An Analysis of Utilizing the Leatherback's Pineal Spot for Photo-identification

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Abstract

Leatherbacks are one of the most endangered species of sea turtles. Their global population size had decreased dramatically over the past several decades. Difficulties in applying long-term marking methods on leatherbacks, have significantly hindered our ability to generate an accurate population estimate and to track population changes on the scale of individuals. External marking methods are plagued by high rates of tag loss which compromise the utility of tagging as a means of long-term identification. While having substantially better retention rates, the high cost PIT tags and the specialized equipment necessary to detect them, have limited the expansion of this technique.

This project outlined the need for innovative approaches to identifying turtles and sought to resolve some of the issues plaguing traditional tagging protocols by reinvestigating the feasibility of using photo-identification to recognize individual leatherbacks. Pioneering studies of pink spot photo-identification by McDonald and Dutton (1996) showed that the pineal spot of the leatherback is distinct enough to be used as a unique identifier. This research investigated whether individual turtles could be recognized by their “pink spot” when a large sample (~400) was drawn from a very large nesting population (~ 3,000 nesting annually). It also sought to determine whether identification could be automated using photo-identification software.

The results indicated that the pineal “pink” spot’s form has sufficient variation to be used as a unique identifier in photo-identification studies. Using the Scale Invariant Feature Transform (SIFT) function we were able to successfully automate identification. Through a cascade filtering approach the program was able to achieve one hundred percent matching accuracy, while eliminating the possibility of false negatives. Further studies are required to examine the degree of deviation that may occur within the pink spot over time, and to refine our methodologies to expand the use of this method amongst leatherback researchers.

I. Introduction

The leatherback sea turtle (*Dermochelys coriacea*) is the largest turtle and the largest living reptile (NOAA, 2007). A mature adult can measure approximately two meters in length and over one meter in width, and weigh between 300 – 916 kg (Eckert & Luginbuhl, 1988). There are several unique features which distinguish leatherbacks from other sea turtle species. For instance, they lack a hard, bony shell. Instead their carapace consists of a thin layer of tough skin overlaying a network of loosely connected dermal plates. This and other morphological differences have led to their classification in a distinct taxonomic family the Dermochelyidae, for which they are the only living representative.

Leatherbacks are one of the most endangered species of sea turtles and are currently listed as Critically Endangered by the International Union for Conservation of Nature and Natural Resources. Analyses of published estimates of global population sizes suggest a reduction of over 70% in less than one generation. Pritchard (1982) estimated the number of adult female leatherbacks worldwide to be 115,000, with about half of them nesting along the Pacific coast of Mexico. However, in 1995, a revised estimate incorporating information from 28 nesting beaches throughout the world yielded approximately 34,500 females, with a lower limit of about 26,200 and an upper limit of about 42,900 (Spotila et al., 1996).

Currently, the only available estimates for the world population of leatherback turtles are confined to data based on breeding females. These estimates are approximated from nest counts and our knowledge of intra- and inter-annual nesting periodicities (K. Eckert, 1996). Our ability to generate an accurate population estimate and to track population changes on the scale of individuals is significantly hindered by difficulties in applying long-term marking methods on leatherbacks, which aid in counting the number of nesting turtles at individual colonies. This project seeks to resolve some of these issues by establishing a new means by which we can identify individual leatherbacks and by generating a method of storing and retrieving this information in an interactive database. By utilizing natural markings of the leatherback, specifically its pineal “pink” spot, we evaluated the ability to develop a photo-identification system that is capable of automatically matching re-migrant individuals. This paper will outline the need for innovative approaches to identifying turtles, detail the creation and application of our system, and highlight its abilities to promote conservation in comparison with classic marking methodologies.

II. Marking Animals

A. Historical Marking Methods

The ability to individually identify animals in the field has been an extremely valuable tool in advancing our understanding of the biology and population dynamics of the animals being researched. Marking allows for the measurement of a wide variety of biological and population variables (*e.g.* total number of individuals, reproductive output, longevity, and survival rates).

The practice of marking animals as a means of identification dates back over 3,800 years to the Code of Hammurabi, which inferred the means used in those times for identifying ownership of herds (Blancou, 2001). Since this early record, the reasons and means by which we mark animals have changed and advanced with the development of society and technology. While previous marking systems were developed primarily to manage livestock herds (and still are today) marking protocols have also become critically entwined with the study of animal populations in the wild. In antiquity, three main identification methods were used to identify individuals or herds of animals which belong to the owner. First, were descriptive documents or a certificate referring to the animal’s unique features or any sign/brand that distinguished the animals (Blancou, 2001). This document was held by the person responsible for the animal, as a title. Another method was the creation of a simple mark directly upon the body of the animal via the skin, hooves, paws, beak or horns (Georgoudi, 1990; Leclainche, 1955; MacGregor, 1996). Lastly, they used removable, exterior marks such as a collar or ring, which would be attached to the animal (Finet, 1993; Fleming, 1872; Theodorides, 1986). The utilization of a given technique was often the result of cultural, economic, and individual preference. For example, written certification was more prevalent in advanced societies where reading and writing abilities were more common in trade professions. However, the use of man-made brands (i.e. hot irons), being the most economically efficient, was the most widely used marking technique (Blancou, 2001). Another key advantage that this method possessed was the relative permanence of the mark. Marking systems became more significant and advanced during the 18th century with the advent of epizootics. In Europe, the extensive epizootics of this period brought about the requirement to present written documents certifying the origin of all animals (Reynal, 1873; Winslow, 1980). For example, in 1716, Friedrich Willhelm I, the King of Prussia, issued a decree setting forth measures to avoid the spread of rinderpest. It stated that all animals imported from abroad or moved within the kingdom had to be branded on the right horn with the initials of his majesty (Torner, 1927).

The beginning of the 19th century witnessed a shift in the objectives for marking animals. The Age of Enlightenment which was gripping the world steered marking protocols beyond administrative and animal husbandry purposes, and towards scientific exploration (Voget, 1968). Enlightenment thinkers placed a premium on the discovery of truth through the observation of nature. This led to many innovative studies to examine the complex social interactions that occur within and between animal species. The assertion of the theory of evolution, and the founding of the study of biology by Lamarck spurred this era of research. Experiments in the natural world, however, proved to be fraught with limitations and led to an over reliance, at the time, on laboratory and museum work which was about to be conducted with relative ease (Woodbury et al., 1956). The results of fieldwork often contained large degrees of uncertainty due to the difficulty in controlling or evaluating the dynamic flux of many factors that are continually at work in the wild (Woodbury et al., 1952). The critical problem was in the recognition of the individual animals to be studied. In order to overcome this obstacle, researchers proposed placing artificial marks on the animals in order to aid the identification process. As a result, marking protocols became essential for identifying animals in their natural environment to assist in accurately depicting social interactions.

B. Marking Reptiles and Amphibians

Pioneering studies with regard to reptiles and amphibians were performed on many species of frogs and toads. The earliest of these studies was by Breder and Redmond (1927), who tied numbered tags around the waists of frogs to obtain accurate data about specific activities and behaviors (Breder et al., 1927). Hamilton established another marking technique in his 1934 study of the rate of growth of the American Toad (*Bufo americanus*). He marked the toads by incising one or more toes from the fore feet as a means of identifying individuals. Through this method, later called toe-clipping, he was able to measure and weigh specific individuals at intervals to get accurate information on their growth rates (Hamilton, 1934). Another identification method developed at this time was the use of jaw tags. In 1939, Raney marked over 600 frogs and toads in Albany County, NY using jaw tagging. He was able to obtain specific data on the movements of individuals which yielded pertinent information about sex ratios, behavior patterns of reproduction, daily and seasonal movements, and other natural history information (Raney, 1940). From this study he concluded that jaw-tagging was superior to other methods, such as toe-clipping, which required extensive bookkeeping, and waist collars, which were impermanent. Detractors, however, noted evidence which demonstrated that jaw-tags irritate or impede marked animals (Stebbins, 1954). As a result, after simplifying the tracking records, toe-clipping, became the primary method of marking amphibians and reptiles. This was due to the small expense associated with using this protocol and the large capacity of individuals which could be identified. By clipping 1, 2, 3, or 4 toes on different feet, in different combinations, several hundred lizards could be individually identified (Hamilton, 1934). Its popularity was confirmed through other studies, such as Freiberg (1951), Martof (1953) and Carpenter (1954). Toe-clipping was not without its disadvantages. In particular, the extension of this method to certain amphibian species, such as salamanders was hampered by the ability of many species within this taxon to regenerate their limbs. This put into question the relative permanence of the mark and the reliability of data over large time spans. In general, this method is appropriate for most anurans, but inadequate for urodeles and *Xenopus* in which the regeneration process replaces removed digits (Heatwole, 1961). Other methodologies, such as scale clipping (Blanchard and Finster, 1933) and tattooing (Woodbury, 1948, 1951), were developed for these species and other animals without limbs.

C. Marking Terrestrial Turtles

Due to turtles' unique body characteristics, most notably their shells, new marking protocols were developed to utilize these features. Many species have thick, rough, pigmented skin which makes them difficult to tattoo, although this protocol has been tested (Woodbury et al., 1956). Toe-clipping, while feasible for terrestrial turtles, has been used as an auxiliary to methods of marking the shell (Woodbury et al., 1956). Several protocols have been created to utilize their shell as a means of identification. These marking techniques include painting, carving, branding, and notching. Painting the carapace of land tortoises had long been used by many amateur naturalists and was one of the earliest marking methods used by researchers. Woodbury and Hardy (1948) painted various combinations of plates using an assortment of colors for individual identification. The study, however, uncovered a significant flaw associated with painting; within a year or two the colors began to wear making identification less accurate. As a result, for long-term studies, this method was soon replaced in favor of branding which was more permanent. Branding tortoise shells with a wire heated in a small fire proved to be a practicable way of marking that was more or less permanent if done properly (Woodbury et al., 1956). In this practice, brands are usually placed on various scutes or combination of scutes. There are some dangers inherent with this method and precautions must be taken not to damage the tissue underlying the shell. If the brand is too deep and affects the underlying tissue, it stimulates a slow process of re-generation which after a few years will completely replace the burned area, obliterating the mark (Woodbury et al., 1956).

In addition to branding, John T. Nicholas of the American Museum of Natural History marked box turtles for twenty four years using a system in which numbers are carved into the plates (Nichols, 1939). In correspondence with Dr. Fred Cagle, Nichols stated that such marks remained distinct after ten or fifteen years, but that this method was not suitable on turtles which molt their outer plates (Cagle, 1939). In addition, using this method on tough-shelled turtles would be a slow and difficult procedure if carved by hand (Woodbury et al., 1956). As a result, this led Cagle to formulate a method of marking turtles by making notches in the edge of the marginal plates with scissors and files. In later studies, the use of power grinders or small portable hand grinders were introduced as a much more efficient means of making the notches (Cagle, 1944).

In notching systems, the marginal plates, exclusive to those connected to the plastron, are marked by a rectangular notch, one-third to one-half the width of the plate (Cagle, 1940). The filing requires only a few minutes and does little injury to the turtle, although, occasionally, it may cause the wound to bleed lightly. Care must be taking when marking turtles whose carapace is partially ossified, as the process can cause serious fracture of the carapacial plates, resulting in either death of the individual, or loss of a considerable portion of the carapace (Cagle, 1940). This fact, and the assumption that these notches did not interfere with the normal activity of the turtles, were one of the few negative aspects of using this method (Woodbury et al., 1956). The strength of this marking protocol was the large number of turtles which could be identified with a relatively small number of notches, and the permanence of the mark itself. The use of Cagle's methodology allowed for individual identification of 16 turtles using one notch, 120 using two plates, 560 using three, and 1820 using four, a total of 2516 possibilities (Cagle, 1940). Hildebrand and Hatsel (1926) confirmed that the notches are permanent if made when the shell is well ossified, but that the marks tend to obliterate in juvenile specimens.

III. Sea Turtle Marking Protocols

Despite the success of these protocols with terrestrial turtles, many difficulties exist with translating them to marine species. Their unique morphology, lifestyle, and precarious population status present obstacles in developing successful marking protocols. For example, due to their paddle-like limbs toe-clipping is not a viable marking method. In addition, so-called mutilation tagging is now generally regarded with disfavor and actively discouraged by several professional societies (Putnam, 1995). Notching, the dominant marking method for terrestrial turtles, is also ill-suited for marine species. For one, notches made for identification purposes can be confused or distorted by those that are naturally induced from shark bites, collisions with boats, or other reasons (Andrews et al., 2003). While notching has been used in sea turtles, regular maintenance is required as abrasion and shell growth can obliterate the notch (J. Richardson pers. com). In addition, notching presents some risk of damaging the structural integrity of the shell in small turtles. The small size of their marginal plates increases the risk that notching could damage the underlying anatomy. Lastly, the limited number of notch site combinations makes it difficult to uniquely mark a large number of turtles which is necessary to obtain accurate population estimates and other data (Anon, 2007)

Several other methods mentioned previously, such as bands, carvings, or drill holes, have been utilized with only passable results. Their life history and the general behavior of sea turtles compound the problem of finding a suitable marking system. Sea turtles are highly pelagic individuals, traveling hundreds or thousands of miles in their migration between foraging and breeding habitats. Their shells are often subject to abrasion on hard substrates, and they often rub themselves on hard benthic structures to remove eco-biota. In addition, these animals remain at sea during their entire lives, except adult females who briefly come ashore to nest in the summer months. As a result, marking methods must be able to withstand the hazards of the marine environment, while not impeding their locomotive, reproductive, or feeding abilities. Furthermore, marking protocols can only be carried out on nesting beaches or after capture at sea. This not only creates logistical challenges, but there is also the potential for disturbing turtles during nesting. According to the U.S. Endangered and Threatened Wildlife and Plants List, all seven species of sea turtles are listed as either endangered or threatened. As a result, their delicate status in the world’s oceans makes identification and conservation efforts even more essential (USFW, 2007). However, the use of some marking methods may be considered too risky for depleted populations, because their application may inflict pain, injury, or disrupt important lifecycles. Several technological developments in the past thirty years have helped to uncover new approaches for identifying individuals and groups of sea turtles in the wild. The following marking systems are currently used for the identification of individual sea turtles. These include tissue modification, plastic tags, metal tags (Monel, Inconel, and Titanium), and passive integrated transponders. All of these methods are considered semi-permanent or permanent marking methods.

A. Tissue Modification

In 1980, L.P. and J.R. Hendrickson experimented with four groups of sea turtles to explore the feasibility of producing permanent, recognizable identification marks by tissue modification. Some turtles had scutes surgically removed and exchanged between the plastron and carapace. Because these reverse pieces of tissue grew into the surrounding tissue the effect was to create a darkly pigmented “spot” from a carapacial scute on the plastron and a lightly pigmented spot on the carapace. Two groups were also treated with melanin destroying substances. Although the chemical treatments did not yield clear, long terms results, there were short-lived blanching effects (Hendrickson et al., 1981). Distinct, unmistakably non-natural, recognized areas of contrasting color (light on carapace, dark on plastron) were produced (Hendrickson et al., 1981). This experiment is one of many that have used the surgical

Figure 1: A green turtle with a living tag. On the plastron the tag appears as a dark spot (or streak) and on the carapace, as a light spot (or streak). Photos: Julia Horrocks/BSTP.

exchange of small pieces of tissue between the carapace and plastron to create permanent marks. These marks, sometimes referred to as "living tags," are retained and increase in size as the animal grows to an adult (Balazs, 1999). The long term retention of such marks is debated. The patterns have been proven to remain throughout the life of the animal (Bell and Parsons, 2002), however, in older turtles the marks appear only as spots or streaks. As a result, differentiating these patterns and matching them to unique individuals is extremely difficult.

B. External Tags

Historically, tagging has been the single-most valuable activity in advancing our understanding of sea turtles and their conservation needs in relation to complex life cycles, reproductive migrations, slow growth rates, and delayed sexual maturation (Balazs, 1999). World-wide, many marine turtle research programs use external flipper tags as the method of choice in obtaining information on the life history of nesting females (Broderick et al., 1999). The most commonly used tags on sea turtles are made of metal or plastic that attach to the posterior edges of the flipper. These so called flipper tags, are actually cattle ear tags whose locking mechanisms and application methods have been modified to suit sea turtles.

1. Plastic Tags

Most plastic tags consist of two pieces that require a special applicator to snap the sides together. Once in place, it cannot be taken apart without destroying the tag (Balazs, 1999). Additional tools are sometimes required to pierce a hole in the flipper where the tag may then be applied. One of the advantages of plastic tags, such as Jumbo tags (made by Dalton Supplies Ltd.), is that they can be ordered in various colors, with letters and numbers embossed on both the internal and external surfaces of the tag's plate (see Figure 2) (Balazs, 1999). This allows for easy recognition of the tag and the possibility of recording the tag ID without the need to physically capture the turtle. However, as with all tags, researchers have reported varying levels of success using plastic tags. According to D.L. MacDonald and P. Dutton, after reviewing leatherback sea turtle tagging data in St. Croix from 1979-1992, they concluded that plastic tags had a very low retention rate, with none lasting over three years (Eckert and Eckert, 1989, MacDonald and Dutton, 1994). Gorham et al. (1999) found similar results with Inconel No. 681 having significantly better tag retention rates than Roto-tags on both Green and Loggerhead sea turtles in Florida. Plastic tags' low retention rate may be due to the fact that they are more vulnerable to increased wear, brittleness, and breakage, depending on the type of plastic and the characteristics of the marine environment where tagging occurred (Balazs, 1999). Another disadvantage of plastic tags, revealed in communication with Dr. Scott Eckert, is that in

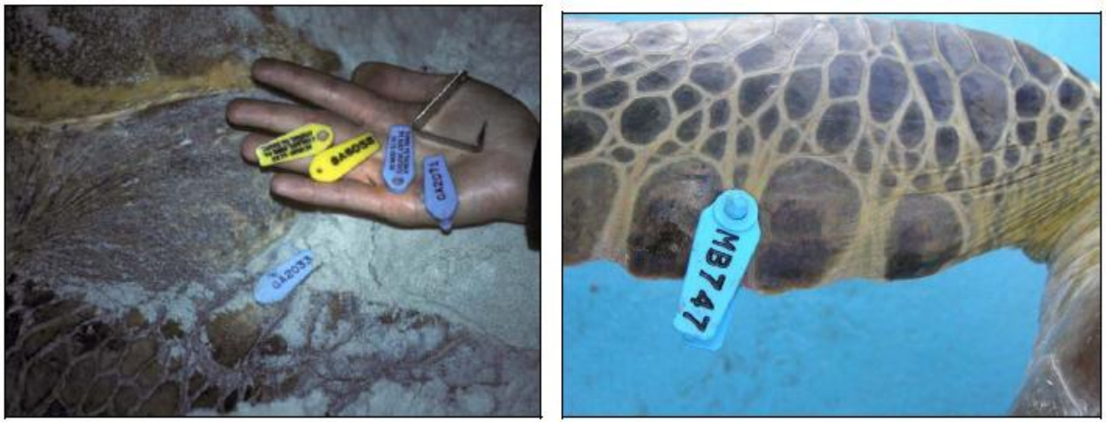


Figure 2: Size and shape of plastic "Jumbo" Rototags and their placement on (a) an adult female loggerhead (Photo: Scott Eckert/WIDECAST) and (b) a juvenile green turtle (Photo: Bermuda Turtle Project).

leatherbacks, they leave no discernable scar, and therefore no way to reveal if the animals had been tagged previously (MacDonald et al., 1994). Lastly, research on Black sea turtle populations in Baja, suggest that plastic roto-tags may contribute to sea turtle by-catch in a variety of fishing net types (Nichols et al., 1998). Their open-ended shape makes them more prove to entanglement in fishing nets, unlike metal tags which are completely closed. However, other studies have reported successes with plastic tagging protocols. Van Dam and Diez (1999) reported that plastic tags outperformed all other tag types (Monel, Inconel, and PIT) with no detectable tag loss (n=42) occurring in the four year period since commencing their application. No heavy bio-fouling of the plastic tags was recorded during the study. In addition, the researchers noted that the plastic tags (jumbo Roto-tags) did not suffer from the reported brittleness or loss of readability through abrasion that had been noted in Allflex plastic tags tested by Limpus (1992) and Alvarado et al. (1988). Green (1979) also found that plastic tags were superior to Monel metal tags on green turtles in the Galapagos as did Alvarado et al. (1988) for east Pacific black turtles. While many plastic tagging protocols have been replaced by metal tags, this method still persists in some areas, due to the tag's inexpensive nature and success with certain population of sea turtles.

2. Metal Tags

There are several types of metal tags which have been employed in sea turtle marking protocols. Metal tags are made primarily of titanium or of stainless steel alloys to reduce the potential for corrosion in sea water. The two most common materials are the nickel alloys Monel and Inconel, made by the National Band and Tag Company (NBTC) (Balazs, 1999). Inconel and Titanium tags were developed in the late 1970's, prior to which, Monel tags were used exclusively.

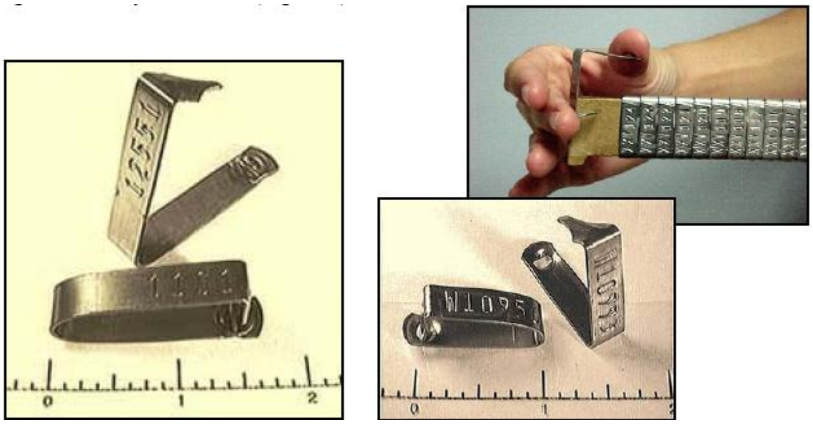


Figure 3: Size and shape of Monel tag style 49 and Inconel style 681, manufactured by National Band and Tag Company (http://nationalband.com). Tag card courtesy of NMFS-SEFSC.

The composition of Monel and Inconel alloys are similar; both are composed primarily of nickel (67% and 60% respectively). Their secondary materials differ slightly with Monel containing copper (31 %) and iron (2.5%) and Inconel containing chromium (23%) and iron (13%) (SPC, 2008). Both contain trace amounts of 5-6 other elements. The slight variations in their composition provide each type of tag with unique abilities. For example, Monel tags have intrinsic anti-fouling capabilities as a result of their copper-nickel content (WHOI, 1952). However, their composition also makes them more vulnerable to corrosion and pitting in extreme conditions (*e.g.* water with high salt content). Monel tags' highly variable rates of corrosion have been noted between geographic locations and on different turtles tagged at the same study site (Balazs, 1999). Despite this fact, Monel tags are still prevalent in tagging protocols because of their availability, relatively low cost, and metallurgical properties which make them easy to fashion into tags. Inconel tags have more resistance to corrosion as a result of their chromium content, an element typically added to alloys as an anti-corrosion agent (Jones, 1996). Titanium tags are equally superior at withstanding corrosion due to the element's ability to resist oxidation. However, the surfaces of titanium tags are prone to mass settlement of barnacles (*Chelonibia lepas*) (Parmenter, 1993). It is common to observe nesting turtles with titanium tags carrying high barnacle loads (solid masses up to 10 cm in diameter) (Parmenter, 1993). Such barnacle masses can cause tearing or cutting, ultimately resulting in the tag wearing through the flesh and leading to increased tag loss. While it is evident that there are advantages to each type of tag, overall, their retention remains relatively poor. Numerous studies have examined the problem of tag loss (Balazs, 1982; Henwood, 1986; Bjorndal et al., 1996), and the search for the ideal tag design and material has been elusive. Conflicting and highly variable assessments of tag loss abound in the literature. As a result, it is likely that the relative performance of different tags varies with the species of turtle, the size class of turtles within a species, and the various environments the tags are exposed (Gorham et al., 1998).

Contradictory results comparing Monel and Inconel tag retention rates are widespread in sea turtle literature. For example, Troeng et al. (2002), observed consistently lower retention between seasons for Inconel No. 681 tags (year 1998-1999) when compared with Monel No. 49 tags (year 1996-1997) for green turtles at Tortuguero, Costa Rica. Lower tag loss for Inconel No. 681 tags were attributed to the corrosive nature of Monel tags, and an easier to check locking mechanism for Inconel No. 19 tags. Inconel No. 681 tags have “through the hole” locking mechanisms, while the No.49 tags lock by bending the lock-tab of the tag through a thin metal stirrup. This latter style tag is often referred to as the “tamper-proof design” by many companies. Yet findings by Bjorndal et al., (1996) comparing Monel No. 49 and Inconel No. 681, on Green sea turtles at the same nesting beach concluded that between season (in 1989) probabilities of tag loss for the two types of tags were not significantly different. TA. Henwood’s (1986) tag retention results on Loggerheads offer more conflicting evidence. In research conducted with the National Marine Fisheries Service, a plot of the probability of tag loss indicates that the regression slope of the No. 681 tags (Inconel) was approximately double that of the No. 49 (Monel) tags (Henwood, 1986). McDonald and Dutton (1994) also had similar results in St.

Croix with leatherback sea turtles, noting that both titanium and Monel rear tags have consistently higher retention rates than both Inconel and Reise-type plastic tags. Results demonstrated that Monel flipper tag retention, particularly on the rear flippers, was fairly high for the first two or three years (85% and 67% respectively) but that it was quite low in subsequent years (less than 12% over five years) (McDonald and Dutton, 1994). There is little explanation for these trends especially given that Monel tags have higher corrosion rates. However, one must suspect that the increased complexity of the Monel locking mechanism may have contributed to its increased retention, or that corrosion was not a significant factor at these sites. Studies with Flatback sea turtles by Parmenter (1993) noted that for periods up to three years (the most frequent inter-nesting time interval) Monel tags outperformed titanium tags applied in the same position (17.2% loss vs. 29.8% loss). Over longer periods, the titanium had superior retention (at 8 yrs Monel: 90% loss vs. Titanium: 41.2% loss). Figure 4: Displays a summarization of important tag loss studies.

Study	Type of Tag	Species	3-Yr Loss Rate	4-Yr Loss Rate
Limpus (1992)	Monel	Green Turtles	0.875 +/- 0.229	0.591 +/- 0.205
Bjorndal (1996)	Monel	Green Turtles	0.201 +/- 0.033	0.354 +/- 0.114
McDonald et al. (1994)	Monel	Leatherback Turtles	~ 0.33	
Parmenter (1993)	Monel	Flatback Turtles	~ 0.172	
Balazs (1983)	Inconel	GreenTurtles	0.23 +/- 0.14	
Bjorndal (1996)	Inconel	Green Turtles	0.14 +/1 0.13	
Gorham et al. (1998)	Inconel	Green Turtles	~0.500	

In addition to between season tag losses, some researchers have investigated within season tag retention rates. While this trend has been studied to a far lesser degree, highly variable rates have also been reported. Most literature contends that the majority of tag loss occurs soon after tagging. Retention rates then increase while at sea but eventually decrease with time (Chevalier and Girondot, 2000). This time-dependent nature of tag retention has been called tag senescence (Nichols and Hines, 1993).

Several other factors affect tag retention besides the tag's construction materials. These can include the skill of the person applying the tag, the circumstances or environment of tagging, and the methods of application. All metal tags require a special applicator for proper attachment. Unlike plastic tags, pre-punching a slit or hole through the tissue of the flipper is usually not needed due to the self-piercing design of metal tags (Balazs, 1999). The application of metal tags can be done improperly. Researchers have remarked that the tags can incompletely seal or the point prematurely bend-over before passing through the hole or lock mechanism (Balazs, 1999). Incorrect settings of the No. 19 applicators have been shown to result in partial bending and locking of tags (Henwood, 1986). Malfunctions can also result from applicators that are rusted, clogged with sand or other debris, or are worn from heavy use. Less than optimal conditions can also affect tagging success. For example, tagging that occurs at night or aboard vessels can reduce the chance of adequate attachment, or make inspection of the tags difficult (Henwood, 1986). Tissue necrosis is another factor which undoubtedly contributes to differences in shedding rates. Metal tags that are attached too far into the flipper or skin can inhibit the full range of free movement of the tissue within the tag (Balazs, 1999). This can result in abrasion of the skin

Figure 5: A Monel style 49 metal flipper tag correctly loaded (left) and cinched (right) in the application pliers. Always align the base plate of the tag flat against the pliers. Note the tine bent over and completely through the stirrup. Source: http://www.nationalband.com/nbt.pdf

which may result in infection or the tag eventually tearing through the skin. Skin or tumors can often grow over the surface of the tag as well, obscuring the markers readability. Overall, the undetermined and sometimes high rates of tag loss and illegibility of tag numbers compromise the utility of tagging as a means of long-term identification.

C. Internal Tags

The development of Passive Integrated Transponder (PIT) tags in the 1980's and their use in sea turtles were seen as a solution to the tag loss problems experienced by externally mounted marking devices. PIT tags are electronic microchips that are encased in biocompatible glass. The glass casing protects the electronic components of the microprocessor while preventing tissue irritation (Gibbons and Andrews, 2004). Prior to the addition of the glass casing, tagged organisms encountered high rates of tag failure (*e.g.* 30 percent) (Fagerstone and Johns, 1987). These chips serve as permanent coded markers enabling long-term identification of individual animals. PIT tags used on sea turtles range in size between 10 to 14 millimeters in length and approximately 2 millimeters in diameter (Gibbons and Andrews, 2004). Their size roughly correlates to their transmitting strength, with larger sizes able to be read from greater distances (Balzas, 1999). About the size of a grain of rice, each microchip is injected using a 12-gauge needle or inserted by surgical incision under the animal's skin, usually into muscle or the

Figure 6: PIT tags shown for size comparison (left) and with its applicator (right). Source: http://biomark.com/RFID-tags.htm

body cavity (Whitfield and Andrews, 2004). While tagging locations vary by species, it is recommended that hard shelled sea turtles be injected into the triceps muscle complex of the turtle’s flipper, and that leatherbacks be injected into the turtle’s shoulder muscle (SEFSC, 2007). These injection sites are ideal because the muscle mass reduces the chance of the tag migrating to another location.

Figure 7: Insertion of a PIT tag into the triceps muscle complex of a juvenile sea turtles flipper (left) and into the recommended PIT tagging site for leatherback turtles (right). Photos: NOAA/SEFSC and Matthew Godfrey

PIT tags remain dormant within the subject until activated by a hand-held reader, with which the researcher scans the animal (Gibbons and Andrews, 2004). The reader generates a close-range, electromagnetic field that energizes the tag. Once energized, it responds by transmitting a unique alphanumeric code to the scanner via radio waves (Gibbons and Andrews, 2004). Detection of this code permits a tagged individual to be distinguished. A limiting factor to this protocol is that PIT tags and readers must function on the same radio frequency for the code to be communicated. Because turtles may be encountered over large ranges by a variety of observers, lack of compatibility in tag readers can be a problem (Eckert and Beggs, 2006). Currently, PIT tags are produced in many different transmitting frequencies (125 kHz, 128 kHz, 134.2 kHz, and 400 khz), though readers operating at 400 khz are being phased out (Balazs, 1999, Epperly et al., 2005). They are available from several companies including Avid (Norco, California, USA) Destron-Fearing (South St. Paul, Minnesota, USA) and Trovan Ltd. (Koln, Germany). Presently, most readers store tag numbers in memory for download to a computer, allowing researchers a secondary record in addition to written documentation. Standardization and compatibility of tags and readers within the industry will allow superior access to all individuals.

The use of PIT tags in biological wildlife studies began with fisheries studies to determine the efficacy of the method for measuring fish movement (Prentice and Park 1983). Research that used PIT tags for mark-recapture of animals in field situations expanded over the years to include studies on mammals (Brady et al. 2000), birds (Ballard et al. 2001), reptiles (Mills et al. 1995), amphibians (Perret and Joly 2002), and invertebrates (Pengilly and Watson 1994). The major advantage to PIT tagging is that due to the internal nature of the tag there is relatively low tag loss associated with this method. In addition, they offer virtually no negative impacts to the animal, provided that the organism is of sufficient body size, and show little evidence of influencing the growth rates, mating performance, predator susceptibility, or swimming speed of a PIT tagged animal (Gibbons & Andrews, 2004). Overall, PIT tags have the ability to provide valuable information on many aspects of sea turtles' life history including growth rates, migration patterns, and reproductive history.

In contrast to external markers, there is low variability and relatively high retention of PIT tags within all sea turtle species. According to McDonald and Dutton (1996) the retention rate of PIT tags within leatherback turtles during a four year window was close to one hundred percent. In studies with flatback turtles, Parmenter (1993) found that only 8% of PIT tags injected into the shoulder became unreadable after two years. Godley et al. (1999) analyzed the success of PIT tagging protocols in both green and loggerhead sea turtles. The proportion of PIT tags detected in green turtles was 93% (94 of 101), with 100% re-identification of individuals.

The proportion of PIT tags detected in loggerhead turtles was 89% (27 of 31) with 94% of loggerheads re-identified on the strength of PIT tags alone (Godley et al., 1999). Interestingly, Van Dam and Diez (1999) observed a steady decline in probability of PIT tag retention with time during their five year monitoring period. They noted that this trend seemed to indicate that PIT tags continued to be lost or failed through time, a phenomenon shared by external markers. Several other studies have also commented on the time-dependent relationship that PIT may exhibit. For example, it is possible that an increased failure rate may occur with time as a result of circuitry destruction. This can occur if the glass capsule succumbs to the physiological challenge of the surrounding tissue or breaks as a result of externally applied crushing forces as the turtle bumps into obstacles (Parmenter, 1993). Currently, most tag losses or failure have been identified to result from improper implantation (Gibbons and Andrews, 2004). Tag loss can occur soon after injection if the tag exits through the opening caused by the needle (e.g., Feldheim et al. 2002, 12.1 percent), but this can often be prevented by applying a topical substance on the injection wound to speed healing.

A major detractor associated with this marking protocol is the high expense of tagging equipment. The cost of each tag is between US $4-10 dollars (Balazs, 1999). PIT tag readers cost between US $800-1500 dollars (Gibbons and Andrews, 2004). Typically, more expensive readers have greater sensitivity in their ability to detect tags. These expenditures limit the expansion of this technique to large populations and in areas with limited financial means. An additional problem with PIT tagging in some species is that the tag can move within the body of the animal if applied improperly. This migration has been documented for many animal groups, including mammals (big brown bats; Barnard 1989), birds (turkey poults; Jackson and Bunger 1993), and reptiles (Camper and Dixon 1988). Such migration has also incurred in sea turtles

(Van Dam and Diez, 1999). Tag migration complicates identification because an investigator may not be able to find the tag and will interpret it as lost. This can be particularly problematic in larger animals (*e.g.* Leatherback sea turtles) for which detection is already compromised by the individual's bulk. The other major disadvantage (excluding cost and application effort) apparent in PIT marking is that they are only "visible" to the research team that applies them (Parmenter, 1993). Without a scanner (that matches the tag frequency) there is no way to visually identify individual turtles. This makes the technique is inappropriate for studies that address questions such as dispersal patterns where data acquisition relies heavily on tag reportage from the general public (Parmenter, 1993). However, when used in conjunction with other marking methods, PIT tags can markedly reduce the problem of identification loss.

IV. Photo-identification

As discussed previously, individual recognition may be achieved either by applying an artificial mark to an animal or by using an animal's natural markings (Whitehead et al., 2000). The former technique is pervasive in ecological studies and has been used on both marine and terrestrial species of vastly different sizes (*e.g.* Auckland et al., 2004; Watkins et al., 1993). However, several complications can arise from placing artificial marks on wild animals. The marking process itself may be disruptive (Bateson, 1977) due to the necessity of handling and restraining for mark application (Ogutu, et al., 2006). Marking an animal risks injury to the study subject (Mowat et al., 1994), can disrupt its activities and relationships to other individuals (Cuthill, 1991), potentially modifies behavior and physiology (Hindell et al., 1996), can affect survivorship (Daly et al., 1992), and is not practical with large populations. The loss of marks over time (Bradshaw et al., 2000) and the non-reporting of retrieved marks (Schwarz and Seber, 1999) can also compromise the estimation of demographic parameters. Additionally, a host of ethical and welfare issues can arise from the application of permanent or temporary marks (Wilson and McMahon, 2006; McMahon et al., 2006). Lastly, it is not always possible to mark (tag) certain types of animals, either because they are problematic to tag, or they are protected by law from disturbance (*e.g.* cetaceans, Hammond et al., 1990).

As an alternative, it is often possible to identify individual animals from variations in natural marks and (or) polymorphic color patterns (Frisch and Hobbs, 2007). Recognition by natural variation avoids many of the problems commonly associated with traditional mark-recapture studies. These techniques avoid handling individuals, and thus are convenient when animals are difficult to capture, or when non-invasive techniques are required. They are particularly advantageous in studies of threatened and endangered species (Forcada, Aguilar, 2000). The use of natural marks is often preferred over conventional tagging approaches because it is un-stressful, inexpensive, and reliable over a much longer period, features that are particularly suited to species that are of conservation interest (Van Tienhoven et al., 2007).

One of the more popular techniques of recording natural markings of an animal is photo-identification, as this allows storage of photos in a library for subsequent cross-matching. In order to utilize natural markers, photographs are taken in lieu of the application of “marks,” during mark-recapture studies. Identification is achieved by comparing photographs (of individual animals) that were taken at different points in time. An increasing number of long-term studies have shown that natural marks can be used to identify individuals of numerous long-lived species using a photographic file index (Kelly, 2001). In fact, natural body markings have been used to successfully identify individual animals in both terrestrial and aquatic environments from a range of species. Terrestrial studies have included geese (Lorzenz, 1937), zebras (Peterson, 1972), giraffes (Foster, 1966), African elephants (Douglas-Hamilton, 1973), lions

(Schaller, 1972), chimpanzees (Goodall, 1986), wild dogs (Frame et al., 1979), and cheetahs (Caro, 1994). Photo-identification has become the standard research method in the study of marine mammals (McConkey, 1999). For example, it has been used to successfully identify Sirenians (Beck and Reid, 1995; Langtimm et al., 1998), Pinnipeds (Hiby and Lovell, 1990; Yochem et al., 1990; McConkey, 1999; Forcada and Aguilar, 2000;), sharks (Anderson and Goldman, 1996; Arzoumanian et al., 2005; Castro and Rosa, 2005, Van Tienhoven et al., 2007) and over twenty seven species of cetaceans (Wursig and Wursig, 1977; Katona and Krauss, 1979; Bigg, 1982; Hammond et al., 1990; Jones, 1990; Mizroch et al., 1990, Wursig and Jefferson, 1990). The majority of these studies use manual categorization of fluke shape (in cetaceans), scars, or other natural markings, to match individuals in photographs (Hastings et al., 2001). The more prevalent natural markings in marine animals include features such as callosities and fluke patterns (Whitehead et al., 2000), as well as tears, marks, and notches in fins and tail flukes (Wursig and Jefferson, 1990; Dufault and Whitehead, 1995). In Leatherback sea turtles, the usage of photo-identification was first investigated by McDonald and Dutton (1996) at Sandy Point National Wildlife Refuge in St. Croix, US Virgin Islands. During the 1986-1995 field seasons, photographs were taken of the pink spot on the dorsal surface of the head of each adult, located above the pineal gland. This pioneering study established that the pineal spot is distinct enough to be used as a unique identifier, and that its appearance persists over at least four years (McDonald and Dutton, 1996).

Figure 8: The pineal or “pink” spot on an adult leatherback sea turtle (left) and an arrow indicating the position of the pink spot relative to the animals head. Photos: courtesy of Danielle Buonantony.

While many photo-identification catalogues have been in use for over two decades, most rely on visual matching of individuals. However, as the number of photos in a library increases beyond a person’s capacity to process the suite of candidate matches manually, the development of faster, automated techniques to compare new photographs is mandated (Mizroch et al., 1990). The availability of large data sets has rendered manual photo-identification unfeasible, motivating the development of computer-aided techniques for scanning photographic catalogues accurately and efficiently (Arzoumanian et al., 2005). Recent computer-aided efforts for marine mammals have focused on the characteristic shapes and coloring of fins and flukes; these include EUROPHLUKES (Evans, 2003), DARWIN (Wilkin et al., 1998), The Dolphin Project (Lapolla, 2005) and the Mid-Atlantic Bottlenose Dolphin Catalogue (Urian, 2005). The Dolphin Project and the Mid-Atlantic Bottlenose Dolphin Catalogue use the Finscan software (Hillman et al., 2003) to identify individuals by the shapes of their dorsal fins. Many automated matching algorithms have been tested with some success, generally, they are highly specialized and target a particular taxon or morphological feature of the species in question (e.g. dorsal fin shape) (Speed et al., 2007). As a result, the use of photo-identification has not expanded to the extent that exterior marking protocols have. This is especially true for sea turtle species for which automated matching has yet to be undertaken.

The remainder of this paper will examine the feasibility and reliability of using the leatherback's pineal spot as a unique identifier for photo-identification studies. It will also review the accuracy and validity of the automated photo-analysis tools that were developed for this study to match pineal spot images.

V. Pineal Spot Identification

Variable and often high rates of external tag loss coupled with deficiencies in PIT tagging protocols have prompted us to reinvestigate the feasibility of using photo-identification methods to recognize individual leatherbacks. Pioneering studies of pink spot photo-identification by McDonald and Dutton (1996) showed that the pineal spot of the leatherback is distinct enough to be used as a means of identification. These early studies also found that variation of the pink spot's appearance remains low over many years, and thus the pink spot can provide a unique identifier over the long term. However, several limitations associated with this initial study have constrained the expansion of photo-identification as a commonly accepted tool by leatherback researchers. Foremost was the limited sample size available for analysis. The research site at Sandy Point NWR, St. Croix offers 2.4 km of beach for nesting activities, but hosts a relatively small population (220 individuals over the 1986 - 1995 time frame of the McDonald and Dutton study). With a limited choice of possible candidates it was recognized that the potential variation of pink spot form within this population was restricted. Whether the pink spot would remain a unique identifier at larger sample sizes or within larger populations was unknown. Another limitation of the McDonald and Dutton study was the lack of quantitative methods to evaluate the pink spot form. Most pink spots were characterized manually. In other words, a single investigator would visually determine whether two pink spot photos were identical. While valid for small sample sizes, and when combined with other identifying characteristics, it would be more useful if the identification of an individual pink spot could be made by quantitative means. This would also allow for the automation of identification of individual turtles. No computer-matching programs are completely automated; all potential matches for any species must be inspected visually to determine if two animals are a true match, with the final decision resting on the researcher (Whitehead, 1990). However, the development of partially automated systems would decrease the time and effort necessary for identification as well as making identification more subjective.

VI. Methods

This project, sought to test whether individual turtles could be identified using the shape of the de-pigmented area on the dorsal surface of the head, known as the “pink spot”. Other studies have suggested that each turtle has a unique pink spot, but those studies were conducted on small populations and limited sample sizes may not have offered enough variation within the sample to be conclusive. Further, previous research used a qualitative evaluation scheme, and is thus difficult to replicate. Our objective was to apply a more rigorous evaluation of the pink spot method and assess whether the pink spot’s uniqueness would be retained when a large sample (400) was drawn from a very large nesting population (approx. 3,000 nesting annually) and whether identification could be automated using photo-recognition methods.

Data was collected over a two month period (May-July) in Trinidad, which is the southernmost Caribbean nation located off the coast of Venezuela. Trinidad offered the ideal research location for this study as it supports more than 80% of the total leatherback population nesting in the insular Caribbean Sea, with an estimated 6,000 turtles nesting annually (Fournillier and Eckert, 1999, Eckert, 2006). Matura Beach, located on the east coast of Trinidad, hosts over 150 turtles per night and has an annual nesting population of 3,000 – 5,000 individuals. The turtles used in the present experiments were encountered when they emerged to nest at this study site. The population at this site has been studied since 1992 by the local conservation group Nature Seekers. Included in their research is the identity tagging of nesting females using both flipper (Monel style 49) and Passive Integrated Transponder (PIT) microchip tags and monitoring their return to re-nest, within and between reproductive seasons. Nightly beach patrols are maintained to locate nesting females.

For this project photographs of each turtle's pineal spot was recorded at a standard height and angle (3 feet, directly overhead) using a 35mm digital camera (Canon Powershot SD800IS) mounted to a Samsonite tripod (model: Triton 1100). The tripod was located in front of the animal's head (approximately 2 – 5 cm away) so that the camera was positioned directly above the pink spot, and parallel to the ground (Figure 9).

Figure 9: Camera setup during data collection.

This method ensured a standard height +/- 2 inches, minimized photographic distortion, and was optimal in preventing glare from the flash obscuring the pink spot. A 4.5x magnification, achieved through a combination of optical and digital zoom, was utilized to focus solely on the pink spot without any other objects in the frame. In order to clearly see the "pink" spot, the head was cleared of sand using a soft bristle painter's brush. The camera was set to manual exposure with a 1 f-stop reduction less than metered as this resulted in optimal exposure at this close range. Included in the photo was an ID card with a unique code for each turtle made up of an amalgamation of the date (mm/dd/yy) and the number for each consecutive turtle encountered for the night. Thus, the first turtle recorded on June 1st, 2007 would be given a code: 0601071.

Any subsequent pictures of the same turtle would not include the ID card, but later all file names for a given animal were edited to include their unique ID.

All measurements and photographs were taken during the laying stage of the nesting process to minimize the effect of the data collection methodologies on the animal's typical nesting behavior. Photographs were cataloged daily, and information specific to each turtle was linked with each photo file in an access database. Because female leatherbacks nest several times during a nesting season, typically at 8-12 day intervals, I was able to obtain multiple photographs of the same females during re-encounters.

VII. Data Analysis:

Following the collection of the data, image matching methodologies were developed in collaboration with researchers at the Center for Mathematics and Computer Science in Amsterdam, Netherlands to process the pineal spot photographs. All pre-processing and analysis procedures were completed using the software program Matlab, a numerical computing environment which utilizes *C-code* to execute its functions.

Prior to analysis, all photographs went through a series of pre-processing steps to refine the quality of the image. The pink spot was isolated by cropping the image manually to direct our analysis and reduce the dimension of all images, thus speeding the subsequent processing. This selection procedure introduced some arbitrariness as it is not always obvious to what extent "satellite" spots and marks should be included. However, as long as the main salient parts are retained, the resulting classification is quite robust. Cropping is the only manual intervention that was required.

Images were smoothed with a Gaussian filter to remove superfluous detail, and converted from a jpeg to a rectangular data matrix. This conversion allowed each images' pixels to be transformed from a color to a gray-value, enhancing the contrast of within each image and easing the matching process. This allowed each images' pixels to be transformed from color into a gray value. This transformation was accomplished using the following formula, $K = R - 0.5(G + B)$, which converted each pixel (whose color is defined by the intensity value of the red (R), green (G), and blue (B) component) into a gray-scale value (K) from 0 to 1. The primary analysis tool used was the Scale Invariant Feature Transform (SIFT) algorithm (Lowe, 2004). This four stage process identifies distinctive features within the "pink" spot image, known as key-points. Key-points are local points of interest furnished with location, best fitting scale, and orientation with respect to the gradient. Along with each key-point comes a *key-point descriptor,* which is a feature vector summarizing local gradient information. Key-points are selected in a strict manner through a cascade filtering approach (Lowe, 2004). The features are defined such that they are invariant to image scaling and rotation, and to a considerable extent, invariant to changes in illumination and 3D camera viewpoint. Moreover, they are well localized in both spatial and frequency domains, reducing the probability of disruption by occlusion, clutter, or noise (Pauwels et al, 2008).

Scale-space extrema detection

The first stage of key-point detection identifies points which are extremas in scale-space. The image $I(x, y)$ is convolved with a difference of Gaussian function which computes the difference of two nearby scales (separated by a constant factor k).

$$D(x, y, \sigma) = (G(x, y, k\sigma) - G(x, y, \sigma)) * I(x, y)$$

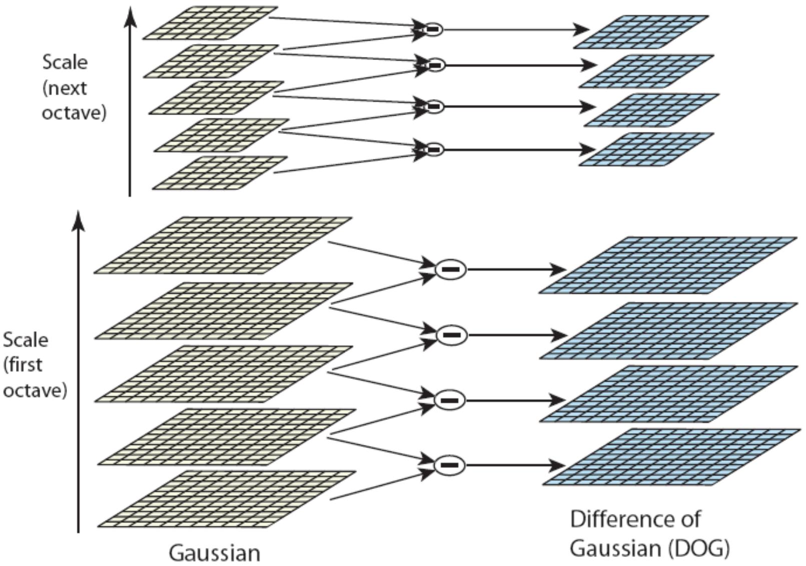


Figure 10: For each octave of scale space, the initial image is repeatedly convolved with Gaussians to produce a set of scale space images shown on the left. Adjacent Gaussian images are subtracted to produce the difference of Gaussian images on the right.

In order to detect the local maxima and minima of D(*x*, *y*, σ), each sample point is compared to eight neighbors in the current image and nine neighbors in the scale above and below (see Figure 11). It is only selected as a candidate key-point if it is an extrema within the scale-space (i.e. X is larger than all of these neighbors or smaller than all of them). This step tends to produce an excess number of candidate key-points, some of which are unstable. The next step in the

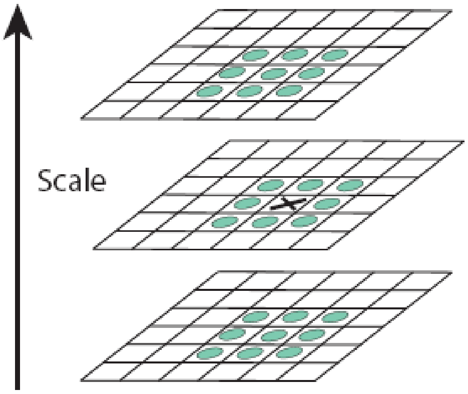


Figure 11: Maxima and minima of the difference of Gaussian images are detected by comparing a pixel (marked X) to its 26 neighbors in 3x3 regions at the current and adjacent scales (marked with circles).

algorithm is to perform a detailed fit to the nearby data for accurate location, scale, and ration of principal curvatures. This allows points to be rejected that have low contrast (are easily influence by noise) and which although having strong edge responses have poorly determine locations (Lowe, 1999).

Key-point localization

To accurately determine the candidate key-point's position, interpolation of the nearby data is done using the quadratic Taylor expansion of D(x, y. σ), with the candidate key-point as the origin and X= (x, y, σ) is the offset.

$$D(\mathbf{x}) = D + \frac{\partial D^T}{\partial \mathbf{x}}\mathbf{x} + \frac{1}{2}\mathbf{x}^T \frac{\partial^2 D}{\partial \mathbf{x}^2}\mathbf{x}$$

The expansion around the extremum helps to detect key-points with low contrast, which are deemed unstable and ultimately rejected. The difference of Gaussian function leads to candidate key-points which have strong responses along edges occurring in the original image I(x, y), even if they are unstable to small amounts of noise. In order to increase the stability of future matches, these points need to be eliminated. These points are detected when the principal curvature across the edge would be much larger than the principal curvature along it. Higher absolute differences between the two principal curvatures indicate the key-point is poorly localized and it is rejected (Lowe, 2004).

Orientation assignment

In the third step, each key-point is assigned one or more orientations based on local image gradient directions. This is the key step in achieving invariance to rotation as the key-point descriptor can be represented relative to this orientation. The magnitude and direction calculations for the gradient are done for every pixel in a neighboring region around the key-point (see Figure 12). An orientation histogram with 36 bins is formed, with each bin covering

10 degrees. Peaks in the histogram correspond to dominant orientations, which are then assigned to the key-point.

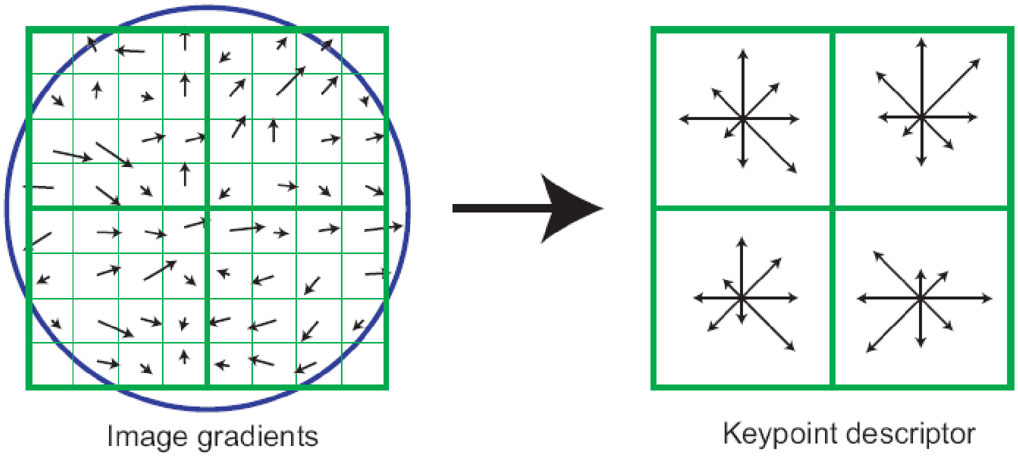


Figure 12: A key-point descriptor is created by first computing the gradient magnitude and orientation at each image sample point in a region around the key-point location, as shown on the left. The region is defined by Gaussian window, indicated by the overlaid circle. These samples are then accumulated into orientation histograms summarizing the contents over 4x4 subregions, as shown on the right, with the length of each arrow corresponding to the sum of the gradient magnitudes near that direction within the region.

Key-point descriptor

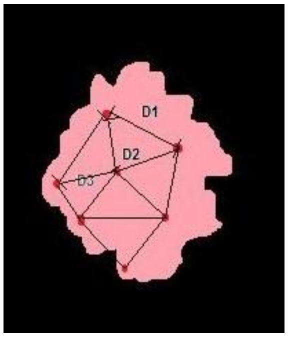
Lastly, descriptors assigned with a corresponding rotation to their key-point. The key-point descriptor is a 128-dimensional feature vector summarizing local gray value information. These descriptors are determined by sampling for local information around the key-point using a 4x4 matrix and multiplying that by the 8 directional variables of the pixels surrounding the key-point.

Defining a match

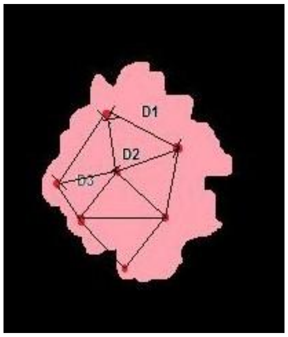
The similarity between two images is determined by the number of bi-directional (nb) matches, which are SIFT key-point matches that persist when the query and reference image are interchanged. To determine whether images are declared to be matching or non-matching, we defined two thresholds: an upper threshold $nb_{high} = 10$ and a lower threshold $nb_{low} = 3$. These thresholds were chosen in an effort to reduce the chance of overlooking a genuine match (i.e.

false negative), while maintaining a high degree of accuracy. The lower threshold, in particular, was derived from past research (Lowe, 2004) regarding the use of the SIFT algorithm for photo-identification which revealed that at least three features must be correctly matched from each image for reliable identification. Image pairs with greater than ten bi-directional matches (exceeding nb_{high}) were considered to be a high quality match and was declared thus in the database. Those with less than three bi-directional matches ($nb < nb_{low}$) were considered dissimilar and were not declared a match. Image pairs achieving a score between these two thresholds underwent secondary testing before a match could be determined.

As an additional test, the distances between all points within an image were calculated for both the query and reference image. Because the deformation between different images of the same pink spot are moderate (as a result of the SIFT), we can assume that if key-points in the query image are correctly matched to their counterparts in the reference image, the distance between any pair of key-points in the query image should be the same as the distance between the corresponding points in the reference image (see Figure 13 below).



e.g. Query Image



e.g. Reference Image

Thus regressing the distances in the reference image over the distance in the query image would define the quality of the match. If the regression fit was low this indicated the point matches were erroneous and the images were classified as non-matching. If the regression fit was satisfactory, we conclude that the point matches, though relatively few in numbers, showed a consistency that indicated a true similarity of the images. The image pairs were therefore classified as a potential match, and presented to the human expert for final validation or rejection.

VIII. Results

In this experiment 398 encounters were recorded which represented 385 individuals and 13 animals which were seen an additional time. At each encounter turtles were marked with flipper tags (Monel style #49), PIT tags, their dimensions were measured, and pineal spot photos were recorded at a standard height and angle. During the data collection, as each turtle was encountered a series of photographs were taken to ensure optimal image quality. While typically only one image was chosen to represent each animal, when multiple high-quality pineal spot photos were captured we sometimes retain more than one. As a result, the database comprises several individuals for which we have two quality images that were taken in quick succession (*i.e.* a few seconds to minutes apart). These pairs, called *alternate images,* were labeled to reflect that they were the same individual, but were given an additional variable (*i.e.* an A or B) to signify that they were separate versions of the "pink" spot. These *alternate images* were very valuable as they furnished us with a secondary dataset, besides the 13 turtles which were seen multiple times, to test the accuracy of the matching algorithms. This dataset was used to check minimal performance measures (*e.g.* whether the number of false negatives among these *alternate matches* was zero). In addition to these *alternate images,* we also obtained several repeat encounters, where the same individual was photographed on different nights, during different nesting attempts. These types of images were termed *replicate images,* and any resulting matches were termed *replicate matches*. In the current database, 13 such individuals were discovered based on current tagging methodologies (*i.e.* flipper and PIT tags). The matching algorithm, as outlined above, was challenged with identifying all true matches (*i.e.* both alternate and non-trivial matches), while simultaneously minimizing the number of images that need to be confirmed manually. Sample matches identified are depicted in Figure 14.

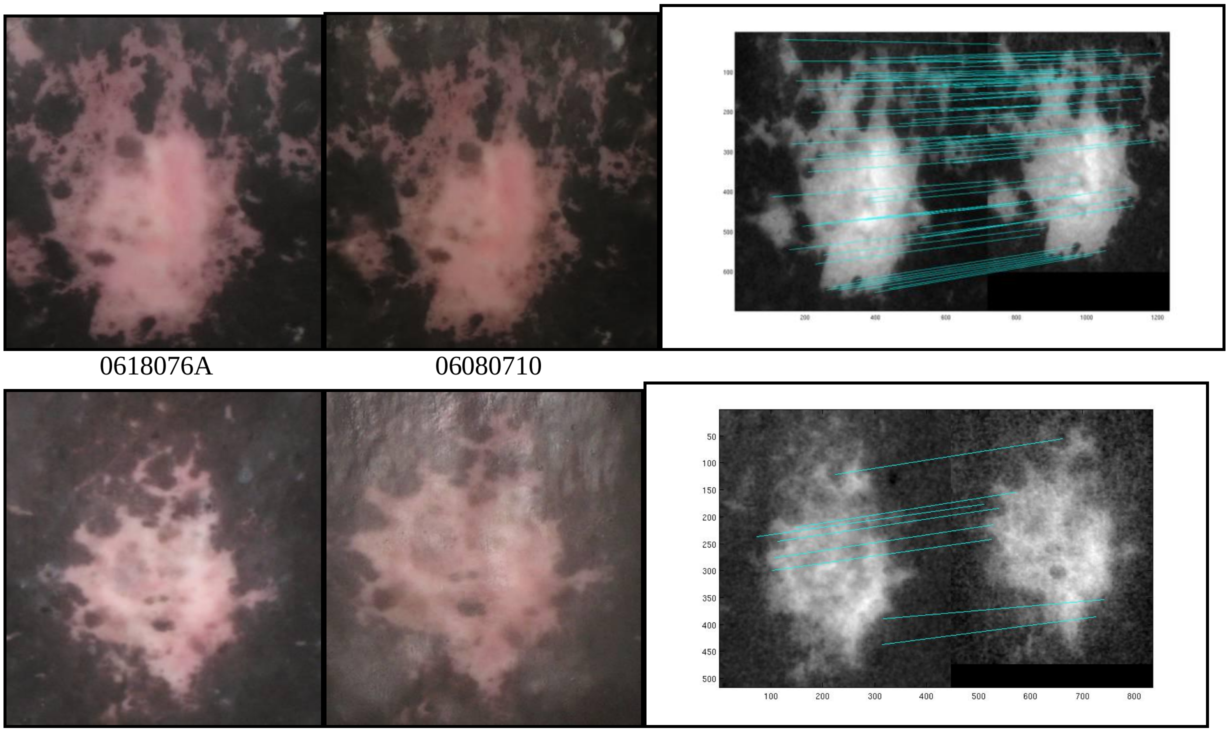


Figure 14: Image pairs identified by the SIFT algorithms as matches by the number of bi-directional key-point pairs within these images.

The algorithm analyzed 613 images, which represented 187,578 image pairs (613* 612÷2). All data was reviewed blindly by the software. The above outlined decision strategy exhibited one hundred percent accuracy at successfully detecting all true matches (*e.g.* replicate and alternate images), while simultaneously ensuring no false negatives occurred. Of all the image pairs analyzed, only 73 pairs were designated by the algorithm as potential matches (*i.e.* fell within the bi-directional matching thresholds) which required manual user confirmation. This amount represented less than 0.04% of all key-points. Once images were cropped, the time required to process an image and determine if a match existed with the database was approximately 5 seconds.

IX. Implications

The ability of the algorithms to define distinct points within the pineal spot for robust image matching proved the reliability of the pineal spot as a unique identifier. Furthermore, the accuracy that the system was able to attain demonstrated that identification could be successfully automated. These findings emphasize that photo-identification methodologies can be valuable alternatives to traditional tagging protocols. Furthermore, the technique outlined in this paper is able to overcome many of the flaws associated with traditional marking protocols for sea turtles, specifically leatherbacks.

Firstly, because this method does not require the capture of the animal for data collection, it is a much less-invasive process. Higher stress levels associated with handling and capture have been shown to adversely affect normal behavior in many sea turtle species. Gregory and Schmid (2001) reported that juvenile kemps ridley's exhibited acute handling stress during sexing research. In another study, Murphy (1985) described that disturbances can cause loggerhead turtles to shift their nesting beaches, delay egg laying, or select poor nesting sites. Lastly, research assessing the biological impacts of tagging and eco-tourism at Tortuguero National Park, indicated that a third fewer nests were laid by green turtles during times of increased disturbance from crowds and research activities. They also noticed an increase in the number of false crawls and non-nesting events (Jacobson and Figueroa Lopez, 1994). While precautions are taken to reduce the stress and disturbance associated with traditional marking protocols (*e.g.* timing of tag placement), the inherent fact that these tags pierce the animal's skin make them more traumatic to the individual. Furthermore, because photo-identification eliminates the need to capture or handle the animals for identification, this method can also be utilized on individuals encountered in the open seas or in habitats other than the nesting grounds.

In addition to being a more benign marking protocol, the use of natural markers excludes the issues associated with varying, but often high levels of tag loss. As described earlier, despite a variety of external tagging methods (*i.e.* Monel, Inconel, plastic tags), none have been found capable of long-term identification. While having varying levels of success, most are typically lost within 3-5 years of their application. Because this statistic corresponds to the average re-nesting period of many female sea turtles, there is the distinct probability that although being previously tagged a large proportion will return un-marked. The misclassification of remigrant turtles as first time nesters can lead to inflated and highly inaccurate population estimates. This complication limits our ability to measure important biological variables (*e.g.* reproductive output, longevity, and survival rates) at the level of individuals, thereby inhibiting the efficiency of management strategies.

Furthermore, the low cost associated with photo-identification is a distinct advantage over traditional tagging methods. The cost of buying tags and their associated applicators is an important consideration when choosing the appropriate method for each study site. Monel tags cost about US$300 per 1,000 tags while tags made of Inconel and titanium cost US$750 and US$2200 per 1,000 respectively (Balazs, 1999). The applicators for metal and plastic tags range from US$15-70 each (Balazs, 1999). These prices reflect the basic cost of the tag; additional fees are typically incurred to imprint supplementary information, such as an address where tags can be sent for recovery. Passive integrated transponder tags, while having greater accuracy, are also more expensive. Each PIT tag typically costs US$4-10, while their readers cost between US$300-1250 (Balazs, 1999).

Due to the deficiencies in both external and internal markers, study sites often use a combination of both methods to ensure accurate tagging records. For instance, the study site used in this project uses both Monel and PIT tags for their data collection, which greatly increases their expenditures. In communication with Dr. Eckert, National Brand and Tag gave a cost quotation of US$2,700 for 6,000 Monel style 49 tags and US$580 for 30 tag applicators. The cost of PIT tags was US$5.50each (US$16,500 for 3,000 tags), and US$490 for each Destron reader ($10,290 for 21 readers). The total cost of the marking methodologies needed for sufficient coverage at this nesting site is approximately US$30,000. In comparison, instituting photo-identification would represent a significant reduction in operating costs. The cost of the camera model used in this study and its accessories totaled ~US$400 each, representing an expense of ~US$6,000 to outfit a comparable number of tagging teams.

The inexpensive nature of this technique, coupled with the reliability of the natural mark has the implication to significantly improve the quality of data and increase the number of marking projects currently being undertaken. Moving forward, further studies are required to assure the accuracy of this method. Additional datasets are currently being examined to replicate our initial findings. These datasets are also being used to assess any variation that may occur within the pink spot form over time, which would reduce successful identification (i.e. migration of the spot, scarring, etc.). Ultimately, it is the goal of this research to release a free-standing or web-based software package which could be distributed to all leatherback researchers and conservationists.

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