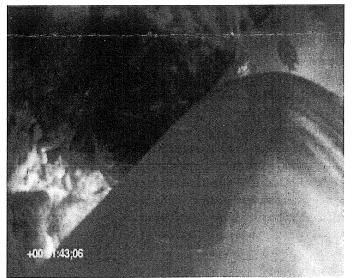
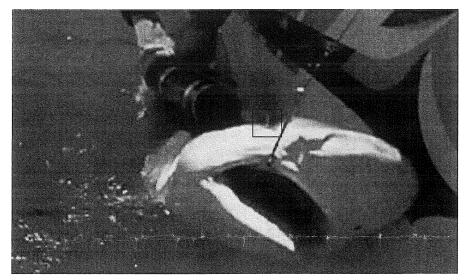


FIGURE 2

Still image captured from $C_{\text{RITTERCAM}}$ video footage showing "nose to tail" group swimming behavior of grey reef shark GR0201.

FIGURE 4

Still image captured from video footage of grey reef shark GR0203 prior to release; note hook point (box) piercing orbit of shark's right eye.



Discussion

Animal-borne imaging technology, like CRITTERCAM, provides a useful tool for directly observing the behavioral response of grey reef sharks to capture stress. When compared to the physical and physiological effects of the capture event, these observations indicate that the former may influence the post-release behavior of grey reef sharks more than the latter. Using imagery collected by CRITTERCAM, we conclude that grey reef sharks subjected to short fight and handling times during handline capture are likely to exhibit natural behavior upon release unless exposed to extensive physical trauma. Moreover, this capture technique did not cause significant physiological perturbation that impacted post-release behavior.

Although studies on sharks are limited, it has been demonstrated that fish subjected to exhaustive exercise associated with capture exhibit physiological disturbances that are manifested in blood biochemistry. In general, exhaustive exercise associated with capture typically causes: an increase in blood lactate levels; the marked decrease in blood pH resulting from metabolic (H*) and respiratory (pCO_2) acidoses; and the disturbance of ionic, osmotic, and fluid volume homeostasis with hemoconcentration and increased plasma electrolytes (reviewed by Pickering, 1981; Adams, 1990; Wood, 1991; Milligan, 1996; Wendelaar Bonga, 1997; Kieffer, 2000; Skomal, 2007).

In contrast, blood acid-base status, as indicated by pH and gases, were not influenced in grey reef sharks subjected to short bouts of handline capture (Figure 1A, B). Although blood lactate, a significant end-product of anaerobic metabolism and exhaustive exercise, did increase with capture duration, blood bicarbonate levels were sufficient to buffer the potential impacts of the concomitant proton load, thereby preventing metabolic acidosis. These results differ markedly from other studies conducted on sharks and can likely be attributed to capture method as well as inherent interspecific differences in physiology. Piiper et al. (1972) and Holeton and Heisler (1978) stimulated spotted dogfish (Scyliorhinus stellaris) with electric shocks until fatigued and observed a significant drop in pH coupled with a rise in blood carbon dioxide and blood lactate. Cliff and Thurman (1984) examined changes in blood biochemistry in dusky sharks, Carcharhinus obscurus, caught on rod and reel and found that blood pH and bicarbonate declined, while carbon dioxide, metabolites (glucose, lactate), and electrolytes increased. Hoffmayer and Parsons (2001) found significant increases in blood lactate, while pH declined in serially sampled Atlantic sharpnose sharks, Rhizoprionodon terraenovae, after rod and reel capture. Spargo (2001) subjected sandbar sharks, Carcharhinus plumbeus, to 10 minutes of rod and reel angling and found significant changes in blood acid-base status. Similarly,

Skomal (2006) found significant disturbances in acid-base status in blue (*Prionace glauca*), shortfin mako (*Isurus oxyrhincus*), and spinner (*Carcharhinus brevipinna*) sharks subjected to rod and reel capture.

It is important to note that our ability to quantify physiological change in response to stress is hampered by our lack of baseline data. This is an inherent problem with this study and many of the aforementioned field studies during which the very act of handling and blood sampling the animal induces physiological stress. Given this methodological constraint, which is not likely to be rectified, we must acknowledge this potential limitation to the study.

Based on CRITTERCAM footage, we conclude that group and solitary swimming behaviors exhibited by five of the six grey reef sharks in this study were natural swimming patterns. Visual observations of free-swimming animals in Enewetak Atoll, Marshall Islands, allowed McKibben and Nelson (1986) to characterize three activity patterns in grey reef sharks: solitary individuals, loose aggregations, and polarized schools. By their definition, a polarized school consisted of more than two dozen closely-spaced sharks swimming just above the bottom, occasionally forming circular milling groups. Three of the sharks in the current study joined a school after release. While it was difficult to ascertain the number of sharks in each group, it appeared to be numerous individuals. McKibben and Nelson (1986) characterized lone individuals as solitary sharks usually found over shallow reefs and near lagoon pinnacles, much like the solitary behavior exhibited in this study.

We conclude that the post-release behavior of a single grey reef shark (Figure 3) that settled on the bottom for two minutes was not natural and likely associated with tissue damage from the hook (Figure 4). In general, sharks are obligate ram ventilators and there are no data to suggest that grey reef sharks differ from this physiological constraint. Images of GR0203 collected by the Crittercam system clearly show that this shark collided with coral rubble, stopped swimming, settled on the bottom, and lost equilibrium. Based on the behavioral patterns reported by McKibben and Nelson (1986) and those observed in the other five sharks in this study, it is likely that this particular shark

was impacted by the capture event. The physiological biochemistry of this individual was not indicative of excessive stress, yet this particular shark was the only tracked shark that was severely wounded by the capture event. Hence, there is strong evidence to suggest that physical trauma likely caused this behavioral response.

Although acoustic telemetry and satellitebased tagging have been used to assess the impacts of physical trauma on post-release survivorship in sharks and billfishes (Domeier et al., 2003; Gurshin and Szedlmayer, 2004; Horodysky and Graves, 2005), neither method provided direct observations like those obtained in this study using animal-borne imaging. However, it should be emphasized that the relatively short duration of these tracks allows us to draw conclusions as they relate only to the acute effects of capture stress on post-release behavior. As such, the ultimate fate of GR0203 could not be determined due to the short duration of the track. However, improved methods for attachment coupled with newer models of CRITTERCAM, and other animal-borne systems, now allow for longer tracks (eight hours), which increase the utility of this approach.

Assessing the impacts of capture and handling methods on shark behavior is difficult. When coupled with direct observations of post-release behavior, qualitative and quantitative information about physiological stress and physical trauma allows researchers to examine causative factors associated with that behavior. The use of CRITTERCAM, and other animal-borne systems, provides a useful tool for collecting direct observations of post-release behavior so that fishery managers and researchers can better assess the impacts of various capture techniques.

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