"Living Tags" For Sea Turtles

Report to SW Fisheries Center

National Marine Fisheries Service

Honolulu, Hawaii

Contract #80-ABH-00062

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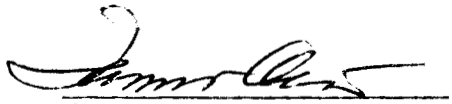
Since this report has been prepared under contract, the statements, findings, conclusions, and recommendations herein are those of the contractors and do not necessarily reflect the view of the National Marine Fisheries Service.



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by

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Introduction

This report describes work carried out under NMFS Contract No. 80-ABH-00062 in Honolulu, Hawaii during the period 9 September to 15 October 1980. The work, with hatchlings of the green sea turtle (Chelonia mydas), was authorized under U.S. Fish & Wildlife Service Sea Turtle Permit No. PRT2-6842 and was done under the supervision of Technical Monitor William Gilmartin who made the basic decisions on types of treatments, numbers of animals in each treatment group, and times when the Contractors performed the operations. Maintenance regimens are determined by NMFS under a separate contract with Sea Life Park where the subject animals were established and where they will be held for a post-operative year of monitoring. Aside from the post-operative inspections by the Contractors, reported here, all monitoring will be handled by NMFS staff. By agreement, this present paper constitutes the final written report on Contract No. 80-ABH-00062.

Starting date on this contract was delayed beyond the proposed date of 1 September 1980 because the first group of animals was not available until September 8.

Background Information

For the past 30 years, the present Contractors and other students of sea turtle ecology have been unable to develop a clear understanding of many aspects of the life history of these animals because of our inability to mark the 20-30 g hatchlings so that they can be identified years later when they may weigh as much as 100 kg.

Almost every conceivable device and method has been tried. No ordinary, externally applied tag or band can meet the need because of the long span of time and the animals' enormous change in body mass. Mutilation markings do not serve because of variable healing, regeneration, and replication by natural accidents. Hughes (1975) notched the shells of 17,000 baby loggerhead sea turtles and reported 6 "probable" returns; Bustard (1979) reports a single positive return from 65,000 green sea turtle hatchlings marked in a massive program. Tattooing is soon absorbed or obscured. Branding by either heat or freezing is unsatisfactory because wound-healing processes stimulate melanocyte re-invasion and growth obscures the branding sites. Magnet implants did not work for Carr (1967). Radioactive tagging methods have not proved to be practical. Injection of rare metals for later detection by neutron activation analysis has been tested (Forbes 1972), but has little practical promise. One of the present investigators worked with immunologists (See Benedict and Pollard 1972) exploring, without success, the possibility of developing

an immunological "living tag" analogous to the long-lived smallpox immune bodies induced in humans by vaccination. We remain about where we started, with no direct approach to the problem.

The prime requirement of the needed marking system is that it endure over a long period, remaining clearly visible and accurately identifiable under field conditions despite great increases in animal body size. Obviously, the mark must also have minimal unfavorable effect on the behavior, mobility, dispersal, health, and other elements of the life history of the marked individual (Plummer 1979, Delaney 1978).

Some of the most important remaining gaps in our understanding of sea turtle biology, potentially resolvable given the existence of an efficient method of lifetime tagging and critically important to decision-makers in conservation management programs, are the following:

1) We are unable to test the hypothesis that sea turtles "imprint" on their natal beach and return there to nest as adults. This has much bearing on national policies toward native turtle populations, etc.

2) We have no direct means for determining the distributions of subadult populations relative to their beaches of origin.

3) We have almost no "hard" quantitative data on growth rates or maturation times in the wild--a basic need for evaluation of assets.

4) We cannot, at present, gauge the success of otherwise-promising "headstart" programs, now being carried out,

intended to reduce juvenile mortality and augment natural recruitment rates.

Much of what we do know about turtle behavior and movements in the sea has been derived from the chance retention of metal tags which have a high, variable, and unpredictable rate of loss (Green 1979). The total expenditure in time, labor, and dollars for each datum obtained is very high; efforts must be made to reduce unit costs and increase information retrieval. If proven usable, the "living tag" approach should go far toward improving this situation.

Materials and Methods

All work described here was done at Sea Life Park, Makapuu, Oahu, Hawaii. The hatchlings are presently installed in six holding tanks measuring 72 in x 22 in, and a circular tank measuring 72 inches in diameter. They are kept in no more than 8 in of water (4-8 in) to insure that they are able to see and reach the food at the bottom of the tanks.

Fifty-three hatchlings were brought to Honolulu on 8 September. The group consisted of one clutch of identifiable siblings (hatched on 6 September 1980), plus a larger number of animals salvaged by digging into recent emergence sites for individuals which had failed to reach the surface with the main body of their siblings. This larger group of culls came from nests which the field party considered to have hatched during 5, 6, and 7 September. For purposes of age notation in this report, all are considered to have begun life

on the surface on 6 September 1980. Because many of the animals appeared weak and unlikely to survive, it was decided to postpone initiation of experimental work for about one week in order to allow them a period of stabilization.

A second shipment of 182 hatchlings arrived on 19 September. This group consisted of 110 siblings that emerged on the night of 18 September from a marked nest laid by tagged, wild female #200, and 72 siblings from an unmarked nest which was discovered while the young were emerging (also on 18 September). On their first night in Honolulu, the tank holding the clutch of 110 siblings overflowed; one hatchling drowned in the tank drain, and 14 others were washed out with the overflow. Two of the 14 were never recovered; the 12 recovered individuals were inadvertently placed with the smaller group of siblings from the unmarked nest. This second shipment of animals, in contrast to the first, appeared to be normally active and robust. The first grafts on these were done four days after their arrival.

Numbered metal tags (National Band and Tag Co. Monel metal tags, series #1005, size 1) were affixed to the last, right marginal scute of all experimental animals. The animals were divided into three treatment groups and one tagged-control group. Sixty-three animals were reserved as controls; 59 were given reversed-plug grafts; 50 were given disk grafts, and 50 were given gouge grafts (see below). All graft wounds were treated with Neosporin (an antibiotic ointment produced by Burrows Wellcome Co., N. Carolina) before

the animals were returned to the water. A "register" (Appendix I) is attached to this report and presents, in tabular form, details of treatment for each individual animal and dates of mortalities through 15 October 1980.

Tagged controls. At the start of the grafting work, on 17 and 18 September, 13 animals from the first shipment were designated as controls. These were not randomly selected, but were those animals left after selection of the 30 most vigorous individuals for treatment.[1] The 50 controls from the second shipment were randomly selected, as were all of the animals from this shipment, whatever their treatment designation.

Reversed-plug grafts. A cutter (2 mm internal diameter) from an ordinary leather punch was machined to a reduced wall thickness so as to create as straight-walled a cylinder as possible. The cutting edge was finely honed and the cutter was mounted in a handle made from a short length of slender PVC pipe. In performing the grafting operation, the animal was held upside down, with the dorsal surface of the scute to be cut resting on a firm cutting surface. The cylindrical cutter was then used to cut a plug of tissue from that scute by holding the cutter approximately perpendicular to the ventral surface of the scute while rotating it back and forth through an arc of about 180°. Care was always taken not to

1. Procedure requested by NMFS Technical Monitor.

apply a crushing pressure on the tissues, but to use only enough driving force to cause the sharp cutting edge to excise a cleanly cut plug. The plug was then reversed dorsoventrally and reinserted into its own hole. Some of the animals received double grafts; i.e., a second plug was cut out of the same scute and reinserted as described. A number of the second plugs were cut immediately after the first, which was thereby pushed farther into the cylinder, both ending up encased within the cutter with the first plug on top of the second. Both were removed without difficulty by demounting the cutter from the PVC handle and pushing against the first plug with a blunted hypodermic needle. In turn, each was removed, reversed, and inserted into its own hole. The grafts were then sealed with a fast-drying cyanoacrylate surgical adhesive and treated with antibiotic ointment.

All but two reversed-plug grafts were made on the last, left marginal scute. Animal #47 was grafted on the penultimate, left marginal scute because the last marginal scute was too small to take the two grafts done on this animal. Animal #124 was mistakenly grafted on the penultimate, left marginal scute. All of the 30 animals experimentally treated from the first shipment were given reversed-plug grafts--15 single and 15 double grafts. Of these, eight of the double, and seven of the single, grafts were sealed with Permabond (Polysciences, Inc., Warrington, PA), a veterinary surgical adhesive. Seven double, and eight single grafts were sealed with Histoacryl blau, a cyanoacrylate adhesive made by B.

Braun Melsungen, W. Germany, for use in human, internal surgery but not yet approved for use in this country. It was thought that this was a good opportunity to test the relative effectiveness of the two adhesives because, whereas Histoacryl blau had been used before and found effective by the Contractors, Permabond is much cheaper and more readily accessible in this country.

Twenty-nine hatchlings from the second shipment were given reversed-plug grafts on 30 September 1980. Of these, 15 received single grafts and 14 received doubles. All of these were sealed with Permabond. (See register for details of treatment given each individual animal.)

Disk grafts. A 3 mm diameter Keyes dermal punch (K/S Instrument Corp., Clifton, NJ #46003 0), held perpendicular to the keratin surface, was rotated to cut a circle through to the subdermal tissue at a selected site on the plastron. The animal was then inverted in the operator's hand and a similar circle was cut in the carapace at the site selected for grafting. The carapace disk was cut free using a small scalpel (Bard-Parker 15C or 15), set aside for later use, and the plastral disk was then similarly cut free and immediately transferred to the prepared site on the carapace. After firm pressure was applied on the scion with the pad of a finger, or using an absorbing tissue if there was fluid to be cleared, the graft seam was sealed with a cyanoacrylate adhesive (see register). The disk removed from the carapace was then placed

in the plastral wound and the seam was sealed in the same manner as for the dorsal graft.

Graft site (carapace) and scion site (plastron) selection was based on the hypothesis that scute growth occurs mainly, if not entirely, at the seams. Therefore, scions were taken from a single scute from an area tangential to a seam, or from two scutes across a common seam. Consideration was also given to selecting a donor site, on the plastron, where the application of vertical pressure on delicate internal organs could be avoided, guarding against causing undue compression that might be damaging. For the most part, the lateral plane of the second left plastral scute was used. On the carapace the graft was generally placed across the seam between right costal scutes 1 and 2, or within right costal scute 2 at a site tangent to its seam with right costal scute 1 (see register). All disk grafts were done on 23 September 1980.

Gouge grafts. A 5 mm diameter Keyes dermal punch (K/S Instrument Corp., Clifton, NJ #46005 5) was employed with an angular orientation instead of perpendicular to the keratin surface as in making disk grafts. A gouge was cut from the carapace by rotating the punch in alternating arcs of approximately 90° while applying sufficient forward force to slice through the tissue. The wound was then filled with a similar gouge of tissue cut from the plastron in the same manner. The same considerations in selecting dorsal and ventral treatment sites apply as discussed under "Disk grafts." On 18 of the animals treated with this type of graft, the plastral wound

was covered with surgical adhesive only. This was done to compare healing rates of wounds covered with the animal's tissue and those sealed only with adhesive (see register). All gouge grafts were done on 24 September 1980.

Post-operative inspections. The Contractors made two post-operative checks, at which time they examined each animal individually. The first check, on 30 September, was on the 12th- and 13th-day post-operative for the 30 animals with reversed-plug grafts from the first shipment, and the 6th- and 7th-day post-operative for the gouge and disk graft groups respectively. At this time the last 29 reversed-plug grafts were also done, and the 50 controls from the second shipment were designated. The second check was done on 15 October 1980.

Results and Discussion

Effects of grafting on survivorship. A major concern in this work was the question of whether or not the grafting operations would affect survival in the young turtles. For purposes of the experiment, it was assumed that significant ill effects from grafting (deaths, apparent general morbidity, infection foci at graft sites, and biting damage near the contrastingly shaded grafts) would be detectable by comparison with the 63 control animals. These controls included representation from both shipments and they were distributed through all the holding tanks during post-operative maintenance. Because of the different histories of the two shipments from French Frigate Shoals, we will consider them separately.

As explained previously, the first shipment included a number of obviously disadvantaged animals. Ten individuals died between the time of arrival in Honolulu and the beginning of our work on 17 September. By 15 October (27th/28th day post-operative), nine more animals had died--5 of 30 (16.7%) reversed-plug grafted animals and 4 of 13 (31%) tagged controls.[2] None of the survivors were noted as being particularly emaciated or showing other signs of morbidity. Almost all (both grafted and control individuals) showed some degree of biting damage on the tail and trailing edges of the flippers, but there was no apparent correlation of biting damage with graft sites. There were no conspicuous fungal infections on shell or skin.

Five of the animals from the second shipment had also died at the time of the second post-operative inspection on 15 October (15 to 23 days post operative).[3] Three of the dead individuals had been tagged, but not yet assigned an experimental status; the other two had received gouge grafts. Therefore, we calculate the mortality in this group of experimental animals as 4% for gouge grafts and 0% for disk grafts, reversed-plug grafts,

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2. Contrary to what might have been expected, mortality was higher in the group of siblings from the emerging nest than in the miscellaneous group of non-emerged hatchlings rescued from older nests. Seven of the nine deaths mentioned above were in the group of siblings (4 controls and 3 grafted animals). Two of the 10 deaths which occurred after arrival in Honolulu, but before work began, were also from these siblings, and 4 deaths had occurred before departure from French Frigate Shoals. The field party reported that the nest contained only about 40 eggs, of which approximately half hatched--further indications of a generally weak clutch.

3. All five deaths occurred in the holding tank population which included the 12 animals rescued after a tank overflow entailing a severe drop to the floor and an undetermined number of hours out of water. It is possible, but unprovable, that all five deaths could have been from this stressed group of individuals.

and controls. No emaciation or morbidity was noted, there was no apparent fungal infection, and no conspicuous biting damage.

A comparison of the condition of the animals in each shipment group, made from notes taken during the two post-operative inspections, is presented in Table 1.

In summary, there does seem to be a real difference in survivability between the animals in the two different shipments, with the hatchlings from the large, normal nests being the more vigorous, but there is no clear evidence from either shipment group of increased mortality risk due to the grafting treatment.

Graft success. Of 25 animals in the first shipment which had been given reversed-plug grafts and were still surviving on 15 October (almost a month after surgery), 8 (32%) showed complete or nearly complete loss of their grafts. Six of the eight failures were double plug operations--almost half of the 13 surviving animals which had been given double plugs. The other two failures were from the group of 12 surviving animals given single plug operations.

Of 29 animals from the second shipment given reversed-plug grafts (15 single and 14 double plugs), all were surviving on 15 October (15th day post-operative). None had lost their grafts and only four were noted as showing any signs of possible future graft loss.[4]

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4. In almost all of these animals the pale, upper surface of the plug graft(s) had darkened so markedly that there was no longer any marked color contrast between graft and adjacent host tissues (more contrast remained on the underside of the shell). Whether the darkening of the pale, plug keratin is due to tissue degeneration or to normal pigment cells, and, if normal, whether it will persist or return to a lighter shade at a later time, cannot be determined at this time. Sacrifice of one or two animals for histological studies would shed some light on this matter.

Table 1. Comparison of Two Groups of C. mydas Hatchlings Following Grafting.

	SHIPMENT I 12/13 DAYS POST-OPERATIVE (on Sept.30,1980)	SHIPMENT II 15/22/23 DAYS POST-OPERATIVE (on Oct.15, 1980)	SHIPMENT I 27/28 DAYS POST-OPERATIVE (on Oct.15, 1980)
Total mortality (non-accidental) since arrival	14 of 53 (26%)	5 of 182 (2.7%)	19 of 53 (36%)
Mortality since beginning of experiment	4 of 43 (9%)	2 of 179 (1.1%)	9 of 43 (21%)
Deaths, Controls	3 of 13 (23%)	0 of 50 (0%)	4 of 13 (31%)
Deaths, Rev.-Plugs	1 of 30 (3.3%)	0 of 50 (0%)	5 of 30 (17%)
Deaths, Disk Grafts	-----	0 of 50 (0%)	-----
Deaths, Gouge Grafts	-----	2 of 50 (4%)	-----
Number noted with debility, fungus, biting damage, etc.	6 of 39 (15%)	0 of 179 (0%)	21 of 34 (62%)

At this stage it is impossible to say whether the differences noted between the two different reversed-plug groups is a function of time following the operation, or of the condition of the animals. It does seem reasonable to predict that double plugs in close proximity are a poorer risk than are single plugs.

The results obtained with reversed-plug grafts here are superior to those obtained with a group of loggerhead (Caretta caretta) hatchlings in Florida earlier this year. In the latter case, the punching was done with an unmodified leather punch, without rotation of the cutting edge and causing considerable compression in the plug tissue. This made the plug tapered, and it had a poor fit when replaced in the hole from which it was cut. The machined, thin-walled cutting tool, employed separately from the plier mechanism and capable of rotation while cutting, clearly does a better job and produces a better fit of the plug scion in its hole. It should be noted, however, that the tool employed in this case, machined to minimum wall thickness with the above features in mind, showed two problems: there was a tendency for the extremely thin cutting edge to double over after repeated use on the tough carapace tissues, and the necessary sharpening at intervals tended to produce a wavy cutting edge which required full 360° rotation in order to cut the plug free.

All 50 disk-grafted animals showed retention of both dorsal and ventral scions, and the 48 surviving gouge-grafted animals showed equally good retention of scions, as well as uncomplicated healing of the 18 plastral wounds which were left with only a

cement seal. No particularly noteworthy details were observed in the cases of the black carapacial scions on the pale plastral surfaces, but the pale scions on the animals' backs commonly showed differentiation into lighter and darker areas. It is our impression that the darker areas represent rapid tissue growth, visible through the lightly pigmented tissue of the graft scion. Whether this represents "take" of the graft and not wound-healing activity from surrounding tissues cannot be determined at this stage without sacrifice of a few animals for histological studies.

Disk or gouge plastral scions cut to include a seam between scutes and placed in a comparable cross-seam wound in the carapace, so that scion and host seams were aligned, seemed to show signs of splitting in the grafted plastral scion to match a degree of differential growth between the host-site carapace scutes. If this interpretation is accurate, and if there is the inferred predominance of keratin production from the anterior and medial edges of a carapace scute, one might expect the portion of the pale scion growing on the posterior edge of right costal scute 1 to erode away with time and disappear, while the other portion incorporated into the leading edge of right costal scute 2 might continue to produce pale tissue. Such a differential in growth rates, comparable to nail growth in humans, might then produce a pale streak, instead of a pale spot, on the carapace (assuming that the paleness of the grafted scion continues to be expressed throughout life).

Operation timing. Another feature of this process, which will be important if this technique is to be used in the field, is the

time necessary to complete each grafting operation. To be meaningful, thousands of newly emerged hatchlings would have to be treated, preferably on the nesting beach and under conditions of darkness, blowing sand, etc., always striving toward the ideal of handling a hatchling quickly, then setting it down to continue its normal procedure of sea-finding without further molestation. With this in mind, we kept time records of several short series of grafts. Admittedly, the laboratory conditions under which we worked are far easier than may be expected under normal beach conditions, but the exercise at least served to check on the possibility that the operations were too time-consuming, even under ideal conditions, to permit consideration of a massive field program.

A number of reversed-plug operations, timed individually, took from 1.5 to 5 minutes each. It is our impression that, with experience, 20 to 30 single-plug grafts per hour may be accomplished with ideal cutting tools.

Disk and gouge grafts (all reciprocal, involving two sites) were timed individually and in group series. In each test series, one Contractor did all animal-handling for the grafts, picking up the subject, making the cuts, transferring the scions, blotting, cementing, treating with ointment, and returning the subject to a container; the other Contractor affixed tags (not part of the projected field process) and served as recorder. Seven, 10, and 25 disk grafting operations took 13, 16, and 51 minutes (averages of 1.9, 1.6, and 2.0 minutes per hatchling). Thirteen gouge grafts took 28 minutes (average 2.2 minutes per hatchling). Every

effort was made to work at "usual" speed; in the case of the 25-animal series, the decision to record time was made at the end of the series when it was realized that the beginning time was known and the group could be used for this purpose.

We have every reason to believe that, with experience, more than 30 animals could be handled per operator per hour, allowing a 10-minute relaxation break each hour.

Concluding Remarks

We offer the following tentative, preliminary "conclusions":

1. There is no evidence that the grafting operation increases mortality rates or in any other way significantly disadvantages green turtle hatchlings.
2. The reversed-plug type of graft seems clearly less useful than disk or gouge grafts. Double reversed-plugs in close proximity to each other are less desirable than are single plugs.
3. There is no significant difference between Permabond (available in the U.S. and cheaper) and Histoacryl blau (available only by special import, more expensive) in drying time or efficacy as a wound-sealer.
4. The unfilled plastral wound left in a single-graft operation, sealed with the same surgical cement used to hold the scion in place on the carapace, presents no complications. It is not necessary to fill the plastral wound with a scion from the carapace, and this will save operation time. On the other hand, the complete, reciprocal operation takes little

longer and one can readily visualize the value of being able to confirm that a pale-spotted animal was a grafted individual by finding a dark spot on the plastron.

5. Without objective evidence from controls, the Contractors believe that smearing germicidal ointment over all treated areas is a desirable insurance measure in captive situations where the bacterial load to which the animals are exposed may be high.

6. It is strongly recommended that the monitoring through the coming year include weekly "in-hand" examination of each experimental animal, with replacement of all lost tags so that a complete history of each individual may be compiled.

7. Two animals from each treatment group of the population from the second shipment (19 September 1980) should be sacrificed to provide material for detailed histological examination of the grafts at their present stage. The Contractors are prepared to do this work, using a special fixative to prepare the tissues for electron microscopy. We would send the material to a colleague in Scotland who is an expert in both light and electron microscopy. There would be no cost to NMFS for this.

8. The operations can be performed with sufficient speed to accommodate the needs of a field marking program.

Acknowledgements

We wish to thank Dr. Wm. Gilmartin of NMFS, Honolulu for the courteous, considerate treatment extended to us, for seeing that the animals for this work were made available, and for providing transportation to and from Sea Life Park. For assistance with the work, and for chauffeur service, we thank Anjanette Perry who will continue to monitor the animals during the next 12 months.

We extend our aloha to the members of the Sea Life Park Reef Tank staff for their help and unfailing patience and good cheer through all the interference and obstruction that our work in their facility surely produced for them.

We wish to mention, especially, the Bishop Museum, where office space and equipment were made available to us for those aspects of our work which required them. We thank Dr. Creutz and his staff for their courtesy.

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Literature Cited

Benedict, A. A., and L. W. Pollard. 1972. Three classes of immunoglobulins found in the sea turtle, Chelonia mydas. Folia Microbiol. 17:75-78. "Brief Reports."

Bustard, H. R. 1979. Population dynamics of sea turtles. Pages 523-540 in M. Harless and H. Morlock (eds.), Turtles: Perspectives and research. John Wiley & Sons, NY. 695 pp.

Carr, A. 1967. So excellent a fishe. Natural History Press, Garden City, NY. 248 pp.

Delaney, M. J. 1978. Introduction: Marking animals for research. Pages 2-10 in Stonehouse, B. (ed.), Animal marking: Recognition marking of animals in research. Macmillan Press, Ltd., London. 257 pp.

Forbes, A. M. 1972. Distribution of injected europium in hatchling Chelonia mydas L. and its considerations as a tag. Master's thesis, University of Rhode Island.

Green, D. 1979. Double tagging of green turtles in the Galapagos Islands. Marine Turtle Newsletter IUCN/SSC No. 13:4-9.

Hughes, G. R. 1975. The marine turtles of Tongaland. 8. Lammergeyer 22:8-18.

Plummer, M. V. 1979. Chapter 2. Collecting and marking. Pages 45-60 in M. Harless and H. Morlock (eds.), Turtles: Perspectives and research. John Wiley & Sons, NY. 695 pp.

Pig (1)	Type of Treatment (2)	No. of Grafts (3)	Dorsal Scute(s) Treated (4)	Adhesive (5)	Date of Operation (6)	COMMENTS
4	R. Plug	D	last LM	H	9/17/80	Dead 10/1/80
5	R. Plug	S	last LM	P	9/18/80	Dead 10/11/80
6	Control	-	-----	-	9/17/80	Dead 9/18/80
7	R. Plug	S	last LM	P	9/18/80	
8	Control	-	-----	-	9/17/80	
9	Control	-	-----	-	9/17/80	Dead 9/18/80
10	R. Plug	S	last LM	P	9/18/80	Dead 9/20/80
11	Control	-	-----	-	9/17/80	Dead 9/19/80
12	Control	-	-----	-	9/17/80	Dead 10/13/80
13	Control	-	-----	-	9/17/80	
14	Control	-	-----	-	9/17/80	
15	R. Plug	S	last LM	H	9/17/80	
16	Control	-	-----	-	9/17/80	(note 7)
17	Control	-	-----	-	9/17/80	(note 7)
18	R. Plug	S	Last LM	H	9/17/80	
19	R. Plug	S	last LM	P	9/18/80	
20	R. Plug	D	last LM	P	9/18/80	
21	Control	-	-----	-	9/17/80	
22	R. Plug	S	last LM	H	9/17/80	
23	R. Plug	S	last LM	H	9/17/80	
24	R. Plug	S	last LM	H	9/17/80	
26	R. Plug	S	last LM	H	9/17/80	
27	R. Plug	S	last LM	H	9/17/80	
28	R. Plug	D	last LM	P	9/18/80	
29	R. Plug	D	last LM	P	9/18/80	
30	Control	-	-----	-	9/17/80	(note 7)
31	R. Plug	D	last LM	H	9/17/80	
32	R. Plug	S	last LM	H	9/17/80	Dead 10/2/80
33	R. Plug	S	last LM	P	9/18/80	
34	R. Plug	D	last LM	P	9/18/80	
35	R. Plug	S	last LM	P	9/18/80	
36	Control	-	-----	-	9/17/70	
37	Control	-	-----	-	9/17/80	
38	R. Plug	D	last LM	P	9/18/80	
40	R. Plug	D	last LM	H	9/17/80	
40	R. Plug	D	last LM	P	9/18/80	
41	R. Plug	D	last LM	H	9/17/80	
42	R. Plug	D	last LM	H	9/17/80	
43	R. Plug	D	last LM	H	9/17/80	(note 8)
44	R. Plug	S	last LM	P	9/18/80	(note 9)
45	R. Plug	D	last LM	H	9/17/80	Dead 10/8/80
46	R. Plug	D	last LM	P	9/18/80	(note 10)
47	R. Plug	D	last LM	P	9/18/80	(note 11)
48	Disk	R	RC2	H	9/23/80	
49	Disk	R	N2/RC1/RC2	H	9/23/80	
50	Disk	R	RC1	H	9/23/80	
51	Disk	R	RC1	H	9/23/80	
52	Disk	R	RC1	H	9/23/80	
53	Disk	R	RC1	H	9/23/80	
54	Disk	R	RC1/RC2	H	9/23/80	
55	Disk	R	RC1	H	9/23/80	
56	Disk	R	RC1	H	9/23/80	
57	Disk	R	RC1	H	9/23/80	
58	Disk	R	RC1	H	9/23/80	

Tag No. (1)	Type of Treatment (2)	No. of Grafts (3)	Dorsal Scute(s) Treated (4)	Adhesive (5)	Date of Operation (6)	COMMENTS
59	Disk	R	RC1	H	9/23/80	
60	Disk	R	N2/RC1/RC2	H	9/23/80	
61	Disk	R	RC1	H	9/23/80	
62	Disk	R	N2/RC1/RC2	H	9/23/80	
63	Disk	R	RC1	H	9/23/80	
64	Disk	R	N2/RC1	H	9/23/80	
65	Disk	R	RC1	H	9/23/80	
66	Disk	R	RC1	H	9/23/80	
67	Disk	R	RC1	H	9/23/80	
68	Disk	R	RC1	H	9/23/80	
69	Disk	R	RC1	H	9/23/80	
70	Disk	R	RC1	H	9/23/80	
71	Disk	R	RC1	H	9/23/80	
72	Gouge	R	RC1	P	9/24/80	
73	Gouge	R	RC1	P	9/24/80	
74	Disk	R	RC2	H	9/23/80	
75	Gouge	R	RC1	P	9/24/80	
76	Gouge	S	RC1	P	9/24/80	
77	Gouge	S	RC1	P	9/24/80	
78	Gouge	R	RC1	P	9/24/80	
79	Gouge	R	RC1	P	9/24/80	
80	Gouge	R	RC1	P	9/24/80	
81	Gouge	R	RC1	P	9/24/80	
82	Gouge	R	RC1	P	9/24/80	
83	Gouge	R	RC1	P	9/24/80	
84	Gouge	R	RC1	P	9/24/80	
85	Gouge	S	RC1	P	9/24/80	
86	Gouge	R	RC1	P	9/24/80	
87	Gouge	S	RC1	P	9/24/80	
88	Gouge	R	RC1	P	9/24/80	
89	Gouge	R	RC1	P	9/24/80	
90	Gouge	R	RC1	P	9/24/80	
91	Gouge	S	RC1	P	9/24/80	
92	Gouge	R	RC1	P	9/24/80	
93	Gouge	S	RC1	P	9/24/80	
94	Gouge	S	RC1	P	9/24/80	
95	Gouge	R	RC1	P	9/24/80	
96	Gouge	S	RC1	P	9/24/80	
97	Gouge	S	RC1	P	9/24/80	
98	Control	-	-----	-	9/26/80	
99	Control	-	-----	-	9/26/80	
00	Control	-	-----	-	9/26/80	
01	R. Plug	S	last LM	P	9/30/80	
02	Control	-	-----	-	9/26/80	
03	R. Plug	D	last LM	P	9/30/80	
04	R. Plug	D	last LM	P	9/30/80	
05	R. Plug	S	last LM	P	9/30/80	
06	Control	-	-----	-	9/26/80	
07	Control	-	-----	-	9/26/80	
08	R. Plug	D	last LM	P	9/30/80	
09	Control	-	-----	-	9/26/80	
10	R. Plug	S	last LM	P	9/30/80	
11	R. Plug	S	last LM	P	9/30/80	

Pig No.	(1) Type of Treatment	(2) No. of Grafts	(3) Dorsal Scute(s) Treated	(4) Adhesive	(5) Date of Operation	(6) COMMENTS
12	R. Plug	D	last LM	P	9/30/80	
13	R. Plug	D	last LM	P	9/30/80	
14	Control	-	-----	-	9/26/80	
15	R. Plug	S	last LM	P	9/30/80	
16	R. Plug	D	last LM	P	9/30/80	
17	R. Plug	S	last LM	P	9/30/80	
18	Control	-	-----	-	9/26/80	
19	R. Plug	D	last LM	P	9/30/80	
20	Control	-	-----	-	9/26/80	
21	Control	-	-----	-	9/26/80	
22	R. Plug	S	last LM	P	9/30/80	
23	R. Plug	D	last LM	P	9/30/80	
24	R. Plug	S	penult. LM	P	9/30/80	
25	R. Plug	D	last LM	P	9/30/80	
26	R. Plug	D	last LM	P	9/30/80	
27	Control	-	-----	-	9/26/80	
28	R. Plug	S	last LM	P	9/30/80	
29	Control	-	-----	-	9/26/80	
30	R. Plug	S	last LM	P	9/30/80	
31	R. Plug	S	last LM	P	9/30/80	
32	R. Plug	D	last LM	P	9/30/80	
33	R. Plug	S	last LM	P	9/30/80	
34	Control	-	-----	-	9/26/80	
35	Control	-	-----	-	9/26/80	
36	Control	-	-----	-	9/26/80	
37	Control	-	-----	-	9/26/80	
38	R. Plug	D	last LM	P	9/30/80	
39	R. Plug	S	last LM	P	9/30/80	
40	R. Plug	D	last LM	P	9/30/80	
41	Control	-	-----	-	9/26/80	
42	R. Plug	D	last LM	P	9/30/80	
43	Control	-	-----	-	9/26/80	
44	R. Plug	S	last LM	P	9/30/80	
45	R. Plug	S	last LM	P	9/30/80	
1	Disk	R	RC1/RC2	H	9/23/80	
2	Disk	R	RC1/RC2	H	9/23/80	
3	Disk	R	RC1/RC2	H	9/23/80	
4	Disk	R	RC1/RC2	H	9/23/80	
5	Disk	R	RC1/RC2	H	9/23/80	
6	Disk	R	RC1/RC2	H	9/23/80	
7	Disk	R	RC1/RC2	H	9/23/80	
8	Disk	R	RC1/RC2	H	9/23/80	
9	Disk	R	RC1/RC2	H	9/23/80	
10	Disk	R	RC1/RC2	H	9/23/80	
11	Disk	R	RC1/RC2	H	9/23/80	
12	Disk	R	RC1/RC2	H	9/23/80	
13	Disk	R	RC1/RC2	H	9/23/80	
14	Disk	R	RC1/RC2	H	9/23/80	
15	Disk	R	RC1/RC2	H	9/23/80	
16	Disk	R	RC1/RC2	H	9/23/80	
17	Disk	R	RC1/RC2	H	9/23/80	
18	Disk	R	RC1/RC2	H	9/23/80	

Tag No. (1)	Type of Treatment (2)	No. of Grafts (3)	Dorsal Scute(s) Treated (4)	Adhesive (5)	Date of Operation (6)	COMMENTS
19	Disk	R	RC1/RC2	H	9/23/80	
20	Disk	R	RC1/RC2	H	9/23/80	
21	Disk	R	RC1/RC2	H	9/23/80	
22	Disk	R	RC1/RC2	H	9/23/80	
23	Disk	R	RC1/RC2	H	9/23/80	
24	Disk	R	RC1/RC2	H	9/23/80	
25	Disk	R	RC1/RC2	H	9/23/80	
26	Gouge	R	RC1/RC2	H	9/24/80	Dead 9/28/80
27	Gouge	R	RC1/RC2	H	9/24/80	
28	Gouge	R	RC1/RC2	H	9/24/80	
29	Gouge	R	RC1/RC2	H	9/24/80	
30	Gouge	R	RC1/RC2	H	9/24/80	
31	Gouge	R	RC1/RC2	H	9/24/80	
32	Gouge	R	RC1/RC2	H	9/24/80	
33	Gouge	R	RC1/RC2	H	9/24/80	
34	Gouge	R	RC1/RC2	H	9/24/80	
35	Gouge	R	RC1/RC2	H	9/24/80	
36	Gouge	R	RC1/RC2	H	9/24/80	
37	Gouge	R	RC1/RC2	H	9/24/80	
38	Gouge	R	RC1/RC2	H	9/24/80	
39	Gouge	R	RC1/RC2	H	9/24/80	
40	Gouge	R	RC1/RC2	H	9/24/80	
41	Gouge	R	RC1/RC2	H	9/24/80	Dead 9/29/80
42	Gouge	S	RC1/RC2	H	9/24/80	
43	Gouge	S	RC1/RC2	H	9/24/80	
44	Gouge	S	RC1/RC2	P	9/24/80	
45	Gouge	S	RC1/RC2	P	9/24/80	
46	Gouge	S	RC1/RC2	P	9/24/80	
47	Gouge	S	RC1/RC2	P	9/24/80	
48	Gouge	S	RC1/RC2	P	9/24/80	
49	Gouge	S	RC1/RC2	P	9/24/80	
50	Gouge	S	RC1/RC2	P	9/24/80	
51	Control	-	-----	-	9/26/80	
52	Control	-	-----	-	9/26/80	
53	Control	-	-----	-	9/26/80	
54	Control	-	-----	-	9/26/80	
55	Control	-	-----	-	9/26/80	
56	Control	-	-----	-	9/26/80	
57	Control	-	-----	-	9/26/80	
58	Control	-	-----	-	9/26/80	
59	Control	-	-----	-	9/26/80	
60	Control	-	-----	-	9/26/80	
61	Control	-	-----	-	9/26/80	
62	Control	-	-----	-	9/26/80	
63	Control	-	-----	-	9/26/80	
64	Control	-	-----	-	9/26/80	
65	Control	-	-----	-	9/26/80	
66	Control	-	-----	-	9/26/80	
67	Control	-	-----	-	9/26/80	
68	Control	-	-----	-	9/26/80	
69	Control	-	-----	-	9/26/80	
70	Control	-	-----	-	9/26/80	

Tag No.(1)	Type of Treatment(2)	No. of Grafts(3)	Dorsal Scute(s) Treated(4)	Adhesive(5)	Date of Operation(6)	COMMENTS
1	Control	-	-----	-	9/26/80	
2	Control	-	-----	-	9/26/80	
3	Control	-	-----	-	9/26/80	
4	———	-	-----	-	9/26/80	Dead 9/29/80(see note 1)
5	Control	-	-----	-	9/26/80	
6	Control	-	-----	-	9/26/80	
7	Control	-	-----	-	9/26/80	
8	———	-	-----	-	9/26/80	Dead 9/30/80(see note 1)
9	Control	-	-----	-	9/26/80	
10	Control	-	-----	-	9/26/80	
11	Control	-	-----	-	9/26/80	
12	Control	-	-----	-	9/26/80	
13	Control	-	-----	-	9/26/80	
14	———	-	-----	-	9/26/80	Dead 9/30/80(see note 1)

NOTES:

1. Tags #1, #2, #3, and #25 not used. First shipment of hatchlings received tags numbered 47 and below. Tags #48 through #145 used on larger clutch, second shipment (from female #200; all these hatchlings are siblings). Tags #146 through #200 not used. Tags #201 and above used on remainder of second shipment (mostly siblings, with accidental admixture of 12 individuals from female #200's clutch). Hatchlings #274, #278, and #300 died before being assigned to either control or treatment status. Contractors tagged #48-#97, #201-#250; NMFS staff tagged others.
2. "R. Plug" = Reversed-Plug Graft.
3. "D"= "Double" (2 reversed-plugs side by side in same marginal scute).
   "S"= "Single" (either single reversed-plug or single graft of plastral scion to carapace wound).
   "R"= "Reciprocal" (2 grafts per animal, with plastral scion to carapace and carapacial scion to plastron).
4. "LM"= "Left Marginal"; "N2"= 2nd Neural (from anterior); "RC1"= 1st Right Costal; "RC2"= 2nd Right Costal. Multiple entries indicate that the graft crossed suture line(s) between scutes (e.g. N2/RC1/RC2).
5. "H"= Histoacryl blau; "P"= Permabond
6. In the case of Controls, date entered indicates date tag was affixed.
7. When work began on 17 Sept., #16 and #17 isolated from others, not eating well and appeared weak; #30 isolated, with apparent fungus infection on shell and skin.
8. Last left marginal scute abnormally small; the two plugs were touching.
9. Congenital indentation at tagging site; tag precariously affixed at junction between last right marginal and right pygal scutes.
10. The two plugs were touching (operator's error)
11. Last right marginal abnormally small; tag on penultimate right marginal.