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MODELING LARGE WHALE ENTANGLEMENT INJURIES: AN EXPERIMENTAL ANALYSIS OF THE INFLUENCE OF TISSUE COMPLIANCE, LINE TENSION, AND DRAW-LENGTH ON EPIDERMAL ABRASION RESISTANCE

By

Jeremy Paul Winn

B.S. University of Maine, 2003

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Marine Bio-Resources)

The Graduate School

The University of Maine

August, 2006

Advisory Committee:

John Riley, Professor of Marine Sciences, Advisor

Michael Peterson, Associate Professor of Mechanical Engineering

Michael Moore, Research Specialist at Woods Hole Oceanographic Institution

MODELING LARGE WHALE ENTANGLEMENT INJURIES: AN EXPERIMENTAL ANALYSIS OF THE INFLUENCE OF TISSUE COMPLIANCE, LINE TENSION, AND DRAW-LENGTH ON EPIDERMAL ABRASION RESISTANCE

By Jeremy Paul Winn

Thesis Advisor: Dr. John Riley

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Masters of Science
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August, 2006

Two test systems were developed to evaluate the influence of draw-length and tissue compliance on entanglement-induced epidermal abrasion in humpback (*Megaptera novaeangliae*) and right whale (*Eubalaena glacialis*) tissue samples. Under straight pull abrasion tests an adult right whale fluke required 3.7 times the load and 15 times the draw-length of a right whale calf flipper to induce epidermal failure while a humpback fluke was intermediate between these extremes. Epidermal thickness did not appear to be the cause of this difference in abrasion resistance. Epidermal thickness averaged 8.0±0.2 mm for the calf flipper, 4.9±0.4 mm for the humpback fluke, and 5.1±0.1 mm for the right whale fluke. It is unknown whether the difference in abrasion resistance is a function of species (right vs. humpback whale), age (adult vs. calf), or body region (fluke vs. flipper). Repeated tests with different line materials (new and used float vs. sink) did

not show a significant difference in the rate of epidermal abrasion. However, line diameter does appear to influence tissue abrasion rates. Thinner (6.4 mm diameter) lines cut substantially deeper than thicker (9.5 mm diameter) lines under similar loads and draw-lengths. Tests with the oscillatory abrasion system revealed that draw-lengths that exceed the tissue compliance limit resulted in substantially increased rates of tissue abrasion. Draw-lengths below the compliance limit did not penetrate the epidermis while those exceeding the compliance limit deeply cut into the underlying dermis. In actual entanglement situations, the relative draw-length to tissue compliance ratio may be the critical component that determines if the line will cut into the body or benignly press against the skin. Investigations into the potential for reducing line modulus to allow lines to stretch with flexure of the body and thus minimize sliding relative to the skin may be beneficial in reducing the mortality associated with entanglement injuries.

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I would like to thank John Riley for being willing to take me on as a graduate student and for supporting me through the process.

I would also like to thank the necropsy team members for obtaining samples from NEAq Eg 1004, Eg NEFL 0602 and VAQS 2006 1007 Mn with members from the Virginia Aquarium, UNC Wilmington, and Woods Hole Oceanographic Institution. In particular, thanks to Sue Barco, Bill McLellan, and Charley Potter for their aid in the acquisition of tissue specimens. Thanks also to Scott Landry for providing the sketch of NEAq Eg 2301 and to CT Harry for his help with the necropsy.

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Chapter 1

BACKGROUND

THE ENTANGLEMENT PROBLEM

Entanglement in commercial fishing gear is one of the leading anthropogenic causes of serious injury and mortality in baleen whales (Johnson *et al.* 2005, Knowlton and Kraus 2001, Robbins and Mattila 2001). Reduction and the eventual elimination of these threats is essential to the preservation of five endangered baleen whale species in the western North Atlantic (blue, fin, sei, humpback, and northern right whales). With only 300 animals remaining in the population, the North Atlantic right whale is of particular concern due to its critically low abundance (NMFS 2005). Photographs from the New England Aquarium's photo-ID catalog indicate that 75.6% of all photographed right whales show evidence of scarring indicative of rope and net cuts around the tail stock, while the rate of entanglement appears to be increasing (Knowlton and Kraus 2001, Knowlton *et al.* 2005). Additionally, 88% of humpback whales studied in 1999 showed some signs of entanglement-related scarring about the peduncle (Knowlton and Kraus 2001, Robbins and Mattila 2001). Continued monitoring of the humpback population suggests that 8-25% of the population receives new scars each year (Johnson *et al.* 2005). The extent of entanglements involving other baleen whale species is not known. Although their more pelagic distribution may decrease their exposure to commercial fishing gear, the probability of identifying entanglements when they do occur is also greatly reduced due to a reduction in sightings of pelagic species and the low probability of animals washing ashore in the event of a mortality.

In the western North Atlantic, right and humpback whales have been found entangled in all major types of fixed fishing gear (Johnson *et al.* 2005). Preventative efforts have included fishing restrictions, mandatory gear removal, deterrent devices such as pingers, and fishing area closures (Johnson *et al.* 2005). Fishing gear modifications such as break-away links, knotless splices, and pop-up buoys are also under development to help reduce entanglement risks (NMFS 2005). Disentanglement remains a costly and logistically difficult retroactive defense against entanglement mortalities. However, model predictions indicate that preventing the deaths of two female right whales per year would allow population recovery (Fujiwara and Caswell 2001). Therefore, minor improvements may have a significant impact.

To enhance the effectiveness of gear modification efforts, the factors that govern entanglement injuries must be more fully understood (Johnson *et al.* 2005). Analysis of entangling lines and the resulting tissue damage on free swimming whales is extremely difficult to characterize. Entangling gear on stranded animals is often removed by the gear owner preventing in-depth analysis during necropsy events (Moore *et al.* 2005). As a result, no design criteria presently exist upon which to base future gear modifications or to verify the effectiveness of existing ones.

EPIDERMAL STRUCTURE IN CETACEA

In addition to selection for hydrodynamic performance, the cetacean epidermis has also evolved as a defensive structure. The cetacean epidermis is thick and hairless, and is often mistakenly referred to as the skin of the whale even though it only composes the outermost layer of the integument (Haldiman and Tarpley 1993). It has an extremely smooth, rubbery texture bordering on velvety (Geraci *et al.* 1986a, Haldiman *et al.* 1985, Ling 1974). These surface properties reduce frictional forces and provide resistance against biofouling by marine organisms (Baum *et al.* 2003, Geraci *et al.* 1986b). Natural abrasive stresses on the cetacean epidermis may include but are not limited to hydrodynamic friction during swimming (Giacometti 1967), attacks by other ceteaceans, kelp gulls (Rowntree *et al.* 1998), and other marine organisms, as well as contact with sediment, ice in the case of northern species, or other marine debris. Physical contact with other, sometimes barnacle covered, individuals of the same species would also occur. These interactions would include mother/calf interactions, mating, and competitive physical contact during mate selection in some species. Therefore, the evolution of a hydrodynamicaly smooth and yet durable epidermis would be essential to survival in the aqueous environment. However, the extent to which these adaptational defenses against natural stresses are significant as a defense against abrasive impacts from entangling fishing gear has not been examined.

Only limited data are available on the cetacean integument. The dermal layer is composed of an extensively cross-linked network of collagen fibers that may function as an elastic recoil mechanism during swimming activities (Pabst 1996b). A limited number of studies have looked at the mechanical properties of the cetacean dermis (Hamilton *et al.* 2004, Pabst 1996a, Pabst *et al.* 1995). However, to date the only structural studies on cetacean epidermis have been measurements of epidermal thickness (Table 1) and evaluations of surface properties, including surface roughness and resistance to biofouling (Baum *et al.* 2002, Baum *et al.* 2003, Baum *et al.* 2000). Epidermal thickness has been reported in 5 species of baleen whales, with thickness ranging from a minimum of 1.4 mm to a maximum of 25 mm in fin (*Balaenoptera physalus*) and bowhead whales (*Balaena mysticetus*) respectively. There have been no published studies on the mechanical properties of the cetacean epidermis.

Epidermis Thickness:							
Species	**Gender Length (cm)**	**Maximum Thickness (mm)**	**Source**	**Gender Length (cm)**	**Minimum Thickness (mm)**	**Source**
Balaena mysticetus	- -	**25.0**	Haldiman *et al.* 1985	- -	**8.6**	Haldiman *et al.* 1985
Balaenoptera acutorostrata	- -	**3.0**	Singarajah 1984	- -	**3.0**	Singarajah 1984
Balaenoptera acutorostrata	**F 708**	**3.0**	Sokolov 1982	**F 708**	**1.4**	Sokolov 1982
Balaenoptera physalus	**M 1565**	**3.9**	Sokolov 1982	**F 1600**	**2.5**	Giacometti 1967
Eubalaena glacialis	**M 1075**	**13.2**	Sokolov 1982	**F 1630**	**8.6**	Sokolov 1982
Megaptera novaeangliae	**M 857**	**5.6**	Jones and Pfeiffer 1994	**M 857**	**4.3**	Jones and Pfeiffer 1994

TABLE 1. Comparative epidermal thickness ranges reported for baleen whale species.

The cetacean epidermis is composed of three primary strata as opposed to the five of terrestrial mammals (Geraci *et al.* 1986b, Harrison and Thurley 1974, St. Aubin *et al.* 1990). These include: the stratum externum, stratum intermedium, and stratum germinativum (Figure 1). The stratum externum is formed of the outermost 12-60 (0.25-1 mm) cells of the epidermis. These cells are typically flattened and keratinized forming a semi-hardened exterior (Haldiman *et al.* 1985, Pfeiffer and Rowntree 1996, Sokolov 1982, Spearman 1972). Beneath the stratum externum, the stratum intermedium is by far the largest portion of the epidermal tissue and may extend as much as 10 mm above the distal end of the papillae in the bowhead whale (Haldiman *et al.* 1985). Epidermal cellular production originates at the germinitive layer, which is composed of a single layer of cells lying adjacent to the basal lamina. This layer is highly convoluted, forming regular parallel lines of serial dermal papillae that extend distally upward through the epidermis. These papillae may reach lengths of 80% of the overall thickness of the epidermal layer. When compared with other mammals, cetaceans have an extremely thick epidermal layer. Increased rates of cellular replication combined with higher relative basal area to skin surface area ratios may contribute to the thickness of the cetacean epidermis (Brown *et al.* 1983, Palmer and Weddell 1964, Sokolov *et al.* 1969, St. Aubin *et al.* 1990).

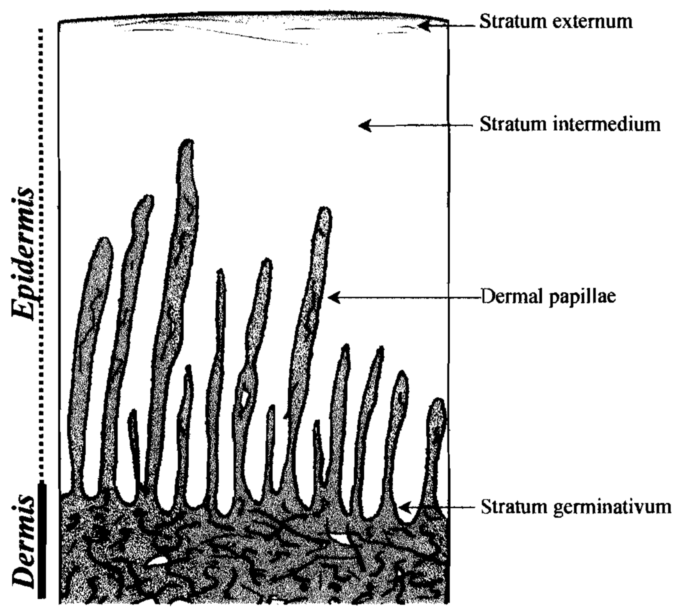

FIGURE 1. Diagram of the epidermal layers visible in a cross section. The epidermal layers, dermal papillae, and the underlying dermal layer is shown. Increasing keratinization is represented by the shading gradation through the epidermis (Greater keratinization is represented by a darker shade).

Major arteries in the cetacean skin enter the dermis, branch to an increasing extent as they approach the epidermis, and turn to run within the dermal bed. The dermal papillae are well vascularized with capillaries lying against the basal lamina (Haldiman *et al.* 1981, Harrison and Thurley 1974, Sokolov 1982). Injuries that result in the penetration of the protective epidermal layer can easily lead to infection (Oen 1990) and occasionally death due to bacterial entry into this highly vascularized region. In all cases in which right whale entanglement mortalities have been examined though necropsy during the period of 1970-2002, regions of epidermal penetration were noted (Moore *et al.* 2005). Microorganisms including diatoms and bacteria that inhabit depressions in the epidermis have been reported to penetrate deeply into the spinous layer particularly when necrotic tissue is present as a result of injury (Haldiman *et al.* 1985, Henk and Mullan 1996). As a result, gear modifications that can help to minimize the penetration of the epidermis are of high priority.

EXPERIMENTAL MODELING OF ENTANGLEMENT INJURIES

In 2006, Woodward *et al.* created a reciprocating load generator to model large whale entanglement injuries. This system was designed to load a line in an oscillatory manner, periodically causing the line to go taut and then slack. In effect, this simulated an entanglement scenario in which a line connecting wraps about a flipper and the tail fluke is loaded and unloaded by the up and down movements of the tail as the whale swims. The reciprocating load generator used a gear motor and a slider crank mechanism to pull up and down on an abrading line. An aluminum frame and set of guiding pulleys positioned the abrading line such that it passed from the slider crank mechanism and motor, over the leading edge of a fluke tissue sample clamped in a static seawater tank, and on to a tensioning weight suspended on the far side (Figure 2). The up and down motion produced by the slider crank mechanism and the return force provided by the tensioning weight generated an oscillatory load that simulated the abrasion experienced by a free swimming whale under controlled laboratory conditions.

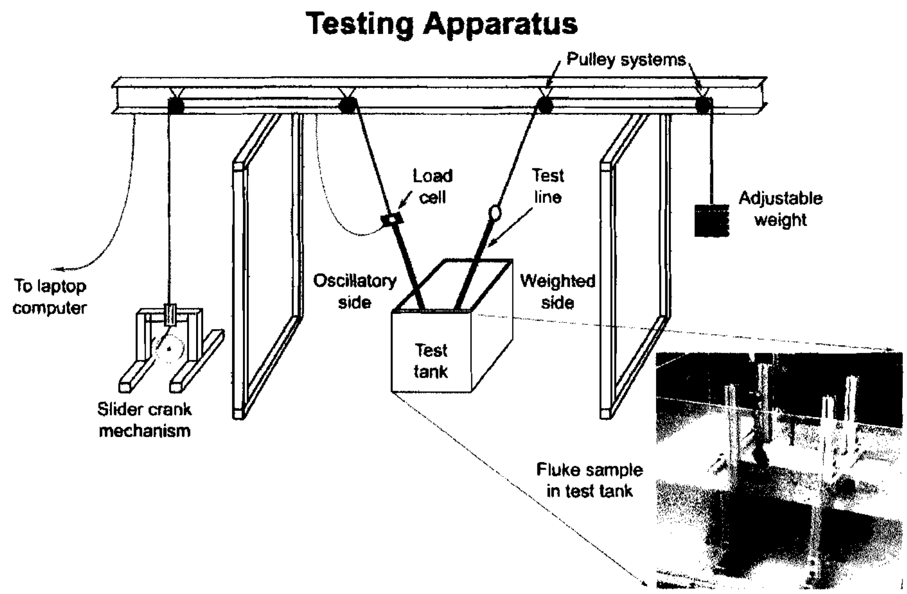

FIGURE 2. Diagram of the reciprocating load generator used by Woodward *et al.* (2006). The primary system components are illustrated and a photograph of the sample clamping technique is shown (lower right).

Abrasion tests were conducted on tissue samples collected from the leading edge of the fluke of an adult right whale (NEAq Eg 1004). Both new and used float (polypropylene) and sink (polyester/polypropylene) lines of twisted 3-stranded construction were tested as well as a hollow braided polypropylene line. All lines were 9.5 mm in diameter. Lines were tested for up to 24 hours using a 9-kg load at an accelerated loading rate of 60 strokes/minute. Maximum line tension was 267 N. These tests failed to penetrate the epidermis but succeeded in producing furrows that closely resembled marks found on entangled whales during necropsy. It was also determined that the line material, construction, and age affected furrow depth and appearance, suggesting a potential forensic analysis application of experimental modeling studies (Figure 3).

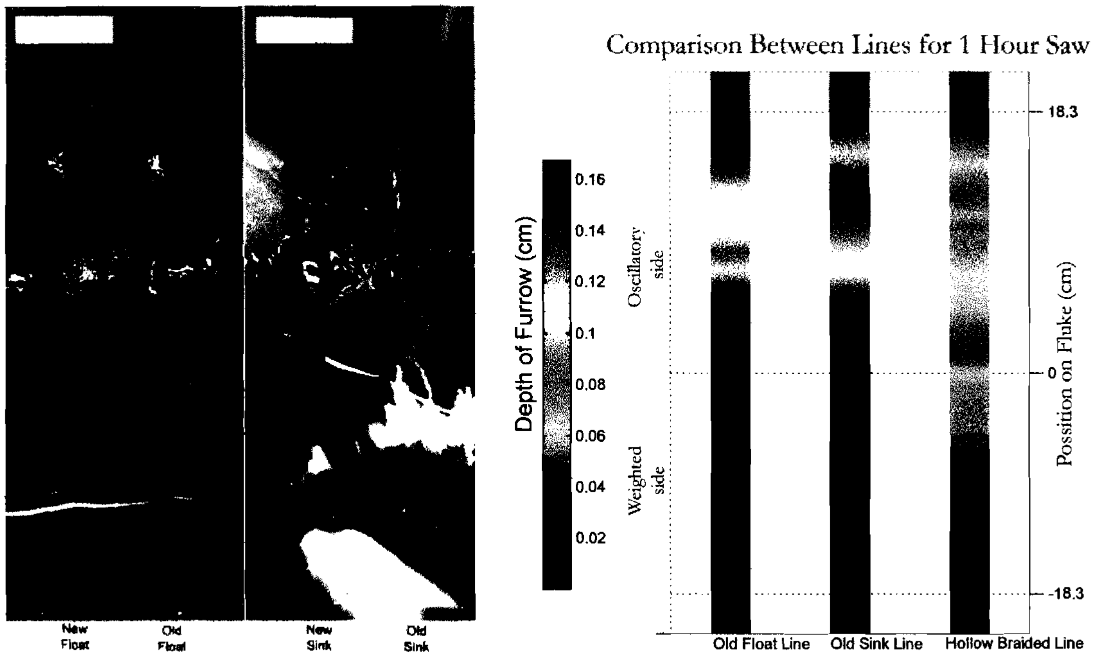

FIGURE 3. Comparative differences in furrow appearance and depth due to line type. Photographs of furrow appearance (left) and a graphical comparison of depth measurements (right) following 1-hour tests with different line materials are shown (Woodward *et al.* 2006).

PROJECT OBJECTIVE

Although the Woodward *et al.* (2006) test system was effective in producing compression furrows similar to those observed on entangled whales, these marks could not be compared to entanglement scenarios where the line cut through the epidermis. Understanding what causes some lines to cut while other do not is essential to preventing injury and mortality due to entanglement. An evaluation of the physical conditions leading to epidermal penetration is needed in order to make recommendations for future gear modifications. During the initial testing, it was noted that the fluke tissue exhibited a large degree of lateral flexure, or compliance, in response to the pull of the line. This flexure was thought to absorb a portion of the test system's abrasive impact by helping to prevent the rope from sliding across the epidermal surface. It is hypothesized that the tissue compliance is essential to the abrasion resistance capacity of the whale skin.

To further examine this theory, two new test systems were developed based upon the original design by Woodward *et al.* (2006) and used to compare line abrasion on right and humpback whale skin samples under controlled conditions. Three factors were examined: 1) the compliance of the skin tissue under load, 2) the relative skin abrasion following a known length of line pulled under a known tension across the skin, and 3) the interaction between tissue compliance and line draw-length as they relate to the rate of tissue abrasion.

Chapter 2

METHODS

Tissue specimens were opportunistically collected at necropsies along the East Coast of the United States and included samples from the leading edge of a right whale calf flipper (Eg NEFLO 602), the fluke of an adult right whale (NEAq Eg 1004), and the fluke of a sub-adult humpback whale (VAQS 2006 1007 Mn). These flipper and fluke regions are known to be regularly associated with entanglement injuries in large whales (Johnson *et al.* 2005, Moore *et al.* 2005, Woodward *et al.* 2006). Samples were wrapped in plastic to avoid hydration change, transported on ice to a storage facility (a process of several days) and stored in a –20° C freezer until testing was possible. The fluke sample from Eg1004 had been thawed during the prior Woodward *et al.* (2006) study and refrozen following a 4 day period of refrigeration and testing. For the current study, samples were thawed for 24 h in seawater prior to testing. When tested, tissue specimens appeared in good condition with the epidermis remaining firmly attached to the underlying dermal tissue.

Two test systems were developed to examine fishing line related entanglement injuries in large whales. These systems were designed to independently evaluate straight pull abrasion and oscillatory abrasion in relation to tissue compliance.

STRAIGHT PULL ABRASION SYSTEM (SPA)

A straight pull abrasion (SPA) system was designed to measure the depth of epidermal penetration following a unidirectional pull over a standard draw-length. This system allowed for control over factors such as line material, line diameter, pressure, contact length, velocity, and pull distance. It has been shown in the tribology literature that controlling for pressure and velocity allows for the evaluation of the mechanical interaction between the abrading material and the abraded substance (Arnell *et al.* 1991). The SPA system utilized a set of two line-positioning pulleys and a tensioning pulley mounted on an aluminum frame to position and tension an abrading line (Figure 4). The abrading line was drawn by a sailing winch connected to a ¼-hp gear motor (Model 6ML51, Dayton Electric Manufacturing Company, Lincolnshire, IL). The motor pulled a sliced loop of line in one direction across a test sample clamped in a static seawater tank. Tension on the line was maintained using an adjustable weight connected to a tensioning pulley. The tensioning pulley was free to slide along a rail to absorb any stretch of the test line and maintain a constant tension in the system. A load cell (Model LC101, Omegadyne Engineering Inc., Stamford, CT) placed between the tensioning pulley and the adjustable weight allowed constant evaluation of tension during testing, and a rotating shaft counter mounted on the tensioning pulley measured the distance the line was pulled across the test sample (±1 cm).

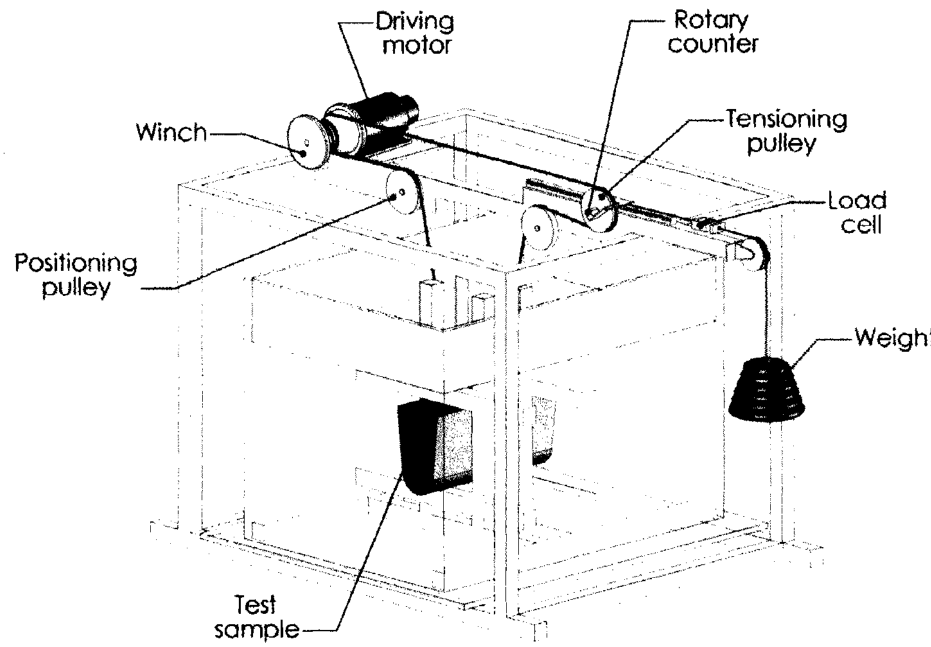

FIGURE 4. Diagram of the straight pull abrasion (SPA) system.

Test samples were submerged and clamped in a static seawater tank. The clamping system uprights were spaced 7.6 cm apart to minimize lateral flexion in the sample and maintain the sample's vertical orientation. The abrading line was then pulled across the test sample. In order to ensure accurate pull distances across the sample regardless of tissue compliance, tension was first applied to the sawing line by adding weight to the tensioning pulley. The line was tensioned by manually turning the winch until the compliance of the tissue was reached and the line began to slide across the epidermis. At this point the rotary counter was zeroed. A standard draw-length (3.1 m) of line was then pulled across the fluke sample at a continuous rate of 1.8 cm/s.

The line samples used for the abrasion tests were all of 3 stranded twisted construction. Both new and used polypropylene float and polypropylene/polyester blend sink lines were tested. Float line diameters included both 6.4-mm and 9.5-mm samples while only 9.5-mm diameter sink line was tested. The "used" rope samples were cut from the same line used in the Woodward *et al.* (2006) study. These lines were previously used for lobstering (Southwest Lobster and Fish Unlimited, Southwest Harbor, ME) and contained ingrained mud and fraying rope fibers. In order to create a continuous loop of line, a 3.5-m section was cut and the ends joined using a 15-cm splice. The ends of the splice were coated with Skotch 667 tape to create a smooth transition across the splice. Three sets of tests were conducted with the SPA system.

1) *Point of epidermal penetration for each test specimen*

   A standard draw-length of 3.1 m was used with new 9.5-mm diameter float line to determine the load and draw length combination necessary to penetrate the epidermis for each of the tissue specimens: right whale calf flipper, right whale fluke and humpback whale fluke. Load on the system was increased in 2.3-kg increments from 2.3 kg to 31.8 kg. Epidermal failure was defined as substantial cracking of the epidermis allowing the underlying dermal material to become visible. If epidermal failure did not occur, the line was moved to a new test location and run with an additional load. If the 31.8-kg load failed to penetrate the epidermis, the draw-length was increased in 3.1-m increments until failure was achieved.

2) *Relative line abrasion between line types*

   Four types of 9.5-mm diameter line were used to examine the relative rate of abrasion following a standard 3.1-m draw-length with 31.8-kg load. Three tests of each line type (new and used float and sink) were conducted in random order on samples from the humpback fluke. The results were compared using a pair- wise Bonferroni corrected Wilcox-Mann-Whitney test at the $\alpha = 0.05$ level.

3) *Influence of rope diameter and sample curvature on relative abrasion*

Both 6.4-mm and 9.5-mm diameter new float lines were drawn 3.1 m across humpback fluke specimens using a 31.8-kg load. Tests of each line diameter were repeated twice: once on the most proximal and once on the most distal portion of the leading edge of the fluke, representing the widest range in curvatures available using tissue samples from the same specimen. In this manner, both the relative influence of line diameter and sample curvature could be compared based on the rate of epidermal abrasion.

OSCILLATORY PULL ABRASION SYSTEM (OPA)

An oscillatory pull abrasion (OPA) system was designed to measure both the tissue compliance in a test sample and experimentally model abrasion rates with draw-lengths below, at or above this predetermined tissue compliance limit. The rate of epidermal abrasion as a result of this load/draw-length combination could then be identified.

The OPA system utilized two lower positioning pulleys to orient the abrading line across the test sample, as well as an upper positioning pulley and a tensioning pulley that translated lateral motion of a driving slider into a back and forth sawing motion of the abrading line (Figure 5). The line between the upper positioning pulley and tensioning pulley was securely clamped to the base of the slider. The same ¼-hp gear motor used in the SPA system was used to drive the slider using a slider crank mechanism to convert rotary motion of the motor into the linear motion of the driving slider. The slider's motion in turn drove the lateral sawing motion of the abrading line across the test sample. The draw-length of the abrading line could be adjusted by changing the throw of the slider crank mechanism. Tension in the line was maintained using an adjustable weight connected to the tensioning pulley. Movement of the tensioning pulley absorbed any stretch in the line while insertion of a cam cleat in the loading line maintained the position of the tensioning pulley during the return stroke of the slider. A position transducer (Model PT101, Celesco Transducer Products Inc, Chatsworth, CA) was used to measure the precise distance of linear travel in the line, while two load cells (Model LC101, Omegadyne Engineering Inc., Stamford, CT and Model MLP100, Transducer Techniques Inc., Temecula, CA) were inserted in either side of the line system to simultaneously measure load on both sides of the tissue sample.

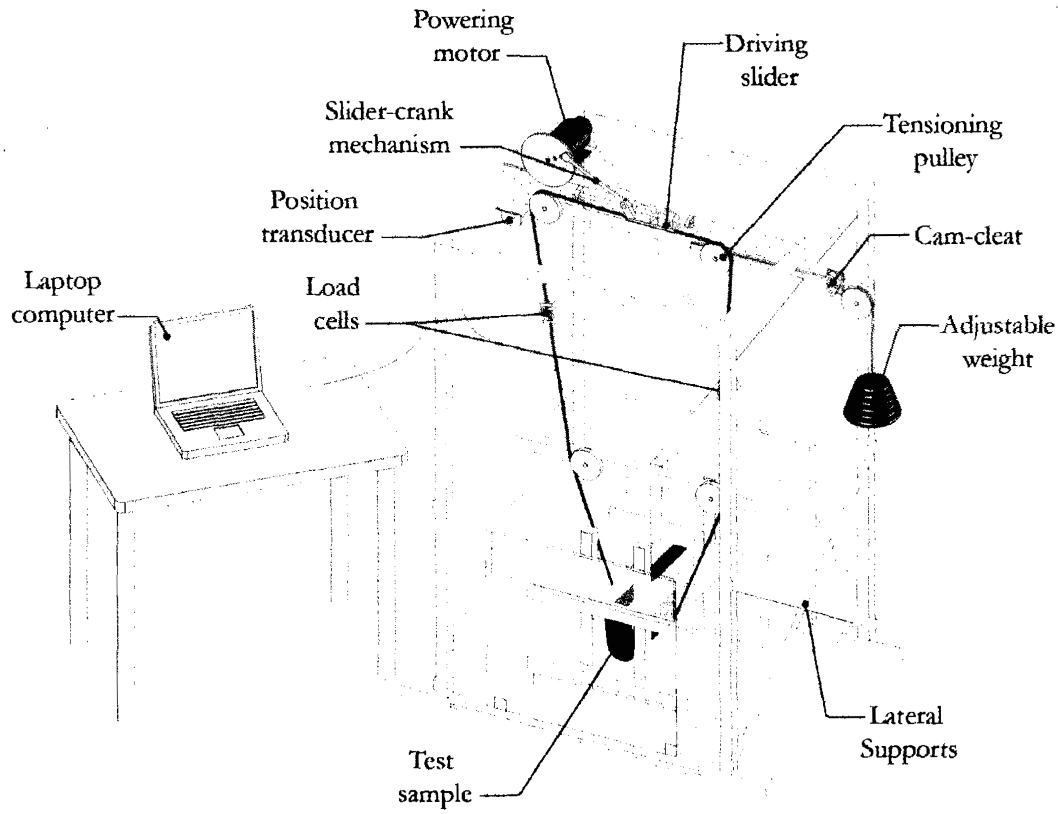

FIGURE 5. Diagram of the oscillatory pull abrasion (OPA) system.

In order to simulate the natural compliance of the fluke and flipper samples as closely as possible, a new clamping technique was developed for use with the OPA system. In the SPA tests, the tissue sample was securely clamped with bars pressing along both the sides and upward against the leading edge of the sample, limiting sample movement as much as possible. In the OPA tests, however, only the base of the sample was clamped leaving the leading edge free to flex without any form of lateral restraint. Three 6.4-mm diameter threaded rods were inserted through the base of the sample and secured to two pieces of 90° angle irons (3.8 cm wide and 61 cm long). The ends of these angle irons were then securely bolted to the original clamping frame within the seawater tank (Figure 6).

FIGURE 6. Clamping technique for the OPA system: (a) angle irons and threaded rods clamping right whale calf flipper sample, (b) sample clamped in static sea water tank.

The OPA system was used to test the influence of tissue compliance on abrasion using samples from the flipper of the right whale calf and the humpback whale fluke. The compliance (total lateral flexural due to line pull) in the test sample under a particular load was determined by applying tension to the line system. A unidirectional load was applied via the slider until static friction was overcome and the rope started to slide across the epidermal tissue. The motion of the slider and the force applied to the line were recorded simultaneously on a laptop computer using the position transducer and load cells. The linear displacement of the line was calculated at the point when the maximum load was applied prior to overcoming static friction. The direction of pull was then reversed until static friction was overcome in the opposite direction. The distance between these two flexural extremes was recorded as the tissue compliance limit of the sample. The test was repeated 6 times to determine the average tissue compliance for each test sample used in the OPA testing.

Prior to oscillatory testing the tissue compliance was determined for each specimen using a 9.5-mm new float line. Loads were selected to be 2.3 kg above the load needed to penetrate the epidermis with a 3.1-m pull in the SPA system. Two 2-hour oscillatory tests were then conducted: one with a draw-length below the tissue compliance limit and a second with a draw-length above this limit. The relative abrasion resulting from the different draw-length tests was then compared for each specimen.

COMPARISON OF LINE ABRASION

As in the Woodward *et al.* (2006) study, individual test sites on the samples were separated by a minimum of 2 cm to isolate test sites and maintain the integrity of the tissue. The resulting tissue indentation and/or damage was assessed following each test. Line furrow patterns were photographically documented and the furrow depth was measured every 2 cm along its length using a set of digital calipers (±0.02 mm). The midline of the leading edge was marked and designated as the zero point for these measurements. Positive numbers denote proximity to the motor in all measurements. Relative dermal abrasion was compared in two ways: 1) maximum depth of dermal penetration and 2) the length of epidermal removal. This length measurement was taken along the curve of the sample with a flexible ruler (±1 mm). It included the region of exposed dermis but excluded cracked epidermis on either end of the furrow. This cracking could have represented an additional length of epidermal failure but was not objectively measurable.

Chapter 3

RESULTS

STRAIGHT PULL ABRASION (SPA) TESTING

Among the three specimens, the right whale calf flipper had the thickest epidermis (8.0±0.2 mm). Both the right whale and humpback whale flukes had similar epidermal thicknesses, measuring 5.1±0.1 mm and 4.9±0.4 mm respectively. Despite differences in thickness, the mode of epidermal failure was similar in each of the tissue samples. Prior to abrasion tests the epidermal surface was typically smooth with a slick rubbery exterior. Once these exterior layers were removed by an initial period of abrasion a rougher sponge-like tissue was revealed beneath, presumably the stratum intermedium. As abrasion continued, cracking of the epidermal material began. Initially, crack formation occurred directly beneath and in the same direction as the abrading line. Subsequent cracking then appeared at approximately 45° to the direction of line travel, creating a patchwork of cracked epidermal material reflective of the twisted three stranded line construction. With continued abrasion this cracking of the epidermis intensified and was followed by the epidermis being pulled out in chunks, separating from the underlying dermis at the dermal/epidermal interface (Figure 7).

(a)

(b)

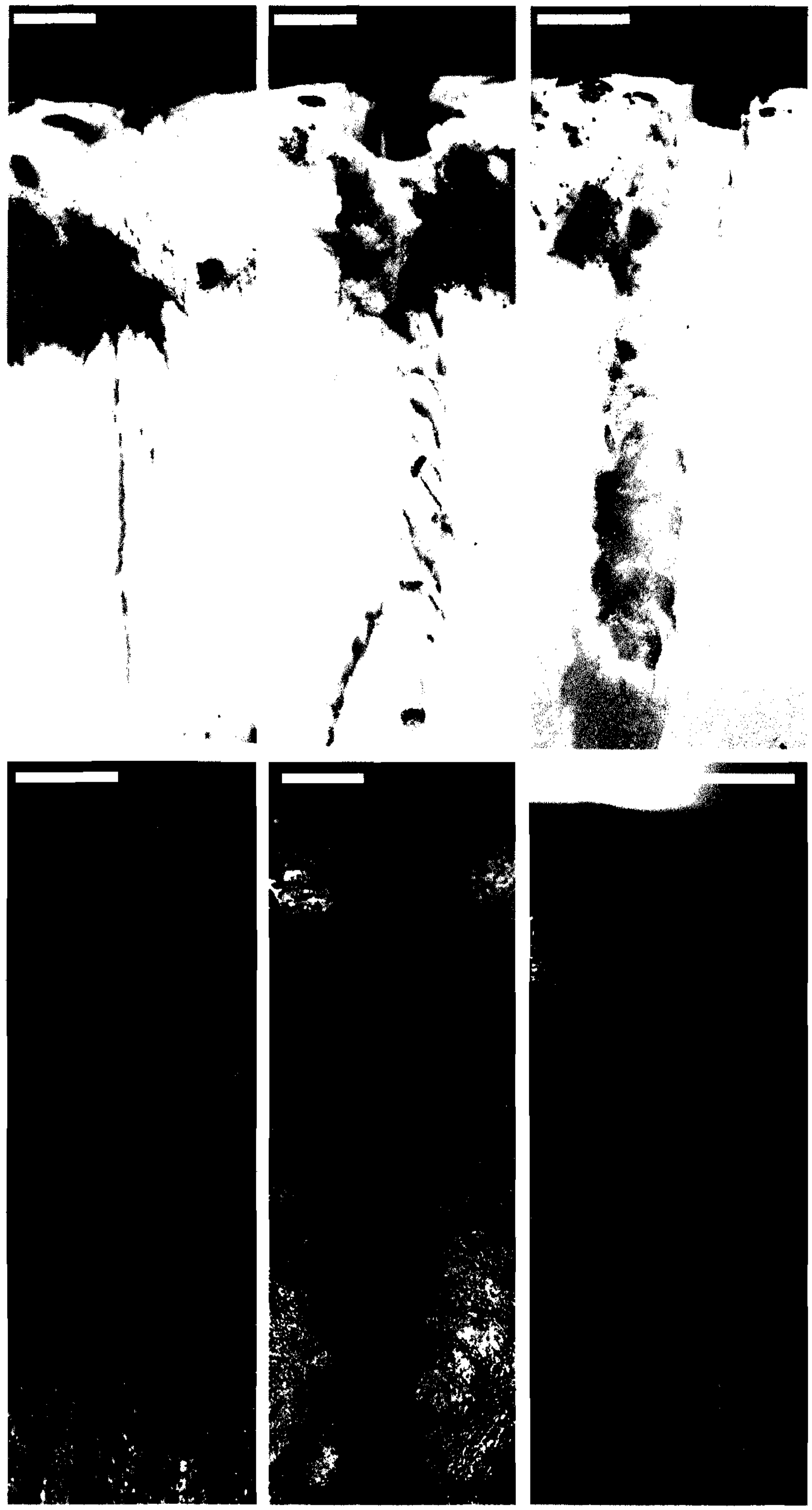

FIGURE 7. Progression of epidermal failure following line abrasion: (a) humpback whale fluke, (b) adult right whale fluke. Sequence progresses from left to right. Scale bars represent 1 cm.

A substantial difference in epidermal abrasion resistance was found between the 3 test specimens. A 3.1-m draw-length with a 6.8-kg load produced epidermal cracking on the right whale calf flipper specimen, while an 11.3-kg load produced a region of epidermal removal 15.76-mm long with a maximum depth of 8.44 mm. Thus, the point of epidermal penetration on the calf flipper occurs somewhere between 6.8 and 11.3 kg using a 3.1-m draw-length. Using the same draw-length (3.1 m), the humpback whale fluke withstood a 27.7-kg load prior to epidermal penetration. The right whale fluke specimen showed an even greater resistance to abrasion. A 31.8-kg load and a 45.7-m draw-length (requiring the splice to pass over the sample 14 times) only produced the initial stage of epidermal cracking beneath the abrading line.

A second series of tests were conducted on the humpback fluke to determine if statistical differences in abrasion rates exist when using various line materials. A standard 31.8-kg load was applied to the test system using a 3.1-m pull. Four test materials were examined, each with a 9.5-mm diameter: new and used polypropylene float and polypropylene/ polyester sink lines. Each line type was tested 3 times. The resulting maximum furrow depth and length of epidermal removal are shown in Table 2. No statistical difference in abrasion rates between line types was found at the 0.05 level (Wilcox-Mann-Whitney rank sum test). However, the new float line had the smallest average furrow depth and length of epidermal removal (5.4 mm and 33 mm) while the new sink line had the largest furrow depth and length (6.8 mm and 79 mm). Both used float and new sink were intermediate to these extremes.

Line Material Comparison								
	New-float		*Used-float*		*New-sink*		*Used-sink*	
	Depth	*Length*	*Depth*	*Length*	*Depth*	*Length*	*Depth*	*Length*
Test1	5.0mm	44mm	6.6mm	64mm	7.6mm	94mm	7.3mm	66mm
Test2	5.3mm	26mm	5.5mm	84mm	7.1mm	68mm	6.6mm	73mm
Test3	5.0mm	29mm	6.2mm	69mm	5.8mm	76mm	6.6mm	71mm
Mean	**5.4mm**	**33mm**	**6.3mm**	**72mm**	**6.8mm**	**79mm**	**6.8mm**	**70mm**
SD	*0.5*	*9.6*	*0.6*	*10.4*	*1.0*	*13.3*	*0.4*	*3.6*

TABLE 2. Comparison of abrasion rates in relation to line material. The maximum furrow depth and length of epidermal removal according for each line type are provided.

The influence of line diameter and specimen curvature was examined in two regions on the humpback fluke. Both 6.4-mm and 9.5-mm diameter lines were tested on adjacent regions of the most proximal (widest) and most distal (narrowest) portions of the fluke. The 6.4-mm line caused the greater depth and length of epidermal removal in both cases (Table 3).

Line Diameter and Sample Curvature Comparison				
	Wide Proximal Sample		*Narrow Distal Sample*	
Line Diameter	*Furrow Depth*	*Length of Removal*	*Furrow Depth*	*Length of Removal*
6.4 mm	7.24 mm	147 mm	6.49 mm	117 mm
9.5 mm	5.65 mm	44 mm	4.02 mm	61 mm

TABLE 3. Comparison of abrasion rates relating to line diameter and sample curvature. The maximum furrow depth and length of epidermal removal for each trial are provided.

OSCILLATORY PULL ABRASION (OPA) TESTING

Epidermal abrasion as a result of oscillatory loading of the tissue was examined in the right whale calf flipper and the humpback whale fluke using the OPA system. With an 11.3-kg tensioning load applied to a 9.5-mm new float line, the compliance limit in the calf flipper was determined to be 2.4±0.08 cm. The compliance limit in the humpback whale fluke was determined to be 4.45±0.10 cm using a 71.8-kg load on the test system (Figure 8).

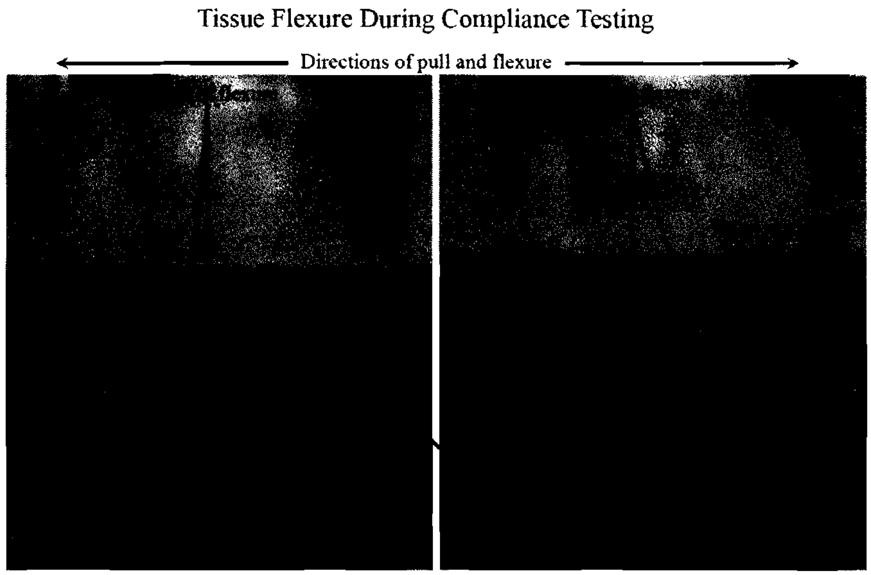

FIGURE 8. Tissue flexure during compliance testing of the humpback fluke specimen. Short line shows a vertical orientation of the sample under a no-load condition. Longer line shows the midpoint of the leading edge once the sample has been loaded. Angular difference shows flexure of the tissue as the load is applied.

Two oscillatory tests were then conducted on each specimen using new 9.5-mm float line: one with a 2.5-cm and one with a 7.6-cm draw-length. These draw-lengths were selected to be below or above the compliance limit of the tissue. Test loads were determined based up on the compliance testing: 11.3-kg for the calf flipper and 71.8-kg for the humpback fluke. In each case, the 7.6-cm draw-length cut deeply into the underlying dermis while the 2.5-cm draw-length did not break the epidermis (Figure 9). In the calf flipper, the 7.6-cm draw-length test resulted in a maximum furrow depth 5.9 times as deep as that of the 2.5-cm draw-length (3.78 mm compared to 22.15 mm). It also left a 139-mm long region of epidermal removal whereas the 2.5-cm draw-length did not penetrate the epidermis. The difference in furrow depth was even greater in the humpback whale fluke. The 7.6-cm draw-length caused a maximum furrow depth 8.5 times as deep as the 2.5-cm draw-length (3.9 mm compared to 33.1 mm), and left a 169-mm length of dermal tissue exposed. The characteristics of the furrow also differed between the 2.5-cm and 7.6-cm draw-lengths in each specimen. The shorter draw-length (2.5 cm) produced a shallow furrow showing extensive streaking and a deeper three-stranded dimpled pattern representative of the 3-stranded rope construction as noted in the Woodward *et al.* (2006) study. The longer draw-length (7.8 cm) left a smooth furrow without dimpling on either the epidermal or dermal tissue.

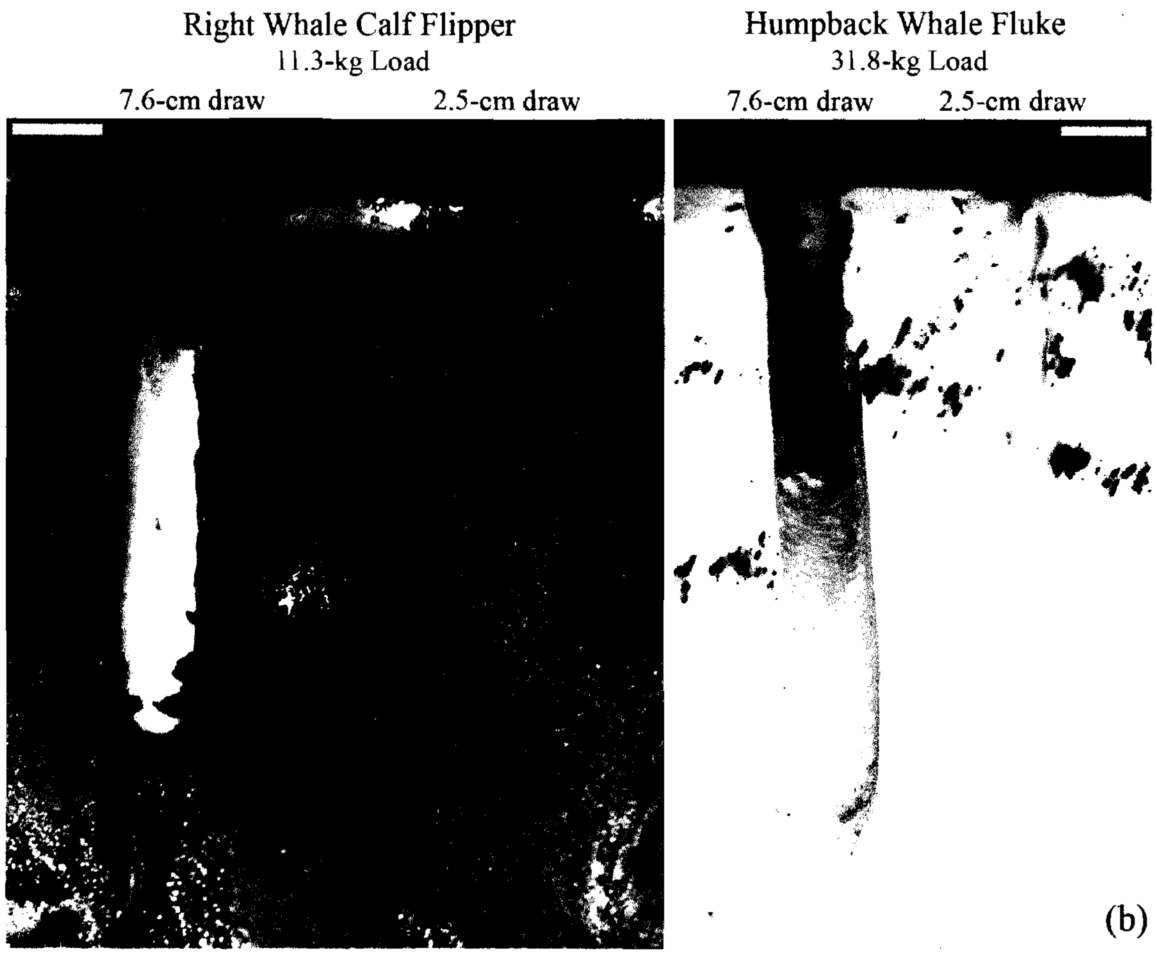

FIGURE 9. Comparison of oscillatory abrasion furrows using different draw-lengths: (a) right whale calf flipper, (b) humpback fluke. The 7.6-cm draw-length cut significantly deeper into the skin tissue for both tissue specimens. Scale bar represents 1 cm.

Chapter 4

DISCUSSION

COMPARATIVE ABRASION RESISTANCE BETWEEN SPECIMENS

Epidermal abrasion resistance varies according species (humpback vs. right whale) sample location (flipper vs. fluke) and age (adult vs. calf) of the animal. However, it does not appear to be related to epidermal thickness. Of the three specimens, the right whale calf flipper had the thickest epidermis (8.0±0.2 mm) but required the lowest load (8.6 kg) and draw-length (3.1 m) combination to penetrate the epidermis following tests with the SPA system. The two fluke specimens had similar skin thicknesses (4.9±0.4 mm for the humpback and 5.1±0.1 mm for the right whale) but required different loads (27.7 kg vs. 31.7 kg) with dramatically different draw-lengths (3.1 m vs. 45.7 m) to generate epidermal failure. At the two extremes, the right whale fluke withstood 15 times the draw-length (3.1 m vs. 45.7 m) with 3.7 times the load (8.6 kg vs. 31.7 kg) of the calf flipper prior to epidermal failure. Potential factors that may influence differences in the abrasion resistance among the three specimens include: the age of the animal from which tissue samples were collected, species specific variations in mechanical properties of the epidermis, differences in dermal structure between body regions (flippers vs. flukes), curvature of the tissue and contact length of the line on the sample, and any freezing/thawing effects on the tissue. The presence of these factors make it difficult to isolate the influence of individual factors on the observed differences in epidermal abrasion resistance between samples, but the overwhelming conclusion from this study is that regardless of age, species, or sample conditions, compliance in the tissue is the critical factor.

Due to the necessity of opportunistic sample collection at necropsy events, consistency in age, species and body region could not be maintained across test specimens. A substantial difference in age existed between the different whale specimens. Right whale Eg 1004 was a mature adult at least 24 yrs old. Humpback whale VAQS 2006 1007 Mn was a minimum of 3 yrs. old, and right whale Eg NEFLO 602 was less than 1 year old (probably 2-3months). The tensile properties of skin in other vertebrates are known to change with age, typically increasing in strength and decreasing in elasticity through middle age (Cloete *et al.* 2004, Cua *et al.* 1990, Vogel 1994). It is unknown whether similar age effects may affect skin properties and abrasion resistance capacity in cetaceans. Oil content in the dermal tissues was also markedly different between specimens. The adult right whale fluke sample was substantially oilier than either the humpback fluke or the right whale calf flipper. The oil content may be indicative of an age-related difference in tissue properties within a species or between species. In any event, it likely influenced the coefficient of friction between the epidermal surface and the abrading line, helping to minimize abrasive injuries.

DIFFERENCES IN EPIDERMAL MECHANICAL CHARACTER

Differences in the structure of the epidermis between body regions (flipper vs. flukes) may exist. Although the stages of epidermal failure appeared consistent across specimens, the rate at which this failure progressed differed between body regions. In the calf flipper, epidermal removal followed quickly once the first signs of cracking appeared. However, in the fluke specimens the progression from initial crack formation to epidermal removal occurred at a much slower rate with a patchwork of interlocking cracks spreading across the furrow. This advanced cracking stage could be compared to a rug-like structure with stalks of epidermal tissue remaining firmly attached to the dermal/epidermal interface beneath, but no longer being attached to adjacent fibers (Figure 10).

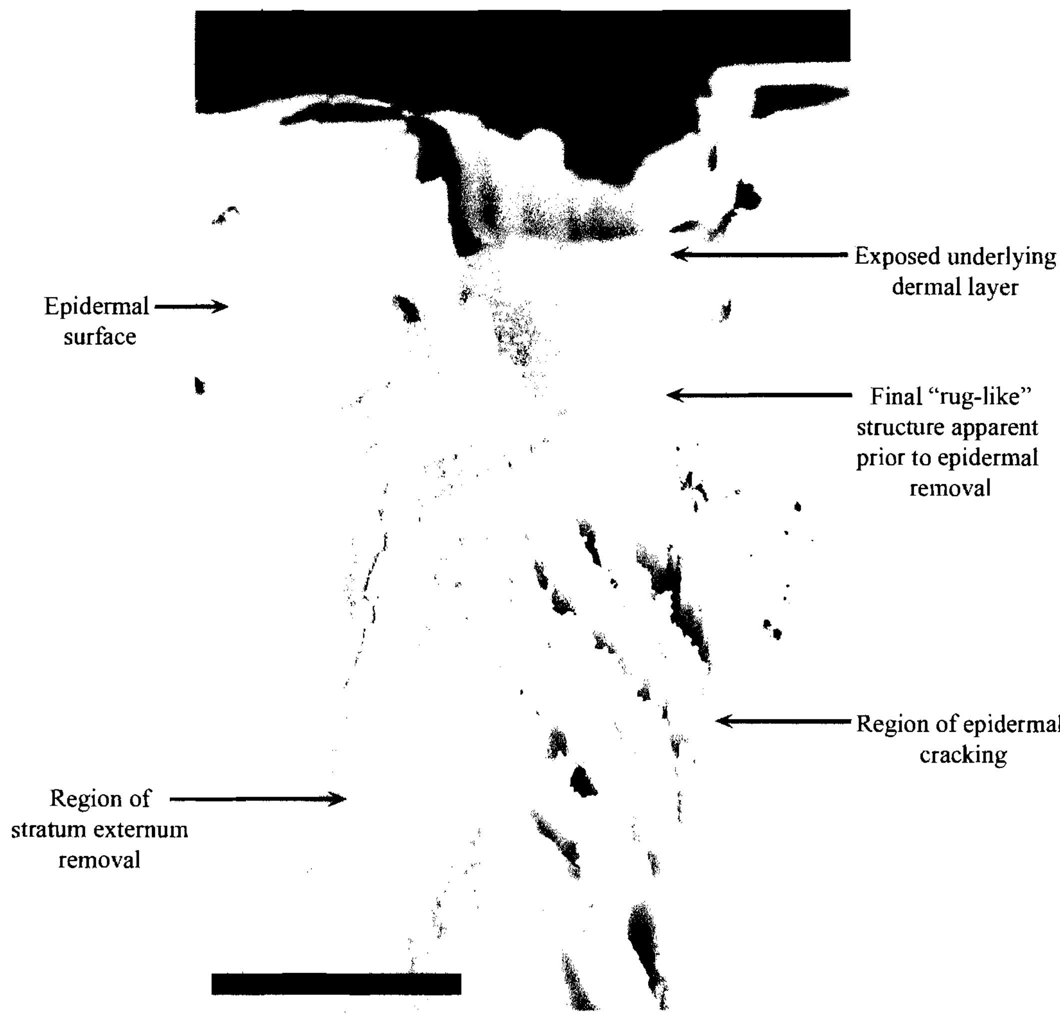

FIGURE 10. Close up image of the stages of epidermal failure. Scale bar represents 1 cm.

There appears to be a vertical orientation in epidermal structure within the cetacean integument. It has been suggested that the presence of epidermal rods provides support to regions of thick (>4 mm) epidermis without deep papillae penetration in bowhead whale skin (Haldiman *et al.* 1981, Haldiman *et al.* 1985). In these regions, epidermal cells encircling the sides and ends of the papillae form solid keratinized structures separated by epidermal cells with long axes parallel to the epidermal surface. Henk and Mullan (1996) observed that superficial lacerations that do not penetrate to the dermal papillae follow a progressive change in epidermal surface properties during the healing process. The first stage of this process is keratinization of the epidermal rods followed by a progression of keratinization within the stratum intermedium beneath the necrotic tissue, which is then shed. Keratinocyte rosettes have been documented in right whale calf epidermis, suggesting the presence of similar epidermal features in right whales (Reeb *et al.* 2005). However, the structural functions of vertical features within the epidermis have not been evaluated for any baleen whale species.

Observations from both bowhead and right whales indicate that the primary strength in the epidermis is oriented in a vertical direction while laterally the structure is more elastic with lower strength. From a structural standpoint, this type of fiber integration would have several mechanical advantages. Strong vertically oriented fibers would both increase the abrasion resistance of the epidermis and may help to decrease the compressibility of the layer (Figure 11). As a lateral abrading force is applied to the surface of the epidermis, the strong vertical fibers would start to bend, orienting in the direction of the applied load. This flexure would allow the load to be applied in line with the vertical fibers that are anchored through the entire epidermal layer. Retaining elastic elements between these vertical fibers would maintain the flexibility needed in the epidermis allowing the epidermis to stretch during normal swimming activities. This elasticity may be graded through the stratum intermedium as the degree of keratinization and flattening of the epidermal cells has been noted to increase with distance from the stratum germinativum (Haldiman *et al.* 1985).

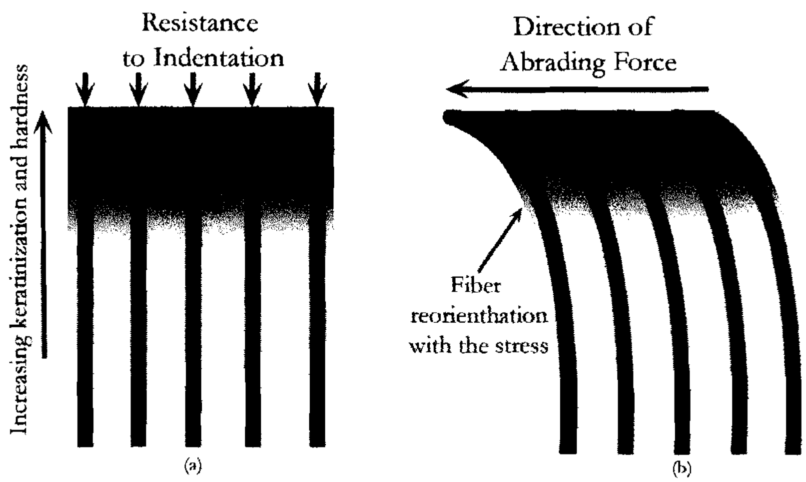

FIGURE 11. Structural advantages of a vertical fiber orientation within the epidermis. Fibers provide resistance to: (a) indentation and (b) lateral abrasion.

The depth of penetration and character of the dermal papillae may also substantially influence the strength of the epidermis as a unit. Assuming that a more multidirectional orientation of collagen fibers in the dermal material enhances the tensile properties of the dermis, the volume of this dermal material interwoven within the epidermis would substantially influence the structural characteristics of the layer.

Differences between the right whale flipper and humpback fluke were most noticeable in the later stages of epidermal failure. The primary failure mechanism following cracking appeared to be removal of the epidermis in chunks at the dermal interface. Three potential factors may account for this difference:

1) The epidermal/dermal connection could be stronger in the fluke.
2) The depth of penetration of the dermal papillae and/or the shape of the distal tips of the dermal papillae could be greater in the fluke tissue, increasing connection surface area and hence increasing attachment strength.

3) Assuming that the dermal papillae are removed along with the epidermis during epidermal failure, a difference in the degree of collagenization of the dermal papillae and a subsequent difference in the tensile properties could exist regionally between the flippers and flukes.

Future studies examining the relative differences between the flipper and fluke structure from the same animal and multiple individuals from the same species would be helpful in determining if the observed differences in abrasion resistance are related to the biomechanical structure of adult versus calf epidermal tissue or if flippers have a lower abrasion tolerance than flukes. Several types of tests are needed. An abrasion study examining the comparative durability of the flipper and fluke samples should be conducted. Second, mechanical evaluation of the tensile properties of the dermis from the two regions should be made. And finally, an analysis of the 3D structure of the dermal papillae should be made. Knowlton *et al.* (2005) noted that a higher percentage of new entanglement scars are observed on juvenile versus adult animals. Assuming the difference in abrasion resistance is due to age alone, it may be that benign entanglements (e.g. non-injury producing and self disentangled) are occurring in adults without scar formation to a greater extent than has been previously reported. Conversely, if this difference in observed entanglement scaring is due to variations in epidermal abrasion resistance between flippers and flukes rather than age, then entanglements involving flipper wraps may warrant even greater concern than was previously believed.

DIFFERENCES BETWEEN INDIVIDUAL SAMPLES

Variations in sample curvature may also contribute to variations in abrasion resistance. Straight pull abrasion tests yielded longer but shallower furrows on the right whale fluke and shorter but deeper furrows on the calf flipper. With the wider curvature and greater thickness of the leading edge in the right whale fluke (Figure 12), the load from the abrading line is distributed over a greater surface area, which would tend to mitigate abrasive impacts. On the other hand, the narrow leading edge of the calf flipper would tend to concentrate the load at a particular point, accelerating the abrasion process. It is interesting to note that tests with 6.4-mm and 9.5-mm diameter float line on the proximal (widest) and distal (narrowest) regions of the humpback fluke showed a greater depth and length of abrasion on the wider sample with both line diameters (Table 3). Although the range in curvatures represented by the proximal and distal ends of the humpback fluke is not as great as that presented by the right whale fluke and calf flipper, these results suggest that the mechanical properties of the skin may have a greater influence on abrasion resistance than the curvature of the sample.

FIGURE 12. Visual comparison of the differences between test specimens. A photograph of representative test samples: right whale calf flipper (left), humpback fluke (center), and adult right whale fluke (right) is shown.

Variations in the condition of the sample may also play an important role in abrasion resistance. The influence of the freeze/thaw process on whale skin has not been examined but is known to increase variability in mechanical tests on human skin (Millington and Wilkinson 1983). An evaluation of the mechanical properties of integument before and after a freeze/thaw cycle are needed in order to verify the real world application of data obtained from experimental modeling studies.

THE INFLUENCE OF LINE MATERIAL ON THE RATE OF ABRASION

Overall, it appears that the abrasion resistance of the skin is more a function of epidermal characteristics than of the abrading rope material. No statistical differences were found in relative abrasion rates between new and used float and sink lines. Sink lines appeared to be slightly more abrasive than float lines. Abrasion increased with wear in the float lines but decreased slightly with sink line materials. The apparent difference between new and used line was greatest in float lines presumably as a result of fraying of the polypropylene fibers that increased the abrasiveness of the line. Larger sample sizes are needed for further statistical comparisons. On the other hand, the diameter of the abrading line does appear to be an important factor in determining the rate of epidermal abrasion. Comparisons between 6.4-mm and 9.5-mm diameter new float lines revealed that the smaller diameter line consistently cut deeper into the fluke tissue and had a greater length of epidermal removal in both tests.

OSCILLATORY ABRASION AND ENTANGLEMENT INJURIES

In regards to oscillatory loading, tissue compliance appears to be a primary factor in mitigating the abrasive impact of rope on whale skin. Tests with the OPA system revealed that a draw-length above the tissue compliance limit (7.8 cm) cut deeply into the dermal tissue while draw-lengths below the compliance limit (2.6 cm) did not penetrate the epidermis in either the right whale calf flipper or the humpback whale fluke (Figure 9). This result suggests that although characteristic indentation furrows such as those found in the Woodward *et al.* study are produced as lines are pressed into the skin in an oscillatory manner, tissue removal does not occur until static friction is overcome and the line slides across the epidermal surface. Once this sawing and subsequent tissue removal process begins, the rate of abrasion increases substantially. In actual entanglement situations, draw-length may be the critical component that determines if the line will cut into the body or benignly press against the skin.

Differences in wound generation are exemplified by the case of right whale NEAq Eg 2301. Two entangling lines were woven through and fixed in the baleen, exited the right side of the animal's mouth, passed over the rostrum and blowhole and terminated in wraps of line about the animal's left flipper (Figure 13).

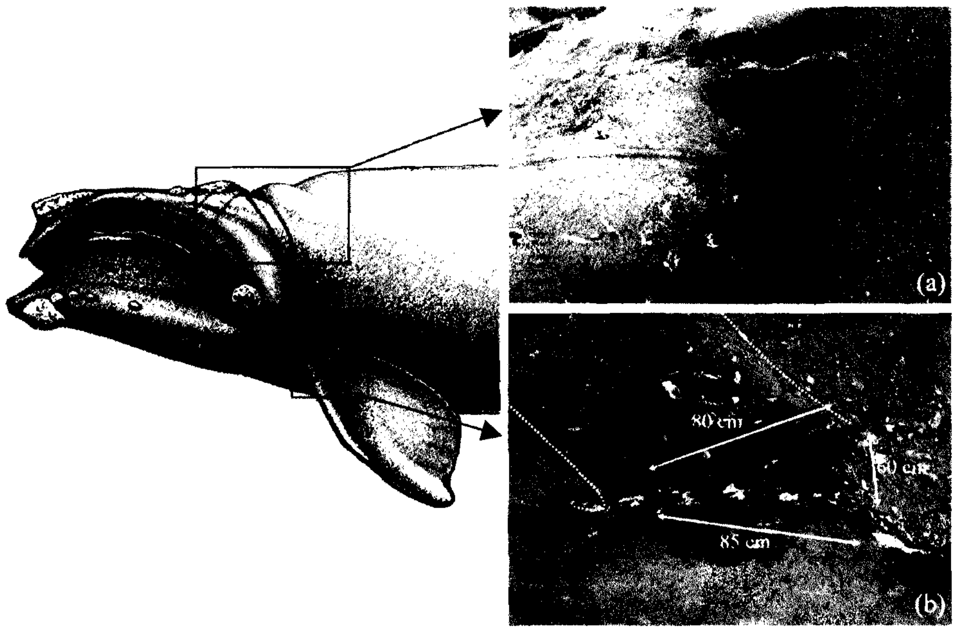

FIGURE 13. The entanglement of NEAq Eg 2301. Artist's rendition of the location of entangling lines as observed from photographs of the animal (left) and photographs of the abrasion marks on the head (a) and left flipper (b) are shown. Drawing by Scott Landry of the Center for Coastal Studies.

During necropsy, indentation furrows were visible on either side of the head, across the rostrum and blowhole of the animal. Although the epidermis was no longer attached due to an advanced stage of decomposition, marks from the entangling lines remained imprinted in the underlying dermis. On the head, substantial penetration of the dermal tissue was not apparent except where one of the lines crossed the left blowhole. Instead, indentation patterns following the 3-stranded twist of the entangling line were visible in the dermis (Figure 13a). On the other hand, a severe laceration penetrating to the bone was observed on the leading edge of the left flipper over the proximal third of the humerus as a result of the flipper wraps (Figure 13b)(Moore 2005 Necropsy Report). The oscillatory abrasion results from this study suggest that in the case of Eg 2301, the movement of the flipper during normal swimming motions, and presumably the opening and closing of the blowhole exceeded the tissue compliance and accelerated abrasion rates in these two locations. In contrast, the movement of the lines passing over the rostrum and along the side of the animal's head did not exceed the skin compliance and as a result did not cut into the dermal tissue beneath.

CONCLUSIONS

This study suggests that reducing line movement across the surface of the epidermis is the most significant factor in preventing serious entanglement injuries such as the case of Eg 2301. Changing draw-lengths from 2.5 to 7.6 cm could mean the difference between a deep laceration and a dimpled furrow that does not penetrate the epidermis. Lines with decreased modulus could be developed to stretch and absorb a portion of the potential abrasion during entanglement. For a line anchored at both ends, if the rope flexes with the whale's body, it will help prevent slippage of the line relative to the skin. Investigations into the potential of decreasing line modulus (or increasing the line "stretchiness") may provide clues that could lead to a reduction in serious entanglement injuries. Development of such a line, however, would have to incorporate a reduced recoil speed to retain operational safety for the fisherman. Future studies are needed in order to resolve these questions and to evaluate the practicality of decreasing line modulus.

RECOMMENDED FUTURE WORK

- ◊ Mechanical tensile testing of cetacean integument
  - Mechanical evaluation of the tensile properties of the dermis evaluating both regional variation and directionality due to tissue fiber orientation
    - ➢ A method for consistently removing a uniform thickness of dermal tissue just beneath the epidermis and cutting it into dumbbell shaped test samples is needed.
    - ➢ An effective clamping technique must be developed to securely clamp and align the samples without compromising the test tissue.
  - Mechanical evaluation of the tensile properties of the epidermis
    - ➢ Due to the complex 3-D nature of the dermal/epidermal junction, an ultrasonic method may be the best means of determining the tensile properties of this layer.
- ◊ Analysis of the influence of the freeze/thaw process on the mechanical properties of cetacean integument
- ◊ Additional straight pull abrasion testing
  - Evaluate the abrasion resistance of the flipper and fluke from the same animal and a series of animals from the same species.
  - Evaluate of age related differences in abrasion resistance within a species.
  - Evaluate of differences between cetacean species.
- ◊ Compressive load analysis
  - Determine the influence of large (50 to 1,000 kg) loads on the mechanical behavior of cetacean skin.

    - Design and construct a test system to evaluate loading necessary to cause a laterally static compressive load injury leading to epidermal failure.
    - Determine the influence of large loads on the rate of abrasion, tissue compliance, and draw-length prior to epidermal failure.

- ◊ Development of a surrogate synthetic whale skin
  - Following tensile testing and mechanical evaluation of cetacean skin, a synthetic material should be selected with similar properties to that of the cetacean epidermis.
    - This would eliminate the primary problem of obtaining skin tissue samples and allow statistical evaluation of mechanical tests and proposed gear modifications.
    - It would also provide a means to test other whale research tools such as tag attachments techniques, drug administering devices, and biopsy darting practices.
- ◊ Potential gear modification analysis
  - Evaluate the potential for decreasing line modulus.
    - Modify the Oscillatory Abrasion System to allow evaluation of the influence of line modulus on abrasion resistance in a test sample.
    - Estimate the effective line modulus necessary to prevent oscillatory abrasion in an entangled whale during normal swimming activities.
    - Determine if a line modulus change would be practical for the fishing industry and address any safety concerns associated with this change.

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Jeremy Winn was born in San Diego, California on December 11, 1977. He spent his childhood in the Rocky Mountains living predominantly in New Mexico and Montana and graduating from Darby High School, Darby, Montana in 1996. Jeremy attended the University of Montana for 3 years in the wildlife biology program but then took two years off from school to pursue other interests. These included completing a wilderness emergency medical certification course from the Airie School of Backcountry Medicine, becoming a certified Baja California, Mexico kayaking guide and eventually becoming a skipper for the Coastal Ecosystems Research Foundation in British Columbia, Canada, conducting whale research and ecotourism. He came to The University of Maine in 2001 to pursue his interest in marine biology and received a B.S. in Marine Sciences in December 2003. Jeremy is a candidate for the Masters of Science degree in Marine Bio-Resources from The University of Maine in August, 2006.