An Investigation into the use of Phototherapy for the treatment of Pododermatitis (Bumble-foot) in Coastal SeaBirds.

Submitted for The College Of Animal Physiotherapy Diploma in Animal Physiotherapy

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Definitions

Blue

A "Blue" is an African penguin chick that has lost its (down) feathers and is blue-grey in appearance, prior to the brown Juvenile markings.

LLLT

Low Level Laser Therapy (also known as laser biostimulation or laser therapy) is whereby wavelengths via low power light sources are applied to treat medical conditions athermally.

Wavelength

"A wavelength is the distance between one peak of electromagnetic energy and the next. They are expressed in nanometers (nm)..." (Porter, 1998).

Abstract

Pododermatitis (bumble-foot), a multifactorial condition, is common in a variety of sea bird species in rehabilitation environments and in housing situations. Various treatment options have been previously described and treatment is often required promptly as left untreated, pododermatitis can result in complex infections and bone erosion. The study was investigating largely, the African penguin, a species endemic to Southern Africa and Vulnerable to Extinction. The use of phototherapy was investigated for the treatment of this pododermatitis – a therapeutically beneficial for the treatment of various acute and chronic wounds, musculoskeletal conditions and bone injures without heating the tissues significantly. Five cases studies were chosen from a rehabilitation Centre. Phototherapy units can be designed to administer various chosen light wavelengths with different wavelengths being researched as having altered therapeutic benefits. The results were varied with the birds having altered causative factors to the development of this condition, in addition to altered lesion development. Through this study, future areas for research have been defined for more information to be obtained to the area of phototherapy for the healing of bumble-foot.

INTRODUCTION

Pododermatitis

Pododermatitis, also known as bumble-foot, can be defined as "a chronic inflammation, ulceration, and hyperkeratosis ... on one or more footpads" (Kahn & Line, 2005). Presenting in a variety of different species including felines and rodents, this contact dermatitis commonly occurs in various avian species whereby calluses and pressure sores occur on the ball of the foot or along the plantar surface of the digits (Vogelnest, 1994). The tarsal (hock) joint is occasionally affected.

Contributing factors

A number of factors contribute to the development of pododermatitis including penetration of the foot, trauma, obesity, increased sedentary behavior, unilateral limb problems caused by disease (degenerative or neurological etc), poor sanitation and prolonged standing on hard, abrasive surfaces often with excess moisture (pressure necrosis). More recent literature has indicated a link between pododermatitis and protein levels, dietary protein sources and the sex of the patient (Nagaraj, 2005).

Description of pododermatitis and the development thereof

Presenting unilaterally or bilaterally, pododermatitis is commonly characterised by accompanied lameness and inflammation (including heat and swelling) of the footpad due to a localised bacterial infection.

In the developing stages, a small area is affected and may be seen on the foot as a superficial lesion. At this stage, to prevent further development of the lesion, husbandry techniques should be altered (if pressure necrosis is the causative factor) to prevent lesion development. Certain topical agents may be applied.

Once bumble-foot has developed further and a deeper ulcer is present, the condition is more serious and often penetration has occurred. At this stage, with penetration creating a transfer path for bacteria, infection and subsequent sequelae are potential concerns and a systemic antibiotic is required. Without a suitable treatment regime, distortion of the foot and possible permanent damage can result.

Infections and sequelae

Once the epithelium has been compromised, secondary, opportunistic bacterial infections may set in, with lesions facilitating entry for bacteria. Often, Staphylococcus aureus (MRSA) is isolated from the lesion but many other micro-organisms have been implicated, for example, Brucella abortus has been isolated from a bumble-foot case in Germany (Boden, 2005). Viral infections have also been implicated with proliferative pododermatitis, with the presence of papovavirus-like particles being identified in gannet species (Daoust et al, 2000). Bacterial infections are common in superficial dermal layers with deeper soft tissue injury and infection, osteomyelitis and deep granulomas being less common. Involvement in deeper layers has the potential to cause possible erosion of the foot bones (Coles, 2007). Other chronic cases of infection may result in degenerative joint disease, tendonitis, lymphadenopathy and amyloid accumulations in organs such as the kidney, liver, spleen, pancreas and adrenal glands (Kahn & Line, 2005). Severe cases can result in septicaemia and amyloidosis, whereby at this stage amputation (at the sacrohumeral joint) is often the resultant response. Amputation may also be recommended for cases unresponsive to treatment, in order to slow the development of amyloidosis which can be fatal (Kahn & Line, 2005). Furthermore behaviourally, affected birds may experience a pain-induced reduction in appetite (Nagaraj, 2006) in addition to higher tonic mobility indicating an increased fear response.

Diagnosis

Early diagnosis of pododermatitis is vital for the prevention of complicated developments. Diagnosis of such condition is based upon clinical signs and bacterial cultures, preferably in conjunction with each other. Initial examination of such cases should include an evaluation of conformation and gait, behavior and an additional examination of the area for scab formation, epithelial thinning, lacerations, drainage, swelling, heat and or redness. Radiographic investigation is recommended to determine bone involvement and the extent thereof.

Treatment

The aims of various treatment regimes include protection of the affected area from further damage, systemic and local treatment and prevention (from injury re-formation). Treatment may include the administration of systemic antibiotics, commonly administered if swelling,

heat and drainage are present. The choice of antibiotics for treatment is dependent on the bacterial sensitivities as indicated by bacterial cultures undertaken during diagnosis. Local antibiotics may be used with or without Dimethyl Sulfoxide (DMSO). Worth noting is long-term (topical) exposure to DMSO may cause "a transient irritation in the skin" (Stashak, 1995). Surgical removal and cryotherapy may be used in addition to wrapping (in conjunction with topical agents and intermittent debridement). This wrapping may prove helpful to wound healing as additional padding is provided, minimising pressure on the wound. Similarly, "boots" constructed to support this idea have been used with some success. If the wound is not closed (surgically), "...the wound site should be kept moist to encourage granulation" (Ellis & Branch, 2005).

Some other less commonly used methods of treating bumble-foot have been explored with varying degrees of success. These include chamomile tea footbaths and (topical) banana skin tannin gel. (Reisfeld *et al*, 2010). Orthovoltage radiation therapy has been used for treatment, under anaesthesia, in select cases (Brobowski *et al*, 1995).

Basis for Research

The African Penguin, Spheniscus demersus

The African Penguin, *Spheniscus demersus*, is endemic to the Southern African coastline however; presently this species is considered according to the IUCN Red Data species listing. "Between 2001 and 2009, the population of the African penguins declined by 60%..." (Samm, 2010), a drop attributed to anthropogenic factors including oil spills, increased pollution, altered climatic conditions and reduced fish stocks. For conservation and educational purposes, the African Penguin is housed at more than five aquaria and zoological gardens in the Southern African region along with a dedicated aquatic bird rehabilitation centre based in Cape Town- the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB).

SANCCOB is a non-profit organisation dedicated to the rehabilitation of various sea bird species. With a mission aimed at the conservation and protection of seabirds, SANCCOB treat approximately 2000 oiled, injured and sick coastal birds releasing a majority of birds back into wild breeding populations each year. In addition to rehabilitation of African Penguins, another species commonly rehabilitated at the Centre is the Cape Gannet, *Morus*

capensis. "The African penguin population is proven to be 19% higher due to the efforts of SANCCOB than it would be in the absence of rehabilitation efforts" (Ryan, 2002). Pododermatitis formation in seabird species is common after a period of spending a period of time at the facility.

Photo therapy

Phototherapy has been explored for more than 30 years with few research studies on the topic being conducted in Africa. In 2006, only 0.17% of all phototherapy studies worldwide had been conducted on the continent (Hawkins & Abrahamse, 2007). Thus, the need for more research on phototherapy is important as Africa is ecologically dynamic with many endemic animals present and potentially species-specific health conditions.

Cells of the body may become compromised through injury, inflammation, trauma etc. Once compromised, the cell will not function to its optimum ability and cell function is impaired. Phototherapy works based on light energy being converted to biochemical energy, energizing compromised cells and increasing blood flow to the area by using coherent light of a highly beneficial wavelength. Through this process, cell death is reversed resulting in quicker, more optimised healing and recovery post- acute, or chronic, injury. A wavelength penetration depth of 5-10mm (with maximum penetration being dependent on the wavelength) can be achieved, thus deeper and superficial layers are stimulated (Walsh, 1997). "The effects of photon energy are cumulative, with repeated doses having more impact than a single, extended application. Depth of penetration is not determined by the treatment time" (Porter, 1998).

Phototherapy is considered as "...athermic with no appreciable heat transfer (<0.65°C) so the photonic energy is transferred directly to the target cells and thermal damage is avoided" (Hawkins & Abrahamse, 2007). The prevention of tissue heating makes phototherapy a useful tool when treating acute conditions as there is "no danger of aggravating the inflammatory phase or increasing intertissue bleeding" (Porter, 1998).

"A biostimulatory window has been found; the parameters of the window include wavelength (nm), power of intensity (mW), and energy density or dose (J/cm²). If powers and doses are

too low, they are ineffective; if too high, they are damaging." (Dyson, 2007). Thus, a "therapeutic window" exists where a plateau of biostimulatory effect is achieved as evident in Figure 1.

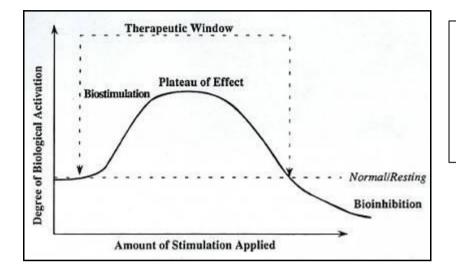


Figure 1: Change in direct and indirect biological activation compared with the amount of stimulation applied to the cells. (Photizo)

Positive effects of Infra Red Light Wavelengths

With infrared light wavelength therapy, chromophores (cytochrome C oxidase) in the cellular mitochondria function as photon acceptors for infrared light wavelengths (Dyson & Tafur, 2007).

Phototherapy has been used for pain relief, for trigger-point therapy or for the treatment of chronic and acute wounds, inflammatory conditions, joint degenerative disease and for various acute and chronic musculoskeletal injuries including fractures (refer to Appendix 1). Positive effects from phototherapy can be divided into various categories: primary, secondary and tertiary (see Figure 2).

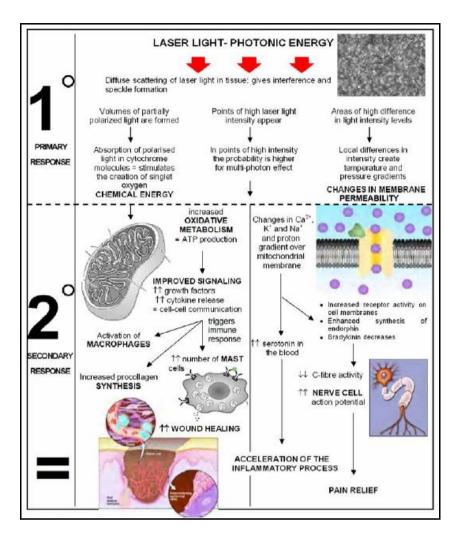


Figure 2: "The primary mechanisms related to the interaction between photons and molecules in tissue, while the secondary mechanisms relate to the effect of the chemical changes induced by the primary effects" (Hawkins and Abrahamse, 2007).

Primary effects include the direct effects from biostimulation: altered membrane permeability, improved signalling between mitochondria and nuclei and increased oxidative metabolism. Secondary effects are based upon altered cell functions and therefore are stimulating factors to tissue repair and healing. Tertiary effects are effects with systemic benefit. The secondary and tertiary benefits include:

- Accelerated wound healing (refer to Appendix 2)
- Accelerated cell growth by up to 150-200% (with exposure to infra-red light therapy) (Sussman & Bates-Jensen, 2007)
- Reduced scar tissue formation and softened scar tissue
- Increased (rate of) collagen synthesis.

- Stimulated ATP production to prevent cell necrosis and reverse cell death. This has been stated as one of the most important positive effects resulting from phototherapy
- Increased lymphatic system activity
- Promoted expulsion of pus and natural wound debridement
- Reduced nerve conduction speed and "excitability" and regeneration of peripheral nerves (Anders *et al*, 2004)
- Increased endorphin and serotonin levels
- Regeneration of mucosa and return to functional barrier (Nazaroglou *et al*, 2009)
- Enhanced autoimmune response in immune-compromised cases (Hawkins & Abrahamse, 2007)
- Increased bone stiffness by forming smaller, stronger bone calluses during the bone healing process (McGowan *et al*, 2007).

The application of phototherapy makes used of various wavelengths. Blue light application, with the emittance of wavelengths in the region of 400-500nm, has been found to "…exert a phototoxic effect on *Porphyromonas gingivalis* and *Fusobacterium nucleatum*" (Feurstein *et al*, 2004). Research has also shown blue light to be effective in the treatment of cutaneous and subcutaneous infections. (Eurekema *et al*, 2009). Infra- red phototherapy has not been found to affect bacterial activity and thus, the concurrent use of different wavelengths may be effective. The use of green light for therapeutic benefit is being researched (Porter, 1998).

Application of phototherapy

Phototherapy can be applied either as a continuous or a pulsed mode. Continuous application is whereby the wavelength (radiation) is applied at a constant rate during application. Some light therapy diodes have the potential to produce too much heating to the tissues; therefore the unit is run under pulsed application. During a pulsed application, the light output is interrupted at a cyclical interval or frequency as determined by the light therapy manufacturer, commonly with a duty cycle of 50%. Having the application of phototherapy,

either under pulsed for the full duration or for part of, "...appears to make the energy more acceptable to the cells" (Porter, 1998).

Of particular success with the treatment of phototherapy, is body surface wounds (such as those in the case of pododermatitis) which fail to heal due to factors such as poor circulation, infection, recurrent injury and poor healing processes. The phototherapy will trigger the repair response, decreasing recovery time. Photon therapy has the potential to stimulate the healing process from the first treatment (Porter, 1998).

Contra-Indications

With the safety of phototherapy well established, the minimal contra-indications that occur are either applicable to operator use or not applicable to the patient in this specific study. Caution, such as the use of phototherapy on acupuncture points or over the uterus in pregnant animals, does not apply. Much debate still surrounds presently as to whether cancer lesions should be treated with phototherapy, thus far no correlation has been fully established between cancer and photon energy. Drug administration should be discussed with the Veterinarian and caution should be taken not to expose the beam to the operator or patients' eyes as corneal damage is possible.

Photizo Light Therapy Device

The Photizo (Model 641) was the light therapy unit used. This unit produces light (only) of the desired/most effective/beneficial wavelengths. Additionally, a single-point (pen-style) 100mW/150mw diode and the 1200mW (cluster) diode are available for application, with maximum output achieved with minimal treatment time. Incorporating various protocols, the Photizo unit is manufactured with six different (pre-set) treatment protocols (Wounds 0-21days, Wounds 21+ days, Seroma Hematoma, Tissue Trauma, Abscess and Osteo). For the treatment of pododermatitis, the following protocols could be used: Wounds (case-dependent), Tissue Trauma and Abscess. The medical conditions indicated for treatment with the Photizo Unit are listed in Appendix 1.



Figure 3: The Photizo 641 Unit (<u>www.photizo.net</u>) used for the study, showing the six pre-set protocols and the two diodes: 1200mW diode (A) and the 100mW/150mW diode (B).

The Abscess protocol is advised, by Photizo, for the treatment of pododermatitis, this protocol is recommended for infectious conditions. However, due to varying stages of development of pododermatitis in this study, standardising the treatment would be optimum (to eliminate variables) and thus the Tissue Trauma protocol is the protocol of choice. Under infectious conditions, antibiotics will be co-administered as the light therapy will assist with absorption of the medication (Venter, *pers.comm.*). This treatment, as pre-set by Photizo, will entail an application of (initial) 30% pulsed and the following 70% of the treatment time, continuous. Wavelengths of 640nm (635nm) and 820nm (850nm) are administered with a dosage of 6 Joules per treatment (under the Tissue Trauma protocol). For 0-21 day wounds, 2 Joules are administered, 4 Joules for 21day+ Wounds and 8 Joules for Abscess (Venter,

pers.comm.). Additionally, it has been observed that lower dosages are superior in wound healing than higher dosages (Hawkins & Abrahamse, 2007) with higher dosages being recorded as inhibitory for cell proliferation (Nazaroglou *et al*, 2009).

Treatment was administered three times weekly with a minimum of 48 hours between treatments. All treatments lasted 6 minutes. All case studies and lesions were chronic and with chronic cases, treatments up to three times a week are considered maximum (Hawkins & Abrahamse, 2007). For all five case studies, both feet were treated, with the exception of "325" (unilateral lesions). Additionally, all treatments were undertaken with a minimum of 1 hour post- feed, taking into account handling directly post- feeding may result in regurgitation.

Variables and the Control Thereof

The medication administered to penguins affected with pododermatitis included a combination of Metacam (Meloxicam), Dimethyl Sulfoxide 90% (DMSO), Baytril (Enrofloxacin) and Kortiko (Dexamethazone). DMSO is an anti-inflammatory and analgesic with free radical scavenging properties (Roby & Southam, 1998). "Its greatest use is in reducing acute swelling of non-infectious origin" (Stashak, 1995). Additionally, due to potent solvent properties, DMSO acts as a "vehicle" carrying drugs (such as Metacam and Kortiko) through the skin to penetrate tissues. Metacam and Kortiko are both anti-inflammatory agents while Baytril functions as a broad- scale antibiotic.

Treatment may be administered systemically with Baytril being administered (bi-daily) at a dosage of 20mg/kg penguin body weight. In conjunction with the Baytril, Metacam may be administered, once daily, at a dose rate of 0.4mg/kg (day 1) then at a dosage of 0.2mg/kg for the following five days (this may be extended to a course of ten days, case- dependent). Topical treatment may also be administered in a 1:1:2 formula of Baytril (50mg/ml 5% injectable), Kortiko (2mg/ml injectable) and DMSO respectively (Parsons, *pers.comm*.).

Delivery of the photon dose can be considered as a variable as various factors can affect the amount of light penetrating the desired tissue. In order for maximum penetration to be achieved, the treatment head must be kept in close, stationary contact with the tissue and the light (beam) must be centred over the wound in a position to strike the tissue at a

perpendicular angle. For the study, the aim is to minimise scattering, delivering maximum dose per treatment. Additionally, "it is worth mentioning that, since LED emissions are divergent, the power output falls dramatically as the distance from the source to the power meter probe is increased..." (Al-Watban *et al*, 2003). For the purpose of this study, a maximum distance, between diode and wound, of 1mm was maintained. Additionally, due to the size of the phototherapy diode (150mW) and the size of the lesion, both the peripheral edges of the wound and the wound centre were treated simultaneously.

For the prevention of major photon scatter, a penguin box was designed to ensure maximum penetration, with minimised movement of the bird under least restraint (and least stress). This box design was constructed taking into account where and how long the bird would remain for the period of treatment, while minimising stress to the penguin, ensuring proper hygiene standards, maintaining adequate access to the feet for treatment (Figure 3) and while minimising scatter. The penguins appeared to be calmer in the box, when the lid was removed with light from above (Parsons, *pers.comm.*)



Figure 4: Penguin box (dorsal view) illustrating the design of the box. Note the (plastic) substrate covering the access hole at the bottom of the box (allowing for maximum access to the feet). The dimensions of the box are 36.5x31x54cm.

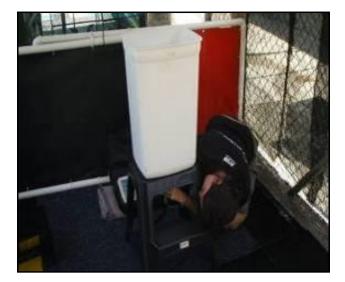


Figure 5: The penguin box was secured onto a (plastic) stand which enabled access to the feet, from below, for the phototherapy.



Figure 6: The Gannet was too large to fit into the penguin box and was therefore held for the duration of the treatment. The Gannet was receiving treatment to a chest wound in addition to phototherapy on the feet.

Photo supplied by Marlei Martins (SANCCOB).

Measuring the outcome

Wound healing was measured by: 1) actual measurements of the foot lesion (mm), 2) changes in healing measured as a percentage of the original wound size and 3) a grading of the wound. There are various grading systems that exist for the evaluation of bumble-foot. A three-point system was used in the Nagaraj study (2006) to assess the incidence and severity of pododermatitis based on the lesion size. An alternate scoring system was used by Cooper, defining a Type 1 lesion as mild and localised, being either degenerative or proliferative. A Type 2 lesion is more extensive and develops from a Type 1 lesion. At this stage, the lesion is defined as chronic with the presence of infection. Once the lesion is degenerative, with deeper layers affected, a Type 3 description is applied (Reidarson *et al*, 1999). The scores for this study were measured on a tri- weekly basis over the full investigation period. A scoring system was developed for the purpose of this study:

- 0 : no lesions.
- 1- : developing lesions or heat development
- 1+ : mild lesions of <1.5cm in size
- 2- : lesions of >1.5cm
- 2+: deep lesions of >1.5cm with heat and or swelling.
- 3 : lesions with oozing (pus) and altered behavior and gait

Haematological investigation as an indicator for systemic benefits

Changes in haematological values were observed over the period of the study in addition to comparisons with baseline haematological data. The purpose of measuring the outcome of changes in haematology is to determine the extent of the systemic effect of phototherapy. "Phototherapy also produces systemic effects on the vascular, immune, endocrine, and nervous systems, which permit it to affect deep targets indirectly without photons actually reaching these targets" (Dyson, 2007). Haematological data was collected on the same day each week (limiting the variables) - a day upon which phototherapy treatment did not happen and with phototherapy not having being applied for a minimum of 48 hours prior to blood collection.

The blood parameters recorded on a consistent basis, and used for investigation were Total Protein (TP) and Packed Cell Volume (PCV). Total Protein, a measure of the concentration of total protein, is a variable that is an indicator for hydration and inflammation. A decrease in the TP may be indicative of over-hydration, haemorrhage, protein deficiency and heart failure while an increase in the TP value may be an indicator for dehydration. Total Protein should remain within the acceptable range of 4–6 g/l; however Total Protein levels as low as 2g/l are occasionally recorded in weak birds and can be rectified by additional dietary protein. High Total Protein levels may be indicative of chronic stress and are usually recorded in chronic bumble foot cases (Parsons, *pers.comm.*).

Packed Cell Volume (PCV) is the proportion of blood volume occupied by red blood cells and is thus an indicator of anaemia. At SANCCOB, any PCV value recorded <38 results in early treatment for anaemia (Parsons, *pers.comm.*). The decrease in the PCV may be a result of haemorrhage, a breakdown of erythrocytes in circulation or a lack of erythrocyte production from the bone marrow. Conversely, an increased PCV value may be an indicator for dehydration (Thurman & Van der Elst, 1995).

Various other haematological investigations were done, predominantly for parasitic load assessment, however for this study, the data is not applicable.

Expected outcomes

It is expected that there should be an improvement in wound healing with increased granulation tissue visible within 1-4 days. A definite improvement, if the therapy is effective, should be notable within 10 days (Venter, *pers.comm.*). With regard to changes in blood parameters, for the investigation of systemic effect, it would be expected that the PCV value remains within non-anaemic range (>38) during the therapy application (study) period unless there is an underlying disease and or ailment. The TP value is expected, if high to drop down and ideally to remain closely within the acceptable range (4-6 g/l).

Case Studies

African Penguin, Number 220



Figure 7: African Penguin, Number 220

Description

Number "220", an African Penguin in the "Blue" life stage, weighs 3.5kg and is on a daily food limit in order to control the body weight. There is a healing wound on the dorsal side of the neck.

<u>History</u>

"220" was admitted to SANCCOB on the 11th August 2010 with an open neck wound. Treatment of this neck wound has included an antibiotic prescription of (oral) Amoxicillin and (topical) Flamizine with weekly wound debridement.

Bumblefoot development

On the 9th October 2010, the topical bumblefoot treatment was commenced with the bumblefoot having developed within a 6 week period of being at the Centre. On the 4th November 2010, phototherapy treatment commenced 3 weeks post-bumblefoot development.



Figure 8: Initial state of "220"s feet indicating bilateral developed bumblefoot.

Upon initial investigation of "220", the bumblefoot lesions presented bilaterally and were developed with a score of 1+ (Figure 8). As treatment progressed, the wound healing was noted as minimal (Table 2).

	Wound Size (mean)	Percentage of Original Wound Size	Score
Commencement of study	13mm	100%	1+
Post- Treatment 1	12mm	96%	1+
Post- Treatment 2	12mm	94%	1+
Post- Treatment 3	12mm	92%	1+
Post- Treatment 4	12mm	92%	1+
Post- Treatment 5	12mm	92%	1+

Table 1: Changes in the wound size and bumblefoot classification, for "220".



Figure 9: Treatment session 2 with "220".



Figure 10: Treatment session 4 with "220".



Figure 11: Treatment session 5 with "220".



Figure 12: Treatment session 6 with "220" (final treatment pre-release).

<u>Haematology</u>

The haematology values for "220" indicated a PCV that remained within range (>38) until the 8 November when there was a drop from 43 to 34. The Total Protein levels however remained constant and within slightly elevated levels of 5.8-8 when compared with acceptable ranges. Preliminary anaemia treatment commenced.

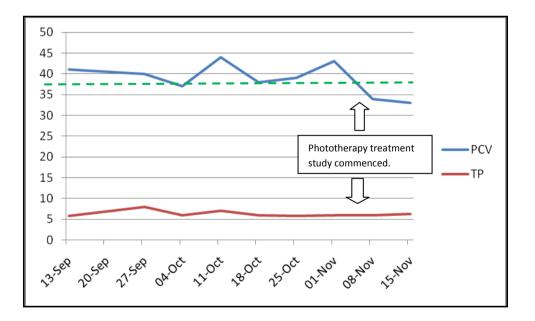


Figure 13: Changes in haematological parameters for "220" since arrival at SANCCOB on the 11 August 2010. The acceptable range for the PCV is indicated (---).

African Penguin, Number 314

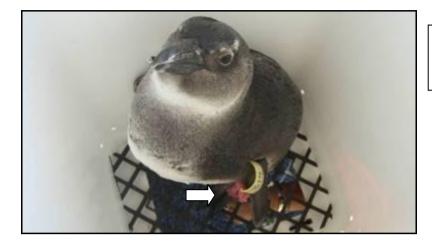


Figure 14: African Penguin, Number 314

Description

Number 314, a "Blue" African penguin is identifiable at the Centre by a piece of red fabric around the left flipper for easy identification of the damaged flipper. "314" weighs 3.4kg.

<u>History</u>

"314" arrived at SANCCOB on the 25th September 2010 with an injury sustained to the left flipper at the "elbow" joint. "314" was admistered an oral 10-day course of Baytril and additionally is being supplemented with zinc (effective 22 October) due to the condition of the feathers. Supplemented zinc has the potential to aid with the growth of down feathers (Parsons, *pers.comm*).

Wound history

On the 1st October, "314" was observed not using the left flipper. On the 4th October, the open wound on the left flipper indicated a poor prognosis for release. The flipper was swollen on the 8th October, with healing of the wound on the 19th October and subsequent stability of the "elbow".

Bumblefoot development

On the 2nd November 2010, the topical formula for bumblefoot treatment was commenced as bilateral bumblefoot was noted in the early stages of development (bilateral lesions were of

similar size). Bumblefoot development was noted 5-6 weeks post arrival at the Centre. Phototherapy was commenced 48 hours after topical treatment commenced.



Figure 15: "314" on initial investigation. The bumblefoot are presenting bilaterally and are still in developing stages.

With "314", the bilateral lesions, noted as developing, have not progressed further although minimal improvement in depth or size was recorded. Of note is the lesions develop along a line from the original lesion to the "toe" (bilaterally). This can be indicative of possible gait abnormality as the pressure lesions lend towards a "toe-in" gait.

	Wound Size (mean)	Percentage of Original Wound Size	Score
Commencement of study	6mm	100%	1-
Post- Treatment 1	6mm	100%	1-
Post- Treatment 2	6mm	95%	1-
Post- Treatment 3	6mm	95%	1-
Post- Treatment 4	6mm	90%	1-
Post- Treatment 5	6mm	90%	1-
Final evaluation	6mm	90%	1-

Table 2: Changes in the wound size and bumblefoot classification for "314".



Figure 16: The lesions on the feet of "314" after one treatment session.



Figure 17: The lesions on the feet of "314" after three treatment sessions.



Figure 18: The lesions on the feet of "314" after four treatment sessions.



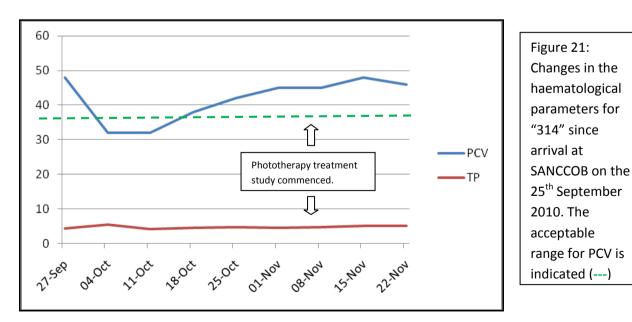
Figure 19: The lesions on the feet of "314" after five treatment sessions.



Figure 20: The lesions on the feet of "314" three weeks after phototherapy treatment commenced.

Haematology

The haematological parameters for "314" since arrival at SANCCOB indicated a drop in PCV down to 32, a week after arrival at the Centre. The PCV value then remained low returning to within acceptable range on the 18th October. Total Protein remained within acceptable ranges.



African Penguin, Robyn



Figure 22: Robyn, an adult African Penguin residing at SANCCOB.

Description

Robyn is an Adult Resident bird at SANCCOB with visual impairment in the right eye. The affected eye presents as cloudy in appearance. Robyn weighs 4.0kg.

<u>History</u>

Robyn arrived at SANCCOB on the 11th May 2010, from Robben Island. Upon arrival, Robyn presented with a "chesty" sounding respiratory tract and ulcers in the mouth. Robyn was moved from the rehabilitation pens to the resident area, Home Pen, on the 18th June 2010. Robyn has since settled and is now free feeding (unassisted), has moulted and is pairing up with Milo, another of the Resident African penguins.

Bumblefoot history

Presently, Robyn does have bumblefoot although the topical bumble-foot formula has not been prescribed. The bumblefoot is presenting unilaterally (right foot) with a well developed, large deep lesion. The left foot does appear callused with possible evidence of a healed lesion. Phototherapy commenced on the 4th November 2010. For the resident birds at SANCCOB, it is uncommon for bumblefoot to develop due to the different substrate when compared with the temporary holding pens. The behavior of Robyn however, has previously been largely sedentary, contributing to pressure on the feet.



Figure 23: The bumblefoot on Robyn's right foot on initial day of treatment. The bumblefoot presented unilaterally. There did appear to be a callus on the other foot.

After Treatment 1 (see Table 5), even though the wound size remained the same (diameter), it was clear at the start of treatment session 2 that there was an alteration of lesion depth with signs of healing from deeper layers. From the second session to the session on the 16th November (9 days later), minimal improvement was seen. On the 16th November, the day of treatment number 5, a change in the periphery of the wound was noted and there was a distinct improvement in the wound healing. The periphery of the wound indicated a change in colour from yellow to white and the actual lesion size was 25 % smaller than the original size.

	Wound Size	Percentage of Original Wound Size	Score
Commencement of study	9mm	100%	1+
Post- Treatment 1	9mm	90%	1+
Post- Treatment 2	9mm	90%	1+
Post- Treatment 3	9mm	80%	1+
Post- Treatment 4	6mm	75%	1+
Post- Treatment 5	6mm	75%	1+
Final evaluation	6mm	75%	1+

Table 3: Changes in wound size and bumblefoot classification for Robyn.



Figure 24: The bumblefoot on Robyn's right foot is appearing smoother in texture with changes in lesion depth although minimal changes in size. This image was taken after three treatments.



Figure 25: The bumblefoot on Robyn's right foot from treatment three to four indicates a change in size and texture.



Figure 26: The bumblefoot on Robyn's right foot is decreasing in size and is about 20% smaller and visible as contracting.



Figure 27: Robyn's feet three weeks postphototherapy treatment commencement.

Haematology

Haematological assessments for Robyn were undertaken since her arrival at SANCCOB on the 11th May 2010 but due to Robyn being a Resident bird blood assessments are not completed weekly. The blood data available was for the period 11th May to 2nd August 2010 and thus, even though baselines could be evaluated, no correlation could be made before and after the phototherapy study commenced. Therefore, Robyn's blood data was not available for comparison of systemic effect from phototherapy treatment. The data for Robyn has not been included here as is the data is not applicable for the purpose of haematological investigation (and systemic effect investigation). The data for Robyn has been included in Appendix 3 for review (and for possible future research).

African Penguin, Number "325"



Figure 28: African Penguin Number 325. Note the altered foot position.

Description

Number "325" is a juvenile African Penguin, weighing 3.0kg. "325" has previously sustained trauma and musculoskeletal damage to the left limb. The extent of damage and origin (soft tissue or bone) has not been confirmed. When swimming, "325" does not use the left limb and the leg hangs limp (drags) behind the body. In some cases with small animals with neurological impairment, the affected limb use in-water is much altered from limb use when weight-bearing. The non-use of the limb, for "325", is possibly linked to musculoskeletal and neurological implications caused by trauma. No radiographs have been undertaken thus full confirmation of such is not possible.

<u>History</u>

Number "325" arrived at SANCCOB on the 12th October 2010 with a fractured left flipper, an altered gait (due to wounds on the feet, especially the left foot) and in a state of "arrested moult". "325" has been prescribed and administered an antibiotics (Synulox) course with additional (supportive) wound debridement. Range-of-motion exercises were undertaken for a 2 week period.

Bumblefoot history

Topical bumblefoot treatment was commenced on the 2nd November 2010 with phototherapy commencing on the 4th November 2010. This was a period of 3 weeks post arrival at the Centre. An interesting case, "325" has pressure sores presenting on the dorsal side of the left foot due to the altered gait- "325" walks with the dorsal side of the foot ventral and thus in contact with the ground.



Figure 29: The left foot of "325" was affected with bumblefoot (unilateral), with the pressure sores presenting on the dorsal side of the foot, due to the altered foot position. Table 4: Changes in wound size and bumblefoot classification for "325" with development of the study

	Wound Size	Percentage of Original Wound Size	Score
Commencement of study	20mm	100%	2+
Post- Treatment 1	19mm	95%	2+
Post- Treatment 2	19mm	90%	2+
Post- Treatment 3	18mm	90%	2-
Post- Treatment 4	15mm	75%	2-
Post- Treatment 5	15mm	70%	2-
Final evaluation	14mm	60%	2-



Figure 30: The affected left foot of "325" after one treatment session.

On the 11th November, staff at SANCCOB noted that there was an improvement in the wound healing of "325". The measurements taken revealed no change in lesion size however on the 16th November a decline in wound size was recorded along with a change in depth of the wound visible and a change in colour of the healing tissue (from yellow, to pink).



Figure 31: The affected left foot of "325" after three treatment sessions. It was at this session the SANCCOB staff noted a visible improvement.



Figure 32: The affected left foot of "325" after four treatment sessions. It was at this session that measurements confirmed wound improving.



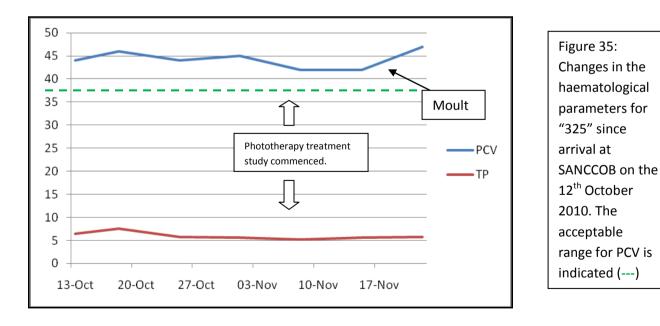
Figure 33: The affected left foot of "325" after five treatment sessions. A colour change in the healing tissue, along with a change in depth of the wound is now prominent.



Figure 34: The large lesion on the left foot of "325". A scab has formed in the centre of the wound.

Haematology

The haematological parameters (PCV and TP) for "325" remained consistent across the period of 13 October to 15 November. The PCV value exceeded 38 at all times and the Total Protein remained within the range of 5.2 - 7.6g/l. The Total Protein although a little higher than acceptable ranges (4 – 6g/l) was relatively stable. Of interest is on the 18th October, both the PCV value and the TP value were at their highest. "325" commenced a moult at the end of the phototherapy study.





Cape Gannet, Morus capensis, Super-Duper Number One

Figure 36: Super Duper Number One, an adult Cape Gannet, *Morus capensis*

Description

Super Duper Number One is an adult Cape Gannet with an extensive chest wound. The present weight for this bird is 3.12kg.

<u>History</u>

Super Duper Number One, arrived at SANCCOB on the 27th September 2010, in a weak condition, with a chest wound which appeared deep and extensive with possible penetration to the chest wall musculature. Super Duper Number One has been administered with a combination of antibiotics including Baytril and Amoclan (both oral) and topical Flamizine has been applied to the wound.

Bumblefoot history

On the 26th October 2010, the SANCCOB staff noted that Super Duper Number One's feet (bilateral) were warm to the touch and it was noted that inflammatory changes in the feet were forming with possible bumblefoot lesions to follow. Historically, bumblefoot lesions in gannets have developed with an increase in tissue temperature. Bumblefoot does not present in gannets as that of penguins with bumble-foot in gannets responding in altered ways to bumble-foot treatment when compared with penguins (Parsons, *pers.comm.*). This altered response to treatment could be due to the difference in gait of gannets when compared with penguins. The topical bumblefoot treatment was commenced on the 3 November 2010, a

week after the changes in foot temperature were noted. Phototherapy was commenced on the 4th November 2010.



Figure 37: The ventral side of the right foot. This picture was taken on the 4 November 2010 at commencement of study.

Measuring of outcome with the Gannet

Due to there being no actual lesion and rather a tissue temperature change, it was not possible to use the bumblefoot score or measure any lesion to ascertain improvement. It was noted that, on the 11th November, there was a distinct difference in the temperature of Super Duper Number One's feet with the feet being cooler. These cool feet were consistent with the thermal "feel" on the 16th November, 5 days later. On the 16th November however, bilateral splitting on the ventral surface of the feet was present (Figure 36) and on the 18th November, the feet were again warm to the touch. On the 25th November, the feet were cool to the touch. Additionally the behavior of Super Duper Number One had improved, possibly attributed to the addition of another gannet to the Centre.



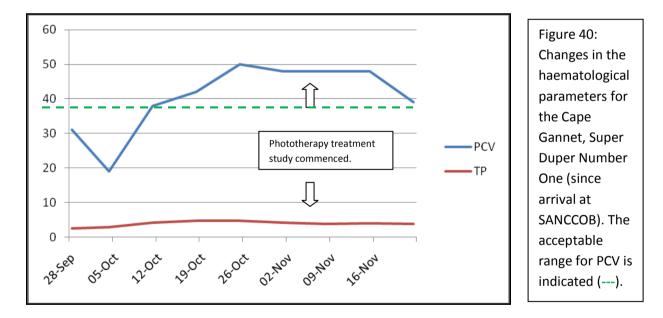
Figure 38: The ventral side of the right foot. Visible in this image is the split epidermis.



Figure 39: The split on the ventral side of Super Duper Number One's left foot a week after the wound was first observed.

Haematology

Super Duper Number One's haematological parameters have remained unstable with the Packed Cell Volume dropping as low as 19 on the 4th October and reaching levels as high as 50 on the 25th October. The Total Protein levels remained within the range of 2.4 - 4g/l; however this is lower than the acceptable ranges.



Conclusion

Development of pododermatitis

The occurrence of pododermatitis post-arrival at the Centre is consistent with development occurring within a period of 5-6 weeks. The exception of this case was for the African Penguin, "325", who had developed the pressure lesions in response to trauma and a subsequent altered gait. Gannets develop bumblefoot much earlier than this 5 to 6 week period and their "window" prior to inflammatory changes is approximately 3 weeks (Parsons, *pers.comm.*). Factors such as wound healing (the chest in the case of Super Duper Number One) result in birds not always being released within the "window" prior to the lesion development occurring.

Wound healing changes with the application of phototherapy

It was expected that a difference in wound healing would be noted as early as 1-4 days but certainly within a 10 day period if an effect (positive) was to be seen. The time it took to see effect in the case studies was delayed in comparison with the expected. This is likely attributed to the fact that the feet are dark in colour (black) and "when treating through a dark complexion, adipose or highly vascular tissue, more light will be absorbed with reduced penetration to the target tissue, so a greater irradiance will be required in order to achieve the same biological effect" (Hawkins & Abrahamse, 2007). A positive was the lack of hair at the site of phototherapy application as hair will absorb and scatter part of the photon energy dose.

Investigation of the systemic effect

The investigation into systemic effect was an aim of the study with blood parameters analysed. Ultimately, more extensive blood "pictures" would give a more accurate analysis but conclusions were drawn on data present. The fact that systemic effects were investigated is important due to, in many studies, "...the systemic effects are not considered by investigators" (Dyson, 2007).

The investigation of the blood parameter, Alkaline Phosphatase (ALP) would be preferable, particularly if bone involvement exists. The effect and changes of Alkaline Phosphatase parameters have been investigated in previous studies undertaken (Nazaroglou et al, 2009) with this parameter being confirmed as a useful indicator for changes in inflammatory

response and stimulated osteoblast activity. Positive effects noted by phototherapy were correlated with an increase in the level of Alkaline Phosphatase. Increased Alkaline Phosphatase levels are indicative of increased bone deposition and corticosteroid administration.

In the investigation of haematological data for the five case studies, the Total Protein (TP) and Packed Cell Volume (PCV) were monitored for changes post- phototherapy treatments. Robyn's haematological data was not used for an investigation of systemic effect as Robyn did not have blood taken during the course of this study. Further investigation is required for conclusive evidence of systemic effect, a fuller "blood picture" would assist in achieving more conclusive data. With the PCV, it was expected that values remain >38 (non- anaemic range). Before the phototherapy treatment study commenced, 25% of the cases ("325") were consistently within acceptable range (>38). Once the phototherapy treatment had commenced, only 25% of the cases remained lower than 38 ("220"). The PCV remained stable, post- treatment, in 50% of the birds, dropped in 25% of the cases and did not change in 25% of the cases. The acceptable range for TP was 4-6g/l, The TP levels remained within acceptable range for the case studies after the phototherapy study had commenced. Prior to the study, 50% of the birds had TP values within acceptable range. Of note, is "325" underwent a moult at the end of the phototherapy study. A moult is a stressful time for a bird and despite this, the TP remained stable and the PCV increased but remained still, within acceptable range.

Control of Variables

The aim throughout the study design and conduction was to keep as many variables as possible consistent. "When analyzing healing among wounds, it would be beneficial if the wounds were as alike as possible; therefore, the differences in healing could be attributed to the treatment and not to other factors, such as wound variability" (Hopkins, *et al*, 2004). The purpose of the study was to investigate the treatment of bumble-foot, in varying stages, as opposed to altered light therapy protocols. Therefore the Photizo protocol was kept constant with a constant dose being administered as this is the "most important parameter that should be maintained is the dose or the energy density" (Hawkins & Abrahamse, 2007).

Of the five case studies, four cases were pressure related lesions with none of the penguins being obese even with the one being on a fish/diet limit (all weighed within 0.9kg of each other to aid in eliminating variability). Additionally, all were similar ages/life stages thus degenerative joint disease was possibly at similar stages, if at any stage of development, although radiographs would confirm such. The fourth case study ("325") was a trauma related case with musculoskeletal and possible neurological long-term problems. This was a case of bumble-foot development on the dorsal surface of the foot and hence was included in the study for interest purposes and for future research. The Gannet was included in the study as this was an interesting case in terms of earlier pododermatitis development and greater conflict with treatment options once gannets developed the condition.

The one variable which was kept constant was a factor which could contribute to optimised wound healing. The birds remained in the same environment for the duration of the study, an environment of substrate which comprised of a combination of duckboards, felt and towels. Swimming sessions took place daily and consistently. For the treatment of bumble- foot, by changing the environment to support their behavior may contribute to facilitate healing. Even though the birds were being treated, the substrate remained the same and thus pressure on the feet was consistent prior to, during and post phototherapy treatment. Optimally, an altered substrate would be the solution however this is not a viable option.

The Case Studies

• "325" and foot damage from historical trauma

"325" was an unusual case due to the presence of the lesions on the dorsal side of the foot. The causative factor of such lesions was the altered gait compensating for a historical trauma. With probable musculoskeletal implications, it was noted by SANCCOB staff that cases such as these are observed commonly upon admission of birds to the Centre for rehabilitation. The fact that "325" cannot rectify the foot implies neurological damage however this is unconfirmed. Other cases seen at the Centre are capable of walking with correct foot position, if the foot is rectified to a "normal" position. Regarding potential for release, penguins do not use their feet for propulsion while swimming and thus this foot trauma long-term poses no reason for prevention of release. "325" was later released.

• The Gannet, Super Duper Number One

Super Duper Number One had sustained a deep, extensive wound to his chest region upon arrival at the Centre. As a result of the wound, Super Duper Number One could not be released within a few weeks' period post- arrival and inflammatory changes took place in his feet. These inflammatory changes are believed to be precursors to bumble-foot. During the course of the study, the feet did change in thermal range although thermography could have been useful for confirmation of tissue change in the feet. The feet did split open to form a narrow wound (bilateral). Gannets are renowned for developing bumblefoot within a shorter period whilst in rehabilitation environments, when compared with penguins. The treatment of bumble-foot has also proven to be difficult in gannets with regard to the patient's response to treatment. It is possible, based on research done on Northern Gannets, that the bumblefoot in this species of bird is susceptible to a viral particle overload, as opposed to a bacterial infection. Thus, the antibiotic response is not as effective. Super Duper Number One was released.

• "314" and possible benefits of zinc supplementation

"314", the only case which was being administered zinc additionally to the diet, showed no definite improvement in the bumblefoot lesions although no further development of lesions. Zinc is a mineral which aids in protein synthesis, energy metabolism, regulation of the immune system and wound healing. Thus it would be anticipated that this case showed an improvement with the supportive phototherapy. Zinc is present in large quantities in the (white and red) blood cells. Investigation of haematological data yielded a stability of the Packed Cell Volume (PCV) after zinc supplementation with PCV results remaining within acceptable range. "314" was released.

• "220"

"220" showed minimal wound healing of the bilateral bumblefoot lesions. The lesions were well developed at the time the study commenced. It was this case that had bumble-foot lesions for 3 weeks prior to phototherapy. The lesions did not deteriorate further. "220" was released at the end of the treatment study, a week prior to final evaluation.

• Robyn

Robyn showed an improvement in lesion healing through the course of the phototherapy study. The behavioral patterns also changed from sedentary activity to more active with altered social behavior. An important factor in bumblefoot treatment is a change in environment (substrate or stimulated change in behavior) to aid in lessened pressure on the foot, if pressure necrosis is the cause. This case indicated the effect of altered substrate and activity (if the causative factor is pressure necrosis) in conjunction with (photo) therapy. Robyn remains a Resident at SANCCOB; the poor vision in the right eye does not bode well for release.

Difficulties with the Project

There are few scores present in literature for bumble-foot or pododermatitis classification at the different stages of lesion development. Nagaraj developed a score for pododermatitis in chickens and it was upon this score, that a basis for this study's classification scores was developed. The complexity exists in the fact that wound scores often rate lesions on (peripheral) size while depth of a lesion is important to note. Depth is a factor of wound healing that should be accounted for, however techniques for measuring the depth of a wound need to be investigated further. The reason for mention of such is in the five case studies, wounds were measured weekly and sizes scored (Tables 1-4). This often did not reflect a change in the lesion however visibly; there was a definite change in the lesion depth with a "filling in" from the depths of the wound. Thus lesions were noted to have filled in from the depths often prior to peripheral edges showing a closing in. The wound healing of such "pressure sores" was altered in wound contracture when compared with general wound healing principles.

"The open area of the wound should receive a lower dose than the periphery" (Hawkins & Abrahamse, 2007). This was difficult to achieve with the size of the lesion and the size of the probe. In some cases, specifically "220", the lesion and the probe were approximately the same size. This was a flaw in the study as maximum penetration with minimised scatter was desired, however differentiation between the wound centre and the periphery were not possible to acknowledge, in some cases, with regards to treatment.

Tissue colour change is commonly used as an indicator for wound healing with changes in tissue colour proceeding from dark red to pale pink (Hopkins, 2004). This colour change was

not as reliable in this study with the lesion existing as a yellow colour. When filling in from the depths and closing in from the periphery, the new tissue existed as a grey colour (with a slight pink colour).

Control

It was decided against the presence of a Control and comparison against. The reasons behind doing so were the introduction of too many variables. A control could have existed in one of two ways, a) Untreated- a bird with bumblefoot lesions (preferably in developing rather than developed stages) with no phototherapy application, or b) A bird with bumblefoot that received treatment to one foot and not the other. The variability and concerns with treating birds and comparing to an untreated bird is the large degree of variables. The untreated bird may heal differently due to underlying illness, time at the Centre, activity and behavior, altered weight, increased parasitic load, etc. Additionally causative factors differ significantly and thus comparing bumblefoot in cases with more than one causative factor is inconsistently comparable. The alternate control was to treat one foot and not the other, however with phototherapy having a systemic effect, the untreated foot would be receiving "indirect" effects of the treatment. Therefore, for consistency, a control was decided against.

Future Considerations

Research has been conducted, by Daoust *et al* (2005), on proliferative pododermatitis in (Northern) Gannets with a confirmed viral association. The record of such viral involvement is vital to the treatment of Gannets in rehabilitation settings and when housed in aquaria etc. Pododermatitis has commonly shown association with specific bacteria presence with infection; seldom has viral presence been noted. This particular papovavirus-like particle observed in Northern Gannet species could be Gannet specific and thus treatment needs to be adjusted accordingly. Additionally, with Super Duper Number One, a chest wound was present upon arrival at the Centre and this wound and slow healing was a contributing factor to the extended time this case spent at SANCCOB. A suppressed immune system could have been in place due to this extensive wound on the chest. Pododermatitis (bumble-foot) in gannets is definitely an area for future research with phototherapy possibly being applied to

the foot, prior to inflammatory changes commencing to potentially slow the dermatitis development.

For future treatment of pododermatitis, phototherapy (infra red wavelengths) can be supported by the additional use of blue light therapy and additional physiotherapy techniques. Pulsed electromagnetic field therapy (PEMF) could be concurrently used and would possibly aid in a case such as "325" as amongst many benefits, PEMF aids in regeneration of (peripheral) nerves and in increased collagen synthesis. Ultrasound additionally aids in an increased rate of collagen synthesis (Sutton, 2003). Regardless of the therapy type, in cases with acute inflammation and infection, use of antibiotics (topical or oral) with the application of phototherapy is advised. Thus future research in the area of blue light therapy for treatment of pododermatitis is required.

Concurrent use of radiographic analyses and thermography would assist with further investigation. Radiographs would contribute to assessment of complex cases, such as "325", even post- bone fusion, to investigate bone erosion, incomplete fusion etc. Thermography could aid in slowing the time to lesion development as inflammatory changes detected could lead to early treatment and control of lesion development prior to the lesion forming.

Conclusion

As a non-surgical aid, with few negative effects or side- effects, phototherapy certainly has been noted and can be relied on as a tool for the supportive treatment of various conditions. The principle that phototherapy operates without tissue heating renders phototherapy a desired treatment as lack of tissue heating will additionally, prevent exacerbation of acute inflammation. Commonly, phototherapy investigations have been recorded on acute wounds and musculoskeletal conditions, rather than those in chronic state. With chronic bumble-foot, treatment would possibly be more successful if applied during the acute phase of lesion development. The developmental stage of bumblefoot lesion formation, prior to a small superficial visible area, is often not clear. Thus, the supportive use of thermography could be of assistance, and with a change (increase) in tissue temperature, phototherapy could be applied, aiming to slow bumble-foot development. Additionally, the causative factors for pododermatitis need to be a focus with altered housing conditions to aid in relieved pressure

on the feet. Further research is required to investigate the health of sea birds in general, with scope for further investigation of phototherapy.

Appendix 1: Photizo

phot		The Photizo [™] 641 Veterinary Unit						
	a letter a series a	Protocols	Chart					
Condition	Wounds 0-21 Day	Wounds 21+ Day	Seroma Hematoma	Tissue Trauma	Abscess	Osteo		
A				0				
Acupuncture points Amputation sites	0			0				
Acral lick granuloma				0				
Arthritis				4		0		
В	and the second second							
Back pain						0		
Burn wounds	0			9 S.				
Bursitis	222.2			0		19 1		
Bite wounds	0				0			
С						0		
Cartilage injuries	Contractor Report				0	0		
Cellulites		6 5		0	0	0		
Chronic pain			-	0				
Circulatory problems	All the second s		0		0			
Cysts								
De-gloving wounds	Ö			-	1			
Degenerative joint disease		1		2	Sector Sector	0		
Dermatitis	0	4. 				1.1		
Disc (intervertebral) lesions				ò		0		
E								
Eczema					0			
Edema			0					
F						The later of the second		
Fasciitis				0				
Fibromyalgia				0				
Fractures	and the second second				Sector Constants	. 0		
Fungal Infections	Contraction of the				0 1			
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Herpes infections					0			
Hot spots		1	0					
Hygromas	The second s	· · · ·	0					
Hyaloma tick bite		2 4			٥			
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Infection			and an and the second		0			
Infected wounds	1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 -	k.			0			
Inflammation			0	0				
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January January								
Joint inflammation	and the second second					0		
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Joint pain					Bern Harris	0		
Joint swelling								
Keratosis		0		Contraction of the second	2000 (1990) (199			

Condition	Wounds 0-21 Day	Wounds 21+ Day	Seroma Hematoma	Tissue Trauma	Abscess	
L						<u></u>
Ligament injuries	and the second second			0		
Lip wounds	0			- 10 T		
Lymph nodes					0	
м						
Metaplastic cells		0	•			
Mastitis		* · · · ·	0			
Mucositis	0					
Muscle pain				•		
Muscle spasm				0		
Muscle injury				0		-
Myalgia				0		
N						1
Neck injury				0		-
Neuralgia				0		
Neuronal Conditions				0		
Non-union fractures			N. State of the State			0
Nail bed infection				A REAL AND	0	
0						
Osteo arthritis						0
Othematomas	North Contraction	<u>k</u>	0			
Over-use syndromes				0		
P						
						0
Painful joints				0		
Painful muscles			Consequences of the second second	0		6) () ()
Painful nerve conditions				0		
Peripheral nerve injuries				0		
Post-operative pain	and the local data and		0	0		2
Post-operative swelling			No. of the second s		2002 (C. 1997)	
Pressure wounds	the second second	0	Sec. 1			2
Pododermatitis		-			0	
Repetitive injury syndrome	and the property of the state of the state of the			0	and the second second	0
Rheumathiod arthritis			and the second second	in the second		0
Rhinopathy/rhinitis	an star was				0	1
Rodent ulcer		0				<u>.</u>
S						1
Sinovitis	Carl State Street	2	ALC: NO. OF THE OWNER OWNER OF THE OWNER	0		1
Seroma		2	0			<u></u>
Sprains				0		
Skin grafts	0					8
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Sinusitis/snuffles			1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.		0	
Spinal cord injuries				. 0		0
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Vasculitis		the second se				
W				1999 (Procession)		
Warts					0	1

Appendix 2:

Table 2: Possible mechanisms involved in the acceleration of wound healing by LLLT (Walsh, 1997)

Fibroblasts

Proliferation

Maturation

Locomotion

Transformation into myofibroblasts

Reduced secretion of PGE2 and IL-1

Enhanced secretion of bFGF

Macrophages

Phagocytosis

Secretion of fibroblast growth factors

Fibrin resorption

Lymphocytes

Activation

Enhanced proliferation

Epithelial cells

Motility

Endothelium

Increased granulation tissue

Relaxation of vascular smooth tissue

Neural tissue

Reduced synthesis of inflammatory mediators

Maturation and regeneration

Axonal growth

Appendix 3- Haematological data

Packed Cell Volume (PCV) - acceptable range >38

	13 Sep	27 Sep	4 Oct	11 Oct	18 Oct	25 Oct	1 Nov	8 Nov	15 Nov	22 Nov
220	41	40	37	44	38	39	43	34	33	Released
314		48	32	32	38	42	45	45	48	46
325				44	46	44	45	42	42	47
SDNO*		31	19	38	42	50	48	48	48	39

	17 May	24 May	31 May	7 June	14 June	2 Aug
Robyn	39	33	35	40	42	37

Total Protein (TP) - acceptable range 4-6g/l

	13 Sep	27 Sep	4 Oct	11 Oct	18 Oct	25 Oct	1 Nov	8 Nov	15 Nov	22 Nov
220	5.8	8.0	6.0	7.0	6.0	5.8	6.0	6.0	6.2	Released
314		4.4	5.4	4.2	4.5	4.8	4.6	4.8	5.0	5.0
325				6.4	7.6	5.8	5.6	5.2	5.6	5.8
SDNO*		2.4	2.8	4.2	4.8	4.8	4.2	3.8	4.0	3.8

	17 May	24 May	31 May	7 June	14 June	2 Aug
Robyn	6.4	8.0	8.2	10.0	9.8	8.0

*SDNO= Super Duper Number One, Cape Gannet

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