ASSESSMENT OF RESIDUAL VASCULARIZATION OF THE LIMB AS A PROGNOSTIC FACTOR TO AVOID SEA TURTLE FLIPPER AMPUTATION

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ABSTRACT: Entanglement occurs when a marine turtle becomes trapped within anthropogenic materials such as debris or fishery gear, inducing strangulation of anatomical parts such as flippers or the neck, causing deep lacerations, maiming, amputation, or choking. Often, severely entangled flippers in captured or stranded turtles are removed surgically. Turtles with flipper impairment have difficulty in swimming, diving, and feeding. Our aim was to use color doppler ultrasound and multi-detector computer tomography to evaluate residual vascularisation or neovascularization in severely entangled flippers of loggerhead sea turtles (*Caretta caretta*) to assess viability of flippers, even in the absence of limb sensation ~~sensitivity~~. We studied 12 turtles with either unilateral (*n*=8) or bilateral (*n*=4) involvement. A total of 14 flippers were severely entangled whilst two flippers were spontaneously amputated. Only two out of the 14 entangled flippers had to be removed surgically. For 12 entangled flippers, after surgical curettage, the treatment protocol was based on

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Commentato [DMM5]: Author: "...were spontaneously." What?

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the use of a plant-derived commercial dressing. The animals were monitored and treated for 1 to 3 mo, until the soft tissue defects were completely healed by secondary intention. Interestingly, in the treated animals the healing flippers steadily recovered motility and ~~sensitivity~~sensation, restoring the complete functionality of the flipper. Vascularization of the limb was found to be critical to prevent amputation of entangled flippers, preserving the flipper and its functionality with conservative therapy, and avoiding amputation as much as possible. Our study showed that in cases of entanglement, amputation does not need to be performed immediately but can wait for non-viability to declare itself following conservative therapy and should be reserved as a last-resort treatment.

Key words: *Caretta caretta*, CT, entangled, flipper, ultrasound

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INTRODUCTION

Entanglement of marine turtle occurs when a marine turtle becomes entwined or trapped within anthropogenic materials, the majority of which are plastics accumulating on the surface of the seas, in the water column, and on the seabed (Thompson et al. 2004). Fishery gear, most notably monofilament and multifilament lines and nets, have been documented as the most significant source of entanglements for sea turtles, marine mammals, coastal and marine birds, and fish.

Marine turtles caught in fishing gear frequently suffer devastating injuries because the fishing lines and nets tighten around their flippers, and sometimes around their necks. Entanglement has the potential to cause serious wounds, leading to maiming, amputation, increased drag, restricted movement, or choking (Votier et al. 2011, Barreiros and Raykov 2014, Lawson et al. 2015).

The constriction of flipper tissues causes alteration of the vascularization with consequent ischemic areas that may progress to necrosis of soft tissues and bone segments. If the constriction is not quickly resolved it may cause deep lacerations of soft tissues, but also fractures, bone loss, and bone exposure; secondary infections are always present, and osteomyelitis is often observed. Spontaneous a~~A~~mputation of part or all of a forelimb are common sequelae, and affected turtles have greater difficulty diving and require greater expenditure of energy to swim. Severe flipper lacerations are usually treated with partial or complete surgical amputation of the affected limb (Church et al. 2009).

Commentato [DF9]: Yes, however it is a citation from Church et al 2009. We added "spontaneous" and "surgical"

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When evaluating a sea turtle affected by flipper entanglement, the veterinarian often decides to amputate the limb, which is often connected to the distal stump only by a shred of tissue. Sea turtles retain remarkable swimming and manoeuvring abilities after the loss of one flipper although swimming and diving ability or mobility are affected and eating and reproduction activities (i.e., nesting), may be impaired. Forelimb loss in males or hind limb loss in females can negatively affect reproductive capabilities of marine turtles (Church et al. 2009).

Our aim was to use color doppler ultrasound (CDU) and multi-detector computer tomography (MDCT) to evaluate residual vascularisation or neovascularization in severe entangled flippers of loggerheads, even in the absence of limb ~~sensitivity~~sensation, as a predictive factor to evaluate viability of flippers. When residual vascularization was present, we analysed the vessels involved in angiogenesis and monitored the healing process under a conservative therapy regimen. We provided valuable baseline information to improve the decision-making step for treatment of rescued entangled turtles, to understand if amputation of entangled flippers could be avoided, preserving their functionality with conservative therapy.

MATERIAL AND METHODS

Between June 2016 and May 2018, 12 loggerhead sea turtles (*Caretta caretta*) were referred to the Sea Turtle Clinic (STC) of the Department of Veterinary Medicine of University of Bari, Italy for flipper lesions caused by entanglement. The majority of the animals were found either drifting at sea or stranded on the coast.

Upon admission, physical examinations were performed of each turtle, including morphometrics (curved carapace length notch-to-tip, curved carapace width, and weight), as well as core body temperature measured with a temperature probe inserted about 10 cm into the cloaca. Blood samples were collected to measure plasma biochemical and hematological values in order to assess the general health status.

To evaluate the extent of tissue strangulation and tissue involvement, we carefully examined of the flipper affected by entanglement. The movement of the affected limb in and out of water, as well as turtle's ability to use the flipper for swimming were evaluated. An accurate flipper neurological examination was performed. A neurologic examination was performed to assess the functionality and sensitivity sensation of flippers. The withdrawal reflex and deep pain sensation were evoked by compressing the flipper margins with hemostatic forceps, starting from the cranial margin up to the entire caudal margin. The turtles underwent radiographic examination of the total body and of the flippers.

All the turtles were subjected to ultrasound (US) examinations, using a portable US device (MyLab Alpha, Esaote,), , Esaote SpA, via Melen, 77, 16152 Genova, Italy) with a linear multifrequency probe (10-14 MHz; -Esaote SL 1543) with longitudinal and transverse scans optimized for anatomical and vascularization study of the entangled flipper. When present, the contralateral flipper served as a healthy control.

We used bidirectional CDU to assess the blood flow in the affected flippers. We visualized the intestinal canal using coelomic US, to check for fishing lines ingested by turtles in the attempt to get free from entanglement (Franchini et al. 2018). During US examinations, the turtles were placed in ventral recumbency on a foam mattress.

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When better visualization of residual vasculature and assessment of the bone damage were necessary, a total-body MDCT was performed with a 16-slice MDCT scanner (Somatom Emotion, Siemens, Forchheim, Germany). The technical scan and reconstruction parameters were: 110 kVp, 180 mAs, 1-mm slice thickness, pitch of 0.8, 0.6 s/rotation, 0.5 mm reconstruction interval and standard (soft tissue) acquisition algorithm. Three-dimensional (3D) multi-planar reformatted (MPR), maximum intensity projection and volume-rendered images were obtained using a dedicated 3D software (Pixmeo OsiriX DICOM-viewer®, Pixmeo, CH-1233 Bernex, Switzerland). The computed tomography (CT) images were acquired before and after the manual injection of iodinate contrast medium (600 mg I/kg; Iopamigita® Insight Agents GmbHR, Heildeberg, Germany; Wyneken 2013) in the external jugular vein through a 22 gauge (in two turtles, all < 5kg) or an 18 gauge (in three turtles, all > 20 kg) intravenous (IV) catheter. For MDCT, all the turtles underwent general anesthesia with propofol (Propovet® Zoetis Italia srl) (5 mg/kg, IV) and self-adhesive elastic bandage to cover the eyes was applied.

In case of total absence of vascularization, the therapeutic protocol provided was the amputation of the entangled flipper (Di Bello et al. 2017). In the case of residual presence of vascularization, even when this was minimal, the flipper was subjected to surgical curettage and daily treatment with 1 Primary Wound Dressing® (Brevetto ENEA; Endospin Italia srl Phytoceuticals Ltd, CH-8008, Zurich, Switzerland~~Phytoceuticals~~) (1PWD) ~~, —)~~ and petroleum jelly as water proofing (Franchini et al. 2016).

All the surgical procedures on loggerheads were carried out after general anesthesia with 5mg/kg IV tramadol before 5 mg/kg IV propofol (Ciccarelli et al. 2018). After induction, the turtles were intubated and anesthesia was maintained with oxygen and sevoflurane 2-3% (SevoFlo, Esteve SpA, Milano, Italy,).

Before surgical curettage, precise wound cleansing of the flipper lesions and disinfection of the surgical areas was done with povidone-iodine. All the necrotic and infected tissues, including necrotic or loose bone fragments, were removed until bleeding from live tissue occurred. After

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surgical curettage, microbiological swabs were taken. Samples were inoculated on Columbia blood agar and McConkey agar (Liofilchem, Teramo, Italy) and incubated for 48 h at 37 C.

Following surgical curettage, the treatment protocol included rinsing of the wound with sterile saline solution and the exclusive use of the plant-derived dressing ~~1 Primary Wound Dressing® (~~1PWD, ~~Phytoceuticals AG)~~ applied on the wound surface daily until the end of treatment (Franchini et al. 2016). Every day, before the application of 1PWD, the wounds were cleaned with gauze and forceps. Turtles subjected to curettage were not treated with antibiotics. Tramadol (5 mg kg intramuscularly/d) was used as analgesic in the first 2 wk. To assess neovascularization, imaging follow-up (CDU and/or MDCT) was performed again after 1 mo.

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RESULTS

We subjected 12 severely entangled turtles to clinical evaluation and treatment. The animals presented with either unilateral (*n*=8) or bilateral (*n*=4) involvement. A total of 14 flippers were severely entangled and two flippers were spontaneously amputated. The right front flippers were involved in four turtles, the left front flippers in four turtles, both front flippers in three turtles, and in one turtle the right front flipper was missing at presentation (Figures 1A, 1B~~, 1C~~).Both caudal flippers were entangled in one turtle, with the absence of the right flipper (Table 1~~, Figure 1D~~).

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Curved carapace length notch-to-tip ranged from 22 to 71 cm (~~average~~ mean 41 cm, SD15.35), curved carapace width was 20.5 to 67 cm (~~average~~ mean 37.4 cm, SD 14.63), and weight was 1.35 to 35.0 kg (~~average~~ mean 11.1 kg, SD 11.86). On clinical evaluation, 10 of 12 loggerhead sea turtles were in a poor state of nutrition. The constriction was at the level of the humerus in all 11 turtles with cranial flipper entanglements. In the turtle with the caudal flipper entangled, the constriction was at the level of the femur level. All the lesions were very severe with deep lacerations and loss of soft tissues, bone exposure, necrosis, and severe secondary infections. In two turtles, there was a prior spontaneous amputation of the distal portion of the flipper.

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Please do not use single sentence paragraphs.

On neurologic examination, the withdrawal reflex and deep pain for nine front flippers were absent on the caudal (all or only proximal part) and distal margins, while for four front flippers the withdrawal reflex and deep pain were completely lost. The entangled caudal flipper did not show withdrawal reflex or deep pain along the margins, although minimal ~~sensitivity~~ sensation was retained on the ventral surface. Other specific neurological signs are reported in Table 1.

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Fractures were observed in radiographs of 11 of the 14 entangled flippers. Humeral fractures with severe bone loss were present in 10 flippers, (Figure 2). Of these, three turtles had lost the metacarpal bones and one of these also showed radial and ulnar fractures. The turtles with entanglement of the caudal flipper presented femoral fracture with severe bone loss (Table 1).

Upon CDU examination, in two front flippers the vessel flow was completely absent and the flippers were subsequently removed surgically. In 11 entangled front flippers, the flow of the brachial artery and part of the radial artery was clearly visible (Figure 3A), whilst flow in the ulnar artery was not identified. However, flow in small subcutaneous vessels was still visible proximally to constriction of those flippers. In seven of those turtles there was no blood flow detected in the most distal part of the flipper downstream of the constriction. In all the examined front flippers, the biceps muscle and extensor carpus appeared completely transected. In the entangled caudal flipper, the blood flow was still retained in the distal portion of the limb. After one month, the US follow up highlighted the presence of angiogenesis of superficial collateral vessels in the residual muscle and of subcutaneous tissues in all 12 entangled flippers (Figure 3B). In addition to external entanglements, two loggerheads had sonographic findings of intestinal linear foreign bodies with plication of the small intestine and colon, consistent with ingested line.

We examined five turtles with MDCT. The normal contralateral front flipper was present in three turtles and were used as controls. In the normal flippers, the vasculature was clearly identified in the small digital arteries that were tributaries of the radial and ulnar arteries, to the brachial artery, to the subclavian artery, up to the brachiocephalic trunk (Figure~~.~~ 4A). Upon MDCT analysis,

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Twelve flippers (in 10 turtles) with residual vascularization at imaging (CDU and MDCT) underwent surgical curettage and treatment with 1PWD and vaseline. All the soft tissue defects were completely healed by secondary intention and swelling was substantially resolved within 24-85 d, with a mean ~~n ±SD~~ of 34~~57~~ d, SD 19.12 ~~d (~~ (Figures 6-A, 6B) and during this time span, two to three surgical curettages were required for healing. The flippers steadily recovered almost complete motility (range 30-90 d, mean 63~~±~~ d, SD 18.34) and sensation (90-150 dys, mean 122 d; SD 18.85 Table 1).

Seven species of Gram-negative bacteria were isolated from deep lacerations of soft tissues of the entangled flippers. The bacteria were identified using the Api System (Biomérieux Corporate, Ponte a Ema, 50012 Bagno a Ripoli (FI), Italy) as *Pseudomonas putrefaciens*, *Acinetobacter calcoaceticus var. anitratus*, *Morganella morganii*, *Enterobacter cloacae*, *Aeromonas* spp., *Vibrio* spp., and *Citrobacter freundii*. Susceptibility to a panel of 13 antibiotics was determined by the disk diffusion method, following EUCAST guidelines (Ahasan et al. 2017). The isolates exhibited

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resistance to β-lactam antibiotics included imipenem, (7/7) followed by tetracyclines (6/7) and quinolones (5/7). Two strains were found to be resistant to all the antibiotics. In the two turtles with US evidence of long fishing line located inside the intestine (~~Brannian 1984, Gould et al. 1992, ~~Di Bello et al. 2006, Franchini et al. 2018) m~~. M~~ultiple enterotomies were necessary to remove the fishing line. Eleven of the 12 turtles were discharged from STC after about 8-16 wk and finally released into the sea after a rehabilitation period at the Sea Turtle Rescue Centers. One turtle, with spontaneous amputation of the right front flipper and a stump of the left front flipper, died at the Sea Turtle Rescue Center 8 wk after being discharged from STC.

DISCUSSION

Sea turtles are particularly vulnerable to entanglement due to their behaviour. Young sea turtles tend to seek shelter under floating objects to avoid predation (Carr 1987, Stevenson 2011). Our study included 11 animals that were presented with a total of 14 entangled flippers. Three flippers had been already lost when the animals arrived at the STC, due to complete (*n*=2) or partial (*n*=1) amputation. Entanglement occurs in all life stages (pelagic juveniles, neritic juveniles and adults) across all species (Hamann et al. 2011) but a high entanglement incidence is observed in the pelagic juvenile stage (Duncan et al. 2017). Hatchlings and young sea turtles are particularly susceptible to getting tangled in lost or discarded fishing gear or floating debris. Interestingly, 10 out of the 12 turtles hospitalized at STC were juvenile individuals (Curved carapace length - CCL was 23-46 cm and curved carapace width - CCW was 2243) and only two animals were subadult (median CCL was 68 cm and CCW was 60 cm), thus confirming that young animals are more prone to these problems. Sea turtles very frequently suffer severe injuries involving flippers and often the therapy is based on amputation.

More of these injuries involved the front flippers, possibly because they are longer and more exposed than the hind flippers, which are protected to some extent by the carapace (Di Bello et al.

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2017). Moreover, sea turtle forelimbs function as the major thrust generation and turtles with forelimb injuries have a greater likelihood to stranding. These data were confirmed in our study, as the flipper most frequently involved in entanglement was the front flipper at the level of the humerus (11/12 turtles). At this level, vascularization is provided by the brachial artery that runs antero-dorsally and by the axillary vein. Continuous constriction from debris or fishery gear causes ischemia and gangrenous necrosis of the affected flipper due to complete occlusion of arterial and venous vascular trunks. In addition to the involvement of tissues and vessels, this phenomenon affects the nerve trunks, causing loss of pain ~~sensitivity~~ sensation and reduction or loss of motility.

Usually entangled flippers display infected and necrotic margins, with bone exposure, significant loss of soft and bone tissues and either partial or complete absence of the withdrawal reflex. We used neurological examination of entangled flippers to determine the neurologic damage at the first physical assessment. All flippers showed the absence of deep pain and loss of the withdrawal reflex of the caudal margin. In some cases, the neurologic deficist affected the whole flipper.

The neurologic deficit was monitored during the hospitalization period. Surprisingly, we observed a progressive recovery of the deep pain and withdrawal reflex during or after complete healing of the soft tissue lacerations. In the freshwater D'Orbigny's slider (*Trachemys dorbignyi*) cell proliferation seems to form a cellular scaffold that supports the navigation of sprouting axonal branches, which cross the damaged spinal cord region (Rehermann et al. 2009). Accordingly, freshwater turtles would be a unique amniote model system occupying a peculiar intermediate place between anamniotes, with potential complete regenerating capabilities, and mammals, with highly restricted regenerating capabilities (Rehermann et al. 2011). Likewise, sea turtles have been shown to have astonishing capabilities to heal, if given proper supportive care and husbandry (Marin and Norton, 2019).

On the basis of our observational findings, we hypothesized that if vascularization is still preserved, even in a small proximal part of flipper, sea turtles can steadily regenerate blood vessels through neovascularization and anastomosis, promoting, at the same time, re-innervation of the limb. Indeed, in the 10 flippers subjected to conservative therapy, we could observe neovascularization after a follow-up imaging (CDU or MDCT) after 1 mo. Also, upon neurologic examination, we observed that flippers gradually recovered almost complete sensation in 3-5 mo. Rapid assessment of the vascularization status in entangled flippers was possible using CDU, suggesting the correct therapeutic approach. Moreover, coelomic US allowed for a thorough evaluation of the turtle bowel and development ofa correct surgical plan to remove any possible fishing lines that were not visible at radiographic examination. In entangled sea turtles, the presence of fishing lines ingested in the course of entanglement should always be considered as they may cause severe lesions, perforations, necrosis, and plication of the bowel loops, that can lead animals to death (Franchini et al. 2018). Ultrasound examination does not require anesthesia and the equipment is not as expensive as CT. On the other hand, MDCT allowed studying more in depth the course of new collateral vessels and the anastomosis. Angiography by MDCT can help to visualize large anatomical segments with greater spatial resolution and many more details of the vasculature. In our observations, MDCT angiography revealed two patterns of collateral circulation able to effectively restore flipper vascularization: deep, large collaterals vessels with a peri-humeral course and small, thin collateral vessels with a superficial subcutaneous course. Moreover, MDCT allowed studying in detail the bone fractures, even if incomplete, that were not well shown on radiographic examination and to evaluate the characteristics of the humeral bone loss. Despite trauma being a frequent cause of admission to sea turtle rehabilitation facilities, there are few published case studies and limited research on the healing processes of sea turtles (Mette et al. 2017), hampering the development of alternative therapeutic approaches to flipper amputation. During healing of muscle injuries, damaged myofibers are phagocytized by leukocytes, and progenitor cells (myosatellite or satellite cells) activate to form new fibers. More extensive destruction of tissue architecture by trauma or infection results in scarring or replacement of muscle by fibrous connective tissue. Infections or injuries involving bone tissue can lead to osteolysis and formation of reactive or woven bone and like other chelonians, sea turtles readily undergo avascular necrosis and formation of sequestra (Mette et al. 2017). The plant-derived dressing, 1PWD, may have a marked capacity to promote wound healing in mammals and sea turtles, even in cases of difficult management (Läuchli et al. 2012, Carnevali et al. 2014, Franchini et al. 2016). Although at clinical presentation the entangled lacerations were severe and infected, the soft tissues defects were completely healed by secondary intention after about 1-3 mo of treatment. Before the use of 1PWD, surgical curettages were performed until tissue bleeding, along with daily cleansing. These practices were considered helpful to promote healing.

Promotion of wound healing by 1PWD might be accounted for by the antimicrobial activity of the fatty acids contained in the spray formulation (Debois and Smith 2010), and the balanced, moist environment obtained by the semi-occlusive layer created by the oil (Sharman 2003). Therefore, although the tanks used to house turtles were a contaminated environment, the bacterial load in healing tissues remained under control (Franchini et al. 2016).

We performed bacteriological investigations from the swabs made after surgical curettage of flipper tissues. Seven bacterial isolates were made that were subsequently subjected to evaluation of their antibiotic resistance profile. Resistance appeared common to β-lactam antibiotics (7/7) and quinolones (5/7), with almost 30% of the strains resistant to all the antibiotics. Interestingly, all the isolates were also resistant to imipenem, an antibiotic that is registered for use in humans only. Antibiotic treatment was not contemplated in our therapeutic protocol. This 1PWD-based protocol for treatment of deep lacerations of entangled flippers can be useful in each phase of the healing process, avoiding the need to use antibiotics and cytotoxic disinfectants (~~Noble and Kent 1992,~~ Lloyd et al. 1999, Rantala et al. 2004~~, Paterson et al. 2005~~, Lee et al. 2006, Franchini et al. 2016).

A wound treatment protocol should be easy to apply and remove, prevent dehydration and infections, and promote wound healing. The association of 1PWD with vaseline, an occlusive dressing, was effective in protecting the wound, allowing us to hospitalize the turtles in water. Daily dressing changes were easy and quick, and the animals showed little or no reaction during

Commentato [DMM47]: Author: Six citations is too many for this single point. Please reduce to three or four.

Commentato [sc48R47]: We are reduced four citations treatments. Severe bone fractures of entangled flippers are quite common but limited information is available in the literature about treatment of long bone fractures in sea turtles. In these animals the limbs serve almost exclusively for swimming and, with exception of adult females during the brief period of nesting, the appendicular skeleton does not support the body weight as it does in terrestrial chelonians. This important difference should suggest a different prognostic evaluation in sea turtles because long bone fractures may not necessarily impair the flipper function long term. Indeed, it is not uncommon to observe an asymptomatic fracture undergoing spontaneous healing in sea turtles under observation for other reasons (Di Bello et al. 2017).

All the animals that we treated with a conservative approach regained almost normal mobility of flipper after 1 to 3 mo, despite the fact that the radiographs still showed the presence of a fracture. Injured flippers of sea turtles are often amputated at the hip or at the shoulder joints but when possible, it is important to treat flipper injuries so as to preserve at least partial function and avoid the need for complete amputation (Church et al. 2009). The primary objective should be to preserve as much of the limb as possible because the flipper can always be amputated later if the outcome of conservative treatment is poor (Church et al. 2009). An important consideration during assessment is that bilateral front flipper amputation or amputation of both flippers on the same side of the body would make turtles nonreleasable. Hind flipper amputation may be better tolerated by sea turtles, as caudal limbs are mainly used for steering while swimming, but bilateral amputation for a female would inevitably impair future nesting (Di Bello et al. 2017).

Absence of sensation and deep pain of the entangled flipper is indicative of neurologic damage. Unlike in mammals, this clinical sign in sea turtles is not sufficient to opt for limb amputation. In our study, the turtles that showed partially or totally absent flipper mobility, deep pain and sensation, but that retained even minimal vascularization of the entangled flipper, were all able to recover limb function and sensation. Injured sea turtles presenting with serious entanglement lesions similar to those treated in our study are not uncommon, raising the need for suitable, effective protocols for management of these complicated cases. The therapy with 1 PWD®

facilitated rapid healing of the lesions, preventing secondary infections without requiring systemic administration of antibiotics. Hundreds of entangled turtles are rescued and treated annually in rescue centers. With extensive rehabilitation efforts and given proper supportive care, these turtles may be eventually returned to the wild without the need to perform amputation.

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Commentato [DMM49]: Author: There seems to be many publications on the use of imaging on subjects related to yours.

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Commentato [DF50]: Yes we know item, we have to insert them?

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Commentato [DMM51]: Author: Please add the sponsoring orgainization following the title of the conference. Follow the location with the date of the conference.

ha formattato: Apice

ha formattato: Inglese (Stati Uniti)

ha formattato: Italiano (Italia)

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ha formattato: Inglese (Stati Uniti)

Codice campo modificato

Commentato [DMM52]: Author: Add month, year you accessed this site.

ha formattato: Inglese (Stati Uniti)

Table 1: Neurological, radiographic, ultrasound, CT scan findings, treatments, and follow ups in 12 loggerhead sea turtles (*Caretta caretta*) with flipper entanglement ~~during rehabilitation in Italy~~observed and treated at STC of~~of~~ University of Bari from June 2016 and May 2018.

Turtles	Flipper[a]	Findings				Treatment[f]	Days	
		Neurologic[b]	Radiographic[c]	Ultrasound[d]	MDCT[e]		Soft tissue healing	Return of sensation
1	FR	A, B2, C	F	L	–	PWD~~Cu+1PWD+V~~	45	110
2	FR	D	G1	M	–	AM	–	–
3	FL	A, B1	G2	L	–	PWD ~~Cu+1PWD+V~~	75	125
4	FR	A, B1	G1	K	–	PWD ~~Cu+1PWD+V~~	48	120
5	FL	A, B1	G2	P	Q, R	PWD ~~Cu+1PWD+V~~, CE	85	140
6	FR	A, B1 C	G2	L	Q, R	PWD ~~Cu+1PWD+V~~	75	130
6	FL	A, B1	F	N	G3, S	PWD ~~None~~	40	95
7	FR SA	–	–	–	–	none~~None~~	–	–
7	FL	D	G1, H, I	L	R	PWD ~~PCu+1PWD+V~~	65	135
8	FL	D	G3, I	L	R, S	PWD ~~Cu+1PWD+V~~	55	148
9	FL	D	G1 I	M	–	AM	–	–

Commentato [DMM53]: Author: Your original table had 12 lines of data, but you have 40 abbreviations. Please carefully check through this table to assure that I have not introduced any errors.

I changedthe abbreviations to upper case letters as lower case letters are used to indicate footnotes.

Commentato [DF54]: We are not a rehabilitation center but a Sea turtle hospital

Commentato [DMM55]: Author: Is this the days to when these things happened or the days that you chose to do the follow-up exams?

Commentato [DF56R55]: the days to when these things happened

Commentato [DMM57]: Author: As I noted in the text, "Vaseline" is a trademarked name. You cannot use it as an abbreviation.

Commentato [DMM58]: Author: This abbreviation is too long. Replace it with something simpler. You define it in the footnote, below.

Commentato [DMM59]: Author: Correct?

Commentato [DF60R59]: No was PWD tratment

Commentato [DMM61]: Author: This abbreviation is too long. Replace it with something simpler. You define it in the footnote, below.

10	CR SA	–	–	–	–	n~~N~~one	–	–
10	CL	E	J	O	T	PWD ~~Cu+1PWD+V~~	24	90
11	FR	A, B1	G1	K P	–	PWD ~~Cu+1PWD+~~, ~~V,~~ CE	38	108
12	FR	A, B2	G1	L	–	PWD~~Cu+1PW D+V~~	85	150
12	FL	A, B1	F	N	–	PWD ~~None~~	45	110

[a] F=front; C=caudal; R=right; L=left; SA=spontaneously amputated

[b] A=~~:~~ no withdrawal reflex or deep pain; B1=~~:~~ proximal part of the caudal margin; B2~~:~~ = entire caudal margin; C=~~:~~ distal margin; D=~~:~~ no withdrawal reflex or deep pain in all flipper; E=~~:~~ no withdrawal reflex or deep pain along the margins but l~~. L~~ight sensation of the ventral surface;

[c] F=~~:~~ no fracture; G1=~~:~~ complete diaphyseal fracture of the humerus; G2=~~:~~ complete and fragmentary diaphyseal fracture of the humerus; G3=~~:~~ incomplete fracture diaphyseal fracture of the humerus; H=~~:~~ fractures of radio and ulna; I=~~:~~ loss of metacarpal bones; J=~~:~~ complete diaphyseal fracture of the femur;

[d] K=~~:~~ no flow of caudal margin; L=~~:~~ no flow of caudal and distal margins; M=~~:~~ no flow in the entire flipper; N=~~:~~ no flow of proximal caudal margin; O=~~:~~ no flow in the dorsal margin, vascular calibre reduction on ventral surface;

[e] MDCT=multi-detector computer tomography P=~~:~~ fishline with plication of the small intestine and colon; Q=~~:~~ brachial artery interrupted and presence; R=~~:~~ presence of deep large collateral vessels surrounding and bypassing the bone fracture; S=~~:~~ presence of superficial collateral vessels; T=~~:~~ femoral artery was visible while the sciatic artery was interrupted.

[f] PWD =~~Cu+1PWD+V:~~ curettage and 1PWD =~~:~~ Primary Wound Dressing®+ Petroleum jelly~~Vaseline~~; AM = ~~:~~ amputation; CE = ~~:~~ coeliotomy and enterotomies to remove the fishing line.

Commentato [DMM62]: Author: This abbreviation is too long. Replace it with something simpler. You define it in the footnote, below.

Commentato [DMM63]: Author: Go through all of the foonotes and replace ": " with "=" after each abbreviation (as I did in the first row of footnotes).

Commentato [DMM64]: Author: To what does this statement apply?

Commentato [DF65R64]: applies to the letter E

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Figure legends

Figure 1. Entangled flippers at presentation (A) Juvenile loggerhead sea turtle (*Caretta caretta*) 5: clinical presentation of severe entanglement of the left front flipper~~.~~; (~~C~~B) ~~-~~Loggerhead sea turtle turtle 1: appearance of the wound on arrival.

Figure 2. Radiographic appearance of entangled fractured flipper. Loggerhead sea turtle (*Caretta caretta*) 5: dorsoventral radiographic view of the left front flipper showing complete humeral fractures with severe bone loss.

Figure 3. Color Doppler ultrasound examination and follow up of an entangled flipper in a ~~(A)~~ l~~L~~oggerhead sea turtle (*Caretta caretta*) ~~1~~(A-) Turtle 1~~:~~: ~~Color Doppler ultrasound examination.~~ The flow of blood in the brachial artery and in part of the radial artery was clearly visible, while flow in the ulnar artery was not identified; (B) Turtle 1: Color Doppler ultrasound follow up after 1 mo of treatment, showed the presence of angiogenesis of superficial collateral vessels in the residual muscle and subcutaneous tissues.

Figure 4. MDCT findings of the normal and entangled flippers (A) Loggerhead sea turtle (*Caretta caretta*) 8: Volume rendering MDCT angiography of the normal front flipper. (B) Loggerhead sea turtle 5: Volume rendering MDCT angiography of the left entangled flipper: the main trunk of the brachial artery was interrupted (arrows); (C) Loggerhead sea turtle 5: Volume rendering MDCT angiography follow up after 1 mo of treatment: deep large collaterals surrounding and bypassing the bone fracture with an evident peri-humeral course (arrow). (D) Oblique sagittal MPR MDCT angiography follow up after 1 mo of treatment: small, thin collateral vessels surrounding the narrowing of the flipper with a superficial subcutaneous course (arrows).

Figure 5. Dorsal MPR MDCT angiography of an entangled flipper (A) Loggerhead sea turtle (*Caretta caretta*) 10: Dorsal MPR MDCT angiography before treatment. (B) Loggerhead sea turtle (*Caretta caretta*) 10: Dorsal MPR MDCT angiography follow up after 25 d of treatment.

Commentato [DMM67]: Author: Figure (and table) legends must “stand alone” in that they each contain sufficient information (who, what, where, when, why, how) that the reader is not forced to continually refer back to the text to understand what is being presented. Please improve yours. Include the common and scientific names in each.

Begin each legend with a sentence presenting an overview (what are going to show), then identify the panels and state what each of them shows.

Commentato [DMM68]: Author: Two images of the same lesion are not needed.

Commentato [sc69R68]: ok

Figure 6. Follow ups of the entangled flippers after the conservative treatment (A) Loggerhead sea turtle (*Caretta caretta*) 5: Appearance of the wound at 70 d after the first surgical curettage, 1 PWD® and ~~vaseline~~ petroleum jelly therapy (B) Loggerhead sea turtle 1, Appearance of the wound at 45 days after the first surgical curettage, 1 PWD® and petroleum jelly ~~vaseline~~ therapy.