A. Title: Application for Permit for Scientific Purposes under the Endangered Species Act (ESA) of 1973 as amended.

B. Species: Pacific Sea Turtle Populations of:

Loggerhead, Caretta caretta

Olive ridley, Lepidochelys olivacea

Leatherback, Dermochelys coriacea

Green turtle / Black turtle, Chelonia mydas

C. Date of Permit Application: April 27, 2001

D. Applicant Identity:

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The field personnel for this research activity will be fishery biologists, biological technicians, fishery observers, vessel operators and crew of Hawaii-based longline fishing vessels. The principal investigator and principal field supervisor will recruit fishery technicians to supervise experiments conducted on contracted fishing vessels. The names of these individuals are pending and will be supplied when available, prior to implementing the experiments. Fishery observers working for the National Marine Fisheries Service (NMFS) Pacific Islands Area Office (PIAO) may also supervise experiments. Their names and letters indicating their authority to supervise fieldwork will be supplied prior to implementing the experiments. No experiments will be conducted by vessel operators without supervision by a NMFS

employee or contracted fishery biologist, biological technician, or fishery observer.

Vessel operators will be chosen through an interview process conducted by NMFS that will focus on aptitude, adherence to rules, understanding of technical requirements, and motivation. Vessel operators under consideration will participate in a workshop covering the fishing technology and contract requirements of the experiments and will be tested on their comprehension to ensure understanding of the experiment, the terms and conditions of their contracts, and the role of their field supervisor. The name of each vessel operator and a copy of the contract requiring compliance with all permit conditions will be provided prior to that vessel initiating activities under the permit. Field supervisors working for NMFS (fishery biologists, biological technicians, or fishery observers trained in turtle handling procedures) will oversee all turtle takes by each fishing vessel and terminate participation by any vessel operator or crew member who does not adhere to research protocols and turtle handling procedures.

Tissue sampling, disposition of samples, and turtle tagging will be covered under separate permits held by NMFS Honolulu Laboratory and NMFS Southwest Region. Transport and holding of deeply hooked hard shelled turtles will be the responsibility of the PI (Balazs) and the project veterinarian (to be named) who will provide a written certification of the criteria for transporting and maintaining the animals prior to initiating the work.

F. Project Description, Purpose, and Significance:

This application is for a NMFS sea turtle scientific research permit to take sea turtles while conducting experiments on methods for reducing sea turtle take by longline fisheries, and to import living, deeply hooked sea turtles for treatment to alleviate hooking damage and to monitor the progression of ingested hooks. The need to develop fishing methods that reduced sea turtle bycatch is urgent because the great majority of sea turtle takes result from foreign fisheries unaffected by new restrictions on the domestic Hawaii-based longline fishery. The goal is to develop methods to reduce turtle take that retain viable fishing performance, so that foreign fisheries in the region can be encouraged to adopt these fishing methods.

Choosing gear modifications for the fishing experiments

A variety of pelagic longline fishing gear and fishing operation modifications have been suggested to reduce the take and mortality of sea turtles (Balazs and Pooley, 1994; Williams et al.1996; Hoey, 1998; Hoey and Moore, 1999; Kleiber and Boggs, 2000). Ideas for fishery modifications to reduce turtle takes (Table 1) have come from fishermen, from logical consideration of fishing mechanics and turtle behavior, and from analysis of observer data on sea turtle takes. The choices of initial take

reduction measures for testing in the proposed experiments are based on these

Table 1. List of fishing gear and operations that could reduce sea turtle bycatch mortality. Sources for the ideas are given with each measure (in parentheses, with source details listed at the end of the table). This list was the subject of review at the Technical Gear Workshop to Reduce the Incidental Capture of Sea Turtles in the Atlantic Pelagic Longline Fishery, January 17-18, 2001. See also "1999" (below, = Kleiber and Boggs, 2000) for a similar list.

Sea Turtle Protection Measure	Prospects for Improved Sea Turtle Survival	Expected Fishery Impact
Fishery Harvesting Strategies		
Avoid warm core eddies (Captain's)	Not Determined	Not Determined
Avoid water temperatures greater than 68 degrees F when possible (Scoping, Captain's)	Not Determined	Not Determined
Coordinate rolling closures that will close areas of high concern at times there are many turtles (Scoping)	Not Determined	Not Determined
Delay setting until after 9 p.m. (Captain's)	Not Determined	Not Determined
Inform fishermen on a real-time basis about sea turtle migration patterns and areas of possible and/or current interactions (1994)	Not Determined	Not Determined
Limit the number of sets each vessel can make per month via lottery (Scoping)	Not Determined	Not Determined
Move if you catch a turtle (2000)	Probability of catching a turtle increases if one turtle has already been caught	Depends on number of vessels in the area
No new entrants in the fishery (1994)	Not Determined	Not Determined
Reduce total fishing effort (Scoping, 1999)	Very effective	High impact - (proportional to reduction)

Sea Turtle Protection Measure	Prospects for Improved Sea Turtle Survival	Expected Fishery Impact
Season - area closures/restrictions (1999)	Probably effective; issue of fixed vs. adjustable space or time boundaries	Depends on degree of segregation between turtles and target species - modeling and tracking research needed
Shorten soak time (1999)	Unknown, but potentially high	Tuna: high Swordfish: moderate?
Time of set and haul vs. daylight (Scoping, 1999)	Some effectiveness, needs research	Depends on scenario, needs research
Underwater set (2000)	Unknown	Unknown
Gear-Related Considerations		
Deeper hooks (Scoping, 1999)	Probably effective - needs more research	Moderate - depending on depth of hook
Set main line slack with line setter (Scoping)	Not Determined	Not Determined
Branch lines longer than float lines (2000, 1999, 1994)	Unknown, but makes sense	Swordfish: low Atlantic Tuna: Higher Hawaii tuna: impractical
Colored (blue) bait (2000, 1999)	Unknown	Possibly increase swordfish catch
Corrodible hooks and/or crimps (2000, 1999)	Unknown, but makes sense	Low
Different color of floats (Scoping, 1999)	Unknown	Unknown
Eliminate lightsticks (2000, 1999)	Evidence to date not promising - more analysis of Atlantic data needed	Pacific: high impact on swordfish, low of tuna Atlantic: unknown

Sea Turtle Protection Measure	Prospects for Improved Sea Turtle Survival	Expected Fishery Impact
Examine different types of bait (mackerel vs. squid) (Scoping, 1999)	Unknown, some	Unknown - could be low impact or even an improvement
Guarded hooks (2000, 1999)	Could be very effective	Depends on degree of depth segregation between turtles and target species
Move branch lines away from floats (2000, Scoping)	Many turtles caught on branch line adjacent to float	Could reduce number of hooks fished per set
Use of odors as a deterrent (1999, 1994)	Unknown (Probably Low)	Unknown
Reduce number of hooks per set (Scoping)	Not Determined	Not Determined
Remove strobe lights (1999)	Unknown	High
Replace "J" hooks with circle hooks (2000, 1999, Captain's)	Could increase survival by decreasing foul hooking	Effective in Canadian fleet for catching tuna and swordfish
Set four or more hooks between floats and try longer gangions and droppers (Captain's)	Not Determined	Not Determined
Acoustic deterrent devices (1999, 1994)	Low	Unknown
Use lead weights or swivels (Scoping)	Not Determined	Not Determined
Use an attractor on floating buoys (Scoping)	Not Determined	Not Determined
Sea Turtle Handling		
Develop a rescue program to recover turtles trapped in decaying warm eddy cores (Scoping)	Not Determined	Not Determined
Make guidelines outlined in Balazs et al. 1995 into regulations (1999)	High - depending on compliance	Low to moderate (specified in Balazs et al. 1995)

Sea Turtle Protection Measure	Prospects for Improved Sea Turtle Survival	Expected Fishery Impact
Release methods (2000)	High	Low
Dehooking device to be carried and used on pelagic longline vessels (Scoping)	Not Determined	Not Determined
Tool to cut line short (Scoping, 1999)	High - depending on compliance	Low
General Research Needs		
Develop and ground truth predictive models based on physical oceanography and sea turtle biology, sea turtle distribution patterns by species and size class, in order to close fishing areas where sea turtle densities are high (1994)		
Experiment both in lab and field with various gear/bait assemblies to reduce or eliminate attractiveness to sea turtles (1994)		
Implement tracking studies to determine fate of longline released sea turtles (1994)		
Initiate gear research projects to develop release and de-hooking techniques (1994)		
Research and education to avoid concentrations of sea turtles, such as they find on warm water bends on the Grand Banks (Scoping, 1994)		
Study gear: mono, color and size, buoys, high flyers, radio beacons, polypropylene rope, crimps, snaps, leads, glow beads, plastic squids and skirts, rattlers, and other ornaments (1994)		

Abbreviations:

2000 - Working Group on Reducing Turtle Bycatch in the Hawaii Longline Fishery. Report of First Meeting. Los Angeles, September 12 - 13, 2000.

Scoping - Suggestions and Submission from Biological Opinion Scoping Workshops, July 31 - August 17, 2000. (No assessment of prospects or impacts provided)

1999 - Workshop on Reducing Sea Turtle Takes in Longline Fisheries. Miami, August 31 - September 1, 1999 (= Kleiber, P. and C. Boggs. 2000. Workshop on reducing sea turtle takes in longline fisheries. Southwest Fisheries Science Center Administrative Report H-00-09 16 p.)

Captain's - Captain's Report Multi-Species Catch Characteristics for the U.S. Atlantic Pelagic Longline Fishery. Hoey and Moore. August 1999. (No assessment of prospects or impacts provided)

1994 - Pelagic Longline Fishery - Sea Turtle Interactions. Proceedings of an Industry, Academic and Government Experts, and Stakeholders Workshop held in Silver Spring, Maryland, 24-25 May

1994. (No assessment of prospects or impacts provided)

documents and on reviews by three expert working groups including scientists, commercial fishermen, and conservationists (Anon. 2000, 2001a, 2001b).

Some suggested take reduction methods show evidence of efficacy in reducing turtle bycatch while retaining viability in catch rates for target species based on fishery observer data, experimentation with captive turtles, or fishing experiments. The latter have the highest priority for fishing experiments. For other methods additional research and development or laboratory testing on captive turtles is being undertaken first, as the efficacy of these methods seems too speculative to incur turtle takes in fishing experiments without better justification. Some methods suggest promise for bycatch reduction but have unknown performance in catching target species. The target species viability of such fishing methods may be tested in fishing experiments with minimal turtle takes because a relatively small number of experimental fishing operations are required to demonstrate viable target species CPUE. More fishing operations and turtle takes are required to test bycatch reduction than to test CPUE viability because turtles are taken very infrequently compared to target species.

Two modifications to fishing practices which have been determined to have great promise for reducing turtle takes while having only minor impacts (if any) on fishing performance (target species CPUE) are the use of squid bait dyed blue with food coloring and the removal of branch lines attached to the main line closest to the

float line attachment points. Blue-dyed squids have been shown to reduce the bycatch of seabirds (Boggs 2000) and possibly increase the catch of swordfish. In field testing on seabirds, no turtles were taken on blue-dyed bait, although turtles were captured in the same tests with regular bait (Kleiber and Boggs, 2000). A higher proportion of loggerhead and leatherback turtles are taken on the branch line closest to floats than on other branch lines (Kleiber and Boggs, 2000). A typical distance between branch lines on a set of longline gear targeting swordfish is 37 fathoms but one branch lines is usually attached much closer to the floats than this. The take reduction technique would be to require that the nearest branch lines be at least 40 fm from the nearest float line. These two techniques are proposed to be applied together as a single experimental fishing treatment which will test the efficacy of the combined method for reducing turtle bycatch in contrast to standard (control) fishing operations. This experiment will entail the highest turtle takes.

Reducing the visibility of longline gear to sea turtles by using “stealth” longlines with major gear modifications (countershaded floats, dark lines, dulled hardware, down-welling narrow-frequency lightsticks, and blue dyed bait) is proposed for testing viability in maintaining target species CPUE in both swordfish-style (shallow set, nightime) and tuna-style (deep set, daytime) fishing operations in comparison with standard swordfish- and tuna-style operations. Similar testing of target species CPUE is proposed for deep-set swordfish fishing. This propsed method would target swordfish deep where they descend during the day using swordfish-type bait

and lightsticks in areas where near-surface nightime swordfish abundance is high. Every effort will be made to avoid taking any turtles in the stealth and deep swordfish fishing tests for target species CPUE. No takes are needed for initial tests of these methods, which are intended to demonstrate viable CPUE, although some loggerhead and a few leatherback, olive Ridley, and green turtle takes are anticipated.

Changes in the time and depth of setting, the soak duration, and the time of hauling longline gear could possibly reduce turtle bycatch but no reliable trends have been documented that are not confounded with fishing style and fishing area (e.g. high bycatch swordfish-style shallow-set night soaking longline at higher latitudes versus low bycatch tuna-style day soaking longline at lower latitudes) . Measuring trends in the time and depth of turtle captures could reveal particular time intervals or depths of longline operations to which turtles are most vulnerable, suggesting modifications to fishing operations for future testing. The use of hook timers in conjunction with time-depth recorders (Boggs 1992) is proposed for this purpose. Substantial turtle takes will be required to successfully document trends in the time or depth of capture. Past work indicated practical methods for increasing gear selectivity for tuna and decreasing gear selectivity for gamefish (Boggs 1992).

One method for reducing the severity of turtle injury from longline hooking is the use of 16/0 circle hooks instead of the more typical J hooks used in swordfish-style

longline operations. Experiments comparing circle and J hooks in the Azores (Bolton and Bjorndal 1999) and North Pacific (LaGrange 2001) indicate a much lower frequency of deep hooking (defined as hook ingested past mouth cavity - in esophagus or deeper) as opposed to hooking in the mouth. Presumably, delayed mortality of mouth hooked turtles is lower than that of deeply hooked turtles. Circle hooks (16/0) do not decrease the turtle take rate, but do reduce the target species CPUE by 30-50%. Both Bolton (personal communication) and LaGrange (personal communication) have suggested that larger (18/0) circle hooks could increase the viability of target species CPUE. Testing of large circle hooks is proposed as a piggyback project during the hook timer measurements.

Returning Deeply Hooked Turtles to Port for Rehabilitation and Monitoring

Most other measures suggested to reduce post release mortality of turtle bycatch (Balazs and Pooley, 1994; Williams et al.1996; Kleiber and Boggs, 2000) have already been implemented. The need for understanding more about the fate of deeply hooked turtles has been made clear by expert working groups, and recommendations have been made to return deeply hooked sea turtles to port for determination of the degree of injury and progression of hooks through the digestive tract (Balazs and Pooley 1994). While permitting the development of treatments and rehabilitations for hooked turtles this activity will also provide the opportunity to better understand the mode of injury, and prognosis for recovery of deeply hooked

turtles.

Benefits Compared to Impacts of the Take

The U.S. domestic Hawaii-based longline fishery is a small segment of the total amount of longline fishing that occurs in the Pacific Ocean compared to the international fleet (e.g., Japan, Korea, and Taiwan). The applicants believe that an Endangered Species Act Section 7 Consultation on this application will show that fishing experiments are critical to developing gear technologies and fishing strategies for reducing sea turtle capture rates throughout the Pacific Ocean. Developing a gear technology or fishing strategy that is capable of significantly reducing sea turtle capture rates by longline vessels is essential if the U.S. is going to cultivate an open dialogue between the international community to formulate collaborative efforts to address the incidental sea turtle interaction problem. Examples of such efforts to reduce international takes of protected species include the adoption of dolphin-safe fishing methods in foreign tuna purse-seine fisheries, the export of turtle excluder devices (TEDs) to foreign trawl fisheries, and the use of trade sanctions to encourage foreign nations to adopt such methods.

The potential long-term benefits of developing a new fishing strategy or modifying existing gear technologies to significantly reduce sea turtle interactions throughout the Pacific Ocean could outweigh the harm to turtle population status due to experimental takes over the short term because of the future savings that will occur

when these strategies and measures are instituted internationally. Conducting fishing experiments on sea turtle take reduction methods may ultimately increase the likelihood of survival and recovery of the sea turtle populations by reducing takes and mortalities in international longline fisheries and by increasing sea turtle conservation awareness in the international community through the development of sea turtle saving technologies.

Several protective measures are necessary in order to minimize the impact of the experiments on sea turtle populations. All experiments will be supervised by NMFS employees or contracted biologists, technicians, or fishery observers. All measures that have been implemented by regulations in the commercial fishery to reduce the mortality of the bycatch will be used in the experiments. A rigorous experimental design will be followed in which the number of turtles taken by modified fishing gear (treatment operations) are contrasted with turtle taken by normal fishing gear (control operations). The number of control operations will equal the number of treatment operations, and control operations will follow a rigid standard that will not vary among vessels or at the discretion of vessel operators. In addition, takes of sea turtles in the experiments will be reported to NMFS on a real-time basis using the satellite vessel monitoring system. If at any time NMFS determines that the take levels for the experiment are being exceed or are likely to be exceeded in the control and treatment fishing operations required by the experimental design, then NMFS will cease the experiment.

The success of the experiment will be judged by the effectiveness of the gear modifications in reducing bycatch, their viability in catching target species, and their exportability and enforceability. If the results indicate that a 50% or greater reduction in the level of sea turtle bycatch can be achieved while maintaining viable catch rates for target species, then the export of this methodology could reduce the international sea turtle take rate by half. This level of reduction is far more valuable than the reduction that would be achieved in the U.S. domestic longline alone since the U.S. fleet represents a small portion of the international longline fleet. Past incidental take levels in the Hawaii-based domestic longline fishery were around 716 sea turtles annually prior to 2000. New regulations will reduce this incidental take to an estimated 110 sea turtles per year according to NMFS 2001 ESA section 7 biological opinion (BO) on the fishery. In contrast, the longline fishery of Japan (one of the three important longline fishing nations, the others being Korea and Taiwan) was estimated to have taken 21,200 sea turtles in the western Pacific and South China Sea in 1978 (Nishimura and Nakahigashi, 1990). If this foreign fishery were induced to adopt a 50% effective turtle bycatch reducing gear modification then incidental longline takes could be reduced by 10,600 turtles per year; an amount equivalent to 96 years of estimated incidental take by the current Hawaii-based domestic fishery. Similarly, adoption of the bycatch reduction method by 10% of the Japan fleet could save as many turtles in one year as the Hawaii-based fishery is estimated to take over the next decade.

The combination of preferred experimental designs chosen in this application would take a maximum of 121 turtles (88 loggerhead, 17 leatherback, 10 olive ridley, and 6 green) per year (Table 2) and utilize about 1,280 shallow-set longline operations and 90 deep-set longline operations (60 deep-set tuna operations and 30 deep-set swordfish operations). These directed takes will be in addition to the incidental take expected in the commercial fishery. Based on the Japan longline turtle take estimated by Nishimura and Nakahigashi (1990) the annual incidental take estimated for the Hawaii-based fishery combined with the annual take in the proposed experiments could be saved in 8 days if the Japan fishery could be induced to adopt a 50% effective bycatch reduction method. And if only 10% of the Japan fishery adopted the method this number of turtles could be saved in three months.

Experimental Design

Sets will serve as the experimental unit for testing any treatment that involves the attractiveness of the longline to turtles or target species. Alternating treatments with controls along a single longline will not result in independence between control and treatment if the control sections (e.g. highly visible undyed squid) attract turtles to the adjacent treatment sections. An exception to this design would be when testing of different hook types which differ only in their mechanical effects after a turtle interacts with the hook. In that case treatment and control can be applied

independently on the same set without pseudo-replication.

A variety of experimental designs for each proposed experiment are reviewed in this application and a set of preferred designs is chosen (Table 2). Fishing vessel operators will be contracted to use their fishing vessels to conduct the fishing experiments under this permit under the direction of the field supervisors. The catch of target species will be sold to help defray the cost of the contracted fishing operations. The largest experiment in terms of turtle take and fishing operations will be the testing of minor gear modifications with great promise of reducing turtle take, and which are also known to have minor impacts on target species CPUE: blue bait and removing branch lines near floats. These minor gear modifications will be most economical to test because with near normal CPUE the catch will defray a significant part of the contract expense.

The limiting condition of this experiment will be the need to minimize the take of endangered sea turtles while retaining the statistical power to detect a significant effect of the bycatch reduction treatment. Turtle takes are rare events in the fishery and they have the statistical properties of a Poisson distribution, in which the standard deviation is as large as the mean. In such circumstances the statistical power of a controlled experiment depends on the number of turtles taken in control and treatment operations (attachment 1) and not on the number of fishing operations (sets). Therefore, to increase the power of the experimental tests it is

best to use the fishing style with the greatest turtle take rate.

Table 2. Preferred experimental designs

		Turtle takes per year				All species	Sets	
Experiment	Years to complete	Leatherback	Loggerhead	Olive Ridley	Green	takes/year	per year	Vessels
Minor gear modification blue bait + move branch line	3	12	65	6	4	87	1039	9 *
"Stealth" and deep fishing	1	2	8	2	1	13	150	3 **
Hook timers to measure capture time + depth	2	3	15	2	1	21	181	2 *
Alternative hook design piggyback on hook timer test	1	0	0	0	0	0	0	0
Totals		**17**	**88**	**10**	**6**	**121**	**1370**	**14**

* Full-time fishing vessels
** Three vessels operating at the same time

In the commercial fishery, shallow-set gear uses < 10 hooks between floats to reach a maximum main line depth of < 100 m (typically 28 m). The target species of this gear configuration are swordfish or a mix of swordfish and tuna species, and this configuration is now prohibited to commercial fishermen because it has a much higher take rate of sea turtles than deep-set gear designed to target bigeye tuna. However, this type of fishing is the best for testing sea turtle take reduction measures because it will have a higher take rate will provide more rapid statistical confirmation of bycatch reduction by contrasting control and treatment operations. Control operations for comparison with gear modifications will strictly adhere to the parameters given in Table 3 for experiments with swordfish-style, and tuna-style fishing respectively. The only experiment that is planned for both styles of fishing is the testing of stealth fishing gear for the viability of target species CPUE.

A power analysis was conducted to scope out a variety of sample sizes required to detect a bycatch method that has different degrees of effectiveness in comparison with the control fishing method (attachment 1). The first example given is for leatherback turtles, arguably the species for which results are needed most badly due to the presumed dire status of the population Table 4. Take numbers required to detect a 25% effective treatment are much higher than those required to detect a 50% effective treatment because of the lower signal-to-noise ratio when the treatment is closer to the control method (Table 4). The higher the type I (alpha, attachment 1) and type II (beta, attachment 1) error

Table 3. Average of fishing gear parameters for the Hawaii-based longline fishery using two styles of fishing: Swordfish-style fishing and tuna-style fishing. Control fishing operations in most of the experiments will strictly adhere to the parameters given below for swordfish-style fishing. The exception will be that for stealth gear tests in the tuna fishery, tuna-style parameters will be used in control fishing operations.

Gear/Trip Type	Swordfish Fishing	Tuna Fishing
Area Fished	North of Hawaiian Islands	South of Hawaiian Islands
Main line Length	42 miles	34 miles
Shooter Used	No	Yes
Vessel Speed	7.8 knots	6.8
Lightsticks Used	Yes	No
Branch Line Length	17 meters	13 meters
Float Line Length	8 meters	22 meters
Number of Hooks	820 hooks	1,690 hooks
Number of Hooks per Float	4 hooks	27 hooks
Number of Floats	189 floats	66 floats
Type of Hook	J-shaped	Tuna
Type of Bait	Squid	Saury
Target Depth	28 meters	167 meters
Gear Soaks	Night	Day
Soak Time	20 hours	19 hours

rates that can be accepted , the lower the sample sizes required.

Because the take of this endangered species is so controversial the take numbers required to detect an effective bycatch reduction treatment have been spread out over three years to keep the annual directed take rate low in comparison to the level of incidental take allowed in the Hawaii-based fishery. This can best be accomplished by accepting the highest level of type I and II error rates that the investigators can accept, and by anticipating that the treatment will be at least 50% effective in reducing take. Thus the preferred design will take a total of 36 turtles spread out over 3 years (Table 4). If the treatment is 50% effective 12 of these turtles will be caught by treatment fishing operations and 24 will be caught by control operations and the results will be statistically significant at the alpha = 0.10 level. One third of the required take is 12 turtles per year, the number given in the summary of designs (Table 2) Fractional numbers are raised to the nearest whole integer in summarizing annual takes (Table 2), so these numbers are sometimes one integer higher than the rounded turtle numbers given for individual experiments (e.g. Table 4).

Equal numbers of treatment and control operations (sets) will be conducted but the total number of sets listed is just an estimate based on historical capture rates of turtles by swordfish style fishing gear (Table 4). Again, the statistical properties of Poisson-distributed data are such that the number of sets is not critical to the test,

and the experiment will be limited to the number of turtle takes

Table 4. Minor gear modification experiment: with significant leatherback findings in 3 years
Several techniques combined in one fishing style
(blue dyed squid bait, keeping branch lines 40 fathoms from float)

*preferred experimental design

25% Effective Fishing Method (reduces turtle take by 25% compared with control sample)

Error Levels		Leatherback turtle takes				Sets needed				Full time	Concomitant takes per year (other)			All species
Alpha	Beta	Control	Treat.	Total	Per year	Control	Treat.	Total	Per year	vessels	Loggerhead	Olive Ridley	Green	(total per year)
0.05	0.10	236	177	413	138	15325	15325	30649	10216	85	741	70	39	988
0.10	0.10	169	126	295	98	10946	10946	21892	7297	61	529	50	28	706
0.05	0.20	158	119	277	92	10278	10278	20557	6852	57	497	47	26	663
0.10	0.20	116	87	203	68	7532	7532	15065	5022	42	364	34	19	486

50% Effective Fishing Method (reduces turtle take by 50% compared with control sample)

Error Levels		Leatherback turtle takes				Sets needed				Full time	Concomitant takes per year (other)			All species
Alpha	Beta	Control	Treat.	Total	Per year	Control	Treat.	Total	Per year	vessels	Loggerhead	Olive Ridley	Green	(total per year)
0.05	0.10	41	20	61	20	2641	2641	5281	1760	15	109	10	6	146
0.10	0.10	36	18	54	18	2338	2338	4675	1558	13	97	9	5	129
0.05	0.20	34	17	51	17	2208	2208	4416	1472	12	92	9	5	122
0.10	0.20	24	12	36	12	1558	1558	3117	1039	9	65	6	3	86 *

Sets per year per vessel

120

Historical takes per year

	Leatherback	Loggerhead	Olive Ridley	Green	All species
Takes per set	112	418	146	40	716

(control rate, assume all take is reduced in treatment)

Leatherback	0.0154
Loggerhead	0.0829
Olive Ridley	0.0078
Green	0.0044

required, not the number of sets estimated. If more sets are needed to reach the required number of observed turtle interactions, additional fishing operations will be contracted. The estimated total number of sets per year will be 1039, a third of the 3117 sets that may be required over three years (Table 4). Spreading the take out over time may be more cosmetic than of real benefit to the turtle population. Minimizing the number of experimental turtle takes per year delays the process of acquiring the number of observations needed to reach some level of statistical confidence in the efficacy of results. There may be no benefit to the delay if turtle populations are declining, because the relative population impact of a take increases as the population declines.

The estimated number of fishing operations required for finding a statistically significant effect of a bycatch reduction measure that is 50% effective for leatherback turtles will have a concomitant take of other turtle species, based on the historical rate of interactions with those species by the type of fishing operations that will be used in the experiment (Table 4). It so happens that the requisite number of operations for the preferred leatherback experimental design will probably result in a take of loggerhead turtles (65 per year, Table 4) sufficient at an alpha level of 0.05 and a beta level of 0.1 to find a significant effect of a 50% effective bycatch reduction method in only 1 year (61 per year, Table 5). Over three years this take will reach 195 loggerhead turtles which is enough to find a significant effect of a treatment that is only 30% effective at a lower, but acceptable error level.

Since this design dovetails so well with the leatherback

Table 5. Minor gear modification experiment: with significant findigs for loggerheads in 1 year
Several techniques combined in one fishing style
(blue dyed squid bait, keeping branch lines 40 fathoms from float)

*Preferred experimental design

25% Effective Fishing Method (reduce turtle take by 25% compared with control sample)

Error Levels		Loggerhead turtle takes			Sets needed			Full time	Concomitant takes per year (other)			All species
Alpha	Beta	Control	Treat.	Total (annual)	Control	Treat.	Total	vessels	Leatherback	Olive Ridley	Green	(total per year)
0.05	0.10	236	177	413	2847	2847	5694	47	77	39	22	551
0.10	0.10	169	126	295	2033	2033	4067	34	55	28	16	393
0.05	0.20	158	119	277	1909	1909	3819	32	51	26	15	369
0.10	0.20	116	87	203	1399	1399	2799	23	38	19	11	271

50% Effective Fishing Method (reduce turtle take by 50% compared with control sample)

Error Levels		Loggerhead turtle takes			Sets needed			Full time	Concomitant takes per year (other)			All species
Alpha	Beta	Control	Treat.	Total (annual)	Control	Treat.	Total	vessels	Leatherback	Olive Ridley	Green	(total per year)
0.05	0.10	41	20	61	491	491	981	8	11	6	3	81 *
0.10	0.10	36	18	54	434	434	869	7	10	5	3	72
0.05	0.20	34	17	51	410	410	820	7	9	5	3	68
0.10	0.20	23	11	34	273	273	547	5	6	3	2	45

Sets per year per vessel

120

Historical takes per year

Loggerhead	Leatherback	Olive. Ridley	Green	All species
418	112	146	40	716

Turtle takes per set
(control rate, assume all take is reduced in treatment)

Leatherback	0.0154
Loggerhead	0.0829
Olive Ridley	0.0078

Green	0.0044

design it is incorporated the preferred design for leatherbacks (Table 2).

It has been suggested that rather than conducting control operations for comparison with the treatment operations in these experiments, the historical take rates for this type of fishing be utilized as the control baseline for comparison. The problem with that idea is that there is so much inter-annual variability that it would be difficult to determine whether the change in an experiment were due to the treatment or an oceanographic change. Oceanographic features such as the number and intensity of frontal structures and changes in ecosystem productivity from year to year would make it impossible to know for certain whether a treatment were really effective without a synoptic control. A synoptic control might be combined with historical control data in a Bayesian approach which would have some power to reduce th sample size needed for the experiment especially if the oceanographic change were intense. But ironically, if the oceanographic change were small, the synoptic control might need to be even larger to eliminate the hypothesis of an annual change than it would need to be to detect the efficacy of the bycatch reduction method.

The possibility that historical data could lend statistical power to the experimental design will be explored further as part of an evaluation of results from the first year. This evaluation will also include a risk assessment that will examine whether the

accumulated results indicate a method that is much more or less effective than anticipated. A much more effective method might be detected by a smaller sample size, allowing the experiment to be completed early. A treatment that appeared much less effective might be supplanted with a newly developed or discovered technique. Laboratory experiments with captive turtles are underway, as well as field experiments (attachment 2) that may reveal promising new techniques during the coming year. Similarly, If multi-variate analyses of the results indicated no significant difference in the effectiveness of a take reduction method between species, significant results could be achieved sooner, with fewer turtle takes. For some techniques there may be no reason to expect differences between species. Any change in the design of the experiment would require an permit modification request.

Experiments with major modifications to fishing gear such as stealth fishing gear and deep daytime fishing for swordfish will have very low levels of take and so they will be completed in a single year (Table 6). None of the more limited design options were preferred for these experiments because a better take reduction method will need to be ready for bycatch reduction testing in the near future if the minor gear modification experiments do not look promising after the first year. The stealth and deep day swordfish experiments will be conducted synoptically with three vessels: one conducting control operations to demonstrate high near-surface abundance of target species, another conducting stealth tests, and the third

conducting deep daytime fishing for swordfish. Thus there will be some economizing of the control operations to serve two purposes. In testing

Table 6. Major Gear Modification Experiments

*Preferred experimental design

"Stealth" and deep daytime gear tests to demonstrate viability for target species

Need at least 3 fishing trips (30 sets), with controls, for credible demonstration; Needed first year

	Number of sets			Synoptic	Takes per year				All spp
Fishery & test	Control	Stealth	Deep day	vessels	Loggerhd	Leathbk	O.Ridley	Green	(per year)
Sword stealth	30	30	0	2	5	1	0	0	7
Sword stlth+deep	30	30	30	3	7	1	1	0	10
Tuna stealth	30	30	0	2	0	0	1	0	1
Both stealth	60	60	0	2	5	1	1	0	8
Both stlth+sword d	60	60	30	3	7	2	2	1	11 *

Hook timer experiments to detect trends in turtle capture time or depth

Need 30 observation on a species to see trends
Required number based on fish results, Boggs 1992)
Hook type tests on target species viability will piggyback on hook timer experiment

	Loggerhd	Sets	Full time	Concomitant takes per yr			All spp
Years to results	per year	per year	vessels	Leathbk	O.Ridley	Green	(per year)
1.00	30	362	3	6	3	2	40
1.50	20	241	2	4	2	1	27
2.00	15	181	2	3	1	1	20 *
2.50	12	145	1	2	1	1	16
3.00	10	121	1	2	1	1	13

Sets per year per vessel

120

Turtle takes/trip
(asume control rate in all sets as worst case)

	Swordf	Tuna
Leatherback	0.0154	0.0055
Loggerhead	0.0829	0
Olive Ridley	0.0078	0.0153
Green	0.0044	0.0025

the stealth gear with tuna style fishing there will be only two vessels, as both stealth and control fishing operations will be conducted deep during the day.

The only substantive take levels among the major gear modification experiments are for the hook timer study (Table 6). Research on fish (Boggs 1992) indicated that at least 30 hook timer readings were needed to detect significant trends in the distribution of fish by hook depth and time. To reduce the annual take the preferred design will be stretched out over 2 years.

Estimated potential mortality over the 3 years of the experiment is 6 greens,15 leatherbacks, 85 loggerheads, and 9 olive Ridley turtles. This mortality is calculated by adding the 1) immediate mortality (multiplying annual estimated maximum take (Table 7) by the immediate mortality rate for that species of sea turtle in the shallow-set swordfish fishery, (or in the case of the tuna stealth experiment, the mortality rate in the deep-set tuna fishery) and 2) delayed mortality (multiplying the remaining live animals by the delayed mortality rate of 27 to 42 percent based on mortality rates of deep and light hooking assumed in the BO. This is very likely an overestimate of the mortality, due to using the very conservative mortality assumption used in the biological opinion. The deeply hooked turtles that are returned to Honolulu for observation and potential rehabilitation may have a reduced rate of delayed mortality, which could reduce the number of mortalities below these estimates.

Despite the estimated levels of mortality, the long-term benefits that will be achieved for sea turtle conservation might be much greater than the short term impacts of the experiment. This benefit could far outweigh the harm of the directed take because of the future savings that could occur if successful bycatch reduction measures are instituted internationally.

G. Project Methodology:

It is requested that this permit cover at-sea activities of the NMFS, Southwest Fisheries Science Center overseeing and working with long line captains and crew members selected for a one year trial period, with potential annual renewal of up to 3 years (or longer up to 5 years, depending on the number of takes authorized per year). An evaluation of all results, including the results of a risk assessment to determine the costs and benefits of altering the experimental design to incorporated newly discovered or developed take reduction measures, will occur in consultation with FPR at the end of the first year. The issuance of this permit will allow sea turtle research activities at sea, to better fulfill NMFS ESA responsibilities to protect, conserve, and recover listed species of sea turtles, and better meet the goals and objectives of the U.S. Pacific Sea Turtle Recovery Plans and the requirements of present and future Section 7 Biological Opinions developed for this fishery.

The methodology for transporting deeply hooked sea turtles and maintaining them in Honolulu are described below in section I.

Selected fishing vessel operators judged to be capable of conducting the fishing operations for the proposed research will receive a one-day (8 hour) training course at the NMFS Honolulu Laboratory on the experimental protocols and terms and conditions of participating in the experiment. At the end of the course they will be tested on comprehension of the take reduction treatments as applied to their fishing operations and also on the terms and conditions under which they will be allowed to operate. Testing may be conducted in languages other than English (e.g. Vietnamese). The training will emphasize the need to conduct all experimental operations scrupulously. If not done so, as noted by the field supervisor at sea, continued participation of the vessel in fishing experiments will be terminated. Failure to adhere to protocols will be grounds for termination of the contract for conducting fishing experiments under the permit.

Fishermen participating in the minor gear modification experiment must agree to follow these instructions for setting their gear. NMFS field supervisors will oversee the operations and record results, using the attached Experimental Turtle Bycatch Reduction Trip Plan and Report form (attachment 3) to show that the pre-designed plan for the order of sets on each trip was followed. The two techniques (blue bait and removed branch lines near floats) will be used together on every treatment operation. On about half of the sets (in a random pattern) no turtle take reduction measures will be used (standard sets). Other than these changes, the gear in the treatment and control operations must resemble that of standard swordfish-style fishing (Table 3) except that in keeping all branch lines 40 fathoms away from the float line the branch lines will have to be spaced closer together (25 fathoms apart instead of 37) to result in the same number of hooks being set per float and the same length of main line as the control operation.

At standard setting speeds (8 knots) the proper distance of 40 fathoms between the float line and branch lines in treatment operations can be estimated as 18 seconds of run time. The setting speed is not a critical component of the experimental design, as it can depend on weather and engine performance. As long as the timing is adjusted property to account for speed changes the same gear configuration can be maintained. For example at 6 knots = 40 fathoms is 24 seconds (and 7 knots = 21 seconds, 8 knots = 18 seconds, 9 knots = 16 seconds, 10 knots = 14 seconds). Field supervisors will check the spacing between float lines and branch lines during setting or during haulback. Haulback is generally conducted at slower speeds than setting. At a hauling speed of 6 knots the distance of 40 fathoms between a float line and the closest branch line can be estimated as 24 seconds (5 knots = 29 seconds, 4 knots = 36 seconds, 3 knots = 48 seconds).

The stealth gear experiments will utilize floats that are blue on the bottom and orange on top for treatment sets and orange all over for control sets. Dark grey monofilament will be used for main line, float lines, and branch lines. Battery powered, narrow-frequency, yellow light emitting diode- (LED) based, down-welling (shaded on the upper half) light sticks will be used on stealth gear and regular yellow chemical light sticks will be used on control gear. The metallic shine of the branch line and float line snaps will be removed or they will be painted for stealth gear, and the bait will be dyed blue as described in Boggs (2000).

Deep daytime fishing operations for swordfish will use a depth configuration comparable to that of tuna gear, which will be modified based upon results expected within the next few months from swordfish recently tagged with pop-up satellite transmitting archival tags (PSATs). These tags will report the typical

daytime depth distribution of swordfish. Target depth will be achieved using a main line shooter and a much greater length of main line and greater number of hooks between floats while maintaining the standard swordfish-style number of branch lines per set. Depth will be measured with time-depth recorders to assure target depths are achieved.

Hook timer experiments will be conducted using standard swordfish style gear fitted with hook timers as described by Boggs (1992). No controls are used, and the comparison is between different times and depths within the combined fishing operations. The piggyback hook type experiment will utilize alternating J and 18/0 circle hooks on all hook timer operations.

H: Description and Estimates of Take:

The populations and species of turtles to be taken are listed in item B (above). The maximum number of turtles by species expected to be taken under this permit, and the estimated mortality of these turtles is given in Table 7. Under 100% observer coverage and with real-time reporting of results the experiments will be terminated when these take limits are reached.

Some of the turtles taken and released alive during those experiments may be biopsied and tagged with flipper tags, passive induced transponder (PIT) tags, radio transmitters, or pop-up satellite transmitting archival tags (PSATs), but only if these activities are conducted by authorized personnel using techniques approved for these activities by other permits. This application requests that fishery biologist and biological technicians contracted or employed by NMFS as field supervisors in this experiment be authorized to handle, tag, biopsy and release sea turtles using

techniques authorized by permits 1190 and 1296 after training as described in those permits.

The proposed annual take levels for the experiments are premised on the preferred experimental designs summarized in Table 2, and decrease with the completion of the stealth and deep fishing operations after year 1 and the completion of the hook timer and hook type experiments after year 2. However, additional takes may be requested by application for permit modification in years 2 and 3 to accomplish other testing or to make up for errors. These requested takes would only raise the annual take back up to the annual number for the first year.

The annual estimated maximum incidental mortality (Table 7) is calculated by adding the 1) immediate mortality (multiplying annual estimated maximum incidental take by the immediate mortality rate for that species of sea turtle in the swordfish fishery) and 2) delayed mortality (multiplying the remaining live animals by the delayed mortality rate of 27 to 42 percent based on rates of deep and light hooking for the species in the swordfish-style gear segment of the fishery). The deeply hooked turtles that are returned to Honolulu for observation and potential rehabilitation may have a reduced rate of delayed mortality, which could reduce the number of mortalities below these estimates. Dead turtles will be removed from the marine environment for research purposes, including necropsy and collection of life history data. Tissue samples may be used for lab studies including the following: toxicology, histopathology, and genetic studies to identify nesting origins of incidentally taken turtles.

Table 7. Estimated annual capture and mortality levels in the experiment to reduce bycatch of sea turtles on longline fishing gear.

Species	Annual Estimated Take			Estimated Mortality		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
Green	6	5	4	2	2	2
Leatherback	17	15	12	6	5	4
Loggerhead	88	80	65	32	29	24
Olive Ridley	10	8	6	4	3	2

The location of the authorized take activities requested in this application will be the Pacific Basin, including the high seas and area within the U. S. EEZ. Dates of taking or collection, importation and exportation will be dependent on the encounter with turtles during the period of the experiments. Quarterly reports of activities and pertinent research results will be provided to the Office of Protected Resources. The size and sex of individuals by species taken will vary and cannot be predicted since incidental captures are involved. However, the sizes of all sea turtles so far captured and measured by NMFS fishery observer program are shown in the attached graphs (Balazs and Parker, NMFS unpublished data, attachment 4)

Loggerhead Turtle

The loggerhead turtle is listed as a Threatened species throughout its range. In the Pacific, threatened status is consistent with population levels and trends. The stocks found in U.S. jurisdiction most likely originate from Japanese nesting areas and thus activities in Japan which impact nesting success or foraging turtles in coastal waters are of concern. The United States and Mexico (primarily Baja

California South) support important developmental habitats for juvenile loggerheads. A primary threat to the species in the Pacific is from the incidental mortalities associated with commercial fisheries, particularly long line and net fisheries. This threat must be minimized for recovery of this species.

Olive Ridley Turtle

The olive ridley turtle is listed as Threatened in the Pacific, except for the Mexican nesting population, which is classified as Endangered. This latter classification was based on the extensive over-harvesting of olive ridleys in Mexico, which caused a severe population decline. Since the ban on the harvest of turtles in Mexico, the primary threat to the Mexican nesting population has been reduced and the population appears to be stabilizing. Down listing to Threatened status may be feasible. The primary threats to the olive ridley while in U.S. waters (or by U.S.-based fishing fleets) appear to be incidental take in fisheries and boat collisions. Other primary threats are the harvest of turtles and eggs on Mexican and Central American nesting beaches.

Leatherback Turtle

The leatherback turtle is listed as Endangered throughout its range. In the Pacific, leatherback populations are in severe decline and recovery actions must be given the highest priority. Primary threats to the species are incidental take in coastal and high seas fisheries, and the killing of nesting females and collecting of eggs at the nesting beaches. The United States does not have any nesting of leatherbacks in its jurisdiction in the Pacific, but has important foraging areas on the continental U.S. west coast and near the Hawaiian Islands. It is likely that stocks in U.S. waters

originate in Mexico and Central America, though some may originate from Southeast Asia as well. While not directly classified as a threat, the lack of information on the movement patterns and habitat needs of this entirely pelagic species (leatherbacks are the only species which remains pelagic throughout its life) is severely hampering recovery efforts and must be addressed as a high priority.

Green Turtle

The green turtle is listed as Threatened under the Endangered Species Act (ESA) throughout its Pacific Range, except for the Endangered population nesting on the Pacific coast of Mexico which is covered under the Recovery Plan for the East Pacific green turtle. In reviewing this species' current status, the Recovery Team found that, outside of Hawaii, the green turtle populations have seriously declined and should probably be classified as Endangered. By far, the most serious threat to these stocks is from direct take of turtles and eggs, both within U.S. jurisdiction and on shared stocks that are killed when they migrate out of U.S. jurisdiction (e.g., nesting turtles from American Samoa migrate to Fiji and French Polynesia to feed). In Hawaii, green turtle populations appear to have a somewhat less dire status, probably due to effective protection at the primary nesting areas of the Northwest Hawaiian Islands and better enforcement of regulations prohibiting take of the species. However, the relatively recent increase in the incidence of tumorous disease, fibropapillomatosis, in the Hawaiian green turtle threatens to eliminate improvements in the status of the stock. Another serious threat to green turtle populations throughout the Pacific is associated with increasing human populations and development. In particular, human development is having an increasingly serious impact on green nesting beaches.

East Pacific Green Turtle (Black Turtle)

The East Pacific green turtle, known commonly as the black turtle, is listed as Endangered throughout its range. This regionally important population of the green turtle (*Chelonia mydas* although see Taxonomy), has exhibited an extreme decline over the last 30 years. This decline was undoubtedly caused by the massive over harvest of wintering turtles in the Sea of Cortez between 1950 and 1970, and the intense collections of eggs between 1960 and early 1980 on mainland beaches of Mexico. Primary threats to the species in U.S. waters are from entanglement in debris and boat collisions. Primary threats in Mexico are the (illegal) harvest of turtles and eggs.

I. Transportation and Holding:

Deeply hooked (defined as hook ingested past mouth cavity - in esophagus or deeper) hard shelled turtles taken in the experiments will be transported back to holding facilities for treatment and monitoring of hook progression. Only hard shelled sea turtles of less than 70 cm straight carapace length will be transported, and only if they are captured within an estimated 72 hours return time to Honolulu. Based on the raw observer data for sea turtles caught by shallow set swordfish and mixed target longline sets the percentage that is deeply hooked but alive, and caught within 72 hours of Honolulu (at a vessel speed of 8 knots) is about 7% for loggerhead turtles, 53% for olive ridley turtles, and 20% for green turtles. The data on all turtle takes that have been measured by observers indicates that about 82% of loggerheads, 100% of olive ridleys, and 87% of greens are under 70 cm straight carapace length (attachment 4). These percentages and the estimated number of turtles which may be taken in the experiments were used to estimate the number of

turtles which might be transported to Honolulu (Table 8). These estimate are likely to be higher than the number actually transported as the vessel may not always be prepared to depart immediately for Honolulu from the capture location. However, if more turtles of the appropriate size or condition for transport happen to be captured they is no reason why they also should not also be returned to port, and the numbers in table 8 are not intended to be a limit. The upper limit will be the total annual take shown in Table 7.

Table 8. Estimated annual number of deeply hooked but alive hard-shelled turtles less than 70 cm straight carapace length captured during the experiments within 72 hours of Honolulu that could be transported to Honolulu for treatment and monitoring of hook progression.

Species	Annual Estimated Turtles Transported		
	Year 1	Year 2	Year 3
Green	1	1	1
Loggerhead	5	5	4
Olive Ridley	5	4	3

1. Transportation of a Listed Species:

1.a. The NMFS Honolulu Laboratory has been transporting injured and diseased turtles for many years as part of the Sea Turtle Stranding Network. Transportation from vessels to the veterinary clinic on Oahu by motor vehicle will follow the same procedure used in the Stranding Program to transport injured or diseased turtles on Oahu. Transportation at sea will be provided by captains of fishing vessels participating in the research under the supervision of on-board fishery observers, fishery biologists, or biological technicians.

1.b. Turtles must be caught within an estimated 72 hours from the port of Honolulu and will be brought to port within 8 hours of this estimated time or else they will be released at sea. The Protocol for Sea Turtle Stranding Response (attachment 5) calls for turtle transportation 24 hours a day 7 days a week with a typical response time of a few hours for remote Oahu locations during weekends and after hours. With the advantage of real time reporting from participating vessels and with a veterinarian contracted specifically for this project it should take no more than an hour to transport the turtles from the vessel to the veterinarian. Depending on the turtles condition and any necessary surgery or other treatment turtles will be kept at the clinic for as long as necessary and then be moved again to NMFS holding facility at Kewalo Basin. Turtles which appear to be rehabilitated will be released to the wild (1.c, below) and some turtles may be euthanized when necessary in the opinion of the attending veterinarian (Section 3, below)

1.c. Turtles which eliminate hooks or have had them removed by surgery or other means developed by the veterinary work in this project, and which appear to be fully recovered may be transported back to a vessel and taken to sea for release within a 72 hour radius of Honolulu.

1.d. Same as 1.a.

1.e. Turtles will be transported at sea and in Honolulu using Petmate Vari Kennels, giant size (48" long x 32" wide x 35" high) which will be supplied to each vessel participating in the research. These kennels are wide enough for the largest turtles that will be transported (70 cm strait carapace length = 28")

1.f. Captured deeply-hooked turtles that will be returned to Honolulu will be handled

according to the guidelines developed in NMFS workshops (see Guidelines for Handling Marine Turtles Hooked or Entangled in the Hawaii Long line Fishery, Balazs et al., 1995). These include specified procedures for how to haul the main line, retrieve branch lines which catch turtles, assess turtle condition and hook location, removing lines, resuscitation, and care of turtles on board the vessel. The procedures are those mandated for use by all Hawaii-based longline captains and crew by Court Order and/or Biological Opinion, to dehook and disentangle, and resuscitate turtles, as appropriate and possible. In this study the field supervisor (fishery observer, fishery biologist, or biological technician) will oversee or conduct these procedures, and instead of returning a recovering deeply-hooked turtle to the sea after 24 hours , instead the turtle will be retained on board, covered in a moist cloth in the pet carrier in a shaded area for the 72 hour transit period to Honolulu.

Treatment by the veterinarian in Honolulu will include all measures possible at a fully equipped clinical facility including x-rays, surgery, anesthetics, antibiotics etc... as deemed best by the project veterinarian (to be named). The veterinarian contracted for the project will have a minimum of 5 years experience in the first hand assessment and treatment of injured and diseased sea turtles and will operate from a clinic provided with complete veterinary instrumentation required to treat injured and diseased sea turtles. When a turtle's condition has stabilized and/or all treatments which may improve its chances for survival have been carried out it will be moved to NMFS holding facility at Kewalo Basin. If the turtle is to be returned to the sea for release, the release procedure will again be as specified in the "Guidelines for Handling Marine Turtles Hooked or Entangled in the Hawaii Long line Fishery" (Balazs et al., 1995).

2. Holding of a Listed Species:

2.a and 2.b. Dimensions of pools used to hold sea turtles at NMFS Kewalo Facility and water supply:

2 fiberglass tanks, 2 m diameter, 1 m deep, 20 gal/h, 2 turtles/tank maximum.

2 fiberglass tanks, 2 m diameter, 1.5 m deep, 20 gal/h, 2 turtles/tank maximum

3 fiberglass tanks, 7 m diameter 2 m deep, 200-300 gal/h, 10 turtles/tank maximum

Turtles will be less than 70 cm straight carapace length, of both sexes, and unknown age. Within these broad categories, the size and species of individuals held at any given time will vary and cannot be predicted since incidental captures are involved. The maximum number of turtles per tank listed above is based on assuming a 70 cm carapace length, whereas a larger number of smaller turtles could be kept. Assuming 70 cm carapace length the capacity of the 7 tanks is 38 turtles. The total number and mix of species may be as estimated in Table 2, with a maximum of 11 turtles in the first year, but it would be extremely unlikely that such numbers of turtles would need to be held at one time. Delayed mortality of the deeply hooked turtles is assumed in the BO to be 42% so it would be unlikely that even the total annual numbers estimated to be transported (8 to 11 turtles, Table 8) would ever need to be maintained at one time.

2b. Water quality. Water supply is from a filtered sea water well, no temperature control is needed since ambient temperature is maintained at 25 plus/minus 1 C. Oxygen levels are irrelevant as turtles are air breathers.

2c. Frozen squid (*Loligo*), and or herring (*Clupea*) packed for human consumption, is maintained frozen until day of use, then thawed and fed to turtles once a day *add libitum* (Protocol for care and feeding of Kewalo turtles, attachment 6). Depending on appetite and size feeding turtles are fed 1-15 squid or fish weighing ca.100 g apiece per day.

2.d. Sanitation practices include regular tank cleaning and quarantine of diseased turtles (Protocol for care and feeding of Kewalo turtles, attachment 6). Green sea turtles affected by fibropapilloma tumors are held in separate tanks, fed and cared for after non-tumored turtles have been fed and cared for, and are cleaned with separate brushes. Tanks and brushes used with tumored turtles are disinfected prior to use with non-tumored turtles.

3. Emergency contingencies

Euthanasia will be used when necessary according to the procedure given under University of Hawaii - Institutional Animal Care permits (attachment 7).

NMFS Kewalo Facility has a second sea water well with its own pump as a backup to the primary seawater supply. The facility also has an automated alarm system that notifies key personnel in case of fire or power failure. Emergency power generation and saltwater pumping equipment is available.

K. Previous or Concurrent Activities Involving Listed Species:

MMPA/ESA Permit No. 848-1335 issued in 1997 to take Hawaiian monk seals . The mortality of an adult male monk seal died in September 1999 while being

restrained for epidemiological research sampling. Full information on this mortality is on file with the NMFS Office of Protected Resources.

Certification by Applicant:

"I hereby certify that the foregoing information is complete, true and correct to the best of my knowledge and belief. I understand that this information is submitted under the Endangered Species Act of 1973 (16 U.S.C. 153 1-1543) and regulations promulgated thereunder, and that any false statement may subject me to the criminal penalties provided under the Endangered Species Act of 1973, as amended."

Signature of Applicant:

_____ April 27, 2001

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Attachment 1

Testing for a reduction in the rate of turtle takes

M. McCracken, March 2001

Objective: Test if a treatment reduces turtle takes versus a control.

Experiment: The experiment will continue until a fixed number of turtles are caught. This kind of experiment is frequently referred to as a sequential experiment. Set is our experimental unit. Approximately equal number of sets will be randomly assigned to the control group and treatment group.

Assumptions: We are assuming that turtle takes come from two populations, treatment group and control group. Within each group, we assume that turtle takes at the set level are independent identically distributed Poisson variates. Basically we are testing if the treatment group has a different mean (average rate of take per set) than the control group.

Hypothesis. We are proposing a one-sided composite test where the null hypothesis is that the treatment reduces turtle take by 50% or more versus the alternative hypothesis that the treatment reduces turtle takes by less than 50%.

Helpful definitions:
Pr (Type I error)=alpha=Pr(reject null hypothesis given the null hypothesis is true)
Pr(Type II error)=beta=Pr(accept null hypothesis given the alternative hypothesis is true)
Power=1-beta=Pr(reject the null hypothesis given the alternative hypothesis is true)

Advantages:
(1) Under the Poisson assumption, the test is an exact test versus relying on asymptotic distributions and estimation of the standard errors of our two populations. A transformation to normalize takes at the set level is not an option in our case as takes are too rare.

(2) The test is the uniformly most powerful test. A test is uniformly most powerful of size alpha if it has size alpha and if among all tests of size less than or equal to alpha it has the largest power for all alternative values specified in the alternative hypothesis.

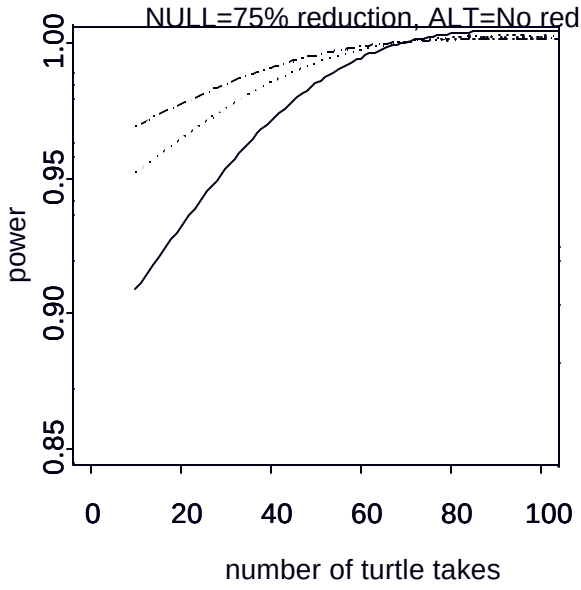
Possible problems:

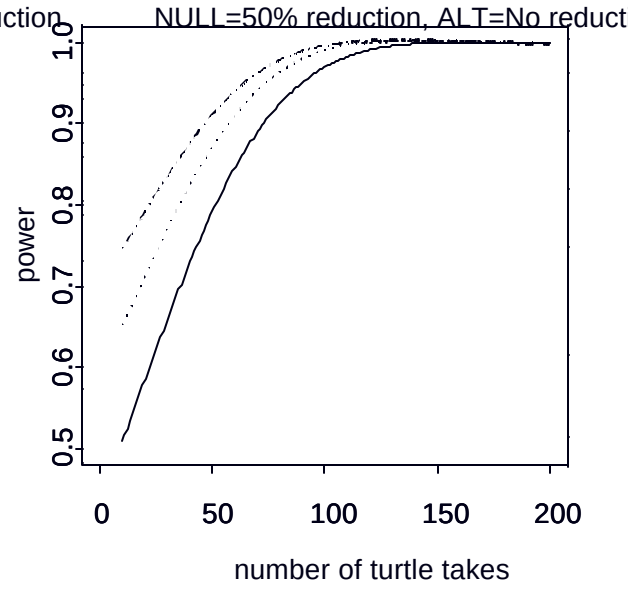
(1) Sets within the same trip may not be independent if the experiment is conducted over several trips. Hopefully with good planning, we can eliminate or reduce the variation between trips so that assuming sets are independent is reasonable. Before doing the test above it is recommended that one addresses the questions if sets are independent.

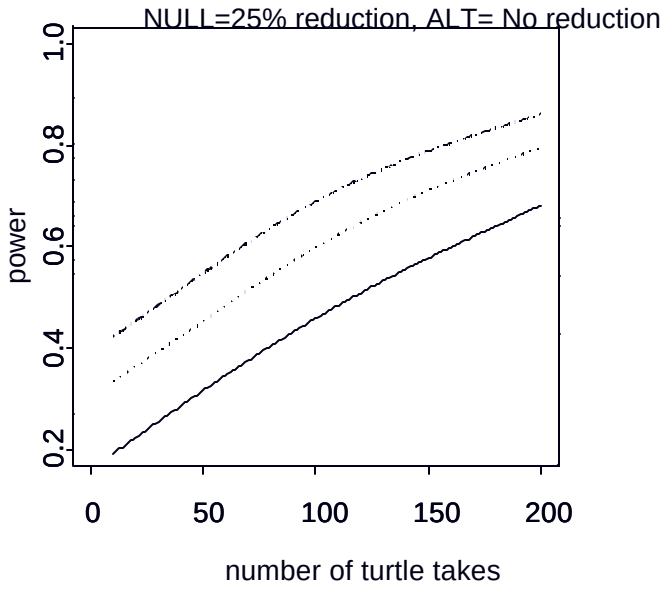
Attachment 1

(2) Loggerhead takes in the observer data show some overdispersion (more variation than expected under the Poisson assumption) at the set level. This could be due to sets within the same trip being dependent or the aggregation of turtles. We would expect the overdispersion to be less than observed in the observer data as we are controlling for some of the variation in the experiment. If takes are overdispersed, a larger turtle take would be required to obtain the same size test (alpha) and beta value. The same test statistics could still be used if there is evidence of overdispersion but the test would be an approximate test as we would need to approximate the size of the test. We are not losing ground here compared to using the asymptotic distribution of the standardization of takes because we would still need to estimate the variance of takes and obtain a large enough sample size that the asymptotic distribution is a good approximation.

Attachment 1







The plots above show the power curve for a fixed alpha. The plots assume a simple hypothesis test where we are testing if the treatment group reduces takes by 25%, 50%, or 75% versus the alternative hypothesis of no reduction. The solid line is when alpha=.05, the dotted line is alpha=.10, and the broken line is alpha=.15. The left end point of power curves are at 10 turtle takes.

Draft Draft

PROPOSAL TO CONDUCT EXPERIMENTS IN THE WESTERN ATLANTIC NORTHEAST DISTANT WATERS (GRAND BANKS) AREA TO EVALUATE SEA TURTLE MITIGATION MEASURES

Background

Possible management measures addressing the incidental take and mortalities of endangered and threatened sea turtle species by U.S. pelagic longline fisheries include research to design, develop, and evaluate gear and/or tactical measures capable of significantly reducing the interaction between sea turtles and longline fishing gear. In relation to international pelagic longline fisheries the U.S. fishery represents a relatively small impact on sea turtle populations and a major emphasis of the U.S. gear development research effort will be to transfer successful technology and encourage the use of practical measures to reduce sea turtle interactions by foreign fleets.

Pelagic longline fisheries that affect U.S. sea turtle populations occur in the Eastern and Western Pacific Ocean, Western Atlantic Ocean, Azores, Caribbean, and Gulf of Mexico. Fishery dependent research to develop and test sea turtle mitigation measures is being conducted in the Western Pacific (Hawaii fishery) by the NMFS Honolulu Laboratory (Boggs, 2000), the Azores (Bolten and Bjorndal, 2000), California (La Grange, 2000), and is planned for the Western Atlantic (Watson, 2001), and Mexico (Boggs per.comm.). Fishery independent research using captive reared turtles, research vessels, and/or contract commercial vessels is also being planned (Watson, 2001). These various research efforts are being coordinated and cooperative research planned to

provide collective expertise and collaboration in order to solve this complex problem. Although there are differences in environmental conditions, gear, and fishing tactics used among the different fisheries, there are common factors that can be evaluated and success in any area will lead to evaluation in other areas with some solutions effective across differences.

Several industry/academia/government workshops have been held to address possible gear and or fishing tactic modifications with potential to reduce sea turtle interactions with pelagic longline gear (Williams et al, 1994; Kleiber and Boggs, 1999; Anon., 2000; Anon., 2001). Pelagic longline observer data have been analyzed to examine gear, environmental, and operating practices associated with sea turtle longline interactions (Kleiber, 1998; McCracken, 2000; Cramer and Adams, 2000; Hoey, 1998, 1999, 2000). The information from these reports is the basis for current and planned research to develop and evaluate potential mitigation measures. A major component of this research is to conduct cooperative experiments with commercial pelagic longline vessels in the Pacific and Atlantic fisheries. This document outlines a research plan for the Grand Banks area in the Western Atlantic.

METHODOLOGY

Research will involve experiments using commercial vessels to evaluate the potential of candidate mitigation measures under actual fishing conditions and will be designed to estimate the reduction in turtle interactions and injury and impact on target species. This research will be conducted in coordination with concurrent research in other fisheries and with fishery independent studies in order to systematically investigate

effectiveness of candidate measures and utilize available resources in the most effective manner. To achieve this goal, potential mitigation measures from reports cited above have been evaluated as to their potential effectiveness based on available knowledge and placed into a matrix outlining the initial methods of evaluation (table 1). The matrix lists possible mitigation measures for initial evaluations with commercial vessels and measures that require fishery independent research to determine potential or to develop more precise methodology before testing in commercial fishing operations. The matrix is designed to best utilize resources to achieve effective results. As research results become available mitigation measures will be moved within the matrix as the results indicate. For example, if fishery independent research demonstrates a potentially highly effective measure it will be moved into fishery dependant evaluation and if measures appear ineffective in fishery dependent studies they may be moved to fishery independent studies or dropped from the research matrix. New measures will be added to the matrix as they are developed. A pelagic longline gear-working group consisting of gear researchers, fishery managers and fishers will be established to make decisions regarding the priority and method of evaluations.

The estimated potential effectiveness of the mitigation measures proposed for research based on current knowledge is given in table 2. Methods proposed for testing in the Western Atlantic Grand Banks fishery are those for which potential effectiveness has been indicated by observer data and which require evaluation in terms of turtle take reduction and affect on target species catch rates in commercial fishing operations. Other methods are proposed to be initially investigated using captive reared turtles in field

experiments and wild turtles using research and contract vessels. Some methods are also proposed for testing in other commercial fisheries. Possible priorities for the Grand Banks fishery are testing the effectiveness of a variety of methods including: 1) use of dip nets, line cutters, and de-hookers to remove longline gear from turtles, 2) moving away from sites where turtle interactions occur, 3) altering the arrangement of branch lines, 4) use of blue dyed bait, 5) collecting data on hooking times and depths for turtles and swordfish, 6) a sea surface temperature (SST) limit,

The experimental design will be to develop a null hypothesis for each experiment and test the treatment against a control by alternating sets or alternating treatment and control within a set depending on the nature of the method being tested. The limiting factors that will determine how many treatments can be tested in a given year is the total effort (number of vessels, number of trips, number of sets per trip, number of hooks per set) and the number of hooks required to provide statistically significant estimates of turtle take reduction rates.

In order to provide an estimate of the effort required and the estimated turtle takes per treatment, observer data collected by the NEFSC and SEFSC for the northeast distant area (NED) from 1991- 1999 were analyzed to estimate the sea turtle CPUE. The total number of turtles taken by longline gear on observed sets between 1991 and 1999 was 376; the number of hooks fished on observed sets was 303,089. The number of loggerhead turtles taken on observed sets was 249, and the number of leatherbacks was 117. CPUE values were 0.00124 for total turtles, 0.00082 for loggerhead turtles and

0.00038 for leatherbacks. For the most recent year available (1999), the total number of turtles taken on observed sets was 76, loggerhead turtles 44, and leatherback turtles 32 for 32237 hooks fished. CPUE values were 0.00235 for total turtles, 0.00136 for loggerheads, and 0.00099 for leatherbacks. The sample size computations, controlling for Type I and Type II errors at pre-specified levels, to detect a reduction of 25% and 50% in CPUE due to treatment effectiveness were performed using the arcsine square root transformation. These computations were done using the 1991 to 1999 series data and 1999 data only. The burden of proof is placed on the treatment being tested and hence the null hypothesis states that the control capture proportion is less than or equal to the corresponding treatment proportion. Estimated sample sizes for various levels of precision for type I and type II error (alpha and beta) are presented in table 3 assuming the treatment is 25% effective and in table 4 assuming the treatment is 50% effective. Using the 1991-1999 time series data, assuming the treatment is 25% effective, and choosing an alpha level of 0.10 and beta of 0.30, sample sizes of 73,241 hooks for all turtles, 110,485 hooks for loggerheads and 235,290 hooks for leatherbacks would be required (table 3). Assuming the treatment is 50% effective 15,311 hooks for all turtles, 23,132 hooks for loggerheads, and 49,232 hooks for leatherbacks would be required (table 4). These projected sample sizes assume that the CPUE in 2001 will be similar to the average CPUE from the observed rates from 1991 to 1999.

Another approach would be to assume that the turtle CPUEs for 2001 would be similar to the most recent year for which data is available (1999). The turtle CPUEs for 1999 are roughly double the CPUEs for the 1991 to 1999 time period. If we use the CPUEs for 1999 to estimate sample sizes the required, choose an alpha level of 0.10 and

beta level of 0.30, and assume the treatment is 25% effective sample sizes would be 37,776 hooks for all turtles, 64,815 hooks for loggerhead turtles, and 90,773 for leatherback turtles (table 3). Assuming the treatment is 50% effective 7,906 hooks for all turtles, 13,564 hooks for loggerheads, and 18,995 hooks for leatherbacks would be required (table 4).

The number of turtles impacted by the experiments can be calculated by multiplying the sample size required (number of hooks) times the turtle CPUE from the observer data. If, for example, we choose to test removing hooks adjacent to floats as the treatment to be tested, and assume the reduction rates for all turtles to be 50%, using the CPUE for the 1991 to 1999 time series the estimated sample size will be 15,311 hooks for the control and treatment for a total of 30,622 hooks. With a turtle CPUE of 0.0012 the maximum expected number of turtle takes would be 37. If the treatment were 50% effective the number of turtles impacted would be 27.

For all experiments the affect on the directed take (swordfish) CPUE will be calculated to determine the impact on the fishery of the turtle mitigation measure being evaluated.

Fishing effort for 2001 was estimated based on logbook data and interviews with the longline industry. During 2001, it is anticipated that between 8 and 10 vessels will fish in the Grand Banks, making an average of 4 trips per vessel. It is estimated that each vessel will make between 14 and 20 sets per trip with an average of 806 hooks per set.

Each vessel would be expected to set between 11,284 and 16,120 hooks per trip and between 45,136 and 64,480 hooks per season. If 10 vessels fish the maximum number of hooks the total effort would be 640,480 hooks. The number of treatments that could be tested on the Grand Banks in 2001 will depend on the effectiveness of the treatment and whether the treatment will be for total turtles, or loggerheads and leatherbacks, with leatherbacks being the limiting factor.

PROPOSED TREATMENTS FOR GRAND BANKS EXPERIMENTS

Note: The proposed treatments in this document are based on analysis of longline observer data and reports of analyses of that data (Hoey, 1998,200; Hoey and Moore, 1999; Kleiber and Boggs, 2000). This is a working document and has not had direct input from the longline industry or fishery managers. This document will be revised and refined at a gear working group meeting scheduled for April 19th in Miami FL.

Effectiveness of dip nets, line cutters, and de-hookers

The harvestings systems and engineering branch and fishers are developing line cutters and de-hooks that will enable fishers to remove longline gear from turtles hooked or entangled by longline gear. In 2000 fishers were allowed to boat turtles using dip nets provided by the SEFSC observer program and remove longline gear from turtles. New gear being developed will allow removal of gear in water for turtles to large to bring aboard. Turtle handling procedures will be developed using these tools and the effectiveness of the procedures and tools evaluated by observers and vessels crews. Observers will record detailed information about the turtle interaction including whether the turtle was hooked and or entangled, where the turtle was hooked and/or entangled, procedure used to remove gear and condition of turtle upon release. Some turtles may be

fitted with pop-up satellite transmitting archival tags (PSTATs) to quantify survivorship of marine turtles after release from encounters with longline gear and the effectiveness of handling and gear removal techniques.

Hook Proximity to Floats

An important effect of hook configuration for swordfish longline gear is that both loggerhead and leatherback turtles are caught with a significantly greater frequency on hooks adjacent to the floats in the Hawaii-based fishery (Kleiber and Boggs 2000). Hawaii and Atlantic (Beideman, pers. comm.) longline fishers fishing for swordfish typically using 3-5 hooks per float place one branch line (hook) on the main line as close as possible after attaching the float line, which makes that the shallowest hook position as well as the position closest to the float. A preliminary analysis of data on swordfish caught in the Hawaii-based fishery by Pierre Kleiber (pers. comm.) indicates that the distribution of hooks that caught swordfish was not much different from the distribution of all hooks available to the swordfish. Hooks adjacent to floats did not appear to have a higher swordfish catch rate compared to other hook positions. Swordfish fishers add the hook adjacent to the float because they believe that the action imparted to the hook by wave motion makes the bait presentation more attractive to swordfish. Historical observer data from the Atlantic does not include information on the position of hooks that caught turtles.

The experimental design for hook configuration will test the removal of hooks adjacent to floats (treatment) versus leaving such hooks in place (control). Participating

vessels will place hooks at least 20 - 30 fathoms (?) from float lines on treatment sets and immediately adjacent to float lines on control sets. Control and treatment sets will be alternated during a given trip. Gear configuration and fishing methods will be the same between control and treatment sets except for placement of hook nearest floats. Participating vessels will carry an NMFS approved observer on board to supervise operations and record results. Observers will collect a suite of data on forms generated by the SEFSC Pelagic Longline Observer Program including the Longline Gear Configuration Log, the Longline Haul Log, and the Individual Animal Log (Appendix I). An additional data log will be generated to collect data specific for each experiment not collected on the above referenced data logs. Hook positions will be numbered between floats in sequence and turtle and target catch will be recorded by hook position relative to float position. Depending on the effectiveness of moving hooks at least 20 – 30 (?) fathoms from float lines a minimum of 4 trips and a maximum of 20 trips will be required to collect sufficient data to determine reduction rates for loggerhead turtle interactions. For leatherback turtles a minimum of 9 trips and a maximum of 42 trips will be required. This experiment will require the cooperation of a minimum of two vessels for one season and a maximum of 10 vessels for one season depending on the effectiveness of the treatment. The expected number of turtles to be impacted during this experiment based on the 1991-1999 observer data will be 29-168 loggerhead and 29-168 leatherback turtles depending on the effectiveness of the treatment. Participating captains, crews and observers will follow NOAA guidelines and permit requirements for handing marine turtles hooked or entangled on longline gear. Turtles hooked or entangled will be brought aboard using dip nets if size permits and all gear removed following recommended

procedures. For turtles than cannot be brought aboard gear will be removed using line cutter and de-hooker prototypes being developed as part of the turtle mitigation research project. Prototype line cutters and de-hookers will be evaluated by crews and observers and information on performance provided to NMFS. All live turtles brought aboard will be tagged with standard flipper tags and released. Turtles that are stressed will be maintained onboard and given the opportunity to revive before release. Some turtles may receive satellite tags to determine life history information and fate.

The estimates of catch rates per hook of control and treatment group will be computed from the sample data. Using these estimates, a one-tailed hypothesis test will be conducted to test if the true catch rate for the treatment group is lower than that of the control group. Since the sample proportions are estimated from a large number of hooks, a test based on asymptotic normality to compare the two binomial proportions will be used here at a pre-specified level of significance. A confidence interval on the difference in the true proportions will also be computed. The Fisher's exact test and the likelihood ratio test will be performed as well and examined.

Move Away From Interactions

Observed takes of sea turtles (all species combined) in the Atlantic Fishery show clear evidence of aggregation, that is, the incidence of taking several turtles on one set (3%) was six times higher than expected due to chance based on rates given by Hoey (NMFS, Contract Report). About 7% of sets interacted with a single turtle so the expectation that two wouldn't be caught together due to chance is only about 0.5% (0.07 x 0.07 = 0.0049). Turtle aggregation appears to be even more pronounced in the Grand

Banks portion of the fishery, where 5% of the sets observed accounted for over 50% of the turtles taken. This result and the experience of many fishermen suggest that avoidance of the areas of sea turtle aggregation could reduce turtle takes. Moving fishing operations away from an area where turtle interactions occur could be a highly effective measure for reducing takes.

This approach was by commercial fishermen in various working groups (Los Angeles, CA in August 2000, Washington, DC in January 2001). The difficulty is in specifying how to move. An early suggestion by fishermen was to move 1 nautical mile, but since the longline gear may extend for 60 nm and can drift an equivalent distance in the course of one operation, such small move seems trivial. It would seem prudent to move as far as possible from the site of the interaction, and in regard to the temperature relationships discussed above, towards the direction of cooler water. However, when swordfish catch rates are high fishermen will want to remain in the general vicinity. Furthermore, fishing operations can often occupy as much as 20 hours per day, leaving little time for transit before making the next set. A top speed of 8 knots is common among many small longline vessels and so a reasonable compromise that has been accepted in concept by some commercial fishermen is to move 30 nm after an interaction with a sea turtle.

The direction of a move away from the site of a turtle interaction could be towards colder water if the temperature structure of the area were known, and especially if real time information on turtle aggregations were collected and disseminated to the

fleet. It has been suggested that the move be made in a direction at right angles to the axis of the set in which the sea turtle take occurred, to prevent any portion of the set overlapping the site of the previous set. This simple operational definition could serve as the basis of the treatment to be tested, with fishermen using any knowledge they might have about temperature structure or turtle distributions to improve the efficacy of the measure, as it would be in their best interests to do so.

Important information on the best direction to move could be related to whether or not they generally attempt to set across or along temperature gradients. If they set across temperature gradients then moving 30 nm away from the axis of the set could place the set in a very similar orientation across the same temperature front. Of course that assumes the front tends to be a linear feature at the scale of longline operations. However, as often as not, such features are part of meso-scale warm-core rings that spin off the Gulf Stream and a perpendicular movement of 30 nm could have unpredictable results. Temperature and primary productivity features of the water masses, which have some bearing on turtle aggregation, are monitored by some fishing vessels and could be monitored by the scientists managing the experimental fishery to provide guidance on the direction of vessel movement after a set. John Hoey (NMFS) has submitted a proposal for funding to integrate satellite derived oceanographic data with fishery dependent longline observations and this activity should be integrated into this experiment. However making the experiment dependent on such a real time information system could introduce serious obstacles if the information system could not be operated reliably. It might better be left to the fishermen to decide where to move within the general constraint that the move be at least 30 nm, again considering that it is in their best interests that the move should be

effective in reducing turtle takes while minimizing impacts to target species catch rates. Satellite data will be used to predict areas of turtle concentration and to evaluate effectiveness of moving away from turtle interactions relative to oceanographic features.

Participating vessels will carry an NMFS approved observer on board to supervise operations and record results. Observers will collect a suite of data on forms generated by the SEFSC Pelagic Longline Observer Program including the Longline Gear Configuration Log, the Longline Haul Log, and the Individual Animal Log (Appendix I). An additional data log will be generated to collect data specific for each experiment not collected on the above referenced data logs. For this experiment fishers will fish in their normal mode of operation until a turtle is encountered. When a turtle is caught on the longline gear the vessel will set in the same location as the previous set (control). When the next turtle is caught on the longline gear the vessel will move a minimum of 30 nautical miles at right angle to the direction of the previous set from the area of the previous set. The captain and observer will determine the best direction of movement based on available information at the time. The basis for the direction of movement will be recorded for later analysis and movement position correlated with oceanographic conditions and subsequent turtle captures. . Depending on the effectiveness of moving away from turtle interactions a minimum of 4 trips and a maximum of 20 trips will be required to collect sufficient data to determine reduction rates for loggerhead turtle interactions. For leatherback turtles a minimum of 9 trips and a maximum of 42 trips will be required. This experiment will require the cooperation of a minimum of two vessels for one season and a maximum of 10 vessels for one season depending on the effectiveness of the treatment. The expected number of turtles to be impacted during this

experiment based on the 1991-1999 observer data will be 29-168 loggerhead and 29-168 leatherback turtles. Participating captains, crews and observers will follow NOAA guidelines and permit requirements for handing marine turtles hooked or entangled on longline gear. Turtles hooked or entangled will be brought aboard using dip nets if size permits and all gear removed following recommended procedures. For turtles than cannot be brought aboard gear will be removed using line cutter and de-hooker prototypes being developed as part of the turtle mitigation research project. Prototype line cutters and de-hookers will be evaluated by crews and observers and information on performance provided to NMFS. All live turtles brought aboard will be tagged with standard flipper tags and released. Turtles that are stressed will be maintained onboard and given the opportunity to revive before release. Some turtles may receive satellite tags to determine life history information and fate.

The estimates of catch rates per hook of control and treatment group will be computed from the sample data. Using these estimates, a one-tailed hypothesis test will be conducted to test if the true catch rate for the treatment group is lower than that of the control group. Since the sample proportions are estimated from a large number of hooks, a test based on asymptotic normality to compare the two binomial proportions will be used here at a pre-specified level of significance. A confidence interval on the difference in the true proportions will also be computed. The Fisher's exact test and the likelihood ratio test will be performed as well and examined.

Use of Blue Dyed Squid

Research in Hawaii has shown that blue dyed squids reduce the bycatch of seabirds and possibly increase the catch of swordfish. When field-testing blue bait on

seabirds no turtles were caught while turtles were caught with normal bait during the study (anon, 2000). Laboratory tests have shown that green turtles are reluctant to take blue dyed squid compared to normal squid, but eventually habituate to dyed bait (Brill, pers. comm.). The NMFS Honolulu laboratory is conducting test on commercial longline vessels using blue dyed squid and it may be prudent to duplicate these test in the Grand Banks.

Temperature Limit

Evaluation of observer data from the Grand Banks fishery suggests that turtle takes occur more often in waters with a sea surface temperature (SST) >65° F (Hoey, NMFS Contract Report). One treatment for testing should include making equal numbers of sets in waters with SST below 65° F (< 65°, treatment) and in waters with SST at 65° F or above (65°+, control). Longline fishers contend that the temperature effect is complex and a simple cutoff in temperature would not necessarily be effective. They propose that they use available information on site to determine setting temperature to avoid turtle interactions.

The experimental design for SST effect could test setting longline gear in waters below 65°F (treatment) and in waters of 65°F or higher (control). An alternate design for this experiment could test moving to colder water (temperature determined by captain using real time oceanographic features) when a turtle is encountered. The control would be to set in the same location when a turtle is encountered and treatment would be to move to colder water when a turtle is encountered. Control and treatment sets will be

alternated during a given trip. Gear configuration and fishing methods will be the same between control and treatment sets. Participating vessels will carry an NMFS approved observer on board to supervise operations and record results. Observers will collect a suite of data on forms generated by the SEFSC Pelagic Longline Observer Program including the Longline Gear Configuration Log, the Longline Haul Log, and the Individual Animal Log (Appendix I). An additional data log will be generated to collect data specific for each experiment not collected on the above referenced data logs. Depending on the effectiveness of moving to colder water a minimum of 4 trips and a maximum of 20 trips will be required to collect sufficient data to determine reduction rates for loggerhead turtle interactions. For leatherback turtles a minimum of 9 trips and a maximum of 42 trips will be required. This experiment will require the cooperation of a minimum of two vessels for one season and a maximum of 10 vessels for one season depending on the effectiveness of the treatment. The expected number of turtles to be impacted during this experiment based on the 1991-1999 observer data will be 29-168 loggerhead and 29-168 leatherback turtles. Participating captains, crews and observers will follow NOAA guidelines for handing marine turtles hooked or entangled on longline gear. Turtles hooked or entangled will be brought aboard using dip nets if size permits and all gear removed following recommended procedures. For turtles than cannot be brought aboard gear will be removed using line cutter and de-hooker prototypes being developed as part of the turtle mitigation research project. Prototype line cutters and de-hookers will be evaluated by crews and observers and information on performance provided to NMFS. All live turtles brought aboard will be tagged with standard flipper tags and released. Turtles that are stressed will be maintained onboard and given the

opportunity to revive before release. Some turtles may receive satellite tags to determine life history information and fate.

The estimates of catch rates per hook of control and treatment group will be computed from the sample data. Using these estimates, a one-tailed hypothesis test will be conducted to test if the true catch rate for the treatment group is lower than that of the control group. Since the sample proportions are estimated from a large number of hooks, a test based on asymptotic normality to compare the two binomial proportions will be used here at a pre-specified level of significance. A confidence interval on the difference in the true proportions will also be computed. The Fisher's exact test and the likelihood ratio test will be performed as well and examined.

Setting Time

Loggerhead and Leatherback interactions with longline gear are highest on sets deployed between 4:00 PM and 6:00 PM for the Grand Banks fishery (Hoey, 2000). Analysis of observer data by Hoey, 1998 indicated that there might be a slightly higher sea turtle interaction rate associated with early evening sets as opposed to late evening sets. Delaying start of gear setting until after 9 PM may reduce turtle encounters. More data is required to substantiate the merit of this possible mitigation measure. It is proposed that electronic microchip hook timers be attached to branch lines to record times when turtle interactions occur and times when target species are hooked. This technique has been successfully used in the Pacific to resolve the uncertainty in estimating capture depths and times of fish on pelagic gear (Boggs, 1992). Selected commercial vessels will be outfitted with hook timers to collect this data. This data

collection experiment may be conducted in combination with other treatments and will not result in additional turtle takes. Data collected from this experiment will be analyzed to determine the times of day in which turtles interact with longline gear and the times of day when target species are caught. This data will be used to determine if altering set and retrieval time of longline gear can reduce turtle interactions.

Sea Turtle and Swordfish behavior

Gear researchers and fishers have proposed a suite of possible mitigation techniques for which there is very little or no data to indicate possible effectiveness. These techniques include type of bait (squid, fish), use of stealth gear, hook design, hook guards, turtle deterrents and or attractors, stiffer monofilament, and modified light stick (Table 1). Some of these techniques will be evaluated in other fishery dependant research (Azores) and through fishery independent research in the Pacific and Atlantic. The successful development of conservation gear has historically been dependant on a through knowledge of animal behavior in relation to fishing gear. Knowledge of turtle and target species behavior in relation to longline gear can greatly expedite the development of effective mitigation techniques. This project proposes to develop specialized camera systems that will enable video recording of turtle behavior in an around longline gear including floats, drop lines, mainline and gangions. The same equipment will record target species behavior when encountering longline gear. The camera systems will be used to record behavior and interaction with specific mitigation measures listed above and will expedite decisions on which techniques to evaluate on commercial vessels. The camera systems will be developed and tested on research vessels

and provided to commercial fishers to record and document turtle and swordfish behavior interactions with fishing gear. Cameras will also be used on a chartered commercial vessel that will direct efforts in areas of high turtle concentrations. These cameras will also be used in other areas including the Azores and Pacific in cooperation with other researchers.

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Williams. P., P.J. Anninos, P.T. Plotkin, and K.L. Salvini (Compilers). 1996. Pelagic longline fishery-sea turtle interactions: Proceedings of and industry, academic and government experts, and stakeholders workshop held in Silver Spring, Maryland, 24-25 May 1994. NOAA Technical Memorandum. NMFS-OPR-7. 77p.

Table 1. 2001 longline sea turtle mitigation research matrix

	MITIGATION														
	SST limit	Move from turtle interactions	Setting time	Line Cutters and de-hookers	Hook timers	Move hooks from floats	Bait studies	Fish hooks deeper	Stealth gear	Hook design	Turtle swordfish behavioral interactions	Deterrents Attractors	Hook stoppers & guards	Stiff mono	Mod. light sticks
FISHERY DEPENDENT															
Grand Banks	X	X	X	X	X	X	X			X					
Hawaii		X		X		X	X								
Mexico					X			X	X	X	X				
Azores								X	X	X	X				
FISHERY INDEPENDENT															
Captive Turtles							X		X		X	X	X	X	X
Research Vessels							X		X		X	X	X	X	X
Contract Vessels					X		X		X	X	X	X	X	X	X

Table 2. Estimated potential for mitigation measures proposed.

MITIGATION TECHNIQUE	CURRENT DATA AVAILABLE	ESTIMATED POTENTIAL EFFECTIVENESS
Use of line cutters and de-hooking devices		High potential, need data to quantify reduction in mortality
Upper limit on sea surface Temp (SST)	Hoey, NMFS contract report	Significant reduction for loggerheads and leatherbacks depending on upper limit. *
Moving away from turtle interaction area	Hoey, NMFS contract report	Unknown but could be significant for loggerheads *
Delay setting until after 9 p.m.	Hoey, NMFS contract report	Possible reduction for loggerheads and leatherbacks*
Set 4 or more hooks between floats	Hoey, NMFS contract report	Unknown, review summaries and update
Use of hook timers		Data from hook timers can determine time of turtle hooking and target catch and provide data for more efficient mitigation measures
Move hooks away from floats	Kleiber and Boggs, 2000	Could be significant reduction *
Type of bait		Unknown
Fish hooks deeper		Unknown
Use of stealth gear		Unknown
Hook design (use circle hooks)	Bolton, LaGrange	Significant reduction in hooks ingested by turtles, 48% reduction in swordfish CPUE.
Turtle/swordfish behavioral information		Provide information necessary to design new mitigation measures
Deterrents/Attractors		High potential need basic research to provide direction
Hook guards		High potential need R&D effort
Stiffer monofilament		High potential for leatherback mitigation need R&D
Modify light sticks		Needs controlled experiments to determine potential

* Based on observer data

Table 3. Estimated sample sizes (number of hooks) required for longline experiments in the Grand Banks fishery assuming mitigation technique reduces turtle takes by 25%.

	Based on 1991-1999 Observer Data				Based on 1999 Observer Data Only			
Precision	Alpha 0.10 Beta 0.15	Alpha 0.10 Beta 0.20	Alpha 0.10 Beta 0.25	Alpha 0.10 Beta 0.30	Alpha 0.10 Beta 0.15	Alpha 0.10 Beta 0.20	Alpha 0.10 Beta 0.25	Alpha 0.10 Beta 0.30
All Turtles	120,661	101,231	85,921	73,241	62,233	52,212	44,315	37,776
Loggerhead Turtles	182,017	152,708	129,612	110,485	106,779	89,585	76,036	64,815
Leatherback Turtles	387,625	325,208	276,024	235,290	149,543	125,463	106,488	90,773

Table 4. Estimated sample sizes (number of hooks) required for longline experiments in the Grand Banks fishery assuming mitigation technique reduces turtle takes by 50%.

	Based on 1991-1999 Observer Data				Based on 1999 Observer Data Only			
Precision	Alpha 0.10 Beta 0.15	Alpha 0.10 Beta 0.20	Alpha 0.10 Beta 0.25	Alpha 0.10 Beta 0.30	Alpha 0.10 Beta 0.15	Alpha 0.10 Beta 0.20	Alpha 0.10 Beta 0.25	Alpha 0.10 Beta 0.30
All Turtles	25,224	21,162	17,961	15,311	13,025	10,928	9,275	7,906
Loggerhead Turtles	38,109	31,972	27,137	23,132	22,345	18,747	15,912	13,564
Leatherback Turtles	81,107	68,047	57,755	49,232	31,293	26,254	22,283	18,995

Attachment 3

Experimental Turtle Bycatch Reduction Trip Plan and Report (002)

Vessel Name:_____ Date Trip Began_____

1st Set: = BYCATCH REDUCTION SET Date set started:_____
Branch lines more than 40 fathoms from float lines (check)_____
Squid bait dyed blue (check)_____

2nd Set = NORMAL SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set in the same place again (check)_____
Branch lines put on close to float lines as usual (check)_____
Squid bait not dyed blue (check) _____

3rd Set = BYCATCH REDUCTION SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set at least 30 miles away (check)____
Branch lines more than 40 fathoms from float lines (check)_____
Squid bait dyed blue (check)_____

4th Set = BYCATCH REDUCTION SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set at least 30 miles away (check)____
Branch lines more than 40 fathoms from float lines (check)_____
Squid bait dyed blue (check)_____

5th Set = NORMAL SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set in the same place again (check)_____
Branch lines put on close to float lines as usual (check)_____
Squid bait not dyed blue (check) _____

6th Set = NORMAL SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set in the same place again (check)_____
Branch lines put on close to float lines as usual (check)_____
Squid bait not dyed blue (check) _____

7th Set = BYCATCH REDUCTION SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set at least 30 miles away (check)____

Attachment 3

Branch lines more than 40 fathoms from float lines (check)_____
Squid bait dyed blue (check)_____

Experimental Turtle Bycatch Reduction Trip Plan and Report (continued) (002)

8th Set = NORMAL SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set in the same place again (check)_____
Branch lines put on close to float lines as usual (check)_____
Squid bait not dyed blue (check) _____

9th Set = NORMAL SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set in the same place again (check)_____
Branch lines put on close to float lines as usual (check)_____
Squid bait not dyed blue (check) _____

10th Set = NORMAL SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set in the same place again (check)_____
Branch lines put on close to float lines as usual (check)_____
Squid bait not dyed blue (check) _____

11th Set = BYCATCH REDUCTION SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set at least 30 miles away (check)____
Branch lines more than 40 fathoms from float lines (check)_____
Squid bait dyed blue (check)_____

12th Set = NORMAL SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set in the same place again (check)_____
Branch lines put on close to float lines as usual (check)_____
Squid bait not dyed blue (check) _____

13th Set = BYCATCH REDUCTION SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set at least 30 miles away (check)____
Branch lines more than 40 fathoms from float lines (check)_____
Squid bait dyed blue (check)_____

Attachment 3

14th Set = BYCATCH REDUCTION SET Date set started:_____

Was a turtle caught on the previous set (yes or no)_____

If yes, then set at least 30 miles away (check)____

Branch lines more than 40 fathoms from float lines (check)_____

Squid bait dyed blue (check)_____

Experimental Turtle Bycatch Reduction Trip Plan and Report (continued) (002)

15th Set = NORMAL SET Date set started:_____

Was a turtle caught on the previous set (yes or no)_____

If yes, then set in the same place again (check)_____

Branch lines put on close to float lines as usual (check)_____

Squid bait not dyed blue (check) _____

16th Set = NORMAL SET Date set started:_____

Was a turtle caught on the previous set (yes or no)_____

If yes, then set in the same place again (check)_____

Branch lines put on close to float lines as usual (check)_____

Squid bait not dyed blue (check) _____

17th Set = BYCATCH REDUCTION SET Date set started:_____

Was a turtle caught on the previous set (yes or no)_____

If yes, then set at least 30 miles away (check)____

Branch lines more than 40 fathoms from float lines (check)_____

Squid bait dyed blue (check)_____

18th Set = NORMAL SET Date set started:_____

Was a turtle caught on the previous set (yes or no)_____

If yes, then set in the same place again (check)_____

Branch lines put on close to float lines as usual (check)_____

Squid bait not dyed blue (check) _____

19th Set = NORMAL SET Date set started:_____

Was a turtle caught on the previous set (yes or no)_____

If yes, then set in the same place again (check)_____

Branch lines put on close to float lines as usual (check)_____

Squid bait not dyed blue (check) _____

20th Set = BYCATCH REDUCTION SET Date set started:_____

Was a turtle caught on the previous set (yes or no)_____

If yes, then set at least 30 miles away (check)____

Attachment 3

Branch lines more than 40 fathoms from float lines (check)_____
Squid bait dyed blue (check)_____

21st Set = BYCATCH REDUCTION SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set at least 30 miles away (check)____
Branch lines more than 40 fathoms from float lines (check)_____
Squid bait dyed blue (check)_____

Experimental Turtle Bycatch Reduction Trip Plan and Report (continued) (002)

22nd Set = BYCATCH REDUCTION SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set at least 30 miles away (check)____
Branch lines more than 40 fathoms from float lines (check)_____
Squid bait dyed blue (check)_____

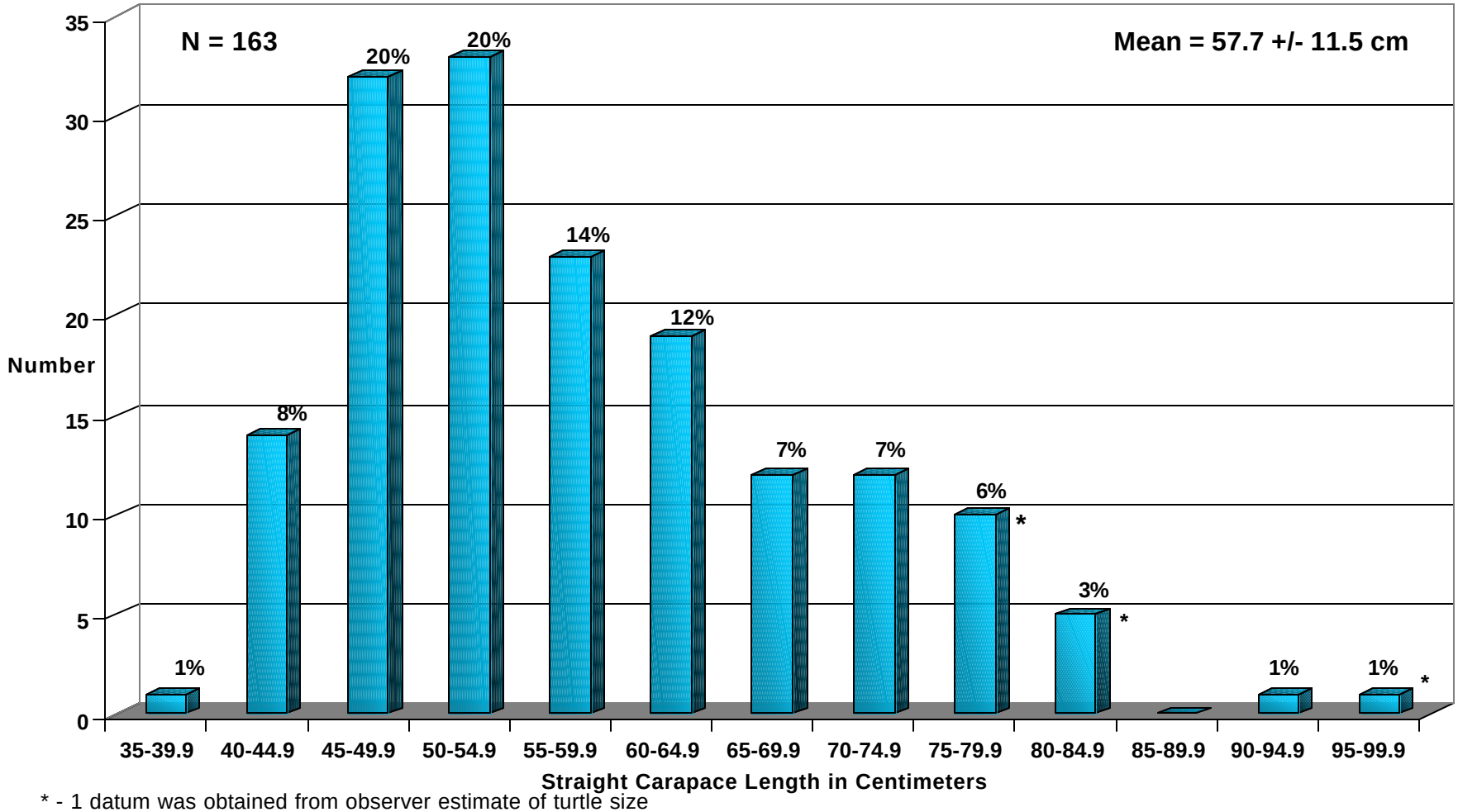
23rd Set = BYCATCH REDUCTION SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set at least 30 miles away (check)____
Branch lines more than 40 fathoms from float lines (check)_____
Squid bait dyed blue (check)_____

24th Set = NORMAL SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set in the same place again (check)_____
Branch lines put on close to float lines as usual (check)_____
Squid bait not dyed blue (check) _____

25th Set = NORMAL SET Date set started:_____
Was a turtle caught on the previous set (yes or no)_____
If yes, then set in the same place again (check)_____
Branch lines put on close to float lines as usual (check)_____
Squid bait not dyed blue (check) _____

Attachment 4

Size-Class Distribution of Loggerheads
Taken as Bycatch in the Hawaii-based Pelagic Longline Fishery
Based on NMFS Observer Data

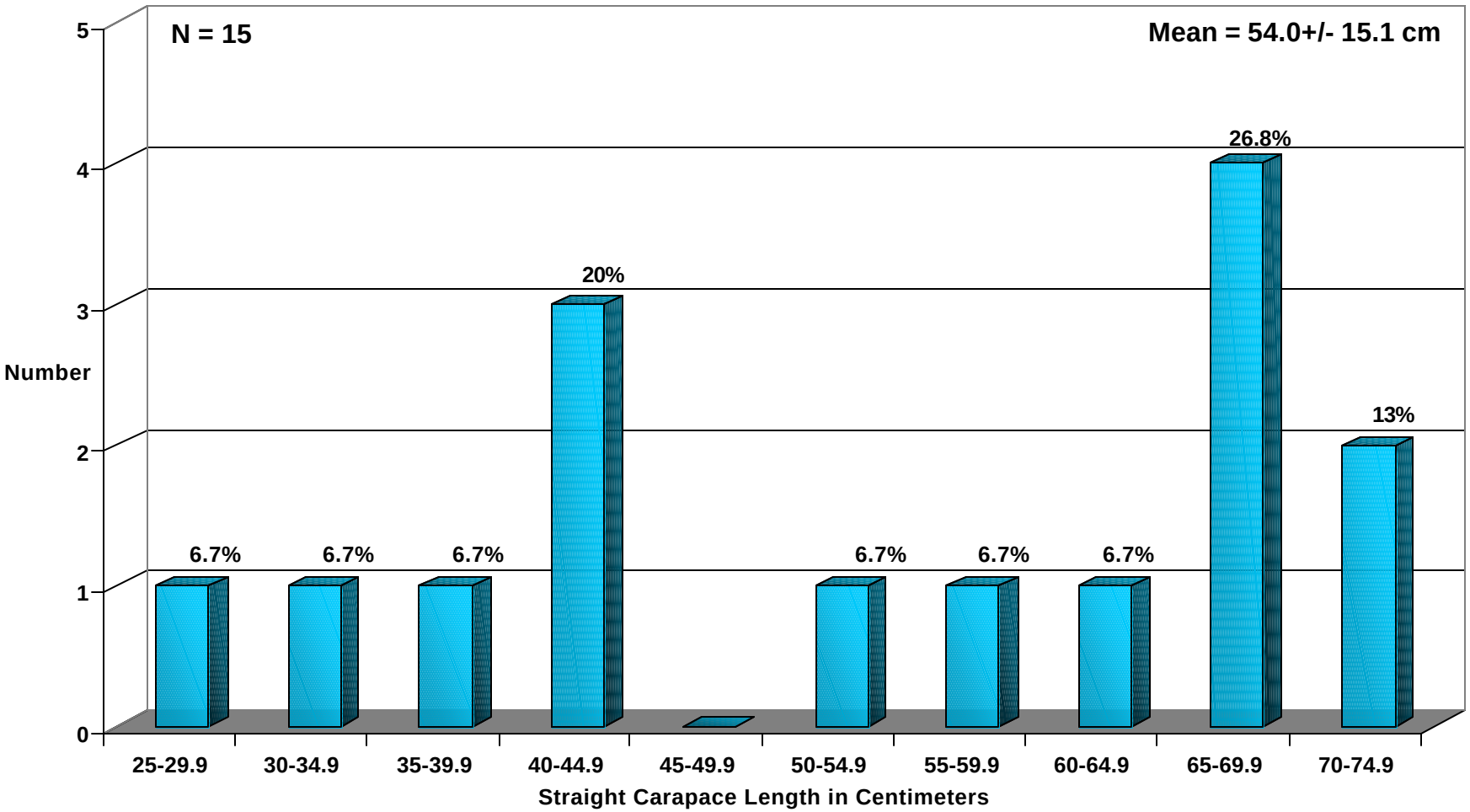


* - 1 datum was obtained from observer estimate of turtle size

Prepared by G. Balazs and D.Parker
SWFSC Marine Turtle Research
1/24/01

Attachment 4

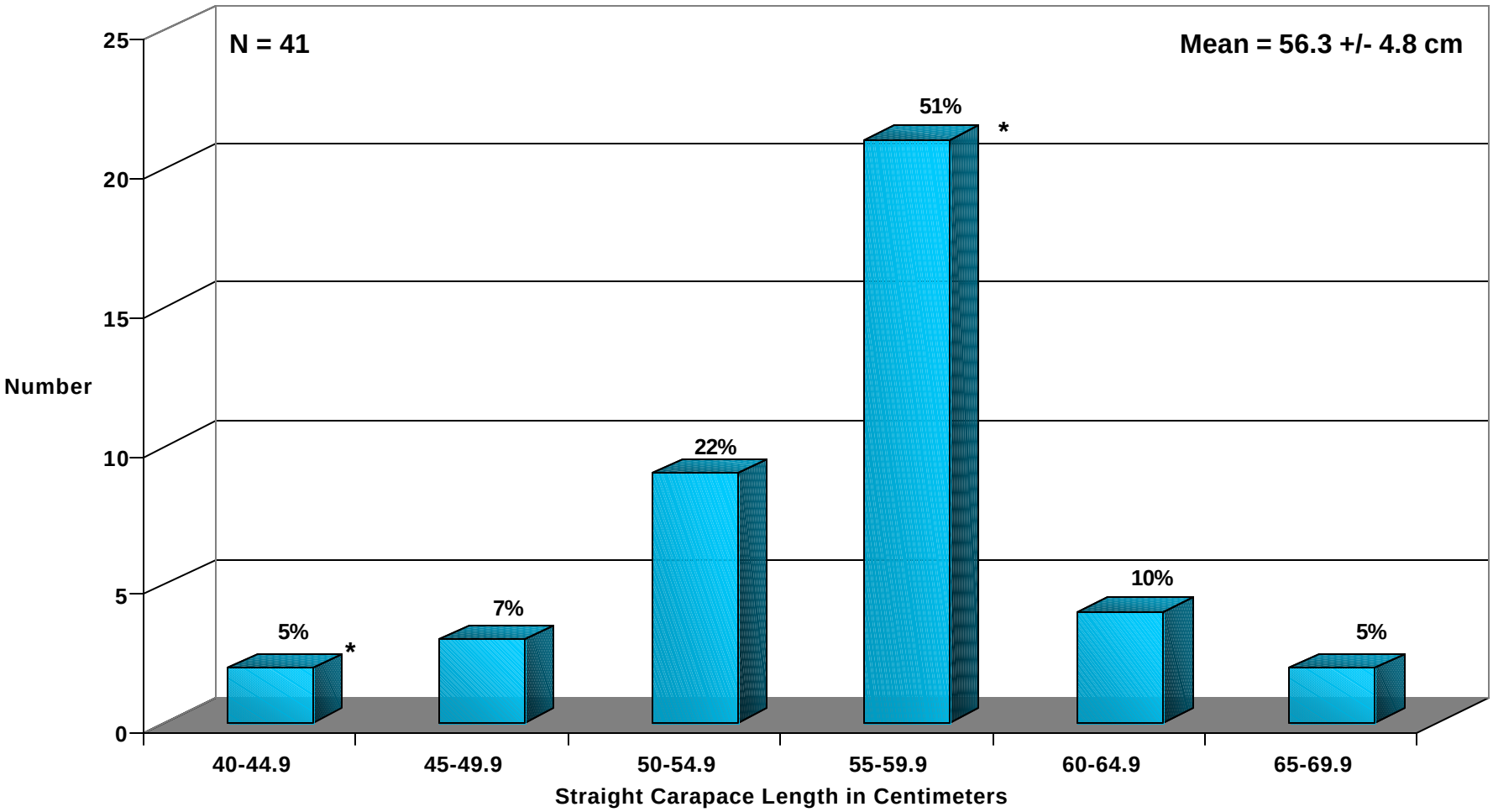
Size-Class Distribution of Green Turtles
Taken as Bycatch in the Hawaii-based Pelagic Longline Fishery
Based on NMFS Observer Data



Prepared by G. Balazs and D.Parker
SWFSC Marine Turtle Research
1/24/01

Attachment 4

Size-Class Distribution of Olive Ridleys
Taken as Bycatch in the Hawaii-based Pelagic Longline Fishery
Based on NMFS Observer Data

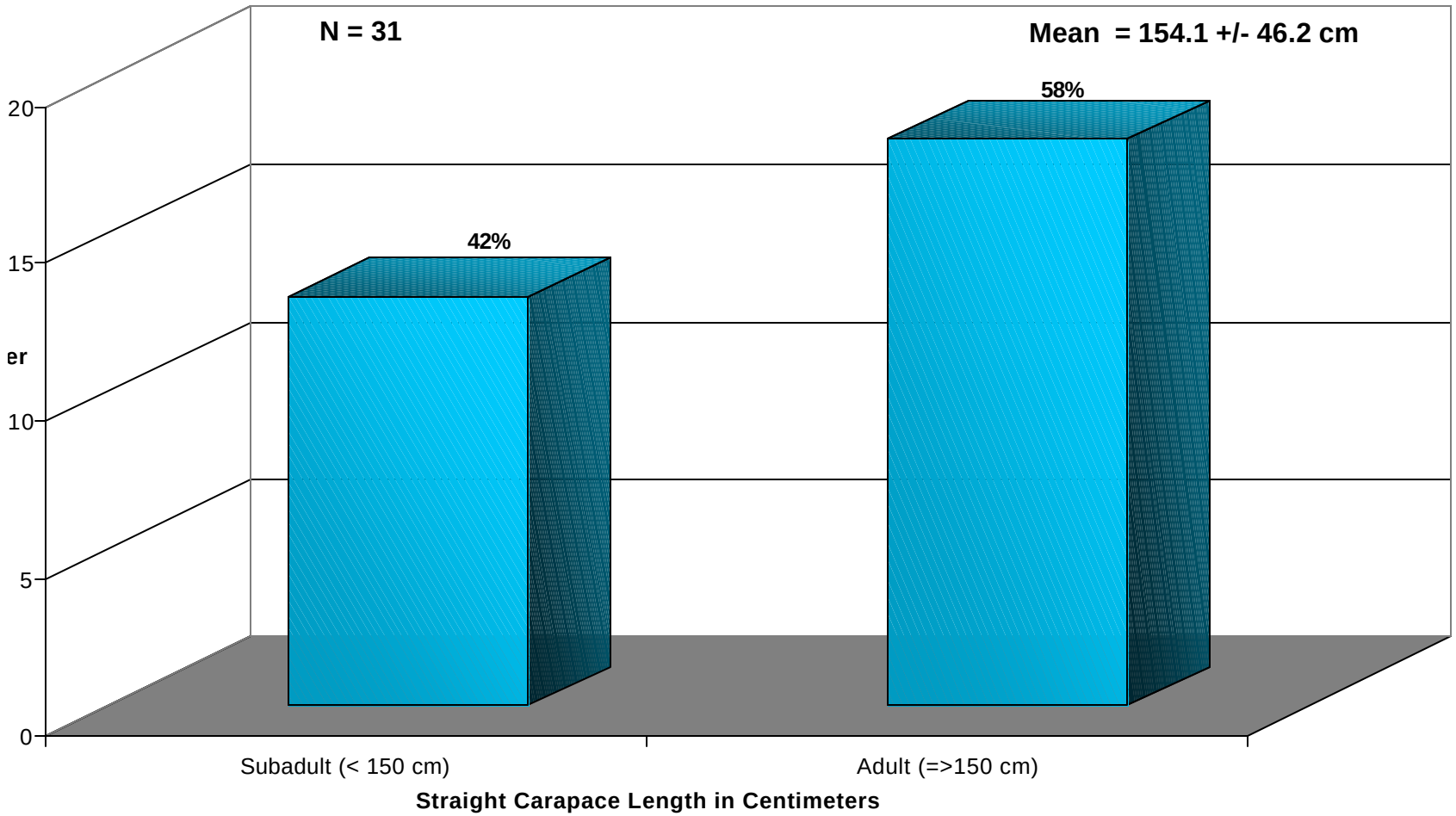


- 1 datum was obtained from observer estimate of turtle size

Prepared by G. Balazs and D.Parker
SWFSC Marine Turtle Research
1/24/01

Attachment 4

Size-Class Distribution of Leatherbacks
Taken as Bycatch in the Hawaii-based Pelagic Longline Fishery
Based on NMFS Observer Data

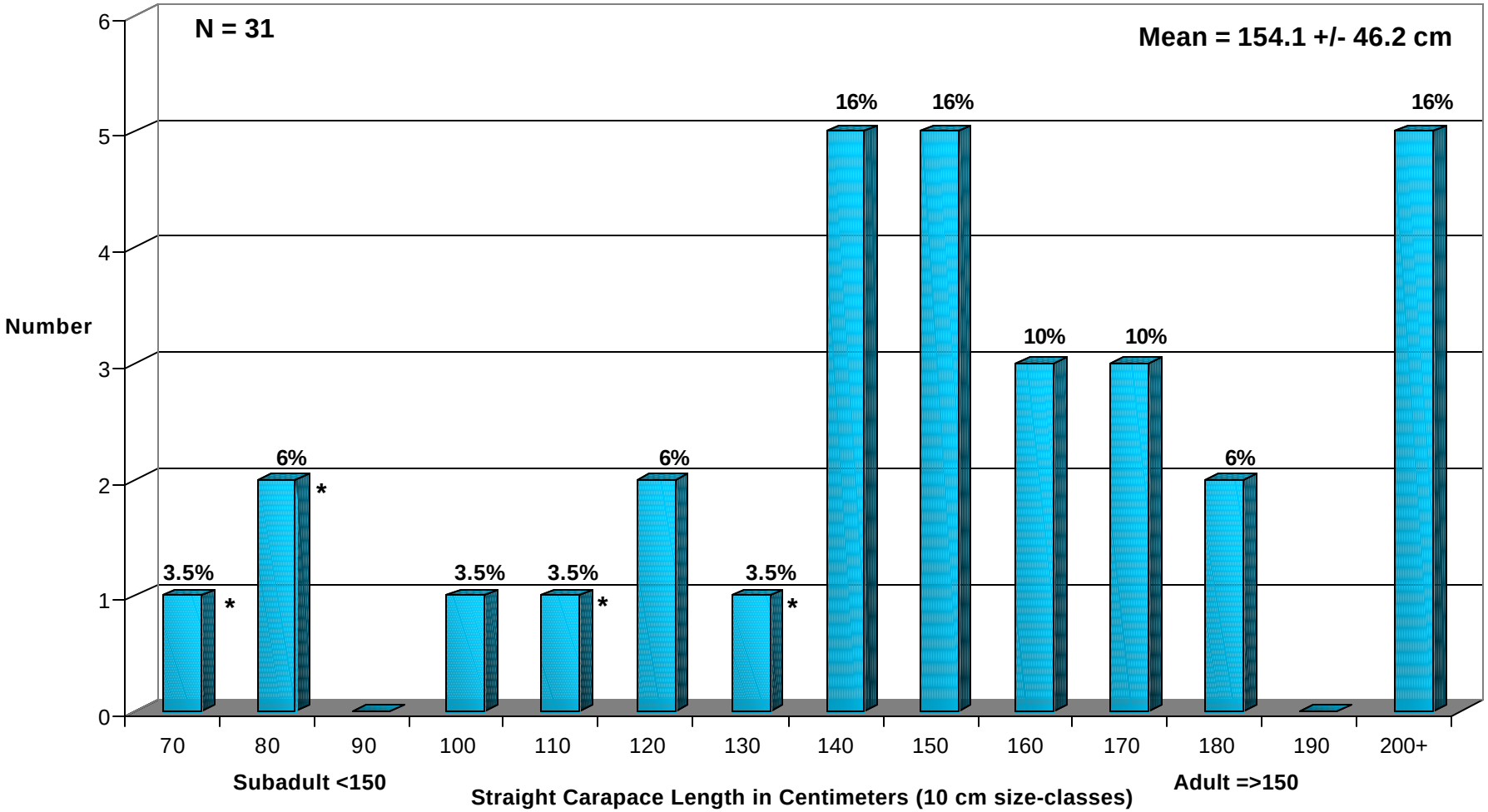


all sizes were determined from observer estimates, except in the case of 5 subadults

Prepared by G. Balazs and D.Parker
SWFSC Marine Turtle Research
1/24/01

Attachment 4

Size-Class Distribution of Leatherbacks Taken as Bycatch in the Hawaii-based Pelagic Longline Fishery Based on NMFS Observer Data

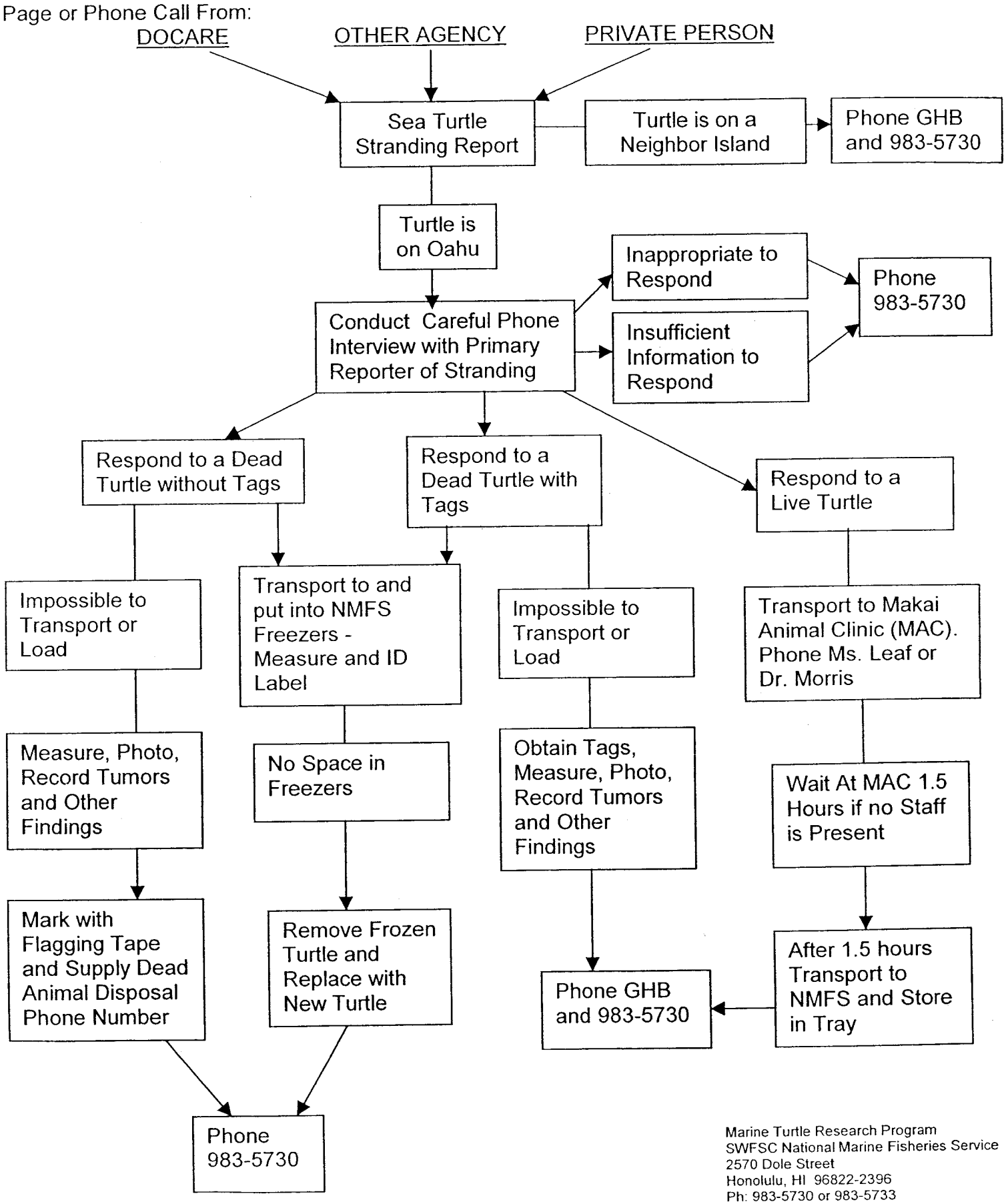


all sizes were determined from observer estimates, except for 5 subadults (*)

Prepared by G. Balazs and D.Parker
SWFSC Marine Turtle Research
1/24/01

Attachment 5

PROTOCOL FOR SEA TURTLE STRANDING RESPONSE BY THE MARINE OPTION PROGRAM ON WEEKENDS, HOLIDAYS AND AFTER HOURS



Marine Turtle Research Program
SWFSC National Marine Fisheries Service
2570 Dole Street
Honolulu, HI 96822-2396
Ph: 983-5730 or 983-5733
Fax: 983-2902

Note: Illegal activities involving sea turtles should be reported to DOCARE (587-0077) and/or NMFS Law Enforcement (541-2727)

Protocol for care and feeding of Kewalo turtles:

#1 Rule: Deal with all non-tumored animals FIRST. Complete all tasks (cleaning, feeding, etc.) with non-tumored animals before taking care of any sick or tumored turtle.

When a new turtle is brought to KRF, be sure to paint the turtle's ID date on the side of its shell in small numbers. There is no need to paint it all across the turtle's shell. Also be sure to write the ID on the side of the tank the turtle was placed in.

Feeding schedule varies with each turtle. If the turtle seems to eat it all, go ahead and feed as much as it will eat (slowly increasing). Just don't let food go to waste. If there's quite a bit left over the next day, cut back on amount of food given. If tanks are becoming too dirty due to fish remains, cut back on amount of fish and give double the amount of vit./ cal. pills.

Suggested:

1) Remove fish/squid from refrigerator to defrost. If fish or squid is needed from the freezer, then take out the day before and place in refrigerator. Then bag accordingly, so we are only using what is needed, and place the rest in freezer.

2) While food is defrosting, clean tanks by:

a) Removing large fecal matter and debris and disposing of in plastic bags, then placing in specified box in freezer. Do NOT leave in nets! If needed, double check to see that all fecal matter has been placed in freezer. The fecal matter needs to brought up to the lab to discard twice a week, recommended on Tuesdays and Fridays and then put in the dumpster on Fridays.

b) Scrub tanks with long handled broom/brush to remove as much algae growth as possible from the bottom and sides of tanks and to stir up smaller material so it will drain. Remove drain for only 2-5 minutes after scrubbing is finished to allow most of material to go down the drain.

c) Once a week (twice if algae is getting bad) tanks should be drained completely and scrubbed thoroughly using fresh water to rinse tanks and drains. Don't forget to include tanks that are not in use. Turtles should also be scrubbed. Tanks with turtles should be refilled after cleaning is done.

3) After food is defrosted (so they are pliable and not cold to the touch), feed non-tumored turtles. Make sure to cut food small enough so it won't clog the drains and hand feed turtles food with vit./cal. pills.

4) Observe animals, record information and report any discrepancies noted (i.e. turtle not eating, major changes in appearance, etc.) Also, use your judgement on the water depth of the tanks. If you think the water level could be either raised or lowered, mention it to any one of us (Shawn, Shandell, or George) and we will decide on what to do from there.

Repeat for tumored animals. Then thoroughly clean up sinks and areas around tanks (spray off table, pick up any trash, check bungee cords on tarp and replace if needed, etc.)

Record all observations in the Log Book. If you note anything of importance or abnormal, again, be sure to mention it to either George, Shawn, or Shandell.

UNIVERSITY OF HAWAI'I AT MĀNOA

Institutional Animal Care and Use Committee
University Compliance Officer

29 September 2000

MEMORANDUM

TO: Dr. Thomas Schroeder
Director
Joint Institute for Marine and Atmospheric Research
Marine Science Building, 312

FROM: Norman K. Magno
UH Regulatory Compliance Officer

SUBJECT: Protocol Renewal for #97-051-3; *"Fisheries Oceanography (Hawaiian Green Sea Turtles - I)."*

Review of our records indicate that this protocol will **expire on 18 November 2000.** The University Policy requires yearly renewals of all animal use protocols. To be in compliance with this Policy, a completed Animal Care & Use Committee Protocol Form or Protocol Annual Follow-up Form is required. If your animal experimentation or use of animals for teaching is to continue, please submit your renewal within 30 days from the receipt of this letter.

If your experimentation has ceased, and no longer require the use of animals for experiment(s) or for teaching, please forward to this office a memorandum or return this notice to confirm this. In closing the file for this application, the committee also requests that you submit a brief project report detailing the past year's animal use activities for this protocol. As a reminder, the use of vertebrate animals beyond the expiration date of the approval letter for this protocol will be considered non-compliant.

If you are renewing this protocol, please use the enclosed form:
[ ] 2000 Protocol Annual Follow-up Form
[✓] 2000 Animal Care & Use Committee Protocol Form (3 year intervals)

If you are NOT going to renew this protocol, please sign/date below and mail this notice back to the Compliance Office with a brief project report as requested above.

	10/25/00
Signature	Date

Your cooperation is greatly appreciated. Mahalo.

c: Douglas L. Vincent, Ph.D., Chairperson, Animal Care & Use Committee
Sylvia Kondo, D.V.M., Interim LAS Director and University Veterinarian
Investigator File - 97-051-3

2538 McCarthy Mall, Snyder Hall 410, Honolulu, Hawai'i 96822
Telephone: (808) 956-4446, Facsimile: (808) 956-4448

An Equal Opportunity/Affirmative Action Institution

Project Protocol Number

UNIVERSITY OF HAWAII -- INSTITUTIONAL ANIMAL CARE & USE COMMITTEE
2000 -- VERTEBRATE ANIMAL USE PROTOCOL FORM

Instructions: The applicant is responsible for providing complete and accurate information. All sections must be addressed. Use additional pages if necessary. Please [✓] the appropriate boxes. If a section does not apply to your study, indicate "does not apply" to avoid leaving blank. The completion of this form is required by Federal Law through the Animal Welfare Act (USDA) and by the Public Health Service Policy (NIH). Handwritten protocols will be returned to the applicant without IACUC review. Submit the **TYPED** original and 14 photocopies of the completed protocol form to this address: **Animal Care and Use Committee, c/o Compliance Office, 2538 The Mall, Snyder Hall Room 410, Honolulu, HI 96822. Phone: 808-956-4446. FAX: 808-956-4448.**

I. ADMINISTRATIVE DATA: Please [✓] the appropriate category for your work.
Animal Project Category: RESEARCH [XX] TEACHING/TRAINING [ ] HERD/FLOCK/BREEDING [ ]

Principal Investigator/Instructor: Dr. Thomas Schroeder Title: Director, JIMAR
Contact Person (and phone no.) if not PI: George H. Balazs, 808-983-5733
Institutional Affiliation: University of Hawaii Department: JIMAR
Building: Marine Science Building Room Number: 312
Phone Number: 808-956-7476 FAX Number: 808-956-4104
E-mail: tas@soest.hawaii.edu

Project Title: Fisheries Oceanography (Hawaiian Green Sea Turtles - I)

Project Type:
[ ] New [ ] Pilot Study [ ] Revision (Major) [XX] Renewal (Full Project Review at 3 Year Intervals)
Project Starting Date: November 18, 2000 Total Project Period: from 11-18-2000 to 11-18-2003.

Project Funding Information:
Project funded? [ ] No [ ] Private Sector [ ] Dept/College [ ] UH Research Council [XX] Extramural
Name of source or funding agency: NOAA, NMFS, Southwest Fisheries Science Center
Proposal submitted to Office of Research Services (ORS)? [XX] No [ ] Yes, date submitted?: _____
Peer Reviewed?: [ ] No [XX] Yes

II. PERSONNEL QUALIFICATIONS:
Please list all personnel on your project by name and title that will be involved with animals. Indicate duties, qualifications (specific course work, training, degrees, etc), and any other training received in animal handling/care procedures. Review of the Animal Use Regulations and Institutional Animal Handling and Procedures Certification is required prior to animal use. Attach copy(ies) of the certificates of listed personnel. Attach additional pages if necessary.

Person: Shawn K. K. Murakawa Title: Research Associate III

Skills Required for this study:

Ability to restrain sea turtles for short periods, to apply Inconel alloy tags to the front and hind flippers and to subcutaneously inject passive integrated transponder (PIT) tags.

Qualifications/Experience:

Bachelor's degree in biology, zoology or Animal Sciences and four years experience handling and marking sea turtles.

Review of Animal Use Regulations and Institutional Animal Handling and Procedures Certification?
[ ] No [XX] Yes when: 1998 & 1999 If No was checked, what is the reason.

PERSONNEL QUALIFICATIONS: (Continued)

Person: Denise M. Parker Title: Research Associate III

Skills Required for this study:
Ability to capture and restrain sea turtles for short periods, to apply Inconel alloy tags to to the front and hind flippers and to subcutaneously inject passive integrated transponder (PIT) tags.

Qualifications/Experience:
Bachelor's degree in biology, zoology or Animal Sciences and four years experience handling and marking sea turtles.

Review of Animal Use Regulations and Institutional Animal Handling and Procedures Certification?
[ ] No [XX] Yes when: 1998 & 1999 If No was checked, what is the reason.

III. PROJECT OBJECTIVES:

Please indicate by [✓] if your use of animals will be for research; teaching/training; or herd/flock/breeding. Please [✓] all that apply, then provide a non-technical explanation for the purpose of animal use.

[XX] Research: Explain in non-technical terms the aim of the study and why the study is important. The abstract from your grant proposal is suitable (**DO NOT** attach your entire grant proposal). Use additional pages if necessary.

[ ] Teaching/Training: Explain the scope and purpose of the class or laboratory, and why animals are necessary for the project. It is highly recommended that a course manual and syllabus accompany the completed protocol.

[ ] Herd/Flock/Breeding: Explain the overall intent for maintaining the herd/flock/breeding colony. Please list the protocol numbers of the research and/or teaching/instruction projects that this application provides animals for.

To provide appropriate veterinary care for stranded, injured, and sick sea turtles obtained by the National Marine Fisheries Service Stranding Research and Rescue Program.

To achieve the recognition of individuals or cohorts for research purposes aimed at obtaining information on reproductive biology, movements, residency and growth rates. Tagging is the single-most valuable tool in advancing an understanding of sea turtles and their management and conservation needs in relation to complex life cycles, population numbers, reproductive migrations, growth rates and sexual maturation.

II. Personnel Qualifications: (Continued)

Person: Shandell Eames **Title**: Data Entry Clerk

Skills Required for this study:

Ability to restrain sea turtles for short periods, to apply Inconel alloy tags to the front and hind flippers and to subcutaneously inject passive integrated transponder (PIT) tags.

Qualifications/Experience:

Studies in Biology, Zoology or Animal Science and three years experience handling sea turtles.

Review of Animal Use Regulations and Institutional Animal Handling and Procedures Certification?

[ ] **No** [XX] **Yes when**: 1999 **If No was checked, what is the reason.**

Person: Kristy Masada **Title**: Student Help

Skills Required for this study:

Ability to restrain sea turtles for short periods of time.

Qualifications/Experience:

Studies in Biology, Zoology or Animal Science and two years experience handling sea turtles.

Review of Animal Use Regulations and Institutional Animal Handling and Procedures Certification?

[ ] **No** [XX] **Yes when**: 2000 **If No was checked, what is the reason.**

Person: Sean Lockerman **Title**: Student Help

Skills Required for this study:

Ability to restrain sea turtles f or short periods of time.

Qualifications/Experience:

College student, experience handling sea turtles.

Review of Animal Use Regulations and Institutional Animal Handling and Procedures Certification?

[XX] **No** [ ] **Yes when**: _____ **If No was checked, what is the reason.**

They will be directly supervised by an authorized person when handling turtles.

II. Personnel Qualifications: (Continued)

Person: Daniel Toibero **Title**: Student Help

Skills Required for this study:

Ability to restrain sea turtles for short periods of time.

Qualifications/Experience:

College student, experience handling sea turtles.

Review of Animal Use Regulations and Institutional Animal Handling and Procedures Certification?

[XX] **No** [ ] **Yes when**:_____ **If No was checked, what is the reason.**

They will be directly supervised by an authorized person when handling turtles.

Person: Russell Sasaki **Title**: Student Help

Skills Required for this study:

Ability to restrain sea turtles for short periods of time.

Qualifications/Experience:

College student, experience handling sea turtles.

Review of Animal Use Regulations and Institutional Animal Handling and Procedures Certification?

[XX] **No** [ ] **Yes when**:_____ **If No was checked, what is the reason.**

They will be directly supervised by an authorized person when handling turtles.

IV. ANIMAL REQUIREMENTS:

List species **(include common name if scientific names are used)**, strain, age, weight, sex, source of animals, animal holding and procedure location (if different from holding location), and proposed numbers of animals to be used. Be sure to check the appropriate pain or distress category(ies) and indicate the number of animals in each. Sum(s) should equal total number of animals requested. [**NOTE: USDA Category C** = minimal, transient, or no pain or distress; **USDA Category D** = pain or distress relieved by appropriate measures such as anesthetics, analgesics, and/or tranquilizers (complete Section VII.); **USDA Category E** = Unrelieved pain or distress. **If animals are indicated in category E, a scientific justification is required to explain why the use of anesthetics, analgesics, sedatives or tranquilizers during and/or following painful or distressful procedures is contraindicated**]. If your project uses more than two species of animals, please **photocopy** this page and provide the information on the other species.

Animal Information	Year 1	Year 2	Year 3
Species:	Green turtle, Chelonia mydas --- same for all 3 years		
Stock/Strain:	Hawaiian Population ---- same for all 3 years ----		
Sex/Age/Weight:	Both sexes, all ages and size classes from hatchling to adult		
Source(s):	Natural marine and terrestrial habitats of the HI archipelago		
Animal Holding Location:	Kewalo Research Facility - (KRF) -- same for all 3 years		
Animal Procedure Site:	Natural marine and terrestrial habitats of the HI archipelago		
Pain or Distress Category:			
Herd/Flock/Breeding			
USDA Category C	XXX	XXX	XXX
USDA Category D			
USDA Category E			
Total Number of Animals:	500	500	500
Species:	Loggerhead, Caretta caretta --- same for all 3 years		
Stock/Strain:	Pacific population - from the Hawaiian Island Population		
Sex/Age/Weight:	Both sexes, all ages and size classes from hatchling to adult		
Source(s):	Natural marine and terrestrial habitats of the HI archipelago		
Animal Holding Location:	Kewalo Research Facility - (KRF) --- same for all 3 years		
Animal Procedure Site:	Natural marine and terrestrail habitats of the HI archipelago		
Pain or Distress Category:			
Herd/Flock/Breeding			
USDA Category C	XXX	XXX	XXX
USDA Category D			
USDA Category E			
Total Number of Animals:	50	50	50

IV. ANIMAL REQUIREMENTS:

List species **(include common name if scientific names are used)**, strain, age, weight, sex, source of animals, animal holding and procedure location (if different from holding location), and proposed numbers of animals to be used. Be sure to check the appropriate pain or distress category(ies) and indicate the number of animals in each. Sum(s) should equal total number of animals requested. [**NOTE: USDA Category C** = minimal, transient, or no pain or distress; **USDA Category D** = pain or distress relieved by appropriate measures such as anesthetics, analgesics, and/or tranquilizers (complete Section VII.); **USDA Category E** = Unrelieved pain or distress. **If animals are indicated in category E, a scientific justification is required to explain why the use of anesthetics, analgesics, sedatives or tranquilizers during and/or following painful or distressful procedures is contraindicated**]. If your project uses more than two species of animals, please **photocopy** this page and provide the information on the other species.

Animal Information	Year 1	Year 2	Year 3
Species:	Olive Ridley, Lepidochelys olivacea -- same for all 3 years		
Stock/Strain:	Hawaiian Is. Population -- same for all 3 years ----		
Sex/Age/Weight:	Both sexes, all ages and size classes from hatchling to adult		
Source(s):	Natural marine and terrestrial habitats of the HI archipelago		
Animal Holding Location:	Kewalo Research Facility - (KRF) --- same for all 3 years		
Animal Procedure Site:	Natural marine and terrestrial habitats of the HI archipelago		
Pain or Distress Category:			
Herd/Flock/Breeding			
USDA Category C	XXX	XXX	XXX
USDA Category D			
USDA Category E			
Total Number of Animals:	50	50	50
Species:			
Stock/Strain:			
Sex/Age/Weight:			
Source(s):			
Animal Holding Location:			
Animal Procedure Site:			
Pain or Distress Category:			
Herd/Flock/Breeding			
USDA Category C			
USDA Category D			
USDA Category E			
Total Number of Animals:			

V. ELECTRONIC DATABASE SEARCHES (FEDERAL ASSURANCES -- USDA POLICIES #11 & #12): Please note that this section must be addressed.

A. Certification for Alternatives to Painful and Distressful Procedures.

Please [✓] the appropriate box.

[X] I am **exempt** from completing this subsection because the animals in this project are for Herd/Flock/Breeding and/or categorized in the USDA Pain or Distress Category C.

[ ] I am **required** to complete this subsection because the animals in this project are categorized in the USDA Pain or Distress Categories D and/or E.

Federal law, **USDA POLICY #12** requires that the applicant provide a written narrative of the sources consulted to determine whether or not alternatives exist to procedures which may cause pain or distress. The applicant must consider alternatives to procedures which may cause pain or distress, and is required to state the sources consulted. Examples of some sources are: Biological Abstracts, Agricola, Medline, and the Animal Welfare Information Center. The narrative must be written such that the IACUC can readily assess whether the search topics are appropriate and thorough. **The minimal written narrative must include the information listed below.** Have alternatives to painful/distressful procedures been considered? [ ] Yes [ ] No If you have not considered alternatives to painful/distressful procedures, please explain. Attach additional sheets application if necessary.

Names of databases searched or other sources consulted:

Date of the search and **years (date range)** covered by the search:

Key words or search strategy used to consider alternatives or descriptions of other methods and sources to determine whether no alternatives are available for painful or distressful procedures that are being proposed:

Please provide a **statement** that non-animal and/or animal alternatives were evaluated during the electronic search. Attach additional sheets to this application if necessary.

B. Certification that the Project is NOT Unnecessarily Duplicated

All projects that have animals categorized in any of the USDA Pain or Distress Categories (C, D, and/or E) must have this subsection addressed. **ONLY** Herd/Flock/Breeding applications are exempt.

It is important that your project does not unnecessarily duplicate previous work. Indicate the referenced literature you reviewed to determine that this work is necessary and not unnecessarily duplicated.
[XX] Review Articles [XX] Meetings or Conferences Attended [XX] Consultations with Colleagues
[ ] Electronic Database Searches: (Provide the names of the databases searched).

Do you certify that the activities involving animals described in this protocol do not unnecessarily duplicate previous work? [XX] Yes [ ] No If no, please explain.

VI. RATIONALE FOR USE OF ANIMALS (Justification of the 3 R's):

This section also pertains to Federal regulations. Please provide details for all subsections.

Replacement - Explain your rationale for live animal use as opposed to in vitro studies, other types of animal models or non-animal methods. The objective of marking and tagging sea turtles in Hawaii is to estimate life history parameter by which we can best describe the poulation dynamics of the WILD population. In vitro studies can not provide this information which is essential to formulate long-term management plans.

Refinement - Scientifically justify the appropriateness of the species selected. What is unique about the animal model(s) chosen? Explain why a different animal model cannot be selected that is lower on the evolutionary scale? The green turtle is a threatened species in the Hawaiian Islands that requires the formulation of management and conservation straegies to restore the species to former levels of health and abundance, consistent with their ecological role. Knowledge of the species and wild populations, as can be achieved by tagging, is basic to developing such strategies.

Reduction - Justify the number of animals needed. Include information about your experimental design, variability, power of analysis and other statistical or mathematical arguments if possible.

Work is being done in natural marine and terrestrial habitats wherethe animals are not being held in captivity but released on site. Recapture rates of animals released into oceanic habitats are usually low so that large numbers of animals need to be tagged in order for life history parameters to be estimated with statistical significance.

VI. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES:

Please [✓] the appropriate subsection category and provide details requested for that subsection.

[XX] **A. Research and Teaching/Training Projects**

Please explain the experimental design (ie. pre-experimental, experimental, and post-experimental procedures). Specify all animal procedures performed. Provide a complete and accurate description which will allow the ACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study. For complex studies, flow-charts are highly recommended. Use additional sheets of paper if necessary.

Discrete nearshore foraging areas used by green turtles throughout the Hawaiian Islands will be sampled for the purpose of tagging for long-term individual identification. The numbers of turtles tagged is limited by personnel resources, assessibility of habitats, and seasonal environmental conditions. Since short-term restraint and tagging are harmless activities, the goal is to mark as many turtles as possible in the wild in order to increase probabilities of future recoveries. However, the number of turtles capable of being marked will not exceed 500 per year unless additional personnel and other resources become available. Based on earlier tagging wrok in Hawaii and elsewhere with sea turtles, this sample size will be adequate for statistically significant numbers of turtles to be resighted and recaptured.

Injections or inoculations (drugs, infectious agents, adjuvants etc.). Indicate dose, injection sites, volume, route of administration, and schedules. Use additional pages if necessary. [XX] Not Applicable

Injections or Inoculations	Dosage/Volume	Injection Site	Route of Administration	Administration Schedule

VI. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES:
A. Research and Teaching/Training Projects (Continued)

Turtles reported by the public found to be stranded, injured or sick throughout the Hawaiian Islands are taken to an experienced sea turtle veterinarian (Dr. Robert Morris, Makai Animal Clinic) for immediate diagnostic assessment and treatment. Turtles, under the direction of the veterinarian, are then taken to holding tanks at KRF for further rehabilitation, therapy, assessment and concomitant associated research. The holding tanks are designed to have a constant fresh flow of saltwater and turtles are cared for, fed, and monitored daily. The turtles stay under direct veterinary care until they are judged suitable for release back into the wild.

VI. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES: (continued)

Blood withdrawals (volume, frequency, withdrawal sites, and methodology). [XX] Not Applicable

Methodology	Volume	Withdrawal Sites	Frequency

Radiation (dosage and schedule). See Section X. [XX] Not Applicable

Methods and length of time of restraint (eg. restraint chairs, collars, stanchions, etc.). Describe and justify the use of any restraint devices employed. [ ] Not Applicable Turtles will be restrained manually by holding their flippers and carapace. No ropes or devices are necessary. Restraint time for flipper tagging will average five (5) minutes and will not exceed 10 minutes.

Animal identification methods (eg. ear tags, tattoos, collar, cage cards etc.).
Flipper tag and PIT tagging will provide extremely long-term, hopefully life-long permanent individual identification of Hawaiian green turtles marked in this study. Natural scar patterns will supplement, but could never replace these technological means of recognition.

Non-survival surgical procedures. [XX] Not Applicable

Other Procedures (eg. survival studies, tail or other biopsies, etc.). [XX] Not Applicable

[ ] B. Herd/Flock/Breeding Projects

Please provide a description of the procedures performed on the animals. Are the animals subjected to any procedures that are likely to cause more than slight, momentary pain or distress: (special agricultural practices like castration, dehorning, tail docking, break or toe-trimming, forced molting, electro-ejaculation, branding, toe-clipping, or ear notching. [ ] No [ ] Yes If yes, please attach copies of the relevant portions of your Standard Operating Procedures (SOPs) for the committee to review.

VII. SURVIVAL SURGERY:

Please [✓] the box, if this section does not apply to your work. [XX] Not Applicable.

If your study involves survival surgery, complete the following.

Describe the surgical procedure(s) to be performed. Include the aseptic methods to be utilized. (Use additional pages if necessary).

N/A

Who will perform the surgery and what are their qualifications and/or experience?

N/A

Where will the surgery be performed? Building and Room: _____ _____.
Is this a dedicated facility? [ ] yes [ ] no. If no, explain.

N/A

Describe post-operative care & procedures required and identify the responsible individual(s).

N/A

Has major survival surgery been performed on any animal prior to being placed on this study?
[ ] No [ ] Yes If yes, please explain and justify the repeat surgery.

N/A

Will more than one major survival surgery be performed on an animal while on this study?
[ ] No [ ] Yes If yes, please explain and justify the repeat surgery.

N/A

VIII. ANESTHESIA, ANALGESIA, TRANQUILIZATION:

Please [✓] the box, if this section does not apply to your work. [ ] Not Applicable.

For animals indicated in Section II, USDA Category D, specify the anesthetics, analgesics, sedatives of tranquilizers that are to be used. Include the name of the agent(s), the dosage, route, frequency of administration, monitoring methods and person(s) responsible.

Name of Agent	Dosage	Route of Administration	Frequency of Administration
Foille Medicated			
Antiseptic/Anesthetic Spray	5% Benzocaine	Topical	Once, shortly before tag application.

Monitoring Method(s):
Respiration and temperature will be monitored before, during and after restraint.

Person(s) Responsible:
Shawn K. K. Murakawa, Denise M. Parker and Shandell M. Eames
George H. Balazs

IX. METHOD OF EUTHANASIA OR DISPOSITION OF ANIMALS AT END OF STUDY:

Euthanasia is the primary means for the disposition of animals at the end of a study; however, in some special cases, animals may be put up for adoption. To determine if adoption is applicable for this protocol, refer to the criteria for adoption as described in IACUC Policy 2.0 The IACUC in consultation with the University veterinarian(s) reserves the right to make the final decision if adoption is applicable.

Requirement: An acceptable method of euthanasia must be provided by the applicant even if the animals are not planned to be sacrificed after the research, teaching/training, or herd/flock/breeding activities. For acceptable euthanasia methods refer to the **1993 American Veterinary Medical Association - Report of the AVMA Panel on Euthanasia**. If the method of euthanasia is not referenced in this Report, please provide a reference or journal article along with scientific justification that supports the use of the proposed method.

Important: Personnel that euthanize animals must be certified to administer the proposed method of euthanasia.

Euthanasia [ ] Adoption [ ] Return to Natural Habitat [XX] Other (please explain) [ ]

Other:

Euthanasia Method: If a chemical agent is used, specify the dosage and route of administration. Please note that even if you do not intend to euthanize the animals, you should provide a method that you would use in the event of unanticipated injury or illness. In the event of unanticipated injury or illness or if deemed necessary by the veterinarians, turtles will be euthanized by an injection of 2 cc/10 lb of Beuthanasia D Special solution by one of the attending veterinarians. This dosage has been determined to be the most humane way for euthanizing sea turtles by veterinarians specializing in sea turtles and reptiles. If any turtle dies unexpectedly/ suddenly during handling or care, a full necropsy will be performed by veterinarians to determine cause of death and any recommendations for changes in handling given by the veterinarians will be implemented.

Method of Carcass Disposal:
The carcass of turtles that have been euthanized will be incinerated at the Halawa Animal Quarantine Facility.

Laboratory Animal Service (LAS) Users - Please indicate if this condition applies to your project. You are a UH Faculty or facility user with research conditions that warrant your own laboratory personnel to euthanize animals because of specific experiment parameters and time-frame for blood and/or tissue collection.

[XX] No [ ] Yes

X. HAZARDOUS AGENTS:

Important: Use of hazardous agents requires the approval of the UH Environmental Health & Safety Office.

Hazardous Agents	Yes	No	List Agents and Registration Document (If Applicable)
Radioisotopes		XX	
Biological Hazards and/or Pathogens		XX	
Hazardous Chemicals and/or Drugs		XX	
Recombinant DNA		XX	
Additional Safety Concerns		XX	

Use additional sheets of paper if necessary.

XI. VETERINARY CARE:

Important: In case of injury to the animal or problems occur where the animal(s) may unexpectedly not respond normally, it is the investigator's responsibility to contact the attending veterinarian for emergency care or euthanasia. List the names and contact numbers for the attending veterinarians providing animal care.

[ ] UH Veterinarian(s): Telephone: (808) 956-8770 or (808) 956-4444
Digital Pager: 577-4045

[XX] Local or Contract Veterinarian(s)

Name: Dr. Robert Morris, DVM Telephone: 808-262-9621 Pager (if available):
Address: Makai Animal Clinic
420 Uluniu Street
Kailua, Hawaii 96734

Name: Dr. Thierry Work, DVM Telephone: 808-541-3445 Pager (if available):
Address: USNBS-NWHC-HFS
P.O. Box 50167
300 Ala Moana Blvd, Honolulu, HI 96850

[ ] Veterinary care is not required. If this box is checked, please provide your justification. The IACUC reserves the right to make the final decision to whether veterinary care is required for this application despite the justification that is provided.

XII. OTHER IACUCs:

Is this application being reviewed by other Animal Care & Use Committees? Yes [ ] No [XX]
If Yes, the IACUC may require that you provide documentation of approval for this activity from the other IACUCs or review committees. Please provide their location, address, and telephone/fax numbers.

Location:

Address:

Telephone/Fax Number(s)

XIII. ENDANGERED SPECIES AND SPECIAL PERMITS:

Are the animals being used in this activity endangered species? Yes [ ] No [XXX]

Are special domestic or foreign government permits required for the study? Yes [XXX] No [ ]
If Yes, the IACUC may require that you provide documentation of the permits for this activity.

Research of the Hawaiian green turtle and the Loggerhead and olive ridley turtles as threatened species is authorized under the State of Hawaii Scientific Collecting Permit SCP 2001 04, National Marine Fisheries Service Permit No. 924 and 50CFR 17.31(b) authorizing Dr. Alonoso Aguirre to be an "agent" of the National Marine Fisheries Service. Special Use Permits are also required by the U.S. Fish and Wildlife Service to conduct research within the Hawaiian Islands and the Midway Atoll National Wildlife Refuges. These permits are issued annually and can be made available if necessary.

XI. VETERINARY CARE

Local or Contract Veterinarians (Continued):

Name: Dr. Alonso Aguirre Telephone: 617-628-5000 Pager (if available):

Address: Tufts University
Env & Population Health - Vet
Medford, MA 02155

XIV. ASSURANCE STATEMENTS:

A. Laboratory Animal Service Resource Manual Assurance Statement.

[X] Yes [ ] No I have received and referred to the appropriate sections of the LAS Animal Resource Manual in the preparation of this protocol review form. I will abide by the policies and procedures stated and I intend to use this informational resource as a guide and aid. If no, please explain.

B. UH Medical Surveillance Requirements Assurance Statement.

[X] Yes [ ] No I have read and understood the nature and purpose of the UH Medical Surveillance requirements (currently LAS Resource Manual pages 3 & 4). I agree to comply fully with this policy and all of the provisions that are related to my research or teaching/training. If no, please explain.

C. Statements for Accuracy and Responsibility for Implementation of the Animal Use Protocol.

[XX] **(1) RESEARCH and/or HERD/FLOCK/BREEDING activities that support research:**
I certify that there is demonstrable evidence that this research will contribute to the future well being of humans and animals, that any discomfort or injury will be limited to that which is unavoidable, and that anesthetics, analgesics and tranquilizing drugs will be used whenever indicated and appropriate to minimize discomfort to the animals. I am aware of current research in my field. The proposed experiments do not unnecessarily duplicate earlier efforts. Painless alternatives to this work either do not exist or would not accomplish my research goals. I further certify that these statements are true and the protocol stands as the original OR is essentially the same as found in the grant application or program/project. The IACUC will be notified in writing immediately of any changes in the proposed project, or personnel, relative to this application prior to proceeding with any animal experimentation. I will not proceed with animal experimentation until approval by the IACUC is granted.

[ ] **(2) TEACHING/TRAINING and/or HERD/FLOCK/BREEDING activities that support teaching/training:**
I certify that the animal demonstrations and labs are an essential part of a well balanced curriculum. The use of animals has been carefully considered. I have considered the use of alternatives to the proposed procedures which cause more than momentary pain or distress to the animals. The minimum number of animals required to provide the quality of instruction necessary will be used in this course. I certify that the information in this application is essentially the same as contained in the course outline and a copy of the laboratory exercises using animals is on file at the Compliance Office.

BAB

Signature: Principal Investigator/Instructor — Date: 10/24/02

As a reminder, have you ...

- ☛ checked that all sections that pertain to your work have been completed, checked-off appropriate boxes, justified and provided detailed answers, and
- ☛ signed and dated the form.

Don't forget...

- ☛ to renew your protocol on a yearly basis, if your work takes longer than 1 year to complete.
- ☛ to inform the IACUC in writing and get approval when you make changes or revisions to the application.

Thank you for your time and cooperation in completing this form.

Submit the original and two copies
Please type or print legibly

Attachment No. 1

Institutional Animal Care and Use Committee

Office of Research Administration Funding Agency Information Form

Please complete this form if this project is being funded.

Principal Investigator:_____

Sponsoring/Funding Agency:__

Agency Mailing Address:_____

Agency Telephone Number:____

NOT APPLICABLE

This animal use protocol is applied

Title of Grant Application/Proposal:_

_____

_____

Grant/Proposal Identification Number

Title of Animal Use Protocol Application:__

_____

_____

Animal Use Protocol Number
(assigned by IACUC Compliance Office)

Signature of Principal Investigator/Instructor

Date

University of Hawai'i at Mānoa

Institutional Animal Care and Use Committee
University Compliance Officer
2538 The Mall · Snyder Hall 410 · Honolulu, Hawai'i 96822
Telephone: (808) 956-4446 · Facsimile: (808) 956-4448

IACUC MEETING DATES FOR CALENDAR YEAR
January 1 - December 31, 2000

Month	Closing Date for Month[1]	IACUC Meeting Date[2]
January	January 12	January 20
February	February 9	February 17
March	March 8	March 16
April	April 12	April 20
May	May 10	May 18
June	June 14	June 22
July	July 12	July 20
August	August 9	August 17
September	September 13	September 21
October	October 11	October 19
November	November 8	November 16
December[3]	November 29	December 7

1 Protocols from PIs are due on the second Wednesday of each month by 3:00 PM.
2 Committee meets on the third Thursday of each month.
3 Earlier closing and meeting dates due to winter semester break and holiday period.

U.S. GOVERNMENT PRINCIPLES FOR THE UTILIZATION AND CARE OF VERTEBRATE ANIMALS USED IN TESTING, RESEARCH, AND TRAINING

The development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals requires in vivo experimentation with a wide variety of animal species. Whenever U.S. Government agencies develop requirements for testing, research, or training procedures involving the use of vertebrate animals, the following principles shall be considered; and whenever these agencies actually perform or sponsor such procedures, the responsible Institutional Official shall ensure that these principles are adhered to:

I. The transportation, care, and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) and other applicable Federal laws, guidelines, and policies.*

II. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.

III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and in vitro biological systems should be considered.

IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.

V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

VI. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.

VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.

VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.

IX. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal care and use committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

*For guidance throughout these Principles, the reader is referred to the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources, National Academy of Sciences.

Click on BACK button above to return to the previous page

Policy #11 --- Painful/Distressful Procedures --- April 14, 1997

References: AWA Sections 13(a)(3), 13(a)(7), 13(e)(2, 3) and 9 CFR, Part 2, Sections 2.31(d)(1)(i,ii,iii,iv), 2.31(e)(4), 2.33(b)(4) and 9 CFR, Part 3, Section 3.6(b)(5,6,7)

History: Replaces letters dated May 8, 1992, November 7, 1991, November 9, 1990, and March 1, 1990.

Justification: Provides requested guidance. Procedures involving animals will avoid or minimize discomfort, distress and/or pain.

Policy:

A painful procedure is defined as any procedure that would reasonably be expected to cause more than slight or momentary pain and/or distress in a human being to which that procedure is applied. The Institutional Animal Care and Use Committee (IACUC) is responsible for ensuring that investigators have appropriately considered alternatives to any procedures that may cause more than slight or momentary pain or distress. A written narrative description of the methods and sources used to search for alternatives must be provided. Where specific testing procedures are required by Federal law, the CFR references or other legal guidelines requiring them should be noted.

Examples of procedures that can be expected to cause more than momentary or slight pain include, but are not limited to, the following:

- **Terminal Surgery** is considered a painful procedure which is alleviated by anesthesia.
- **Freund's Complete Adjuvant** used for antibody production may cause results ranging from momentary or slight pain to severe pain depending on the product, procedure, and species.
- **Ocular and Skin Irritancy Testing.** The dosing procedure itself is generally not painful but the reaction caused by the product being tested may cause pain.

Examples of procedures that may cause more than momentary or slight distress include, but are not limited to, the following:

- **Food or water deprivation** beyond that necessary for normal presurgical preparation.
- **Noxious electrical shock** that is not immediately escapable.
- **Paralysis or immobility** in a conscious animal.

Many procedures, including any of those in the lists above, may cause both pain and distress. An example of a procedure that can be expected to cause more than momentary or slight pain as well as distress would be a study involving extensive irradiation.

Animals exhibiting signs of pain, discomfort, or distress such as decreased appetite/activity level, adverse reactions to touching inoculated areas, open sores/necrotic skin lesions, abscesses, lameness, conjunctivitis, corneal edema, and photophobia are expected to receive appropriate relief unless written scientific justification is provided in the animal activity proposal and approved by the IACUC.

Research facilities must have a mechanism in place for ensuring that animals are reported in the appropriate pain category on the annual report (APHIS Form 7023). Individual animals that do not experience pain/distress from testing procedures should be reported in column C. Individual animals

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Policy #12 --- Written Narrative for Alternatives to Painful Procedures --- April 14, 1997

References: AWA Section 13(a)(3)(B) and 9 CFR, Part 2, Section 2.31 (d)(1)(ii)

History: Provides requested guidance.

Justification: The Principal Investigator must provide a written narrative of the sources consulted to determine whether or not alternatives exist to procedures which may cause pain or distress.

Policy:

Consideration of alternatives to each procedure which may cause pain or distress must state sources consulted, such as Biological Abstracts, Index Medicus, Medline, the Current Research Information Service (CRIS), and the Animal Welfare Information Center (AWIC).

The minimal written narrative should include: the databases searched or other sources consulted, the date of the search and the years covered by the search, and the key words and/or search strategy used by the Principal Investigator when considering alternatives or descriptions of other methods and sources used to determine that no alternatives were available to the painful or distressful procedure. The narrative should be such that the IACUC can readily assess whether the search topics were appropriate and whether the search was sufficiently thorough.

Reduction, replacement, and refinement (the three R's) must be addressed, not just animal replacement.

Click on BACK button above to return to the previous page