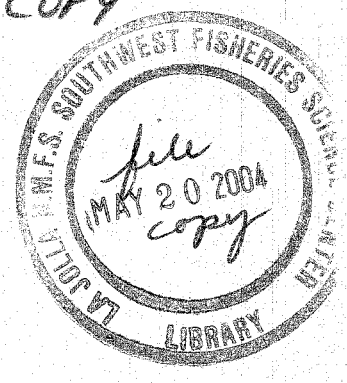


NOAA Technical Memorandum NMFS


NOVEMBER 1981

# AN EVALUATION OF TAGGING, MARKING, AND TATTOOING TECHNIQUES FOR SMALL DELPHINIDS

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NOAA-TM-NMFS-SWFC-16

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southwest Fisheries Center

**NOAA Technical Memorandum NMFS**

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# AN EVALUATION OF TAGGING, MARKING, AND TATTOOING TECHNIQUES FOR SMALL DELPHINIDS

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ACKNOWLEDGEMENTS

Preparation of this report was covered under NMFS Contract 03-7-208-35282 to the Hubbs/Sea World Research Institute. It serves to synthesize results of a coordinated set of studies by the NMFS, HSWRI and other NMFS contractors. The following individuals and institutions participated in the research effort:

David De Blanc, Biological Technician, Mississippi Laboratories, Southeast Fisheries Center, National Marine Fisheries Service, Bay St. Louis, Mississippi.

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Jacqueline Jennings of the Southwest Fisheries Center served as Chief Official Technical Representative for the contract. Referral to products by name does not imply endorsement by the government.

## IN MEMORIAM

The authors wish to particularly acknowledge the invaluable contributions of the late Dr. R. Keith Farrell to the research project. Dr. Farrell, Professor of Cryobiology at Washington State University, was a pioneer in the use of cryogenic marking techniques for terrestrial and marine animals. "La joie de vivre" emanated from Dr. Farrell for his work and the finer things of life. He is sincerely missed.

# INTRODUCTION

Marking and tagging have been recognized as important techniques for collecting data on marine mammals since the mid-1920's, when "discovery type" tags were developed for commercially exploited stocks of whales.

An increasing dependence on marine resources has created social pressures against exploitation of marine mammals and a concern for proper management that has become international in scope. Increased awareness has resulted in the Marine Mammal Protection Act of 1972, and international regulations via the International Whaling Commission, United Nations (FAO) and CITES, are pending to provide blanket protection for all marine mammals.

To obtain important field information for marine mammal management the National Marine Fisheries Service has developed marine mammal programs in biology, technology, and assessment. Visual identification of individuals is essential for this type of research and an important aspect of this program involves porpoise marking, tagging, and subsequent observation using a Porpoise School Impoundment System (PSIS), designed by NOAA's National Fisheries Engineering Laboratory and Southwest Fisheries Center.

The Porpoise School Impoundment System functions as a platform for marking and tagging small pelagic delphinids, primarily spotter porpoise, Stenella attenuata. Specialized procedures and equipment are necessary to process sufficient numbers of animals in an efficient and humane manner to insure adequate sample size.

Under the guidance of Ms. Jacqueline G. Jennings (Leader, Porpoise Tagging Project, Southwest Fisheries Center), the Hubbs/Sea World Research Institute conducted a research program to evaluate marking and tagging techniques and equipment for use with the Porpoise School Impoundment System

(PSIS). This work was coordinated with that of the other NMFS contractors mentioned in the Acknowledgements Section.

Procedures and equipment for tagging were developed according to the following criteria derived from the Marking and Tagging Workshop convened by the Southwest Fisheries Center, National Marine Fisheries Service -- May 1977;

1) Tag design that minimizes tissue trauma.
2) Tag fabrication from biocompatible materials.
3) Tag fabrication from materials durable to field environmental factors for at least one year.
4) Tag application in less than one minute per animal.

Procedures and equipment for marking were developed according to the following criteria:

1) Equipment designed with sufficient coolant capacity and insulation, or pigment reservoir to allow marking large numbers of animals.
2) Equipment designed for marking with multiple symbol capacity.
3) Marking equipment fabricated from materials durable at cryogenic temperatures and in field situations.
4) Marking equipment portable in field situations.
5) Mark application in less than one minute per animal.
6) Legible mark for at least one year.

Experiments were conducted to provide the National Marine Fisheries Service with observations and recommendations based on the proposed criteria, although the time schedule for field operations with the Porpoise School Impoundment System (PSIS) did not allow all tags and marks to be evaluated for the proposed year period. However, tags and marks cited in the report

as "during the study period" were evaluated for at least a full year between October, 1978 and April, 1980.

This report will review research progress in three separate sections:

1) Tagging equipment design and evaluation for small delphinids.
2) Cryogenic marking equipment design and evaluation for small delphinids.
3) The feasibility of tatooing small delphinids.

For the purpose of clarity the following definitions for "tag" and "mark" will be used:

Tag -- A visible object attached to, implanted in or covering the epidermis for the purpose of identification.

Mark -- A visible symbol produced by depigmentation of the epidermis with cryogenic temperatures or its implantation with indelible pigments for the purpose of identification.

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# Section I - Tagging Equipment Design and Evaluation

## Tag Evaluation - Hampshire Pig (Sus scrofa)

Initial tag evaluations were performed with a Hampshire pig (Sus scrofa) maintained at the University of California's Laboratory Animal Facility. The pig's epidermal - fat - muscle layers are anatomically similar to delphinids and they can sustain multiple cutaneous implants without health complications.

Prior to tag implant, the animal was manually restrained and scrubbed with septodyne disinfectant. After tagging, it was monitored periodically by Institute and Laboratory Animal Facility staff.

## Tag Group #1 Hampshire Pig (Sus scrofa)

### Tag Description and Application

1. Floy Spaghetti Tag (Fig. 1)

The Floy spaghetti tag had a thin, flat, pointed type 304 stainless steel barb (35 mm x 8 mm) attached to a flexible monofilament lead with fluorescent polypropylene streamer (14 cm). Stainless steel is a biocompatible material. The tag was applied with a jab stick (Fig. 2). The length of the jab stick's holding pin determined depth of penetration. The anchor barb penetrated the tissue at an acute angle (from verticle plane) and anchored by rotating at a right angle to the entry point when back pressure was applied to the streamer.

#### Design Concept

This tag was originally designed for fish. When applied properly, the tag head swiveled 90° relative to the streamer as shown in Fig. 1.

The barb no longer pointed medially, which was intended to reduce tag shedding. The tag head was flat and thin with a slight curvature, allowing penetration and anchoring in body tissue.

2. Floy Arrowhead Tag (Fig. 3)

Also designed for use with fish, the Floy arrowhead tag had a round, pointed, nylon dart (16 mm) with two adjoining flukes (30 mm). Nylon is a biocompatible material. The dart was attached to a flexible vinyl tubing streamer (14 cm). This tag was applied adjacent to the dorsal fin with a jab stick. Length of the jab stick's tag holding pin determined depth of penetration. The arrowhead anchored when the back pressure was applied to the streamer while removing the jab stick spread the flukes outward.

Design Concept

The pliable twin flukes formed the anchoring mechanism for this tag design. As the flukes penetrated into the body tissue they were pressed against the streamer. Once the tag penetrated to the required tissue depth, back pressure from jab stick withdrawal spread the flukes. The dart's point remained positioned medially in the tissue.

3. Disc-Arrowhead Tag (Fig. 4)

The disc-arrowhead tag had a pointed nylon head (16 mm) with two adjoining rigid flukes (8 mm), and a rigid shaft (22 mm). The shaft united

the flukes and a disc 2.54 cm in diameter. Application was made by incising the tissue with a scalpel and firmly pressing the tag into position by hand.

Design Concept

The disc was designed to rest on the epidermis and control the tag's depth of penetration. It was also intended to act as a pivot point for a highly visible streamer (not tested) while reducing tissue trauma caused by the streamer's flexion. The shaft length was intended to position the flukes in the blubber layer only to determine anchoring ability.

4. Disc-Conehead Tag (Fig. 5)

Fabricated from Delrin, the disc-conehead tag had a pointed, cone shaped anchor head (13 mm x 10 mm). Delrin is a biocompatible material. Application procedures were the same as the disc-arrowhead tag.

Design Concept

This anchor head design was intended to separate rather than cut the tissue during application. It was hypothesized that the tissue's resilient nature may hold this anchor head design more securely.

5. Carbon-Coated Disc-Conehead Tag

This tag was structurally identical to the Disc-Conehead design. Application technique was also the same. A low temperature isotrophic carbon coated all surfaces.

## Design Concept

A low temperature isotrophic carbon coating was used on the tag's surface areas. The tags were coated by the Gulf General Atomic Corp., La Jolla, California. This type of coating has successfully promoted tissue adherence and augmented surgical implant healing cycles in primates and humans. A magnified view of the carbon coating is shown in Fig. 6.

## Results

The tag evaluations are summarized in Table 1a, b.

### Application

The Floy spaghetti and arrowhead tags were implanted with a jab stick and presented no difficulties. Each tag was implanted within one minute. However, all three of the new tag designs were difficult to implant without incising the skin with a scalpel. The increased surface area and rigid nature of their anchor heads may have been responsible. An application tool of specialized design was needed to provide sufficient force for penetration of the tag. Hemorrhaging subsequent to tag application was observed, particularly with the conehead design.

### Durability

No structural failure was observed with any of the tags. The carbon coating used on the disc-conehead tag was absent on the disc's ventral surface and at its junction with the shaft.

## Visibility

The Floy tag streamers (fluorescent orange, yellow) contrasted well against the pig's black skin. No streamers were attached to the other tags, but the white nylon and delrin discs also contrasted well with the pig's skin color.

## Retention Time

Retention times of tag group #1 were as follows:

1) The Floy spaghetti tag remained implanted during the study period.
2) The Floy arrowhead tag was retained for 60 days.
3) The disc-arrowhead tag was retained for 34 days.
4) The disc-conehead tag (uncoated) was retained for 5 days.
5) The carbon coated disc-conehead tag was retained for 5 days.

Retention time of the new tag designs (disc-arrowhead, disc-conehead, carbon-coated disc conehead) were low when compared to the Floy spaghetti and arrowhead designs. The nylon disc-arrowhead tag design may have been retained longer due to its 22 mm shaft length which could have anchored in the fibrous fascia tissue.

## Tissue Trauma

The pig was frequently observed to rub all the tags against it's pen wall, which subjected the tags and tissue to considerable abrasion. The tags' entry hole enlarged until shedding occurred. The Floy

spaghetti and arrowhead tags also displayed an erosion of tissue where the streamers entered the epidermis. Individual tag sites appeared to be healed within 30 days after the tag was shed and no infections or other complications were noted.

## Discussion

Use of a pig for the initial tag implants proved to be important since the animal's rubbing behavior subjected all the tags and surrounding epidermal tissue to trauma similar to the water friction expected in a delphinid environment. Although retention times for disc-conehead, carbon-coated disc conehead, and disc arrowhead tags were lower than the Floy types, the absence of significant structural and biocompatibility problems influenced our decision to implant them all as a group in two common dolphin (Delphinus delphis) specimens.

## Tag Evaluation - Common Dolphin (Delphinus delphis)

Tag evaluation experiments were conducted with two common dolphin (Delphinus delphis) specimens maintained in a 10.3 m x 13 m 240,000 liter pool. This species was selected since they display frequent rapid swimming and aerial behavior, similar to Stenella sp. the tags were being developed for. They are also the third most important pelagic species taken during tuna purse seine fishing. The animals were handled with careful attention to their health and safety. The holding pool was lowered prior to tagging and each animal was placed in a stretcher, then lifted by crane onto a soft

foam rubber pad. The skin was scrubbed with Septodyne disinfectant followed by tag implant. Blood samples were taken before tagging and periodically in the days following to monitor health status. Water quality was checked on a daily basis.

## Tag Group II Common Dolphin (Delphinus delphis)

### Tag Description and Application

The tag designs evaluated in the pig were implanted in two Delphinus delphis. Since blubber thickness of the Delphinus was similar to the pig (>18 mm) shaft lengths of the two arrowhead tags were 22 mm to extend the anchor head into the fascia. A streamer was added to one of the arrowhead tags to observe its influence on tag retention.

The disc-conehead tags were fabricated with a 16 mm shaft to anchor in the blubber. One half the tag number were carbon coated for comparison of tissue reaction and healing cycle times with the uncoated tags. All the tags were implanted using a plastic headed mallet (Fig. 7).

1. Disc-Arrowhead Tag (Fig. 4)

Delrin was used instead of nylon. A yellow polyurethane streamer was attached to the disc.

2. Carbon Coated Disc-Arrowhead Tag

Low temperature isotropic carbon coating. No streamer was attached.

3. Disc-Conehead Tag (Fig. 5)

The shaft was 16 mm instead of 20 mm and nylon was used instead of delrin. No streamer was attached.

4. Carbon Coated Disc-Conehead Tag

Low temperature isotrophic carbon coating. No streamer was attached.

## Results

The tag evaluation results are summarized in Table 1b.

### Application

Application with the mallet was intended to improve tag penetration into the tissue. Each tag was implanted within one minute. Hemorrhaging was observed during the application of all tags and continued for approximately thirty minutes subsequent to tagging. The disc-arrowhead tags were implanted with no difficulty. However, the conehead tags required greater force to implant, and several tags were fractured during application. Conehead and arrowhead tag discs protruded above the epidermis within minutes after the animals were released, leaving a visible gap between the disc and the skin surface (Fig. 8).

### Durability

No structural failure was observed with any of the tags. Carbon coated tags displayed areas where the carbon was removed, primarily on the disc's ventral surface at the junction of the disc and shaft. These

sites probably sustained the most significant mechanical trauma from the animal's body flexion, and water friction.

Visibility

The yellow polyurethane streamer attached to the uncoated disc-arrowhead tag was easily observed against the black skin of the Delphinus. The discs seated on the epidermis were visible due to their white color but their small size would limit useful viewing at a distance. Carbon coated discs could only be viewed with considerable effort at tankside.

Retention Time

Retention times of tag group #2 were as follows:

1) Disc-arrowhead tag (uncoated) remained implanted for 4 days.
2) Carbon coated disc-arrowhead tag remained implanted for 14 days.
3) Disc-conehead tag remained implanted for 6 days.
4) Carbon coated disc-conehead tag remained implanted for 5 days.

Within two hours after application, the disc arrowhead tag (uncoated) with streamer was protruding above the epidermis. A companion animal was observed pulling on the streamer repeatedly. Its subsequent shedding (4 days) may be attributed in part, to this behavior. Disc protrusion of the uncoated conehead tag was also observed. The Delphinus were observed to jump frequently after tagging and usually landed on the tag sites when hitting the water (Fig. 9). Although aerial behavior is common with

this species at sea, these Delphinus had not been observed jumping prior to tag application for several weeks.

The Delphinus implanted with the disc-conehead tags was observed to rub against the tank wall in an apparent attempt to remove them. This behavior appeared to influence retention time of the tags. Once the tag discs protruded above the epidermis (Fig. 8), water friction was able to accelerate tag shedding.

## Tissue Trauma

The mechanical trauma resulting from aerial and rubbing behavior, and pulling by mouth, appeared to retard completion of a healing cycle. The tag entry holes enlarged until they were larger than the anchor heads, and the tags were shed. Water friction was also a significant factor, once the tag discs projected above the epidermal surface. No infection of the tag sites was observed, but some swelling was noted during the period tags were implanted. Once the tags were shed, a healing cycle appeared to be complete within 23 days.

## Discussion

Tissue adjacent to the dorsal fin of the Delphinus appears to be very resilient. Without a scalpel incision, the disc-conehead proved difficult to implant. Our rationale had been that the resilient nature of this tissue would serve to hold the tag in place. Since retention times for this tag type were the lowest of the group tested, its further evaluation was discontinued.

A streamer attached to the disc promotes visibility and prevents tissue abrasion where a constantly flexing lead or streamer may pass through the epidermis (i.e., spaghetti tag). However, the "streamer pulling" behavior exhibited by a companion animal may have had a significant effect on retention times of this tag (4 days). This behavior may have occurred with spaghetti tag streamers, but was not observed; "body slapping" was also observed throughout the time this tag group remained implanted.

Carbon coatings were removed from several tag areas even though tag retention time was brief. Milled delrin and nylon appears to be too smooth for low temperature isotropic carbon to adhere well. Further use of low temperature isotropic carbon coating was discontinued to economize research time and funds. If carbon coating is used for field tagging, the tag's dorsal surface should not be coated to permit better observation.

A tag design that will withstand the mechanical trauma of rubbing, jumping, and pulling by mouth is necessary if field tag retention for at least one year is to be realized. We have yet to observe a complete healing cycle with tags implanted in the Delphinus due to the brief retention times.

The rapid shedding of all prototype designs from the Delphinus indicated a need for further design improvements and subsequent testing with emphasis on reducing the disc size of prototype tags, revision of anchor head design, and continuation of Floy tag evaluation. Once again, two common dolphin (Delphinus delphis) specimens were used as test subjects. Efforts were initiated to design a tag for application to the dorsal fin.

## Tag Group #3 Common Dolphin (Delphinus delphis)

### Tag Description and Application

The Floy tags were implanted with a jab stick and the disc-arrowhead and disc-conehead tags were implanted (after a scalpel incision) with a plastic headed mallet.

1. Floy Spaghetti Tag (Fig. 1)

The Floy spaghetti tag design remained unchanged.

2. Floy Arrowhead Tag (Fig. 2)

The flukes were rounded and their length reduced by 50 percent (14 mm) for improved anchoring.

3. Disc-Arrowhead Tag (Fig. 4)

The disc size was reduced to a 25 mm x 15 mm oval. The shaft was hollowed to allow drainage of body fluids away from the wound.

4. Adjustable Disc-Arrowhead Tag (Fig. 10)

A Floy Co. pointed nylon barb (8 mm x 5 mm) with a single fluke (10 mm) was attached to a 90 mm flexible nylon conduit tie band. A flat polycarbonate disc (25 mm diameter) with the wire tie locking mechanism bonded in its center was designed to thread the wire tie band material and lock in place.

The tag head and flexible wire tie penetrated the tissue via a 5 mm diameter trocar implanted to a predetermined tissue depth. Once the trocar was positioned, the tag head was threaded through it until the fluke cleared the opposite end. After removal of the trocar, the wire tie was pulled to anchor the fluke. The wire tie was threaded through the locking mechanism in the disc to "seat" it on the epidermis. Excess wire tie material was clipped off.

5. Flag Tag (Fig. 11)

This was the first dorsal fin tag design tested, with two 80 mm x 5 mm orthoplast flags secured by a nylon nut and bolt. The dorsal fin was cored manually with a #8 trocar (Fig. 12) at a position intersected by the middle one-third of the fin measured from the dorsal tip and the first one-third measured from the anterior leading edge. A nylon sleeve (23 mm x 5 mm) was inserted in the hole prior to securing the flags on each side of the dorsal fin. Nylon is a biocompatible material. Once in place, the nylon nut and bolt were heat welded with a hot metal spatula. Space between the sleeve and the nut and bolt head allowed 360° rotation of the flags.

## Results

The tag evaluation results are summarized in Tables 1a, b, c.

### Application

No problems were encountered while implanting the Floy tags. Their application required only several seconds. However, the rigid shaft

disc-arrowhead tag was still difficult to implant even with a scalpel incision, prior to seating with a plastic headed mallet. If the tag was not struck on the disc's center, the shaft fractured or popped the entire tag out of the incision, prolonging application time.

The adjustable disc-arrowhead tag was easily positioned but the fluke would not anchor in the tissue without considerable maneuvering. Application time was approximately 2.5 minutes.

Coring a hole in the dorsal fin was not easily accomplished due to the extreme resilience of its connective tissue. This prolonged Flag tag application time (2 minutes). The dorsal fin must be held rigid and considerable leverage must be used to core this tissue. Hemorrhaging was noted but insertion of the sleeve in the hole controlled it effectively after about five minutes.

Durability

Only the Floy arrowhead and flag tags exhibited structural failure. One fluke of the Floy arrowhead tag evidently fractured during implant. After 28 days, one of the flag tag's flags fractured near the post. Absence of water friction during the animal's respiration and aerial behavior caused a forward rotation of the flags and subsequent flexion when the animal returned below the water surface. Repeated action of this type caused fatiguing of the flag and finally fracture.

Retention Time

Retention times of tag group #3 were as follows:

1) The Floy spaghetti tag was surgically removed after 31 days.
2) The Floy arrowhead tag was surgically removed after 31 days.
3) The disc-arrowhead tag was shed in 6 days.
4) The adjustable disc-arrowhead tag was shed in 1 day.
5) The flag tag was intact for 43 days until the animal's death from causes not related to the tag.

Tissue Trauma

The Floy spaghetti and arrowhead tags were removed since raised areas developed around each tag and blood sample analysis indicated potential infection. Photographs of the Floy arrowhead tag indicated a continued penetration into the muscle tissue, as shown by a change in position of the script on the streamer (Fig. 13 a, b). Removal of this tag revealed that one fluke had fractured, possibly during implant. An abcess was present. The Floy spaghetti tag was found to have anchored well in the fascia. The barb was encapsulated and no infection was present.

Both disc-arrowhead tags were shed rapidly, again indicating that water friction against the disc did not allow completion of a healing cycle. Rubbing and jumping behavior was frequently observed, and may also have been a significant factor in the shedding of these tags.

The flag tag was removed only because the Delphinus specimen expired of causes not related to the tag. Sectioning of the post hole revealed

that a healing cycle had been progressing well, with reepithelialization noted adjacent to the sleeve location (Fig. 14).

## Discussion

Although the Floy tag depth of penetration should be limited by the jab stick shaft length, the arrowhead type which fractured appeared to migrate medially (Fig. 13a, b). It is possible that muscle layer flexion where the tag anchored accounted for continued penetration of the barb.

Testing of the Floy arrowhead tag was discontinued in favor of the Floy spaghetti tag. Reduction of tag migration after application, and abrasion at the entry site were important design criteria to be considered.

The disc-arrowhead tag, despite design changes in the disc and shaft, was still difficult to implant and retention time was low. Further evaluation of this tag type was discontinued.

Testing of the adjustable disc-arrowhead design was discontinued, although reducing the diameter of the trocar and improving the method for advancing the barb beyond the trocar may improve anchoring and reduce the time needed for tag implant.

With the exception of the Floy spaghetti tag, the flag tag displayed longest retention time and also the most complete healing cycle. Use of the nylon sleeve appeared to minimize tissue trauma encountered with other tag designs. Selection of a more durable material was of primary importance for further testing of this tag type.

## Tag Group #4 Common Dolphin (Delphinus delphis)

New tag designs developed by the National Fisheries Engineering Laboratory, Bay St. Louis, Mississippi, were tested on two Delphinus specimens. All of the tags were fabricated from biocompatible materials and could be applied in less than one minute, without use of tools. Three of these tags were designed for application to the dorsal fin since the most significant retention time (43 days) to date was recorded for a dorsal fin tag.

### Tag Description and Application

1. Anterior Dorsal Fin Tag with Wrap-around Streamer (Fig. 15)

Spring-loaded stainless steel tines were intended to hold the flag to the anterior edge of the dorsal fin. The element of the clasp that pierces the fin is approximately 2.5 cm posterior to the anterior edge. The flag was a continuous piece of hypalon that wrapped around the lower anterior aspect of the dorsal fin. It was connected to the clasp by a nylon fastener.

#### Design Concept

The thin spring-loaded stainless steel tines were intended to hold the flags against the dorsal fin's leading edge, aided by water flow over the flags. The wrap-around flags were thin and pliable to minimize

mechanical trauma of the dorsal fin. The spring placement and sharp tines allowed rapid tag application.

2. Metal Scoop Tag (Fig. 16)

The anchoring devices were M-shaped type 316 stainless steel wire tines with a 14 cm hypalon streamer fastened at the center angle with a nylon rivet. The 3 cm tines were curved downward in a semi-circle to penetrate the tissue ≃ 2 cm, just posterior to the dorsal fin. Water flow from the forward motion of the animal was intended to hold the anchored tines in position.

Design Concept

This tag's tines were shaped in downward curved semicircles so that once implanted, water flow would augment their anchoring by forcing them "into" the tissue.

3. Anterior Dorsal Fin Tag with Side-mounted Streamers (Fig. 17)

Same clasp as tag #1. Streamers were attached to the clasp on each side of the leading edge without wrapping around the anterior aspect of the dorsal fin as in tag #1.

Design Concept

The flag placement was changed to observe water flow effects as compared to the wrap-around flags.

4. Posterior Dorsal Fin Spring Clip Tag (Fig. 18)

Stainless steel spring-loaded tines (similar to tag #1 and 3) with the tines piercing the fin 3 cm anterior to the dorsal fin's trailing edge. No streamer was attached to this tag.

Design Concept

Positioning was adjacent to the dorsal fin to observe water flow effects as compared to tags #1 and 3.

Results

The tag evaluation results are summarized in Table 1d.

Application

Each of the tags were applied within thirty seconds. Considerable force was needed to penetrate the dorsal fin's connective tissue even though the thin stainless steel wire clasps were sharpened and spring loaded. No tools were required for application.

Durability

The stainless steel wire anchoring mechanisms remained structurally intact. However, all the flags used on the dorsal fin applications were shed within three days, indicating a need for improvement of their attachment mechanisms.

Visibility

While intact the streamers attached to the dorsal fin were highly visible. Yellow appeared to be the color of choice for future evaluation of these design types.

Retention Times

All tags applied to the dorsal fin were removed three to seven days after application. The scoop tag was shed within several hours after application.

Tissue Injury

Constant inward pressure produced by the spring-loaded tines of the dorsal fin tags combined with water friction against thin wire promoted significant tissue abrasion within several days after application. The tags were removed to prevent further tissue trauma and insure the animal's health.

Discussion

The primary advantage of the tags was their relative ease of application without tools, and the visibility of dorsal fin streamers. Since significant reepithelialization of dorsal fin tissue was observed with a sleeved anchor pin in tests, this application technique should be evaluated with these tag designs. An alternative would be to increase the diameter of the stainless steel wire, which may serve to distribute water flow forces more evenly against the tissue.

## Insertion and Withdrawal Tests of Tag Head Designs

Tags designed for implant in the body exhibited low retention times (with the exception of the spaghetti tag). More precise data was needed

to determine the relationship between tag head design, tissue morphology and tag retention. Tests were conducted with specially designed tag heads to measure the pressures ($lbs/in^2$) required for insertion and withdrawal.

## Methods and Materials

Insertion and withdrawal pressures were measured for eight specially designed tag heads and two spaghetti tag heads (Fig. 19a). These tag heads were inserted and withdrawn from the dorso-lateral tissue of a thawed and a fresh Delphinus delphis carcass. A specially designed tag head chuck and spring scale were used to measure insertion and withdrawal pressures in $lbs/in^2$ (Fig. 19b).

The tag head chuck allowed penetration through muscle and fascia tissue, and could be adjusted for blubber penetration only. At least three insertions and withdrawals were made for a tag head in each category and the $\overline{X}$ of each test group was recorded. Surgical removal of some tag heads after insertion was also conducted to observe their positions in the tissue.

## Results

Results for the insertion and withdrawal tests are summarized in Table 2.

The $\overline{X}$ $lbs/in^2$ values are higher for tag head insertion and withdrawal through muscle-fascia tissue when compared to blubber tissue measure values. The freezing and thawing process appeared to impart a change in the tissue's integrity since thawed tissue pressure values were also higher.

Tag heads with low insertion and high withdrawal pressure values had a fluked or barbed head design with less mass. The most significant examples were the spaghetti tag designs. The modified spaghetti tag design was more difficult to insert and neither spaghetti tag design could be withdrawn.

Surgical removal of tag heads revealed that cone head designs often caused enough physical tissue trauma during insertion to allow easier withdrawal, especially if inserted only into the blubber tissue. The spaghetti tags with rounded flukes usually caused less tissue trauma and often anchored in the fibrous fascia tissue (Figs. 20-21).

Discussion

Additional insertion and withdrawal tests should be conducted for evaluation of tag head designs. Fresh carcasses should be used for an evaluation of this type since the freezing and thawing process (in Delphinus tissue) tends to produce distorted $lbs/in^2$ values.

The muscle-fascia layer appears to promote higher withdrawal pressure values which would contribute to longer tag retention time. However, surgical removal of tag heads related that some involvement of muscle tissue was usually observed after insertion since the fascia layer is very thin (compared to the blubber and muscle layers). The extent of this involvement and the physical trauma will affect healing cycle time.

Insertion and surgical removal of spaghetti tags provides a good example. The Standard Floy tags three-fluked barb was easier to insert and rotate but the sharp points cause more tissue trauma. This barb design may prolong the healing cycle. A three-fluked barb (fluke points rounded off) was recommended for further live animal testing.

Tag Group #5 Pacific White-sided Dolphin (Lagenorhynchus obliquidens)

The dorsal fin represented the most visible area of the body to tag and an encouraging healing cycle and retention time had been established with the flag tag design. Implanting body tags had not shown significant healing cycles or retention times with the exception of the Floy spaghetti tag design. Therefore, emphasis was placed on a tag design for the dorsal fin (disc tag) and improvement of the Floy spaghetti tag design. The peduncle tag, a new design concept, was also evaluated.

Tag Description and Application

1. Dorsal Fin Disc Tag (Fig. 22)

This dome-shaped disc tag was designed by the National Fisheries Engineering Laboratory. Each teflon disc was 5 cm in diameter with vent holes and radial and perimeter slits. The self-locking anchor pin consisted of a teflon multi-notched pin on one disc's concave side which inserted into a ridged tube on the concave side of the other disc.

This disc tag was applied by cutting a hole through the dorsal fin, slightly smaller than the anchor pin, with a scalpel. The anchor pin hole was not cauterized. The anchor pin sections of both discs were placed in the hole and snapped together.

Design Concept

The disc's dome-shaped vent holes, and slits were intended to channel water flow between the discs and the dorsal fin. They functioned to keep the disc edge from abrading the tissue. The "male-female" anchor pin facilitated rapid application by snapping together when placed in the dorsal fin hole. The teflon, a biocompatible material, had a smooth surface to curtail fouling growth.

2. Spaghetti Tag - Modified Lead and Anchor Head (Fig. 23)

A 30 x 10 x 1 mm type 316 stainless steel anchor head was attached to a 1.5 mm diameter type 316 stainless steel wire lead. A 21 x 1.27 cm polyurethane streamer was attached to the wire lead by a chrome plated brass McMahon fishing swivel (Fig. 24). The tag was applied with a jab stick.

Design Concept

This tag was designed to minimize stress on the lead and anchor head by means of a pivot point for the streamer. The streamer shape was enlarged and flattened to improve visibility and to observe its hydrodynamic characteristics.

3. Snap Fastener Peduncle Belt (Fig. 25)

The peduncle tag was a 21 x 10 cm band of thin soft red synthetic rubber with a polypropylene male-female fastener at each end. The belt was applied around the peduncle, at the insertion of the tail flukes. The male-female fasteners were overlapped and pressed together to secure the belt in place.

Design Concept

The thin soft synthetic rubber belt was intended to minimize tissue abrasion by flexing with the peduncle and flukes as the animal moved through the water. The polypropylene male-female fasteners could be secured rapidly and allowed adjustment for variance in peduncle girth.

## Results

The tag evaluation results are summarized in tables 1a, c, and e.

Application

Coring the dorsal fin with a scalpel for placement of the disc tag was accomplished with no difficulty in about 30 seconds. Caution in incising the connective tissue must be exercised since the scalpel blade is thin and rather fragile.

Considerable force was needed to penetrate the tissue and seat the new spaghetti tag. The first application attempt did not penetrate the anchor head deep enough into the tissue or toggle it sufficiently to prevent withdrawal. The second attempt was successful requiring several seconds. A sharper point and edge for the anchor head of this tag should be used.

The peduncle tag was applied in a matter of seconds with no problems.

Durability

The self-locking anchor pin of the dorsal fin disc tag failed due to the soft nature of the virgin teflon. The spaghetti tags swivel corroded and finally fractured, shedding the streamer after twenty-three days. The anchor head remained implanted. The polypropylene fasteners used on the peduncle tag were also too soft to withstand water friction and rapidly pulled loose.

Visibility

Of the three tags evaluated, the dorsal fin disc tag was most visible above and below the water surface. The spaghetti tag could only be observed clearly above the water surface during respiration. The peduncle tag was also difficult to observe since it rarely was exposed above the water surface. The red and white color of this tag did not augment visibility.

Retention Time

Retention times of tag group #5 were as follows:

1) The dorsal fin disc tag was shed in less than 1 day.
2) The spaghetti tag with modified lead and anchor head remained implanted during the study period, although the streamer needed replacement.

3) The peduncle tag was shed in less than one day.

Tissue Trauma

The dorsal fin disc tag and peduncle tag were shed in less than one day, causing no tissue injury. A blood vessel ruptured during anchor pin hole coring continued to hemorrhage for approximately twenty minutes after the animal was returned to the pool. The modified spaghetti tag displayed minimal tissue abrasion at the barb's entry site (Fig. 24). This abrasion appeared to heal within eighteen days. No additional tissue trauma problems have been noted with the spaghetti tag.

Discussion

Coring post holes for dorsal fin disc tags in the field with scalpel blades is not advisable since they are fragile and dull quickly. Blood vessels in the dorsal fin's arterial tree may be ruptured by coring a post hole for dorsal fin tags. Prolonged hemorrhaging could attract sharks in a field tagging situation. Tissue constriction by using a slightly larger post and chemical cantery can control this blood flow.

Short retention times for the dorsal fin disc tag and peduncle tag precluded a thorough evaluation. More durable materials for locking mechanisms were definitely needed to continue evaluation of these tag designs. The rigid lead wire and swiveling pivot point of the modified spaghetti tag appeared to effectively reduce tissue trauma and promote a complete healing cycle. However, the streamer was shed due to swivel corrosion and was replaced with a barrel swivel (brass) which also

corroded and fractured after thirty-six days. The streamer was finally attached to the lead with a type 316 stainless wire loop and has remained intact during the study period.

## Tag Group #6 Pacific White-sided Dolphin (Lagenorhynchus obliquidens)

Since the spaghetti tag remained intact (with the exception of the streamer) and no biocompatibility problems were observed, emphasis for tag group #6 centered on further development of the dorsal fin disc tag and the peduncle tag.

### Tag Description and Application

1. Dorsal Fin Disc Tag (Fig. 22)

This dorsal fin disc tag was identical to the design used in tag group #5 except for the anchor pin. The anchor pin of this tag was a 6 mm diameter smooth nylon rod, secured with a zinc coated steel retainer ring. Additional security was provided by heat welding the rod tip with a hot spatula.

#### Design Concept

Nylon is biocompatible and more rigid than teflon. It was hypothesized that a nylon anchor pin would maintain its structural integrity under stress from water flow or the animal's aerial behavior. The new anchor pin design allowed adjustments for variance in dorsal fin thickness.

2. Velcro Rubber Peduncle Belt (Fig. 26)

This peduncle tag was fabricated from a 21 cm x 4 cm band of soft synthetic rubber. A 37 cm x 2 cm x 1.5 mm strip of velcro was sewn to the rubber band with nylon thread. The velcro functioned as a fastener to secure the rubber band around the animal's peduncle.

Design Concept

Velcro (trademark) is a soft non-corrosive nylon fastener material. Its soft flexible nature and holding ability were intended to improve the tag retention time. Velcro is also a manufactured item available in quantity.

3. Ty Rap Rubber Peduncle Belt (Fig. 27)

Thjs peduncle tag was fabricated from a 19 x 8 x 1.5 mm band of soft synthetic rubber. A Thomas and Betts polyethylene Ty Rap wire tie was sewn to the rubber section with nylon thread. The tie functioned as a locking device, holding the rubber band in place on the animal's peduncle. The toothed belt is threaded through a slot on the opposite end which contains a tine that seats between the teeth. The threaded belt is folded over and secured under two posts giving this tag two locking mechanisms.

Design Concept

Ty Raps (trademark) are commonly used to bundle together heavy objects such as cable and pipe. Its locking mechanism is strong, adjustable

and non-corrosive. It is also a manufactured item available in quantity.

4. Nasco Multi-loc Cattle Leg Belt (Fig. 28)

A 34 x 3.2 cm x 3 mm polypropylene cattle leg tag. This tag had a locking mechanism similar in design to the Ty Rap (wire tie). Adjustments could be made on the locking end of the band at 6 mm intervals.

Design Concept

Once again the emphasis was on a strong and durable locking mechanism for the belt. This locking mechanism is similar in design to the Ty Rap wire tie except the teeth are only located on one end of the belt, and the belt is not folded over. The tooth and tine arrangement is the only means of locking. This tag has been successfully used on cattle and can withstand considerable stress without fatigue.

## Results

The tag evaluation results are summarized in Tables 1c, and e.

### Application

The disc tag was applied in the same anchor pin hole used for the tag group #5 disc tag. The peduncle tags were easily applied in a few seconds. There was some question about the need for a loose or tight fit to minimize epidermal trauma. Approximately 1-3 mm of clearance between the epidermis and tag was allowed for both tag types.

Durability

After 80 days, sections of the disc tag on one side of the dorsal fin began to fracture and shed until only the portion around the anchor pin was left (Fig. 29). The zinc-coated steel retainer ring was badly corroded when removed at 100 days. Although no structural change occurred with the velcro peduncle belt, continuous water flow caused the velcro mesh to separate, and the tag was shed. The Ty Rap and Cattle Leg peduncle belts did not exhibit any structural integrity problems.

Visibility

Although the white teflon disc tag contrasted well with the dorsal fin's black skin, algae growth was beginning to establish itself on surfaces of both discs when the tag was removed. Continued growth would have significantly reduced visibility of the discs. The red and black colors of the velcro and Ty Rap peduncle belts were not easily observed above or below the water surface. However, the white color of the cattle leg tag contrasted well with the black skin of the Lagenorhynchus.

Retention Time

Retention times for tag group #6 were as follows:

1) The dorsal fin disc tag was removed from the dorsal fin after 100 days to allow implant of a new disc tag design.
2) The velcro peduncle belt was shed in one day.

3) The Nasco cattle leg belt was removed after five days.

4) The Ty Rap peduncle belt was removed after five days.

Tissue Trauma

An indented ring of darkened tissue around the disc tag's perimeter was observed (Fig. 30), but no apparent tissue damage was noted and the tag was left in place. No tissue injury was observed with the velcro peduncle belt. The cattle leg and Ty Rap peduncle belts were removed after five days due to significant tissue abrasion at the junction of the peduncle and tail flukes (Fig. 31).

Discussion

Use of a smooth nylon post with retainer ring for the dorsal fin disc tag prolonged retention time and reduced tissue injury. Further study was needed of more durable materials for this tag design.

Although the locking mechanism of two peduncle belt designs improved retention times, tissue abrasion prompted removal to safeguard the test animal's health. The belt materials were flat and rigid (cattle leg belt) or flat, wide, and soft (Ty Rap rubber belt). Water flow acting on both these designs seemed to press the belt material down against the epidermis and then lift it upward to create a "grating" action. A very soft, round material may "roll" with the body flexion, and distribute the pressure of the belt on the epidermis.

## Tag Group #7 Hawaiian Spinner (Stenella longirostris)

### Hawaiian Spinner Tag Tests - Spring 1978

During the week of March 15-19, 1978, further evaluation of the dorsal fin disc tag was conducted at Sea Life Park in Hawaii using Spinner porpoise (Stenella longirostris). The spinning behavior of Stenella longirostris and availability of a large 45 m x 45 m 680,000 liter pool provided ideal conditions for evaluating the dorsal fin disc tag design. The initial tagging experiment involved six animals. Safe and humane procedures for handling the animals were followed. The water level was lowered to allow placement of an animal into a stand-supported stretcher in the pool. The dorsal fin was washed with Septodyne before coring was attempted. The tags were secured in place and the animal was returned to the pool.

### Tag Description and Application

#### Modified Disc Tag

The disc tag used in this experiment is described in experiment #5 (Fig. 22). Tag application technique was slightly modified from previous experiments. A 7 mm hole was cored through the fin. The coring tool was notched corresponding to the various teflon post and sleeve lengths. The appropriate sleeve was mounted on the post of the male tag section, the post inserted in the protruding coring tool, and pushed back through the hole. Since the sleeve was slightly larger than the hole (7 mm vs. 6 mm) it served as a compress within the wound to curtail blood flow. The female

side was then affixed to the post and the protruding post material removed with wire cutters.

Design Concept

In an attempt to eliminate previous disc tag application problems the male tag disc section was modified to accomodate different post and sleeve lengths and the coring tool as described.

## Results

The tag evaluation results are summarized in Tables 1a, c.

Tag Application

The tags required much longer to apply than expected (>1 minute). Since the sleeve was slightly larger than the post and cored hole, some difficulty was encountered in its positioning. Each tag was individually machined, causing some variability in the male and female sections. Some holes in the female disc section would not fit over the post.

The teflon post was observed to bend when pressure necessary to affix the female section was applied. After the post and sleeve were inserted through the hole, it was necessary to cut back the post by depending on dorsal fin thickness. Wire cutters used to trim the post flattened the soft teflon, making it difficult to attach the female section. Sharper snips and a harder tag material such as polyurethane should eliminate these problems.

Durability

Spinning and related behavior caused repeated tag structural failures soon after application. Problem areas were determined to be the post,

which may have fractured in two, and the discs which fractured in segments or everted entirely (Figs. 32 and 33). The teflon's pliable nature also caused male and female tag sections to pull apart.

Retention Time

A summary of tag retention times is presented in Table 3. None of the tags were retained for longer than a four-day period.

Tissue Trauma

The apparent mechanical trauma from water friction on the tag post caused erosion and softening of the surrounding tissue. Some inflamation was observed as well as necrotic tissue and lesions (Fig. 34). The presence of one or more of these conditions on all the animals necessitated removal of tags remaining intact after four days.

Discussion

The problems encountered with structural integrity, and tolerances between sleeves, posts and discs, suggested that injection molding of the disc tag's components may improve the tag's application, and retention time by rendering them more durable and consistent in dimension.

Considerable time was needed to core the dorsal fin. Since the dorsal fin is flexible, a small piece of wood placed on the side opposite the coring tool's entry should aid the coring process and sleeve positioning. A larger coring tool handle for more leverage, with a sharp cutting edge, is also desirable.

A harder more durable material is needed to prevent the disc fracture and eversion observed in these tests. Flow tank testing of materials at flow rates relative to the swimming behavior of Stenella (20-30 f.p.s.) were needed to further develop tags before continuing live animal tests.

## Tag Tests - Utah State Water Research Laboratory

It was difficult to accurately observe tag design performance (i.e., structural failure) while the tag was affixed to the animal. This was due to the animal's agility and physical factors such as light reflection off the water. It was necessary to test old and new tag designs in a "laboratory" situation before continuing live animal tests, and utilize the data to improve tag designs prior to a second tagging effort with the Stenella at Sea Life Park.

### Methods and Materials

Flow tank tests were conducted at the Utah State Water Research Laboratory in Logan, Utah. Selected tag designs were observed in a specially constructed 35.6 x 30.4 x 35.6 cm viewing chamber (Fig. 35).

A Stenella sp. dorsal fin was secured to a metal mount within the chamber. Tags representing four different categories (spaghetti tags, disc tags, flag tags, and cattle ear tags) were attached to the dorsal fin and subjected to pump-induced varied water flow rates of up to 30 ft./second at different mount angles. Each test was recorded on film, with a Mitchell Monitor 10 movie camera at 24 f.p.s. The contact of tag surfaces

with the skin and any changes in skin appearance were used as criteria for recording observations.

## Results

Flow tank test data is presented in Table 4. Observations on biocompatibility factors are as follows:

Disc Tags - Polyurethane, polypropylene, and delrin tags were fitted to the dorsal fin using a nylon sleeve and screw-type anchor pin. The flow tank tests revealed several important physical characteristics of the dorsal fin, and tag design factors which may influence tag biocompatibility:

1. The dorsal fin flexed to the right or left, changing the position of the disc relative to the skin surface, as the water flow rates increased (Fig. 36).
2. This change in dorsal fin curvature allowed the water flow to bend or tear a thin-walled, flexible disc tag.
3. A thick-walled, non-flexing disc tag chafed the skin at the top and bottom of the disc as the water flow rate increased.

The edges of all disc tags tested were thin. Since delrin and polyethylene (thick-walled type) discs with equally-spaced holes were found to be the most durable, modifying the edges of tags fabricated from these materials to distribute the discs contact surface with the epidermis may minimize mechanical trauma to the skin.

Flag Tags - The dorso-anterior margin of the flag tags tested displayed a tendency to contact the dorsal fin during flow tank testing. This

contact involved flexion of the dorsal fin, the individual flags, and increased as a function of water flow and dorsal fin flexion. A new flag design was fabricated at the test site with double sleeves and anchor pins. The sleeves projected an additional 3 mm beyond the dorsal fin on each side. This design reduced flag flexion and contact with the skin, except at the posterior edge of the dorsal fin. It also eliminated the possibility of flag rotation forward when the dorsal is out of the water (i.e., respiration or aerial behavior).

Spaghetti Tags - A Floy spaghetti tag and the improved design with modified lead and barb, were each implanted in the dorsal fin. The streamers of both tags appeared to lay flat against the dorsal surface, but there was flexion observed. No significant differences between the tag types at flow rates up to 30 ft./sec were observed. Tissue damage was not evident. However, there was no way of approximating the animal's respiration behavior. The alternating presence and absence of water friction on the streamer during respiration or aerial behavior may account for its spiraling motion observed in live animal tests and subsequent tissue erosion at the skin surface. The improved spaghetti tag version now has a 360° pivot point where the streamer is attached, which appears to have eliminated tissue erosion on the Lagenorhynchus test subject.

Cattle Ear Tags - The cattle ear tag tested, appeared to contact the skin unless positioned toward the anterior margin of the dorsal fin, allowing the flags to project beyond a point of contact. We were able to test a

flexible flat type and no significant change in skin appearance at flow rates up to 30 ft./sec was observed.

Flexion of the dorsal fin relative to the tag was easily observed and photographed during the flow tank tests (Fig. 36). This flexion, in combination with water friction, had a direct influence on tag biocompatibility and retention. The dorsal fin used in these tests had been preserved in formalin. However, we feel the flexibility of this dorsal fin compares favorably with those of live animals previously examined. (Manual flexion of dorsal fins and observation of swimming Stenella through Sea Life Park's underwater view ports.) High speed movie film of tagged Stenella through these view ports would allow a more accurate comparison.

## Tag Group #8 Hawaiian Spinner (Stenella longirostris)

During the week of June 11-18, 1978, additional tagging tests were conducted at Sea Life Park, Oahu, Hawaii, on the six Hawaiian spinner porpoise (Stenella longirostris) tagged previously. Due to infection problems encountered during the previous tag evaluations with these animals, copper sulfate was injected into the pool water and antibiotics were administered to the animals in their food. Blood analysis for each animal was regularly performed and water quality was monitored daily.

### Tag Description and Application

1. Dorsal Fin Disc Tag (Fig. 37)

On the basis of flow tank tests conducted at Utah State University, three types of material were used for the 7 cm diameter disc tags: polyurethane (red), high density polyethylene (orange), and delrin (blue). These discs differed from the original teflon disc tags. They were molded to different thicknesses, disc slits were eliminated, and the number of holes was reduced from six to four. Disc edges were smooth and rounded.

The polyethylene and polyurethane discs were applied using a notched post of delrin (a biocompatible plastic) which was cored to allow for insertion of the applicator tool. The delrin discs were not pliable enough to allow for passage of the post through the locking section of the disc so a 316 stainless steel bolt was used with a teflon sleeve. The locking section was cut away and a 316 stainless steel nut was used to lock the disc in place. The posts were used with and without sleeves to determine sleeve effectiveness.

2. Spaghetti Tags (Figs. 1, 23)

Two types of spaghetti tags were tested. The standard orange Floy spaghetti tag with a 304 stainless steel anchor head and a monofilament lead, and a modified spaghetti tag with a 316 stainless steel dart, 316 stainless steel lead wire extended from the anchor head to a stainless steel swivel which was attached to a polyurethane streamer. The anchor head of the experimental tag was wider, thicker, shorter, and blunter than the standard Floy type.

Earlier investigations have indicated that 316 stainless steel is preferable as an implant material to 304 stainless or flexible plastic

monofilament. In order to minimize mechanical trauma to the wound caused by continuous movement of the streamer, we have tested various types of leads and swivels. The one used on this test animal group was successfully tested on a Lagenorhynchus at Hubbs/Sea World Research Institute. Since the animals could be easily approached and handled, a 15.24 cm hand-held jab stick was used for tag applications.

3. Multi-post Flag Tag (Fig. 38)

The flags were fabricated of Kydex, measuring about 10 cm x 6 cm. The forward edge was secured to the fin by two sleeved nylon bolts, nuts, and sleeves to prevent the tag from pivoting about a single post as was observed in earlier flow tank and captive dolphin tests. If the tag is allowed to pivot, it can become oriented in such a way that the flags are forward of the post. The oncoming water, rather than flipping the flags back, can spread them apart resulting in considerable pressure on the post and often breakage of the flags or post. Two models of the flags were tried: a tag with the trailing edges unsecured, and one with the trailing edges bolted open with nylon bolts, nuts and sleeves about 1.4 cm (Fig. 39). The rationale for bolting the tag open was to assure continuous water flow under the tag and to prevent abrasion of the trailing edge of the fin. Both models were designed to ride about 0.25 cm out from the fin on both sides. The flags were painted a fluorescent yellow color for improved visibility.

Tag application involved placement of a flag against the dorsal fin as a template to mark positions of the post holes. The coring tool was designed with a door knob type handle for improved leverage and used in

conjunction with a small block of wood placed on the opposite side of the dorsal fin to reduce flexibility.

4. Surgical Tubing Peduncle Tag

A new peduncle tag was evaluated, as shown in Fig. 40. It was fabricated from rubber surgical tubing (1.2 cm width) with a nylon wire tie inserted inside to secure the tag around the peduncle. The surgical tubing's rounded surface and flexibility were considered important for minimizing skin abrasion. It was coated with orange latex rubber paint for visibility.

## Results

A summary of test results for the Hawaiian spinners is presented in Tables 1a, c, and e.

### Application

The posts for the disc tags were barely long enough to engage the locking section of the opposing disc. Results of the flow tank tests at Utah State University revealed that the tags had to be snugly fitted to prevent water friction from fracturing or everting the disc. We wanted to determine the degree to which the dolphin's fin could accommodate pressure around the periphery of the disc. The dolphin's fin apparently flexes also causing pressure to be applied at the top and bottom of the tag. The tags were generally not flush to the epidermis due to the curvature of the fin.

Application of the spaghetti tags were not well executed. Lack of pressure behind the jab stick did not allow the anchor heads to rotate properly. The experimental spaghetti tags placed anterior to the dorsal fin on the right side made a larger than expected insertion hole because the animal moved during application. The standard type spaghetti tags placed adjacent to the fin on the left side did not penetrate well (the barbs were showing) and were further inserted by hand.

Durability

A summary of tag durability is presented in Table 1a, c, and e. Durability problems with disc tags were attributed to fracture of unsleeved delrin posts (4), and polyurethane discs (2) (Fig. 41). The multi-post flag tags (2) eventually fractured adjacent to the posts (Fig. 42). Disc and flag tags tested displayed varying degrees of fouling by algae. The flag tag's fouling was extensive enough to obscure its number (Fig. 43).

No structural failures were noted with the spaghetti or peduncle tags. The orange latex paint on the peduncle tag was observed to peel within two days. The spaghetti and peduncle tags were not retained for a long enough period of time to evaluate durability.

Visibility

Of the three disc tag colors (blue, red, and orange) orange displayed the highest visibility above and below the water surface. The yellow and fluorescent orange streamers of the spaghetti tags were visible, but their relatively small surface area limits observation, especially underwater

(Fig. 44). Although placed adjacent to the dorsal fin, these tags rarely were exposed above the water surface.

The large surface area and yellow color of the flag tags displayed the highest visibility of all tags tested, above and below the water surface (Fig. 39). The peduncle tags, with reduced surface area, and rare exposure above the water surface displayed minimal visibility.

Retention Time

Retention times for all tags tested are shown in Table 1a, c, and e. The delrin disc tag with sleeved stainless steel post and the rear posted Kydex flag tag were retained intact for the longest period of time (58 days). A modified spaghetti tag was retained for 233 days but the streamer had shed after 33 days.

Tissue Trauma

Tissue trauma still appears to be a significant problem around the periphery of the disc tag, particularly at the dorsal and ventral aspects (Figs. 41, 45). However, Fig. 45 shows reepithelialized tissue along the posterior periphery indicating some mediation of trauma by the animal. Since some of the tags were removed as a healthy precautionary measure or had posts that fractured, it was difficult to determine if a complete healing cycle would have occurred.

The Floy and modified spaghetti tag designs were not retained for sufficient time to fully evaluate their healing cycles. The application of three spaghetti tags was without sufficient force to seat them properly.

The fourth (Floy type), however, lost its streamer and eventually caused an infection, prompting surgical removal. It was not determined if the barb had migrated medially.

Flag tagged animals sustained abraded areas along the anterior margin of the dorsal fin. This abrasion was not significant enough to warrant removal of the tags.

The peduncle-tagged animals sustained significant tissue erosion at the junction of tail flukes and peduncle (Fig. 31 ). The tags were removed as a health precautionary measure. This tag type needs to be evaluated for a longer period of time to determine if the animal could regenerate enough tissue to mediate abrasion by the tag.

## Discussion

Posts for dorsal fin disc tags are in need of further improvement to reduce application time, and increase retention time. A delrin disc and sleeved stainless steel post combination provided best tagging results but the value of the sleeve is difficult to determine. A sleeveless stainless steel post was not evaluated. If nylon or delrin posts are to be evaluated further, a comparison should be made between posts with and without sleeves.

As shown in Fig. 46, the dorsal fin exhibits continuous curvature in several different planes. Effective placement of a disc tag requires careful adjustment of the post to minimize disc pressure on the epidermis. This is time consuming, but can best be accomplished with a threaded post strengthened by a sleeve. Typical aerial behavior (Fig. 47) resulting in flexion of the dorsal fin may still produce abrasions where contact is made with the tag (Figs. 41, 45). Since Stenella attenuata, the primary animal to be tagged in the field, is not prone to aerial behavior, these factors may not be as important.

The Floy and modified spaghetti tags were not retained long enough for a meaningful evaluation, although the Floy type which lost its streamer eventually caused infection and was surgically removed. Absence of the streamer may have allowed medial migration of the barb. This tag type has shown good retention in previous tests, and can be applied rapidly. We would suggest that they be retested when possible.

Factors limiting the retention of flag tags appeared to be related to materials rather than biocompatibility. Use of sleeved stainless steel pins and smooth delrin or polyurethane flags should improve the durability of this tag and minimize fouling. Four posts should be used rather than two, to reduce flexion of the flags.

Peduncle tags may still warrant further evaluation, using a wider and softer foam type material similar to the lining of delphinid radio tracking packages. This material may reduce the tissue abrasion observed with the surgical tubing (Fig. 40), and improve visibility. The wire tie fastener appears to be an effective locking mechanism.

Although numerous instances of tissue trauma were observed, all the test animals completed tag site healing cycles rapidly. Antibiotics were administered in their food and copper sulfate was added to the pool water. However, it should be emphasized that the open ocean environment is essentially sterile, and therefore water treatment or use of antibiotics should not bias this tagging data enough to limit its value.

## Recommendations

Field tagging should be accomplished using dorsal fin disc tags or flag tags, or spaghetti tags, with improvements in design and

materials as discussed. At this time, it should not be expected that tag application (per unit) can be completed in the 60 seconds requested (except for spaghetti tags), since careful post adjustments are still necessary.

Delrin or polyurethane in bright yellow appear to be the most qualified materials for discs or flags. Stainless steel posts sleeved with delrin should be used for attachment. All these materials are biocompatible. Since tag design progress has not completely eliminated tissue trauma problems, we recommend that a medication be used during field tagging to supplement the animal's adjustment to the tag.

Progress in evaluation of tags during this project has been affected by limited access to test animals specifically designated for tagging research. The success of future work will depend on establishing a group of pelagic delphinids designated for tagging research by permit, in a controlled environment situation.

List of Figures

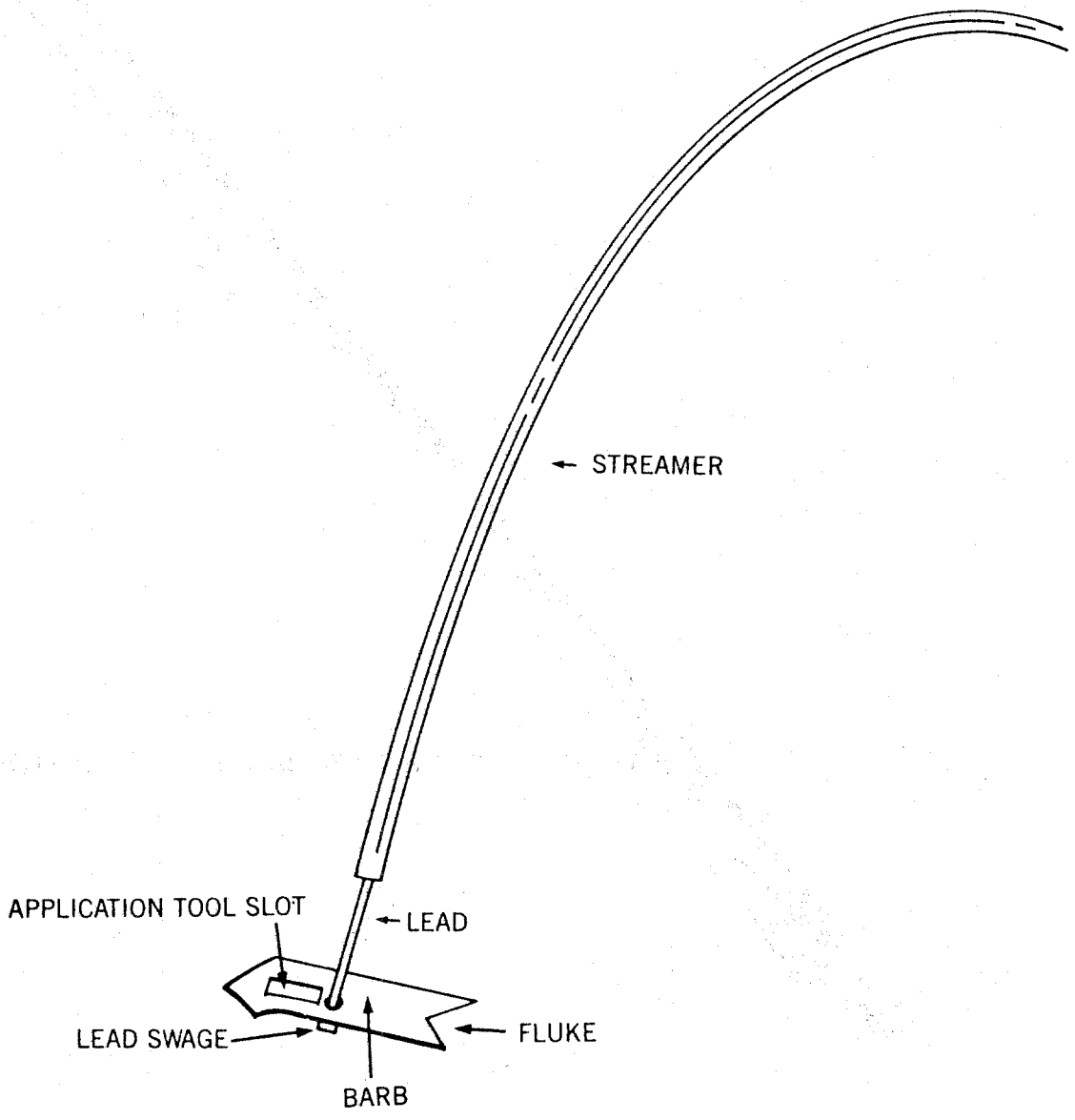



FIGURE 1 — FLOY SPAGHETTI TAG

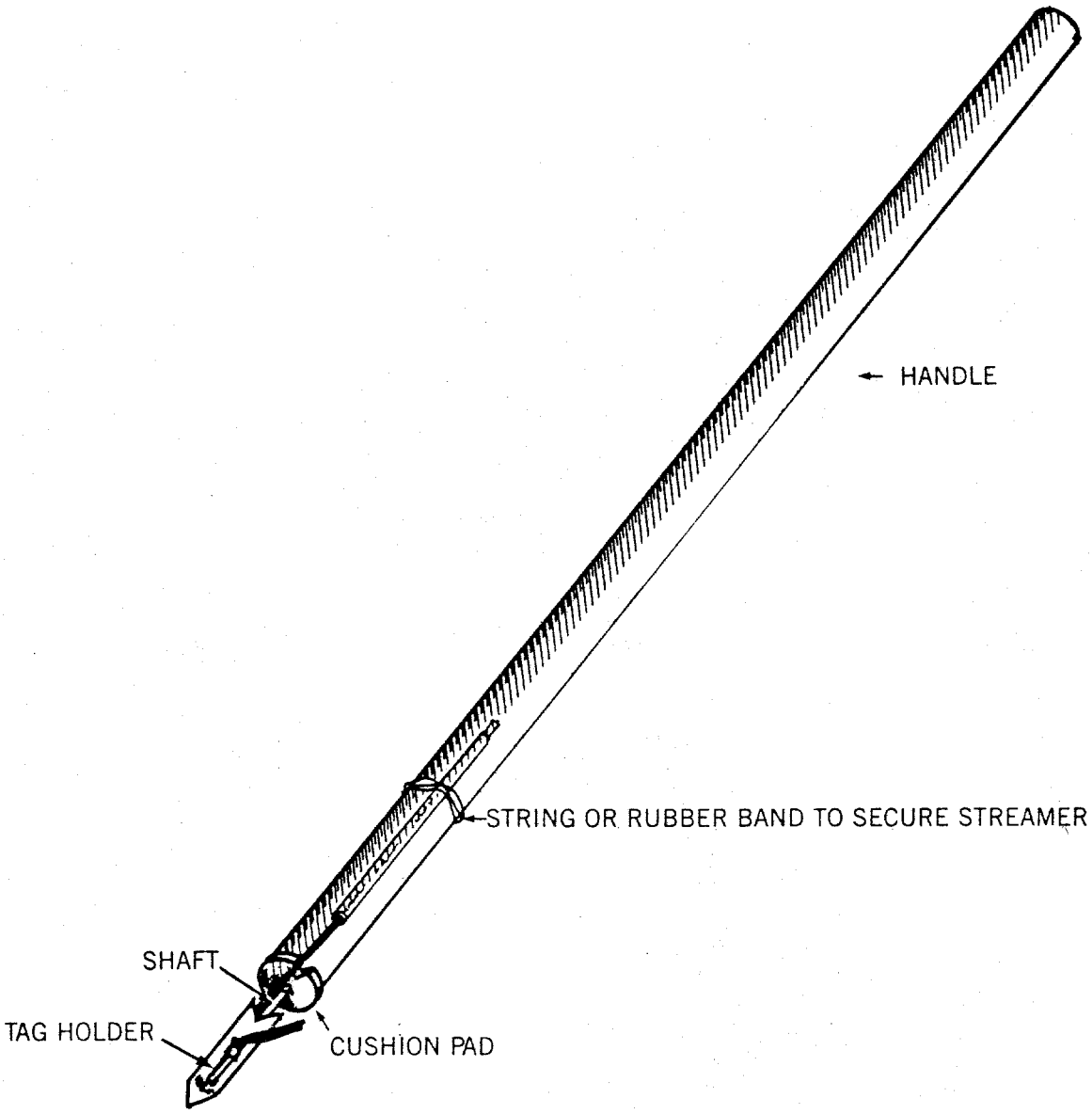



FIGURE 2 — SPAGHETTI TAG APPLICATION TOOL

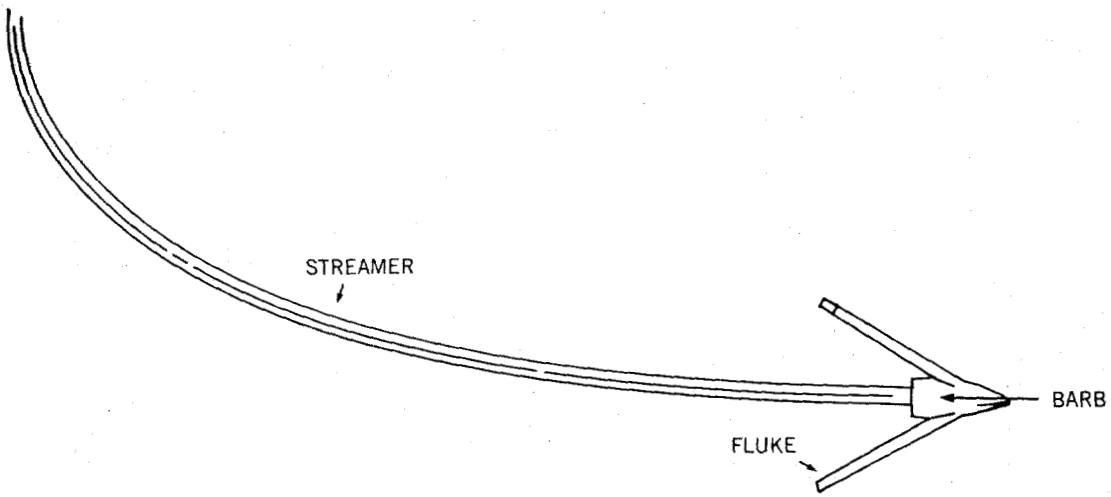



FIGURE 3 — FLOY ARROWHEAD TAG

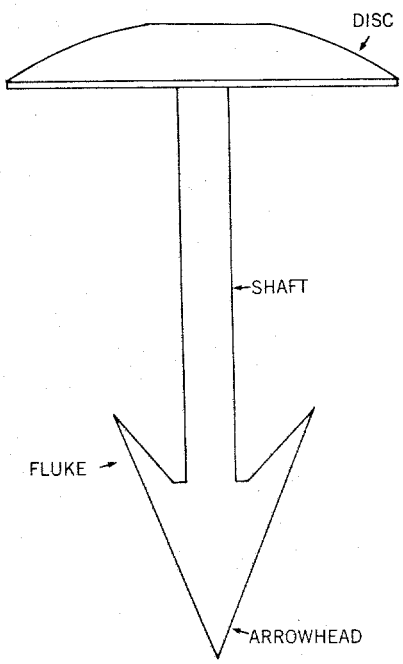



FIGURE 4 — DISC — ARROWHEAD TAG

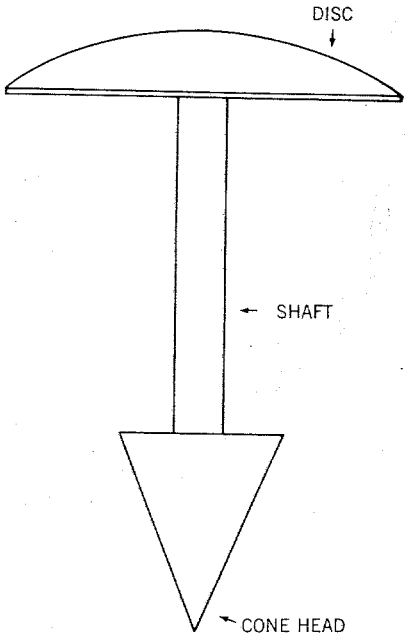



FIGURE 5 — DISC — CONE HEAD TAG

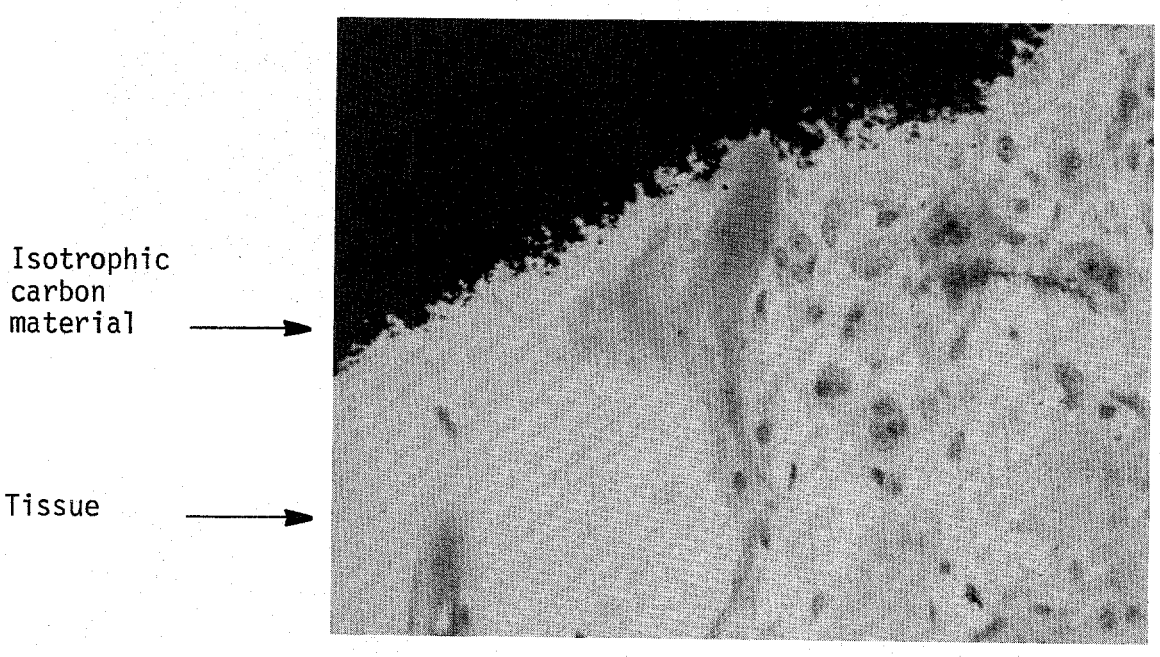



Figure 6 - Magnified view of the interface where the tissue is growing into the low temperature isotrophic carbon.

Photo is courtesy of CarboMetics, Inc.

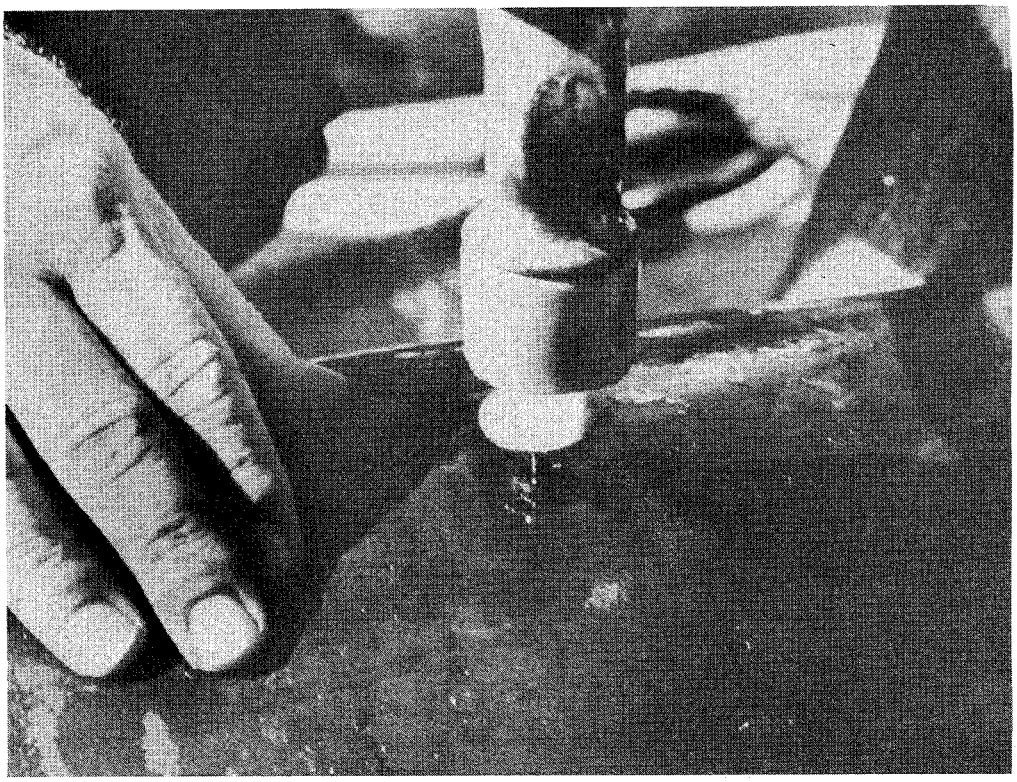


Figure 7 - Tag application with a plastic-headed mallet - Delphinus.

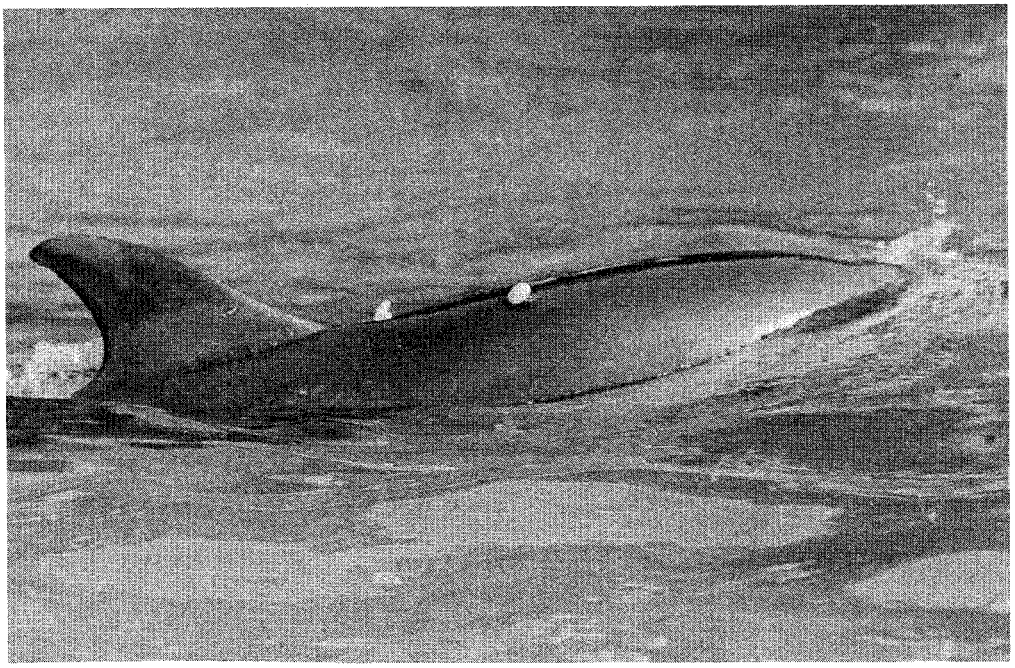


Figure 8 - Protrusion of tag disc above epidermis - Delphinus.

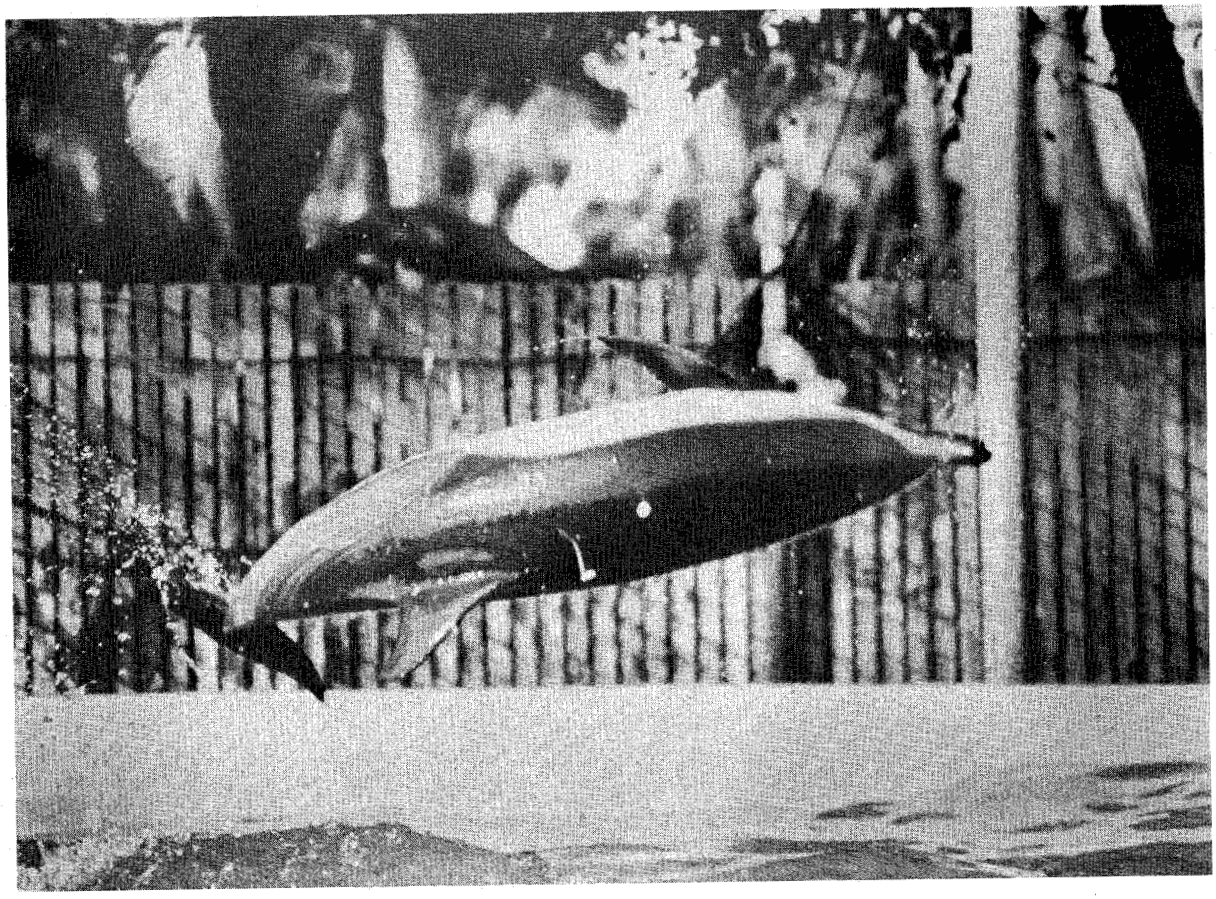


Figure 9 - Aerial behavior of tagged Delphinus.

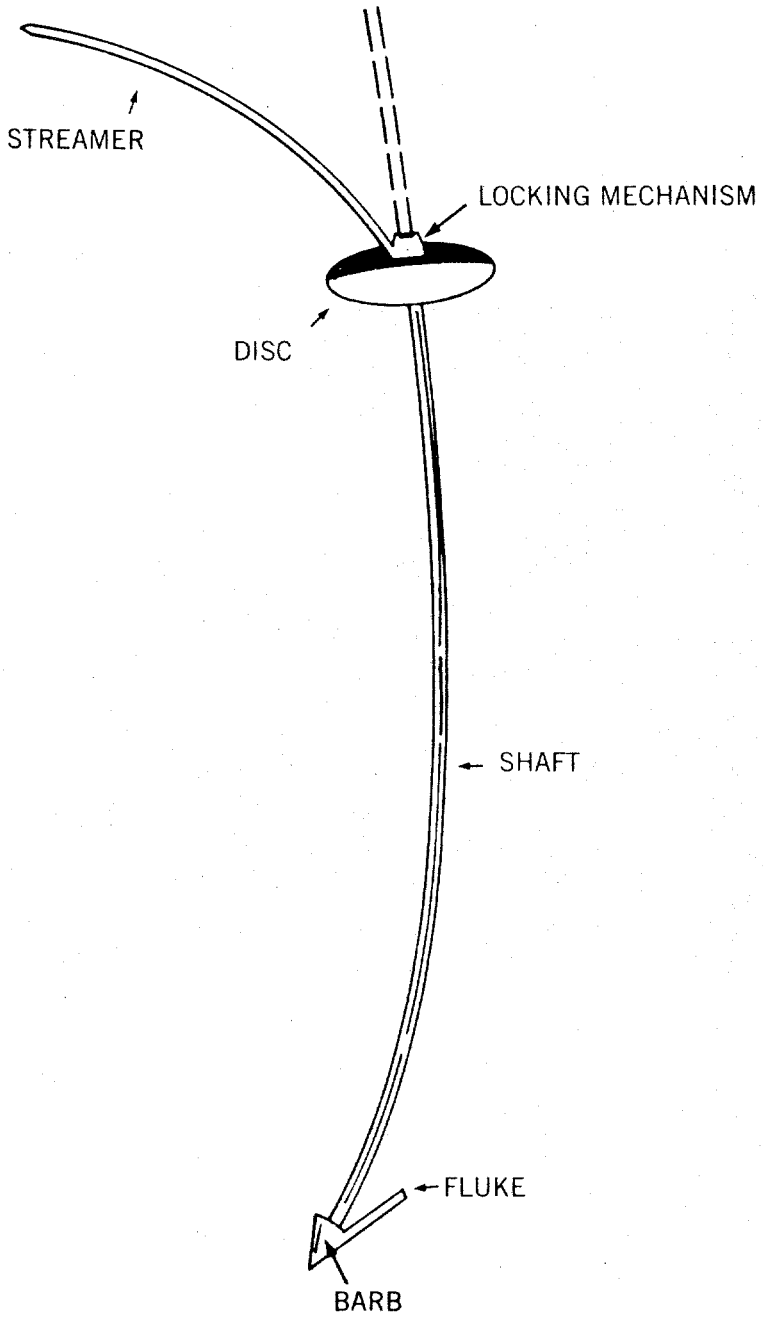



FIGURE 10 — ADJUSTABLE DISC ARROWHEAD TAG

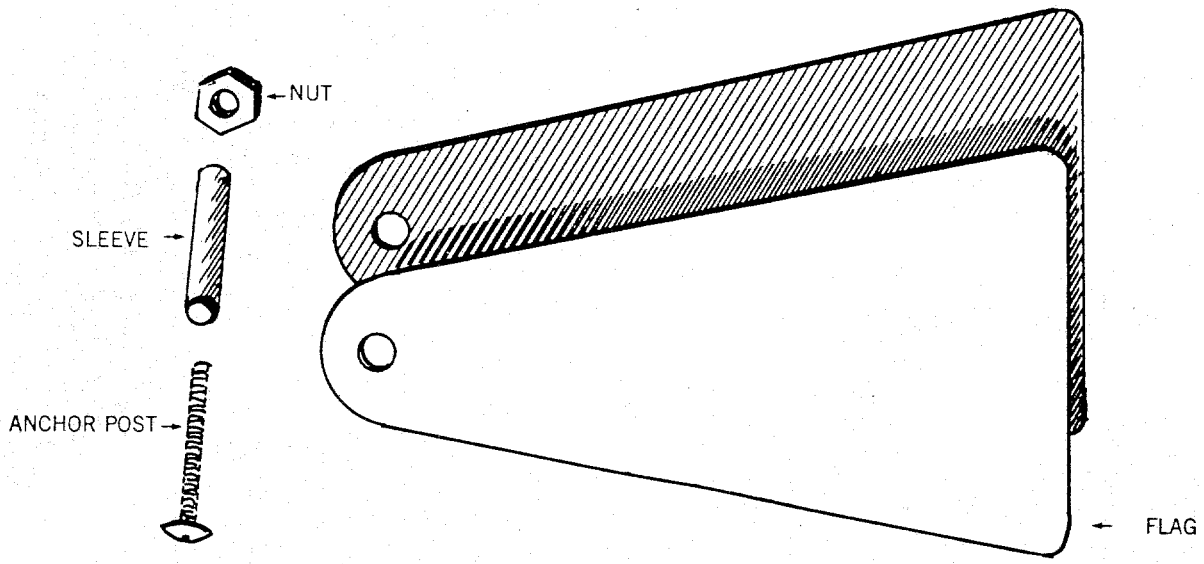



FIGURE 11 — FLAG TAG

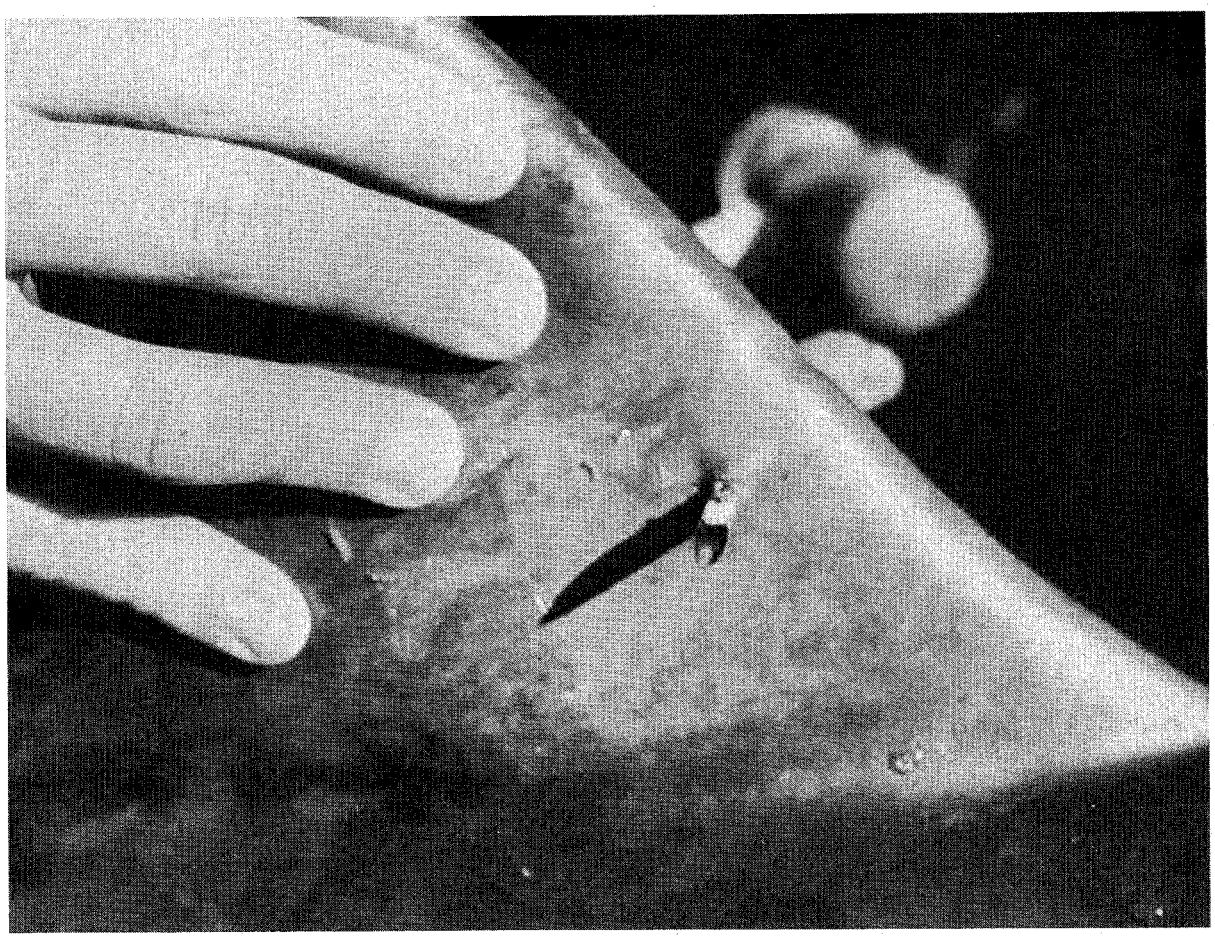


Figure 12 - Trocaring of the dorsal fin - Delphinus.

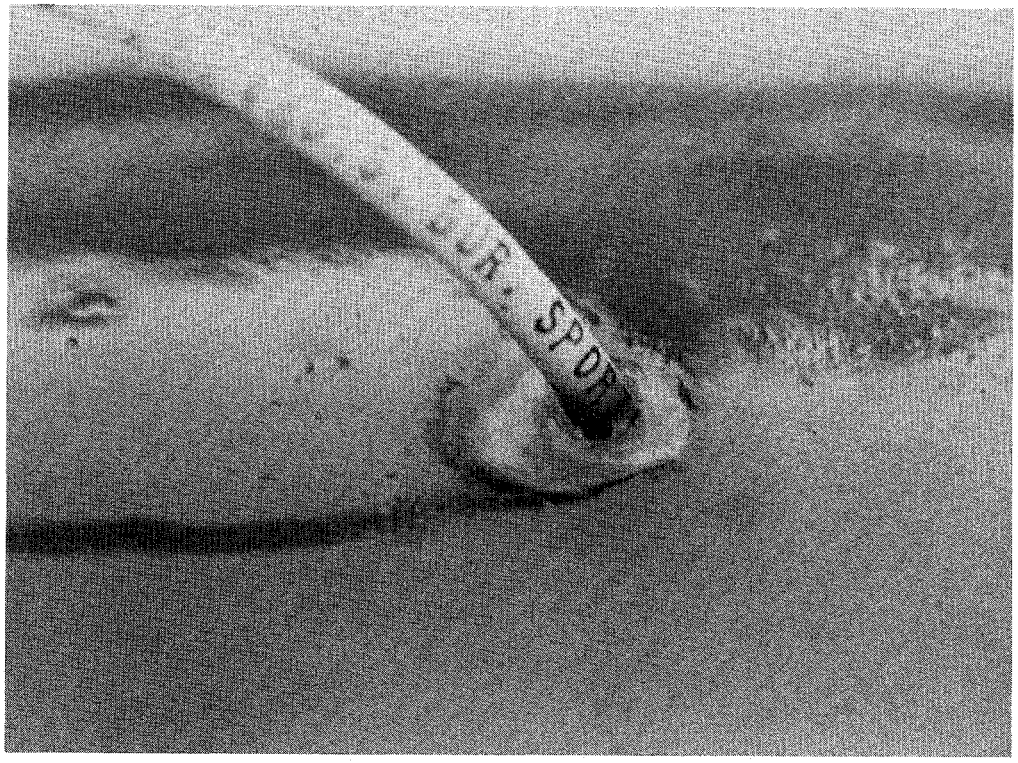



Figure 13a - Floy arrowhead tag - Delphinus.

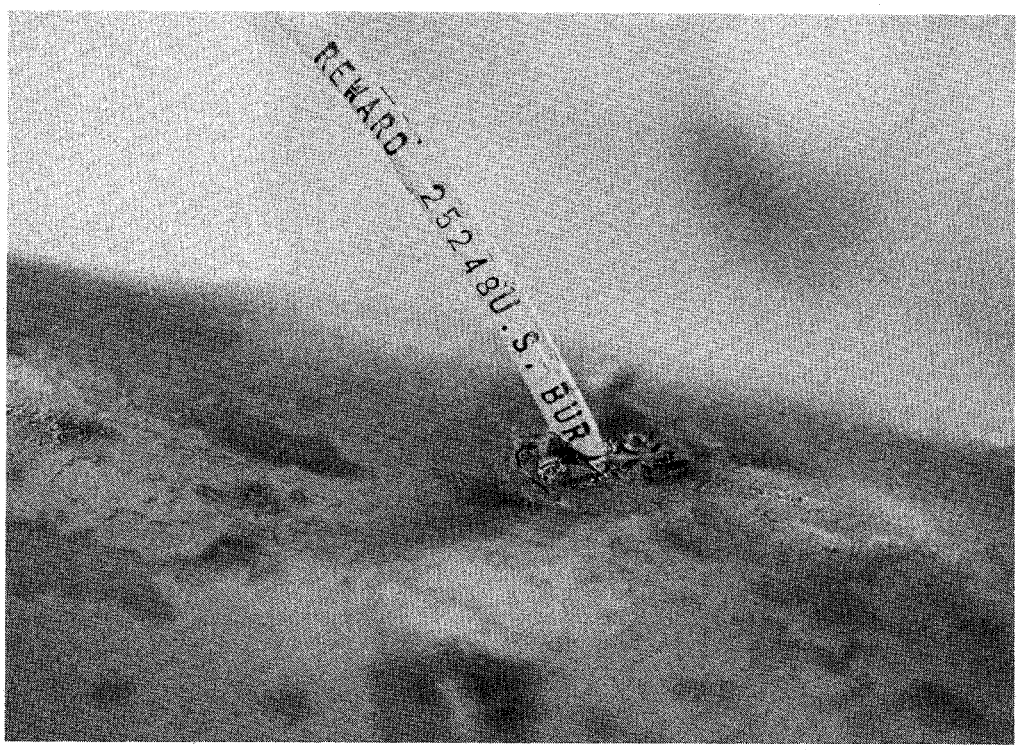



Figure 13b - Evidence of migration in the tissue - Delphinus.
Note "Sport" has disappeared below epidermis.

Figure 14 - Section of anchor post hole in dorsal fin showing extensive re-epitheliazation - Delphinus.

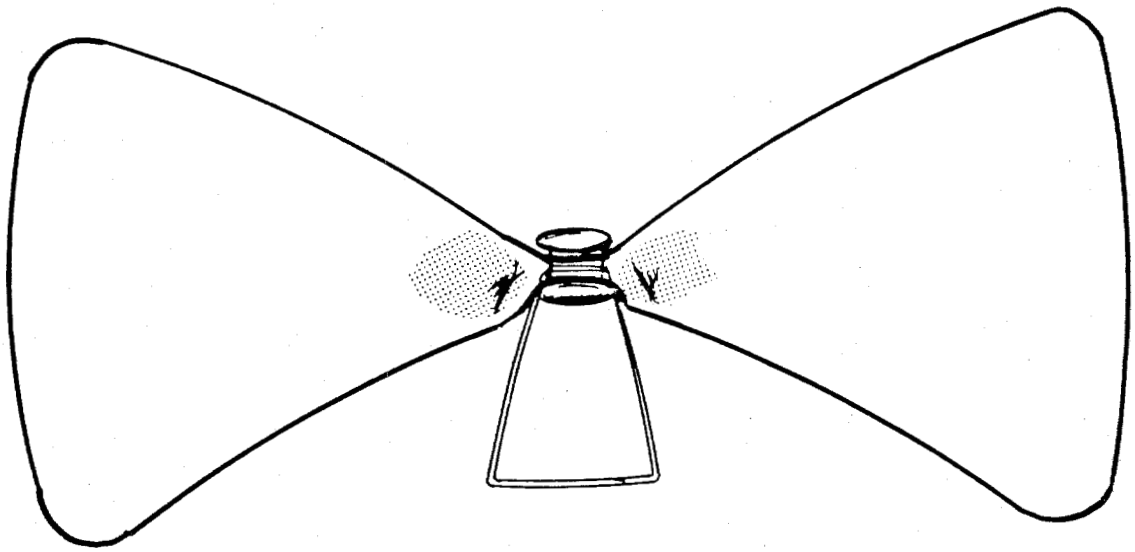


FIGURE 15 — ANTERIOR DORSAL FIN TAG
WITH WRAP AROUND STREAMER

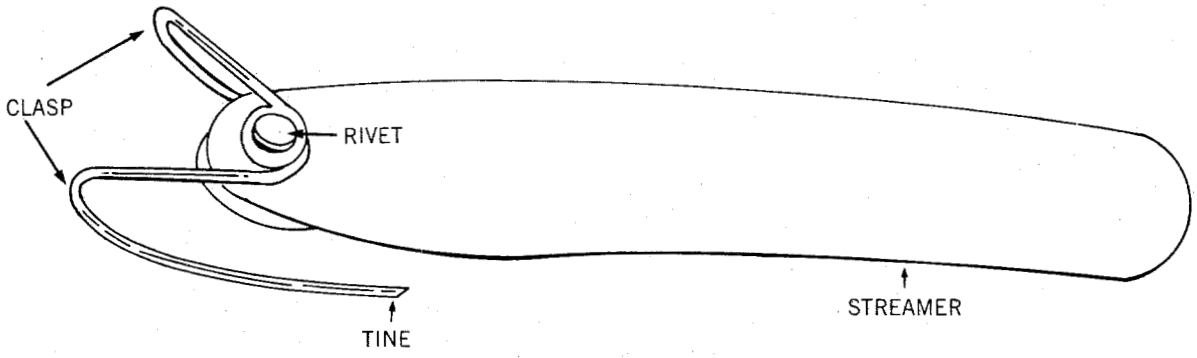



FIGURE 16 — METAL SCOOP TAG

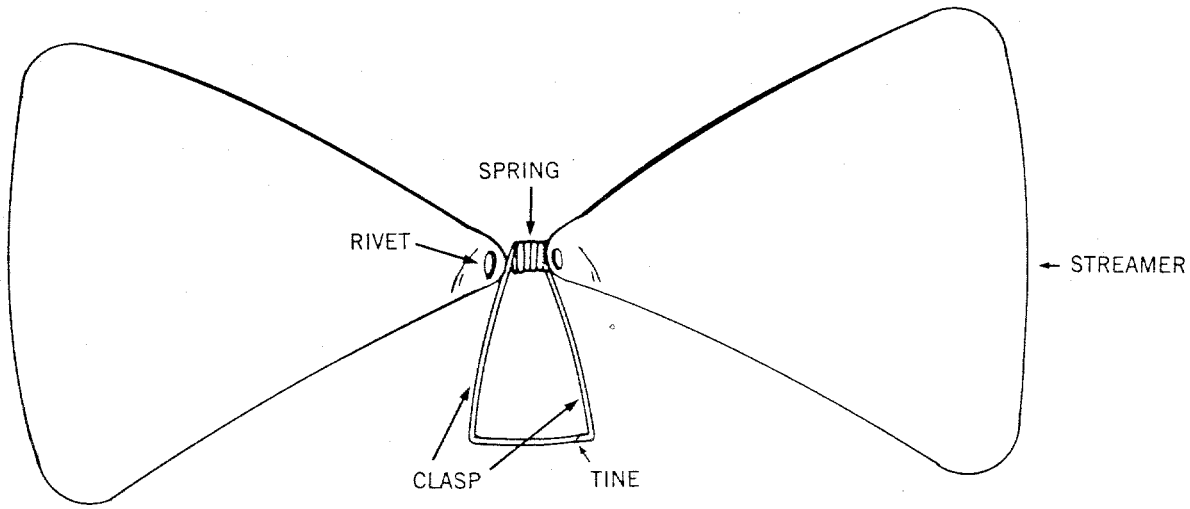



FIGURE 17 — ANTERIOR DORSAL FIN TAG WITH SIDE MOUNTED STREAMERS

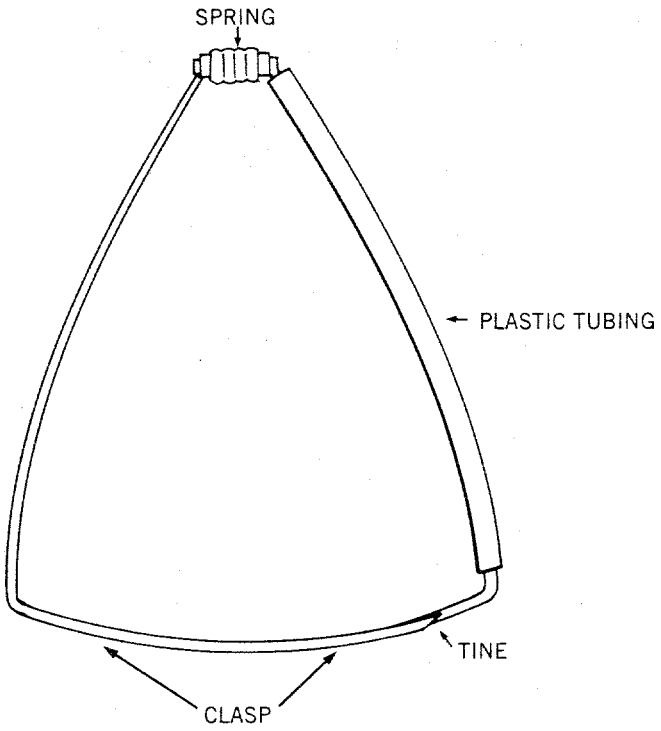



FIGURE 18 — POSTERIOR SPRING CLIP TAG

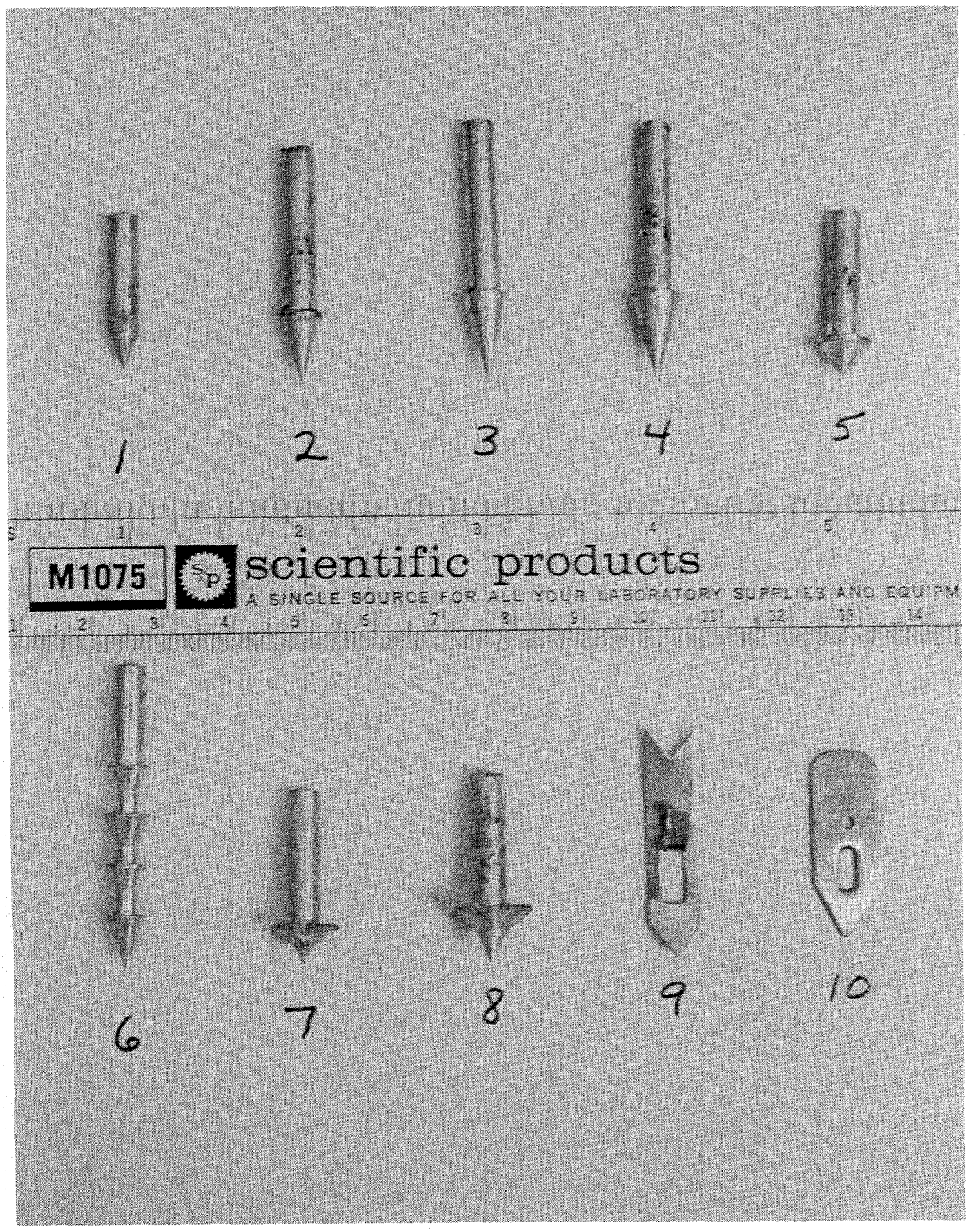



Figure 19a - Tag heads used for insertion and withdrawal tests.

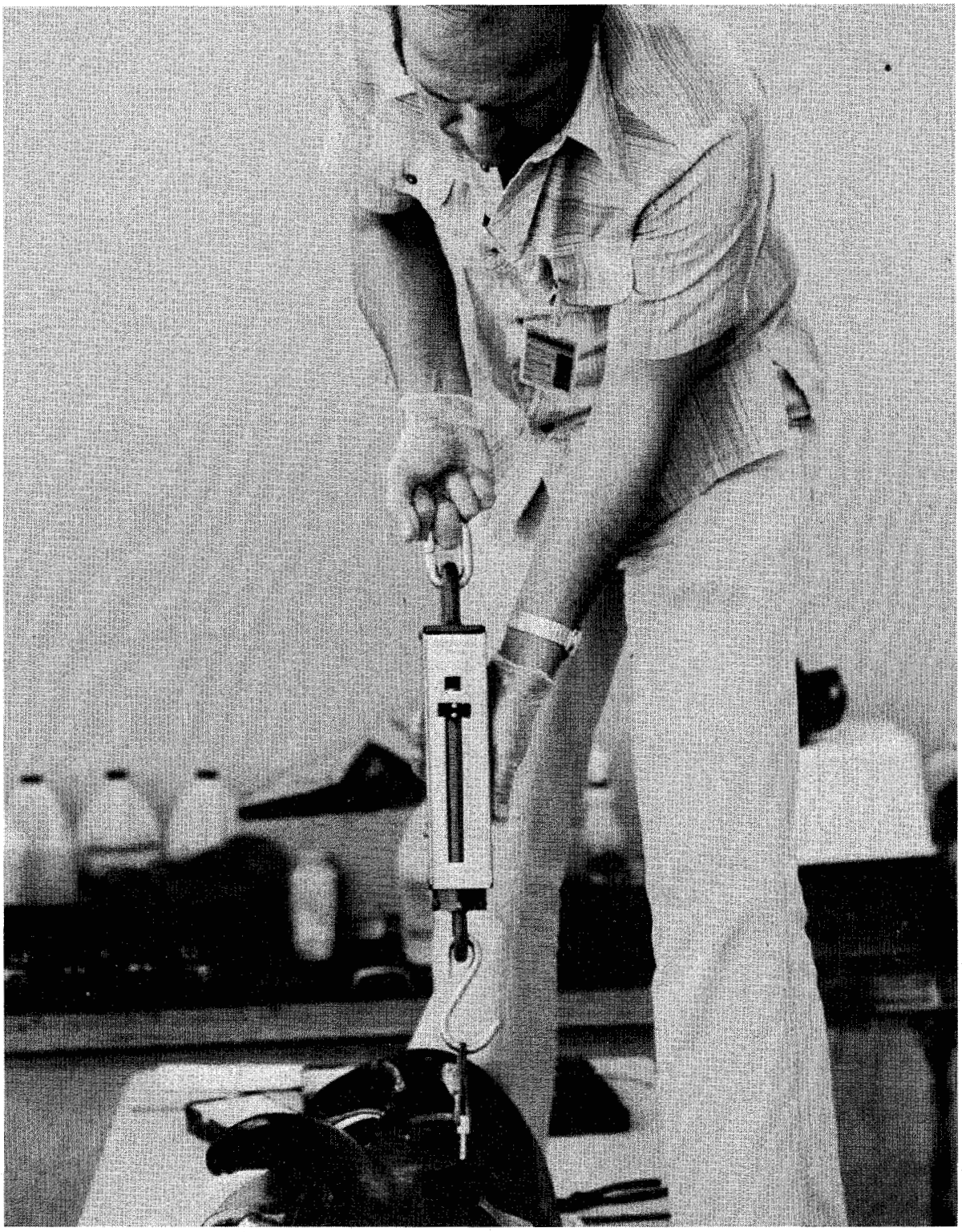


Figure 19b - A specially designed spring scale and tag head chuck for measurement of insertion and withdrawal forces (psi).

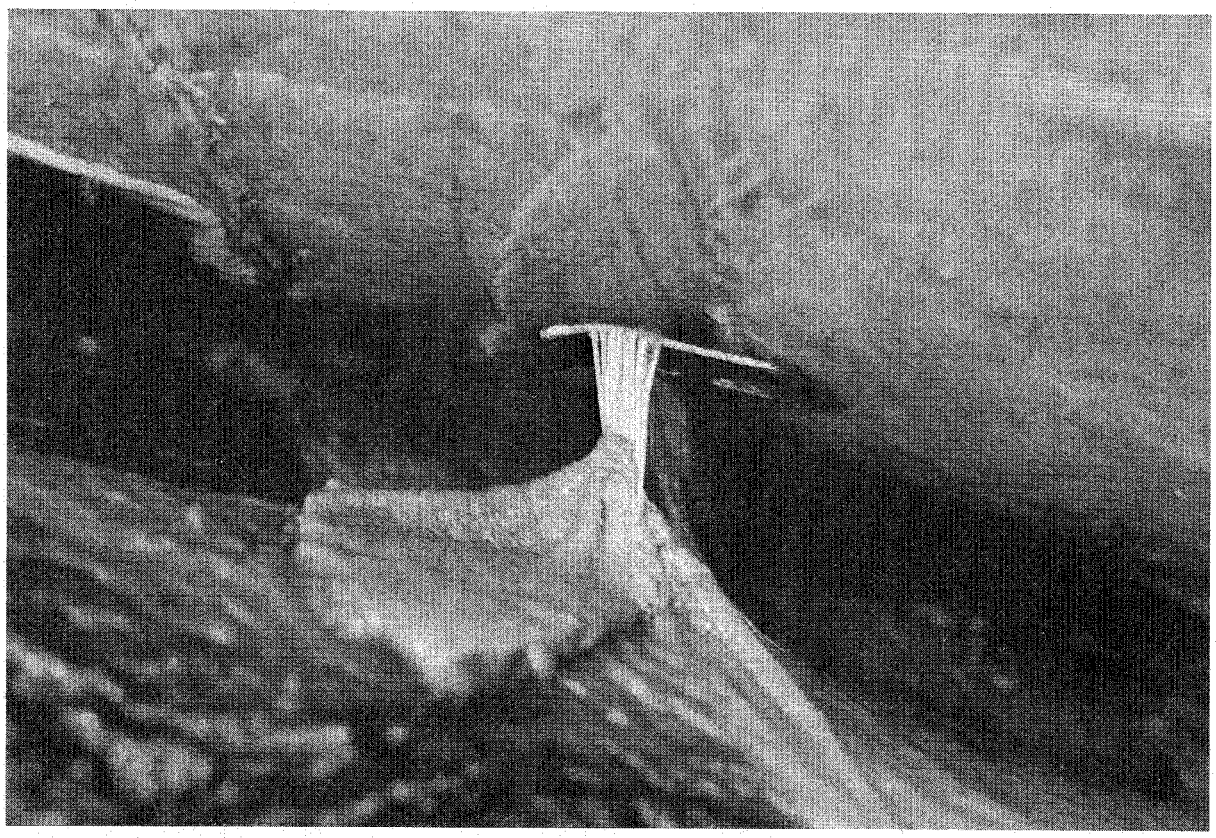


Figure 20 - Spaghetti tag barb anchored in the fascia between muscle and blubber layers. Delphinus.

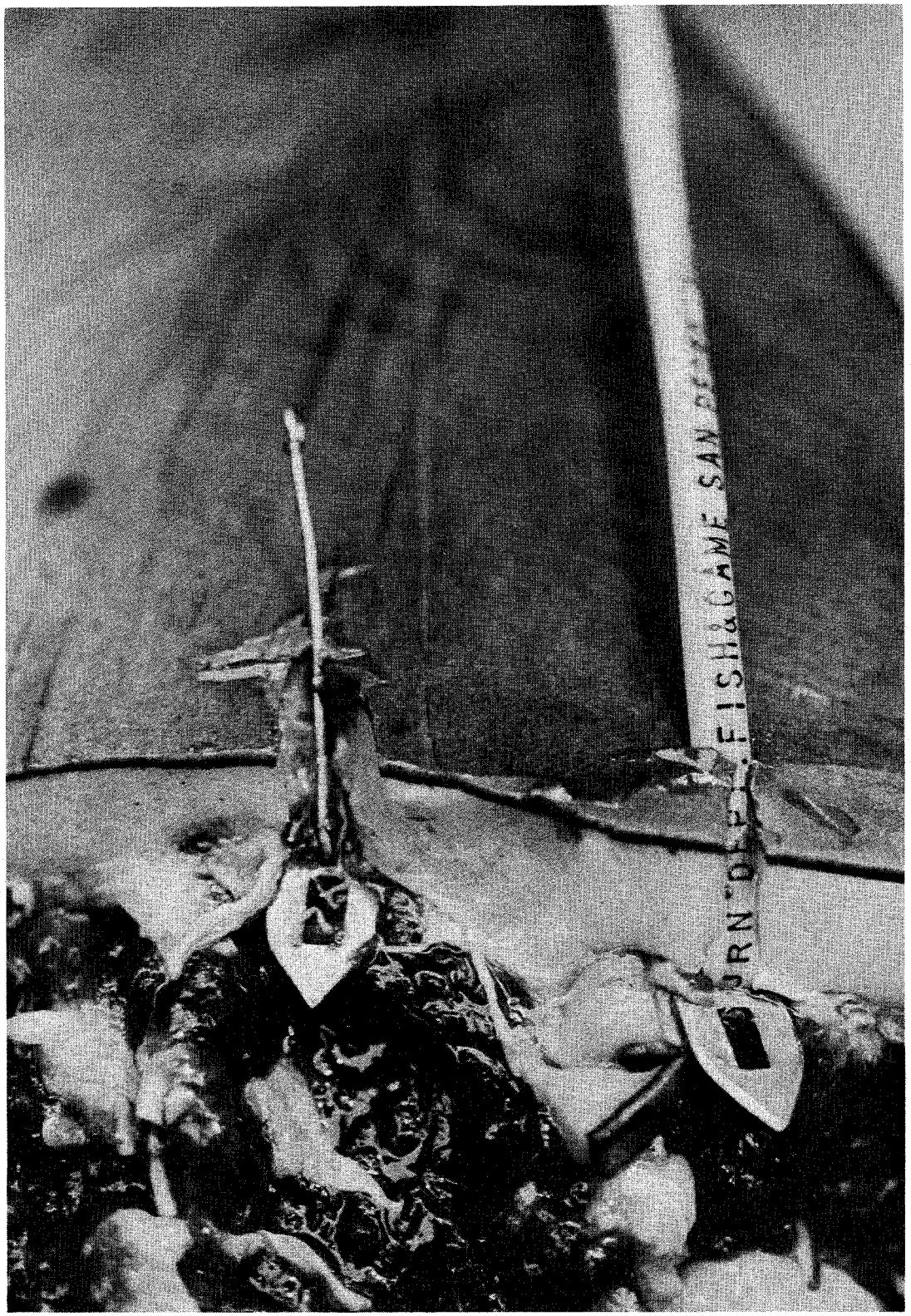



Figure 21 - Dissection showing toggled Spaghetti tag barbs between blubber and muscle tissue. Delphinus.

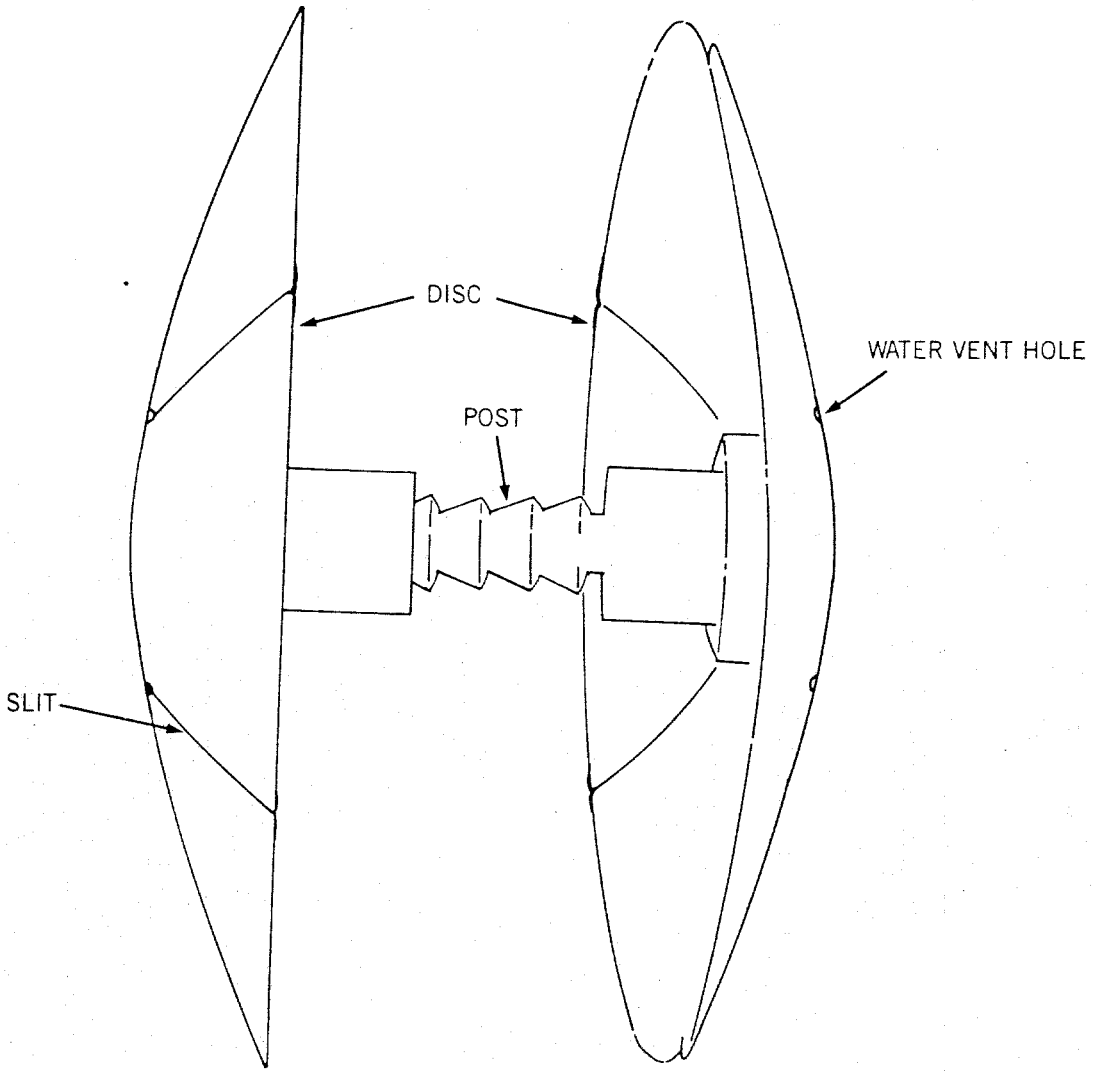



FIGURE 22 — DORSAL FIN DISC TAG

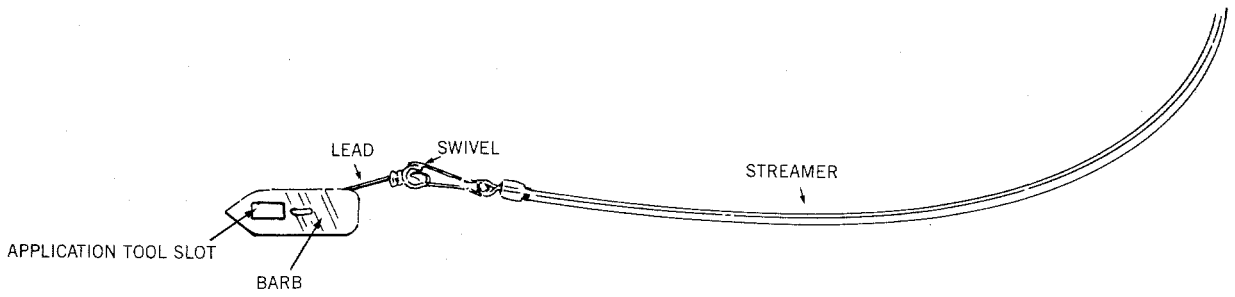



FIGURE 23 — SPAGHETTI TAG — MODIFIED LEAD AND BARB

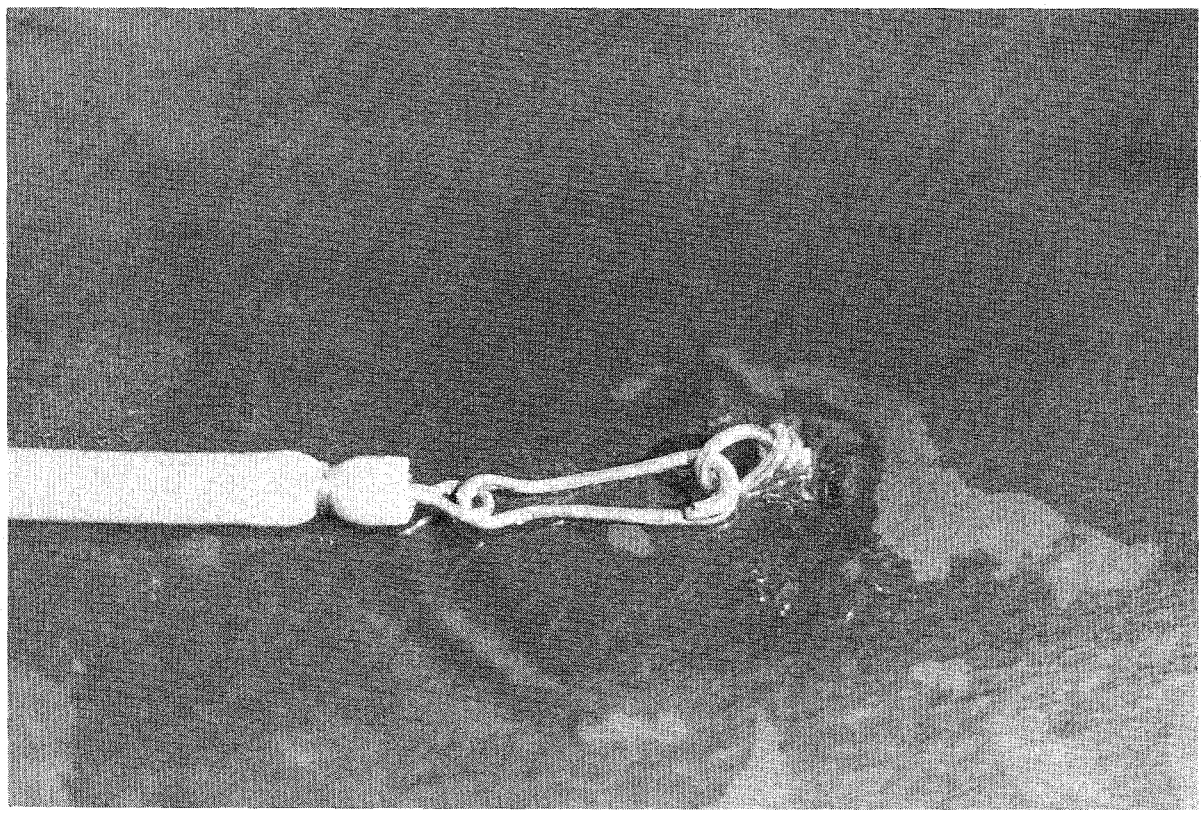


Figure 24 - Spaghetti tag - (modified lead and barb) - showing healed entry site. Lagenorhynchus.

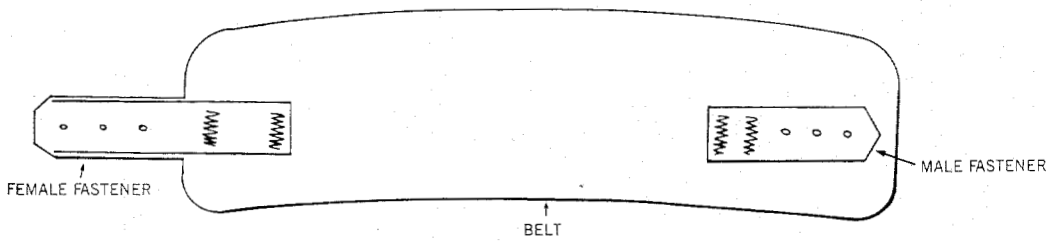



FIGURE 25 — SNAP FASTENER PEDUNCLE BELT

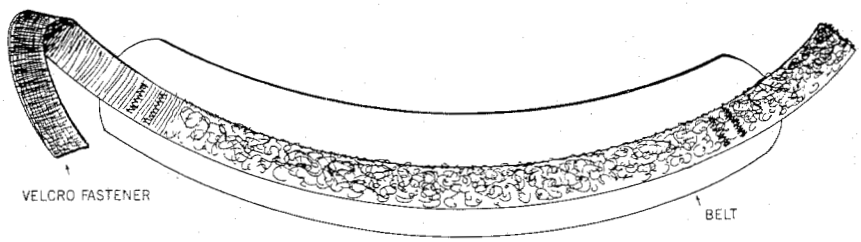



FIGURE 26 — VELCRO RUBBER PEDUNCLE BELT

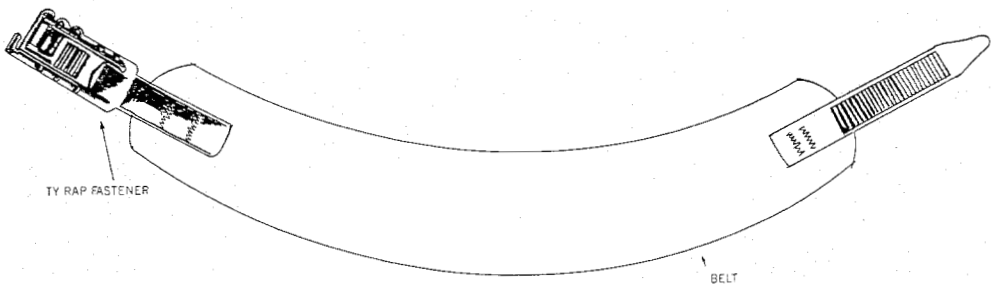



FIGURE 27 — TY RAP RUBBER PEDUNCLE BELT

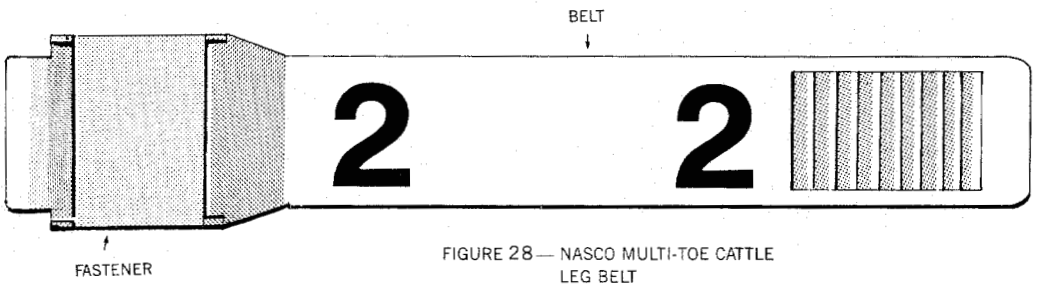



FIGURE 28 — NASCO MULTI-TOE CATTLE LEG BELT

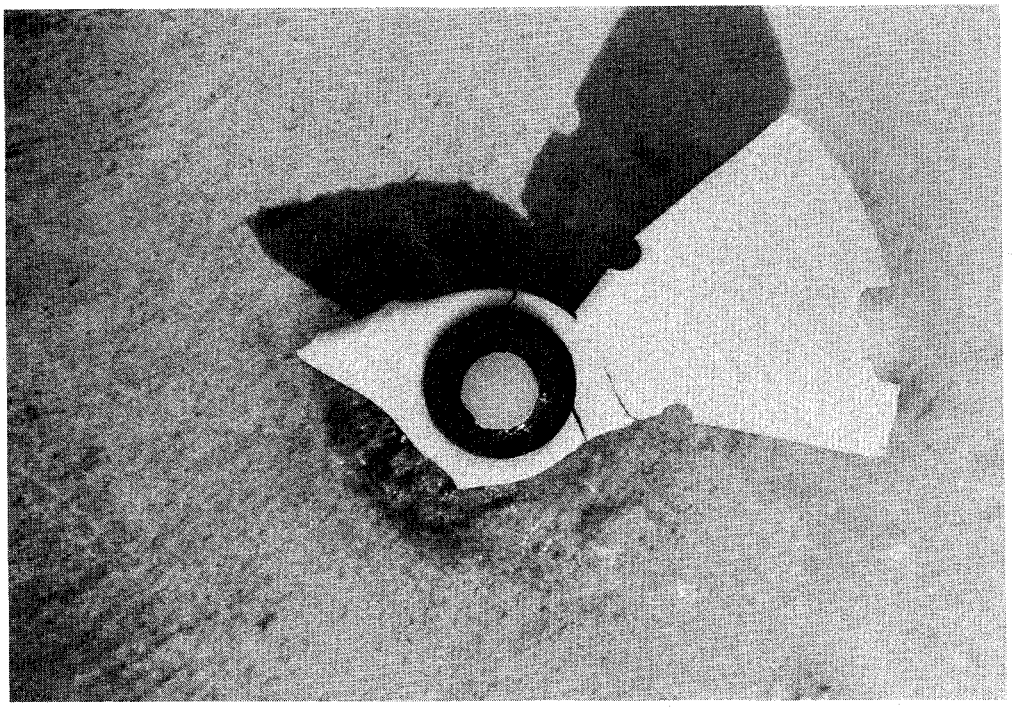


Figure 29 - Dorsal fin disc tag showing fractured disc. Lagenorhynchus.

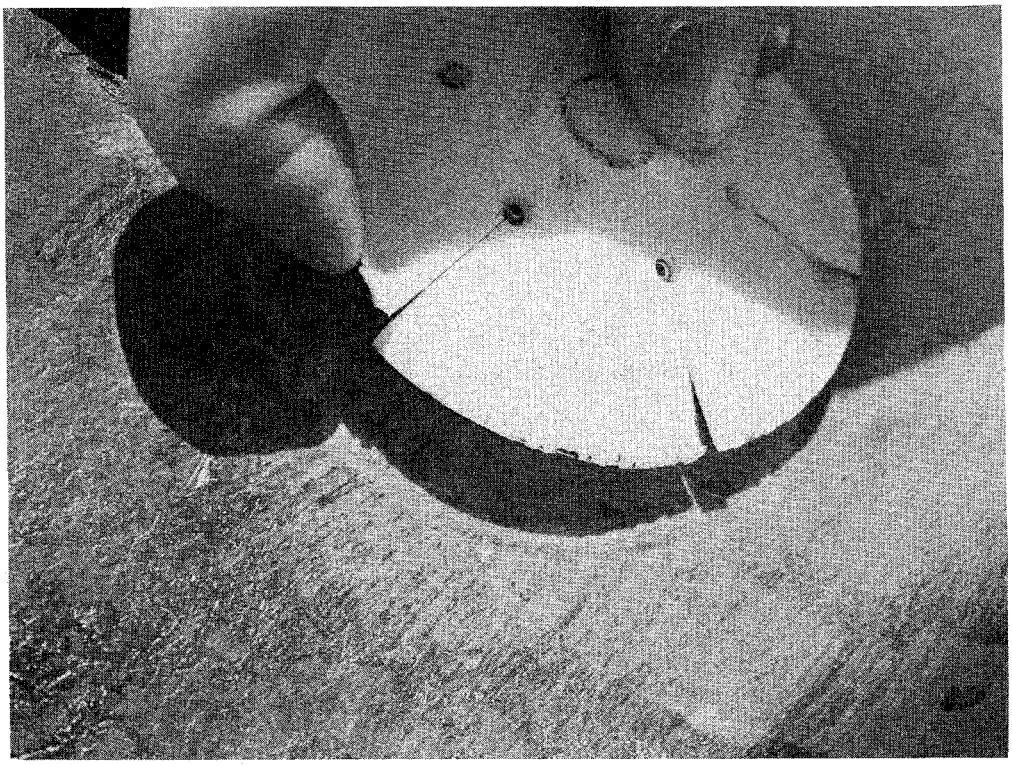


Figure 30 - Indented ring of darkened epidermis around the dorsal fin disc tag's perimeter. Lagenorhynchus.

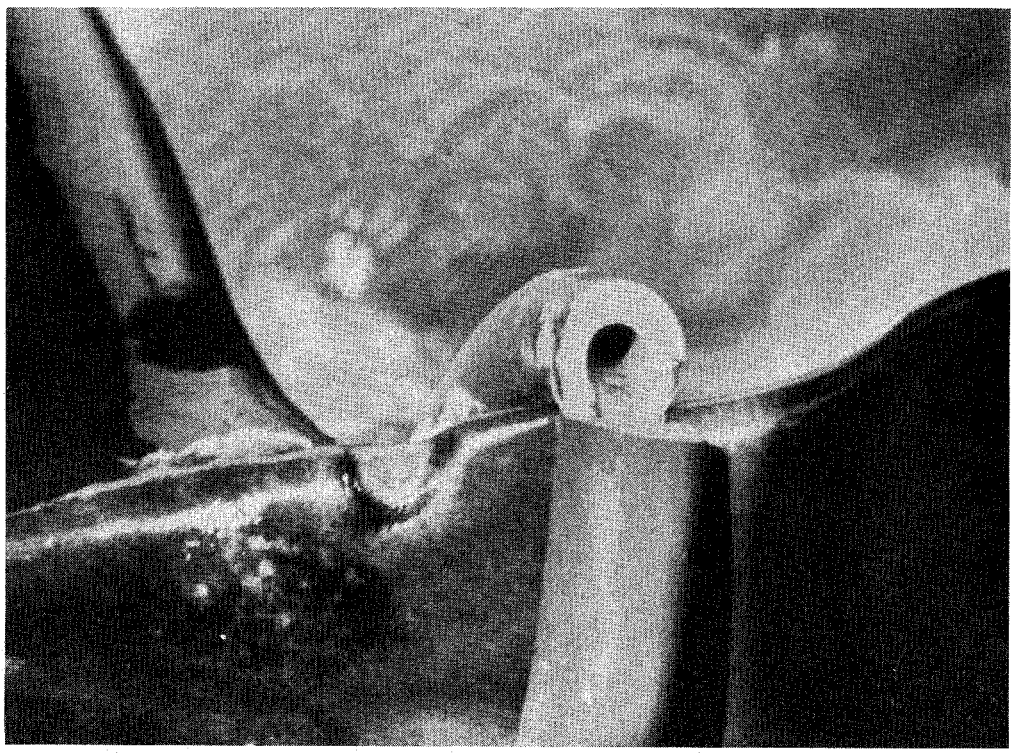


Figure 31 - Abrasion caused by peduncle belt. Lagenorhynchus.

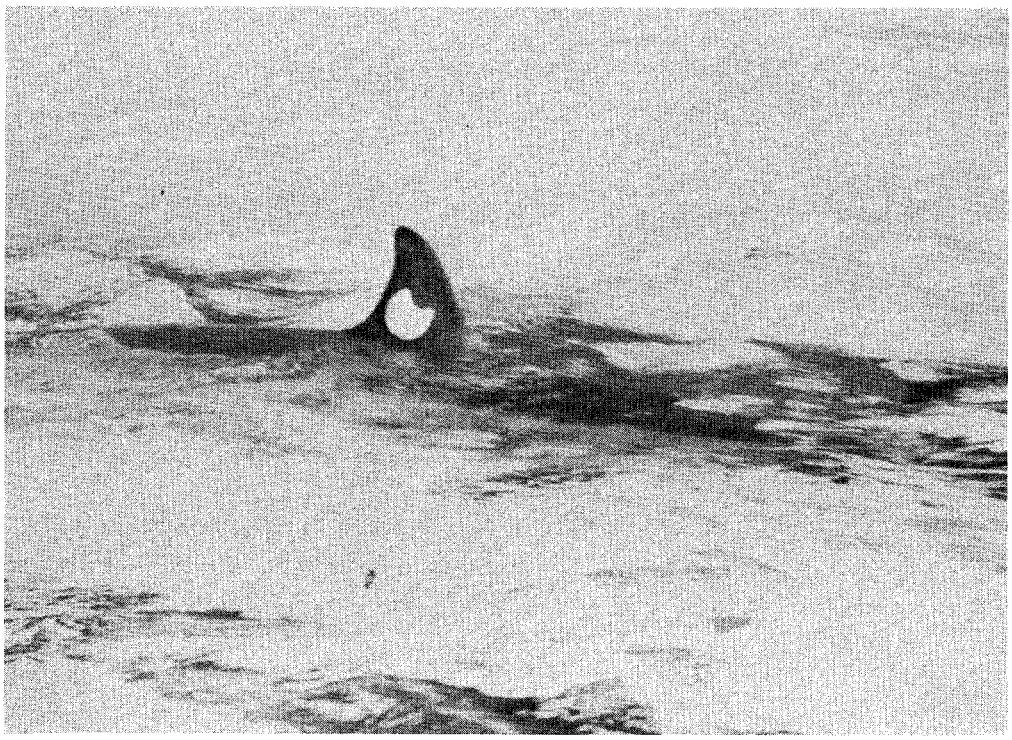


Figure 32 - Dorsal fin disc tag showing fractured disc - Stenella.

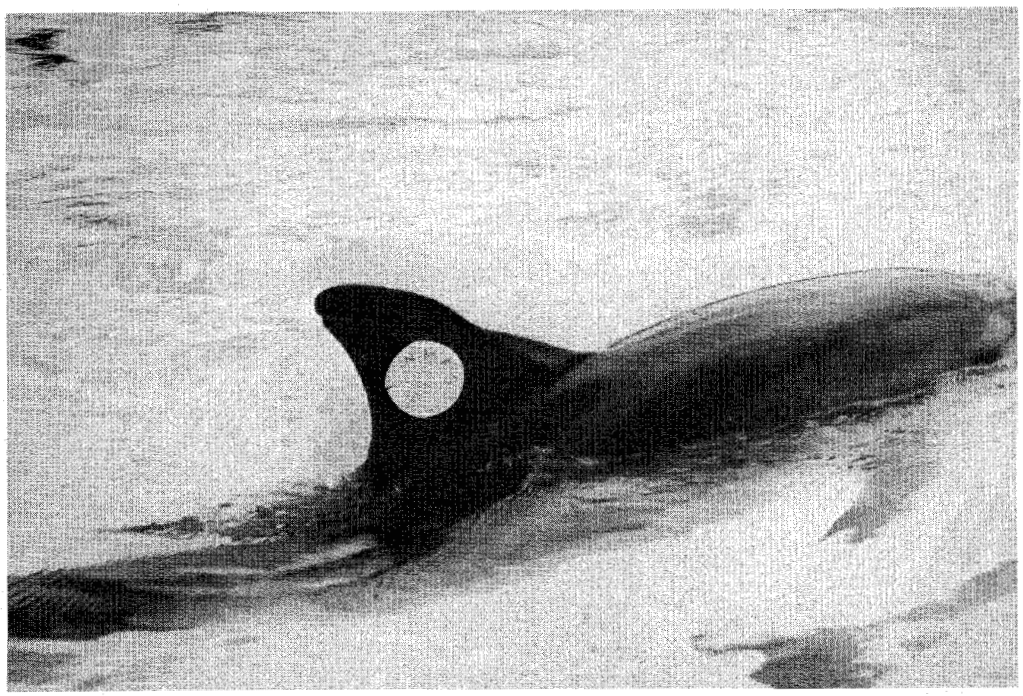


Figure 33 - Disc tag everted by water friction - Stenella.

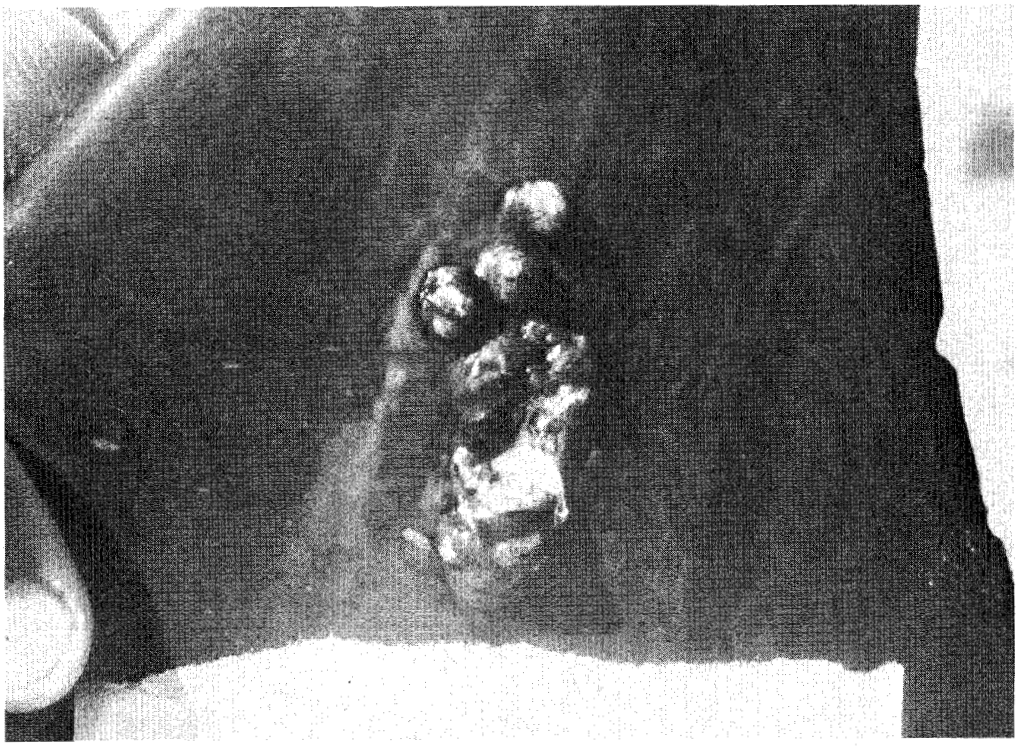


Figure 34 - Inflammation and lesions on disc tagged dorsal fin - Stenella.

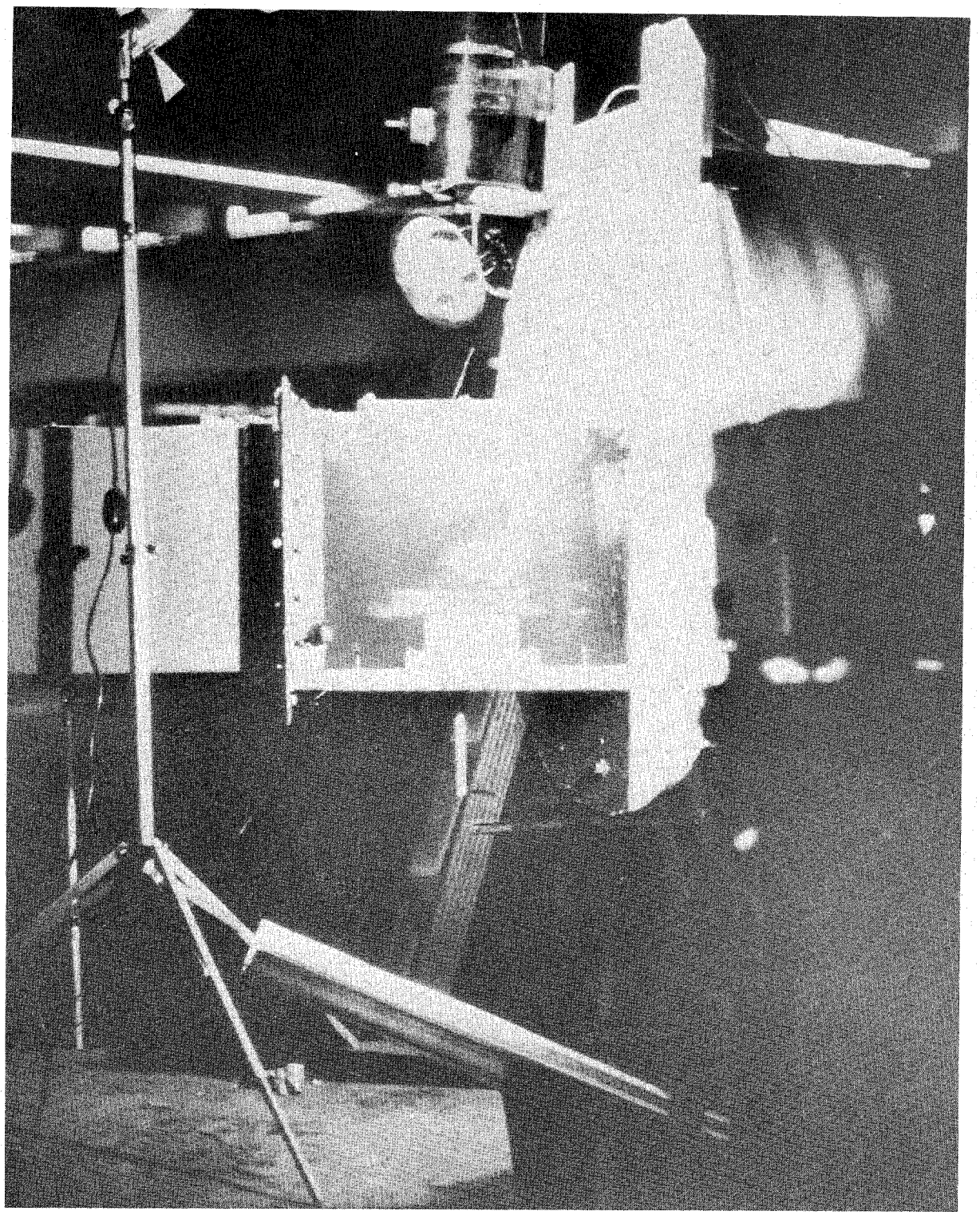


Figure 35 - Flow tank test apparatus for tag evaluation.

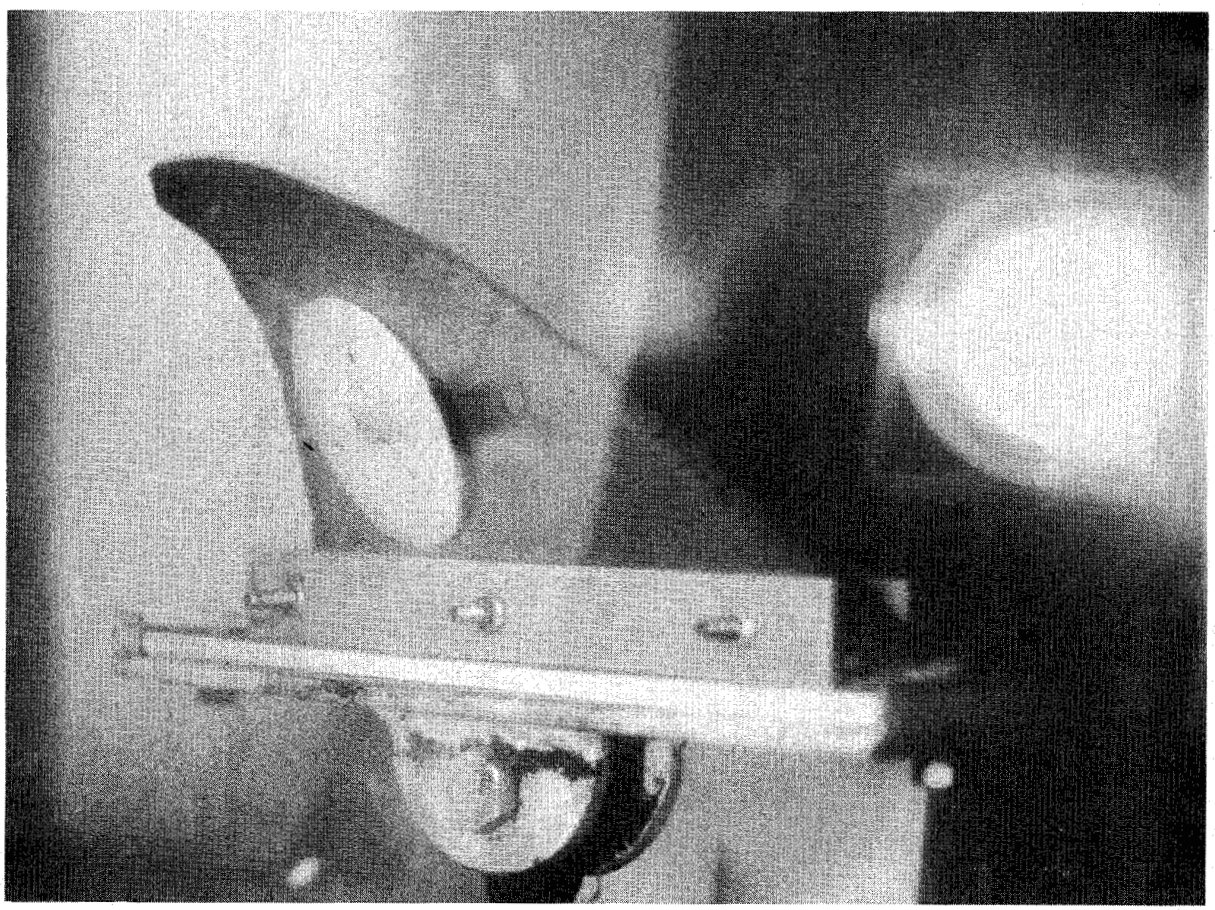


Figure 36 - Flexion of dorsal fin during flow tank test.

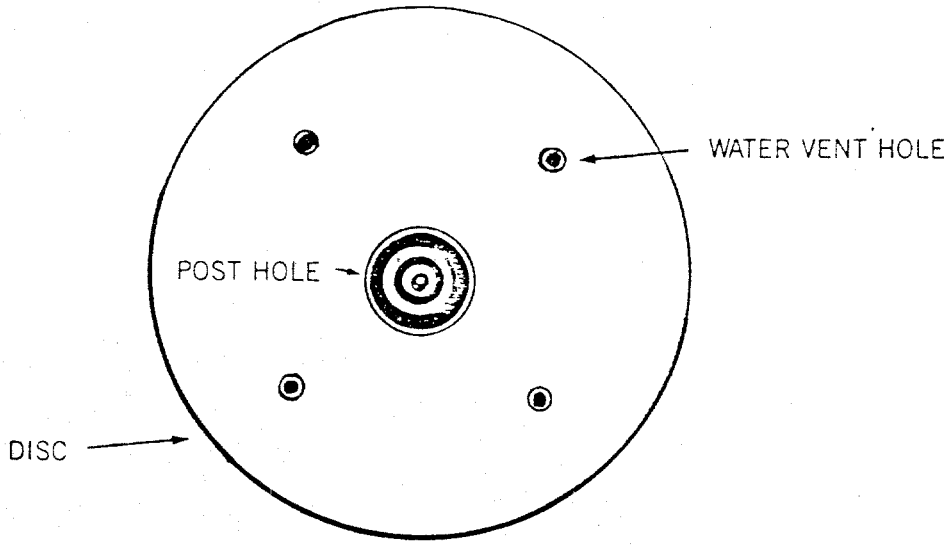



FIGURE 37 — DORSAL FIN DISC TAG

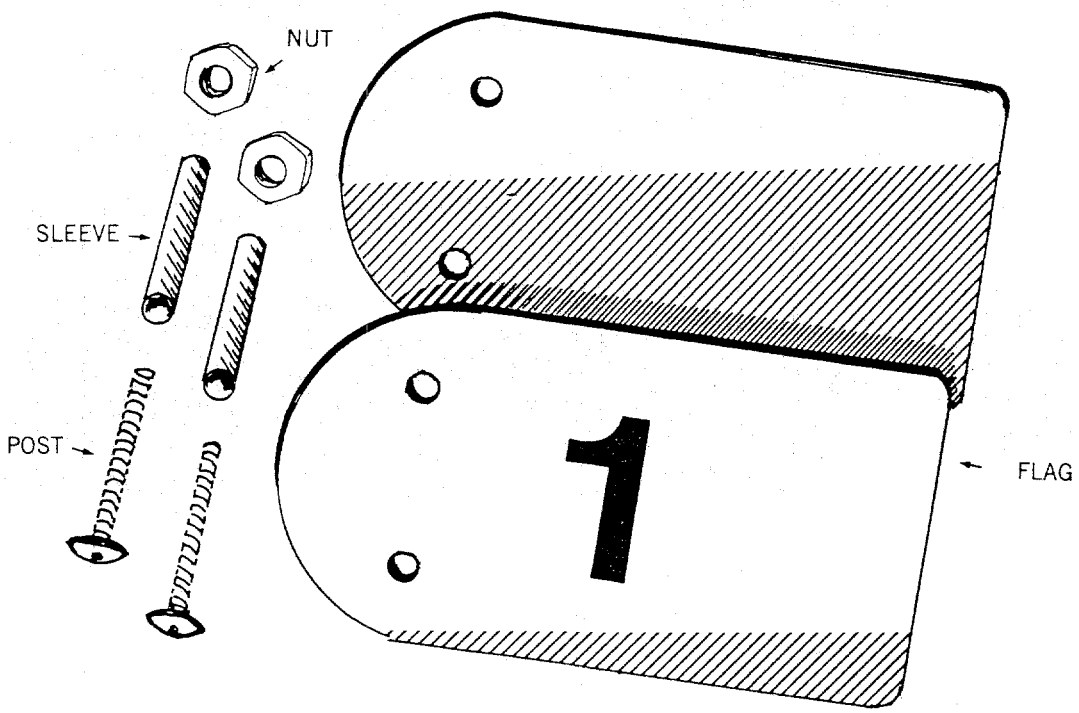



FIGURE 38 - MULTI POST FLAG TAG

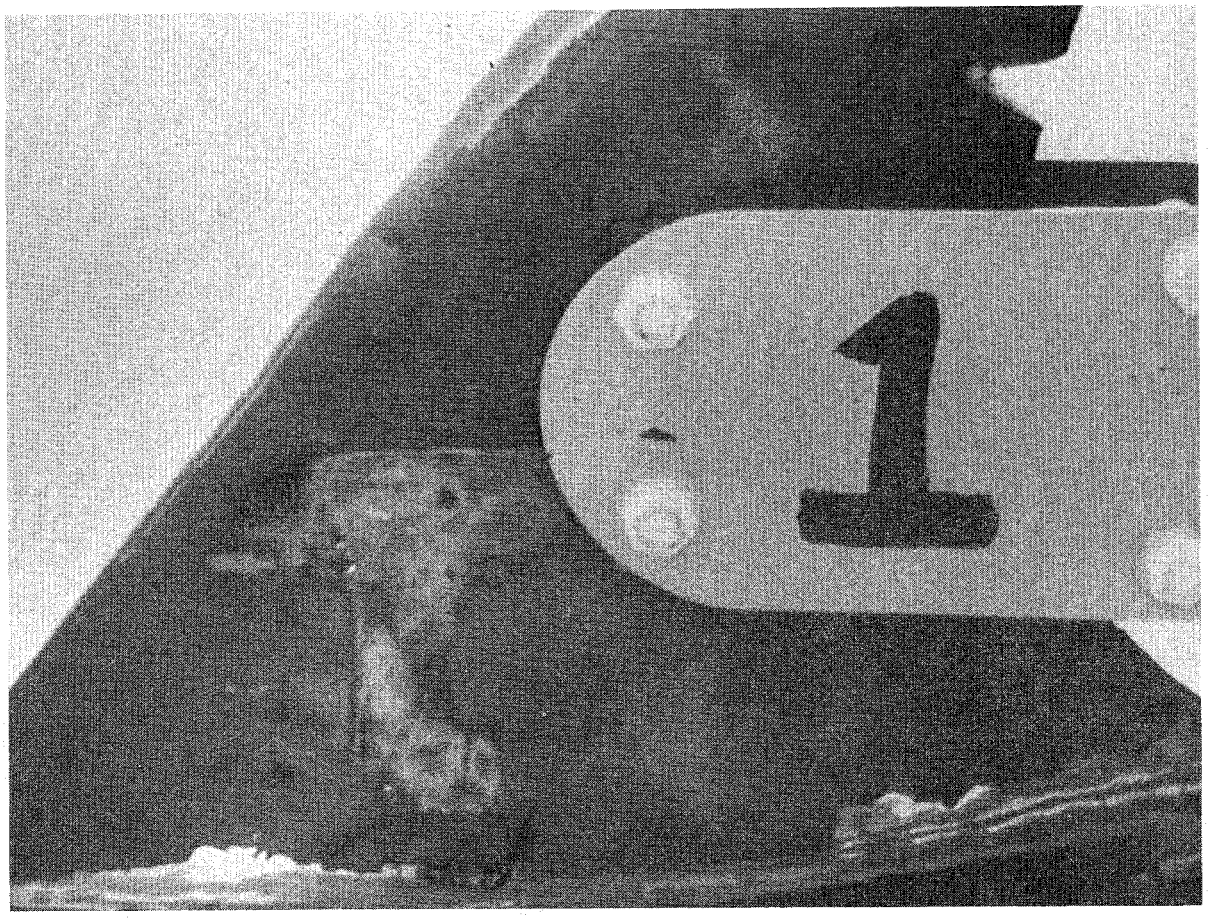



Figure 39 - Multi post flag tag on Stenella dorsal fin.

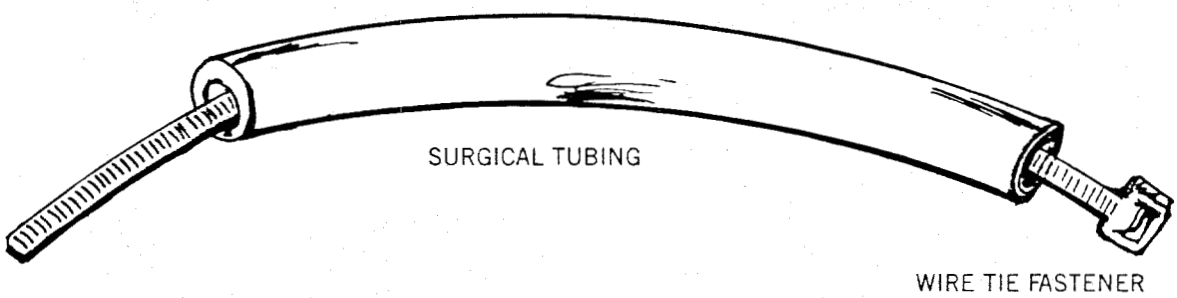



FIGURE 40 — SURGICAL TUBING PEDUNCLE BELT

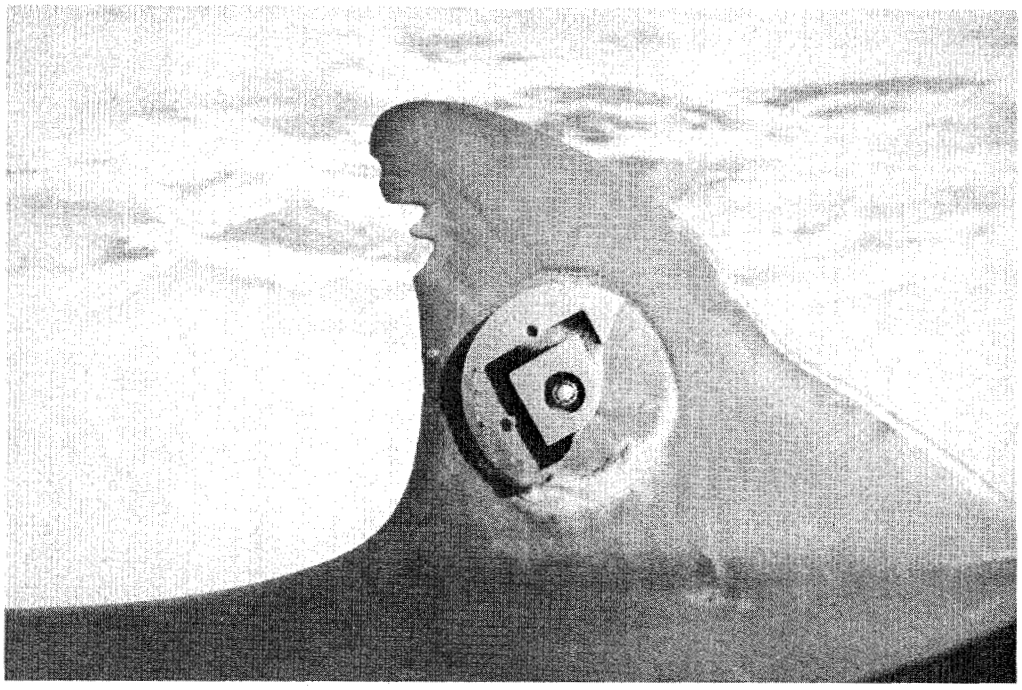


Figure 41 - Fractured dorsal fin disc tag showing healing of abraded tissue - Stenella.

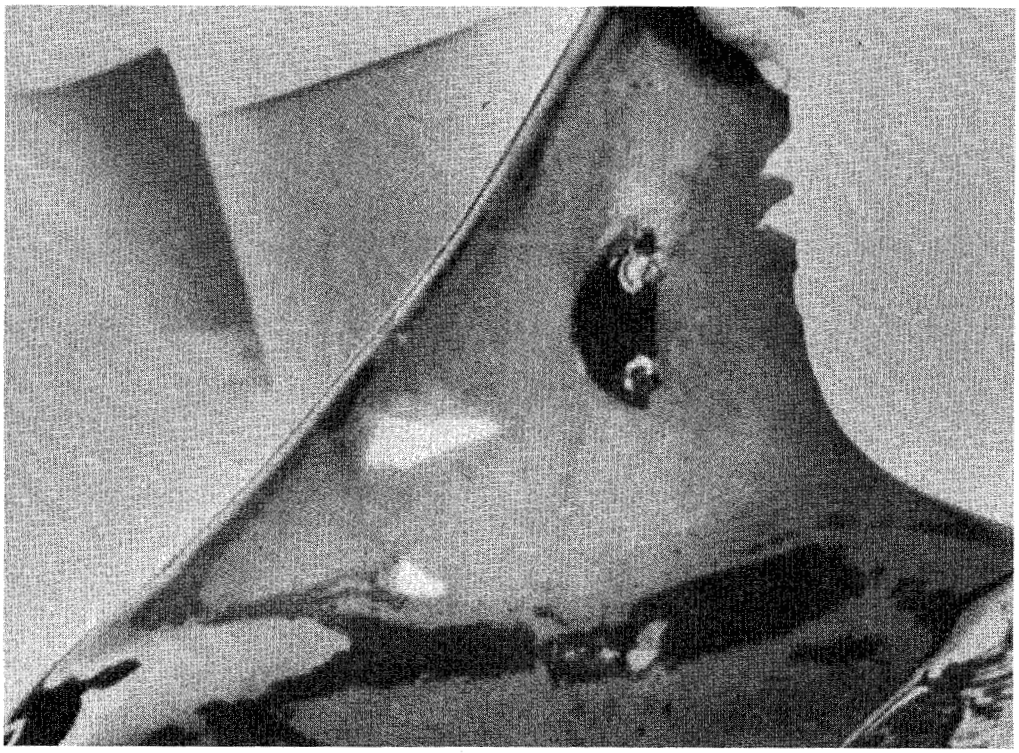


Figure 42 - Fractured multi post flag tag on Stenella dorsal fin.

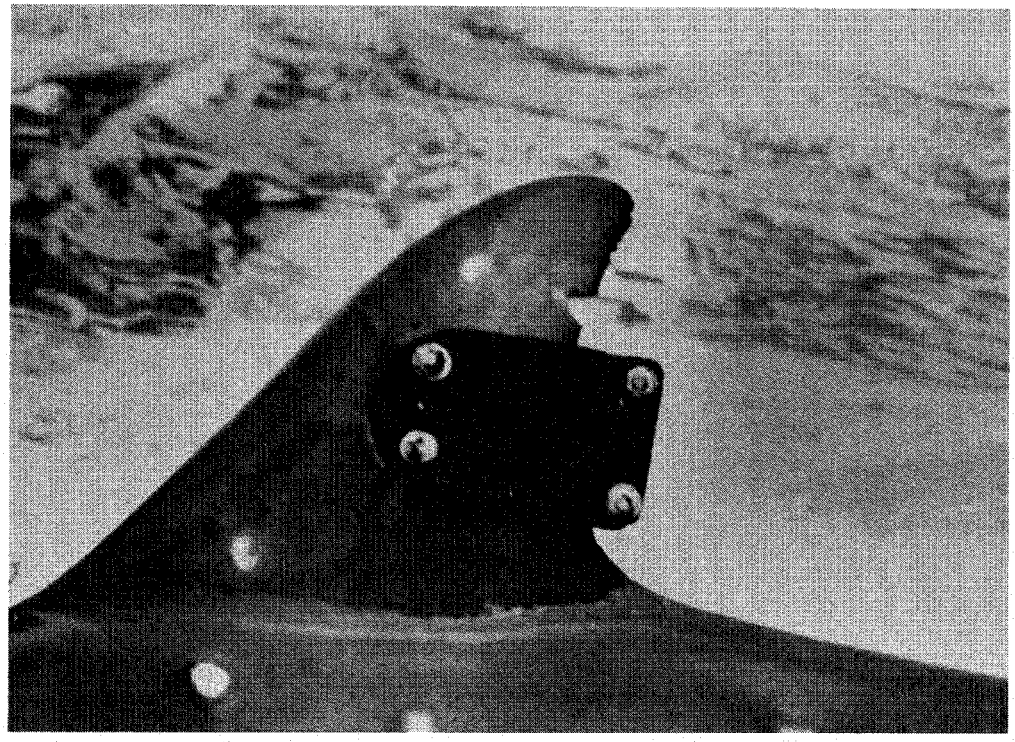


Figure 43 - Algae covered multi post flag tag - Stenella.

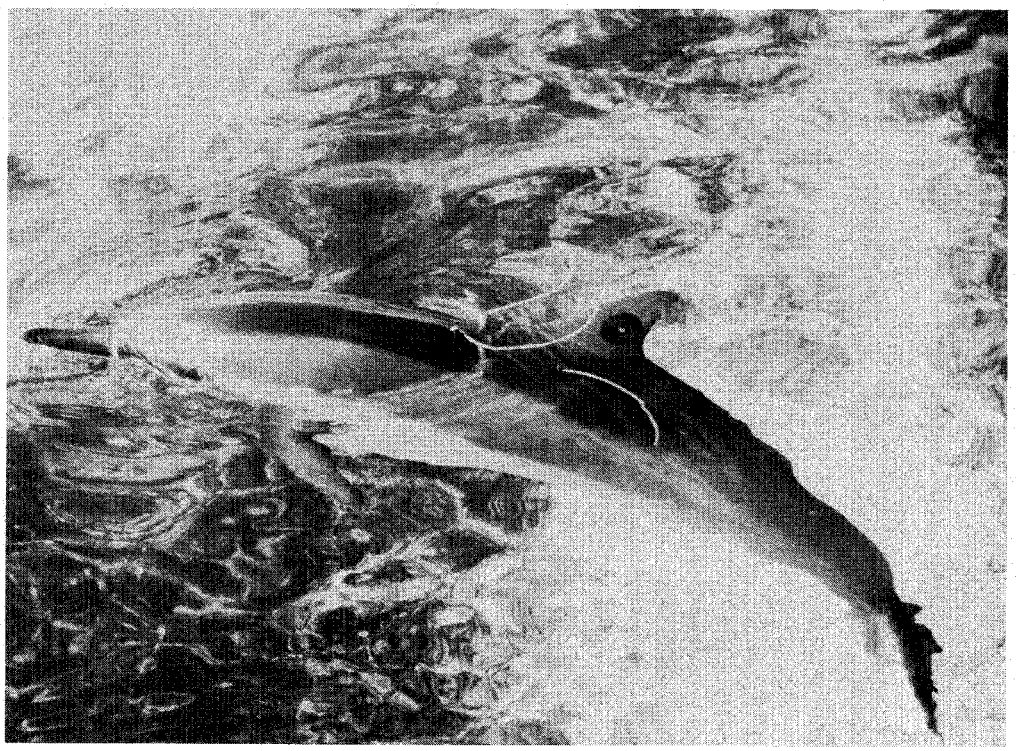


Figure 44 - Spaghetti tags - Stenella.

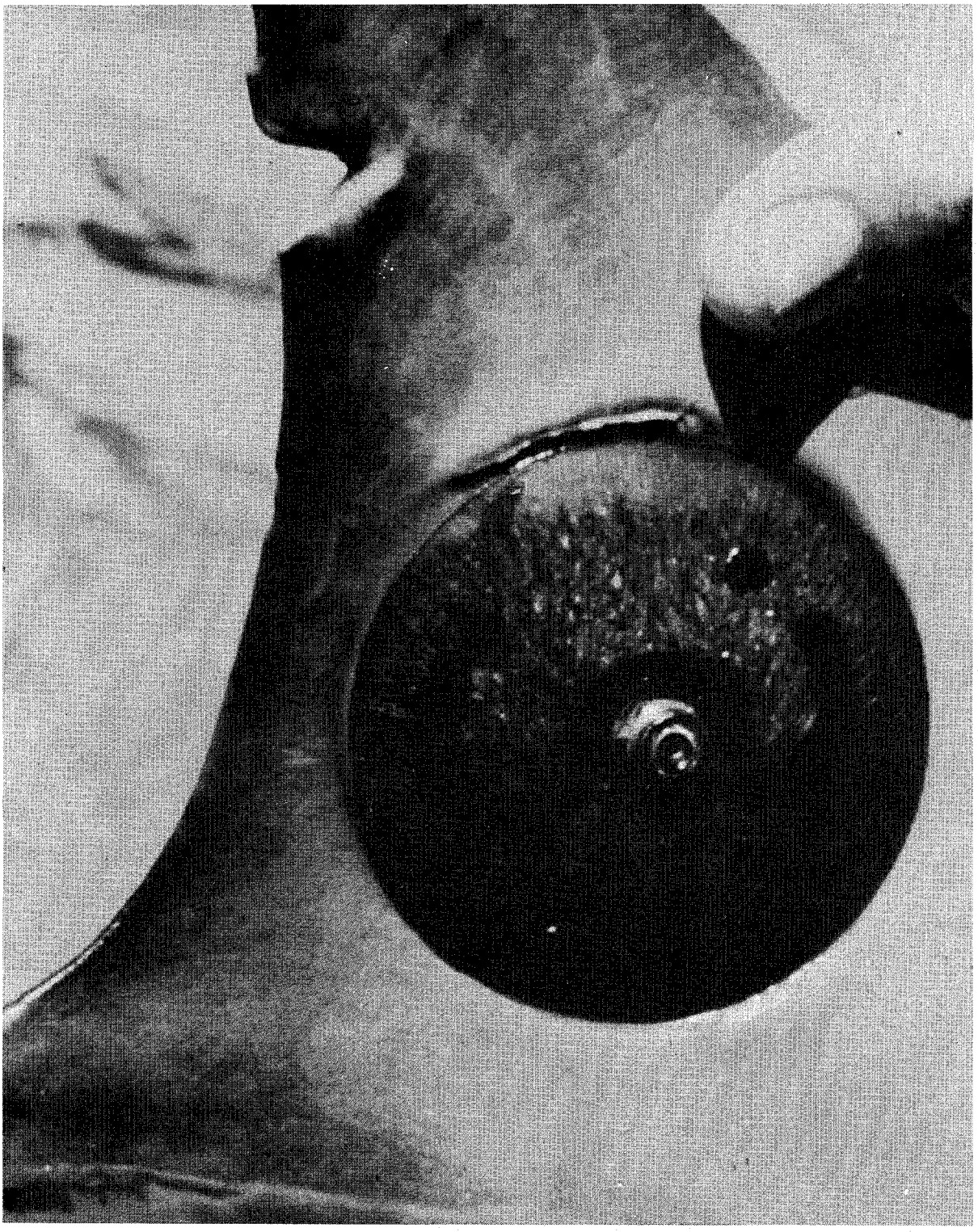


Figure 45 - Dorsal fin disc tag showing primary abrasion points - Stenella.

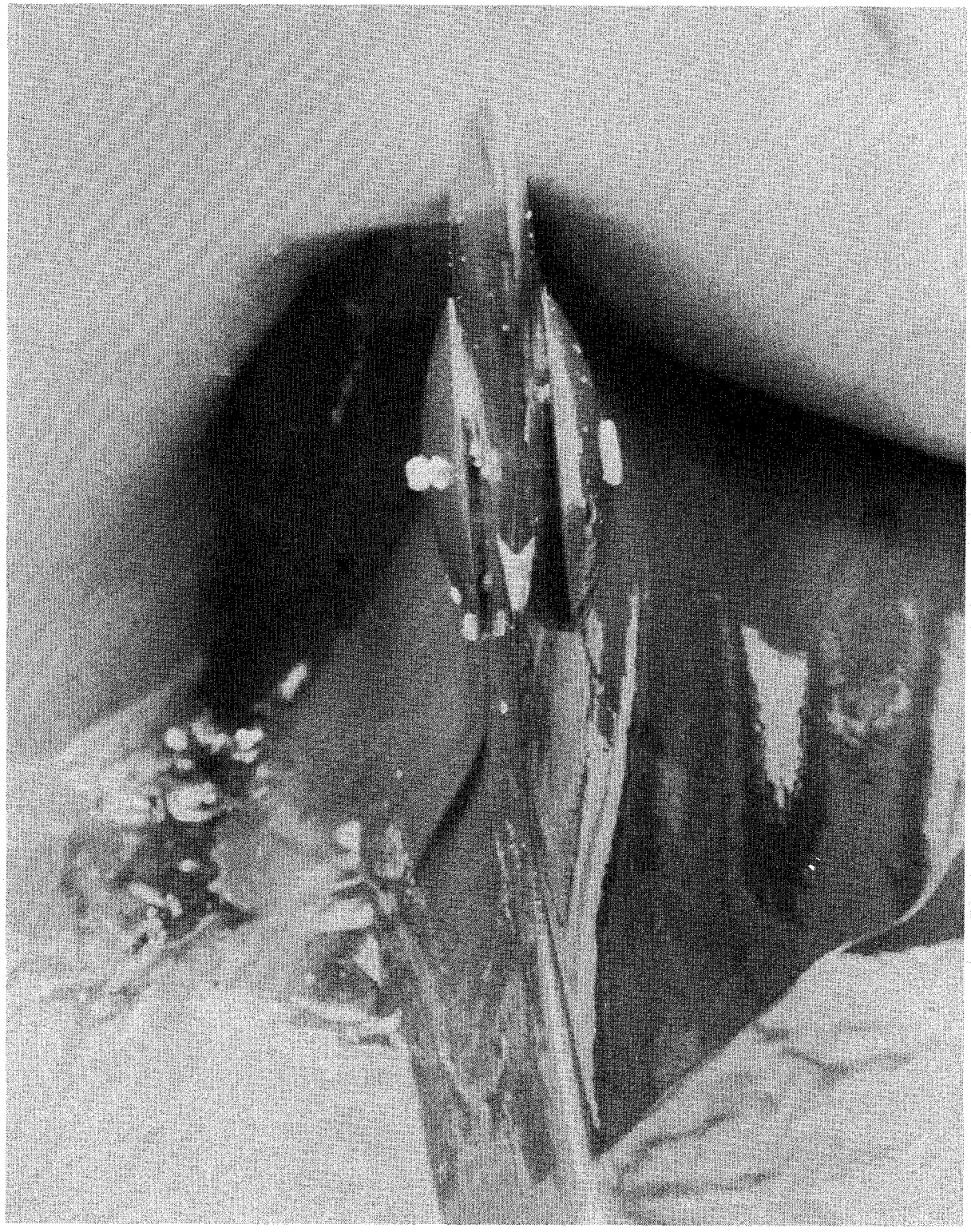


Figure 46 - Dorsal fin disc tag (improved design) showing fit on dorsal fin - Stenella.

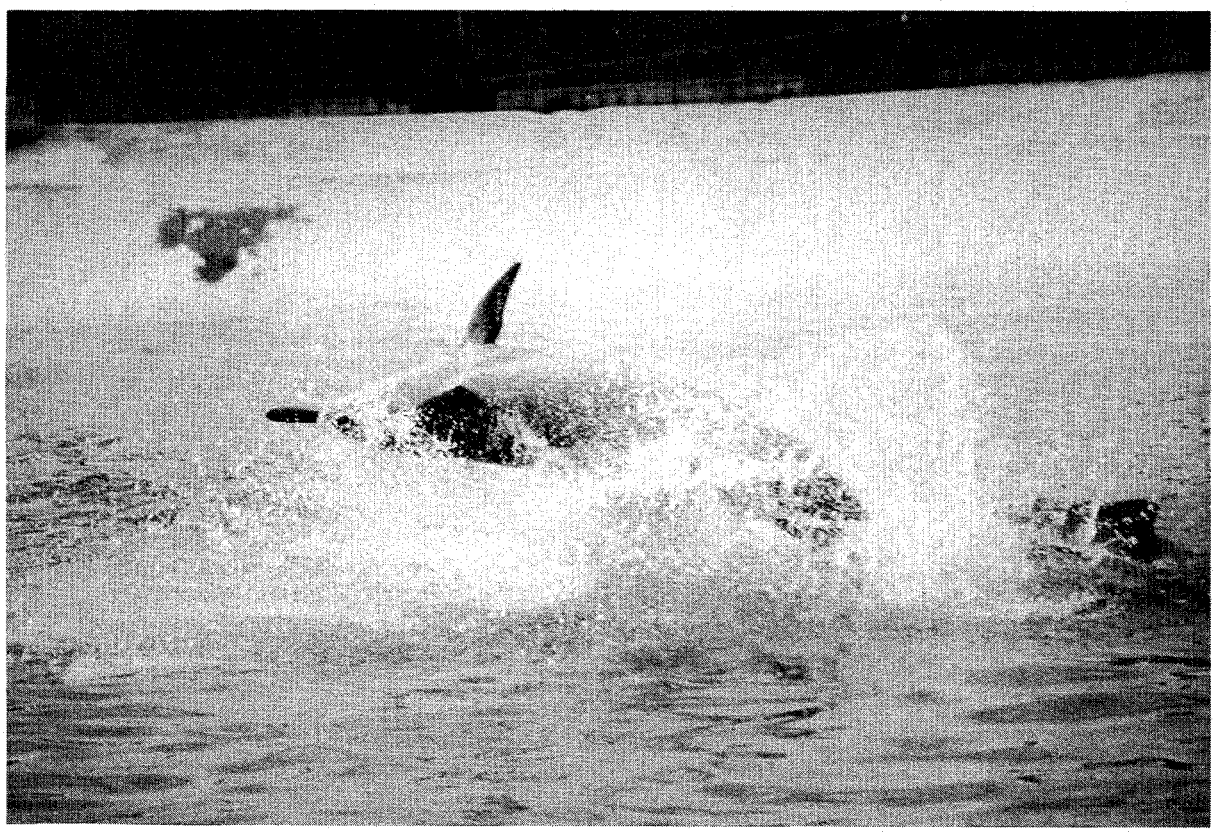


Figure 47 - Disc tagged Stenella - aerial behavior.

Table 1a. Data Summary for Spaghetti Tag Designs

| Fig. # | Tag Type | Description | Test Subject | Application | Retention (days) | Comments |
|---|---|---|---|---|---|---|
| 1 | Floy spaghetti | 8 x 35 mm flat, pointed 304 stainless steel barb, monofilament lead 14 cm fluorescent polypropylene streamer. | Pig | Jab stick | Intact > 365 | Tissue eroded where streamer entered skin. |
| 3 | Floy arrowhead | 16 mm pointed nylon dart, 30 mm adjoining flukes, 14 cm vinyl tubing streamer. | " | " | 60 | " |
| 1 | Floy spaghetti | Same as #1 | Delphinus delphis | " | 31 | Surgically removed. Barb head encysted in tissue. |
| | Floy arrowhead | Same as #3 but flukes rounded, reduced to 14 mm | " | " | " | Surgically removed. Fluke broke. Purulent wound. Tag migration medially. |
| 23 | Modified spaghetti | 10 x 30 mm stainless steel barb, 316 stainless steel wire lead, 21 cm polyurethane streamer, chrome plated brass swivel. | Lagenorhynchus obliquidens | " | Intact > 365 | Swivels corroded twice, streamer replaced twice. |
| 1 | Floy spaghetti | Same as #1 | Stenella longirostris | 15 cm hand held tool, hand insertion | 233 | Streamer off in 33 days. Surgically removed after infection detected. |
| 1 | Floy spaghetti | Same as #1 | " | " | 13 | Insufficient application pressure. Barb did not anchor well. |
| 23 | Modified spaghetti | Same as #23 | " | " | 10 | " |
| 23 | Modified spaghetti | Same as #23 | " | " | 9 | " |

Table 1b. Data Summary for Arrowhead and Conehead Tag Designs

| Fig. # | Tag Type | Description | Test Subject | Application | Retention (days) | Comments |
|---|---|---|---|---|---|---|
| 4 | Disc arrowhead | 16 mm pointed nylon head, 8 mm rigid adjoining flukes rigid 22 mm shaft, 2.5 cm round disc. | Pig | Incision, pushed into place by hand | 34 | Significant bleeding. Animal rubbed tag against enclosure, eventually shed. |
| 5 | Disc conehead | 10 x 13 mm pointed cone-shaped head, rigid 20 mm shaft, 2.5 cm disc. | " | Scalpel incision, pushed in by hand | 5 | Penetration hole too large, insufficient anchoring. Difficult to orient properly for application. |
| " | Disc conehead | Same as #5, but carbon coated. | " | " | 5 | Carbon coating would not adhere to the tag material. No streamer. |
| 4 | Disc arrowhead | Delrin, instead of nylon for tag material, yellow polyurethane streamer. Longer 18 mm shaft. | Delphinus | Tag struck with mallet (Fig. 7) | 4 | Longer shaft to penetrate deeper to fascia, difficult to apply so disc is flush with skin, tag rubbed against wall, streamer pulled on by other animals. Dolphin bled for 30 min. after application. |
| " | Disc arrowhead | Delrin, carbon coated, no streamer. | " | " | 14 | Carbon did not adhere to tag. If not struck properly, popped off skin or fractured. |
| 5 | Disc conehead | Nylon, 16 mm shaft. No streamer | " | | 6 | Penetrated only to blubber, tag rubbed on wall, bleeding. |
| | " | ", carbon coated | " | " | 5 | Carbon did not adhere to tag. |
| 4 | Disc arrowhead | 18 mm shaft, 14 x 25 mm oval disc, hollow shaft, streamer | " | Scalpel incision, pushed in by hand | 6 | Oval disc designed for improved water flow, shaft was to drain wound. Bleeding |
| 10 | Adjustable arrowhead | 5 x 8 mm pointed barb, single 10 mm fluke, 90 mm flexible nylon conduit tie band, 25 mm flat disc with locking mechanism in center. | " | 5 mm trocar implanted, tag head & tie threaded through. Trocar removed, tie pulled, disc threaded onto tie, disc snugged to skin. | 1 | Hole cut by trocar too large for anchoring device, insufficient anchoring. |

Table 1c. Data Summary for Flag and Disc Tag Designs

| Fig. # | Tag Type | Description | Test Subject | Application | Retention (days) | Comments |
|---|---|---|---|---|---|---|
| 11 | Flag tag | Two 5 x 80 mm triangular ortho-plast flags secured by nylon nut and bolt, 5 x 23 mm sleeve. | Delphinus | Dorsal cored with #8 trocar (Fig. 12), sleeve inserted, flag secured, nut welded on bolt with hot spatula. | 43 | Application requires considerable force, bleeding controlled within 5 min. by sleeve. Flags failed 28 days after application. |
| 38 | Multi-post flag | Two fluorescent yellow 60 x 100 mm Kydex flags, 2 nylon posts with sleeves. | Stenella longirostris | Same as above, wood block on opposite side for support of fin. | 42 | Tag broke on bolts on both sides. Algae formed on flag. |
| | " | ", twin nylon posts at rear of flags to hold flags apart. | " | " | 93 | Tag removed. Flags had fractured. |
| 22 | Disc tag | 5 cm dome-shaped discs, teflon, radial & perimeter slits, perimeter holes. | Lagenorhynchus obliquidens | Hole (slightly smaller than pin) cored through fin with scalpel. Slides snapped together. | 1 | Anchor pins failed. |
| 32 | " | ", 6 mm nylon post for connecting mechanism. | Stenella | 6 mm hole cored through fin with tool notched for proper sleeve length. Sleeve put on post, post inserted in tool, pushed back through hole, opposite side secured. | 4 | Several tags tried. Posts broke during application of several. Discs fractured or everted or pulled apart. Removed after 4 days. |
| 37 | " | Red polyurethane discs. Notched delrin posts. No slits, four holes. | " | " | 12, 2<br>21, 2<br>22, 15 | Discs and posts fractured. Fouled by algae. Six tags evaluated. |
| | " | Orange high density polyethylene | " | " | 11, 6<br>60 | Disc and/or post fractured. Some removed due to damage to fin (34 Fig.). Three tags checked. |
| 41 | " | Blue Delrin, stainless steel bolt, teflon sleeve. | " | " | 93 | Tag removed after satisfactory results. |

Table 1d. Data Summary for National Fisheries Engineering Laboratory Tag Designs

| Fig. # | Tag Type | Description | Test Subject | Application | Retention (days) | Comments |
|---|---|---|---|---|---|---|
| 15 | Anterior dorsal fin clip tag | Wrap-around hypalon streamer. | Delphinus | Tag clipped to anterior of fin | 1 | Tines penetrated too deeply and with too much pressure. |
| 16 | " | ", streamer mounted on side of clips. | " | " | 1 | Both tags applied quickly, no bleeding. |
| 17 | Metal scoop tag | 3 cm semicircular tines of 316 stainless steel. Hypalon flag 14 cm. | " | Applied with motion similar to stitches sewing. | 1 | Tines penetrated 2 cm. Tag tilt up, not effective in present configuration. |
| 18 | Posterior dorsal fin clip tag | Clip similar in shape to 15 x 16, no streamer. | " | Clipped to posterior edge of fin. | 1 | Tines pierced the fin 3 cm anterior of posterior edge. |

Table 1e. Data Summary for Peduncle Belt Tag Designs

| Fig. # | Tag Type | Description | Test Subject | Application | Retention (days) | Comments |
|---|---|---|---|---|---|---|
| 31 | Snap fastener belt | 10 x 21 cm soft red synthetic rubber band, polypropylene fastener | Lagenorhynchus obliquidens | Placed around posterior most peduncle. | 1 | Fasteners pulled loose. |
| 32 | Velcro rubber belt | 2 x 37 cm velcro strip sewn to 4 x 21 cm rubber band. | " | " | 1 | Velcro separated. |
| 33 | Ty-Rap rubber belt | Polyethylene Ty-Rap sewn to rubber band 8 x 19 cm. | " | " | 5 | Removed. Abraded peduncle. |
| 34 | Surgical tubing ring | 1.2 cm rubber surgical tubing with internal nylon tie. | Stenella | " | 15 | Removed. Painted with orange latex paint which peeled quickly. |
| 35 | Nasco cattle leg belt | 3 mm x 32 mm x 340 mm polypropylene cattle leg tag. | Lagenorhynchus obliquidens | " | 5 | Removed. Abraded peduncle (Fig. 31). |

Table 2. Summary of Insertion and Withdrawal Pressure Tests

| Test Point | | Dimensions | $\bar{X}$ insertion pressure ($lbs/in^2$) Muscle-fascia | | Blubber | | $\bar{X}$ withdrawal pressure ($lbs/in^2$) Muscle-fascia | | Blubber | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Thawed | Fresh | Thawed | Fresh | Thawed | Fresh | Thawed | Fresh |
| #1 | cone head | 5 x 4 mm | 46 | 20 | 16 | 15 | 14 | 8 | 8 | 8 |
| 2 | " " | 8 x 6 mm | 46 | 22 | 16 | 13 | 28 | 10 | 6 | 5 |
| 3 | " " | 10 x 6 mm | 54 | 28 | 24 | 19 | 30 | 18 | 8 | 7 |
| 4 | " " | 14 x 7 mm | 58 | 30 | 28 | 22 | 50 | 24 | 12 | 14 |
| 5 | " " | 13 x 8 mm | 82 | 40 | 32 | 27 | 68 | 30 | 20 | 19 |
| 6 | multi level shank | 30 x 6 mm | 58 | 22 | 16 | 16 | 64 | 34 | 18 | 15 |
| 7 | four fluke head | 5 x 10 mm | 46 | 26 | 26 | 24 | 178 | 85 | 38 | 34 |
| 8 | four fluke head | 8 x 13 mm | 46 | 22 | 20 | 21 | 200 | 114 | 102 | 98 |
| 9 | Floy spaghetti tag barb | 35 x 8 mm x .3 mm | 26 | 24 | 22 | 19 | force too great to be recorded | | | |
| 10 | modified spaghetti tag barb | 30 x 10 x 1 mm | 34 | 36 | 32 | 29 | force too great to be recorded | | | |

Table 3, SUMMARY OF TAG TEST ON HAWAIIAN SPINNER DOLPHINS (STENELLA LONGIROSTRIS) USING TEFLON DORSAL DISC TAG.

| Tag no. (sleeve size) | Animal activity | Date tag placed | Date tag off | Total time on | Comments on tag | Problems and comments |
|---|---|---|---|---|---|---|
| #1 | - | - | - | - | Rejected, hole too small | Could not snap saucer onto post, post bends (given to E. Shallenberger as model). |
| #2 | - | - | - | - | Rejected, hole too small | - |
| #3 tag (#5 sleeve) | Apiki, very active | 3/15 | 3/15 | 10 minutes | Had to cut post back to 3 exposed notches so post would not bend | Animals do not appear to be bothered very much.<br>Upon release to large tank did fantastic display of spinning. Tag popped off in 10 minutes. |
| #4 (#7 sleeve) | Kehaulani, active, fragile | 3/15 | X | In place on 3/19 | Tag looks good, applied snugly, no signs of rubbing | Animal is not as aggressive as some others so tag not subjected to as much stress. |
| #5 (#6 sleeve) | Maile Sue, very active | 3/15 | X | In place on 3/19 | Tag saucer inverted and saucer 1/4 broken | Tag was more loosely applied and was observed to be about 1/4" away from fin.<br>Only animal reacting and slight bleeding. |
| #6 | - | - | - | - | Rejected--would not fit over post | - |
| #7 (#6 sleeve) | Kahe, very active | 3/15 | 3/15 | 2 hours | Had to enlarge hole to fit. Only female side shed. | Applied loosely to avoid necrosis.<br>Animal did incredible display of spinning before dislodging tag. |
| #8, replaced #4 | Apiki, very active | 3/16 | X | In place on 3/19 | Had newly designed lip on female for locking mechanism. Edges were feathered by cuts 1/8" deep every 1/8". | Prototype tag was used to replace tag #14 but animal was kept in small tank for about 5 hours before being released and did not subject tag to as much stress.<br>Front 1/4 of saucer bent back. |

Table 3. (continued)

| Tag no. (sleeve size) | Animal activity | Date tag placed | Date tag off | Total time on | Comment on tag | Problems and comments |
|---|---|---|---|---|---|---|
| #9 | - | - | - | | Rejected | |
| #10 (#6 sleeve) | Mahealani, fairly active | 3/15 | X | In place on 3/19 | Broken male and inverted female | Saucer inverted and front half of post side broke off in 1 day. Sleeve may have been too large. |
| #11, replaced #16 | Kahe, active | 3/16 | 3/17 | 1 day | Had new locking lip but hole was too small. Hole was overly enlarged. Cut long and short slits. | Not expected to stay on because enlarged hole was too large for post. Saucer side fell off in 1 day whereas post side stayed in for 2 days. Did not spin as much as first test since held 5 hours |
| #12 | - | - | - | - | Rejected, hole too small | |
| #13 (#6 sleeve) | Lioele, inactive (broken back) | 3/15 | X | In place on 3/19 | Tag looks good. | Animal has recovered from a broken back and is relatively inactive so tag was not subjected to many forces. Snug fit. |
| #14, replaced #3 | Apiki | 3/15 | 3/15 | 10 minutes | | Post side of tag stayed in longer than female. |
| #15 experi-mental | - | - | - | - | | (Given to Charlie Hill as model) |
| #16, replaced #7 | Kahe | 3/15 | 3/15 | 1 1/2 hours | Post of original tag #7 may have been deformed. | Replaced only female side of #7 tag. |



Summary prepared by J. Jennings, Southwest Fisheries Center, National Marine Fisheries Service.

## TABLE 4. UTAH STATE WATER RESEARCH LABORATORY FLOW CHAMBER TEST RESULTS

Summary prepared by the National Fisheries Engineering Laboratory

| TAG TYPE | MATERIAL | METHOD OF FABRICATION | VELOCITY (m/sec.) | COMMENTS |
|---|---|---|---|---|
| Dorsal Disc Tag | Teflon | Machined | 4.88 | Disc shattered duplicating experiences in live animal testing |
| Series One* | Delrin (Dupont) | Injection molded | 6.10-7.62 | Disc shattered under extreme flow rates |
| | Polyethylene | (Scientific Plastics, Inc.) | 6.10-7.62 | Inverted and separated from post |
| | (UpJohn) 2103 Polyurethane 80A | (Scientific Plastics, Inc.) | | Inverted and separated from post |
| | 2103 Polyurethane 90A | (Scientific Plastics, Inc.) | | Inverted and separated from post |
| | 2103 Polyurethane 55D | (Scientific Plastics, Inc.) | | Inverted and separated from post |
| Series Two ** | Delrin | (Scientific Plastics, Inc.) | 9.14 | Disc withstood velocities at all angles and inclinations |
| | Polyethylene | (Scientific Plastics, Inc.) | 9.14 | Disc withstood velocities at all angles and inclinations |
| Series Three *** | Polyurethane 2103 75DX | (Scientific Plastics, Inc.) | 9.14 | Disc withstood velocities at all angles and inclinations |

* Series 1 - Median Disc thickness at center is 0.152 cm decreasing to perimeter thickness of 0.038 cm.

** Series 2 - Disc thickness at center is 0.381 cm decreasing to perimeter thickness of 0.076 cm.

*** Series 3 - Disc thickness at center is 0.244 cm decreasing to perimeter thickness of 0.051 cm.

# Section II - Cryogenic Marking Equipment Design and Evaluation

## Cryogenic Marking Evaluation - Phase I

A cooperative cryogenic marking experiment involving representatives from the Hubbs-Sea World Research Institute, Southwest Fisheries Center and the National Fisheries Engineering Laboratory of the National Marine Fisheries Service (NMFS) was conducted at Hubbs-Sea World Research Institute in December of 1977.

### Methods and Materials

Heat sink and evaporative techniques were used for coolant application to the epidermis of a Delphinus delphis specimen. The heat sink techniques required placement of a marking tool cooled to cryogenic levels, on the epidermis. Heat drawn from the warm epidermal tissue to the cooled marking tool resulted in refrigeration of the tissue.

The evaporative technique involved spray application of a compressed gas on the epidermis. As the gas was sprayed, its pressure and temperature decreased allowing heat transfer from the epidermis to the evaporating coolant.

Heat sink marking with liquid $N_2$ was conducted using a self-contained vacuum flask (Foster Froster) modified by the National Fisheries Engineering Laboratory (Fig. 1). A button depressed on top of the cannister released liquid $N_2$ into a hollow brass marking head. The marking head was fitted with a valve for venting $N_2$ gas. An additional heat sink marking iron was used with a polyethylene handle and 2.54 cm diameter copper head. It was immersed in liquid $N_2$ before application to the epidermis (Fig. 2).

For marking by the evaporative technique, a freon (monochlorodifluromethane) applicator designed by Dr. R. K. Farrell of Washington State University was used (Fig. 3). Dr. Farrell personally made the applications in this experiment. The applicator had a hand activated valve to regulate coolant flow through a .025 inch orifice, with high pressure line connected to a cannister containing the coolant. Epidermal temperatures were monitored with a potentiometer and .005 diameter alumel/chromel constantan thermocouple inserted approximately 3 mm into the epidermis.

Our initial cryogenic marking research focused on the physical and biological factors that determine mark legibility. The following factors were evaluated during this experiment:

1) Heat sink and evaporative techniques;
2) Liquid $N_2$ and freon coolants;
3) Specialized application equipment for each coolant;
4) Serial marks with both coolants;
5) Application times ranging from one to twenty seconds;
6) Temperature monitoring equipment to record skin temperature reduction as a function of coolant type and application time;
7) Marking sites to include the dorsal fin and dorso-lateral areas adjacent to the dorsal fin.

The length of time required for "marked" tissue to regain its original integrity with no visual sign of continued swelling, sluffing or repair, was used for healing cycle criteria.

## Results

Results for the cryogenic marking experiment with a Delphinus delphis are summarized in Table I. No infection or other healing cycle complications were observed and the animal's social behavior appeared to be normal after marking.

Dorsal fin marks using liquid $N_2$ (Fig. 4)

Very little swelling was observed after marking. Tissue sluffing was observed within 3 days. A complete healing cycle was observed for all marks within 14 days.

Marks on the dorsal fin with 8, 10 and 12 second application times displayed only slight and diffuse depigmentation. The 14 second mark displayed a pronounced centralized depigmentation, bordered by diffuse depigmentation and was the most distinct of the group.

Dorso-lateral marks using liquid $N_2$ (Fig. 5-top row)

Pronounced swelling of the mark sites was observed within 30 minutes after marking. Tissue sluffing was observed within 3 days. After 51 days, only two mark sites displayed a complete healing cycle (application times: 1 and 3 seconds). Both of these marks displayed a depigmented area shrinkage of $\sim$ 20% as compared to original size. The other mark sites displayed a complete healing cycle in 72 days.

Dorso-lateral marks of 1 and 3 seconds application time also displayed pronounced centralized depigmentation with diffuse depigmentation occurring around the perifery. Marks of 4 and 5 seconds application time show a distinct "pie wedge effect" of pigmented tissue (Fig. 6).

Dorso-lateral marks using Freon 22 (Fig. 5-bottom row)

The most pronounced swelling and tissue sluffing was observed with this group of marks using Freon 22 (evaporative technique). After 39 days

only one mark displayed a complete healing cycle (application time: 10 seconds). The other marks displayed a complete healing cycle in 50 and 76 days respectively (application time: 11 and 20 seconds).

Dorso-lateral marks with 11 and 20 second application time displayed a fairly uniform depigmentation over the entire mark site. Both marks also displayed isolated areas of pigmented tissue. The mark at 10 seconds application time displayed a centralized depigmentation bordered by extensive diffuse depigmentation.

Discussion

The dorsal fin marks were not very distinct (depigmented). The presence of dense connective tissue and an arterial tree, which can act as a heat radiator, may not have allowed for adequate tissue temperature reduction. There also may have been ice on the marking head that acted as an insulator during application. A larger marking head with more surface area and greater mass is also desirable to minimize temperature variance during application.

Dorso-lateral marks using liquid $N_2$ displayed a wide range of variability in terms of depigmentation, and tissue refrigeration, within an application time span of only five seconds (i.e., 1 second application time reaching a temperature of -4.2°C compared to an application time of 5 seconds reaching a temperature of -29°C). The latter appears to have produced excess tissue trauma that could obscure an arabic numeral mark applied in a field situation due to migration of pigmented cells (melanocytes) into the mark site during the healing cycle (Fig. 6).

Lower application times would not leave much margin for error in a field marking situation since the best mark in this group had an application time of only 3 seconds. The most satisfactory mark of the experiment resulted with freon at 11 seconds application time, reaching temperatures of -10°C. However, a freon produced mark with an application time of 20 seconds produced a minimum temperature only 2°C different than the above. This may have been caused by a difference in placement of the thermocouples, and pressure of the marking head on the epidermis. A method to insure consistent placement of thermocouples is needed.

The dorsal fin and dorso-lateral marks were made according to a "predetermined application time". The Freon 22 marks were applied according to a "predetermined temperature level reached" before removal of the coolant source. Results using both techniques show variability in depigmentation of the mark site (Figs. 5, 6). However, the evaporative application technique with Freon 22 eliminates some factors affecting this variability such as marking tool pressure and marking tool mass and surface area. Our next phase of cryogenic marking research focused on eliminating the variables affecting the degree of mark depigmentation and obtained more data on temperature change during the tissue freeze/thaw cycle, as a function of time.

## CRYOGENIC MARKING EVALUATION - PHASE II

Further marking research activity focused on the evaluation of cryogenic marking equipment developed by the National Fisheries Engineering Laboratory (Fig. 7). This equipment was designed for field marking operations with the National Marine Fisheries Service's Porpoise School Impoundment System (PSIS). It consisted of a 30-liter durer connected by two 8-foot long vacuum-lined hoses to a branding tool with a "T"-shaped branding head. The flow of liquid nitrogen was regulated by valves on the durer to flow down one hose, through the head, and the gas was vented out of the other hose. Potentiometers with digital readouts were interfaced with thermocouples placed in the epidermis and branding head for monitoring temperature.

### Methods and Materials

The initial marking experiment was conducted on a Hampshire pig (Sus scrofa) maintained at the Laboratory Animal Facility, University of California at San Diego. Prior to marking, the self-contained cryogenic marking system was assembled and tested at the Hubbs/Sea World Research Institute. A video system was also tested to record marking procedures, temperature changes of the animal's skin and the marking tool head.

In addition to equipment evaluation we also wanted to determine a temperature reduction plateau, which can indicate a state of intra- and inter-cellular ice crystallization in the epidermis. This crystallization is important in producing a well-defined mark, as is the rate of cellular freeze and thaw.

The pig was sedated with Ketamine ("Vetalar" 100 mg/ml). A 15 cm x 15 cm Teflon template with a "T"-shaped silhouette slightly larger than the

brand head was secured around the pig (Fig. 8). This template was designed to stabilize the marking iron head and allow accurate insertion of the thermocouple probes at prescribed depths of 1, 2, or 3 mm. The thermocouples were incorporated into 21 and 31 gauge hypodermic needles, and interfaced with digital readout potentiometers. The temperature changes were recorded on video tape. Due to the firm cornified nature of the pig's epidermis, the thermocouples were inserted to an approximated depth of 3 mm. They were left in place after the marking tool was removed to record the rate of tissue thaw. Application tool pressures of 10 and 20 psi were used.

Results

A data summary for the pig marking is presented in Table I. After removal of the marking tool, the mark site became reddened and edematous within 30 minutes. Due to the amount of sedative given and instrumentation problems, the experiment was extended over a 2 day period for a total of 8 marks. Tissue sluffing was noted after 5 days and all marks completed a healing cycle within 30 days (Fig. 9). No infection occurred but the pig was observed rubbing marked areas against the enclosure walls on numerous occasions.

Figures 10 and 11 illustrate the difficulties of obtaining accurate tissue temperature data. Figure 10 shows that the temperature for mark A dropped at a constant rate to -20°C in 176 seconds, before leveling off. Figure 11 shows a variable temperature reduction for mark B to +18°C with an elapsed time of 157 seconds.

## Discussion

Testing the experimental cryogenic marking equipment on the pig proved to be an informative exercise. Although self-contained, the cryogenic marking system was difficult to maneuver due to the weight of the liquid $N_2$ in the durer and hose stiffening produced by the cryogenic temperatures. The animal's movement was a major problem, which caused repositioning of the template, thermocouples, and marking tool head making it nearly impossible to record accurate application times and temperatures. Frequently, there was interrupted contact between the marking iron and the epidermal tissue and thermocouple probes inserted through the template appeared to shift position in the tissue.

Determination of temperature reduction plateaus was not practical under these circumstances.

The apparent slow temperature reduction during marking tool application (Figures 10 and 11) may have been due to the firm, cornified nature of the pig's epidermis, placement of the thermocouples at too great a tissue depth, or marking tool pressure on the thermocouples.

## Cryogenic Marking of Hawaiian Spinner Porpoise (Stenella longirostris)

Two Hawaiian spinner porpoises (Stenella longirostris) at Sea Life Park were cryogenically marked to provide additional data for the National Marine Fisheries Service field operations.

Methods and Materials

A Stenella longirostris specimen was captured in the holding tank, placed in a stretcher, and taken to a sheltered area out of the sun. The stretcher was secured to a portable metal stand where the epidermal areas to be marked were dried and disinfected. Special attention was given to careful physical restraint of the Stenella test subjects without sedation to insure their health. Serial marking was performed on the body and dorsal fin, using liquid nitrogen and Freon (monochlorodifluoromethane) coolants.

Heat sink and evaporative application techniques were used. For heat sink application with liquid $N_2$, the portable cryogenic marking system developed for field marking by the National Fisheries Engineering Laboratory was tested (Figs. 7, 12). The marking tool and thermocouples were used without the template previously tested on the pig. For heat sink application with freon, a copper headed marking tool was immersed in a coolant bath. Evaporative application of freon was performed with a valved applicator head and high pressure line connected to a freon cannister (Fig. 3).

Each cryogenic mark was made according to a predetermined application time, and temperature change during the freeze-thaw cycle was monitored by a potentiometer, with copper constantan thermocouples inserted in the mark site epidermis. Temperature changes indicated by the potentiometer's digital readouts were video taped to compare temperature change to mark legibility. A total of 21 marks were made with liquid $N_2$ coolant and 12 marks with freon (Figs. 13, 14, 15). Application times ranged from 1-33 seconds.

Results

Results of the cryogenic marking with Stenella are summarized in Tables 2 and 3. All mark sites displayed some degree of swelling within 30 minutes after marking iron removal. Tissue sluffing was observed after 8 days. If the marking iron was applied for longer than 20 seconds, epidermal and dermal skin layers were prone to sluff, sometimes exposing capillary-filled blubber tissue (Fig. 16). Even slight trauma would cause these mark sites to hemorrhage. Mark sites completed healing cycles within 8-51 days

Marks with application times of 12 to 16 seconds and reduced tissue temperatures of between -2°C and -6°C appeared to remain the most legible (8 months to date) with a minimum of tissue trauma. All marks displayed varying degrees of depigmentation and legibility (Figs. 13, 14, 15). Use of marks for comparative data is difficult due to factors such as animal marking tool contact with the epidermal surface, tissue compression and thermocouple sensitivity.

The experiment was shifted from tankside to a covered staging area to facilitate video taping of marking procedures. Transporting the test subject an extended distance combined with serial marking and careful placement of thermocouples, prolonged their out-of-water time.

A review of the video tape and plots of epidermal temperature reduction across time revealed a mark site's temperature reduction was frequently slow or irregular (Fig. 17), which may have resulted from the animal's movement breaking contact between the marking iron and epidermis or the thermocouple may have changed its depth of penetration. Pressure of the marking iron on the epidermis may have compressed tissue and flexed the thermocouple, producing an irregular readout.

The animals position in the stretcher usually dictated that the marking iron was applied at a 45° to 70° angle to the epidermal surface, which may not have allowed even contact (Fig. 17). A comparison of temperature reduction and mark legibility as a function of coolant types was not possible with the variability observed between individual marks.

Due to the out-of-water time, the animal was frequently wet down with a hose to facilitate thermoregulation. Water inadvertenetly covered several sites during marking which also slowed epidermal temperature reduction. Epidermal temperature reduction occurred more rapidly on the dorsal fin due to the presence of dense connective tissue (Fig. 18).

It does not appear that application time can be used as the only criteria to consistently replicate a legible mark. Tissue density and its ability to compress also appears to be important. Note the difference in final internal temperature and thaw times for mark #4 and #7 with liquid $N_2$ on Lioele (Figs. 19 and 20). Both of the marks were made with the same application times. The dorsal fin's dense connective tissue reached a colder temperature and thawed over a longer period of time.

## Discussion

Comparison of each mark in terms of epidermal and marking tool temperatures, application times, pressure, and coolant type was biased by a number of factors such as:

1. Body curvature--Depressions in the epidermal surface formed by muscle bundles may not allow complete marking iron contact.

2. Body movement--Immobilizing the test subject was difficult. Contact of the marking tool with the epidermal surface was often broken by sudden body movements.

3. Tissue compressibility/marking tool pressure--Compression of the tissue by the marking tool effects the rate of freeze and thaw. Increased tissue compression will promote freezing and slow the thawing process because the density increases.

4. Thermocouple position in the tissue--The ability to position and maintain thermocouples at a constant tissue depth in each mark site proved to be difficult, especially with the marking tool compressing the tissue around the probe.

5. The animal's out-of-water time--Since this experiment involved serial marking, each mark was allowed to thaw slowly while additional marks were made. The test animals were out of the water in excess of 40 minutes each. In a field situation this marking time could be reduced to approximately 3 minutes, the rate at which this tissue thaws should also be evaluated in terms of mark legibility.

6. Blood shunting by the animal--Tissue freeze and thaw rates may be influenced by increased or decreased blood flow to the mark site, particularly on the dorsal fin. The animal's overall body temperature and emotional state may produce blood flow changes in a given tissue area.

To fully evaluate these "physiological and anatomical factors", the tissue's reaction to cryogenic temperatures at the cellar level must

be histologically documented. If, for example, the physiological response of the tissue to cryogenic temperatures is too great, excess fibroblasts and collagen fibers may be formed causing a "puckering" of the mark site (Fig. 14). Hyperpigmentation can also result.

At this stage in the research program, we have established a number of factors that appear to influence mark legibility:

1. The temperature of the marking iron as it is applied to the animal's epidermis.

2. The ability of the iron to function as a heat sink while in contact with the epidermis.

3. The amount of time the iron is in contact with the epidermis.

4. The amount of time allowed for cryogenically marked epidermis to thaw before immersion in water.

5. The density and compressibility of the epidermis.

6. Blood shunting by the animal in a given tissue area.

To fully evaluate these physical and anatomical factors, the tissue's reaction to cryogenic temperatures at the cellular level must be histologically analyzed. Cellular activity during the healing cycle such as pigment migration into the mark site and the production of collagen and fibroblasts (which in excess can "pucker" or "shrink" the mark) should be carefully studied.

Further marking should be conducted when marking irons and temperature monitoring equipment have been thoroughly tested on laboratory animals.

A thorough reference search will be required for background information as well as consultation with professional specialists in histology, dermatology and cryogenic marking to realize development of productive experimental design.

List of Figures

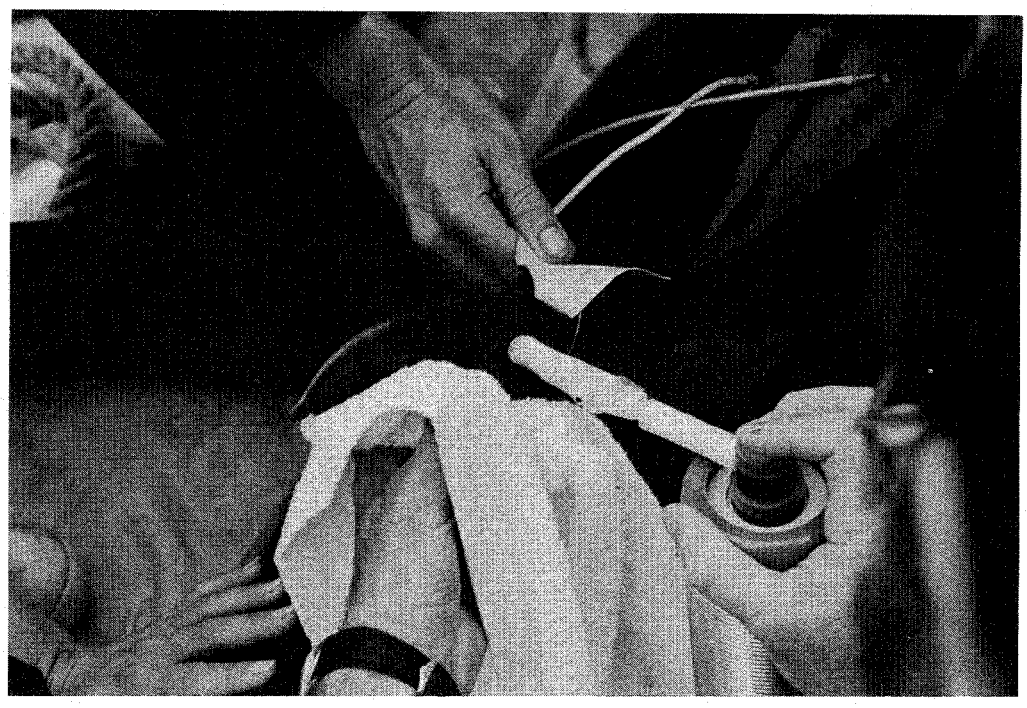


Figure 1 - Foster Froster modified for use with liquid $N_2$ (heat sink technique).

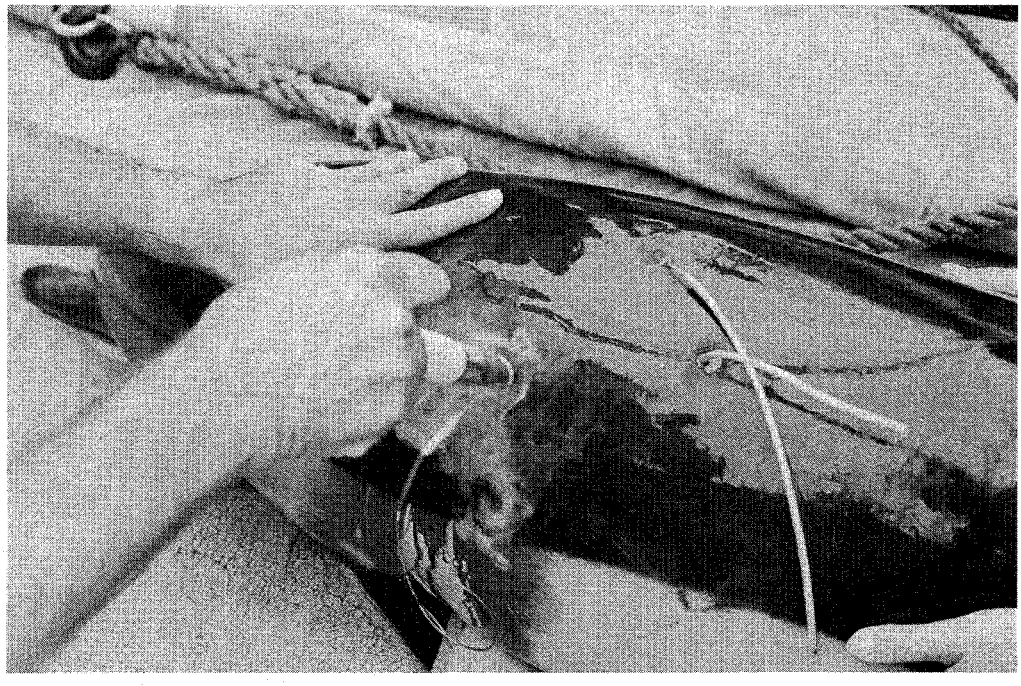


Figure 2 - Farrell heat sink marking tool used with liquid $N_2$.

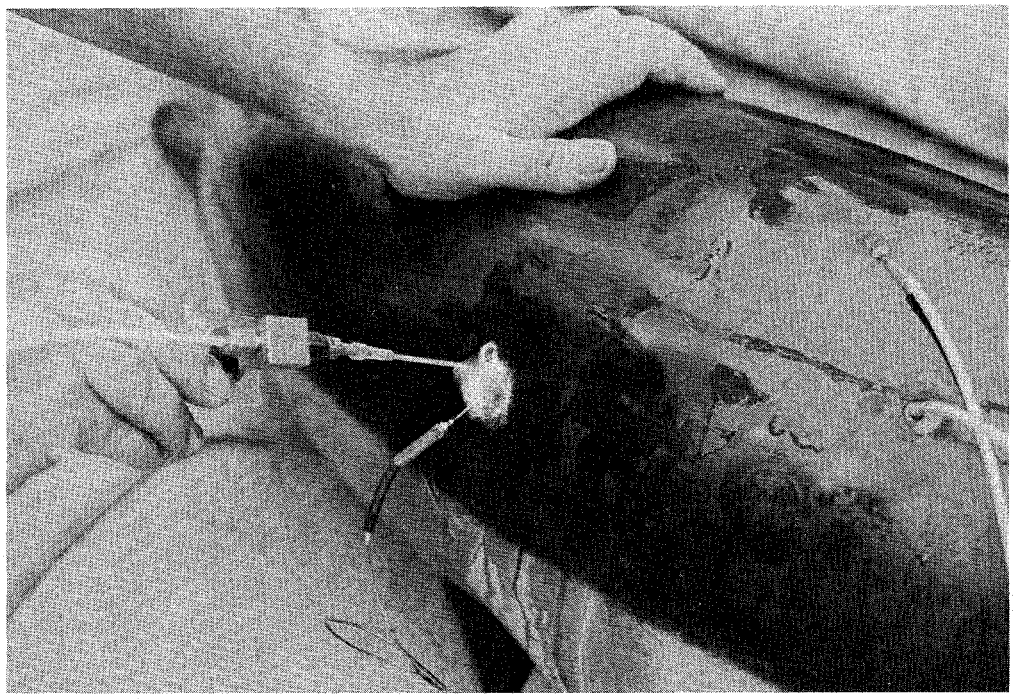


Figure 3 - Farrell freon applicator for evaporation technique spraying of Freon 22 (monochlorodifluromethane).

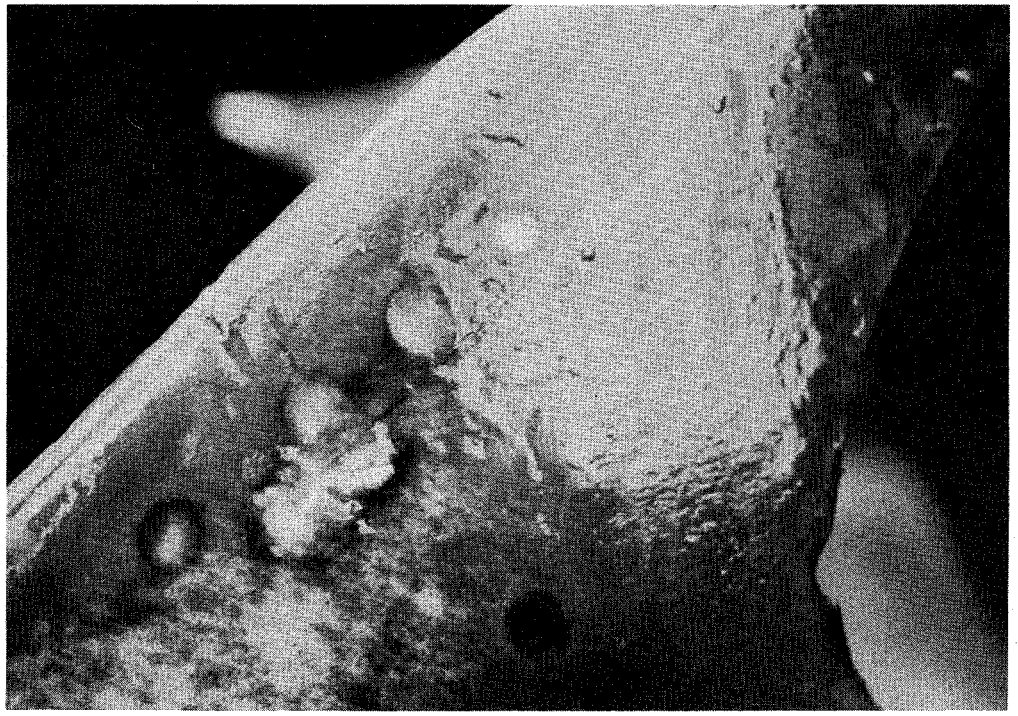


Figure 4 - Cryogenic marks on the dorsal fin (Delphinus delphis). (Branding times from left to right were 14, 12, 10, and 8 seconds.)

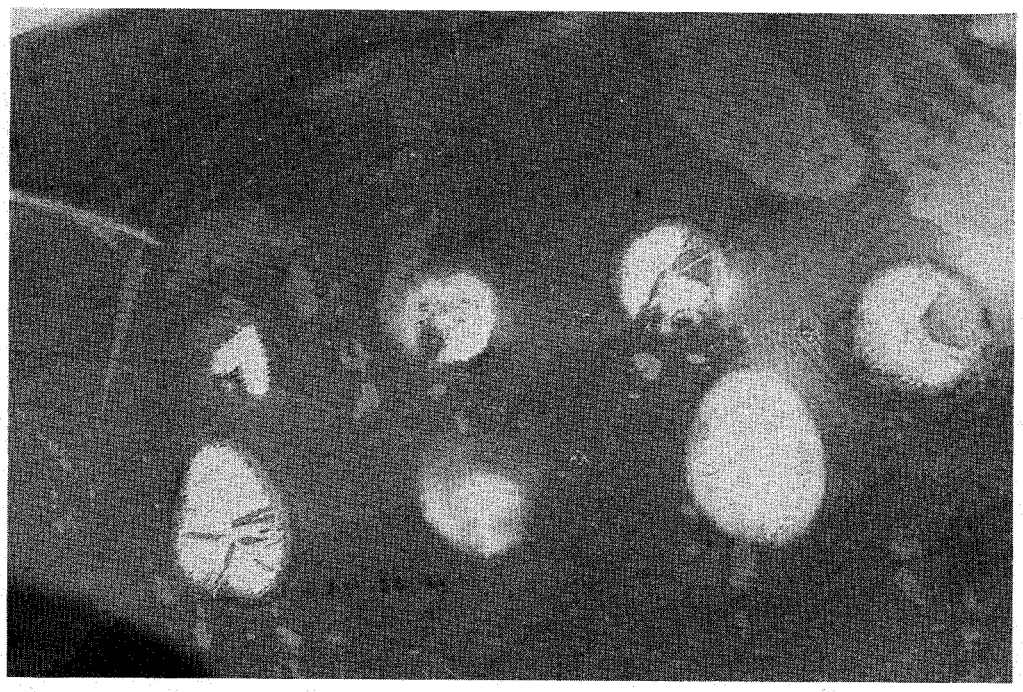


Figure 5 - Cryogenic marks on the dorso-lateral area - Delphinus delphis.

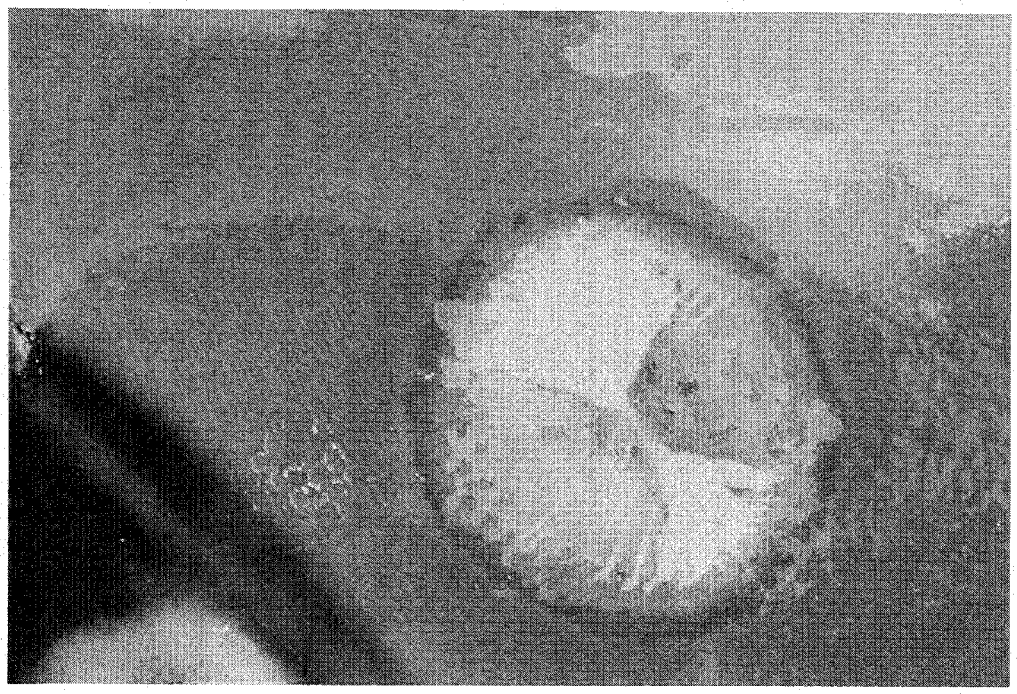


Figure 6 - Migration of pigment cells into the mark site - Delphinus delphis.

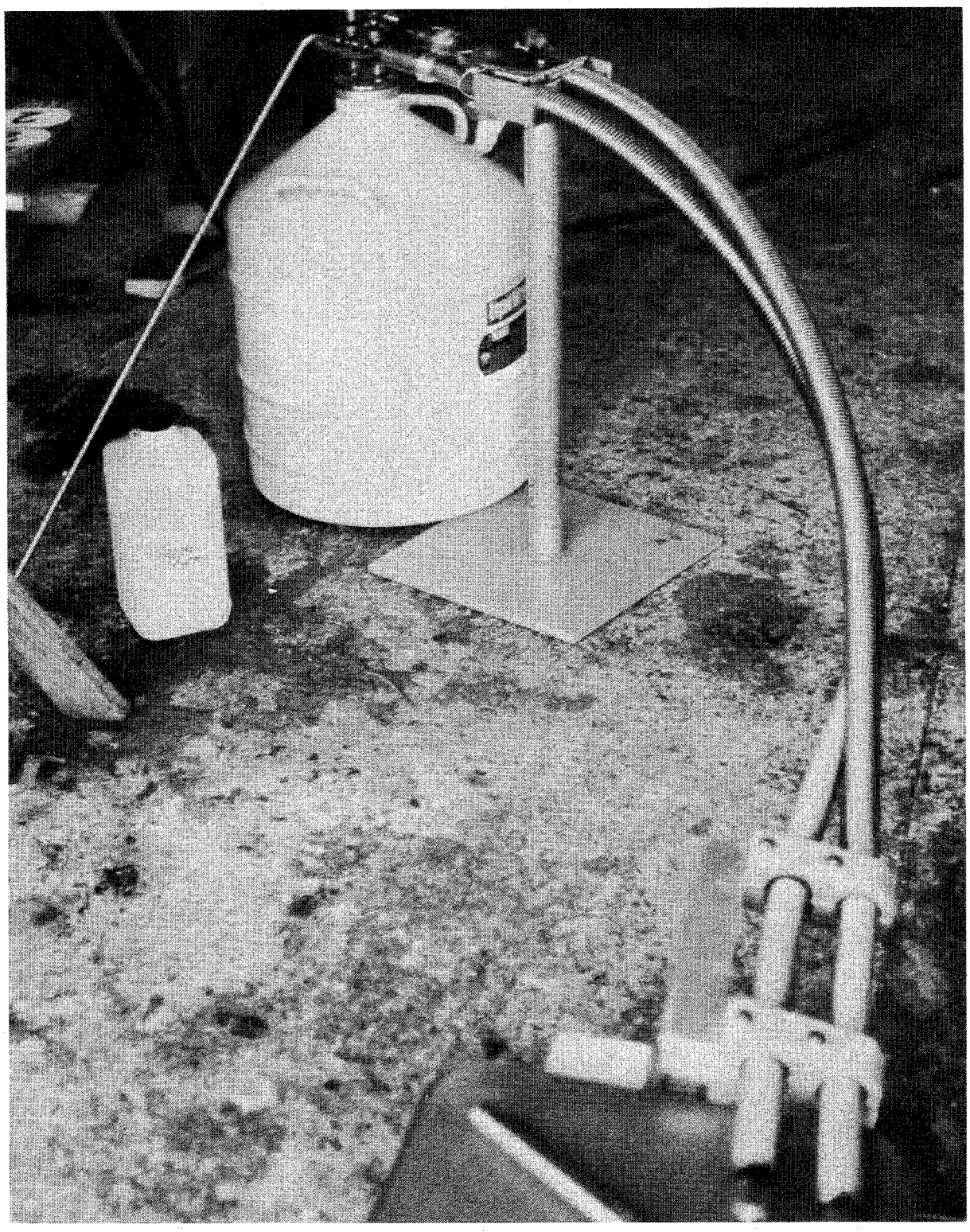


Figure 7 - Cryogenic marking equipment developed by the National Fisheries Engineering Laboratory.

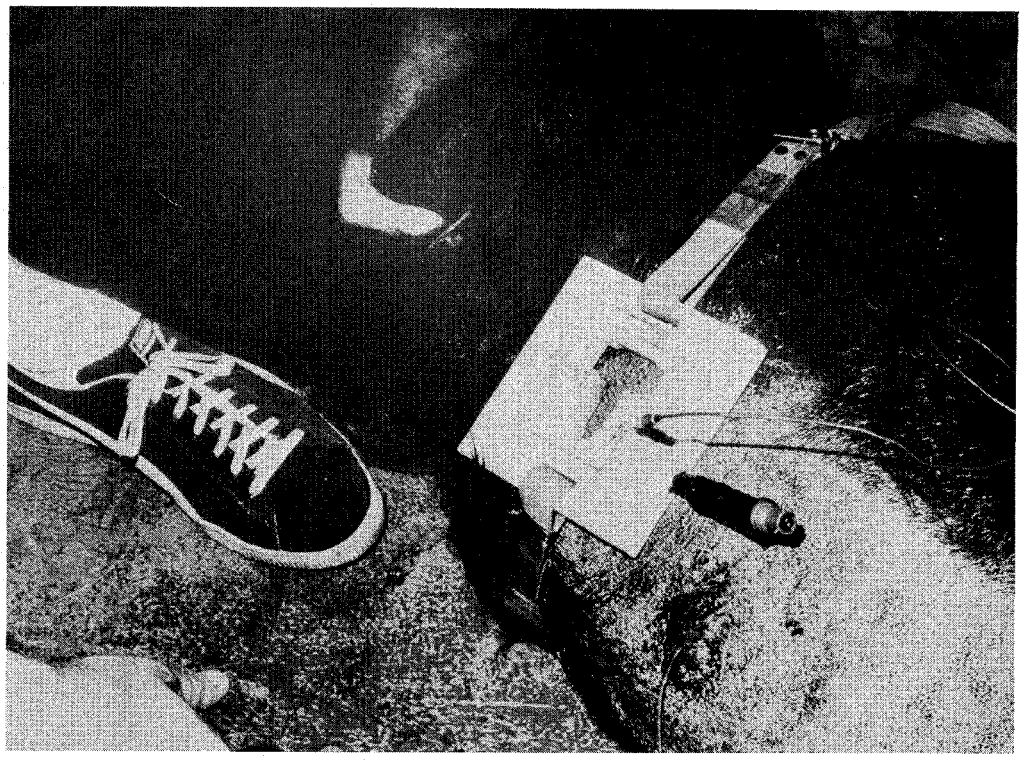


Figure 8 - The teflon template secured around the pig prior to cryogenic marking.

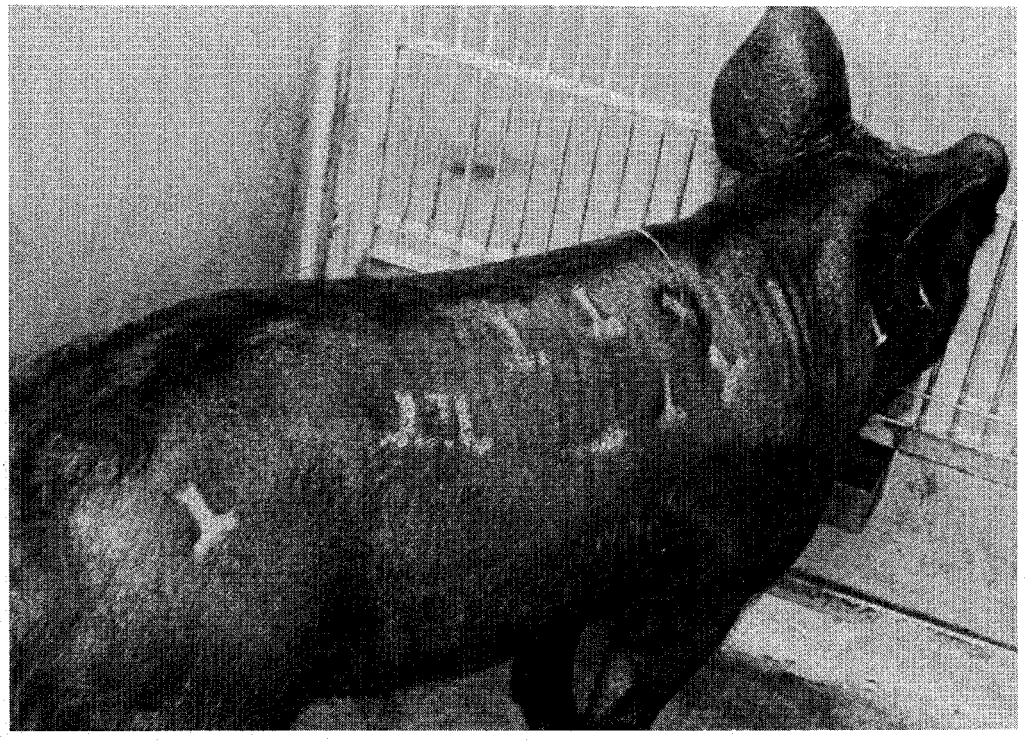


Figure 9 - Cryogenic marks on a pig (Sus scrofa) after completion of the healing cycle.

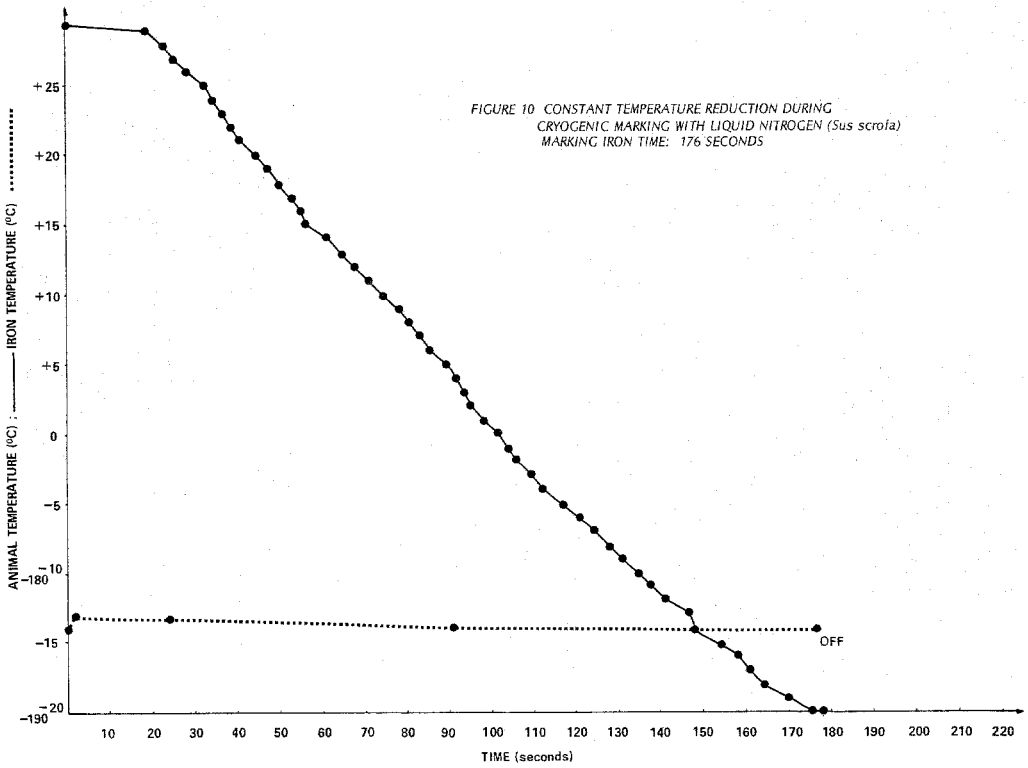



*FIGURE 10 CONSTANT TEMPERATURE REDUCTION DURING CRYOGENIC MARKING WITH LIQUID NITROGEN (Sus scrofa) MARKING IRON TIME: 176 SECONDS*

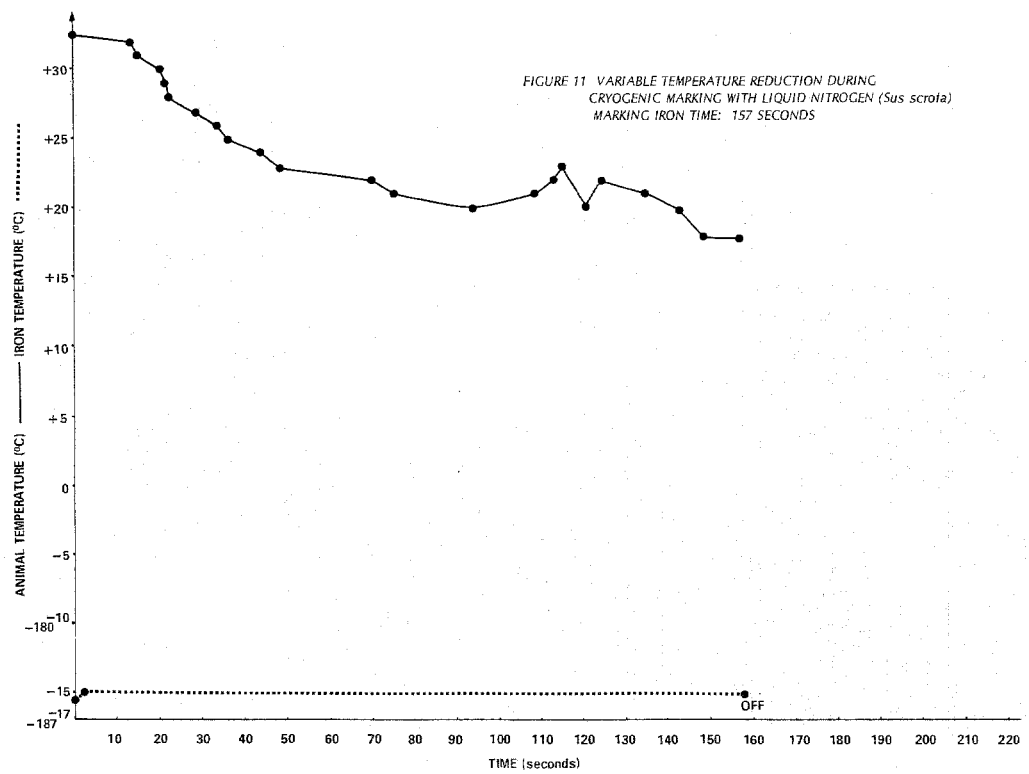



*FIGURE 11 VARIABLE TEMPERATURE REDUCTION DURING CRYOGENIC MARKING WITH LIQUID NITROGEN (Sus scrofa) MARKING IRON TIME: 157 SECONDS*

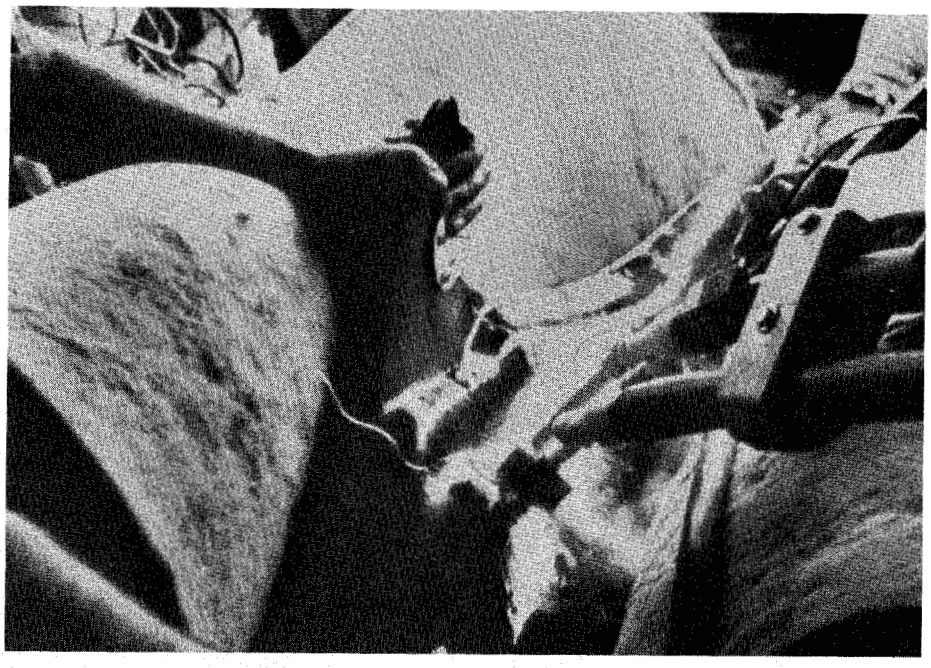


Figure 12 - Cryogenic marking equipment developed by the National Fisheries Engineering Laboratory. Marking a Stenella longirostris. Heat sink technique using liquid nitrogen.

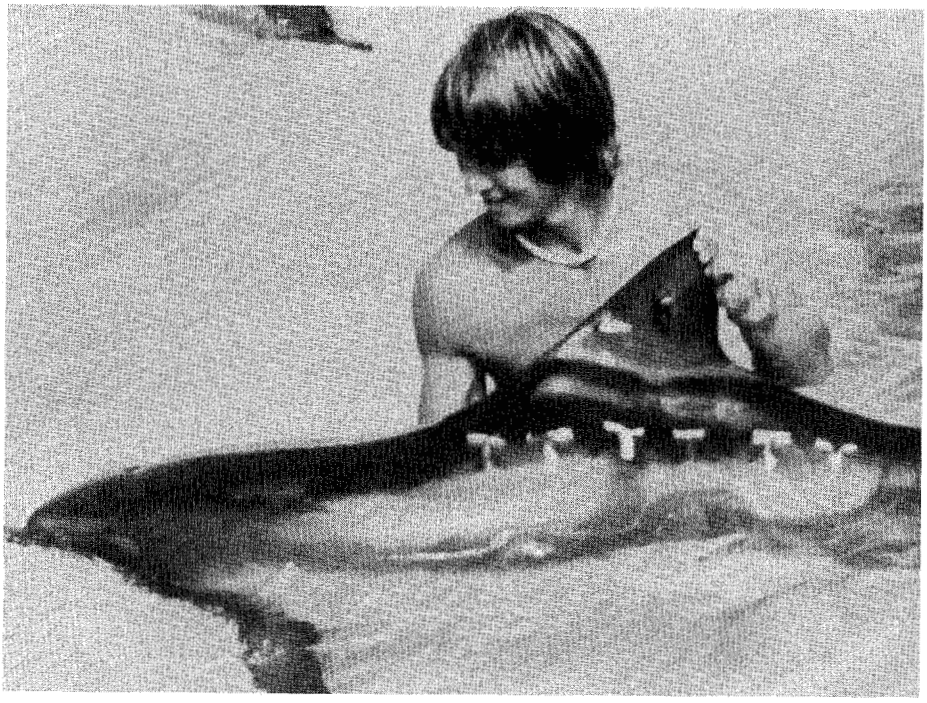
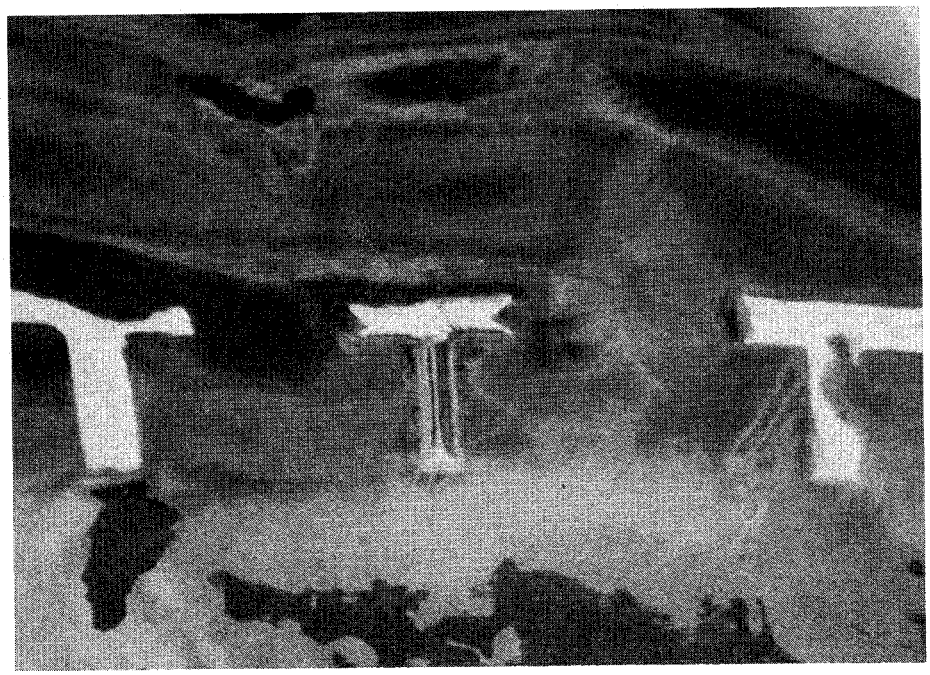


Figure 13 - Example of cryogenic marks (liquid $N_2$) on a Stenella longirostris. Heat sink technique using liquid nitrogen.



F:gure 14 - Close up view of cryogenic marks on a Stenella longirostris showing mark site puckering and uneven depigmentation. Heat sink technique using liquid nitrogen.

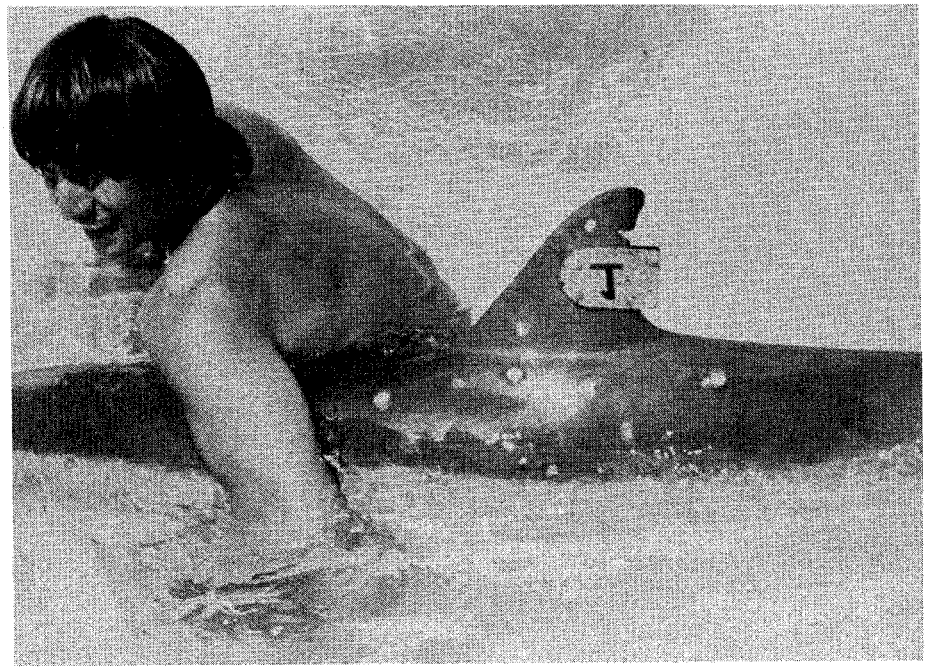


Figure 15 - Examples of cryogenic marks on a Stenella longirostris. Heat sink technique using Freon 22 (monochlorodifluromethane). Bottom row, evaporative technique with same coolant.

Figure 16 - Tissue sluffing of a mark site exposing capillary filled blubber tissue (Stenella longirostris) after cryogenic marking.

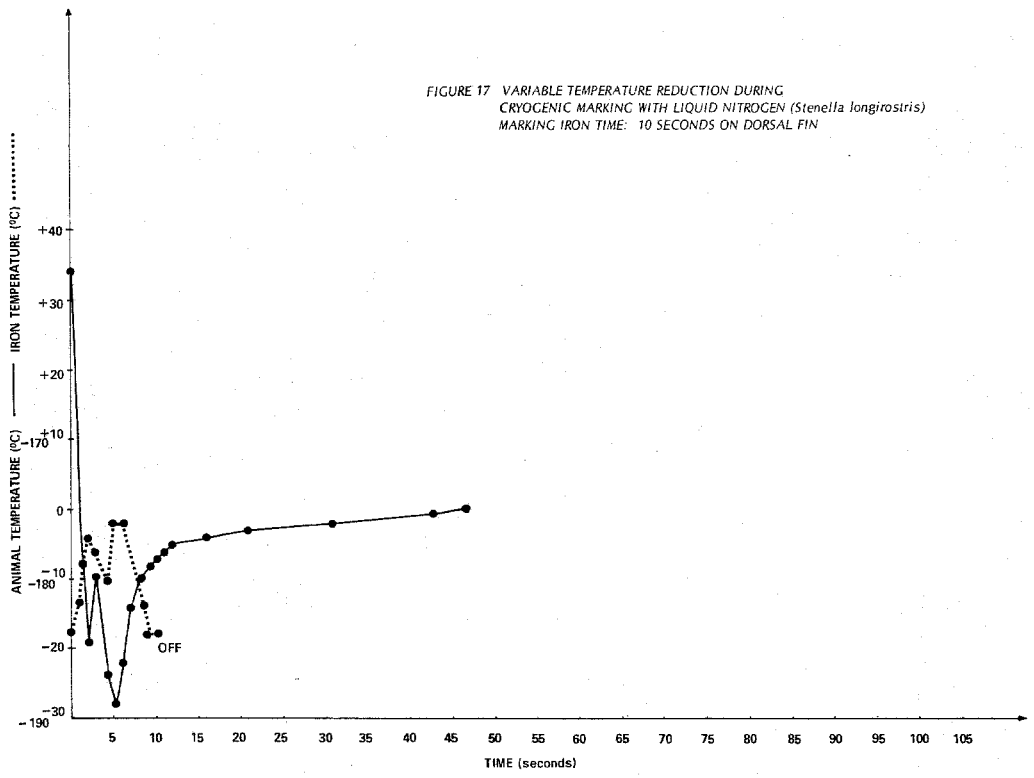



FIGURE 17 VARIABLE TEMPERATURE REDUCTION DURING CRYOGENIC MARKING WITH LIQUID NITROGEN (Stenella longirostris) MARKING IRON TIME: 10 SECONDS ON DORSAL FIN

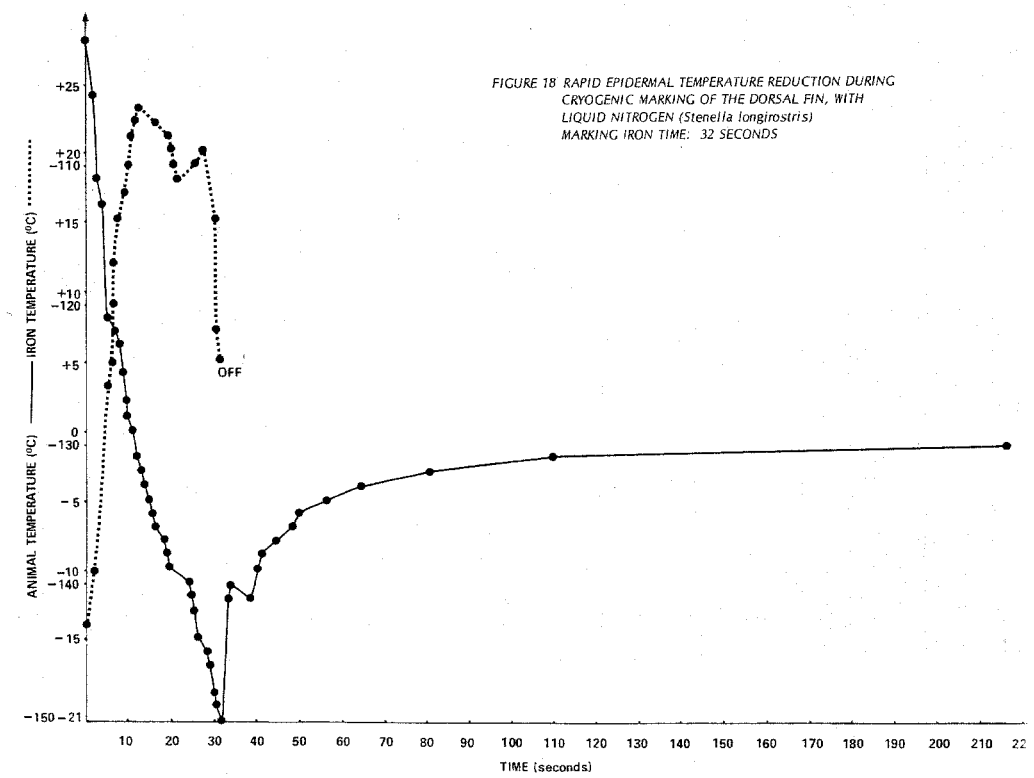



FIGURE 18 RAPID EPIDERMAL TEMPERATURE REDUCTION DURING CRYOGENIC MARKING OF THE DORSAL FIN, WITH LIQUID NITROGEN (Stenella longirostris) MARKING IRON TIME: 32 SECONDS

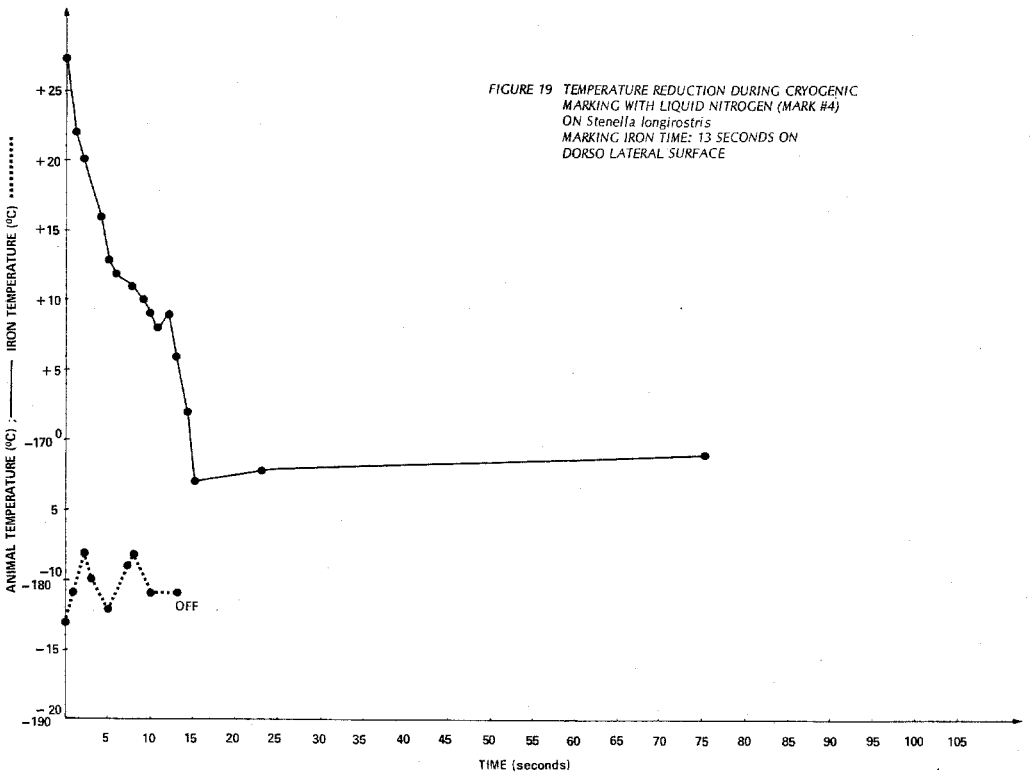



*FIGURE 19 TEMPERATURE REDUCTION DURING CRYOGENIC MARKING WITH LIQUID NITROGEN (MARK #4) ON Stenella longirostris MARKING IRON TIME: 13 SECONDS ON DORSO LATERAL SURFACE*

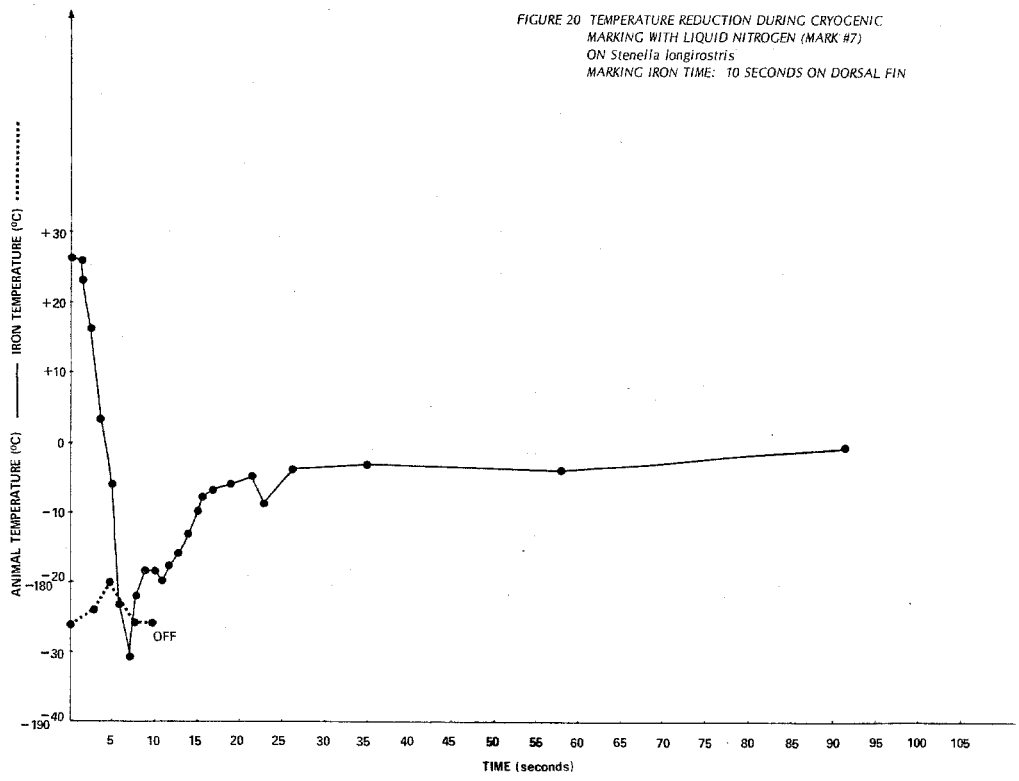



*FIGURE 20 TEMPERATURE REDUCTION DURING CRYOGENIC MARKING WITH LIQUID NITROGEN (MARK #7) ON Stenella longirostris MARKING IRON TIME: 10 SECONDS ON DORSAL FIN*

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Table 1. Cryogenic Marking Data Summary - Liquid Nitrogen, Freon. Hubbs-Sea World Research Institute, Common Dolphin (Delphinus delphis).

| Coolant type | Mark location | Application technique | Application time | Minimum tissue temperature recorded |
|---|---|---|---|---|
| $LN_2$ | dorsal fin | heat sink | 8 | N.R. |
| " | " | " | 10 | " |
| " | " | " | 12 | " |
| " | " | " | 12 | " |
| " | " | " | 16 | " |
| " | dorso-lateral | " | 1 | -4.2° C |
| " | " | " | 3 | N.R. |
| " | " | " | 4 | -19° C |
| " | " | " | 5 | -29° C |
| freon 22 | " | evaporative | 20 | -12° C |
| " | " | " | 10 | -4° C |
| " | " | " | 11 | -10° C |

N.R. = not recorded

Table 2. Cryogenic marking data summary - liquid $N_2$, Sea Life Park, Hawaiian Spinner Porpoise (Stenella longirostris). Summary prepared by J. Jennings, Southwest Fisheries Center, National Marine Fisheries Service.

| Dolphin and Brand Info. | Initial Internal Temp. | Experimental Design (Revised times) | Application Time | Final Internal Temp. | Thawing Time (to-1°C) | Comments |
|---|---|---|---|---|---|---|
| (2) Maile-left side #1 mid-dorsal fin | --- | 5 sec | attempted 5 sec (in contact ~2 sec) | 18°C | --- | Due to movements of the dolphin the brand head was jerked off 3 times, may have been in contact for 2 seconds. |
| #2 front edge of dorsal fin | --- | 7 sec (1 sec) | 1 sec | (down to 12°C) | --- | Brand head missed the thermosensor. Due to failure of #1 brand, experimental design was modified to 1 second to provide lowest endpoint. |
| #3 second in front of fin between #1 and #2 | 29°C | 3 sec (1 sec) | 1 sec | 16°C (down to 11°) | --- | Another attempt to reach lowest endpoint. |
| #4 at posterior edge of dorsal fin | 29°C | 1 sec (3 sec) | 2 sec | ~26°C (down to 19°C) | --- | Design was changed to 3 seconds. Application time was short. Brand pulled off. |
| #5 posterior to trailing edge of dorsal | 29°C | 9 sec (5 sec) | ~1 sec | 25°C (down to 11°C) | --- | Brand jerked off. |
| #6 second behind dorsal | --- | 4 sec (5 sec) | 5 sec | ? | --- | Animal continuously moving. Intermittent branding. |
| #7 | --- | 8 sec | 10 sec | ? | --- | Attempted a 10 sec brand but was probably only in contact 5 sec. Temperature drop fluctuated |

Table 2. Cryogenic marking data summary - liquid $N_2$, Sea Life Park, Hawaiian Spinner Porpoise (Stenella longirostris). Summary prepared by J. Jennings, Southwest Fisheries Center, National Marine Fisheries Service.

| Dolphin and Brand Info. | Initial Internal Temp. | Experimental Design(Time) | Application Time | Final Internal Temp. | Thawing Time (to-1°C) | Comments |
|---|---|---|---|---|---|---|
| (3)Lioele-right side #1-12 cm forward of dorsal | 27°C | 5 sec | 5 sec | 21° (down to 10°C) | --- | The brand head did not make even contact with the dorsal surface. |
| #2 forward of #1 | --- | 7 sec | 7 sec | 23°C | --- | Brand head did not make even contact. |
| #3 12.6 cm behind dorsal fin | --- | 9 sec | 9 sec | -8°C (down to -3°C) | --- | When the brand head was removed at 9 seconds the internal temperature was -8°C. It continued to drop to -3°C. |
| #4 25 cm behind #3 | 28°C | 11 sec | 11 sec | +2°C (down to -2°C) | --- | |
| #5 behind #4 | --- | 3 sec | ~3 sec | 24°C? (down to 7°C) | --- | Erratic rise and drop in temperature initially then dropped as expected. |
| #6 50 cm from dorsal fin | --- | 10 sec | 14 sec | 6°C? (down to -2°C) | --- | Plateau in thaw at -2°C. |
| #7 on dorsal fin | --- | 10 sec | 10 sec | --- | --- | Temperature dropped significantly after the iron was removed. Temperature remained a considerable amount of time at -2°C. |

Table 2. Cryogenic marking data summary - liquid $N_2$, Sea Life Park, Hawaiian Spinner Porpoise (Stenella longirostris). Summary prepared by J. Jennings, Southwest Fisheries Center, National Marine Fisheries Service.

| Dolphin and Brand Info. | Initial Internal Temp. | Application Time | Experimental Design (Final Internal Temp.) | Final Internal Temp. (Brand Off) | Thawing Time (to-1°C) | Comments |
|---|---|---|---|---|---|---|
| (1) Lioele-left side #1 (front of scar) | --- | 33 sec | -20°C | -20°C | ∿250 sec | Looking for plateau in temperature drop. Dolphin moved. Had difficulty holding brand head in place. Temperature remained at -2°C for a considerable time during the thaw. |
| #2 (behind scar) | --- | 25 sec | -20°C | -20°C | ∿172 sec | May have been in contact with thermosensor. Temperature remained at -2°C (eutectic point) during the thaw for a long time. |
| #3 (forward-most mark) | 30°C | 20 sec | -2°C | (+26°C) | --- | Probe placed too deep for measurement. Terminated at 20 seconds (26°C) to get comparison with other times. Continued to drop to 10°C. |
| #4 (second from front) | --- | ∿14 sec | (-4° originally) -2°C | -2°C | ∿81 sec | Same -2°C plateau on thawing. |
| #5 (fifth mark back-first behind fin) | 28°C | ∿14 sec | (-6° originally) -4°C | -4°C | ∿150 sec | Dropped to -6°C. |
| #6 (sixth back-second behind fin) | 29°C | 27 sec | (-10° originally) -6°C | (+8°C) | ∿225 sec | Probe too deep. Slower drop in temperature than normal. Reached -1°C during thaw at 12:36 on the time generator. Dropped to 0°C. Probe moved into brand (-2°C). |
| #7 (base of dorsal fin) | --- | ∿30 sec | -6°C | -70°C | --- | Probe very shallow. Temperature dropped so fast that it was hard to monitor. Thermosensor may have been in contact with brand head. Brand in contact with fin on left and base of "T". |

Table 3. Cryogenic marking data summary - freon 22. Sea Life Park, Hawaiian Spinner Porpoise (*Stenella longirostris*).

| Branding Sequence | Technique | Application Time | Approximate Final Internal Temp. (brand off) | Approximate Thaw Time (to 1°C) | Comments |
|---|---|---|---|---|---|
| 1 | heat sink | 12 sec | -6°C | 47 sec | |
| 2 | heat sink | 9 sec | -17°C | 88 sec | |
| 3 | heat sink | 16 sec | -3°C | 24 sec | Brand head came off after 1 second, after which application time was restarted. Brand site got wet shortly after thawing began causing two types of thawing. |
| 4 | heat sink | 15 sec | -13°C | 83 sec | Had difficulty placing thermosensors. |
| 5 | heat sink | 4 sec | 24°C | --- | Temperature dropped to 7°C. |
| 6 | heat sink | 30 sec | -21°C | 141 sec | Brand head came off after seven seconds. Application time is total time brand head was in contact with the skin. |
| 7 | heat sink (dorsal fin) | 15 sec | -10°C | 27 sec | Initial temperature was 33°C. The rapid thaw may be because the fin is more vascularized. |
| ? 8 | heat sink (dorsal fin) | 20 sec | -15°C | 65 sec | Initial temperature of the dorsal fin was 29°C. The lowest temperature drop was to -16°C. |
| ? 9 | spray | 12 sec | -17°C | 54 sec | On the basis of the freeze, we expected a rapid thaw. |
| ?10<br>?11 | spray through "cookie cutter" | --- | --- | --- | No data. Out of tape. |

# Section III - The Feasibility of Tatooing Small Delphinids

# The Feasibility of Tatooing Small Delphinids

Tatooing may be defined as the implantation of pigment in the skin's dermal tissue, where it is permanently retained and readily visible. It has been employed by various tribal groups as cosmetic adornment for thousands of years. More recently, livestock, dogs, seals, and even fish have been successfully tatooed as a means of identification. To determine the feasibility of tatooing small delphinids in a safe and humane manner, preliminary experiments were conducted at the University of California at San Diego Medical School's laboratory animal facility. The following is a summary report on these experiments.

## Polystyrene Particle Spray

### Methods and Materials

Minute polystyrene particles (biologically inert) coated with fluorescent "day glow" pink pigment were applied to 7" x 5" x 1.5" section of fresh Delphinus epidermis with compressed nitrogen. The spray apparatus is shown in Fig. 1. Tissue spraying was conducted according to the following criteria:

| Test # | Distance (nozzle to target) | Pressure on Tissue | Time |
|---|---|---|---|
| 1 | 6" | 100 psi | 3 sec |
| 2 | 1" | 150 psi | 3 sec |
| 3 | 1" | 125 psi | 3 sec |
| 4 | 1" | 100 psi | 3 sec |

Use of a face mask was necessary during application to avoid breathing the polystyrene particles (Fig. 2). After application, the tissue

was incised across the spray area and examined under ultra-violet light magnification to determine the depth of particle penetration.

## Results

Results of the polystyrene particle spray application are summarized in Table 1. The first test displayed no particle penetration and no tissue erosion. In the other three tests the polystyrene particles appeared to abrade the outer cornified epithelial layer. Only test #2 displayed particle penetration into the epidermis in sufficient quantity to be visually detected. However, tissue damage was sufficient to preclude further testing at higher application pressures. The polystyrene particles appeared to abrade tissue without penetrating sufficiently for permanent retention.

# Schvco Injector

## Methods and Materials

The Schvco Injector (Fig. 3) is designed for subcutaneous injection of liquids used in medicine. A spring-loaded piston is cocked and fired manually, forcing a predetermined amount of liquid from its reservoir into the tissue. No information was available on its psi rating. Carmine red was used for pigment. Injections were made at a fixed pressure into a dog cadaver and a section of fresh Delphinus epidermis. The dog cadaver was injected first, positioning the injector head on the epidermal surface and at .25, .50, and 1 inch above it to determine depth of pigment penetration and surface area. The Delphinus epidermis was injected in the same manner.

## Results

Results of the Schvco Injector evaluation are summarized in Table 1. Pigment penetration was only realized when the injector head was held in contact with the epidermal surface of both tissue types. The surface area of the mark was .02 sq. in. When the dog epidermis was injected, the pigment was forced completely through the epidermal and dermal layers into the blubber. Injection of pigment into the Delphinus tissue appeared to diffuse throughout the epidermal and dermal layers, providing the desired results.

## The Vernitron Hypodermic Injection Apparatus

### Methods and Materials

The Vernitron Hypodermic Injection Apparatus (Fig. 4) is also designed for subcutaneous injection of liquids used in medicine. It is capable of injecting viscous liquids such as penicillin. The unit consists of a self-contained compressor and a piston-driven injection gun. The liquid is conveyed from an inverted rubber capped bottle (60 cc volume) via a stainless steel tube into a holding chamber when the gun's piston is cocked, creating a vacuum. A trigger is pulled and the compressed air driven piston forces liquid into the tissue at 1200 psi.

The dog cadaver, a fresh section of Delphinus epidermis, and Delphinus epidermis with the cornified eipthelium removed, were injected with white Burdizzo tatoo ink paste mixed with normal saline (2 parts tatoo ink paste and 1 part saline by volume). Subsequent tests used a 1:1 ratio by volume. The Schvco Injector tests indicated the injector head must be in contact with the epidermal surface for

pigment penetration. The Vernitron Injector's air pressure was also fixed, requiring the head to be placed in contact with the epidermal surface, or with a material covering the epidermis, for pigment penetration. Several types of materials used to cover the epidermis and regulate the depth of pigment penetration. These materials included single and multiple layers of cellophane tape, adhesive tape, and a .25 inch thick section of closed cell urethane foam.

Results

Test results for the Vernitron Hypodermic Injection Apparatus area are summarized Table 1. Injection of the dog cadaver with no covering allowed pigment penetration into the muscle tissue. Cellophane tape covering the epidermis allowed pigment penetration between the dermal and muscle tissue. Injection of pigment through adhesive tape appeared to distribute it throughout the epidermal and dermal tissue, achieving the desired penetration (Fig. 5.).

Injection of normal Delphinus tissue with an adhesive tape covering produced no pigment penetration. When the pigment mixture ratio was changed to 1 part pigment and 1 part normal saline by volume (used for all remaining tests) the same test was repeated, producing a blister of pigment under the cornified epithelium.

Removal of the cornified epithelium and injection with no covering allowed pigment penetration into the muscle tissue. Addition of an adhesive tape covering again allowed pigment penetration into the muscle tissue. A single layer of cellophane tape on the epidermis (no cornified epithelium) appeared to allow the desired penetration

throughout the epidermal and dermal layers. Two layers of cellophane tape did not allow any pigment penetration.

Discussion

Implanation of polystyrene particles with compressed nitrogen using the equipment shown in Fig. 1 does not appear to be practical. The large quantities of airborne particles which result during application, may jeopardize the animals' health and the tissue trauma observed may produce sufficient scar tissue to obscure the tatoo. The equipment's bulky nature does not lend itself to field use, although the technique should be evaluated further.

The Schvco Injector is designed to inject liquids at a fixed pressure; depth of pigment penetration into the tissue is difficult to regulate. This device must also be cocked manually and its liquid capacity is only 12 cc. It appears that many injections would be needed to mark an adequate surface area on a delphinid (i.e. dorsal fin) for field observations.

Pneumatic injection of indelible pigments using the Vernitron Injector appears to have some potential for field tatooing of delphinids. It is portable, lightweight, can inject pigment rapidly, and has a greater capacity (60 cc). If the pigment is not to viscous, this injection apparatus does not appear to cause significant tissue trauma. It will be necessary to eliminate removal of the cornified epithilium from the animal or cover the epidermis with material to insure required depth of pigment penetration. Air pressure, pigment viscosity and injector nozzle design are all factors that appear to influence pigment penetration and tissue trauma. They should receive further evaluation.

## Recommendations

The biocompatibility and tissue resorption of indelible pigments should be studied with a live animal experiment. Three genera of delphinids (i.e., Tursiops, Delphinus and Stenella) could each be tatooed using the "jailhouse technique", requiring application of the pigment (sterile) to the epidermal surface and placement in the tissue with a needle (sterile), similar to a small pox vaccination. A 1" diameter tatoo would allow sufficient diameter for biopsy and histological evaluation.

Once biocompatibility and tissue resorption of the inedlible pigments has been evaluated, tatoo size and location (i.e. body, dorsal fin) should be determined. When this data is available, further progress can be made to develop portable equipment for rapid, safe and humane tatoo marking of delphinids in a field situation.

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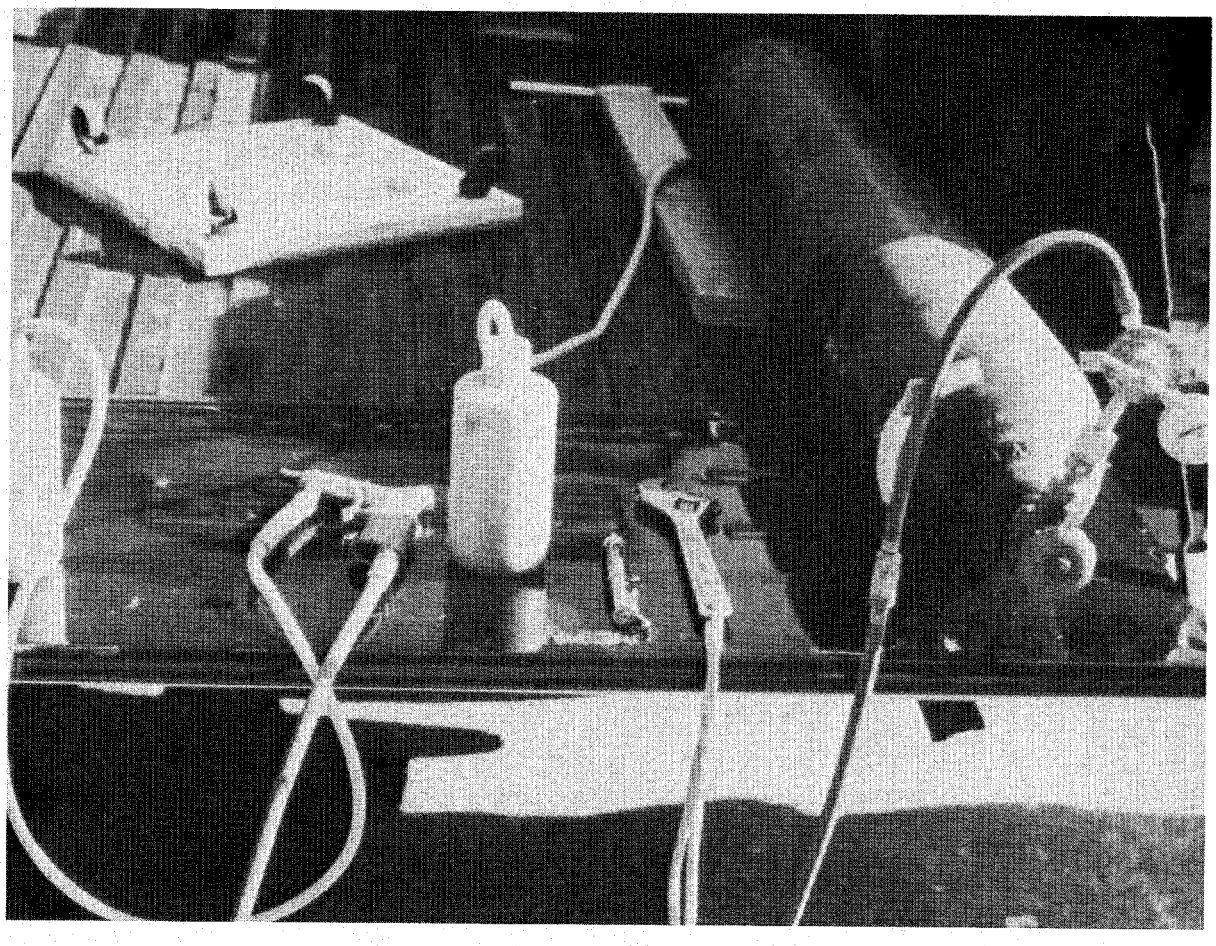

Figure 1 - Spray apparatus for polystyrene particles.

Figure 2 - Spray application of polystyrene particles on Delphinus tissue.

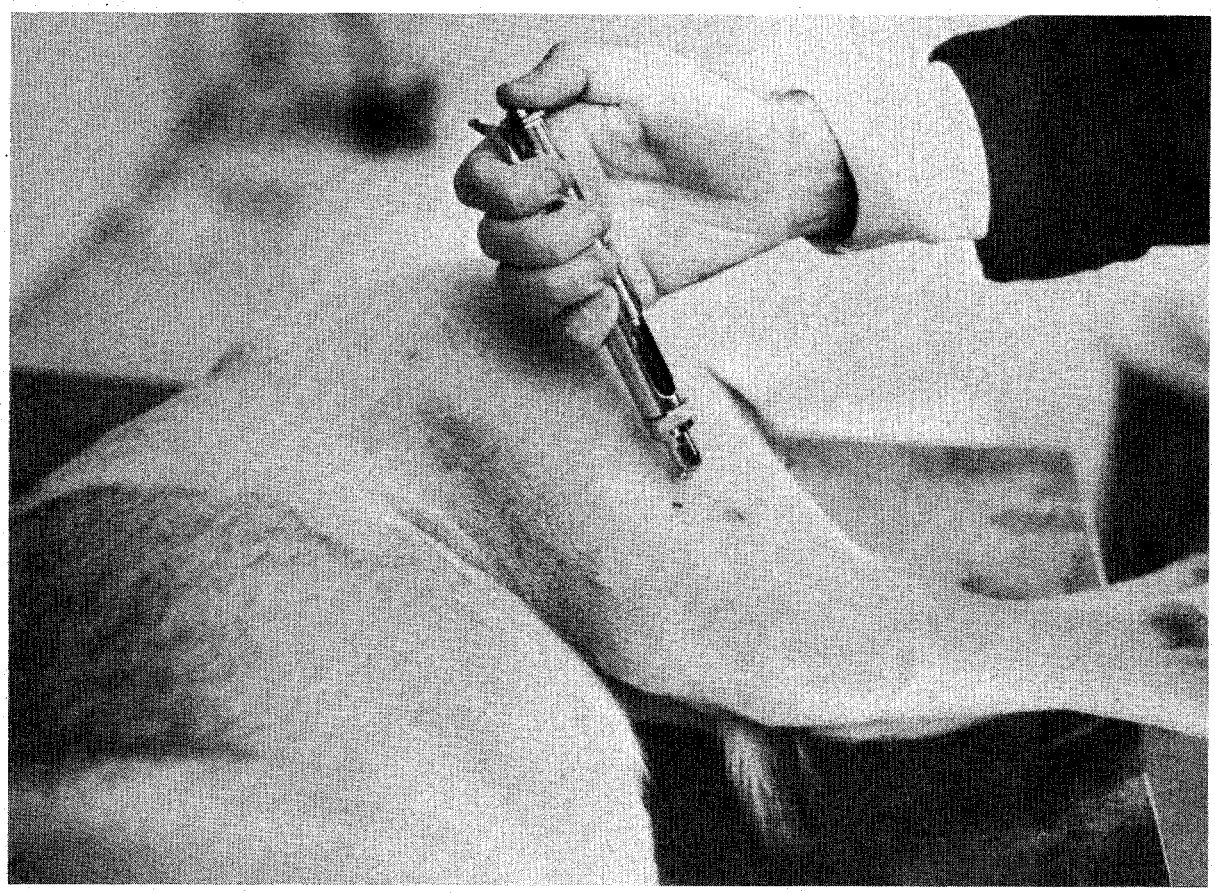

Figure 3 - The Schvco Injector.

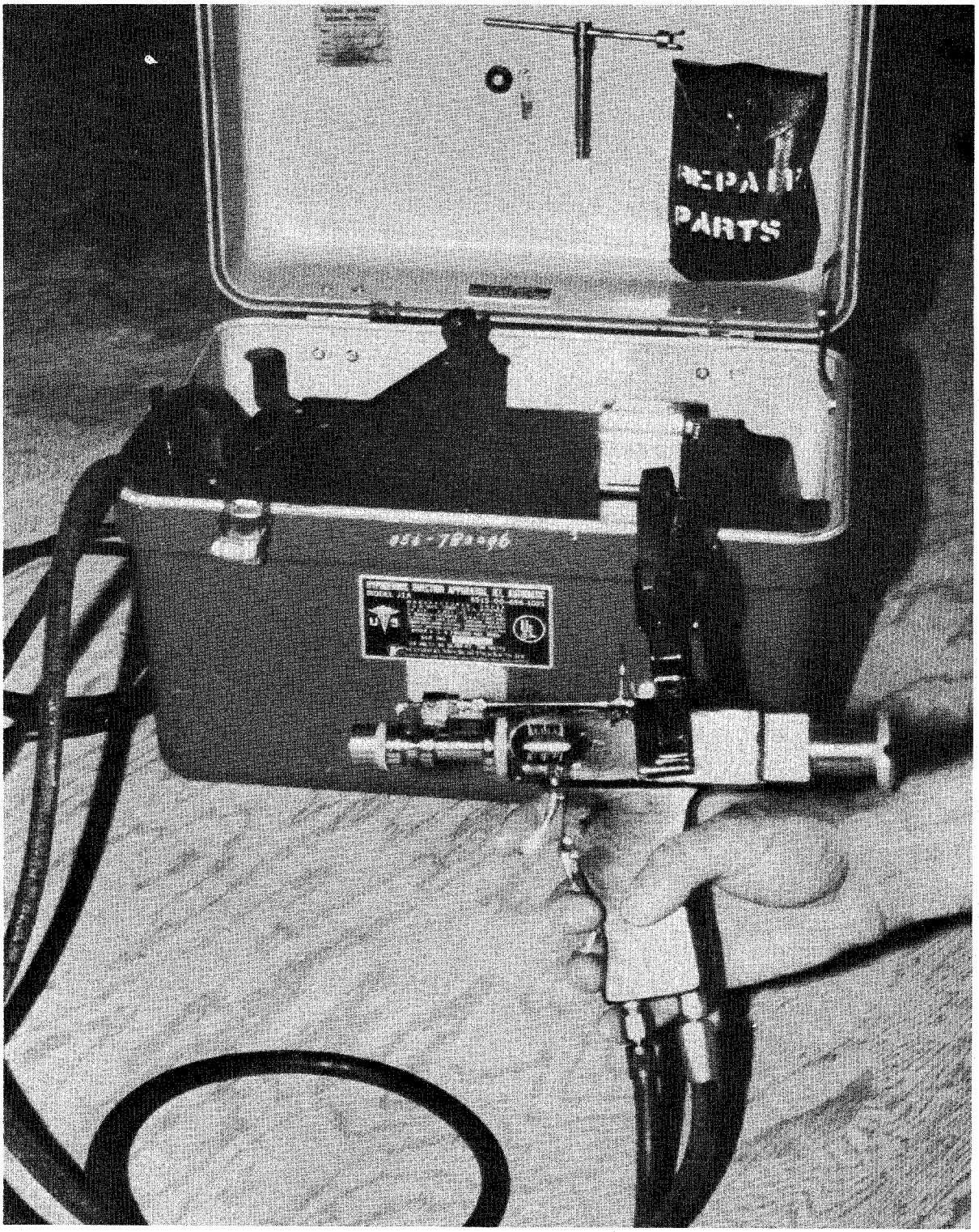


Figure 4 - The Vernitron Hypodermic Injection Apparatus.

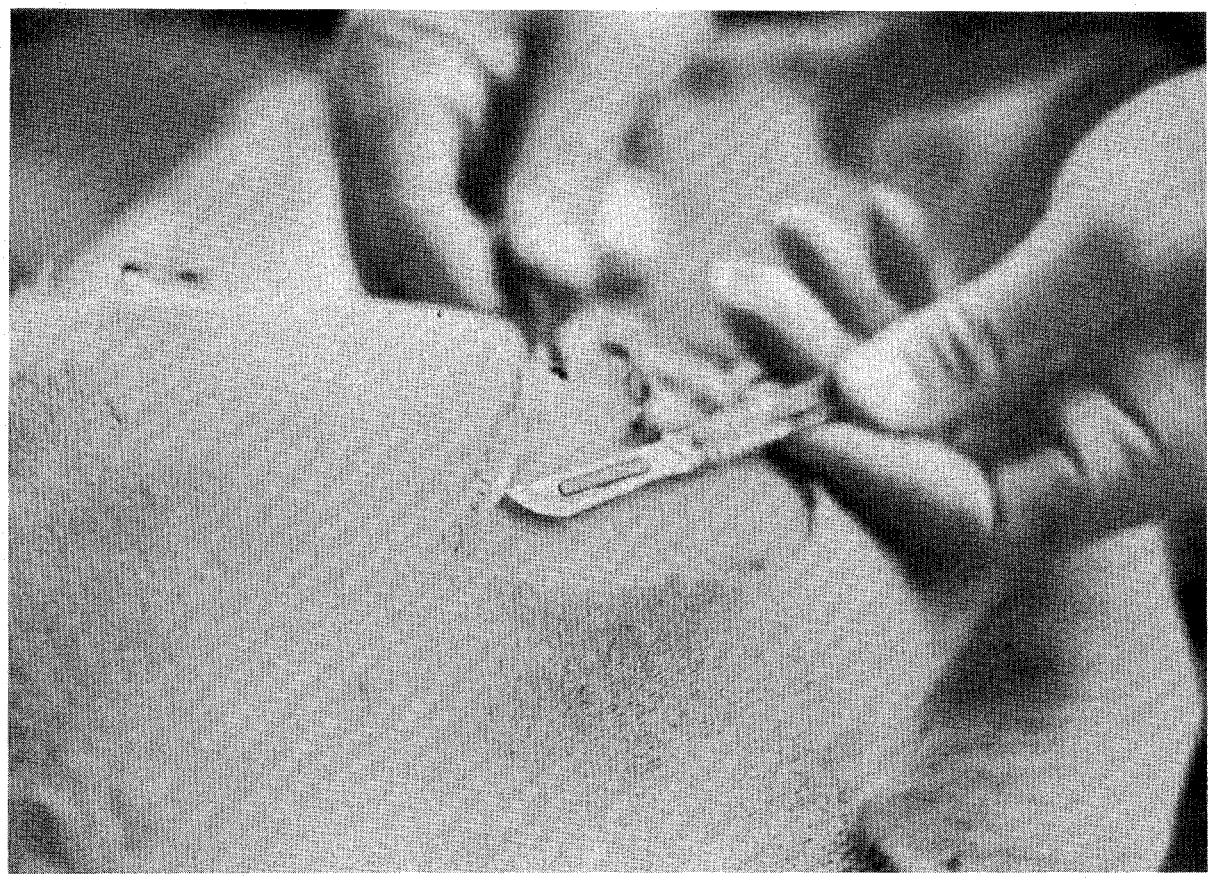

Figure 5 - Injection of dog cadaver with pigment (adhesive tape cover) using the Vernitron Hypodermic Injection Apparatus. Note pigment distribution throughout epidermal and dermal layers at top of scalpel blade.

List of Tables

Table I. Summary of Tatoo Experiment Data

| Tatoo Apparatus | Tissue Type | Nozzle to Target Distance | Injection Pressure (psi) | Injection Time (sec) | Epidermal Covering | Results |
|---|---|---|---|---|---|---|
| Particle Injector $N_2$ | Delphinus | 6" | 100 | 3 | none | No particle penetration or tissue erosion. |
| | | 1" | 150 | 3 | " | Particle penetration into epidermis significant tissue erosion. |
| | | 1" | 125 | 3 | " | No particle penetration or tissue erosion. |
| | | 1" | 100 | 3 | " | No particle penetration or tissue erosion. |
| Schvco Injector | Dog | epidermal contact | ? | - | none | Pigment penetrated completely through epidermis and dermis into blubber tissue. |
| | | .25" | ? | - | " | No pigment penetration. |
| | | .50" | ? | - | " | No pigment penetration. |
| | | 1.00" | ? | - | " | No pigment penetration. |
| | Delphinus | contact | ? | - | none | Pigment diffused throughout epidermal and dermal layers. |
| | Dog | contact | 1200 | - | none | Penetrated into muscle tissue. |
| | | " | 1200 | - | closed cell foam | Produced blister of pigment under cornified epithelium. |
| | | " | 1200 | - | cellophane tape | Pigment deposited between epidermis |
| | | " | 1200 | - | adhesive tape | Pigment diffused throughout epidermal and dermal layers. |
| | Delphinus (normal tissue) | contact | 1200 | - | adhesive tape | No pigment penetration observed |
| | | " | 1200 | - | adhesive tape | Produced blister of pigment under cornified epithelium. |

Table I. (continued)

| | | | | | |
|---|---|---|---|---|---|
| Vernitron Injector | Delphinus (cornified epithelium removed) | contact | - | none | Pigment penetrated into muscle tissue. |
| | | " | - | adhesive tape | Pigment penetrated into muscle tissue. |
| | | " | - | 1 layer of cellophane tape | Pigment appeared to penetrate into epithelial tissue. |
| | | " | - | 2 layers of cellophane tape | No pigment penetration into tissue. |

*Pigment mixture changed from 2:1 to 1:1 (pigment:normal saline).

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