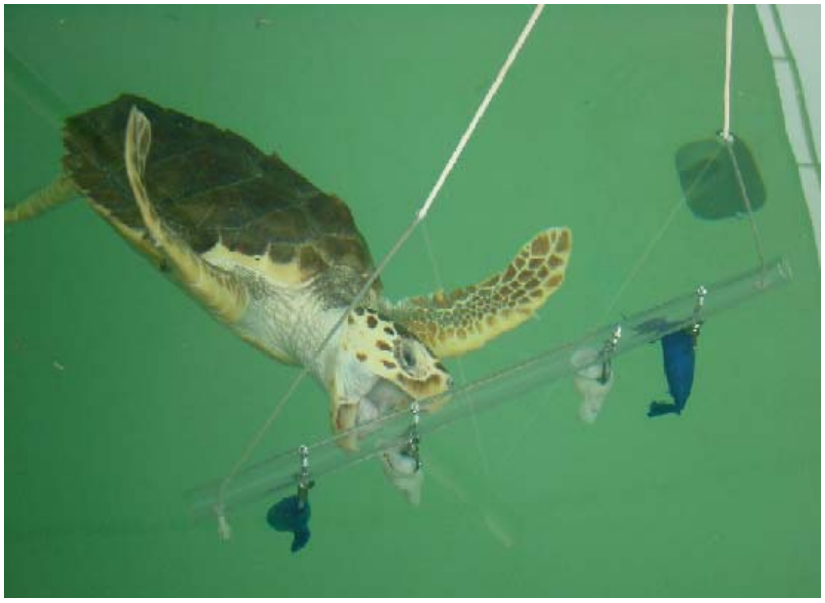
NOAA Technical Memorandum NMFS-PIFSC-7

December 2006

Sea Turtle and Pelagic Fish Sensory Biology: Developing Techniques to Reduce Sea Turtle Bycatch in Longline Fisheries



Compiled and Edited by

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and
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U.S. Department of Commerce

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Cover photo courtesy of Yonat Swimmer. Captive loggerhead (*Caretta caretta*) turtle biting baits during experiments to investigate potential food color preferences at the NOAA Sea Turtle Facility, Galveston, Texas.

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Sea Turtle and Pelagic Fish Sensory Biology: Developing Techniques to Reduce Sea Turtle Bycatch in Longline Fisheries

Compiled and Edited by

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PREFACE

Three of the five sea turtle species that live in the Pacific Ocean (loggerhead, *Caretta caretta*; green, *Chelonia mydas*; and olive ridley, *Lepidochelys olivacea*) are listed under the U.S. Endangered Species Act of 1973 as threatened. The other two species (leatherback, *Dermochelys coriacea,* and hawksbill, *Eretmochelys imbricata*) are listed as endangered. Recovery of all sea turtle populations is severely hindered by multiple factors that may include human-caused mortality from harvesting of adults and poaching of eggs on nesting beaches, natural mortality from disease (e.g., fibropapillomatosis in some populations), destruction of nesting beach habitat, and incidental capture of turtles in fishing gear.

In particular, the high level of incidental capture of sea turtles in pelagic longline fisheries is of great concern to environmental groups, the fishing industry, and fisheries managers in the U.S. and other countries. Regulatory measures to protect sea turtles in both the Pacific and Atlantic Oceans have recently been introduced in U.S. fisheries, including longline gear modifications (e.g., use of large circle hooks) and time-and-area fisheries closures (U.S. Department of Commerce 1999, 2000; Watson et al., 2005).

Most interactions between longline fishing gear and sea turtles occur with shallow-set gear targeting swordfish (*Xiphias gladius*), mahi mahi (*Coryphaena hippurus*) or surface-feeding tunas (*Thunnus spp).* Opportunistic-feeding hard-shelled turtles, such as loggerheads and olive ridleys, generally are caught by biting the baited hooks, whereas leatherback turtles are most often hooked in the flippers or become entangled in fishing line (Witzell, 1996). When handled properly, most hard-shelled turtles released alive after encounters with shallow-set fishing gear appear to survive for at least the first few months after release (Chaloupka et al., 2004; Swimmer et al., 2006). Sea turtles are also caught by deep-set (> 100 m) longline gear targeting bigeye tuna (*T. obesus*) (Ferreira et al., 2001). This type of longline gear, in general, catches fewer turtles, but the incidental mortality rate of turtles is higher (see Gilman et al., 2006). Turtle mortality attributed to deep-set longline gear is usually a result of drowning and occurs when turtles are hooked or entangled at depths that prevent them from reaching the surface to breathe.

Ways to reduce longline-turtle interactions and the mortality caused by such encounters are needed. Solutions may result from research on the behavior, distribution, and sensory physiology of sea turtles and the pelagic fish species targeted by longline vessels. Most promising are studies to define and exploit differences in sea turtle and fish sensory physiology. Sea turtles and pelagic fishes are evolutionarily distinct groups of animals with differences in vision, hearing, and olfaction that may influence the ways in which they interact with fishing gear. The factors that attract sea turtles and target fish species to longline gear and bait are not well understood, but numerous sensory cues may be involved.

In 2001, scientists of NOAA Fisheries created the Sensory Biology Working Group and launched a multidisciplinary, interagency research program to investigate the visual, auditory, and chemosensory abilities of sea turtles and pelagic fishes. The purpose of the research was to identify differences between turtles and pelagic fish species that may be used to develop gear and bait attractive to fish but unattractive to sea turtles or undetectable by

them. The overall plan has been to proceed simultaneously along several tracks employing modern molecular genetic techniques (to identify receptor molecules), standard electrophysiological methodologies (to record responses to specific stimuli and define detection thresholds), and behavioral experiments in several species of sea turtles and commercially important tunas and billfishes. The primary objective of the research is to develop techniques and/or commercially viable devices that eliminate or substantially the interactions of sea turtles with longline fishing gear while not reducing catch rates of the targeted fish species to unacceptable levels.

Research projects have been underway since 2001, supported by funding from the Pacific Islands Fisheries Science Center (PIFSC), NOAA Fisheries, in Honolulu, Hawaii. Because of the complexity of the research, projects have necessarily involved a large and diverse team of scientists. Collaborating scientists have held three meetings to discuss research progress.

This NOAA Technical Memorandum presents scientific work produced by the working group including research discussed at the first meeting, hosted by the PIFSC in January 2003 in Honolulu, Hawaii. The contributions concern the development of gear modification that show promise in reducing interactions of sea turtles with baited longline fishing gear. A PDF of this document is available at http://www.pifsc.noaa.gov/library.

Research progress was also presented and discussed in January 2005 at the 25th Annual Symposium on Sea Turtle Biology and Conservation in Savannah, Georgia, in a special session devoted to sensory physiology studies; contributions from the Savannah symposium will be published in a forthcoming NOAA Technical Memorandum.

A third meeting, in May 2005, was hosted by Ken Lohman at the University of North Carolina, Chapel Hill, NC, to structure future research projects.

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DIFFERENCES IN THE VISUAL CAPABILITIES OF SEA TURTLES AND BLUE WATER FISHES—IMPLICATIONS FOR BYCATCH REDUCTION

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Sea turtles are unfortunate bycatch in the longline fisheries, mainly because they share the same habitat as fish species targeted by this type of fishing activity. Both turtles and blue water fishes are highly visual animals, suggesting that visual attraction plays a role in interactions with longline fishing gear. In our study, we compared the visual capabilities of sea turtles and a number of blue water fishes with the hope of finding differences that might be used to design more species-specific fishing gear. We investigated eye design and optics, sensitivity to light and spatial resolving power, as well as the possibilities for color vision in green turtles (*Chelonia mydas*), tunas (*Thunnus spp*.), and billfishes (*Istiophoridae & Xiphiidae*) (Figs. 1, 2, 3). Compared to blue water fishes, the eyes of sea turtles appear to be better adapted for bright light vision, with a longer focal length (*f*) and a smaller pupil (diameter A) in relation to the size of the eye (Fig. 1).

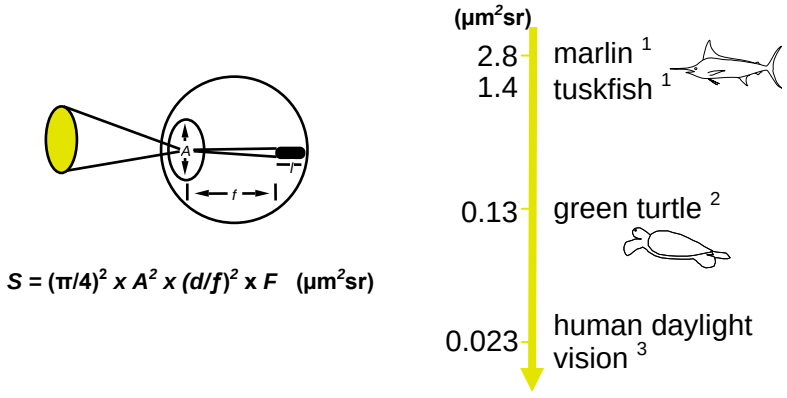

Figure 1. Optical sensitivity among selected vertebrate species. Land's formula considers the optics of the eye and the dimensions of the photoreceptors (diameter d) as well as the fraction of light (*F*) absorbed by the photoreceptors. The larger the sensitivity *S*, the more sensitive the eyes are to low light intensities. Sea turtles have relatively low optical sensitivity compared to billfish (1. Fritsches et al., 2003a; 2. Mäthger et al., in preparation; 3. Land, 1981).

The ability to resolve fine detail was a further visual capability we investigated in sea turtles and pelagic fishes. Based on our anatomical data, it appears that both sea turtles and pelagic fishes have similar abilities to resolve detail in their surroundings. Clearly this visual capability has evolved to suit the pelagic habitat of both groups.

Species	*Spatial resolving power (cycles/deg)*
Sea turtle	5 –11
Blue marlin	9
Yellowfin tuna	16 – 18
Bigeye tuna	11 – 13
Swordfish	7 –10

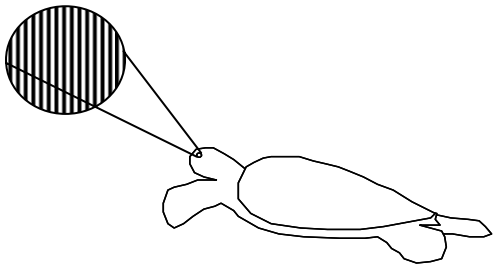

Figure 2. Spatial resolving power in sea turtles and fish, given as the numbers of cycles (one black and one white bar of a grating) that can be resolved within one degree of visual angle. Sea turtle: Anatomical, electrophysiological, and behavioral methods: Bartol, 1999. Pelagic fish: results using anatomical methods, Fritsches and Litherland, in preparation).

Identifying potential differences between sea turtles and pelagic fishes in their ability to detect colors was a further avenue we explored. Determining the potential spectral (color) sensitivity of the fishes' photoreceptors, we found that billfishes and tunas have a smaller sensitivity range (Fig. 3). For instance, longer wavelength (such as red) are invisible to swordfishes but not to sea turtles.

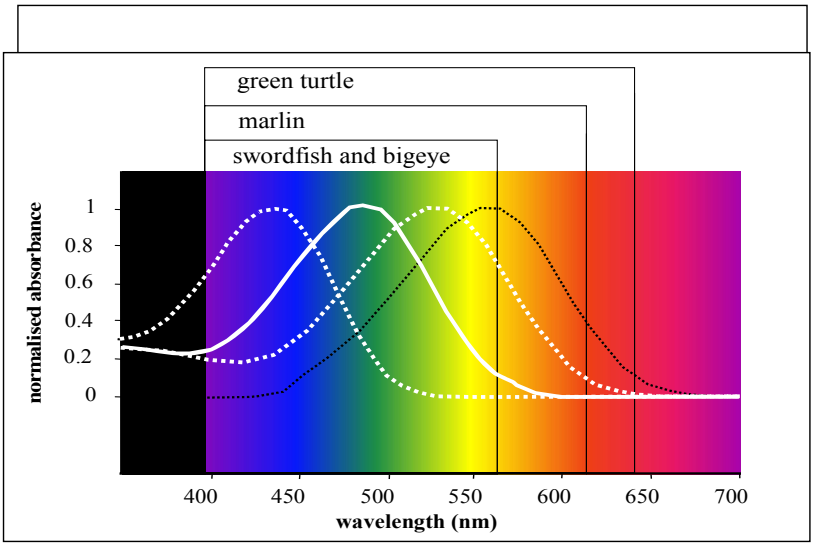

Figure 3. Possible spectral range of pelagic fish and sea turtles. While the photoreceptors of swordfish and bigeye tuna have visual pigments positioned in the shorter wavelength range (400 ~ 560 nm), striped marlin have a third pigment positioned towards the longer wavelength (own observations using microspectrophotometry; Fritsches et al., 2003b). The spectral sensitivity of green turtles (after the results of Liebman and Granda, 1971) shows sensitivities even further into the long wavelength band.

We also found that the lenses of tunas and billfishes block ultraviolet (UV) light, while the ocular media (cornea, lens, and vitreous humor) of green turtles transmit this waveband, potentially allowing these animals to perceive UV light (Mäthger, Litherland, Fritsches, submitted). Our anatomical and optical results indicate that sea turtles can see in the UV waveband, while blue water fishes cannot (Fritsches et al., 2000), thereby suggesting UV light as a possible "secret communication channel" in sea turtles. Our preliminary behavioral experiments in February 2005 indicate that sea turtles (loggerhead hatchlings) actually respond to steady UV light (LED with peak at 370 nm, Fig. 4) when swimming. These experiments take advantage of the natural tendency of the hatchlings to orient towards a light source and clearly showed that these animals can see UV light.

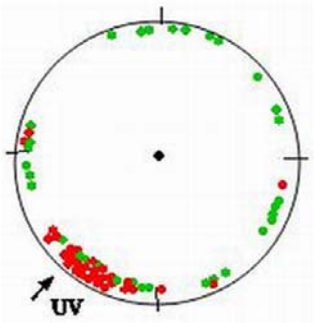

Figure 4. Preliminary data show that hatchling sea turtles can see UV light. The red dots show the orientation of the animal recorded (every 20 sec over 10 min) while the UV LED is on. The green dots show the random positions of the same animal with the UV light turned off.

Depending on the predominant light levels in their habitat, many animals differ in their ability to detect fast-moving objects. We investigated the flicker fusion frequency, a measure of animals' speed of vision, of a number of pelagic fish species and a green turtle (Fig. 5) to identify such species-specific differences (Fritsches and Warrant, 2001; Fritsches et al., in prep.). For instance, animals which predominately remain in the brightly lit surface layers of the ocean, such as the mahimahi and the green turtle, have retinas that are capable of responding to light flickering with a frequency of 60 Hz. Deep divers, such as the swordfish, do not perceive the light as flashing because their retina is not capable of resolving such fast motion (as indicated by the lower FFF). These differences strongly suggest that, depending on the frequency used, flashing lights, such as a flashing lure, will appear differently to a swordfish, sea turtle, and a yellowfin tuna.

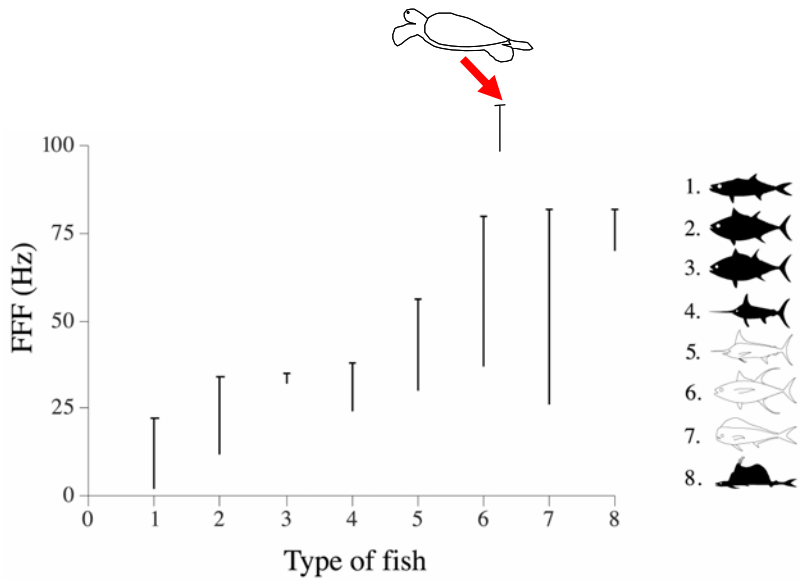

Figure 5. Flicker fusion frequencies of pelagic fishes and green turtle, recorded at a high light intensity. Species of fish tested: 1. Escolar, *Lepidocybium flavobrunneum*; 2. Bigeye tuna, *Thunnus obesus*; 3. Southern bluefin tuna, *Thunnus maccoyii*; 4. Swordfish, *Xiphias gladius*; 5. Striped marlin, *Tetrapturus audax*; 6. Yellowfin tuna, *Thunnus albacares*; 7. Dolphinfish, *Coryphaena hippurus*; 8. Lancetfish, *Alepisaurus ferox*. We recorded the electroretinogram (ERG) of the isolated retina in response to flashing lights and determined the FFF as an indicator for the animal's ability to detect fast flashing lights. Horizontal bars denote the highest FFF reached at this light intensity while vertical bars indicate the range of the responses recorded.

Preliminary results also show that vision in blue water fishes changes dramatically between day and night. The sensitivity to light increases at night and the response to fast flickering stimuli is markedly reduced solely as a function of time of day (Fig. 6). These findings are highly relevant for longline fisheries since fishing often extends through the night-day shift.

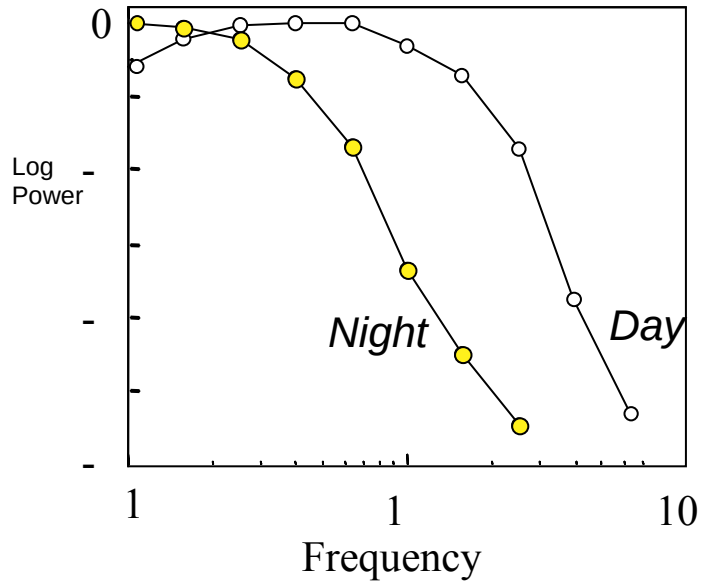

Figure 6. FFF in the yellowfin tuna measured during the day and at night in isolated retinae. The FFF (expressed in log power of the frequency response) in response to the same light intensity is markedly reduced at night, suggesting fundamental changes of visual capabilities between day and night (circadian rhythms).

Based on this range of studies, we conclude that a number of differences in visual capabilities, such as the ability to detect color and fast flashes, could potentially be used for designing more species-specific fishing methods.

Our experiments onboard National Marine Fisheries Service vessels have also shown that warm eyes significantly increase these animals' ability to detect movement (Fritsches et al., 2005), resolving one of the major puzzles in blue water fish physiology. This research allowed us the opportunity to answer the long-standing question of why swordfishes and tuna heat their eyes. Results of this study as well as other work can be found in the publication list below.

ACKNOWLEDGMENTS

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THE SENSORY BIOLOGY OF SEA TURTLES: WHAT CAN THEY SEE, AND HOW CAN THIS HELP THEM AVOID FISHING GEAR?

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BACKGROUND

The increased bycatch of marine turtles in longline fisheries seems linked to chemiluminescent light sticks used by these fisheries to attract fish. Reducing or eliminating the attractiveness of these light sticks might be achieved simply by using wavelengths (i.e., colors) or light intensities that do not appeal to marine turtles but attract the target fish species. Therefore, we initiated a series of studies to determine the light wavelengths that sea turtles could detect. Previous studies of sea turtle hatchlings suggest that they have limited color vision and were behaviorally attracted to blue wavelengths (Witherington and Bjorndal, 1991, Witherington, 1992).

We previously employed flicker-photometric electroretinography (ERG) to examine aspects of the visual sensitivity of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) sea turtles held in captivity at Sea World, San Diego. In this type of ERG, gross electrical changes are monitored at the corneal surface using a conductive contact-lens electrode while the eye is exposed to rapidly flickering monochromatic light (4 − 40 Hz). To determine sensitivity, retinal responses to the monochromatic light are summed for a series of approximately 50 presentations.

The intensity of the light is then adjusted until it elicits stimulation equal to a preset, unchanging value (e.g., 3.2 μV in the green and loggerhead turtle experiments). The relative sensitivity of the eye at each wavelength tested is thus reflected by the amount of light necessary to obtain the desired stimulation level. Sensitivity is thereby determined for each individual turtle at 10-nm increments from 400 to 700 nm. Although the procedure is essentially noninvasive, turtles are given an intravenous injection of general anesthetic, as well as a topical application of local anesthetic to the cornea to minimize any discomfort.

Green and loggerhead turtles were most responsive to rapidly flickering stimuli (16 − 24 Hz) and exhibited at least some sensitivity to lights ranging from 400 nm to 700 nm. Both species had peak sensitivity at about 580 nm (similar to human "yellow"). Also, for both species there was a dramatic drop in sensitivity above 650 nm and below 500 nm, although this cutoff was considerably more pronounced in the loggerhead turtles. The overall shape of the curves indicate that photopic spectral sensitivity for both species is most likely the product of multiple cone photopigment types, as well as the consequence of light-filtering oil droplets known to be present in turtle cone photoreceptors. The results of this work have been described in detail in Levenson et al. (in press), as well as in previous annual reports.

INTRODUCTION

Follow-up experiments were proposed and subsequently conducted to evaluate the spectral sensitivity of leatherback sea turtles (*Dermochelys coriacea*) using flicker-photometric electroretinography (ERG). This technique has been used successfully with a wide range of vertebrate species, including green and loggerhead sea turtles (described above). However, because leatherback sea turtles cannot be held in captivity, these experiments had to be performed in the field on a nesting beach where adult females come ashore to lay eggs. The site chosen for this project was Matura Beach in Trinidad (West Indies), one of the largest nesting colonies of leatherback turtles in the world. Dr. Eckert and the WIDECAST organization have served for more than a decade as the scientific advisor for a long-term population monitoring project at Matura Beach and have a unique history of working with the local community, obtaining permits from Government, etc.

The ERG system of Dr. Crognale was used for these experiments. Dr. Crognale has investigated the visual pigments of a variety of mammalian species, including humans, as well as some marine species (Crognale and Jacobs, 1991; Crognale et al., 1998; 1998a; 1999). The visual stimuli used for testing are derived from a Maxwellian-view optical system consisting of three distinct channels: a test channel, a reference channel, and an auxiliary adaptation channel. All three light channels are ultimately directed with a series of optical grade beam-splitters and achromatic lenses into a final channel. In the setup employed for the leatherback turtles, the light from the final beam was input to a liquid crystal light guide and positioned directly in front of the pupil of the turtle. The final image at the retina subtended over 60 degrees of visual angle. High-speed shutters control the presentation of light stimuli from each channel. For the test channel, monochromatic stimuli are created with a tungsten-halogen source and filtered using an electronic tunable interference filter. A variable neutral-density wheel located in the beam is used to regulate the intensity of the test stimuli. The reference channel shares the same source as the test channel. A separate tungsten-halogen light source is also used in the adaptation channels. Additional neutral-density and interference filters can be used to control the intensity and spectral content of these channels, when necessary.

Experimental procedures were conducted exclusively on nesting female leatherback sea turtles and are identical to those used in the study of green and loggerhead sea turtles with the exception that these procedures were performed at night. Turtles were approached after

the completion of egg laying. They were weighed and then anesthetized with an injectable, partially reversible anesthetic agent, a combination of metatomidine and ketamine (see Table 1). A topical anesthetic was also applied to the cornea.

After anesthetization, a Burian-Allen configuration contact lens electrode was placed against the corneal surface to monitor gross electropotential changes. Testing procedures were fundamentally similar to those of Levenson et al (in press) with some minor exceptions described below. After experiments, the anesthetic reversal agent was injected and the turtles were held from reentering the water until sufficiently recovered from anesthesia.

RESULTS

Experiments were conducted on Matura Beach, Trinidad, West Indies over a 2-week period in May 2004. Fifteen leatherback turtles were evaluated for anesthetization for the project (for details see Table 1). Initial investigations indicated that sensitivity data were best obtained using 4−12 Hz flicker rates. Reliable ERG measurements were successfully obtained from four individuals. All four subjects exhibited peak sensitivity just above 500 nm (roughly similar to human "greens") at about 509 nm. Test results at these frequencies are illustrated in Figure 1. As shown, peak sensitivity was just above 500 nm, and a dramatic drop in sensitivity occurred above 600 nm and below 450 nm. Although explicit attempts were made, no measurable responses could be obtained for any of the turtles beyond the range of data shown in Figure 1.

In addition to evaluating spectral sensitivity, the frequency responses of three turtles were also determined. A 580-nm stimulus was presented to the turtles at a range of flicker rates. Responses were obtained in the 4 − 24 Hz range. Additional frequencies up to 36 Hz were also examined but responses could not be obtained. The specific results of the frequency response testing, as well as those of a similar investigation of green and loggerhead turtles, are shown in Figure 2.

Table 1 - Anesthesia dosages in ketamine (Ket), Metatomidine (Met), and Atipamezole (Ati; Met-reversal agent) for each leatherback turtle used for this experiment. As indicated, in some cases additional doses needed to given to obtain proper levels of anesthesia.

Turtle #	Date	Flipper Tags	PIT	Wt (kg)	CCL (cm)	CCW (cm)	Ket 1 (mg)	Med 1 (mg)	Ket/Med initial dose (mg/kg & µg/kg)	Ket 2 (mg)	Med 2 (mg)	Ket/Med total dose (mg/kg & µg/kg)	Ati (mg)	Ati dose (µg/kg)
1	5/13/04	25864/26137	31892067	369.6	158	112								
2	5/13/04	26848/26083	134814217A	343.8	160	115								
3	5/13/04	21968/25794		295.8	115	111	920	9.2	3 & 30			3 & 30	46	150
4	5/14/04	21369/21975	134836345A	305.2	152	116	1640	16.4	6 & 60			6 & 60	82	300
5	5/15/04	26089/26005	133923767A	273.4	149	114	1350	13.5	5 & 50			5 & 50	67.5	250
6	5/16/04	26839/26024	133924331A	270.6	154	105								
7	5/17/04	26846/26900	133973755A	232.6	147.5	105	1490	14.9	5 & 50	1000	10	8.3 & 83	125	420
8	5/17/04	26293/26298	134409174A	298	163	114								
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10	5/17/04	none	133675130A	256	154	110	1750	17.5	6 & 60	1000	10	9.3 & 93	120	408
11	5/18/04	26891/26384	AVID 044-636-262	294	152	112	1750	17.5	6 & 60	1200	12	9.9 & 99	90	300
12	5/18/04	26850/26361	133631653A	297	150	109	1950	19.5	8 & 80	750	7.5	11.2 & 112	100	413
13	5/19/04	26188/26184	134652644A	242	138	107	2500	25	8 & 80	1200	0	11.9 & 80	125	403
14	5/19/04	26473/26450	AVID 044-796-351	312	158	117	2600	26	8 & 80			8 & 80	130	402
15	5/20/04	26499/26492	134912796A	323.6	157	120								

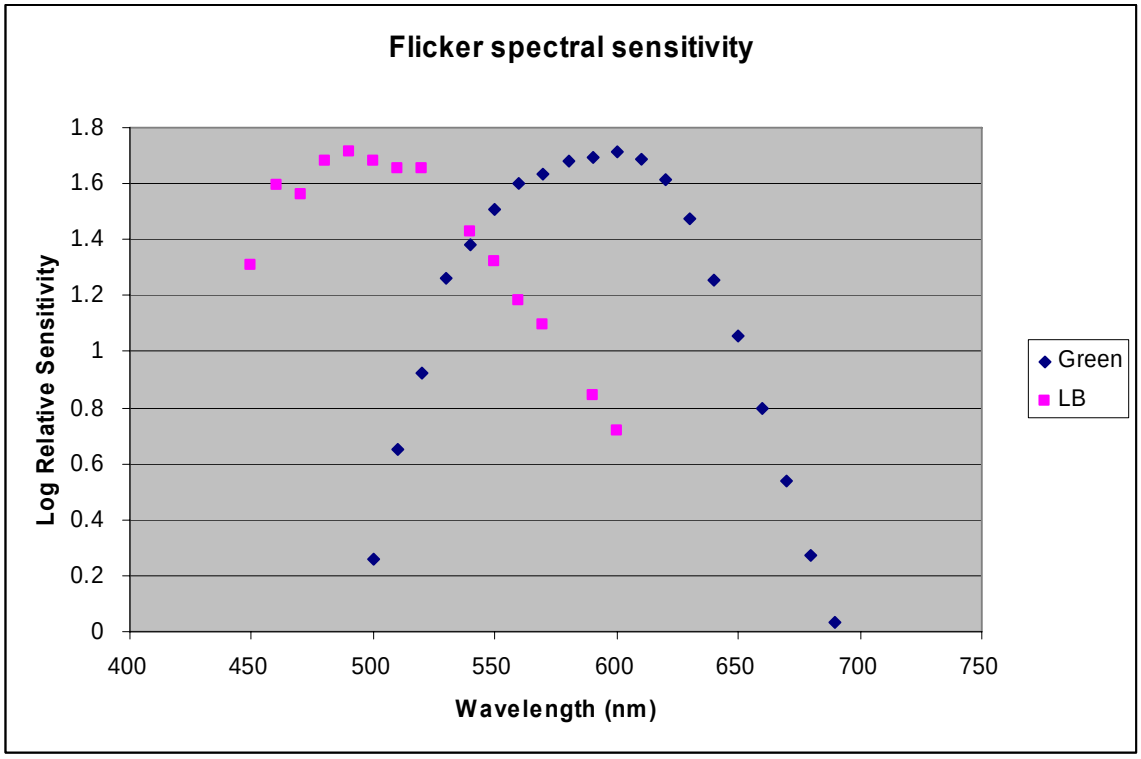

Figure 1. Spectral sensitivities obtained from the leatherback (LB) and green sea turtle (Green) using the ERG flicker photometric procedure. The LB curve represents the average of three turtles run from 4 Hz to12 Hz; the Green curve is the average of four green sea turtles run at 20 Hz (Levenson et al., in press). The curves were normalized at their peaks for comparison.

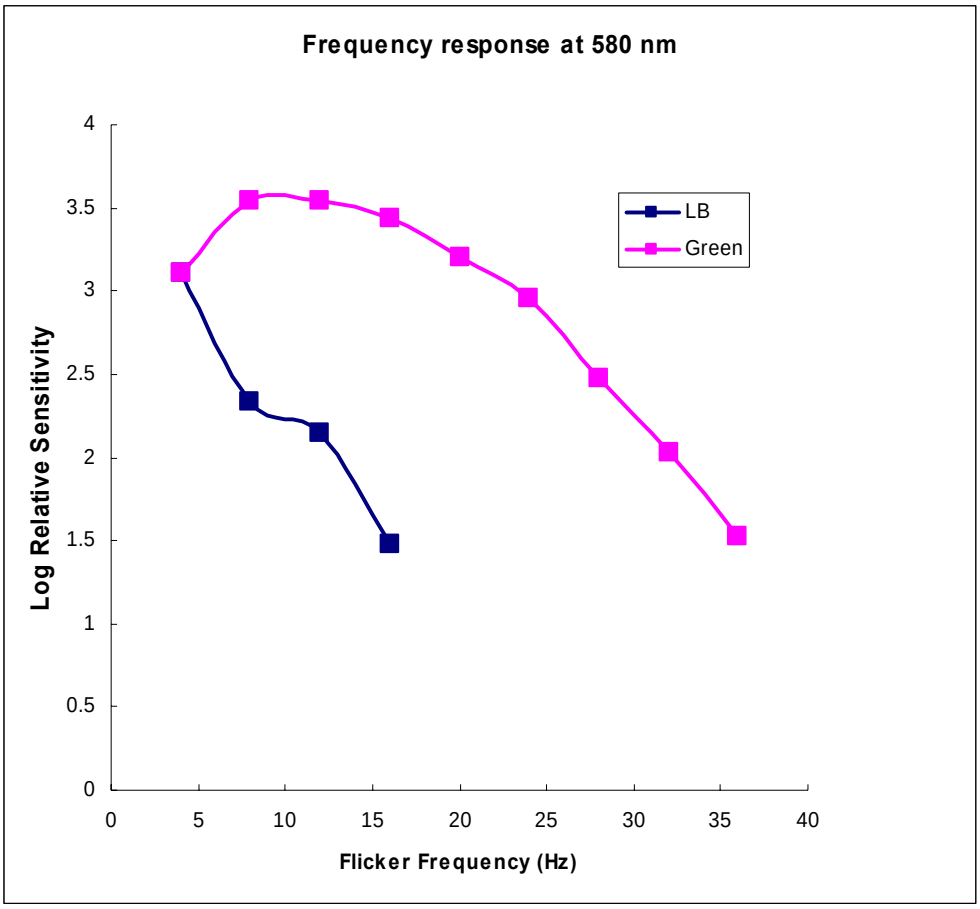

Figure 2. Flicker sensitivity data for three leatherback (LB) and four green sea turtles (Green). The curves were obtained under conditions described above and have been normalized at 4 Hz. Fundamental differences exist between the two species in the fall-off in flicker sensitivity with increases in flicker rate. The leatherback curves are low pass and show a monotonic falloff above 4 Hz, whereas the green turtle curves are bandpass with a peak between 8 and 12 Hz; no simple vertical or horizontal shift in the curves could make them appear similar to each other.

After experiments had been completed, further data analysis was conducted in an attempt to determine the maximum sensitivity (i.e., λ_{max}) of the leatherback photopigment. Overall, the average shape of the leatherback spectral sensitivity curve indicates that their nighttime vision is most likely mediated by a single photopigment type. To determine the spectral position of this pigment, we shifted a standard photopigment absorption function (Dawis, 1981) along a wave number axis to determine the pigment position that best accounted for the array of sensitivity values at all the wavelengths tested. For the three best estimates of sensitivity obtained, average peak sensitivity was determined at 509 nm. A sensitivity profile for a 509 nm λ_{max} photopigment is shown in comparison to the sensitivity data in Figure 3.

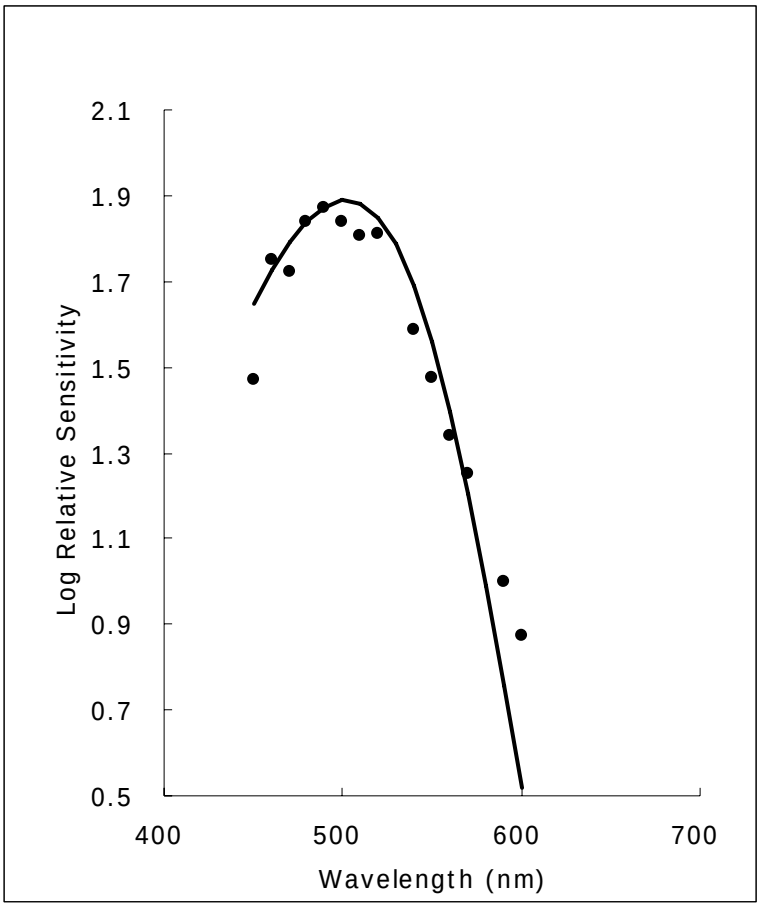

Figure 3. Average spectral sensitivity data at 450 − 600 nm at 10-nm intervals for three leatherback sea turtles measured at 8 Hz. The solid line represents a visual pigment nomogram with λ_{max} of 509 nm, which is near the λ_{max} of the leatherback sea turtle as determined by genetic evaluation of the rod opsin gene (502 nm; Levenson, 2004). Although some variability exists, the leatherback sensitivity peak is clearly different from the 580-nm peak exhibited by the green and loggerhead sea turtle in previous investigations and appears well described by a single pigment nomogram.

CONCLUSIONS

As in previous studies, flicker-photometric electroretinography was demonstrated to be a useful method for determining visual spectral sensitivity in sea turtles. Nighttime spectral sensitivity for the leatherback sea turtle was found to be highest between 500 and 510 nm and to fall off rapidly above 600 nm and below 450 nm. Maximum responses occurred between 4 and 12 Hz. These results are dramatically different than those obtained in daytime evaluations of green and loggerhead sea turtles in previous experiments. In contrast to the cone photoreceptor data obtained from the green and loggerhead turtles, it seems likely that visual responses obtained from the leatherback turtles were representative of their rod photoreceptors. The monotonic shape of their sensitivity curve and its close match to a single pigment nomogram strongly suggest that only a single photoreceptor type was involved. The

very slow frequency responses observed here and the inability to obtain responses at more rapid flicker rates is indicative of rod photoreceptor function (Jacobs et al., 1996). Furthermore, the rhodopsin (rod visual pigment) of the leatherback sea turtle has been shown to have a λ_{max} of 502 nm, which is very similar to the λ_{max} observed here. In fact, it is not uncommon for in vivo scotopic (rod-photoreceptor based) sensitivity to be slightly long-wavelength shifted as a consequence of intra-ocular factors other than the visual pigments alone (e.g., Jacobs, 1993; Jacobs et al., 1993; Yokoyama and Yokoyama, 1996).

Regardless of the underlying photoreceptor type involved, the inter-turtle consistency of sensitivity data indicates that these sensitivity curves are an accurate representation of the nighttime spectral sensitivity of leatherback sea turtles. Results of this project have been submitted for presentation at the 25th Annual Symposium on Sea Turtle Biology and Conservation, February 2005 (see abstract below), and a manuscript to be submitted for publication is in preparation.

SIGNIFICANCE AND FUTURE RESEARCH

Given the significance of this research to federal management issues, further research is clearly merited into the nature of the disparity between the ERG results obtained for leatherbacks and those from our previous study of green and loggerhead sea turtles. Indeed, the development of mitigation measures to reduce the effect on turtles of light attraction devices depends on the resolution of this issue.

Is the visual sensitivity of green and loggerhead turtles dramatically different from that of leatherback turtles, thereby requiring different mitigation strategies for the different species? As the diving behavior of the leatherback turtle is quite different from other sea turtles, it is possible that their differing spectral sensitivities may be the consequence of fundamental differences in ecology. These differences might well have given rise to differences in retinal photoreceptor complements and associated spectral sensitivity as is seen in a wide variety of both terrestrial and marine species (e.g., Jacobs, 1993; Bowmaker, 1995; Levenson, 2004). Alternatively, is this disparity a consequence of differences in testing factors? It is quite possible that the observed disparity in visual sensitivity is a consequence of diurnal changes in visual sensitivity associated with photoreceptor migration, a phenomenon known to occur in a variety of vertebrate taxa, including at least some terrestrial turtle species (e.g., Drenckhahn and Wagner, 1985). In this case, similar mitigation measures would be beneficial for all three species, but would need to be varied between day and night.

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APPENDIX I

ABSTRACT submitted to the 25th Symposium on Sea Turtle Biology and Conservation.

Night-time spectral sensitivity of adult female leatherback sea turtles
Levenson, D.H., Eckert, S.A. Crognale, M.A., Duhamel, P., Kubis, S.A. and Harms, C.A.

Flicker electroretinography (ERG) was used to measure the spectral sensitivity of adult female leatherback sea turtles *in vivo* on a nesting beach on the southern Caribbean island of Trinidad. Individual turtles were selected for examination after the completion of nesting. Four turtles were successfully weighed, sedated, evaluated, and subsequently released without incident. Gross electroretinograms were monitored with a corneal contact lens electrode. Sensitivity was evaluated from 440 nm to 610 nm using flickering (4−12 Hz) monochromatic stimuli. Although testing was attempted beyond this range of wavelengths and flicker rates, measurable responses could not be obtained. Maximum sensitivity for all subjects occurred at or slightly above 500 nm in concurrence with previously reported rod photopigment sensitivity data for this species (maximum sensitivity 502 nm). Results indicate that the rod visual pigments of the leatherback are very similar to those of other sea turtles and are clearly not shifted in sensitivity below 500 nm as seen in many other marine animals active in deep sea environments. Interestingly, the ERG responses of leatherbacks were quite different from those of green and loggerhead turtles when similarly tested. This disparity suggests that further research may be merited into potential underlying differences in retinal organization among these species.

MATHEMATICAL MODEL OF THE VISUAL ABILITIES OF SEA TURTLES AND PELAGIC FISHES

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Sea turtles suffer substantial mortality as bycatch in longline fisheries. While chemical and other cues play a role in luring the animals to the hooked lines, visual cues likely also play a significant role. Because marine visual systems differ, in certain cases it may be possible for an object to be visible to one species yet invisible to another. The current study had two goals: (1) to design fishing gear that was invisible to all species, and (2) to design lures on this gear that were visible to billfish but visually undetectable by sea turtles. Using measured profiles of the optical parameters of oceanic and coastal waters and radiative transfer software, the underwater light field was modeled at a number of depths. These light fields were then used to calculate the reflectance spectra (i.e., color) of perfectly cryptic objects as a function of depth and orientation (Fig. 1).

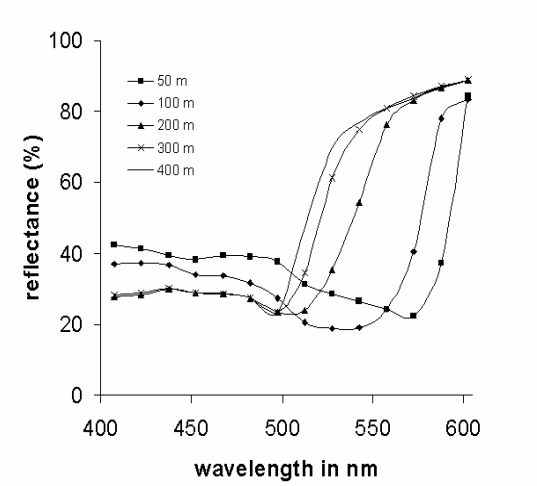

Figure 1: Predicted reflectance spectrum for perfectly camouflaged gear as a function of depth in clear oceanic waters. In this case, the gear is viewed horizontally either in the middle of the day or any time at night. The predictions are slightly different at sunrise and sunset as a result of the strong slanted illumination from the sun. The graph was generated by modeling the underwater light field using measured inherent optical properties from the equatorial Pacific Ocean. The light field was modeled using radiative transfer theory, a well-validated technique. Based on previous research on underwater camouflage (Johnsen, 2002), the reflectance of a perfectly cryptic object underwater must satisfy:

$$R(\lambda) = \frac{\pi L_b(\lambda)}{E(\lambda)},$$

for all wavelengths where there is sufficient light for the predator to see by (R is reflectance, E is the horizontal irradiance, and L_b is the background radiance).

These cryptic reflectance spectra were then combined with the known visual parameters of billfish and sea turtles (Table 1), and various methods were attempted to increase the visibility of the lure to billfish while maintaining its invisibility to turtles.

Table 1: Spectral peaks (from both MSP and ERG methods), spatial resolution (given as minimum resolvable angle), temporal resolution (given as critical flicker fusion frequency) and optical sensitivity for important billfish and sea turtle species.

	rod peak (nm)	cone peaks (nm)	ERG peak (nm)	$\Delta\rho$	CFF (Hz)	s
Green turtle	502	440, 502, 562	580	—	40	0.13
Loggerhead	502	440, 502, 562	520, 580	11	40	
Leatherback	502	—	509	—	15	
Marlin	—	436, 488, 531	—	10	56	2.8
Swordfish	508	440, 490	—	8–10	38	—
Big Eye Tuna	495	488	—	11–12	34	—
Yellowfin Tuna	483	426, 485	—	16–18	80	—

Given current data on the spectral sensitivity of sea turtles and billfish, it appears that the most successful strategy is to increase the reflectance at violet and blue wavelengths (Fig. 2).

However, as can be seen from Table 1, a great deal of overlap occurs in the spectral sensitivities of billfish and sea turtles, particularly at night when the rods alone are used (Fig. 3). This may limit the success of lure-coloring strategies.

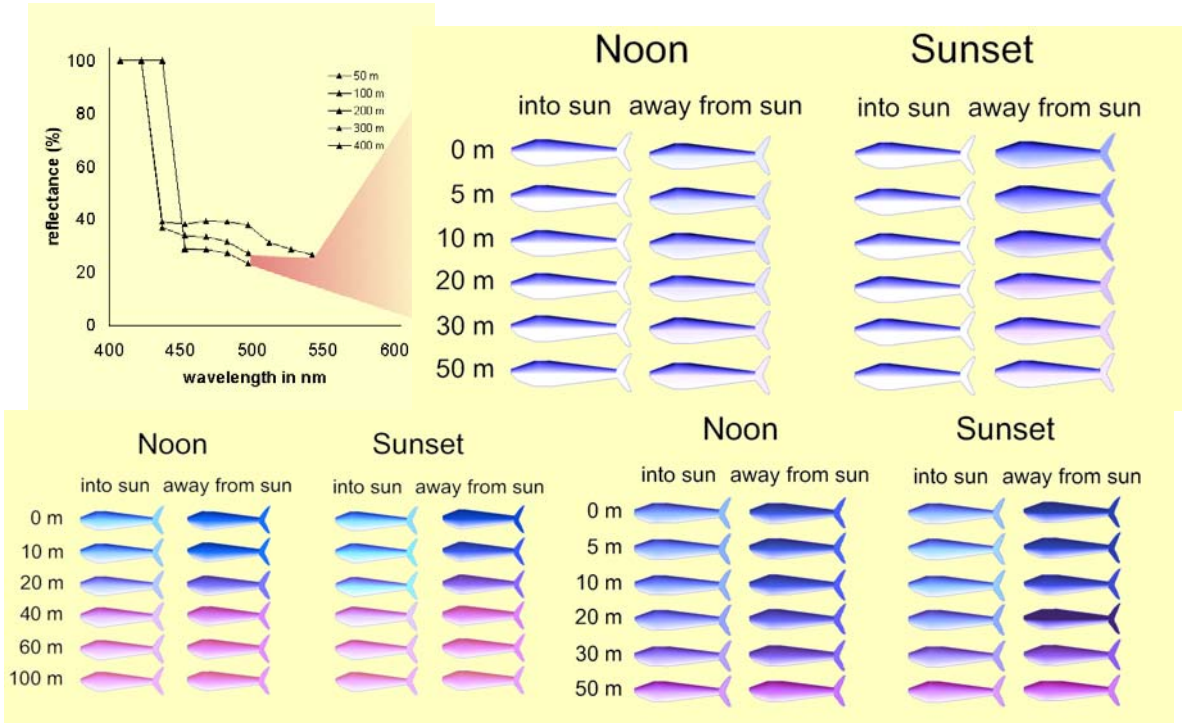

Figure 2: Sea turtles appear to be less sensitive to deep blue and violet wavelengths. One possible solution may be to increase the reflectance at these wavelengths, while keeping the remaining reflectances at a cryptic level (upper left; reflectance at longer wavelengths is unimportant at depth). This results in the following lure colorations for mirrored lures (upper right) and diffusely colored lures in ocean and coastal waters (lower left and lower right, respectively).

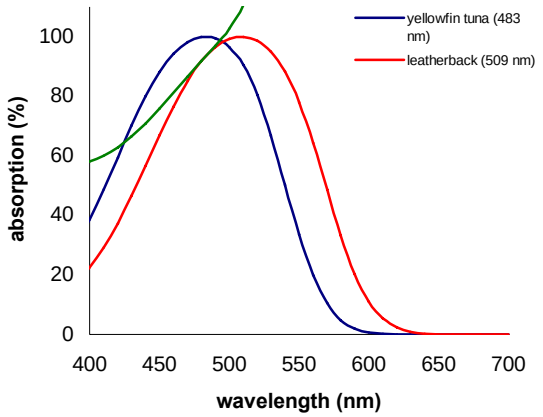

Figure 3: The spectral response curves of the rods of the two species with the greatest difference in λ_{max}. The green line shows the ratio of the quantum catch of the two as a function of wavelength. Even at 400 nm, the ratio is not dramatically different from 1 (100%), suggesting that selective invisibility based on special coloring is not likely to be very effective.

Another possibility is to use lures that take advantage of the slightly different spatial resolutions of billfish and sea turtles (Table 1). This trick is used by striped coral reef fish (Fig. 4). When viewed from a short distance, they appear vividly striped. When viewed from farther away (or by an animal with poor spatial resolution), the stripes blend into a single color that matches the background light.

Figure 4: Coral reef fish viewed by an animal with high (left) and low (right) spatial resolution. Note that the stripes blend to form a color close to the background color.

This may work for yellowfin tuna lures, since this species has a spatial resolution significantly higher than that of sea turtles (Table 1, Fig. 5). In this case, the yellowfin can see stripes that have a spatial frequency of (for example) 15 cycles/degree, while a loggerhead

turtle cannot. Thus, a lure with stripes of this spacing could be visible to the former while invisible to the latter, if the colors of the stripes are chosen so that their mixtures matched the background color. Unfortunately, if the turtle gets close enough to the lure, the angular spacing of the stripes will increase and the lure will become visible.

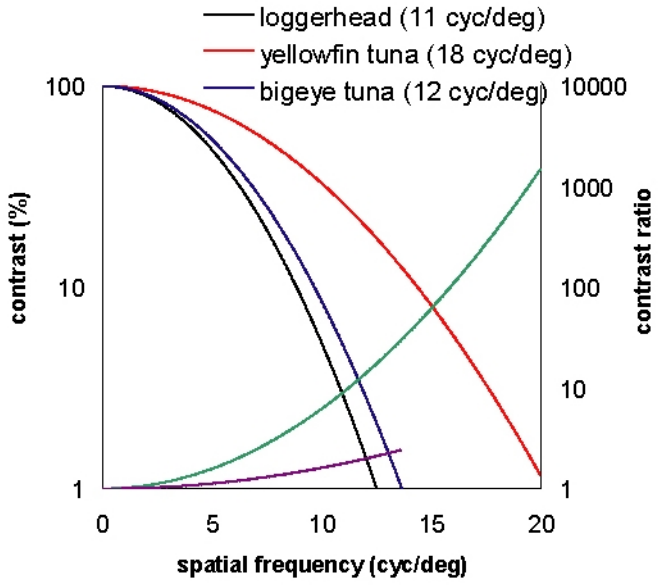

Figure 5: The perceived contrast of details on objects with given spatial frequency for various species. The green line shows the ratio of the curve for yellowfin tuna to the curve for loggerhead turtles. The black curve shows the ratio of the curve for bigeye tuna to the curve for loggerheads. It appears that the ratios are largest at higher spatial frequencies. Most marine species cannot detect contrast levels less than 1%, so the *x*-axis of the graph is a good estimate of the lower bound of visibility.

Perhaps the best solution relies on the differences in the temporal resolutions of billfish and sea turtles. While sea turtles have higher temporal resolution than billfish during daylight near the surface (Fig. 6), they almost certainly have far lower spatial resolution than billfish at night and at depth. First, billfish have much more sensitive eyes, by a factor of about 20 (Table 1). In addition, Kerstin Fritsches has shown that billfish heat their eyes, which results in much higher temporal resolution than otherwise possible.

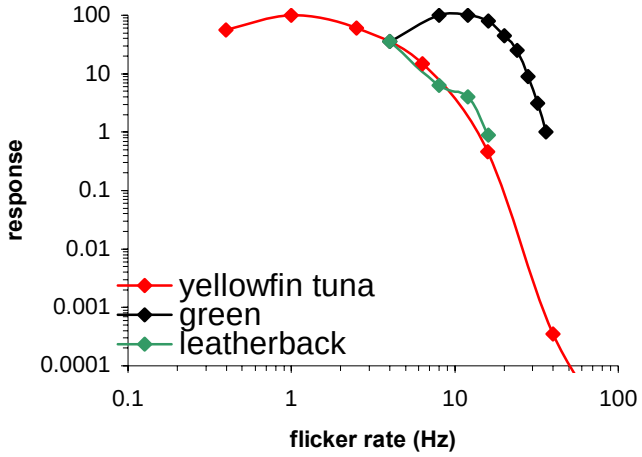

Figure 6: The temporal response curves of several species under bright light.

The best way to capitalize on this is to design flashing lures. Lures used at depth (~ 200 m) during the daytime would flash two different colors that, when blended, produce a light that matches the ocean color background. Lures used at night could have lights that travel rapidly in circles. In either case, an animal with fast vision under low light (e.g., billfish) would see a highly conspicuous target. An animal with slow vision under low light (e.g., turtle), would see nothing. This method is the most likely to be successful of all the ones proposed in this report.

ELECTRORETINOGRAPHIC AND GENETIC EXAMINATION OF SEA TURTLE VISUAL PIGMENTS

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The use of colored "lightsticks" by longline fishing vessels is a relatively common practice employed to lure fish toward baited hooks. However, research indicates that the use of these lights probably also increases the rate of incidental sea turtle bycatch. In an attempt to reduce sea turtle/longline interactions, we have examined sea turtle spectral (color) sensitivity to evaluate whether the spectral output of these lights might be modified to reduce their visibility or attractiveness to sea turtles while maintaining their attractiveness to target fish species. Towards this end, we have employed electrophysiologic and molecular genetic methods to examine aspects of the visual sensitivity of green, loggerhead, and leatherback sea turtles.

Flicker-photometric electroretinography (ERG) was used to evaluate in vivo photopic (bright light or cone-based) spectral sensitivity of four green turtles and six loggerhead sea turtles held in captivity at Sea World, San Diego. Turtles were given an intramuscular injection of general anesthetic as well as a topical application of local anesthetic to the cornea. In this type of ERG (Fig. 1), gross electrical changes are monitored at the corneal surface using a conductive contact-lens electrode while the eye is exposed to rapidly flickering monochromatic light (15 Hz in these expts). To determine sensitivity, retinal responses to the monochromatic light are summed for a series of ~ 50 presentations. The intensity of the light

is then adjusted until it elicits stimulation equal to a preset, unchanging value (3.2 μV in these experiments). The relative sensitivity of the eye at each wavelength tested is reflected in the amount of light necessary to obtain the desired stimulation level. Sensitivity was thus determined for each individual at 10-nm increments from 400 nm to 700 nm. All green and loggerhead turtles exhibited at least some sensitivity to lights ranging from 400 nm to 700 nm. Both exhibited peak sensitivity at about 580 nm (similar to human "yellow"). For both species, a dramatic drop in sensitivity also occurred above 650 nm and below 510 nm, although this cutoff was considerably more pronounced in the loggerhead turtles (Fig. 2). The overall shape of the curves indicates that photopic spectral sensitivity for both species is most likely the product of multiple cone photopigment types, as well as the consequence of light-filtering oil droplets known to be present in turtle cone photoreceptors.

To evaluate scotopic (dim light or rod-based) spectral sensitivity in sea turtles we are using molecular genetic techniques. For these experiments, we are amplifying and sequencing the rod visual pigment genes of green, loggerhead, and leatherback sea turtles. Retinal RNA (reversed transcribed to cDNA) or nuclear DNA is being examined using polymerase-chain reaction (PCR) and dideoxy chain-terminator cycle-sequencing techniques. The primers used in PCR have been designed to amplify the ~ 1050 bp rod pigment genes of these turtles in two or three 300–800 bp fragments. Once sequenced, the fragments will be spliced together to determine the amino acid structure of each species' rod visual pigment protein (opsin). As previous investigations have identified the specific residues on the rod opsin protein to be responsible for "tuning" the spectral sensitivity of resultant visual pigment, evaluation of the primary structure of each species' rod opsins can be used to determine scotopic (rod-based) sensitivity. Unfortunately, at present, only partial gene sequences have been obtained for each species. While previous results using spectrophotometry indicate that green sea turtles have a rod pigment with maximum sensitivity at 502 nm, the comparatively deep-diving nature of loggerhead and leatherback turtles suggests these species may have modified pigments for vision in deeper environments. Thus, it is difficult to draw conclusions about the visual pigments and corresponding sensitivity of these species at this time.

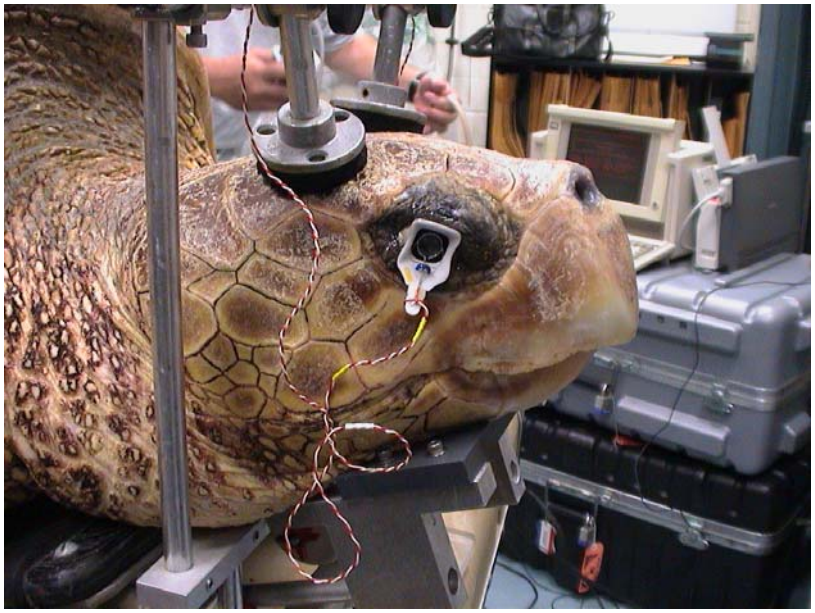

Figure 1. Flicker-photometric electroretinography (ERG) conducted on a loggerhead turtle.

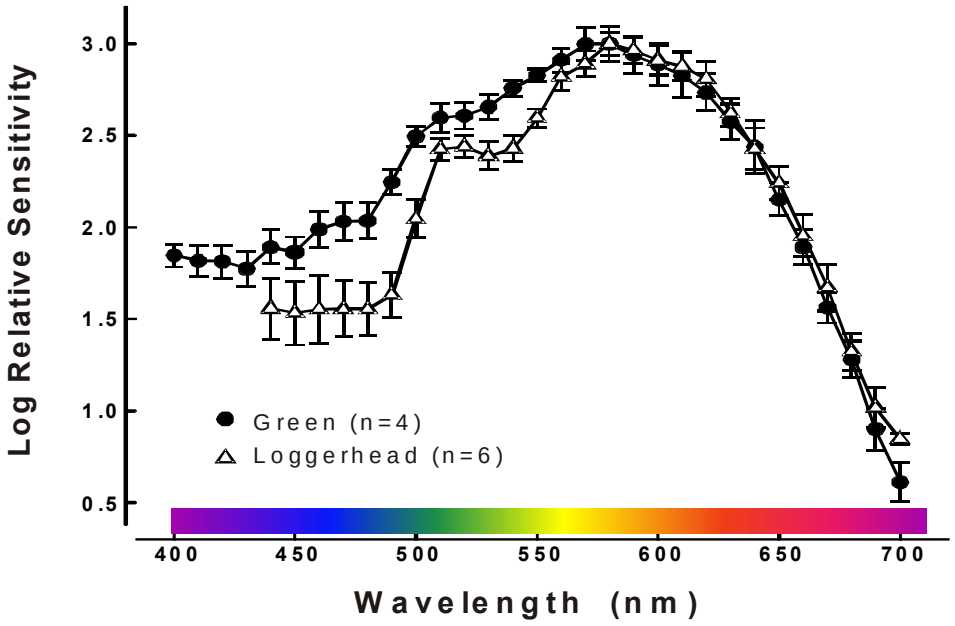

Figure 2. Daytime pectral sensitivity (color vision) in green and loggerhead turtles. Both species showed highest sensitivity around 580 nm, equivalent to human "yellow."

THE IMPORTANCE OF ODOR RECEPTORS TO THE CHEMOSENSORY BEHAVIOR OF SEA TURTLES

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AIM AND SUMMARY

The project's goal was to assess the importance of olfaction in the life history of sea turtles and to use this information to identify deterrent chemicals that might be applied to fish bait. The approach was to identify and characterize odor receptor (OR) genes, determining their relative importance to the life history of the turtles based on evolutionary selection. This approach was entirely noninvasive as appropriate for endangered species, requiring only aliquots of pre-existing blood samples for genetic analysis. Consequently, we successfully identified a subpopulation of OR genes in several sea turtle species. These genes were initially compared among themselves and with OR genes from other species. Subsequently, sequence variation of select sea turtle OR genes was characterized from discrete turtle populations to determine the degree of evolutionary selection acting on these genes. Low sequence variation would be interpreted as indicating positive selection and the importance of these genes to the life history of the species. We identified two OR genes that are highly conserved among two and three sea turtle species, respectively, and observed that a pattern of allelic variation among these and the less conserved OR genes were consistent with our hypothesis. Sea turtles appear to have a reduced complement of OR genes relative to their non-marine relatives, but the OR genes appear to be under positive selection suggesting that the olfactory pathways are indeed important to sea turtle behavior. We are currently performing tests to estimate the strength of the evolutionary selection. These results provide genetic evidence that validates efforts aimed at developing olfactory-based strategies for controlling the behavior of sea turtles, such as repelling them from fishing baits.

BACKGROUND

Sea turtles possess three major chemosensory systems or modalities: ciliary olfactory neurons, microvillus olfactory neurons, and taste neurons. Little is known about what molecules each modality perceives, as there have been only a handful of studies examining chemosensory responses of sea turtles. Since these animals are aquatic, it is possible that all three modalities respond to waterborne stimulants; although there is some conjecture that the ciliary neurons may respond to airborne odorants and that the microvillus neurons may respond to waterborne odorants. Where two modalities might respond to waterborne stimulants, these stimulants might be divided into more or less nonoverlapping chemical classes of molecules. Alternatively, or additionally, the possession of seemingly parallel chemosensory pathways (ciliary, microvillus, and taste) may have allowed these pathways to

evolve to convey information towards different behavioral contexts. In this case, ciliary and microvillar olfactory systems might be used for the detection of airborne or waterborne odors coming from a distant source, while the taste system might be used for the immediate assessment of the suitability of candidate food sources.

A considerable amount is known about the receptor genes of ciliary olfactory neurons; these are referred to specifically as OR genes and they encode the predominant chemosensory receptors involved in attractant type behaviors in vertebrate animals ranging from fish to mammals. The receptor genes associated with ciliary olfactory neurons, the OR genes, were the main focus of our study. The receptor genes of microvillus olfactory neurons were poorly understood at the outset of our project. Microvillus olfactory neurons associate with the vomeronasal organ in mammals and may have roles in pheromone detection and the modulation of social behavior. During the course of our study, others identified classes of these receptors in mammals and one subclass in fish. We used this information to identify such receptors in sea turtles, but have been unsuccessful to date. Microvillus olfactory neurons are abundant in the turtle nasal cavity, along with ciliary olfactory neurons, and thus characterizations of the associating receptors seem desirable. The receptor genes of taste neurons are not sufficiently characterized across vertebrate animals (fish to mammal) to make them suitable for broad scale characterization at this time. However, considering their likely role of assessing the quality of candidate food sources during biting, information of these receptors would as well seem desirable.

I. Identification of Ciliary Olfactory Odor Receptor (OR) Genes in Sea Turtles (manuscript near completion).

We identified OR genes from loggerhead (*Caretta caretta),* leatherback (*Dermochelys coriacea*), and green (*Chelonia mydas*) sea turtles. For comparison, we also identified OR genes from several species of terrestrial turtles ranging in degree of aquatic habitat (musk turtle, *Sternotherus odoratus*; box turtle, *Terrapene carolina*; painted turtle, *Chrysemys picta bellii*; and gopher tortoise, *Gopherus polyphemus*). And, for more distant comparison, we also identified OR genes from the American alligator (*Alligator mississipiensis*). All sequences were characterized for the presence of internal stop codons; such presence identifies them as pseudogenes or nonfunctional ORs. All sequences were also characterized against known OR sequences to determine their phylogenetic relationships among the larger OR gene family.

Our approach was to design Polymerase Chain Reaction (PCR) primers that should universally recognize OR genes in vertebrate animals ranging from fish to mammal and use these to amplify candidate DNA fragments from turtle genomic DNA obtained from provided blood samples. PCR products were cloned (i.e., inserted into bacterial plasmids), resulting in the construction of small DNA libraries enriched with OR genes. Approximately 200 clones were sequenced from each species' DNA library; OR sequences were identified based on significant sequence similarity to previously known olfactory receptor genes. All OR gene sequences have been submitted to and published in the GenBank database.

Sea turtles may have fewer OR genes than freshwater or terrestrial turtles. Table 1 summarizes the number of clones sequenced for each species and the number of unique OR genes encountered among those clones; many of these OR genes were encountered multiple times within the species-specific libraries. Sea turtles yielded fewer OR genes compared to freshwater and terrestrial turtles. This reduction is consistent with at least one report that sea turtles have fewer chemosensory neurons within their nasal epithelium than terrestrial turtles.

Table 1: Summary of olfactory receptor genes identified.

Species	# clones sequenced	# unique OR genes identified	Percent (number) of pseudogenes	Source of tissue
Loggerhead (sw) *Caretta caretta*	260	20	25 (5)	Dr. David Whitaker (South Carolina DNR)
Green (sw) *Chelonia mydas*	160	14	40 (6)	Dr. Richard Brill (NMFS—while at Honolulu Lab)
Leatherback (sw) *Dermochelys coriacea*	285	17	44 (7)	Dr. Peter Dutton (NMFS—La Jolla Laboratory)
Musk (fw) *Sternotherus odoratus*	200	22	9 (2)	Judy Greene (Savannah River Ecology Lab—Aiken SC)
Painted (fw) *Chrysemys picta bellii*	235	63	27 (17)	Judy Greene (Savannah River Ecology Lab—Aiken SC)
Box (terr) *Terrapene carolina*	225	42	2 (1)	Judy Greene (Savannah River Ecology Lab—Aiken SC)
Gopher (terr) *Gopherus polyphemus*	225	45	9 (4)	Judy Greene—(Savannah River Ecology Lab—Aiken SC)
Alligator *Alligator mississipiensis*	200	22	18 (4)	Dr. Travis Glenn—(Biological Sciences, University of South Carolina)

Sea turtles may have a higher percentage of OR pseudogenes than freshwater or terrestrial turtles.

One aspect of the evolutionary dynamics of a gene family is the expansion or contraction of the size of that gene family among species. One expression of this dynamics is the recognizable presence of pseudogenes. In the case of OR genes, these are recognized by the presence of internal stop codons, which would yield truncated and presumably nonfunctional gene products. Humans, for example, have a much higher proportion of OR pseudogenes than mice and consequently a much lower number of functional OR genes

(perhaps 300 compared to perhaps 1000 in mice). The meaning of such a difference is unclear, as olfaction is clearly important to humans. Nevertheless, the greater or lesser presence of pseudogenes suggests something regarding the olfactory capability of a species, perhaps relating to the diversity or complexity of olfactory-related behaviors of that species. In our study, sea turtles appeared to have a considerably higher proportion of OR pseudogenes than freshwater or terrestrial turtles; together with their reduced total number of OR genes, this suggests that odor detection, at least by ciliated olfactory neurons, may be less important to the life history of sea turtles than it is to the life history of freshwater or terrestrial turtles. However, "less important" may be misleading. It may be that sea turtles use ciliated olfactory neurons for airborne odor detection, and the detection of airborne odors may have a more focused or specific role in sea turtle behavior then it does in freshwater or terrestrial turtles. Alternatively, the complexity of airborne odors may be much less over marine environments then it is in terrestrial environments. Again, reduction of capacity does not necessarily mean reduction in importance.

Sea turtle OR genes represent subclasses of mammalian OR genes and perhaps a total number similar to humans (200–300).

Two broad classes of OR genes have been identified in vertebrate animals: one class that ranges from fish to amphibians, and a second class that ranges throughout tetrapod vertebrates, including amphibians, birds, and mammals, but that is not seen in fish. This suggests that an expansion of the OR gene family accompanied the establishment of the tetrapod vertebrate lineage. Within amphibians, the fish-like ORs are thought to detect waterborne odorants while the mammalian-like ORs are thought to detect airborne odorants. The sea turtle OR genes we identified clearly belong to non-fish or mammalian-like class of OR genes, based on sequence comparisons. A survey of the presence or absence of our PCR primers within the mouse OR genome indicates that the sea turtle genes we identified represent a broad range, though not all, of mouse OR gene subfamilies. These numbers and a limited number of studies on the structure of terrestrial turtle olfactory bulbs suggest that turtles in general may have a similar number of OR genes as mammals (perhaps 1000), but that the number of functional OR genes in sea turtles may be similarly reduced as in humans, perhaps to a range of functional 200–300 OR genes.

Two identified sea turtle OR genes are highly conserved between sea turtle species.

Our study identified two genes that are highly conserved between sea turtle species. One gene encodes the identical amino acid sequence in loggerhead, green, and leatherback sea turtles, and a second gene encodes the identical amino acid sequence in loggerhead and green sea turtles. Such conservation is difficult to resolve unless these genes play both a significantly important and significantly similar role in the life history of these species. Given the relatively small sample size in our data set, it is highly likely that more such conserved OR genes are present in these species. It would be of great interest to know what odors stimulate these receptors and how odor detection is used in the behaviors of these species.

II. Population Genetics of Sea Turtle OR Genes: Evidence for Evolutionary Selection (manuscript in preparation).

We tested the importance of OR genes by comparing the sequences of specific genes among individuals among and between discrete populations. The relative prominence of one haplotype over others within a population of animals would suggest that the gene is important to the life history of the animal, its prominence a result of evolutionary selection that favors individuals possessing that haplotype (fitness is dependent on the gene). A more equal representation of multiple haplotypes within a population suggests that the gene is less important to the life history of the animal, the tolerated diversity is the result of a more relaxed evolutionary selection (fitness is less dependent on the gene).

"Haplotypes" are different versions of the same gene, where each nucleotide difference represents an individual "allele." In the evolutionary history of the species, each nucleotide change is presumed to have occurred independently. The generation of two haplotypes differing by, say, four nucleotides means that at least five versions (haplotypes) of that gene must be in existence, the original gene plus a version for each accumulated nucleotide change.

Whether or not a change in gene nucleotide (creating a new allele) results in a gene product (protein) with new properties depends on several things. Each amino acid is encoded by a triplet codon (three nucleotides). Changes in the third nucleotide position of a codon often do not alter the amino acid, while changes in the first or second nucleotide position do often change the amino acid. Nucleotide changes that do not alter the amino acid are referred to as "synonymous," while changes that alter the amino acid are referred to as "non-synonymous." Even if an amino acid is changed, the change may or may not alter the properties of the resulting protein, depending on the exact position of that amino acid within the protein (near or far from a sensitive region), and whether the change is to an amino acid with profoundly different chemical properties (charged to uncharged, hydrophilic to hydrophobic).

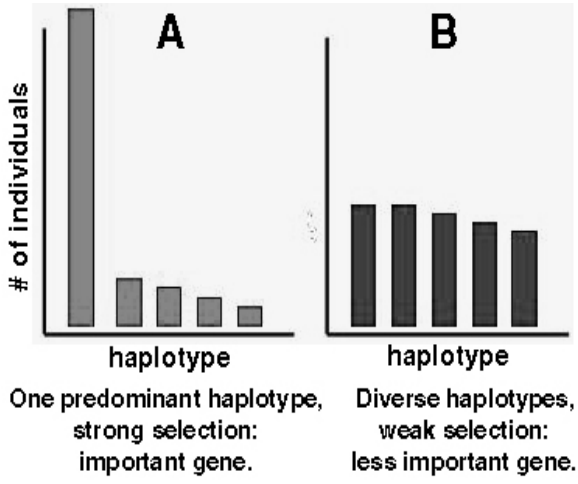

Figure 1. Hypothetical distribution of haplotypes in a population.

In Figure 1, two genes are represented within a population of animals. In both graphs, five haplotypes are shown where the height of the column indicates the proportion of individual animals possessing that allele. In Figure 1A, one haplotype is observed to predominate. In Figure 2B, all five haplotypes are more or less equally represented within the population. The predominance of one haplotype over all others in Figure 1A suggests that that haplotype provides some selective advantage; that individuals possessing that allele tend to "do better" than individuals possessing the other alleles, and the haplotype has emerged as a predominant version of that gene within the population. Such a pattern suggests that the gene is important to the life history of the animal.

Our approach was to examine the sequence variation among several sea turtle OR genes among individuals of defined populations and among different populations. Our hypothesis was that the importance of an OR gene would be reflected in the predominance of an individual haplotype. To test this hypothesis, we compared three sets of genes: one set that we had reason to believe was important, a second set of undetermined importance, and a third set that we had reason to believe was not important. Set 1 included the two OR genes already shown to be highly conserved between sea turtle species. Such conservation is difficult to resolve unless the genes are important to both species, and therefore the Set 1 genes represented OR genes with a high probability of importance. Set 2 included two OR genes for which no obvious orthologues were observed among species, but which also contained no internal stop codons. Set 3 included two to three OR genes which did contain internal stop codons, and which therefore were interpreted as not important based on their presumed nonfunctional status.

Blood samples were obtained for multiple individuals of loggerhead, green, and leatherback turtles from various sources. Four distinct loggerhead populations were characterized (Pacific, Atlantic, Mediterranean, Arabic Sea) while single populations were characterized for green (Pacific) and leatherback (Atlantic). The species and gene studies are summarized in the tables below.

Table 2: Summary of species, populations and numbers of individuals surveyed in the population genetics study.

Species, population, and gene summary	**# of individuals**	**Source of tissue (blood samples)**
Loggerhead—Pacific (LH–P)	10	Dr. Peter Dutton (NMFS—La Jolla Laboratory)
Loggerhead—Atlantic (LH–A)	20	Dr. David Whitaker (South Carolina DNR) Dr. Joe Quattro (University of South Carolina)
Loggerhead—Mediterranean (LH–M)	6	Dr. Peter Dutton (NMFS—La Jolla Laboratory)
Loggerhead—Arabian Sea / Oman (LH–O)	12	Dr. Peter Dutton (NMFS—La Jolla Laboratory)
Green—Pacific (G–P)	18	Dr. Richard Brill (NMFS—while at Honolulu Laboratory)
Leatherback—Atlantic (LB–A)	18	Dr. Peter Dutton (NMFS—La Jolla Laboratory)

Table 3: Summary of OR genes examined for each species.

	Taxon name	Accession number	# nucleotides analyzed	Set #
LH1 (conserved 1)	LHOR11	AY686461	339 (552)*	1
LH2 (conserved 2)	LHOR6	AY686456	329 (549)	1
LH3	LHOR12	AY686462	373 (507)	2
LH4	LHOR15	AY686464	272 (552)	2
LH5 (pseudogene)	LHOR3	AY686453	332 (537)	3
LH6 (pseudogene)	LHOR4	AY686454	299 (522)	3
G1 (conserved 1)	GTOR11	AY686407	339 (552)	1
G2 (conserved 2)	GTOR9	AY686405	329 (546)	1
G3	GTOR1	AY686397	326 (552)	2
G4 (pseudogene)	GTOR6	AY686402	348 (543)	3
G5 (pseudogene)	GTOR14	AY686410	326 (555)	3
LB1 (conserved 1)	LBOR12	AY686445	339 (549)	1
LB2	LBOR15	AY686448	372 (552)	2
LB3	LBOR10	AY686443	385 (552)	2
LB4 (pseudogene)	LBOR5	AY686438	379 (552)	3
LB5 (pseudogene)	LBOR14	AY686447	404 (552)	3

The number of nucleotides analyzed is noted, along with the total nucleotide length under the indicated accession number. Only a portion of the available sequence was used for these analyses because of restrictions in the availability of suitable unique PCR primer sites.

Analysis of Set 1 (conserved) genes—all species/populations.

Highly conserved Set 1 genes show remarkably little variation among populations and species, suggesting they are under strong positive selection and may play an important role in the chemosensory behavior of these animals. Highly conserved genes (Set 1) were represented by only a single haplotype for each population sample set; small but consistent differences were observed among the Pacific, Atlantic, and combined Mediterranean/Arabic Sea loggerhead populations. These OR haplotypes may be useful in large-scale characterizations of loggerhead populations; if so, the presence of common haplotypes in the Mediterranean and Arabic Sea populations suggests these two populations are sharing genetic material, perhaps by emigrational movement through the Suez Canal and Persian Gulf. Sequence variation was less between loggerhead and green than it was between either loggerhead or green and leatherback. This pattern is consistent with the close polygenetic relationship between loggerhead and green turtles and the more distant relationship of leatherbacks.

Table 4: Haplotype distribution and variation for two conserved OR genes found in loggerhead, green, and leatherback sea turtles (Set 1).

A. Conserved 1 (Haplotype Distribution)

	LH-A	LH-M/O	LH-P	G-P
Ha 1	20			
Ha 2		18		
Ha 3			10	
Ha 4				18

C. % difference in sequence (pair-wise comparisons)

Conserved 1	Ha 1 (LH-A)	Ha 2 (LH-M/O)	Ha 3 (LH-P)	Ha 4 (G-P)
Ha 1(L-HA)		*1.8*	*3.7*	*7.3*
Ha 2(LH-M/O)	0.6		*1.8*	*5.5*
Ha 3(LH-P)	1.2	0.6		*5.5*
Ha 4(G-P)	2.7	2.1	2.1	

B. Conserved 2 (Haplotype Distribution)

	LH-A	LH-M/O	LH-P	LB-A	G-P
Ha 1	20				
Ha 2		18			
Ha 3			10		
Ha 4				18	
Ha 5					18

D. % difference in sequence (pair-wise comparisons)

Conserved 2	Ha 1 (LH-A)	Ha 2 (LH-M/O)	Ha 3 (LH-P)	Ha 4 (LB-A)	Ha 5 (G-P)
Ha 1(L-HA)		*1.8*	*0.9*	*0.9*	*8.2*
Ha 2 (LH-M/O)	0.6		*0.9*	*2.8*	*9.1*
Ha 3 (LH-P)	0.3	0.3		*1.9*	*8.1*
Ha 4 (LB-A)	0.9	1.5	1.2		*7.1*
Ha 5 (G-P)	4.7	50.0	4.7	4.3	

Tables 4A and 4B indicate the haplotype distribution among the respective species and populations. Numbers indicate the number of individuals sampled; only single haplotypes were observed in any population for both conserved OR genes. Tables 4C and 4D indicate the percent differences in nucleotide (normal font) and amino acid (italics) sequences, comparing indicated pairs of haplotypes. Since only single haplotypes were observed for each population, these numbers also indicate differences among populations.

OR haplotype variation among four populations of loggerhead sea turtles.

Figure 2 and Table summarize the data for the three populations of loggerhead turtles. In general, the two conserved genes (LH1, LH2), as stated above, do not show haplotype variation within populations; however, they show small variation among populations, the degree of which is also noted above. The two pseudogenes (LH5, LH6) are represented by several alleles, while the two genes that do not contain internal stop codons (LH3, LH4) show an intermediate number of alleles. Assuming these three sets represent a range of importance to the animals' life history, these results appear to support our hypothesis that importance of a gene is reflected in the restriction of allelic variation.

It is noteworthy that the range in variation between haplotype sequences is somewhat similar, regardless of the gene examined (see tables following page). Rather, the prominent difference observed is in the prevalence of minor haplotypes: the conserved OR genes show only single haplotypes, while the presumably nonselected pseudogenes show the greatest diversity of haplotypes.

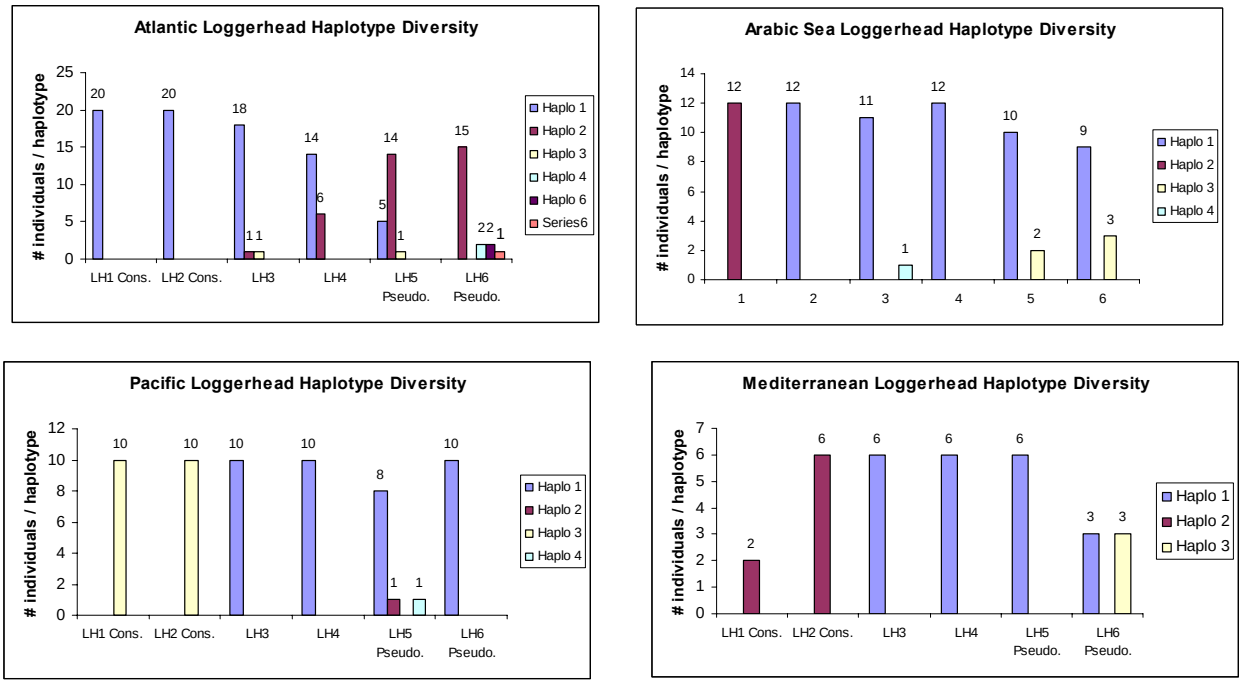

Figure 2. Haplotype diversity of loggerhead OR genes.

Table 5: Table 5 summarizes the data regarding the four non-conserved loggerhead OR genes (Sets 2 and 3).

A. LH3 (Haplotype Distribution)

	LH–A	LH–O	LH–M	LH–P	Total
Ha 1	18	11	6	10	45
Ha 2	1				1
Ha 3	1				1
Ha 4		1			1

E. % difference in sequence (pair-wise comparisons)

	Ha 1	Ha 2	Ha 3	Ha 4
Ha 1		*0.8*	*0.0*	*0.8*
Ha 2	0.3		*0.8*	*1.6*
Ha 3	0.3	0.5		*0.8*
Ha 4	0.3	0.5	0.5	

B. LH4 (Haplotype Distribution)

	LH–A	LH–O	LH–M	LH–P	Total
Ha 1	14	12	6	10	42
Ha 2	6				6

F. % difference in sequence (pair-wise comparisons)

	Ha 1	Ha 2
Ha 1		*0.0*
Ha 2	0.4	

C. LH5−pseudo (Haplotype Distribution)

	LH–A	LH–O	LH–M	LH–P	Total
Ha 1	5	10	6	8	29
Ha 2	14			1	15
Ha 3	1	2			3
Ha 4				1	1

G. % difference in sequence (pair-wise comparisons)

	Ha 1	Ha 2	Ha 3	Ha 4
Ha 1		*1.9*	*0.9*	*0.9*
Ha 2	0.6		*0.9*	*0.9*
Ha 3	0.3	0.3		*1.9*
Ha 4	0.3	0.3	0.6	

D. LH6−pseudo (Haplotype Distribution)

	LH–A	LH–O	LH–M	LH–P	Total
Ha 1		9	3	10	22
Ha 2	15				15
Ha 3		3	3		6
Ha 4	2				2
Ha 5	2				2
Ha 6	1				1

H. % difference in sequence (pair-wise comparisons)

	Ha 1	Ha 2	Ha 3	Ha 4	Ha 5	Ha 6
Ha 1		*1.0*	*0.0*	*2.0*	*1.0*	*3.0*
Ha 2	0.7		*1.0*	*1.0*	*0.0*	*2.0*
Ha 3	0.3	1.0		*2.0*	*1.0*	*3.0*
Ha 4	1.3	0.7	1.0		*1.0*	*1.0*
Ha 5	1.0	0.3	0.7	0.3		*2.0*
Ha 6	1.7	1.0	1.3	0.3	0.7	

Tables 5A − 5D indicate the haplotype distribution among the respective populations. Numbers indicate the number of individuals sampled. Tables 5E − 5H indicate the percent differences in nucleotide (normal font) and amino acid (italics) sequences, comparing indicated pairs of haplotypes.

OR haplotype variation among a Pacific population of green sea turtles.

Figure 3 summarizes data for the green turtles. The same general trend is observed as in loggerheads. The conserved OR genes (G1, G2) show only a single haplotype, respectively; the pseudogenes (G4, G5) show the most allelic diversity. The presumably expressed OR G3 (no internal stop codon) shows an intermediate level of diversity.

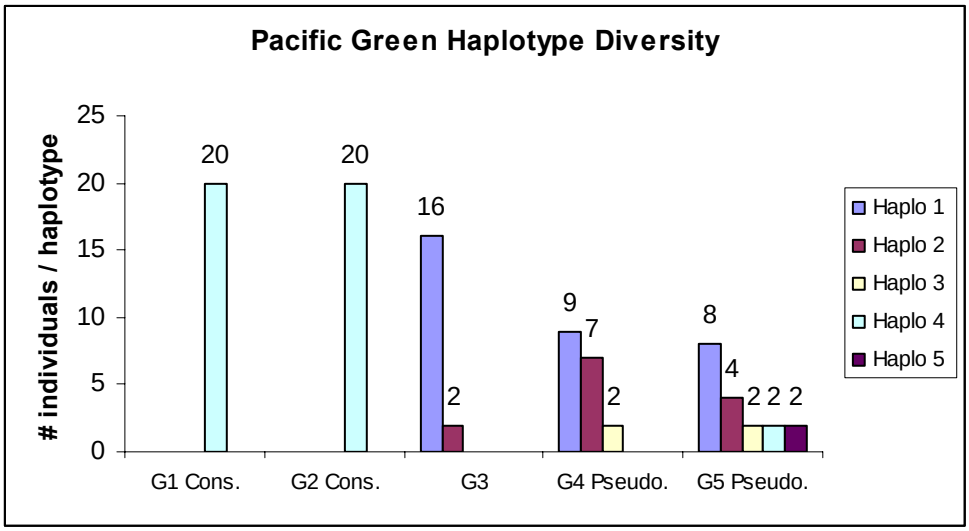

Figure 3. OR haplotype variation among a Pacific population of green sea turtles.

OR haplotype diversity among an Atlantic population of leatherback sea turtles.

Figure 4 summarizes the data for leatherbacks. The presumed expressed sequences (no internal stop codons) show the same trend as described above: conserved LB2 is represented by only one haplotype, while the less conserved LB3 and LB4 show considerably greater allelic diversity. A striking difference among the leatherback data and that of loggerhead and green turtles is the apparent conservation among the pseudogenes; all individuals possessed identical DNA sequences for genes containing internal stop codons. This unquestionably seems quite unexpected if these pseudogenes are indeed without function.

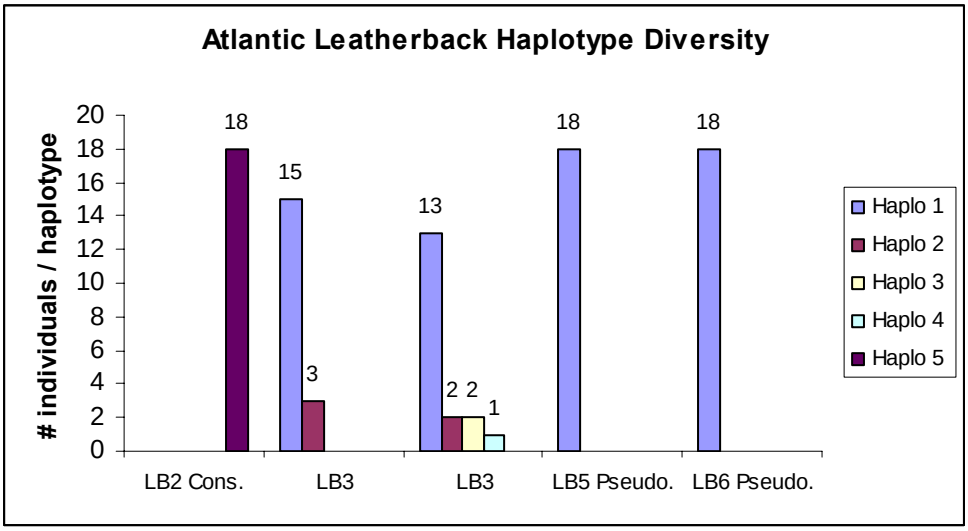

Figure 4. OR haplotype diversity among an Atlantic population of leatherback sea turtles.

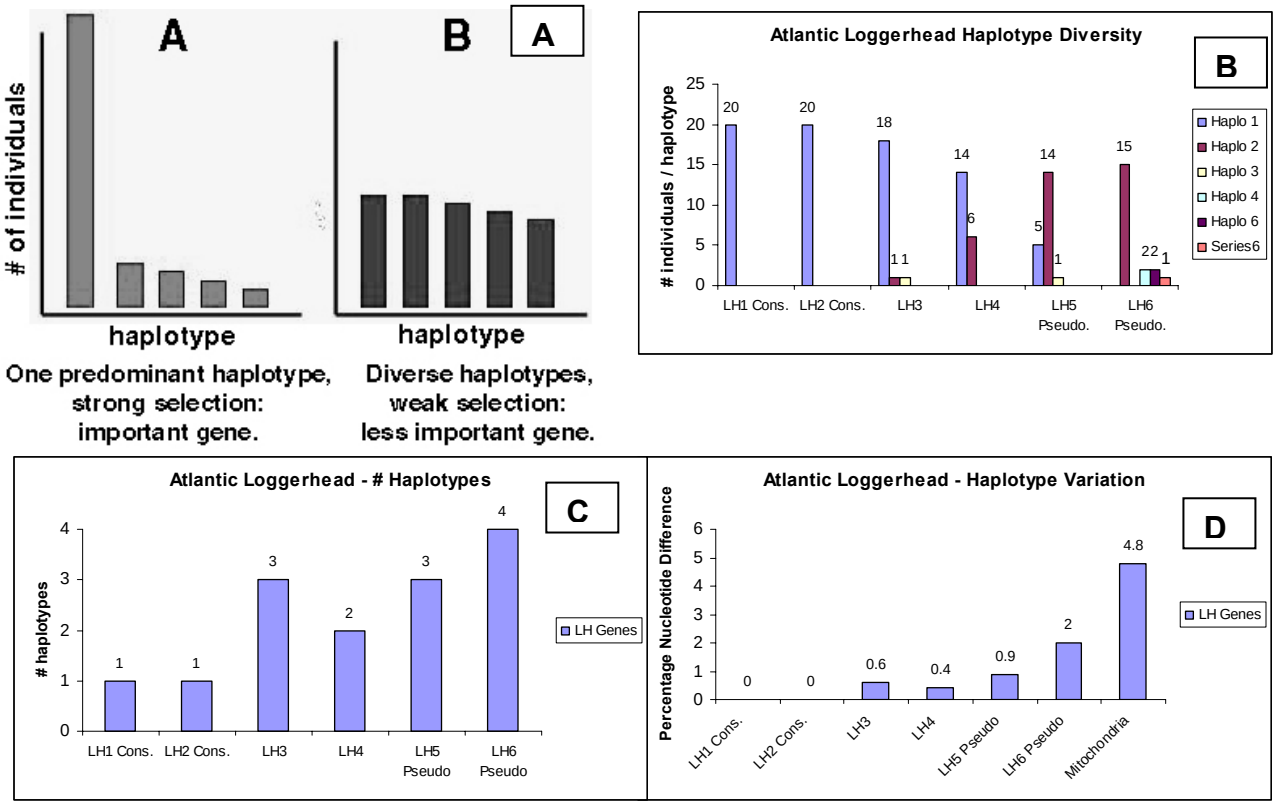

Figure 5. Summary of hypothesis and Atlantic loggerhead results.

Figure 5A illustrates our hypothesis that the importance of a gene may be reflected in the diversity of gene haplotypes. Figure 5B represents our results (previously shown) for the Atlantic population of loggerhead turtles (samples collected off the Carolina and Georgia coasts by the South Carolina Department of Natural Resources). These data appear to support our hypothesis. The two genes we interpret as being important, based on their high degree of conservation among different species, have a single dominant haplotype. The pseudogenes, arguably nonfunctional, show diverse haplotypes, while the two arbitrarily chosen genes (LH3 and LH4) show a somewhat intermediate pattern. Figure 5C indicates the number of haplotypes observed for each gene, and this, too, seems consistent with the hypothesis, with the conserved genes showing less diversity than the less conserved genes. Figure 5D compares the nucleotide differences among haplotypes of the respective OR genes with a mitochondrial gene, all for the Atlantic population of loggerheads. This mitochondrial sequence represents a neutral region of DNA and has been used to characterize the loggerhead populations (Anderson, Quattro, unpublished). Two haplotypes are predominant within the Atlantic population (accession numbers AJ001074 and AJ001075) and they differ in nucleic acid sequence by 4.8%. Because this region is under neutral selection, this variation can be viewed as an upper level variation one might expect. Again, the conserved genes show variation, the pseudogenes the greatest, though considerably less than the mitochondrial genes, and LH3 and LH4 show an intermediate level of variation.

We interpret these data to support our hypothesis that it is feasible to assess the importance of a gene based on its haplotype diversity and variation within a population. And based on the pattern of conservation of the two conserved genes, this study suggests that these genes and the odors their products convey are likely of considerable importance to these animals.

Relative conservation among pseudogenes.

When we began this study, we expected pseudogenes to be highly degenerate, on the verge of being unrecognizable. If they are nonfunctional, then evolutionary selection will no longer be acting on the genes, and they should be accumulating nucleotide replacements. Eventually, they should accumulate so many nucleotide replacements that they will no longer be recognizable. The data seem inconsistent with this point of view; especially with respect to the leatherback pseudogenes (Leatherback tissue samples were taken across a wide region of the Caribbean and therefore not likely from closely related individuals).

One possible explanation is that the pseudogenes have not entirely lost all function. OR genes are known to play a broader role than just detecting odors; they are also known to play a role in axon guidance, directing sensory neurons to their appropriate brain targets during development, and they are known to contribute to the regulation of which OR genes are expressed in a given sensory neuron. It may be that these pseudogenes are still playing a role and that selection is somehow still acting.

Perhaps a more interesting possibility is that these genes have only become pseudogenes relatively recently. In this view, the OR genome would be under constant expansion (gene duplication) and contraction (gene loss). Comparisons of the OR genomes of mouse and human suggest expansion and contraction of OR gene subfamilies. Certainly, we recognize the pseudogenes based on their retained similarity to the presumed functional genes. Perhaps their conservation indicates something of the genetic dynamics the olfactory system is capable of on a relatively short, but nevertheless evolutionary, time scale. Still, the conserved loggerhead pseudogenes remain perplexing.

III. Evidence of positive selection among sea turtle OR genes (under study).

The work described above presents a largely theoretical perspective on the importance of the OR genome in sea turtle behavior. We are currently applying mathematical analyses to these data to confirm (or reject) the proposed hypotheses.

CONCLUSIONS

We set out to determine if one could assess the importance of OR genes in the life history of an endangered species using largely non-invasive methods. We identified and compared the olfactory genomes of three species of sea turtles and, in so doing, identified two highly conserved OR genes suggesting an important and common use for olfactory processing for all three species. We suggested the possibility that airborne odorants play an important role in sea turtle behavior. We established an indirect genetic test of the importance olfaction has in the life history of these animals.

A goal of using these genetic data was to identify odors that might actually be employed to discourage turtles from fishing baits. Perhaps the most valid way to do this would be to take odor receptors which are presumed to be important, and identify the odors that activate them. This is feasible, but techniques are still under development. Perhaps this will be doable in the near future, but right now it remains a technical difficulty. Far easier, cheaper, and faster would be a physiological study recording electrodes on the olfactory nerves of a turtle and screening the types of ecologically relevant odor molecules that the turtle can perceive. Such a study should be coupled with careful behavioral analyses, measuring responses to these odors so that desired answers to these problems may be found.

CHEMORECEPTION IN LOGGERHEAD SEA TURTLES: AN ASSESSMENT OF THE FEASIBILITY OF USING CHEMICAL DETERRENTS TO PREVENT SEA TURTLE INTERACTIONS WITH LONGLINE FISHING GEAR

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ABSTRACT

The mechanisms by which sea turtles are attracted to and become entangled in commercial fishing gear are not well understood. Identification of sensory attractants and repellants may prove useful in developing gear and bait modifications to reduce sea turtle bycatch in commercial fisheries. We conducted experiments to investigate the ability of loggerhead turtles to use chemical and flow cues to successfully locate squid bait and also tested to see if chemical manipulation of squid bait would reduce the turtles' ability and/or willingness to track and locate bait. Captive-reared juvenile loggerhead turtles were placed in a seawater-filled flume tank with a current of 3 – 5 $cm \cdot sec^{-1}$. A nylon bag containing either nylon (control), squid, or squid that had been marinated in 2-phenylethanol or shark-derived compounds was placed in the current upstream from the turtle. Trials were conducted in darkness, and behavior of turtles was monitored and recorded using an IR-sensitive video surveillance system. The presence of squid bait in the tank elicited feeding and searching behavior; however, turtles showed limited ability to locate squid bait in the absence of visual cues. Only 20 – 33% of turtles located and ate the squid bait during the 10-minute trial period. These results indicate that visual cues are important for foraging success in loggerhead turtles, and chemoreception likely plays a secondary role. Treatment of squid with 2-phenylethanol or shark-derived compounds did not prevent turtles from eating squid bait. There was no significant difference in the number of turtles that located and ate bait among control, squid, and chemically-modified squid trials. An effective chemical deterrent for sea turtles has yet to be identified.

INTRODUCTION

The incidental capture of sea turtles in commercial longline fishing gear is an issue of growing concern for fishers, fishery management agencies, and environmental groups. Bycatch of sea turtles in longline gear designed to capture pelagic fish species has been implicated as a significant source of mortality for endangered leatherback and loggerhead turtles and threatened olive ridley turtles (*Lepidochelys olivacea*) in the North Pacific Ocean (Balazs and Pooley, 1994; Spotila et al., 2000). United States regulatory agencies have responded by enacting numerous mitigation measures to reduce or prevent capture of sea turtles by the U.S. longline fleets (National Marine Fisheries Service (NMFS), Office of Protected Resources, http://www.nmfs.noaa.gov/pr/PR3/regulations.html). Swordfish fleets operating out of Hawaii and California have been subject to large time-area closures so that interactions might be avoided. Unfortunately, the U.S. fleet comprises less than 5% of the total longline effort in the Pacific Ocean (Lewison et al., 2004), and fishing fleets from other nations continue to operate in areas deemed by the U.S. government to be high risk for sea turtle interactions. Fishery closures are not a mitigation method that other fishing nations are likely to adopt, so the usefulness of this technique for reducing sea turtle bycatch is limited. For this reason, alternative methods to minimize or prevent sea turtle bycatch in longline gear, such as gear and bait modifications, are currently being investigated.

Sea turtles and pelagic fishes are evolutionarily distinct groups of animals with differences in sensory biology that may influence the ways in which they interact with fishing gear. The factors that attract sea turtles and target fish species to longline gear and bait are not well understood, but numerous sensory cues may be involved. A multidisciplinary interagency collaborative effort was initiated by NOAA Fisheries scientists in 2000 to investigate the visual, auditory, and chemosensory abilities of sea turtles and pelagic fishes to identify exploitable differences that may be used to develop gear and bait attractive to fish but undetectable or unattractive to sea turtles. This paper presents results from a series of studies designed to assess the chemosensory abilities of loggerhead sea turtles and the feasibility of using chemical deterrents to prevent sea turtles from interacting with longline fishing gear.

The term "chemoreception" refers to an organism's ability to detect and differentiate chemical cues in its environment by taste (gustation) or smell (olfaction). Chemical cues may be used for prey detection and location (Zimmer and Butman, 2000), for orientation during long distance migrations (Atema et al., 2002; Doving and Stabell, 2003), or for intraspecific communication related to reproduction and predator avoidance (Weldon, 1990; Hara, 1993; Wisenden, 2003 and references therein). The role of chemoreception in the ecology of marine invertebrates and fishes has been well studied, but relatively little information is available on the ecological importance of chemoreception for marine reptiles.

We were primarily interested in determining the role of chemoreception in foraging behavior and avoidance behavior of loggerhead turtles. Although sea turtles are generally considered visual predators, other sensory cues (tactile, flow, chemical) may also contribute to foraging success. Compelling evidence shows that chemoreception is an important factor in food recognition by post-hatchling and juvenile sea turtles (Grassman and Owens, 1982; Constantino and Salmon, 2003). However, the ability of sea turtles to use chemical cues to

effectively track and locate prey had not been studied. If sea turtles use chemoreception to detect and find food sources in their aquatic environment, then chemicals emanating from squid and mackerel bait may play a role in attracting sea turtles to longline fishing gear. Chemical modifications that make bait less appealing or more difficult for sea turtles to detect may help deter sea turtles from interacting with fishing gear.

Numerous factors can affect the ability of aquatic organisms to use chemical cues to locate prey. Results from studies of chemical tracking behavior in marine invertebrates and fishes show that flow patterns, in particular, play a critical role in successful location of prey in a dynamic and complex environment. Orientation into flow (rheotaxis) is a common component of searching strategies, as the most likely source of an odor would be upstream (Hodgson and Mathewson, 1978; Weissburg and Zimmer-Faust, 1994; Zimmer-Faust et al., 1995; Vickers, 2000; Carton and Montgomery, 2003; Kanter and Coombs, 2003). We designed a flume tank in which squid bait was presented to loggerhead turtles under unidirectional flow conditions to assess the ability of loggerhead turtles to use chemical cues in combination with flow cues to locate a food source in the absence of visual cues. These experiments were designed to gauge the relative importance of chemoreception for successful prey location. The flume tank was also used to test the behavioral responses of loggerhead turtles presented with squid that had been treated with a chemical odor-masking agent, 2-phenylethanol, and squid that had been treated with skin secretions of tiger sharks (*Galeocerdo cuvier*), a known predator of sea turtles, to assess the potential of using predator-derived compounds as deterrents. Finally, we assessed behavioral responses of sea turtles to shark-derived semiochemical compounds purported to have a deterrent effect on feeding sharks but not on teleost fishes. We chose to test shark semiochemicals because these compounds are currently under development to reduce shark bycatch in commercial fisheries, and the manufacturers were interested in the possibility that compounds might deter other bycatch species as well. We hypothesized that loggerhead turtles presented with chemically modified squid would exhibit a lower rate of success in locating bait compared with turtles presented with untreated squid.

MATERIALS AND METHODS

Animals

All experiments were conducted with 2-year-old loggerhead sea turtles at the NOAA Fisheries Sea Turtle Facility in Galveston, Texas, U.S.A., during a 3-week period in September – October 2004. Loggerhead turtles at this facility originate from hatchlings collected from Florida nesting beaches and are captive-reared until 3 years of age, at which time they are released off the Florida coast. Details of animal husbandry at the NOAA-STF are described by Higgins (2003). Briefly, yearling turtles were housed individually in 76 cm diameter (45-cm depth) circular plastic containers with mesh flooring. Containers were suspended in rectangular fiberglass raceways (6.5 x 2.0 x 0.6 m) filled with 6435 L of seawater at 29°C –30°C. The raceways were drained and new seawater pumped in every other day. Ambient air temperature in the facility was regulated at 30°C. Skylights in the facility exposed turtles to a natural light photoperiod that varied with season. At the time of our experiments, local sunrise and sunset were approximately 06:30 and 17:30, respectively.

Fluorescent lights above the tanks were used to supplement natural sunlight and were on from 07:30 to 16:00. Turtles were fed a ration of two squid (approximately 150 – 200 grams) three times a week when experiments were not being conducted. The average mass of turtles used in this study was 8.35 ± 0.14 (S.E.M.) kg and average straight carapace length was 42.08 ± 0.23 (S.E.M.) cm.

Experiment tank

A fiberglass rectangular tank was used to assess the behavior and tracking abilities of juvenile loggerhead turtles presented with untreated squid bait and chemically modified squid bait under steady semiturbulent flow conditions. The working section of the tank consisted of a 0.9 x 0.9 m start chamber separated from a larger (2.1 x 0.9 m) main chamber by a gate attached to a pulley system (Fig. 1). The tank was filled to a depth of 26 cm with seawater at 29°C. A series of three plastic honeycomb baffles (Specialized Metals, Coral Springs, FL) were located on the side of the main chamber opposite the start chamber and gate. During trials, seawater was pumped through these baffles at a volume flow rate of 275 $l \cdot min^{-1}$ and drained out of the tank through a drain grate on the floor of the start chamber. A Marsh McBirney electromagnetic flowmeter (Model 2000) was used to generate flow profiles of the tank under trial conditions. Flow speed was 3 – 5 $cm \cdot sec^{-1}$ in the central portion of the flume and tapered off to < 1 $cm \cdot sec^{-1}$ along the tank walls.

TREATMENTS

Turtles were randomly selected for inclusion in one of three treatment groups: 2-phenylethanol (2-PEA), tiger shark skin extracts (TIGER) or compound VR (VR), and an experimental shark repellent manufactured by Shark Defense, Inc. Each turtle in the 2-PEA and TIGER treatment groups had three trials. In one trial, a nylon bag containing ~ 20–25 g of chopped squid was secured onto the side of the baffle facing into the main chamber. The nylon bag was positioned 12 cm below the water surface along the midline of the tank. In another trial, a nylon bag filled with ~ 20 – 25 g chopped squid marinated overnight in either 0.1 M 2-phenylethanol (2-PEA treatment group) or skin secretions obtained from live wild-caught tiger sharks (TIGER treatment group) was secured to the baffle. The third type of trial was a control trial in which the nylon bag was simply filled with more nylon and secured to the baffle (i.e., no squid, untreated or chemically modified, was presented to the turtle). We conducted the nylon-only control trials to see if flow cues alone could induce the same behaviors that we observed for chemical trials. Trial order was randomized for each treatment group.

Time limitations prevented us from performing three trials for the VR treatment group so the nylon control trial was eliminated. Turtles in this treatment group underwent one trial in which they were presented with untreated squid in the manner described above and a second trial in which compound VR was introduced into the tank alongside the squid. We modified the method of chemical delivery for the VR trials to prevent dilution of the deterrent chemical over the course of the trial. To ensure that a steady concentration of chemical deterrent was delivered throughout the trial, we continuously pumped compound VR at a rate of 5 $ml \cdot min^{-1}$ into the seawater flow stream from a nozzle positioned adjacent to the nylon bag filled with squid.

All chemicals used for these experiments were approved by the NOAA Fisheries Sea Turtle Facility staff veterinarian.

Trial protocol and analysis

Turtles fasted for 36 – 44 hours prior to trials. All trials were conducted between 08:00 and 16:00. A trial was initiated by placing a turtle in the start chamber with the gate closed. The turtle was left in the start chamber for a 20-minute acclimation period in static water, and then seawater flow through the tank was initiated. Seawater flowed through the baffles and the nylon bag, effectively pushing a plume of chemical towards the start chamber. Two minutes after flow began, the gate separating the start chamber from the main chamber was lifted, and the turtle was free to explore both chambers for 10 minutes. All trials were conducted in complete darkness. During the exploration period, the turtle's behavior was monitored and recorded using a system of infrared (IR) spotlights and IR-sensitive cameras interfaced with a digital video recorder (DVR) (A-1 Services Unlimited Inc., Bradenton, Florida).

We were primarily interested in whether or not turtles could use a combination of flow and chemical cues to successfully locate squid bait in the absence of visual cues, and if chemical manipulation of squid bait would reduce the turtles' ability and/or willingness to track and locate bait. Trial videos were analyzed to assess successful location of bait, as indicated by the turtle striking and attempting to consume the nylon secured to the baffle, and the length of time necessary for turtles to locate bait. The amount of trial time spent in the main chamber where the chemical plume originated was also recorded.

During trials, turtles frequently displayed a behavior in which they suddenly stopped swimming, put their nostrils to the tank floor, raised their front flippers to the side of their head, and used rear flippers to paddle backwards or spin in circles around the same spot. This searching behavior is typical of captive turtles feeding in tanks, and we refer to it as "backup" behavior. The frequency with which backups were displayed during trials was recorded.

Within each treatment group, logistic regression for binomial data was used to analyze differences in the number of turtles that located bait among control, squid, and chemically modified squid trials. If data met assumptions for parametric tests, we used one-way repeated measures ANOVA to assess differences between trials in backup frequency and the amount of time spent in the main chamber of tank where the chemical plume originated. If data did not meet assumptions of normal distribution and equal variance, then a repeated measures ANOVA on ranks (Friedman rank test) was used to look for statistical differences between trials.

RESULTS

Turtles showed limited ability to locate squid bait in complete darkness under the flow conditions created in our experiment tank (Fig. 2). During trials in which untreated squid was presented to turtles, only 20 – 33% of turtles successfully located squid within the 10-minute trial period for the 2-PEA, TIGER, and VR treatment groups. Chemical modification of squid

did not alter the ability of turtles to find bait: there was no significant difference in the number of turtles that found bait in squid, chemically modified squid, and control trials for the 2-PEA ($X^2 = 3.632$, df = 2, $P = 0.163$), TIGER ($X^2 = 4.270$, df = 2, $P = 0.118$), or VR ($X^2 = 0.483$, df = 1, $P = 0.487$) treatment groups.

Few turtles actually located the bait, so any meaningful statistical comparison of the length of time necessary for turtles to locate bait during squid, chemically modified squid, and control trials could not be made. Table 1 shows a summary of the time necessary to locate bait for each trial type in the 2-PEA, TIGER, and VR treatment groups. No consistent trend was obvious. For the 2-PEA and VR treatment groups, turtles found chemically modified squid bait 2 – 3 times faster than they found untreated squid bait. In the TIGER treatment group, one turtle successfully located squid bait that had been chemically modified and took twice as long to locate bait compared with turtles in squid and control trials.

Despite their limited ability to actually locate the source of squid bait using chemical cues, the turtles showed alterations in behavior when squid was present in the experiment tank. In the TIGER treatment group, turtles displayed characteristic feeding behavior (i.e., backup behavior) with significantly greater frequency during squid trials and chemically modified squid trials compared with control trials ($X^2 = 12.514$, df = 2, $P < 0.001$) (Fig. 3). There was, however, no difference in backup frequency between squid and chemically modified squid trials, so treating squid bait with extracts from a natural predator (tiger shark) did not deter or alter feeding behavior in captive loggerhead turtles. Likewise, we found no significant difference in backup frequency between squid and chemically modified squid for either the 2-PEA ($F = 2.063$, df = 2, $P = 0.164$) or VR ($F = 0.312$, df = 1, $P = 0.606$) treatment groups (Fig. 3).

There was no significant difference in the amount of time that turtles spent in the section of the tank where the chemical plume was generated (i.e., the main chamber) between trials in the 2-PEA ($F = 1.951$, df = 2, $P = 0.179$), TIGER ($F = 0.346$, df = 2, $P = 0.713$), or VR ($F = 0.202$, df = 1, $P = 0.676$) treatment groups (Fig. 4).

DISCUSSION

Given the difficulties of studying oceanic stage juvenile loggerhead turtles in their pelagic environment, our understanding of their foraging behavior and how their foraging behavior might lead them to interact with fishing gear is rather limited. A recent extensive survey of gut contents of loggerhead turtles captured in the former North Pacific high-seas driftnet fishery has shown that a variety of prey items are consumed, with surface-dwelling pelagic snails, crabs, jellyfish, and tunicates comprising the majority of gut contents (Parker et al., 2005). Non-neustonic prey items that occur at depths up to 100 m are also represented in gut contents, but are less common. Patchy prey distribution in the oceanic habitat likely fosters opportunistic feeding behavior in juvenile loggerhead turtles (Tomas et al., 2001; Parker et al., 2005), and it is hard to imagine that turtles would pass up a meal of longline squid bait should they come across it in their oceanic wanderings. Both sea turtles and other large pelagic predators, such as the target fish species of commercial longline fisheries, are

attracted to oceanographic features such as seamounts and convergent fronts where prey items are concentrated (Polovina et al., 2000). The behavior of sea turtles in relation to oceanographic features increases the chances that turtles will come into close proximity to fishing operations, thus making them susceptible to fisheries interactions. Indeed, satellite-tracking studies have demonstrated that in the North Pacific Ocean juvenile loggerhead turtles congregate at the Emperor Seamounts and along convergent fronts characterized by strong sea surface temperatures and chlorophyll gradients, areas also exploited by longline fishing fleets (Parker et al., 2003; Polovina et al., 2000; Polovina et al., 2004).

The sensory cues used by juvenile sea turtles for orientation and prey finding in the open ocean are not fully understood. Association with oceanic fronts, where prey is more readily available, may be maintained by using temperature and current cues (Polovina et al., 2004). Loggerhead turtles are generally considered to be visual predators, but it is possible that chemical cues associated with high concentrations of prey items in frontal zones contribute to the turtles' ability to detect and locate food in the open ocean. Use of chemical and flow cues in open ocean orientation and migration has been demonstrated in salmon (Doving, 1990; Doving and Stabell, 2003), but has yet to be demonstrated for marine turtles. Results from our laboratory study of chemical orientation in juvenile loggerhead turtles do not provide strong evidence that turtles are effective at using only chemical and flow cues to locate a food source. Flow conditions in our flume tank are necessarily different from what turtles experience in the pelagic environment; however, we attempted to create uncomplicated flow conditions that would be conducive for tracking, i.e., a unidirectional current of low-to-moderate speed with a food source located upstream. Only 20 – 33% of turtles found squid bait presented under these simple flow conditions in the absence of visual cues. Flow conditions in the loggerhead turtle's pelagic habitat are undoubtedly more complex and dynamic than those we could create in the laboratory, and the ability of turtles to use additional cues provided by currents and oceanographic gradients to orient towards a source of attractant chemicals in the oceanic environment warrants further investigation.

Constantino and Salmon (2003) reported that post-hatchling leatherback turtles showed a rheotactic response when food homogenate was introduced into a test chamber through an underwater filter outflow, but we observed no strong rheotactic response to the current created in our flume tank. Behavior during trials was characterized by turtles pacing back and forth between the start chamber and the main chamber along the sides of the tank, with occasional swims down the center of the tank, both facing into and away from the current. The presence of food chemicals resulted in an increase in backup behavior wherein turtles searched the tank floor for the source of food odors rather than a rheotactic response in which turtles searched "upstream." Backup behavior is an artifact of captive rearing; the tank floor is the most likely place that loggerhead turtles will encounter food in their holding tanks. This obviously is not true for wild loggerhead turtles in the open ocean, so it is difficult to assess this species' ability to orient using flow cues based on these captive animal results. In addition to chemical and flow cues, tactile cues may also play a role in bait striking behavior of loggerhead turtles. Five of the nine animals in the TIGER treatment group bit the nylon bait bag even when there was no squid inside (Fig. 2).

Although chemical cues elicit feeding behavior in loggerhead, green, and leatherback turtles (Owens et al., 1982; Grassman and Owens, 1982; Steele et al., 1989; Constantino and Salmon, 2003), the majority of experimental evidence suggests that visual cues are primarily important to foraging success. Constantino and Salmon (2003) found that when visual and chemical cues associated with jellyfish prey were simultaneously presented to leatherback post-hatchlings, turtles ignored the current created by chemical delivery and oriented towards the visual stimuli instead. When tested separately, visual stimuli evoked a more robust feeding response than chemical stimuli (Constantino and Salmon, 2003). Our experiments support the idea that sea turtles are primarily visual predators, as juvenile loggerhead turtles showed a low success rate locating food in the absence of visual cues (Fig. 2). For this reason, it seems likely that use of a visual deterrent, rather than a chemical deterrent, would be a more effective means of preventing sea turtle interactions with longline gear. Researchers with the NOAA Sensory Biology Working Group are currently investigating visual capabilities of sea turtles and pelagic fishes in an attempt to identify visual attractants and repellents (Fritsches et al., 2005; Wang et al., 2005). We must bear in mind, however, that even if an effective visual deterrent is identified and implemented in longline fisheries, bait chemicals in the vicinity of fishing operations may alert turtles to the presence of food and induce a heightened state of awareness and searching behavior. The effectiveness of a visual deterrent will depend largely on whether or not the turtle's aversion response overrides the feeding response, which is fueled in part by chemical cues. Studies investigating the efficacy of various methods for repelling birds show that a combination of both visual and chemical deterrents is more effective than either on its own (Mason and Clark, 1996).

Unfortunately, an effective chemical deterrent has yet to be identified for sea turtles. Previous studies have shown that loggerhead turtles readily consumed squid that had been soaked in lactic acid, urea, quinine hydrochloride, capsaicin, wasabi oil, and natural toxins (ink from *Aplysia* spp.) (J.B. Swimmer, personal communication). One approach we took for the current study was to assess the feasibility of disguising longline bait odor with a novel chemical, such as 2-phenylethanol, as a means to prevent loggerhead turtles from locating and biting squid bait. Loggerhead turtles are capable of detecting 2-phenylethanol, although in previous studies they showed no sign of attraction to this chemical and are unlikely to identify this chemical with a food source at first exposure (Manton et al., 1972; Southwood et al., unpublished data). Treatment of squid bait with 2-phenylethanol did not significantly alter the behavior of loggerhead turtles during trials, so the odor-masking approach using this particular chemical was ineffectual. It is unlikely that results were affected by a decrease in 2-phenylethanol concentration over the course of the 10-minute trial as a result of wash-off in the current, as turtles located and bit squid bait marinated in 2-phenylethanol in less time that it took them to locate untreated squid bait (Table 1).

We were also interested in assessing the behavior of loggerhead turtles in response to chemical compounds derived from sharks, the main natural predators of juvenile and adult sea turtles. Higgins et al. (2005) demonstrated that captive-reared juvenile loggerhead turtles in nearshore holding pens show defensive behavior on encountering a shark-shaped decoy and subsequently avoid the section of the pen where the decoy is present, providing experimental evidence that visual recognition plays a strong role in predator avoidance. The role of chemical recognition in predator avoidance has not previously been investigated for sea

turtles. Terrestrial reptiles and amphibians display avoidance and defensive behavior when presented with skin extracts and rinses from predatory snake species or when placed in environments that have been conditioned by predator presence (Dial, 1990; Weldon, 1990); however, we found no significant difference in behavior of loggerhead turtles between squid trials and trials in which squid had been treated with skin secretions from live wild-caught tiger sharks or semiochemicals extracted from dead shark specimens. If association of a predator's scent with a threat is learned rather than innate, then this may explain why shark-derived chemicals did not alter the behavior of captive-reared loggerhead turtles during bait-tracking trials. Behavioral responses to predator-derived chemicals may be more pronounced in wild-caught sea turtles.

In conclusion, we found that although loggerhead turtles detect and respond behaviorally to the presence of food chemicals in their aquatic environment, they have limited success in tracking and locating a food source using only chemical and flow cues. Visual cues are likely of primary importance to foraging success in this species, with the chemical senses playing a secondary role. Further research is necessary to identify sensory deterrents and evaluate the feasibility of using sensory deterrents to reduce or prevent sea turtle bycatch in longline fisheries.

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Table 1. Length of time necessary for loggerhead turtles to locate squid, chemically-modified squid, and control bait in the 2-PEA, TIGER, and VR treatment groups.

TREATMENT *& trial type*	Time to locate bait (min)*	*N*
2-PEA		
Squid	7.48 ± 0.02	2
2-PEA	2.57 ± 0.94	2
Control	n/a	0
TIGER		
Squid	4.71 ± 1.00	3
Tiger	9.83	1
Control	5.80 ± 1.27	5
VR		
Squid	3.67	1
compound VR	2.16 ± 0.94	2

*Values presented as the $\overline{X}$ ± S.E.M.

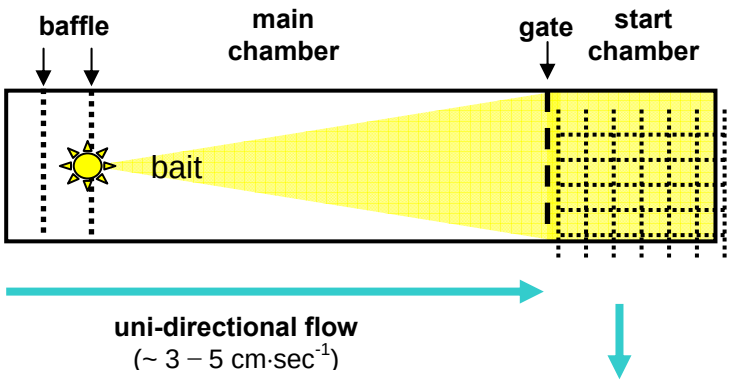

Figure 1. Diagram of tank used in experiments to assess chemical tracking abilities and behavior of 2-year-old loggerhead turtles at the NOAA Fisheries Sea Turtle Facility in Galveston, TX.

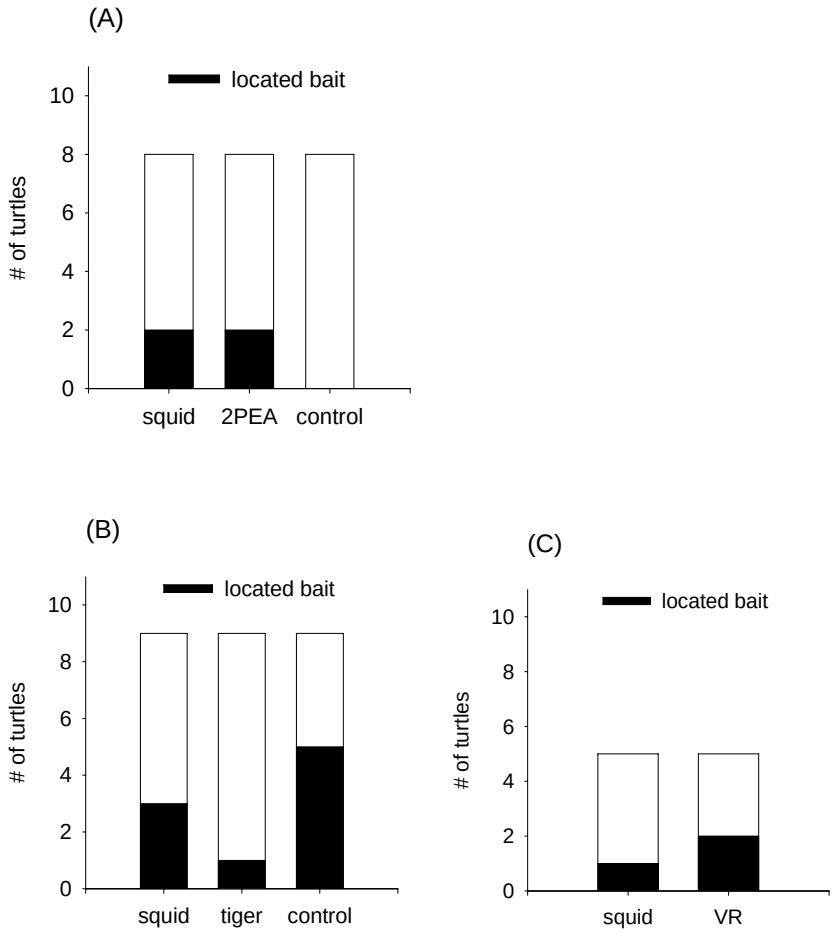

Figure 2. Number of 2-year-old loggerhead turtles that successfully located bait during squid, chemically modified squid, and control trials for the 2-PEA (A), TIGER (B), and VR (C) treatment groups. White bars represent the total number of turtles that participated in trials and black bars represent the number of those turtles that found bait.

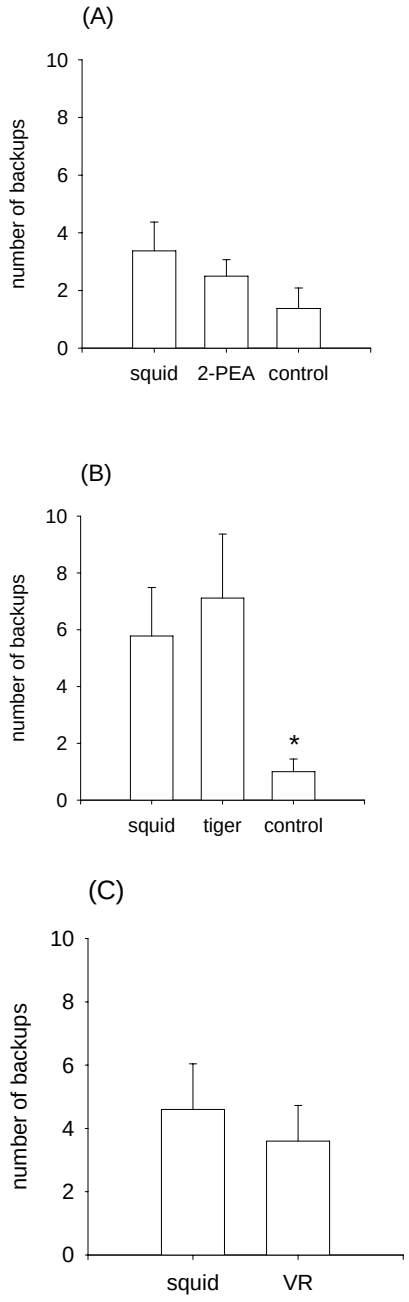

Figure 3. Frequency with which backup behavior was exhibited by loggerhead turtles presented with squid, chemically modified squid, and control bait for the (A) 2-PEA ($N = 8$), (B) TIGER ($N = 9$), and (C) VR ($N = 5$) treatment groups. Turtles in the TIGER treatment group displayed backup behavior significantly more often during squid and chemically modified squid trials compared with control trials (B) (stats).

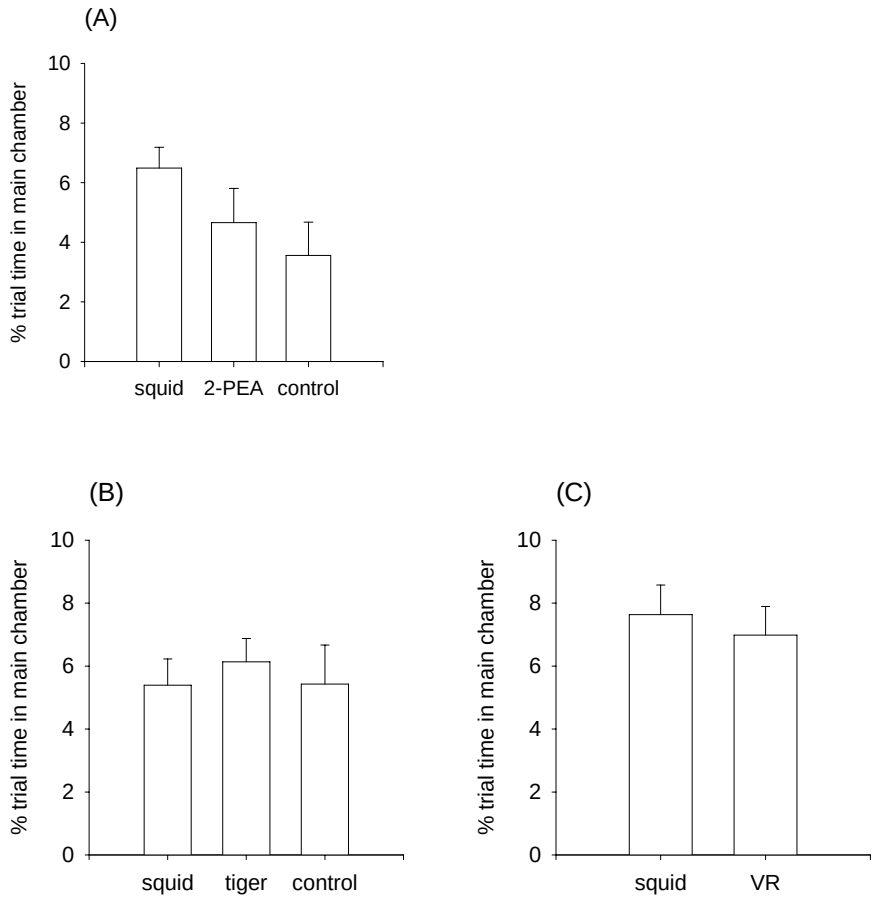

Figure 4. Amount of time that turtles spent in the main chamber of the experiment tank where chemical plume originated during squid, chemically modified squid and control trials for 2-PEA (A), TIGER (B), and VR (C) treatment groups. There was no significant difference between trial types for any of the treatment groups.

TESTS OF REPELLENT BAIT TO REDUCE TURTLE BYCATCH IN COMMERCIAL FISHERIES

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ABSTRACT

We conducted behavioral experiments with captive green turtles (*Chelonia mydas*), yellowfin tuna (*Thunnus albacares*), and skipjack tuna (*Katsuwonus pelamis*) to investigate feeding responses to squid soaked in various chemical compounds with known or suspected properties that repel or conceal food from these species. Our immediate aim was to identify a bait modification that would induce an avoidance response in turtles, but not tunas. Our ultimate objective was to find bait treatments that could reduce the incidental bycatch of threatened and endangered sea turtles in pelagic longline fishing gear. Green turtles and tunas were maintained in captivity and were presented whole squid (*Loligo* spp.) marinated in various chemical compounds (lactic acid, quinine hydrochloride, chlorhexidine gluconate), natural flavorings (garlic, cilantro, Angostura® aromatic bitters, Habanero chili peppers, wasabi oil, and lemon juice), and naturally occurring defensive compounds (100% squid ink and noxious secretions from the sea hare (*Aplysia* spp.) that fed exclusively on the red algae, *Laurencia nidifica*). Turtles and tunas ate all food items presented. While olfaction/gustation likely plays a role in turtles' attraction to bait, we have yet to discover a substance or method that reduces their willingness to bite squid baits. These results are especially important as they help prioritize future research strategies aimed at minimizing turtle bycatch in pelagic longline gear.

INTRODUCTION

The incidental catch of non-target species in fishing gear is one of the most significant issues affecting fisheries management today. Global discards in commercial fisheries are estimated at 27 million metric tons, which represents economic losses in the billions of dollars (Food and Agriculture Organization [FAO], 2002). In addition, the incidental capture of protected marine mammals, sea turtles, and sea birds may be contributing to declines in abundance and to the inability of depleted populations to recover (Dayton et al., 1995; Hillestad et al., 1995; Hayes et al., 2003; Lewison and Crowder, 2003). Marine turtle bycatch in various fisheries has been one of the factors implicated in the precipitous decline of leatherback (*Dermochelys coriacea*) populations in the eastern Pacific Ocean (Spotila et al., 2000). Ecosystem-level effects resulting directly from fisheries bycatch, as well as from bycatch mitigation measures, are also cause for concern (Hall, 1998). Moreover, legally mandated gear modifications aimed at reducing bycatch (e.g., the use of large circle hooks in longline fisheries), as well as time-area closures, have had severe economic consequences (U.S. Department of Commerce, 1999; 2000; Watson et al., 2005).

Fisheries managers are, therefore, eager for information that can be used to develop mitigation and for reducing sea turtle interactions with fisheries. In this paper, we focus on research aimed at reducing the incidental capture of marine turtles with longline fishing gear. Sea turtles interact most frequently with longline shallow-set gear targeting swordfish (*Xiphias gladius*), mahimahi (*Coryphaena hippurus*), and tunas (*Thunnus spp*.). Hard-shelled turtles, such as loggerheads (*Caretta caretta*) and olive ridleys (*Lepidochelys olivacea*), generally bite baited hooks, whereas leatherback turtles are most often hooked in the flippers or become entangled (Witzell, 1999). Hard-shelled turtles are frequently released alive and survive encounters with fishing gear for at least 3 to 6 months after release when handled properly (Chaloupka et al., 2004; Swimmer et al., in press). Sea turtles are, however, also occasionally caught by deep set (> 100 m) longline gear targeting commercially valuable bigeye tuna (*T. obesus*) (Ferreira et al., 2001). Deep-set longline gear generally catches fewer turtles, but with a higher percentage of mortality because hooked or entangled turtles cannot reach the surface to breathe.

Pelagic sea turtles and fishes are evolutionarily distant groups, and differences in their behaviors and sensory biology likely influence the ways in which they interact with fishing gear. Factors that attract sea turtles and targeted fish species to longline gear are not well understood, but several sensory cues are thought to be involved. Based on these ideas, a multidisciplinary interagency collaborative effort was initiated by NOAA Fisheries scientists in 2001 to investigate the visual, auditory, and chemosensory abilities of sea turtles and pelagic fishes. The overall goal of these efforts is to identify exploitable differences in pelagic fishes and sea turtles sensory biology that could be used to design longline gear and/or bait modifications to make gear undetectable or unattractive to sea turtles, while still retaining economically viable levels of targeted fish catch. This paper presents results from a pilot study to find chemical additives that reduce sea turtle detection and attraction to squid baits.

If such a chemical compound could be identified, the benefits to both the fishing industry and the sea turtles could be enormous. Ideally, baits could be soaked prior to packaging and distribution, thereby transferring costly enforcement efforts at sea to less-costly dockside surveillance. In other words, treated longline bait could potentially be labeled "turtle safe," and only treated baits would be allowed on board. This approach is commercially practical, and in achieving this goal, fishing industries could claim products to be turtle friendly, similar to the situation regarding the incidental bycatch of dolphins in the yellowfin tuna industry in the 1970s and 1980s. Such eco-labeling could clearly be economically beneficial to the industry (Wessells et al., 1999). However, despite the success of the modified bait to reduce sea turtle attraction, it must also remain attractive to fish, thereby remaining economically viable and more likely to be adopted by the fishing industry (Gilman et al., 2005). For this reason, we also tested all modified baits on captive tunas. This is the first research of its kind documented in the literature.

MATERIALS AND METHODS

In an attempt to impart sour, bitter, hot, or strong flavors in baits, whole thawed squids (*Loligo* spp.) were soaked for 24 hours in the following chemical solutions: lactic acid (85% solution), urea (10% solution), quinine hydrochloride (10 and 100 mM) (all purchased from Sigma, St. Louis, Missouri, USA), or 25% solution Hibiclens® (= 1% solution chlorhexidine gluconate; Zeneca Pharmaceuticals, Wilmington, Delaware, USA), freshly minced garlic, cilantro, Habanero chili peppers, Angostura® aromatic bitters (Angostura Ltd., Republic of Trinidad and Tobago), Habanero chili pepper extract (Habanero 750; Hot Sauce Harry's, Inc., Dallas, TX), wasabi oil, lemon juice, and 100% squid ink (International Specialty Foods Ltd., Pennsylvania, USA).

To obtain sea hare ink, animals were collected from fringing reefs on the island of Oahu, Hawaii and maintained at the Kewalo Research Facility (KRF) in tanks continuously supplied with seawater. Sea hares were fed exclusively *Laurencia nidifica*, a red algae purportedly responsible for a noxious secondary metabolite excreted in their ink (Carefoot, 1987). Thawed squid were injected with 3 to 5 cc ink secreted from *Aplysia* (opaline not extracted from ink) and presented to captive green turtles at KRF.

Using baits prepared as described above, we conducted behavioral food preference experiments with 23 captive subadult green turtles between September 2000 and April 2003 at the National Marine Fisheries Service (NMFS), KRF in Honolulu, Hawaii. Turtles were captured in Kaneohe Bay on the Island of Oahu. Turtles ranged in straight carapace length (SCL) from 40 to 74 cm (mean = 52.0 cm, SD = 9.0) and in weight from 13 to 65 kg (mean = 32.0 kg, SD = 17.1). Animals were randomly divided into groups of two to three and maintained in round outdoor tanks (7 m diameter x 1.5 m deep). Likewise, yellowfin and skipjack tunas were captured off the Island of Oahu, Hawaii by commercial pole-and-line fishermen and returned to the KRF where they were kept in 8-m diameter tanks. In both cases, tanks were continuously supplied with seawater (25 ± 1^0C, salinity $\approx 34^o/_{oo}$). Tunas weighed approximately 0.8 to 2.0 kg and were fed daily rations of frozen squid (Sea Wave Calamari, Monterey Fish Co., Inc., Monterey, California, USA). Fish procurement, care, and handling procedures are described in Nakamura (1972).

Bait Trials

Food trials were conducted such that an equal number of treated and untreated whole squid (baits) were presented to turtles in test tanks. Baits were suspended from a horizontal plastic rod and were spaced approximately 10 cm apart. Baits were presented on stainless steel clips (which posed no hooking hazard to the turtles) for 5-minute trial periods during which the turtles' behavior was recorded. At the time of experimentation, all turtles had willingly eaten untreated squid. To test captive fishes' willingness to eat modified baits, untreated and treated squid pieces were thrown into the fish tank and feeding responses were recorded. A piece of modified bait was considered accepted if it was eaten (and swallowed) by turtles and fish and considered rejected if bait was either ignored or bitten and spit out of the mouth. Because we were permitted to maintain individual turtles in captivity for a maximum of approximately 1 year, no individual was tested with all the chemical mixtures. Experiments were designed such that turtles' frequency of acceptance vs. rejection of chemically modified baits would be compared using a chi-squared statistic with $\alpha = 0.05$.

RESULTS AND CONCLUSIONS

Feeding behaviors of green turtles and tunas used for screening of modified baits were totally unaffected by the chemicals we presented, as 100% of all modified baits were eaten by nearly all the animals (Table 1). The data were analyzed qualitatively because of very clear data obtained and the simplicity of the experimental design. All turtles ate all modified baits except for one turtle that refused to eat squid marinated in *Aplysia* ink. All tunas were observed feeding on all modified squid that entered the tanks.

This study was initiated to determine if strong flavors imparted to squid baits might be used to reduce green turtles' incidence of biting baited longline hooks. Habanero chili peppers were selected because of the high concentration of capsaicans, the alkaloids responsible for the pungency in chile fruits (Curry et al., 1999) and known to be microbial inhibitors (Dorantes et al., 2000). Other natural flavorings such as garlic, bitters, and cilantro were also tested because of their pungent effects in humans. Additionally, naturally occurring defensive compounds, such as ink extracted from squid and sea hares were used because their secretions are known to deter some predators (DiMatteo, 1981; Carefoot et al., 1987). Despite the variety of substances screened, none proved effective in deterring feeding in green sea turtles.

Sea turtles are generally considered to be visual predators, but other sensory cues may also contribute to foraging success. If sea turtles use chemoreception to detect and find food, then chemicals emanating from longline bait may play a pivotal role in attracting sea turtles to the fishing gear. Chemical modifications that make bait less appealing or more difficult to detect may reduce sea turtle interactions with fishing gear. An effective chemical treatment remains to be identified. Behavioral studies found that loggerhead turtles are capable of detecting 2-phenylethanol. However, they showed no sign of attraction to this chemical (Manton et al., 1972; Southwood et al., in review). Treatment of squid bait with 2-phenylethanol, however, did not significantly alter the feeding behavior of loggerhead turtles during trials, so an odor-masking approach also appears ineffectual (Southwood et al., in review).

Although chemical cues alone can elicit feeding behavior in loggerhead, green, and leatherback turtles (Owens et al., 1982; Grassman and Owens, 1982; Steele et al., 1989; Constantino and Salmon, 2003), the majority of experimental evidence suggests that visual cues are of primary importance. Constantino and Salmon (2003) found that when visual and chemical cues associated with jellyfish prey were simultaneously presented to leatherback post-hatchlings, turtles ignored the current created by chemical delivery and oriented towards the visual stimuli. Likewise, when turtles were tested separately, visual stimuli evoked a more robust feeding response than did chemical stimuli (Constantino and Salmon, 2003).

Researchers with the NOAA Sensory Biology Working Group are currently investigating visual capabilities of sea turtles and pelagic fishes in an attempt to identify visual attractants and repellents (Levenson et al. 2004, in press; Fritsches et al., in press; Johnsen, in press; Wang et al., in press; Southwood et al., in review; in press). The effectiveness of a visual deterrent may also depend on whether or not the turtle's aversion response overrides the feeding response, which is influenced in part, by chemical cues. Studies investigating the efficacy of various methods for repelling birds show that a combination of both visual and chemical deterrents is more effective than either on its own (Clark et al., 1991). In brief, while olfaction/gustation appears be an important component to turtles' attraction to bait and fishing gear, we have yet to discover an effective chemical repellent.

ACKNOWLEDGMENTS

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Table 1. Chemical additives presented to green turtles and tuna. Results for turtles are presented as the number of individuals that willingly ate treated squid on at least one of two attempts/number of turtles tested. Results for tuna indicate whether the individuals ate the treated squid.

Chemical additives	Turtles	Tuna
Citric acid (lemon juice)	4/4	Ate
Quinine hydrochloride	3/3	N/A
Garlic	5/5	Ate
Cilantro	5/5	Ate
Habanero chili pepper	5/5	Ate
Habanero chili extract	5/5	Ate
Lactic Acid	6/6	Ate
Wasabi oil	6/6	N/A
100% squid ink	8/8	Ate
Aplysia ink	6/7	Ate
Angostura® aromatic bitters	5/5	N/A
Hibiclens® Chlorohexidine gluconate	5/5	N/A
Urea	5/5	Ate

DEVELOPMENT OF TURTLE-SAFE LIGHT STICKS FOR USE IN LONGLINE FISHERIES

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BACKGROUND

Over the past 2 decades, many sea turtle populations have declined significantly. In particular, nesting populations of leatherback (*Dermochelys coriacea*) and loggerhead (*Caretta caretta*) turtles in the Pacific Ocean have decreased by 80% to 95% (Limpus and Limpus, 2003; Kamezaki et al., 2003; Crowder, 2000; Spotila et al., 2000). Sea turtle interactions with pelagic longline fisheries are a significant source of mortality and are thought to be a major cause of decline in some populations (Lewison et al., 2004; Spotila et al., 2000).

Pelagic longline fishing involves the use of a single main fishing line that can stretch more than 50 km with thousands of individually hooked lines branching off of the main line. This fishing method, used in every ocean basin, targets bigeye tuna (*Thunnus obesus*), albacore tuna (*T. alalunga*), yellowfin tuna (*T. albacares*), bluefin tuna (*T. thynnus*), and swordfish (*Xiphus gladius*) (Bartram and Kaneko, 2005). In addition to these targeted fish, longline fishing also catches sea turtles (Garrison and Richards, 2004; Yeung, 2001; Yeung, 1999). A recent study estimated that, in the year 2000, pelagic longlines caught more than 200,000 loggerheads and 50,000 leatherbacks globally (Lewison et al., 2004).

Loggerheads are sometimes hooked in the mouth or digestive tract and subsequently drown when they are unable to surface to breathe (Garrison and Richards, 2004; Yeung, 2001; NMFS-SEFSC, 2001; Yeung, 1999). Turtles can also become hooked in their flippers or carapace and become entangled in the lines (Garrison and Richards, 2004; Yeung, 2001; NMFS-SEFSC, 2001; Yeung, 1999). Thus, the potential for injury and death arises as soon as the animal enters the vicinity of a longline set. An improved understanding of the stimuli that induce turtles to approach longlines may therefore greatly aid efforts to minimize the impact of such fisheries on sea turtle populations.

A common practice in the longline fisheries is to attach light sources near the baited hook on the branch lines to attract fish (Bartram and Kaneko, 2005; Witzell, 1999). These

light sources include chemical light sticks and battery-powered light-emitting diodes (LEDs) known as Electrolumes®. As a first step toward determining whether these lights also attract sea turtles, we conducted laboratory experiments investigating the responses of captive-reared juvenile loggerhead turtles to several light sticks used in longline fisheries. Similar tests were also conducted with young pelagic stage post-hatchling loggerhead sea turtles captured in the Gulf Stream near Florida, USA.

METHODS

Tests on juvenile loggerheads were carried out at the National Marine Fisheries Service Galveston sea turtle facility. Juvenile loggerhead turtles were tested one at a time in a 15,000 L fiberglass circular arena filled with sea water to a depth of 1.5 m. The arena was 3.7 m in diameter and located in a darkened room.

Prior to testing, each turtle was placed into a nylon-Lycra harness that encircled the carapace but did not impede swimming. The turtle was then tethered to a rotatable lever-arm attached to an electronic tracking unit (Avens and Lohmann, 2004; Lohmann et al., 2004; Light et al., 1993; Lohmann et al., 1991). This tracking unit was placed over the center of the tank using a wooden crossbar and was wired to a computer in an adjacent room (Fig. 1). Tracking software enabled us to continuously record the direction toward which a turtle swam. To monitor turtle behavior, an infrared (IR) video camera/IR light was mounted directly above the arena. Experiments with wild-caught, post-hatchling turtles were conducted in a similar orientation arena scaled down to accommodate smaller turtles.

During trials, turtles were placed into the tank in the presence of an activated chemical or LED-based light stick that was positioned along the perimeter of the tank. The swimming direction of the turtle was then monitored to determine if the turtle oriented toward the light. All data were analyzed using standard procedures in circular statistics (Batschelet, 1981).

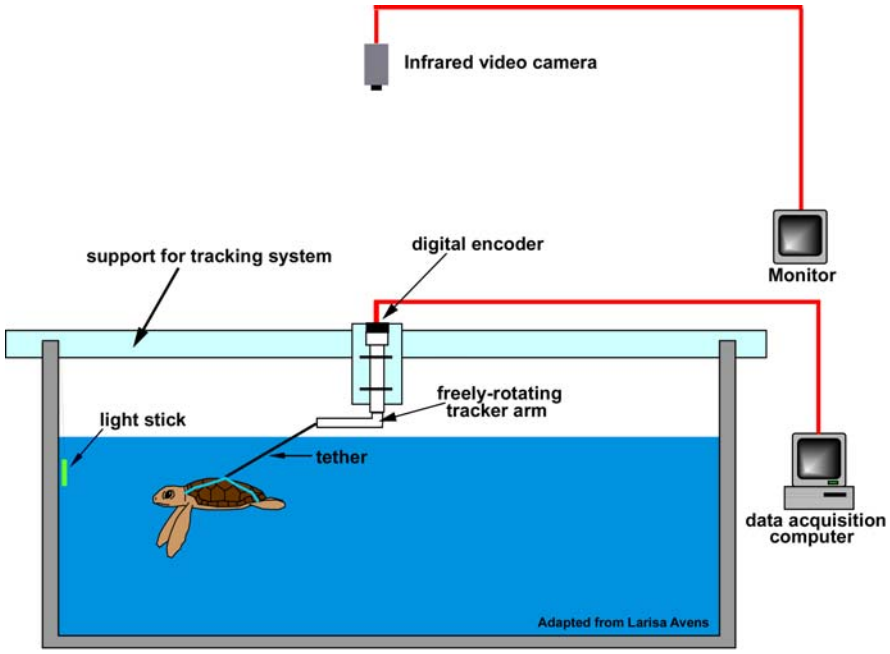


Figure 1. Experimental circular arena.

SUMMARY OF RESULTS

Juvenile turtles oriented towards all light sticks that were tested. The spectral characteristics of each of the light sticks are summarized in Figure 2. Juvenile loggerheads were attracted to green (Fig. 3B), blue (Fig. 4A), and yellow (Fig. 4B) chemical light sticks as well as to battery-powered light sticks composed of either orange LEDs (light-emitting diodes) (Fig. 6), violet LEDs (Fig. 7), or green LEDs (Fig. 8).

While turtles were attracted to all light sticks that were activated and glowing, they did not orient towards light sticks that had not been activated and thus produced no illumination (Fig. 3a). These findings imply that the illumination from the light sticks is the critical attractive feature, and that other potential cues that might be associated with the light sticks (e.g., chemical cues) do not, by themselves, attract turtles.

Results with wild-caught, post-hatchling loggerheads closely paralleled those obtained with captive-reared juveniles (Table 1). These results imply that the responses of the captive-reared juvenile turtles reflect behavioral responses that exist in wild turtles. Thus, the results provide strong evidence that illumination from light sticks is one stimulus that draws sea turtles into the vicinity of longlines.

In an effort to develop new kinds of light sticks that are less attractive to turtles, we conducted experiments with light sticks modified in various ways. One modification included shaded light sticks designed to project light downward towards the deeper water where targeted fish are located instead of upward towards the surface where turtles spend the majority of their time. Captive-reared turtles exposed to these shaded light sticks appeared to

show reduced activity level relative to turtles exposed to normal unshielded LED light sticks (Fig. 8). These results are promising in view of the fact that the studies were conducted in an orientation arena that was only 1.5 m deep. Typically, light sticks are placed far deeper on the baited branch lines, and the greater distance between the lights and turtles near the surface is likely to make such shaded lights more difficult for turtles to perceive.

Other modifications to light sticks that have been examined include flashing lights. Light sticks that flash at fairly fast speeds (e.g., 45 Hz) continue to attract turtles (Fig. 9). Light sticks that flash intermittently, however, failed to attract turtles (Fig. 10). This finding represents a promising avenue for investigation because it may be possible to produce a flashing light that does not attract turtles but attracts fish.

Only one flashing light has been tested so far, one that remained off for several seconds between episodes of blinking. Although this flashing light can hypothetically be tested immediately in the field, some additional laboratory tests may increase the likelihood of success. The main problem is that the light used in initial tests stayed off for significant periods and may therefore fail to attract fish. To find the most promising flashing patterns for field tests, we are preparing to test several different models that differ in the duration of the flash and the duration of the off period. For purposes of the longline fishery, the best light to use is presumably the one that stays on the longest (to attract fish) without attracting turtles. A series of tests should permit us to estimate the optimal pattern of flashing and quickly narrow the range of options that should be considered for field trials.

For daytime fisheries, it may be possible to produce a new kind of light stick that will be conspicuous to targeted fish species but invisible to sea turtles. The design of such a light stick exploits differences in the visual abilities of fish and turtles. Specifically, it takes advantage of the fact that, at the light levels typical of daytime underwater environments, the pelagic fish targeted by the longline fisheries can discern flickering that is imperceptible to turtles. This difference in flicker-fusion perceptual abilities means that light sticks containing two types of LEDs, each producing a different wavelength but flickering synchronously at the right frequency, will be perceived very differently by turtles and fish. To the turtle, the light will appear to be steady and constant and of a single color. Moreover, if the LEDs are selected so that they combine to yield a perceived wavelength matching the background light in the open sea, then the light stick will be essentially invisible to turtles because it will match the normal background. But the fish, with their superior ability to perceive flicker frequency under dim light, will perceive two separate colors of LED blinking on and off, resulting in a highly conspicuous signal.

We are presently working with Sonke Johnsen of Duke University to develop such a light stick. We will begin by obtaining some of the empirical data needed to design and construct such light sticks. Specifically, we will use conditioning techniques developed in our lab to determine the flicker-fusion rate for loggerhead turtles under dim-light conditions approximating those of the open ocean. This information can then be used in designing a turtle-safe light stick.

SUMMARY

Our experiments have provided the first direct evidence that sea turtles are attracted to light sticks used by longline fisheries. The methodology developed in this study is also being used to conduct testing of modified light sticks (e.g., shaded light sticks and flashing light sticks) that may be less attractive to turtles but remain attractive to target fish. These laboratory tests represent a cost-effective way to investigate possible options and identify those that are most promising for field testing.

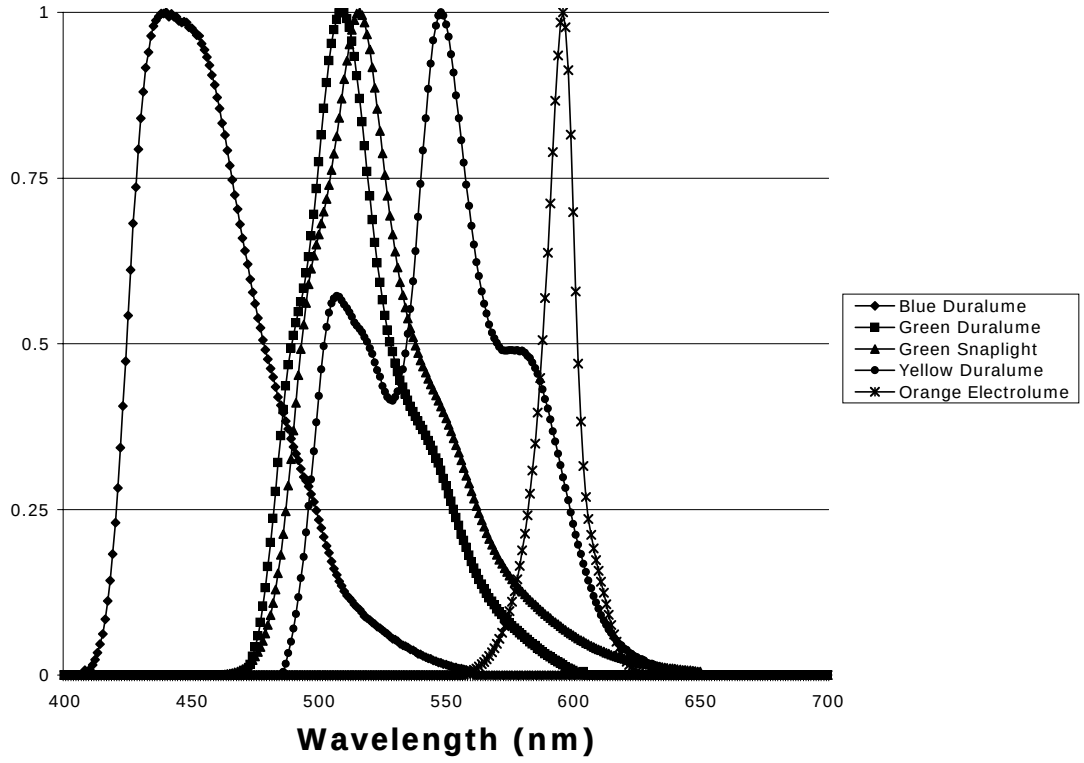
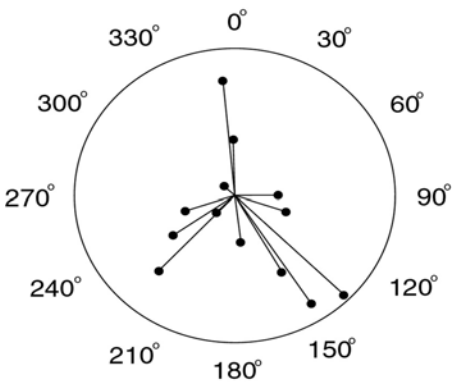


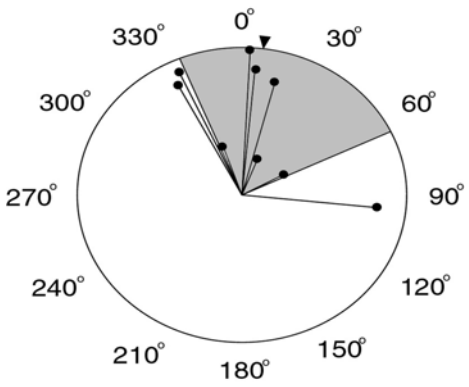
Figure 2. Emission Spectrum for Duralumes (chemical light sticks) and Orange Electrolumes (LED-based light stick).

A. Control (inactivated light stick)



Mean Angle: NS (163.4°)
r-value:0.17
NS ($p > 0.25$)
$n = 13$

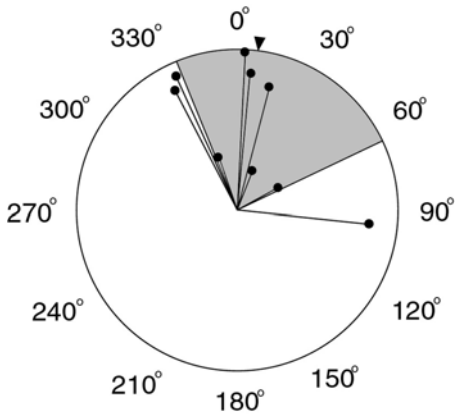
B. Green Duralume® light stick



Mean Angle: 20.2°
r-value:0.42
$p < 0.02$
$n = 15$

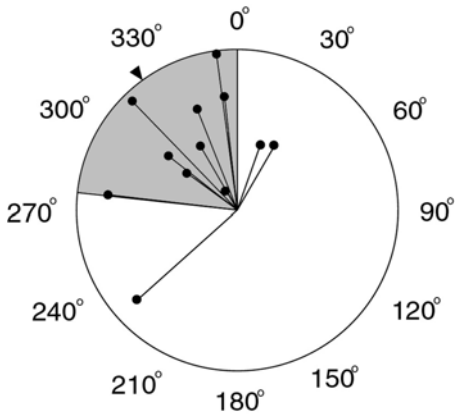
Figure 3. Responses of captive-reared juvenile loggerheads to chemical light sticks. In these diagrams, the small dot represents the direction that one turtle swam. The length of the line reflects the Rayleigh *r*-value (Batschelet, 1981), which varies from 0 to 1, with the perimeter of the circle equal to $r = 1$. Thus, a long line from the center of the circle to the data point indicates a turtle that held a highly consistent heading, whereas shorter lines indicate turtles that had more variable courses. In all diagrams, light sticks were located at 0 degrees. The statistics below each circle represent the analyses for each group. Figure 3A−Responses to light sticks that had not been activated. Turtles were not significantly oriented. Figure 3B–Responses to glowing green chemical light sticks. Turtles generally swam in the direction of the light.

A. Blue Duralume® light stick



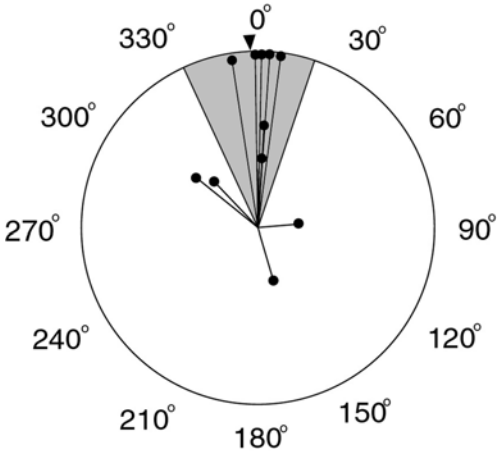
Mean Angle: 7.1°
r-value: 0.54
$p < 0.001$
$n = 9$

B. Yellow Duralume® light stick



Mean Angle: 323.9°
r-value:0.45
$p < 0.001$
$n = 12$

Figure 4. Responses of captive-reared juvenile turtles to chemical light sticks. Figure 4A− Responses to blue chemical light sticks. Figure 4B–Responses to yellow chemical light sticks. Conventions as in Figure 3.



Mean Angle: 358.3°
r-value:0.55
$p < 0.025$
$n = 11$

Figure 5. Responses of juvenile loggerheads to orange LED Electrolume light sticks. Conventions as in Figure 3.

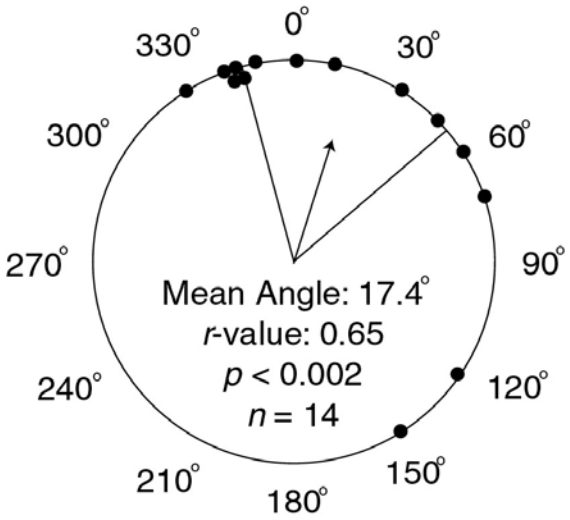


Figure 6. Juvenile loggerhead orientation to Violet LED light sticks. Conventions as in Figure 3.

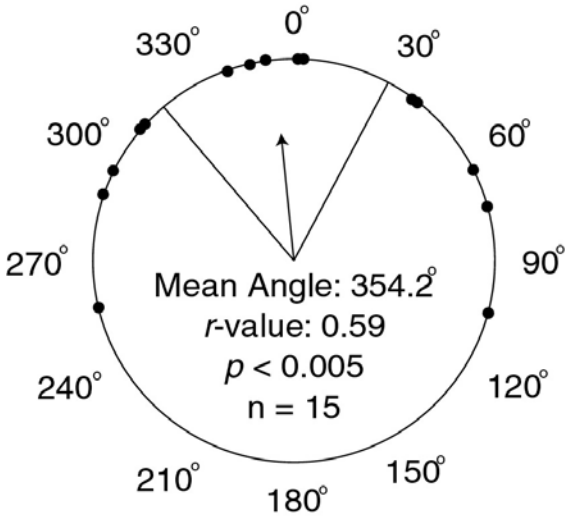


Figure 7. Juvenile loggerhead orientation to green LED Electrolume light sticks. Conventions as in Figure 3.

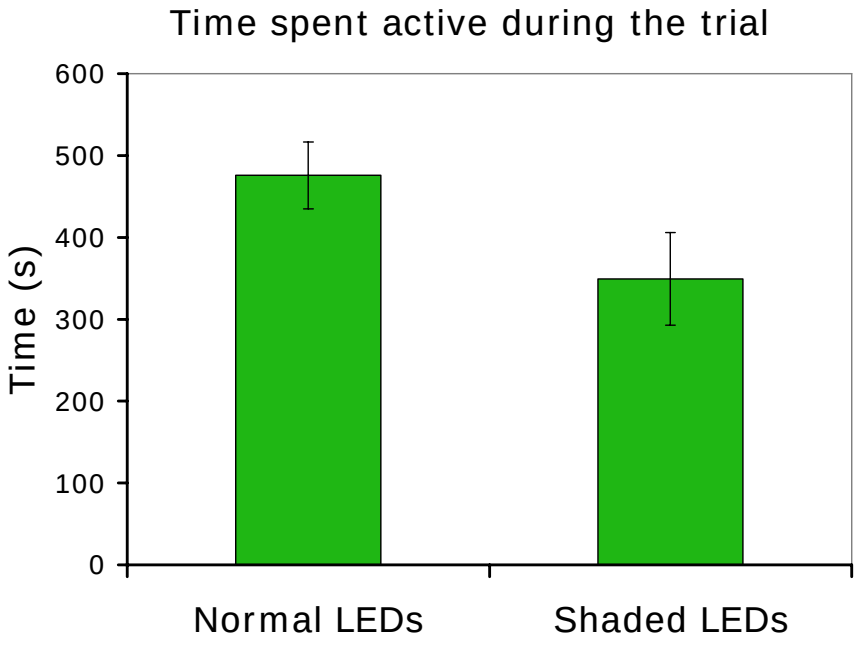


Figure 8. Comparison of time spent active for turtles in the normal green LED Electrolume and shaded green LED Electrolume treatments.

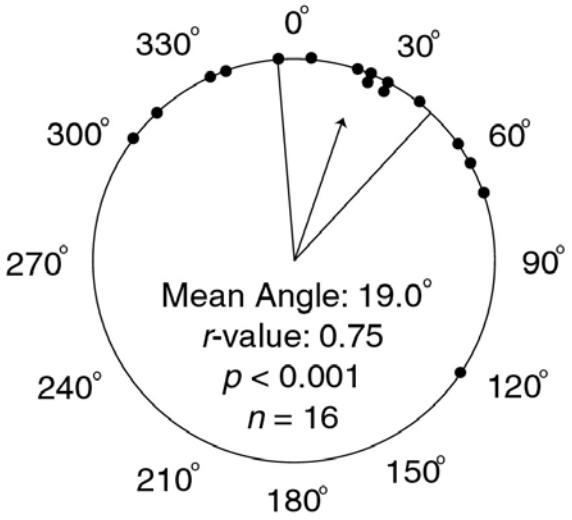


Figure 9. Juvenile loggerhead orientation to flickering blue LEDs at 45 Hz.

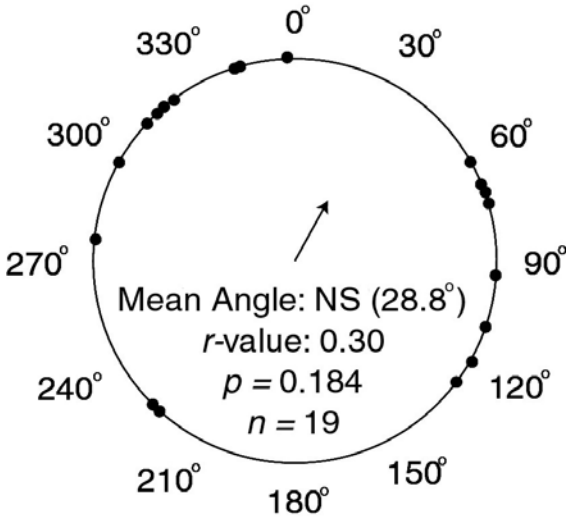


Figure 10. Responses of juvenile loggerheads to intermittently blinking blue LEDs. The turtles did not orient toward the flashing light sticks.

Table 1. Summary of wild-caught post-hatchling responses to green snaplight light sticks.

Experiment	*n*	Mean heading[1]	r-value	Significantly oriented towards the light stick
Post-hatchling indoor control (inactive light stick)	12	270.2°	0.14	No
Post-hatchling indoor green light stick	12	356.3°	0.76	**Yes**[*]
Post-hatchling outdoor control (inactive light stick)	8	150.1°	0.66	No
Post-hatchling outdoor green light stick	8	343.8°	0.81	**Yes**[**]

[1]The data were normalized so that the position of the light stick was considered to be 0°.
[*] $p < 0.005$, [**] $p < 0.001$

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SHARK DECOY EXPERIMENTS

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INTRODUCTION

The bycatch of sea turtles in the pelagic tuna and swordfish longline fisheries is an international problem that has resulted in the closure of some U.S. domestic fisheries. Pelagic loggerheads can become entangled with longlines or ingest baited hooks. The mechanisms used by turtles, however, to encounter the gear are unknown. Solutions for preventing the incidental take of sea turtles need to be identified, developed, and implemented.

While it is known that turtles of all sizes and ages fall prey to sharks, it is not known whether they recognize and actively avoid sharks or whether turtle/shark encounters are simply random acts, and the fate of the turtle rests with the shark. If sea turtles can recognize the threat of a shark and actively avoid them, then some attributes of the shark could be incorporated into/onto longline gear to deter sea turtles.

BACKGROUND

Our preliminary field studies (Hataway and Mitchell, 2001) showed that captive-reared, juvenile loggerheads displayed avoidance behavior when exposed to fiberglass shark replicas. Turtles kept in a 90 ft x 22 ft turtle holding pen at the National Marine Fisheries Service Panama City, Florida Laboratory quickly approach squid when it was suspended below a longline float (Fig. 1). Several 2-year-old loggerheads were placed into the opposite corner and allowed to move about the pen. The turtles showed no avoidance of the float (Fig. 2). However, when a fiberglass replica of a great white shark (Fig. 3) was then placed below the float and squid (Fig. 4) turtles would approach to within 3 ft to 5 ft of the shark then exhibited a dramatic stop, turn, and flee response (Fig. 5). After multiple passes, a few turtles seemed to acclimate to the shark replica, cautiously approached it often, turning vertically (identical to the reaction captured in frame 3, Fig. 11) in the water, with carapace facing the shark as they passed along the pen perimeter. This avoidance behavior was observed on multiple occasions, with different year-class turtles in both June 2001 and September 2002. These observations prompted us to further investigate whether shark characteristics could be used as a sea turtle deterrent.

METHODS

Experiments with a shark replica were conducted in a 20-ft tank consisting of a 12 ft x 3 ft x 3 ft "test" section with a 3-ft diameter, semicircular acclimation/start chamber at one end. A water flow of ~ 100 gpm was delivered from the end of the tank, which flowed

through the test section, and drained out of the tank through a sump located below the starting gate (Fig. 6). Submersible pumps in exterior sumps recirculate water in the tank. The entire tank was shrouded with a cotton canvas curtain to remove outside stimuli (i.e., movements of people, differences in light intensity, shadows, etc.). To track the turtle location in the tank, the bottom of the test section was divided and marked off in 1-ft sections. The fiberglass shark replica is of taxidermy quality, molded from a black tip shark (*Carcharinus limbatus*). It was painted primer grey and fitted with a glass eye. No other attempt was made to make the shark look realistic. The replica was weighted internally to be slightly negatively buoyant. It was tethered with a 30-lb test, clear monofilament line to a clear cross-member (2 in clear PVC pipe) above the tank.

A cylinder shape was also tested to control for just having an object in the tank. The cylinder is an internally weighted piece of schedule 40 PVC pipe painted primer grey. It was also tethered with monofilament to a removable cross-member above the tank. Squid were attached to the bottom of the shark and the pipe with a short piece of monofilament and a modified plastic hair clip.

The juvenile loggerheads used in this study (25 – 30 months old, 8.0 – 8.2 kg, Straight Carapace Length SCL = 37 – 42 cm) were captive reared as described by Higgins, 2003. During the trials, the turtle's regular diet was supplemented with squid, and each turtle was fasted for 24 hours prior to testing. Each turtle was randomly selected and randomly subjected to one of three treatments: squid, cylinder, and shark replica.

Each turtle was given 15 minutes to acclimate in the start chamber with water flow and squid bait in the tank. After acclimation, the starting gate was remotely lifted and a series of video cameras (above and below the water surface) recorded turtle behavior. A 4-channel multiplexer unit was used to allow viewing and recording by multiple cameras at the same time. Trials lasted 15 minutes, which was sufficient time for the turtles to fully explore the tank. For the data to be useable, turtles had to pass the 1-ft marker within 10 minutes of the raising of the starting gate and also pass the halfway point (line at 5 ft) at least once during the trial. Data were collected for:

(1) the time it took for the turtle to eat the squid;
(2) time to pass the starting line (1-ft mark);
(3) number of times the turtle approached the treatment area; and
(4) how long the turtles spent in each end of the tank.
(5) Notes on unusual or interesting behavior were also recorded. Digital video was recorded and archived (Figs. 9–11).

DISCUSSION OF PRELIMINARY RESULTS

If the shark replica evoked avoidance behavior in a turtle, we would expect to see the length of time until eating the squid, with the shark present, to be significantly longer than the time until eating the control squid (squid with nothing else present). The cylinder is roughly the same length and is similar in volume to the shark. If the turtle ate the squid fast with the cylinder present but took significantly longer with the shark, then we might assume that the

turtle is distinguishing characteristics of the shark as threatening and not simply avoiding an object. We do not know what, if any, shark characteristics evoke a threat response, but they may include such things as silhouette, size and placement of triangular fins, eyes, jaws and teeth, color patterns, or a combination of characteristics. Some characteristics may evoke a stronger response than others.

To date, data have been collected from 13 useable turtles. Twelve had "time to eat" (TTE) times that were greater with the shark/squid treatment than with the control squid. However, only four turtles exhibited a behavior pattern that we would expect to see if a turtle was avoiding a shark, which is a large TTE for the shark treatment and short TTEs for both the squid and cylinder treatments. The shark may have influenced the behavior of these four turtles. Five turtles had longer TTE times for the shark than the control squid, but the TTE for the cylinder was also greater than the control squid. This may indicate that the cylinder is also influencing the behavior of these turtles. Four turtles had TTEs that were virtually identical in all three treatments indicating there was no effect of the shark or pipe on their feeding behavior. The means for each treatment are presented graphically in Figure 7.

The Friedman test (a repeated measures analysis of variance on ranks) indicated that there was a significant difference between treatment groups ($P < 0.005$, Chi-square = 10.6, d.f. = 2). Subsequent pairwise multiple comparison procedures (Student-Newman-Keuls test) ($p < 0.05$) showed significant differences between the squid vs. squid/shark treatments, as well as the squid vs. squid/cylinder treatments, but not between the squid/shark vs. squid/cylinder treatments.

An attempt was made to graphically quantify avoidance behavior by examining the movements of the turtle within the tank. Figure 8 shows the number of times each turtle approached and entered the experimental end of the tank during each treatment. If the shark replica evokes an avoidance response, then the number of times the turtle enters the area with the shark should be fewer than the number of times it approaches the other two treatments. Nine of the 13 turtles exhibited more approaches into the experimental end of the tank with squid only present than they did with the shark/squid present. However, only two of those turtles had an approach pattern that would suggest a greater avoidance of the shark than either the squid or squid/cylinder (Fig. 8, RRV044 and RRV161). Five of the 13 turtles had approach figures equal to or less than the approaches for the shark, indicating the cylinder may have evoked a greater avoidance behavior than the shark.

Potentially nonquantifiable, but interesting behavioral data were also collected. We have observed dramatic avoidance and apparently defensive postures for turtles exposed to shark replicas both in the laboratory and the field. Most turtles came directly out of the starting gate and made a direct approach to the squid in the control treatment (Fig. 9), consuming the squid in a short period of time (usually on the first approach to the treatment) followed by a continuous searching of the entire tank throughout the remainder of the test period. Several turtles made multiple approaches to the shark/squid treatment, with dramatic turns and twists, before they eventually consumed the squid (Fig. 11). Once the squid was consumed, searching of the tank was less intense than with the control group. However, some turtles eventually acclimated to the shark and after consuming the squid, bit at the gills, eyes,

and fins of the replica. The turtle interaction with the cylinder was highly variable, including behaviors witnessed with both the squid and shark treatments (Fig. 10).

FUTURE WORK

Testing will continue until a minimum sample size of 25 is obtained. It is possible that a long cylindrical shape may be a threat-evoking characteristic, making a cylinder unsuitable as a control object for the shark. Therefore, we may run a fourth trial using a different object, such as a sphere. If the data indicates that response to the shark is something other than random, we will identify a shark characteristic and incorporate it into another object and test for avoidance behavior.

ACKNOWLEDGMENTS

Thanks to Kenneth Lohmann for his assistance in developing the experimental design. Special thanks to the NOAA/NMFS Sea Turtle Facility Captive Rearing Team for raising the test turtles. Thanks to Roger Zimmerman and Tim Fontaine for supporting this research. Thanks to Tom Minello and the Galveston Fishery Ecology Branch for the use of the laboratory space in the Wetlab Facility. Thanks to FWC for permitting the use of Florida loggerheads for longline-related research. Thanks to the NMFS Pascagoula Laboratory's Harvesting Systems Branch for pursuing the idea of shark replicas as deterrents. Thanks to Rich Brill, Yonat Swimmer, and Chris Boggs for supporting this project financially. Thanks to Jimmy and Glenn Warren of Warren's Taxidermy for providing the shark decoys. Thanks to Alisha Goldberg for her editing and review of the text. Thanks to Jo Williams for printing the poster.

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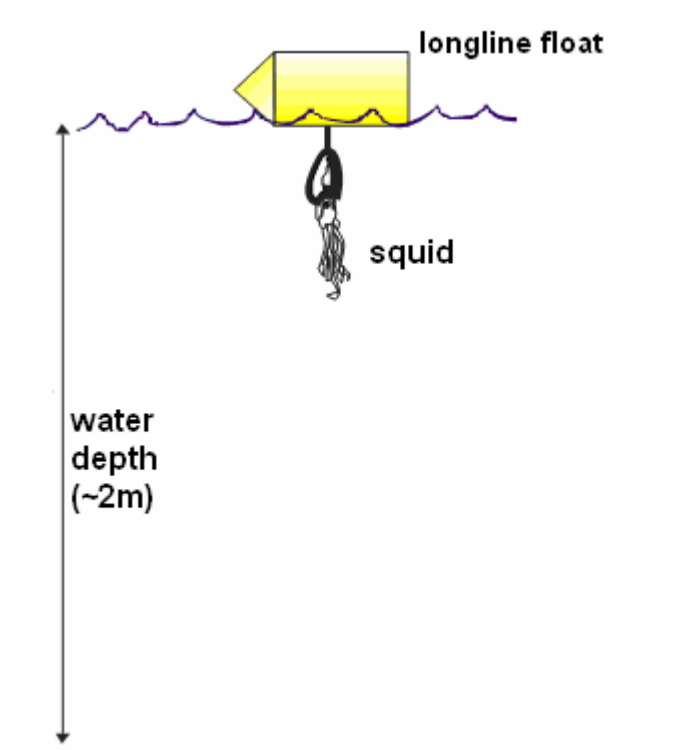

Figure 1. Control setup.

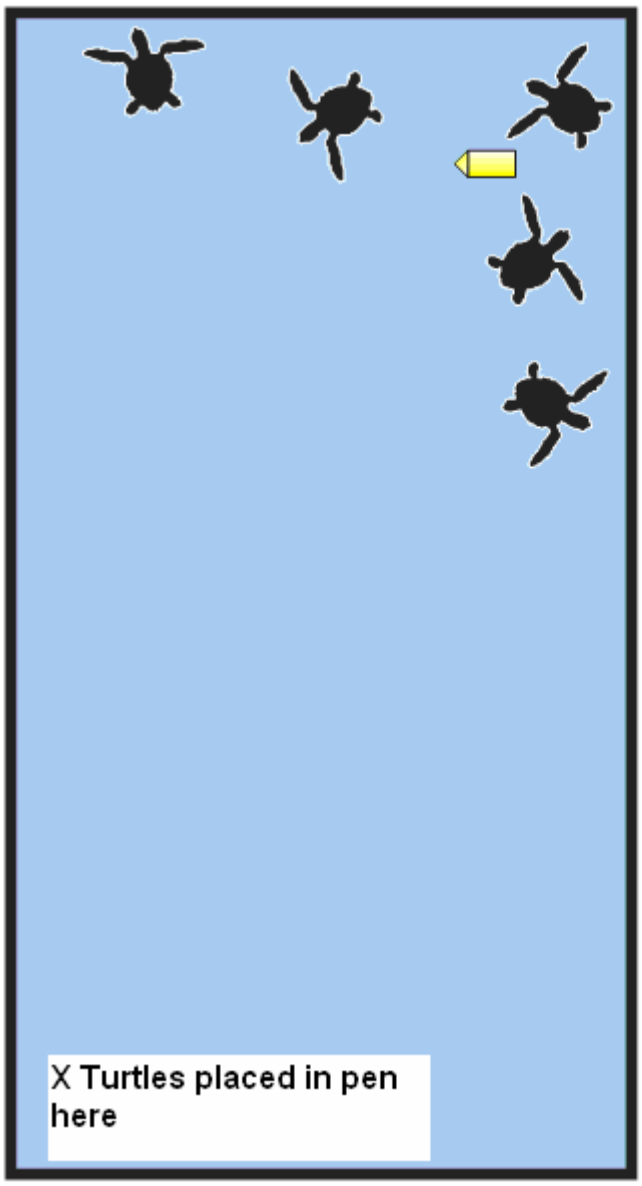

Figure 2. Pen with control setup.

Figure 3. Great white shark decoy.

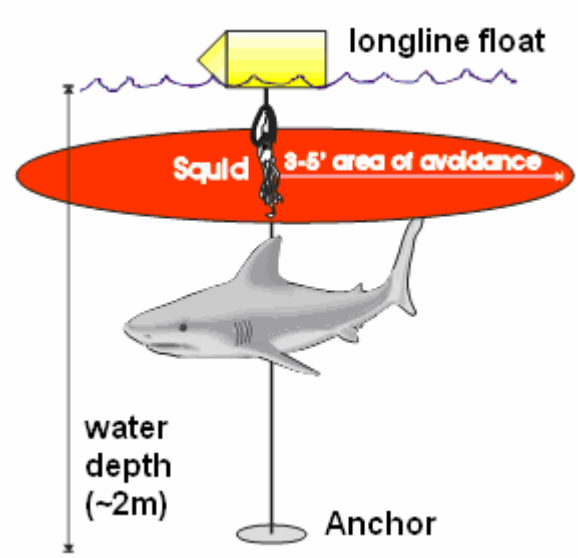

Figure 4. Experimental setup.

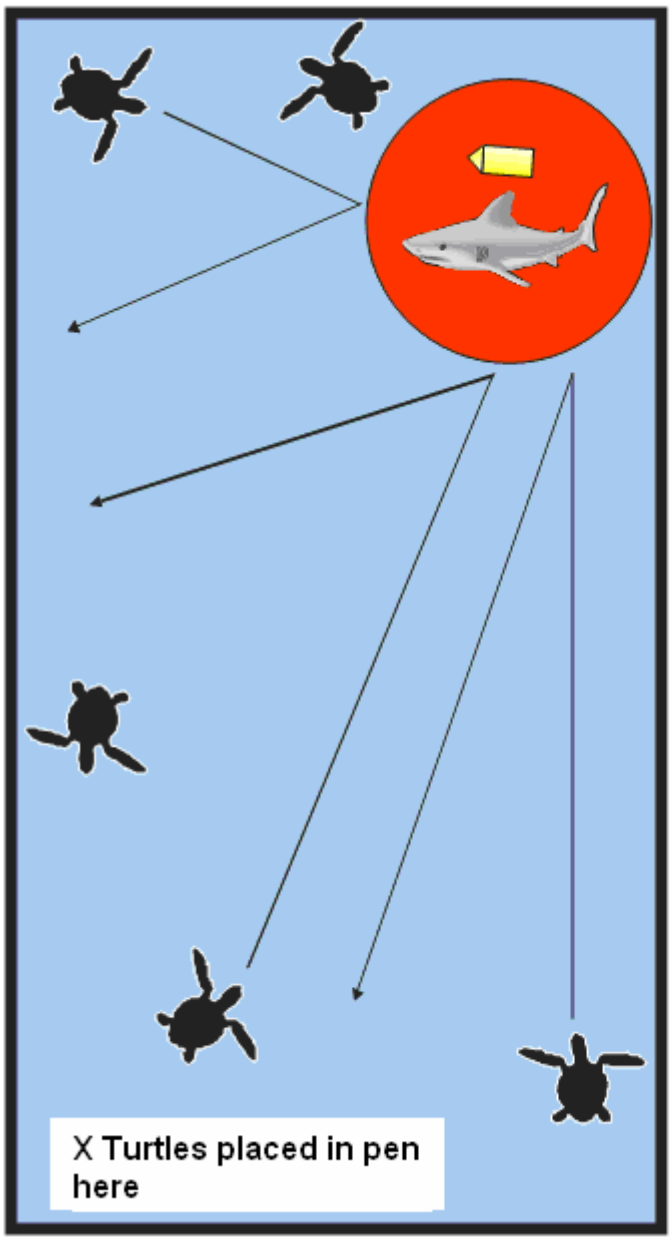

Figure 5. Pen with experimental setup.

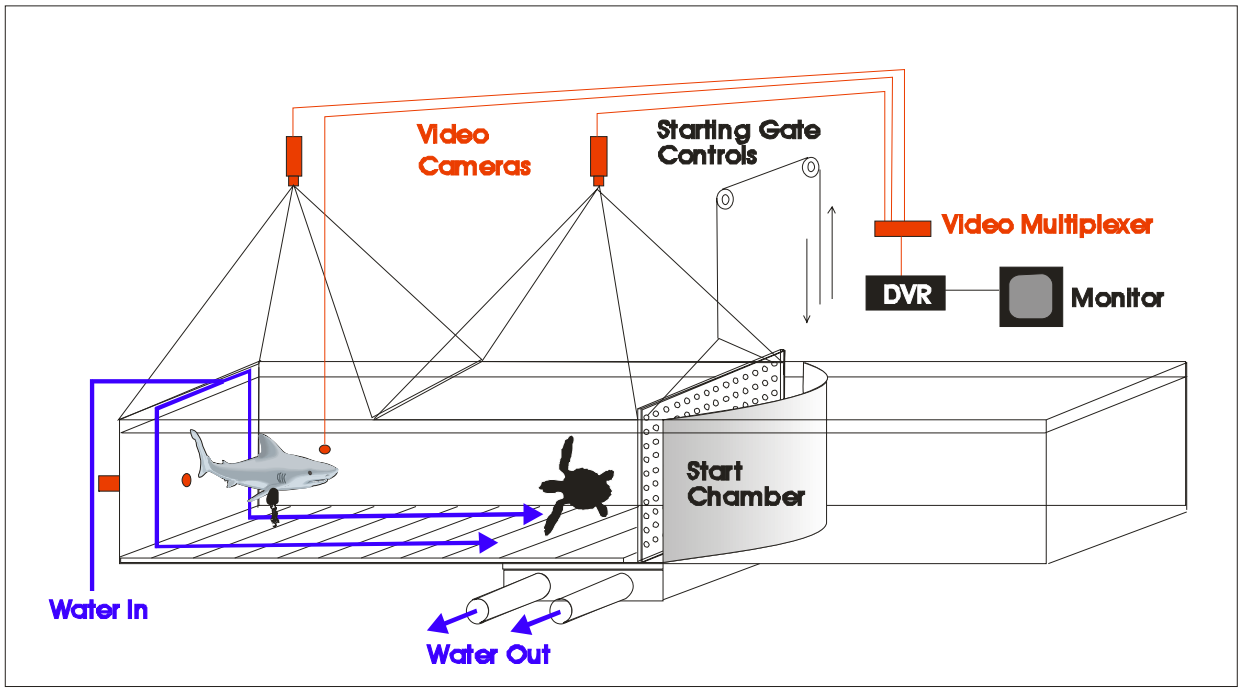

Figure 6. Experimental setup in olfactory tank.

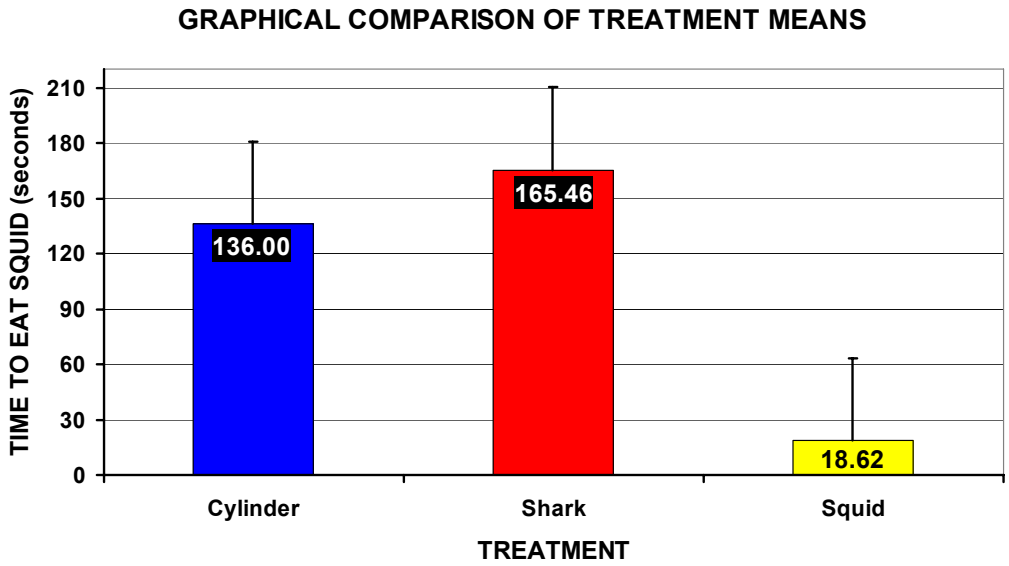

Figure 7. Effect of shark decoy and PVC cylinder presence on time to eat squid.

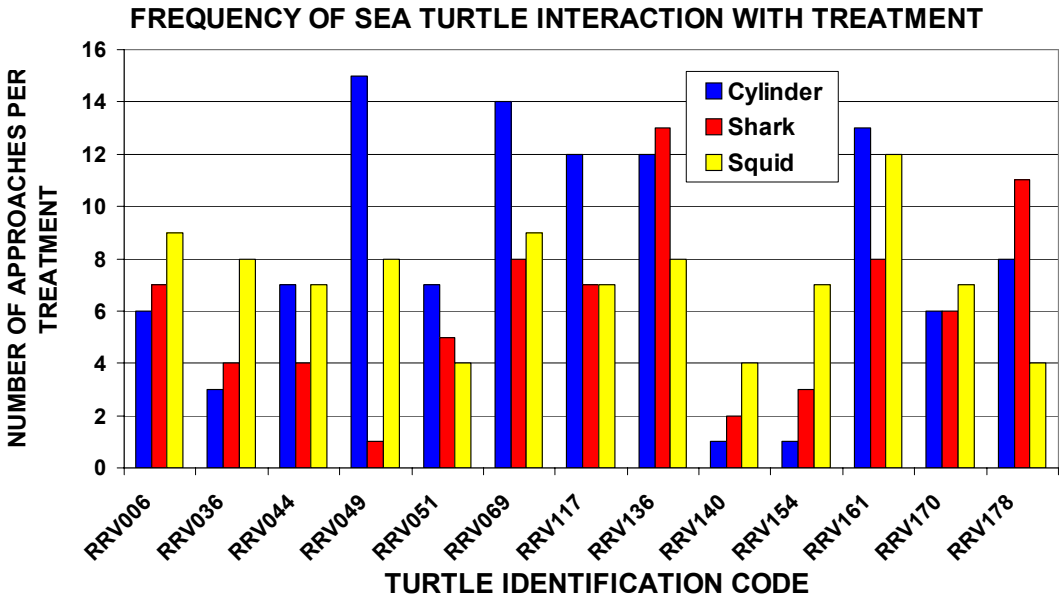

Figure 8. Frequency of sea turtle interaction with treatment.

TESTING MITIGATION MEASURES TO REDUCE SEA TURTLE INCIDENTAL CAPTURE IN LONGLINE FISHING GEAR IN BRAZIL

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INTRODUCTION

Very little is known about the interactions between sea turtles and the pelagic longline fishery in the South Atlantic. The first study on these interactions was conducted between 1994 and 1996 by Achaval and Marin and was presented during the 17th Symposium on Sea Turtle Biology and Conservation. Currently, there are less than 10 publications related to sea turtle-fisheries interactions specific to the South Atlantic Ocean (e.g., Achaval et al., 1997; Azevedo et al., 2000; Barata et al., 1998; Andrés et al., 2002; Achaval et al., 2000), and none involves studies on mitigation measures.

Since 2003, our team has been involved in a variety of studies to identify strategies to reduce the incidental capture of sea turtles in pelagic fisheries. Among these measures are modified bait and fishing gear. With support provided by the University of Hawaii and the National Marine Fisheries Service (NOAA) Pacific Islands Fisheries Science Center, we have undertaken the following experiments: I. Tests of Repellent Bait Modifications Using Captive Turtles; II. Tests of Repellent Bait Modifications Using Semi-wild Turtles; and III. Field Trials to Test Possible Mitigation Measures. This report includes information about the trials with captive turtles. The results on field trials will be reported elsewhere.

I. TESTS OF REPELLENT BAIT MODIFICATIONS USING CAPTIVE TURTLES

In June 2003, Projeto TAMAR–IBAMA, in cooperation with MIX food enterprise/Sao Paulo, accomplished modified bait mitigation measures experiments at a TAMAR base in Comboios–Espirito Santo biological reserve. Tests were conducted on four captive loggerhead (*Caretta caretta)* sea turtles maintained at the TAMAR base since birth. The purpose of these experiments was to assess the efficiency of blue color and the oil/resin scent as potential feeding repellents for captive loggerhead turtles.

METHODS

The tests were conducted inside two 45,000-L. concrete tanks where two captive loggerhead turtles were placed per tank. Sizes and weights of the turtles are shown in Tables 1 and 2.

Table 1. Measurements of turtles used in captive studies in Combois-ES biological reserve.

TANK 1				
SPECIES	TAG#	LENGTH (cm)	WIDTH (cm)	WEIGHT (Kg)
Caretta caretta	25454	88	72.5	78
Caretta caretta	20976/20977	86.5	76	76

Table 2. Measurements of turtles used in captive studies in Combois-ES biological reserve.

TANK 2				
SPECIES	TAGS	LENGTH (cm)	WIDTH (cm)	WEIGHT (Kg)
Caretta caretta	9637	93	86	108
Caretta caretta	25437/25438	89.5	76.5	84

Sardine and squid (*Illex argentinus*) were offered as bait because both types of bait are used by the Brazilian longline fishing fleet. Squid is the preferred bait type and most frequently used. The squid bait was modified (color and scent) as follows:

1) Natural squid with odor
2) Blue dyed-squid
3) Blue dyed-squid with odor

Sardines and a different kind of squid were offered each single day, so that the turtles could choose between sardine and only one kind of squid at the same time.

The blue dye (Special Blue Food Color For Fishing—Code 373) was the same that was developed by Mix Enterprise, Inc. in cooperation with Instituto Albatroz (www.projetoalbatroz.com.br). It has been successfully used by the national longline fishing fleet as a mitigation measure for albatrosses and petrels. The scent of oil/resin was also developed by Mix Enterprise specifically for these experiments. This specific odor was not

chosen on any scientific basis, but only because it was unpleasant for the turtles. The efficiency of a mitigation measure is directly related to the decrease of incidental capture, thus it does not affect the capture of target species.

The tests were conducted on 6 separate days, June 17 – 20 and July 1 – 2, 2003. The tests were conducted in tanks solely with conditioned turtles that had successfully eaten squid and sardine baits prior to the trials. We believe it will be very important to replicate these types of studies aboard longline fishing vessels to obtain data more reflective of turtles' behavior in their natural environment.

RESULTS

On the first day of tests, three of the four turtles chewed and spat the squid several times before swallowing them. On the following days, they ate the squid immediately and on the days following the trials, the turtles did not eat fish after eating squid. All turtles that ate the squid did so as soon as the squid were placed in the tank, making no distinction between treatment. Because of this, we decided to stop the tests.

Turtle 9637 did not eat squid or fish during all test days.

Despite the results, we should consider that the tests were completed indoors with captive turtles that are conditioned to eat anything that falls into the tank. We believe it is very important to conduct these experiments under the most natural conditions as possible to better predict turtles' responses to modified baits in the open ocean.

II. TESTS OF REPELLENT BAIT MODIFICATIONS USING SEMI-WILD TURTLES: SEASON 1.

During October 2003, TAMAR started feeding behavior experiments with sea turtles in floating pens in the ocean. This section reports on activities conducted during Season 1, from October 2003 to March 2004. These experiments were conducted in a semi-wild environment, whereby turtles that had been incidentally caught in longline fishing gear were brought into captivity and were maintained in floating pens in an enclosed oceanic bay. We believe this is a more natural environment than using turtles born and raised in captivity; the results are more likely to be similar to how turtles respond in the open ocean. Once again, turtles' feeding responses to baits modified by different odors were tested to determine if we could identify a repellent bait to reduce the incidental capture of loggerhead turtles in longline fisheries.

METHODS

To accomplish the tests, Fundação Pró-TAMAR established a partnership with Instituto Arruda Botelho-IAB (www.institutoarrudabotelho.org.br/) which donated a net-tank for the experiments. IAB has been working on the Paraty area for almost 10 years, with Projeto Robalo, whose main objective is to establish a sustainable development for the local fishermen through donations of net-tanks to increase their income.

The net-tank used by TAMAR is a 14 mts. long and 8 mts. wide galvanized iron tank, supported by 18 of the 200 lts. plastic barrels, using polyester net covered by PVC with a

2.5 cm mesh size (Pictures 1 and 2). The tank is located in Enseada do Sítio Canhanheiro, Paraty – Rio de Janeiro at 23° 13' 15.1"S e 44° 41' 02.5" W.

Picture 1. Floating pens in Paraty (Rio de Janeiro) used in semi-wild experiments.

Picture 2. Net mesh size of floating pens.

Squid — *Illlex argentinus* is the favorite bait used by the Brazilian longline fishing fleet. The experiments were accomplished using *Loligo plei* because this species was found in waters where the tank was located in Ubatuba–SP and Paraty –RJ. The tested repellents were produced and supplied by MIX food enterprise–SP. This company produces the blue dye that is used to dye the squid, and this practice has been spontaneously adopted by some fishing companies as a mitigation measure against incidental capture of albatrosses and petrels on longline fisheries.

In cooperation with ITAFISH company (Santos-SP), three loggerhead turtles captured by *Oceano Brasil* fishing vessel were brought for tests during late 2003 to early 2004. The first turtle arrived on November 27, 2003 and the other two turtles arrived on January 5, 2004. All turtles were measured and tagged using model 681 INCONEL tags. Skin samples were collected for DNA and organochlorines concentration tests. Blood samples were also collected, every 20 days, to assess the health of the turtles and their blood biochemistry and its variations during the experiments. The blood tests have been analysed in the laboratory of Santa Casa de Paraty (local public hospital). We will report on the turtles' blood chemistry values in a separate report.

The first tested odor was sea turtle feces, and trials were conducted on January 17, 2004. The squid were completely immersed in a solution containing the odor for at least 40 minutes. After that, they were tied alongside natural squid, on a snap 1 meter distant from each other. The snap was attached to a nylon mono-string and hung between the shortest edges of the tank. Prior to setting the bait in the tank, the turtles were confined to one of the

edges of the tank, separated by a net from the rest so that even after the bait was put in the water, the turtles could only reach the bait after the net was removed.

Turtles' feeding behavior (accept, reject, or ignore) was recorded for each bait presented. During the test periods, data about sea surface temperature and water transparency were collected using a thermometer and Secchi disk, respectively.

RESULTS

The turtles' carapaces measured between 61 and 65 cm long. The turtles ate all the squid without making distinction between the modified and the natural ones. It is probable that the turtles had gotten conditioned to the food that had been offered before. For this reason, the animal could have been induced to feed on supposedly unpleasant food. The level of knowledge about these animals should be carefully observed in the sequence of the experiments.

Through observation of the turtles' behavior inside the floating pens, we realized that after ca. 20 days, turtles began to demonstrate at least some conditioning. For example, if we did not keep them on one side of the tank, they swam towards the location of the food. Additionally, they began to associate people with food, and therefore swam towards them. Therefore, we believe that in the future, experimental turtles should not remain in the tanks for more than 30 days, because after this time they start to show strong evidence of conditioning. The first week in captivity is the best time to decide if turtles will accept the modified bait. For the next tests, data about the tide, current, and wind direction should be collected because they contribute to the spread of the odors/tastes in the floating pens.

III. TESTS OF REPELLENT BAIT MODIFICATIONS USING SEMI-WILD TURTLES: SEASON 2.

INTRODUCTION

This part of the report documents activities developed by Projeto TAMAR/IBAMA, through the TAMAR Foundation, on work involved with testing loggerhead turtles' responses to baits modified by various smells. All experiments were conducted during Season 2, from November 2004 to March 2005. Objectives and methods are nearly identical to those reported during Season 1 (above).

METHODS

These experiments were conducted with two loggerhead turtles incidentally caught by a commercial fishing vessel, *Oceano Brasil* (ITA FISH Co.), during fishing trips in February and March 2005 in the south Atlantic Ocean.

The first turtle, herein referred to as turtle nº 39071/39072 (same as tag numbers), was captured at approximately 30º 02´ S/45º 20´ W (Fig. 1) and brought to the floating pens at

Paraty (Rio de Janeiro) on March 9, 2005. It had a curved carapace length (CCL) of 73.2 cm. Prior to its release on April 4, 2005, we performed 50 feeding behavior tests on this animal.

The second turtle, herein referred to as turtle nº 31790/31791, was captured at approximately 29º4.087'S/46º31.885'W (Fig. 1) and had a CCL of 62.5 cm. This turtle was brought to the floating pens at Paraty on March 30, 2005 and participated in 18 tests prior to being released on April 4, 2005.

All tests conducted with these two loggerhead turtles were performed with squid baits infused with tutti-frutti smell, an odor developed by food scientists at Mix Industries, Inc. in Sao Paulo, Brazil. These trials were conducted because previous pilot studies with captive turtles at TAMAR bases suggested that the odor is repellent to turtles.

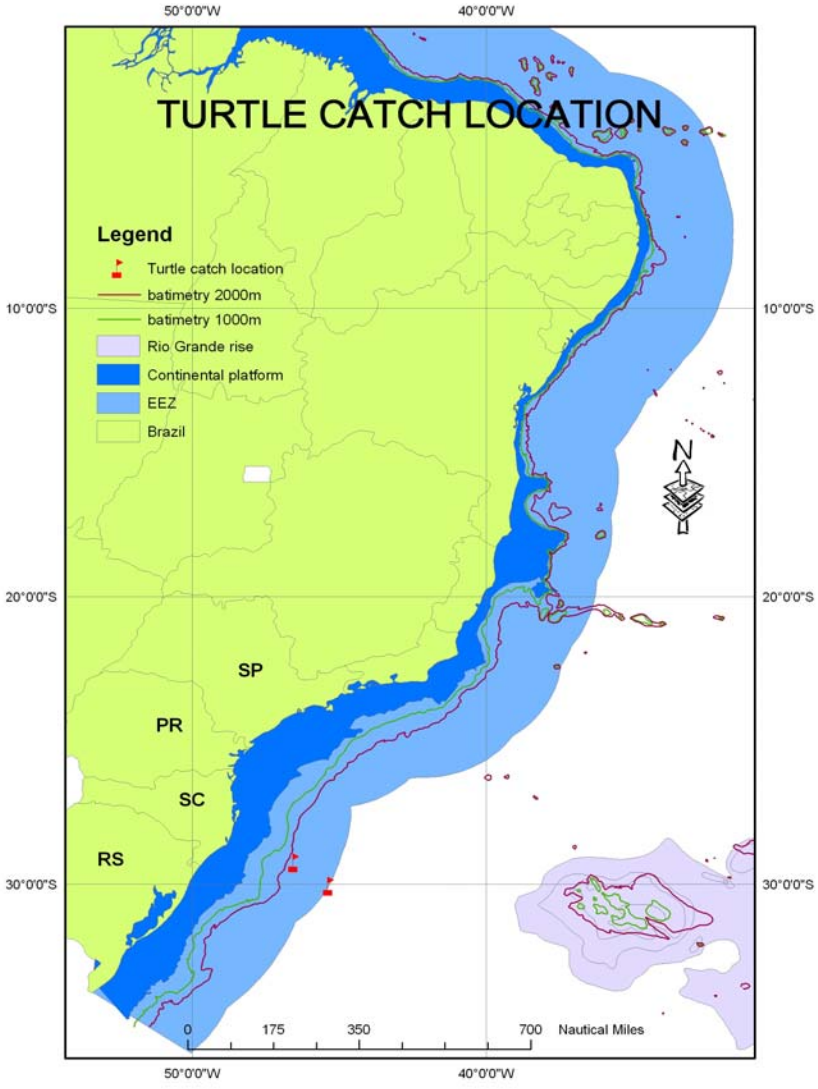
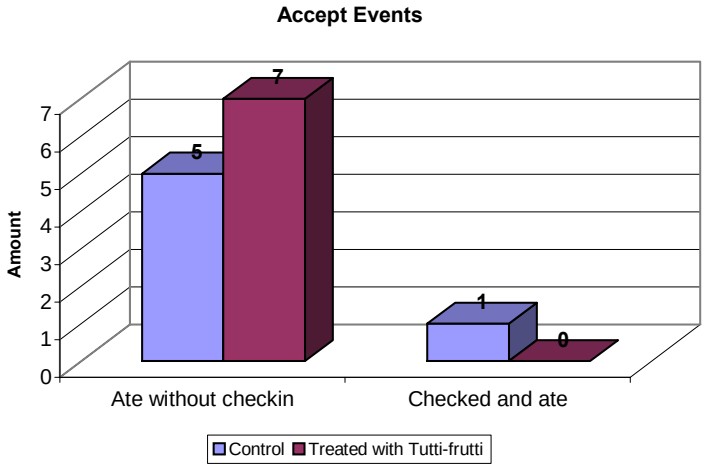
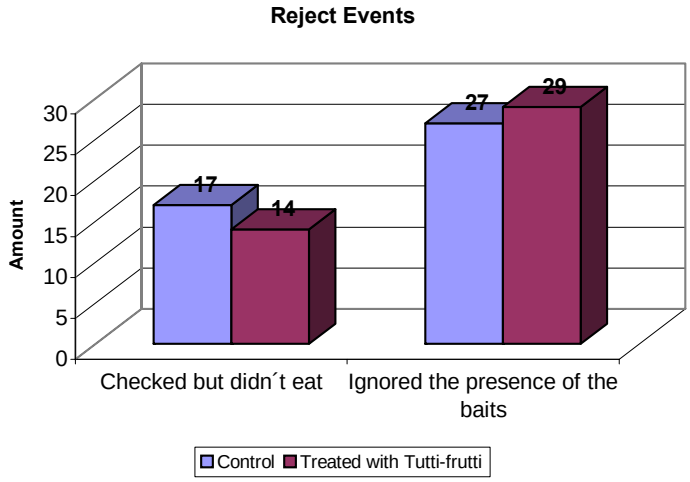


Figure 1. Locations of two loggerhead turtles captured in longline fishing gear and used during semi-wild experiments.

RESULTS

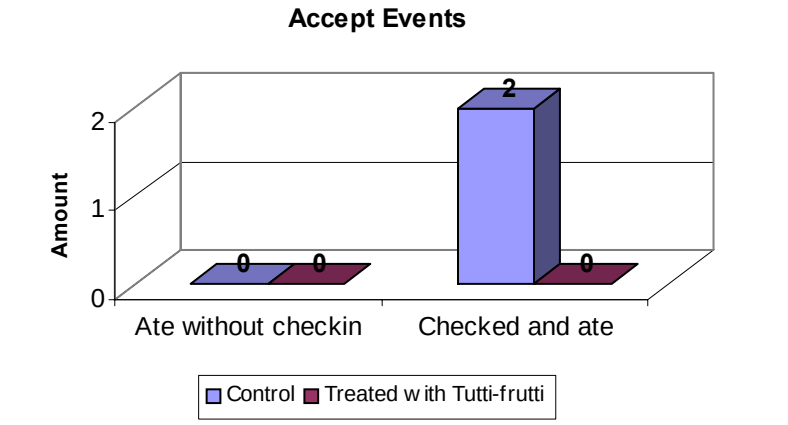
Turtle nº 39071/39072 rejected both the control and the treated baits for the first 22 days, starting only to eat by March 31, 2005 (one day after the arrival of the second turtle, turtle nº 31790/31791). On the first day he started eating, this turtle (nº 39071/39072) consumed both control and treated baits without investigating them. The turtle's feeding responses to modified baits were recorded and graphed (Graphs 1 and 2).

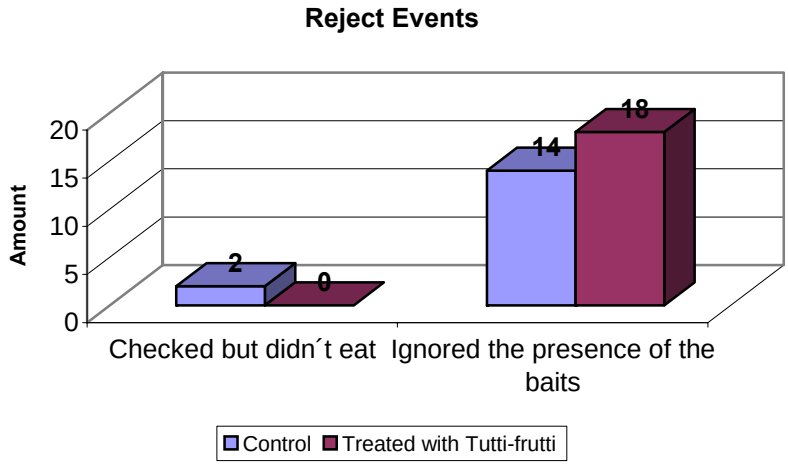




Graphs 1 (top) and 2 (bottom). Feeding responses of turtle nº 39071/39072 to squid treated with tutti-frutti odor.

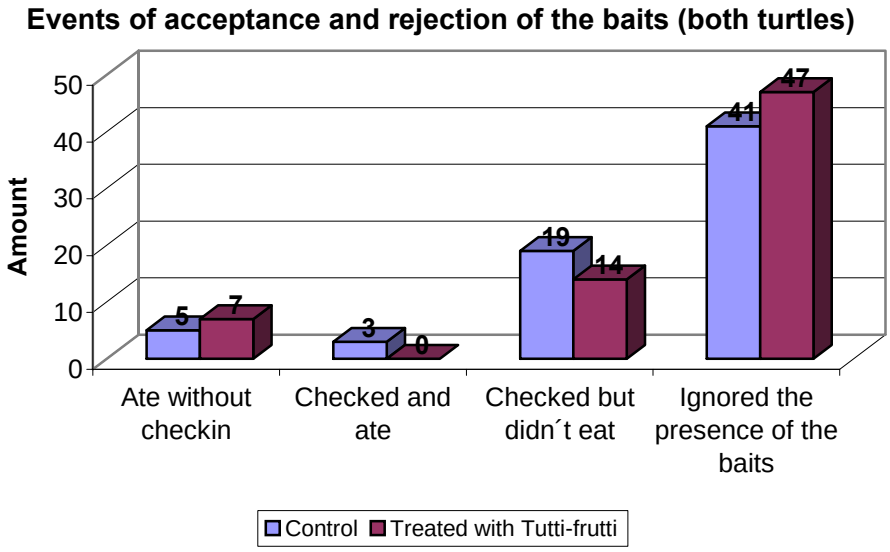
Turtle nº 31790/31791 rejected untreated and treated baits far more often than it ate baits. Of the two baits that the turtle ate, both were controls (Graphs 3 and 4). More tests should be conducted with this turtle.





Graphs 3 (top) and 4 (bottom). Feeding responses of turtle nº 31790/31791 to squid treated with tutti-frutti odor.

In general, both turtles ignored the baits independent of treatment more often than doing anything else (Graph 5).



Graph 5. Combined feeding response results for two loggerhead turtles presented with squid modified by tutti-frutti odor.

FINAL CONSIDERATIONS

We believe it is important to continue the tests with the turtles that are already in the tank and to try to integrate other sea turtle individuals. We must also define the appropriate time to keep animals in captivity given the fast addiction time (around 15 days). This complicates the data analysis and interpretation. We plan to develop new smells for the tests. The smells with positive preliminary results should be tested indoors with standard methods for this type of procedure. If these tests perform well, field tests should be performed during operations by the longline fishery.

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TURTLE AND TUNA HEARING

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INTRODUCTION

Sea turtles are incidentally captured by the longline fishery, and this fishery may be a significant cause of mortality for many species of sea turtles. In this ongoing study, we are investigating the feasibility of using sound stimuli to deter sea turtles from approaching the longlines. However, little is known about either the hearing ability of sea turtles and pelagic fishes or their dependency on sound for survival cues. This project used electrophysiological techniques, specifically auditory evoked potentials, to determine the hearing capabilities of sea turtles and a commercially important pelagic fish, the yellowfin tuna, to assist with development of strategies to reduce turtle bycatch in U.S. longline fisheries without affecting commercial catch rates.

Electrical audiometric techniques are highly suitable for studies with turtles and tuna; they are noninvasive, rapid, and require no overt training of the subject animals. Auditory evoked potentials (AEPs) are the most widely accepted technique for measuring hearing, particularly in situations in which normal behavioral testing is impractical. AEPs reflect the synchronous discharge of large populations of neurons within the auditory pathway and so are useful in monitoring the functioning of the throughput of the auditory system. Fundamentally, the technique entails presenting an acoustic stimulus to the subject and recording the evoked neural responses from electrodes on the surface of the head. Most AEP research has concentrated on the use of responses occurring within the first 10 ms following the presentation of a sound stimulus. This response has been termed the auditory brainstem response (ABR) and consists of a series of distinctive waves associated with the sequence of auditory events occurring in the brainstem in response to perceived sound.

Corwin et al. (1982) recorded AEPs from five classes of non-mammalian vertebrates (including the red eared turtle, *Pseudemys scripta elegans* and several species of fishes) and found the response, recorded outside the brain, to be congruous with the criteria for conventional auditory brainstem responses (ABRs). Furthermore, these ABR recordings were found to be consistent within each species and similar across vertebrate classes in general form and origin, regardless of auditory apparatus (Corwin et al., 1982). Only two attempts to collect electrophysiological data from sea turtles have been successful: one study performed on juvenile green sea turtles (Ridgway et al., 1969) and one study on juvenile loggerhead sea

turtles (Bartol, 1999). Both studies suggest that these sea turtles detect a limited frequency range (200 –1000 Hz) with best sensitivity at the low tone region of about 250 – 400 Hz. However, neither study employed conventional underwater sources as inputs. Consequently, the underwater hearing abilities of turtles are not yet known.

This project was designed to test the hearing ability of sea turtles and tuna underwater. To accomplish this objective, we developed a methodology to directly measure sea turtle hearing and responses to underwater sound stimuli using brainstem evoked potential techniques.

METHODS

During the experiments, the animals were either physically restrained (sea turtles) or anesthetized (fish), and the ear remained underwater while the top of the head was always above water. Subdermal platinum recording electrodes were implanted over the ear, along the midline of the skull and a ground electrode was placed in the water (Image 1). Implantation of the electrodes did not require surgery. A stimulus of known frequency was delivered by the computer system (Tucker Davis Technologies) to a sound source located above the animal. The stimulus was modified to account for ambient noise frequencies and pure tones were presented to the animal. Presentation of the acoustic stimulus elicits synchronized responses of neurons within the auditory system. As noted above, a three-electrode array was used to record the evoked responses. The electrodes served as input to a low noise differential amplifier. The amplified analog signal passed through an anti-aliasing filter and led to an A/D converter. Two channels, left and right, of electroencephalographic (EEG) activity were amplified (x20k) and filtered (5 – 3000 Hz). In general, the digitized response was digitally filtered, written to a memory buffer on a DSP board (Tucker-Davis Technologies), tested for the presence of unwanted signal artifacts, added to the buffer containing the responses to the previous *n* stimulus presentations, tested for signal-to-noise-ratio, and finally averaged based on the number of stimulus presentations.

Image 1. Green turtle with electrodes at Kewalo Research Facility, Honolulu, Hawaii.

RESULTS AND DISCUSSION

ABR data have been analyzed for 10 turtles (juvenile and subadult *C. mydas* and juvenile *L. kempi*) and 2 tuna (*Thunnus albacares*) using a correlation technique to identify the response to sound. Traditionally, a major weakness in the analysis of ABR data is the subjective manner at which threshold is determined (i.e., when the response can no longer be seen by the observer). However, ABR tracings have been found to be very consistent for an individual and repeatable at a specific frequency and decibel level. By always recording two tracings at each frequency and intensity, the two tracings should result in a high correlation if a response is present. Furthermore, a low correlation should occur between a stimulus trial and a control (trial with no sound). By comparing the equality of these two correlations we effectively took the guesswork out of the ABR analyses.

For both species, ABR waveforms were clear at suprathreshold levels and were repeatable between trials. An auditory peak in the brainstem recordings occurred at approximately 5 – 7.5 milliseconds after stimulus presentation. Subadult green sea turtles detected frequencies between 100 – 500 Hz; their most sensitive hearing was between 200 – 400 Hz (Fig. 1). However, we found that the two juvenile green turtles tested in Maryland have a slightly expanded range of hearing when compared to the subadult greens tested in Hawaii. These juveniles responded to sounds ranging from 100 – 800 Hz, with their most sensitive hearing range from 600 – 700 Hz. The two juvenile Kemp's ridleys had a more restricted range (100 – 500 Hz) with their most sensitive hearing falling between 100 – 200 Hz (Fig. 2). Tuna responded optimally to sound frequencies between 200 – 700 Hz, with their most sensitive hearing occurring between 400 – 600 Hz (Fig. 3).

Both sea turtles and yellowfin tuna appear to be low–frequency specialists. We had hoped to use this research to develop strategies to reduce the bycatch of turtles by U.S. longline fisheries without reducing the catch of the target fish species. However, from these preliminary data, we now suspect that tuna will also hear any deterrent noise that may be heard by sea turtles.

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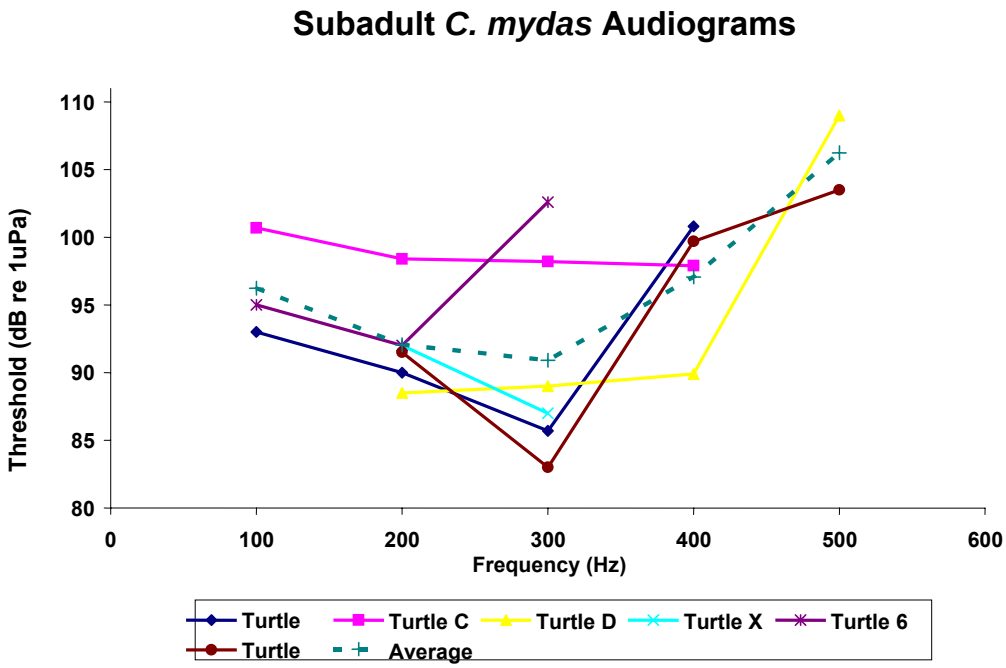

Figure 1. Audiograms for six subadult green sea turtles (*C. mydas*) tested at the National Marine Fisheries Service Kewalo Research Facility. Thresholds were obtained at each frequency from auditory brainstem responses to underwater tonal stimuli. An average threshold audiogram for all turtles combined is also displayed.

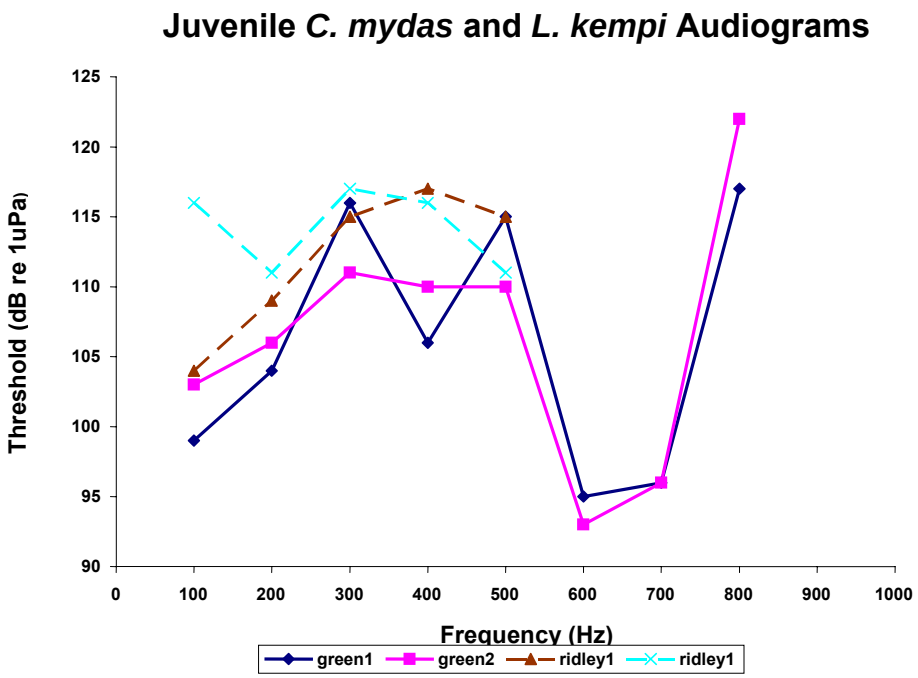

Figure 2. Audiograms for two juvenile green sea turtles (*C. mydas*) and two juvenile Kemp's ridleys (*L. kempi*) tested at Woods Hole Oceanographic Institution in conjunction with the New England Aquarium. Thresholds were obtained at each frequency from auditory brainstem responses to underwater tonal stimuli.

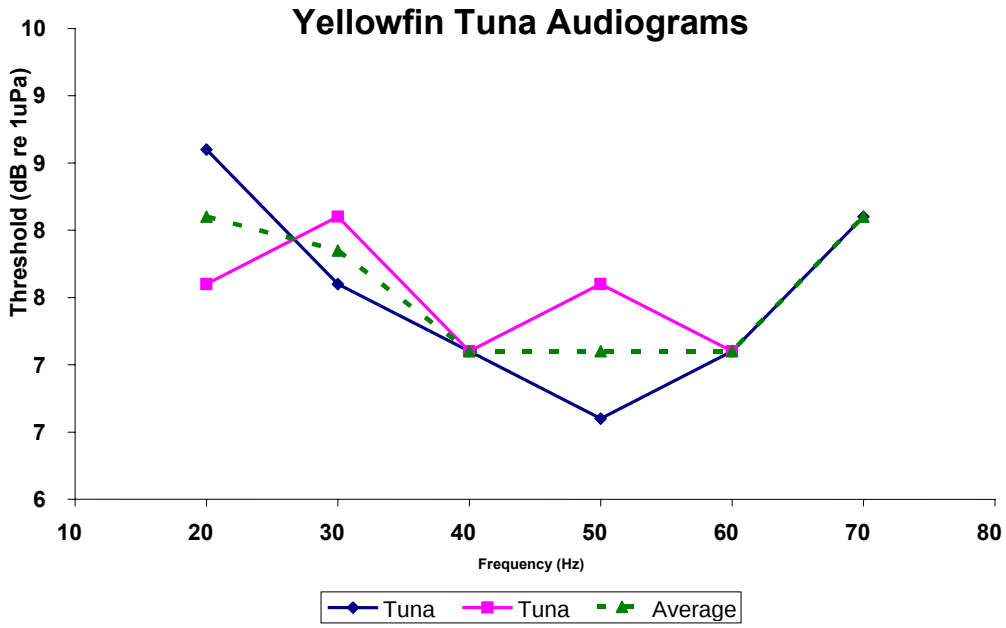

Figure 3. Audiograms for two yellowfin tuna (*Thunnus albacares*) tested at the National Marine Fisheries Service Kewalo Research Facility. Thresholds were obtained at each frequency from auditory brainstem responses to underwater tonal stimuli. An average threshold audiogram for both tunas is also displayed.

OTHER PUBLICATIONS

In addition to the contributions in this volume, projects receiving funding through the Pelagic Fish and Sea Turtle Sensory Biology Working Group have produced articles in the peer-reviewed literature and various informal outlets. To date, the list of articles includes:

Brill, R., K. A. Bigelow, M. K. Musyl, K. A. Fritsches, and E. J. Warrant. 2005. Bigeye tuna behaviour and physiology. Collected Volumes of Scientific Papers ICCAT 57(2):142–16l.

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SCIENTISTS INVOLVED IN RESEARCH UNDER THE PELAGIC FISH AND SEA TURTLE SENSORY BIOLOGY WORKING GROUP, 2002–2005

Christofer Boggs	NOAA Fisheries, Pacific Islands Fisheries Science Center, USA
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Kerstin Fritsches	University of Queensland, Australia
Gerald Jacobs	University of California, Santa Barbara, USA
Michael Crognale	University of Nevada, Reno, USA
Ben Higgins	NMFS, Galveston, Texas, USA
Darlene Ketten	WHOI / Harvard Medical School, USA
David Levensen	Scripps Institute of Oceanography, UCSD, USA
Kenneth Lohman	University of North Carolina, Chapel Hill, USA
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Jess Deegans	California State University, Bakersfield, USA
Richard Vogt	University of South Carolina, USA
Eric Warrant	University of Lund, Sweden
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