

Universidad Autónoma de Querétaro
Facultad de Ciencias Naturales

Salud y síndrome alopécico en lobo fino de Guadalupe (*Arctocephalus townsendi*)

Tesis individual

Que como parte de los requisitos para obtener el grado de

Licenciada en Biología

Presenta

Ariadna Arely Guzmán Solís

Santiago de Querétaro, Qro. Mexico

14 de abril del 2026

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Facultad de Ciencias Naturales

SALUD Y SINDROME ALOPECICO EN MACHOS JUVENILES DE LOBO FINO DE GUADALUPE

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Licenciada en **Biología**

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Centro Universitario
Querétaro, Qro.
13/04/2026
México

Microscopic exploration of alopecia in Guadalupe fur seals from the San Benito Archipelago, Mexico

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Abstract

Until recently, there were no sightings of alopecia in pinnipeds in Mexico. However, juvenile male Guadalupe fur seals (*Arctocephalus philippii townsendii* Merriam, 1897) with patchy ventral alopecia have been sighted at the San Benito Archipelago, in the Mexican Northern Pacific. Given that the species is classified as endangered and considered of priority for conservation under Mexican law and has experienced population declines exceeding 50% in certain years, exploring factors that could be indicative of health problems is relevant. We captured 13 juvenile male fur seals at West San Benito Island during 2017 and 2018 and collected skin scrapings and fur samples for routine dermatological analysis, scanning electron microscopy, and X-ray spectroscopy. Guard fur showed loss of structural integrity, medullary damage, and perforations, while damage to the underfur was less common. We found no evidence of dermatophytes or ectoparasites commonly associated with alopecia. We suggest that fur damage might be caused by nutritional or metabolic alterations secondary to an exogenous stressor, but further studies are needed to confirm this possibility. This is the first microscopic exploration of alopecia in pinniped in Mexican waters.

Key words: alopecia, *Arctocephalus philippii townsendii* Merriam, 1897, fur, Guadalupe fur seal, hair loss, metabolic disorder

Résumé

Jusqu'à récemment, aucune alopecie n'a été observée chez les pinnipèdes au Mexique. Cependant, des otaries à fourrure de Guadalupe (*Arctocephalus philippii townsendii* Merriam, 1897) mâles juvéniles présentant une alopecie ventrale éparse ont été observées dans l'archipel de San Benito, dans le Pacifique Nord mexicain. Étant donné que l'espèce est classée comme en voie de disparition et considérée comme prioritaire pour la conservation en vertu de la loi mexicaine, et qu'elle a connu un déclin de sa population de plus de 50% certaines années, il est pertinent d'explorer les facteurs qui pourraient être révélateurs de problèmes de santé. Nous avons capturé 13 otaries à fourrure mâles juvéniles sur l'île ouest de San Benito en 2017 et 2018 et collecté des grattages de peau et des échantillons de fourrure pour des analyses dermatologiques de routine, une microscopie électronique à balayage et une spectroscopie à rayons X. La fourrure de garde présentait une perte d'intégrité structurelle, des dommages médullaires et des perforations, tandis que les dommages au sous-poil étaient moins fréquents. Nous n'avons trouvé aucune preuve de dermatophytes ou d'ectoparasites communément associés à l'alopecie. Nous suggérons que les dommages à la fourrure pourraient être causés par des altérations nutritionnelles ou métaboliques secondaires à un facteur de stress exogène, mais des études supplémentaires sont nécessaires pour confirmer cette possibilité. Il s'agit de la première exploration microscopique de l'alopecie chez les pinnipèdes dans les eaux mexicaines. [Ceci est une traduction fournie par l'auteur du résumé en anglais.]

Mots-clés : alopecie, *Arctocephalus philippii townsendii*, fourrure, otarie à fourrure de Guadalupe, perte de cheveux, trouble métabolique

Introduction

Alopecia syndrome has been reported for various marine mammal species, including the polar bear, *Ursus maritimus* Phipps, 1774 (Atwood et al. 2015), grey seal, *Halichoerus grypus* (Fabricius, 1791) (Stack et al. 1993; Pugliarres-Bonner et

al. 2018), northern elephant seal, *Mirounga angustirostris* (Gill, 1866) (Beckmen et al. 1997), Australian fur seal, *Arctocephalus pusillus doriferus* Schreber, 1775 (Lynch et al. 2011, 2012), and Subantarctic fur seal, *Arctocephalus tropicalis* (Gray, 1872) (Reisfeld et al. 2015). This syndrome, first reported in 2015 for

the Guadalupe fur seal *Arctocephalus philippii townsendii* Merriam, 1897 is characterized by the thinning and subsequent loss of hair (McCue et al. 2021).

As with any syndrome, alopecia does not have a single etiology. However, different factors have been correlatively associated with this condition in humans and animals. In marine mammals, these factors include ectoparasites, such as lice and mites, which can lead to over keratinization, destroy the epithelium, and damage the hair follicles directly or indirectly when they lead to anemia (Dailey and Nutting 1980). Fungi (Guillot et al. 1998; Reisfeld et al. 2015), bacteria, and viruses can also be associated with alopecia if they cause dermal lesions, abscesses, or vesicles (Hadlow et al. 1980; Stack et al. 1993). However, the development of alopecia has also been related to other factors such as polychlorinated biphenyls (PCB) pesticides (Beckmen et al. 1997) and other persistent organic pollutants (Taylor et al. 2018), toxins (Debier et al. 2005), immune dysfunction (Dierauf and Gulland 2001), endocrine alterations (Frank et al. 2003), and nutritional deficiencies that stunt growth and damage the integrity of hair and skin structures (Engelhardt and Geraci 1978; Puls 1994; Goldberg and Lenzy 2010; O'Connor and Goldberg 2021).

Cases of alopecia began to be observed in pup and juvenile Guadalupe fur seals (hereafter, GFSs) stranded along the Oregon coastline during the atypically high sea surface temperature (SST) event of 2015 (McCue et al. 2021), which coincided with the start of the unusual mortality event (UME) recorded for the species and that continued until 2021 (NOAA 2022). Sightings of juvenile GFS with alopecia were also reported in the San Benito Archipelago (Norris and Elorriaga-Verplancken 2019). This is concerning, given that GFS were considered commercially extinct less than 100 years ago. Also, the species is currently listed as threatened under the Endangered Species Act of 1973, and depleted under the Marine Mammal Protection Act of 1972, although the Mexican government currently considers it as endangered and a priority for conservation (SEMARNAT 2018). Although the GFS population has increased in size and in distribution, with an estimated abundance of between 34 000 and 44 000 individuals (García-Capitanachi et al. 2017), following a dramatic recovery of less than 20 individuals (Hubbs 1956), most of its population and breeding colonies are located on a single island, Guadalupe Island, in the Mexican northern Pacific (Juárez-Ruiz et al. 2022), with non-reproductive recolonization of other areas, such as the San Benito Archipelago (Elorriaga-Verplancken et al. 2016). This site is the second-largest Guadalupe fur seal colony located, with a maximum documented abundance of 5633 individuals, with yearly fluctuations, prone to significant declines (Serrano 2023). One such instance was observed in 2015, when the colony experienced a population decline of over 50%, related to the warm SST caused by El Niño and The Blob, which negatively affected primary productivity in the area.

GFS are known to be susceptible to climatic anomalies (Elorriaga-Verplancken et al. 2016; Gálvez et al. 2020, 2023), and given that thermoregulation is mainly dependent on their double layer of fur (McCue et al. 2021), conditions that

affect the pelage could impact their fitness, body condition, and health, as has been reported for the polar bear (Atwood et al. 2015).

Alopecia might be problematic for GFS, as fur seal blubber thickness is modest compared to that of other pinniped species (Rosen et al. 2007), making them dependent on their pelage as their main thermoregulatory mechanism. Fur seals have a thick double layer of fur that controls heat conduction and convection between the body and the environment (Holmér and Bergh 1981; Lynch et al. 2011; Erdsack et al. 2013). When air is trapped within the fur layers, it forms a shield between the highly irrigated skin and the environment, blocking harmful solar UV radiation from reaching the skin (Øritsland and Ronald 1978) as well as aiding flotation (Ling 1970). Thus, it is plausible that alopecia could impact various aspects of GFS physiology. In this sense, studying the syndrome is relevant and timely for the species.

We have conducted a microscopic examination of fur samples collected from alopecic juvenile GFS from the San Benito Archipelago, a recolonization site that harbors the second largest colony of Guadalupe fur seals (up to 3700 individuals) after Guadalupe Island (Elorriaga-Verplancken et al. 2016). We investigated whether there was evidence of structural alterations in the fur and whether it was possible to detect common microorganisms and ectoparasites that could be associated with the condition.

Materials and methods

Permits and bioethics approval

All sampling and animal handling were conducted by approval of the Bioethics Committee and IACUC of the Autonomous University of Queretaro (Approval No. 59FCN2021). Sampling was conducted under permits SGPA/DGVS/00091/17 and SGPA/DGVS/02460/18 issued by the Dirección General de Vida Silvestre, Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT).

Animal captures and sampling

During July of 2017 and 2018, 13 juvenile male Guadalupe fur seals (*Arctocephalus philippii townsendii*) were captured during routine monitoring of the species at the San Benito Archipelago (28°18'N, 115°32'W) in the western region of the Baja California Peninsula. Individuals were captured using hoop nets and were physically restrained. Captures were opportunistic. Nine of the captured individuals had diffuse patches of alopecia of varying size (ranging from 6 cm² patches to 25 cm²; see Fig. 1 for examples) and four had no observable patches of alopecia.

Prior to collecting the fur samples, visual inspection of the alopecic patches in the ventral surface of the chest was conducted by a trained veterinarian. Namely, the skin was inspected for areas with dermal alterations that could be associated with ectoparasites, such as erythematous patches, nodulation, scaly patches, ulceration (Neuber and Nuttall 2017), or the actual presence of ectoparasites. We performed superficial skin scrapings in the ventral surface of the chest with a

Fig. 1. Examples of alopecic patches in Guadalupe fur seal (*Arctocephalus philippii townsendii*) juveniles from the San Benito Archipelago. (a) Irregular patches on the ventral neck and dorsal area. These areas were the most commonly affected by alopecia. (b) A severe case of alopecia in a GFS (not sampled). Extended alopecia can be observed in the caudal dorsal area (foreground), hairless patches on the snout (left), and a fur seal with decreased capillary density on the snout and head in general (right). (c) Hairless patch in the pectoral area and snout. (d) Lack of fur in the pectoral and axillary area (right) and hairless patch in the snout (left). (e) Absence of fur in the mid-caudal dorsal area. (f) Hairless areas are evident in an animal that is next to fur seals without alopecia.



dull surgical blade that was swiped three times perpendicularly to the skin surface of four of the GFS; two with alopecia and two without alopecia. For the individuals with alopecia, two sets of skin scrapings were performed, one in the center of a patch and one in the periphery. For the fur seals with no alopecia, only one superficial skin scraping in the ventral surface of the chest was performed. The cellular debris collected was placed in the middle of a microscope slide in which a drop of mineral oil had been placed. A coverslip was placed, and the slide was kept in a polypropylene container, transported to the field lab, and examined under a low power microscope (Carson MicroBrite plus) at 10X amplification to search for the presence of mites.

Fur samples (1.5 × 1.5 cm) were collected from alopecic patches found in the ventral surface of the chest. We used sterile scissors for collection and kept the samples in small sterile paper envelopes. Nitrile examination gloves were used throughout the procedure. For individuals that did not show detectable alopecia, fur samples were collected from the same body region and using the same methodology. In the field lab, and less than 30 min following collection of each sample, the fur was washed gently with sterile bi-distilled water using a wash bottle, air-dried at ambient temperature, and kept in new sterile paper envelopes.

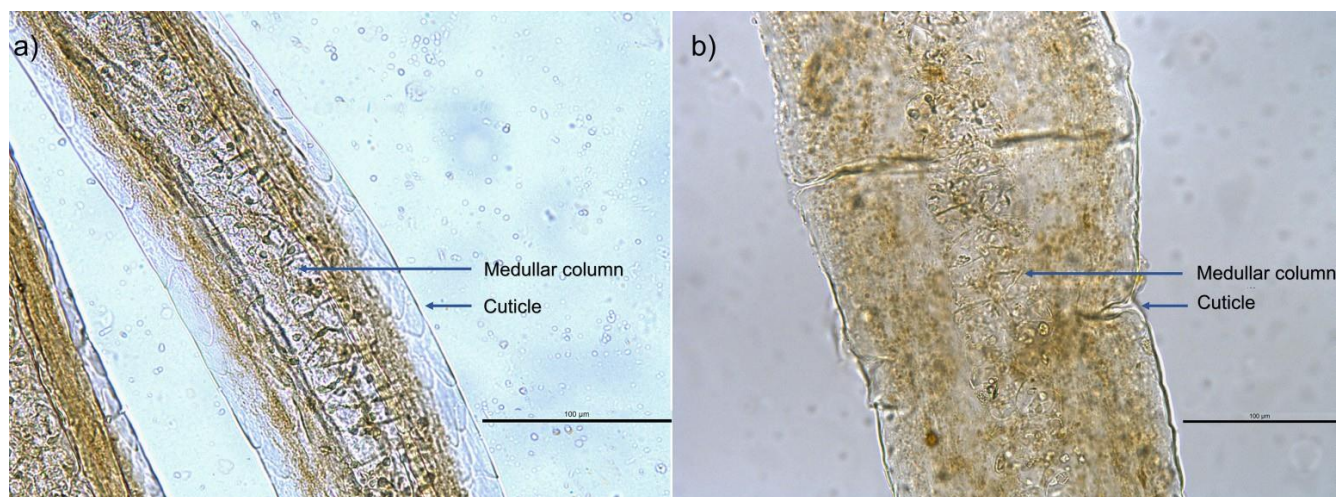
Microscopic examination of samples

A subset of each sample (comprising eight to ten guard hairs and several secondary hairs per individual) was deposited on a clean glass microscope slide and examined using an optical microscope at 10X and 40X. Next, drops of potassium hydroxide (KOH) solution (containing 10% KOH and 40% dimethyl sulfoxide (DMSO) in sterile deionized water) were added until the entire sample was covered to clarify fur structures and allow the visualization of fungi. Within 10–20 min of staining, each slide was examined under a compound microscope (B490, Amscope) at 10X, 40X, and 100X magnification to search for structures typical of fungi like yeast cells, fungal spores, or filamentous fungal structures (conidia, hyphae, sclerotia, or arthrospores). Next, additional fur strands were placed in Petri dishes to be examined by long-wavelength UV light under a binocular stereoscopic microscope (ZM190, UNICO) as a second method to detect the presence of dermatophyte fungi or ectoparasite eggs.

Electron microscopy analyses

To examine hair shaft ultrastructure, scanning electron microscopy (SEM) images were taken of four to six guard hairs per individual, as well as several (approximately 10 per individual) secondary hairs using a EVO50 (Carl Zeiss AG) SEM

Fig. 2. Microscopy images of Guadalupe fur seal (*Arctocephalus philippii townsendii*) guard hair treated with potassium hydroxide. (a) Guard hair showing preservation of cuticular scales at the periphery and a well-organized medullar column. (b) Guard hair showing remains of a disorganized medullary column and splintering of the cuticle. Scale bar: 100 μ m. Magnification 20X.



with variable pressure. Acceleration voltage was 20 kV, magnification at 40X and 100X. To identify the chemical composition of the fur, we performed X-ray spectroscopy (EDX) on every sample that showed ultrastructural damage along with encrustations under SEM, using SE1, back-scattered electron detector (BSD), and EDX detectors. Analyses were conducted at the Laboratory of Scanning Electron Microscopy of the School of Natural Sciences (<https://fcn.uaq.mx/index.php/extension/servicio-de-microscopia-de-barrido>) at the Autonomous University of Queretaro (Mexico). All samples were handled with sterile gloves and new instrumentation to avoid any contamination.

Results

None of the captured GFSs ($n = 13$) had macroscopic lesions in the skin or ectoparasites, and there were no mites in any of the skin scrapings examined in the field. Upon microscopic examination, we failed to find evidence of fungal spores, conidia, septa, asci, yeast, parasites, or parasite eggs, neither associated with the guard hairs or the secondary fur.

Guard hairs collected from GFS with alopecic patches showed moderate to severe structural alterations along the shaft in comparison with most of the guard hairs collected from non-allopecic individuals. Namely, normal guard fur showed cuticular scales that were well preserved on the periphery and had an organized medullar column (Fig. 2a), while those collected from alopecic zones had marked medullary disorganization and areas of breakage or splintering of the hair (Fig. 2b). None of the GFS without alopecia exhibited damage to the guard hairs that was observable under light microscopy. Secondary hair (underfur) mostly showed no observable anomalies, with only a few secondary hairs of GFS with alopecic patches revealing loss of structure and scales (Fig. 3). UV illumination did not reveal any sign of fluorescence, as would be expected by the presence of fungi or most dermatophyte bacteria associated with fur loss.

SEM of samples collected from GFS with alopecic patches revealed guard hairs with a conserved and well-organized cuticular structure of juxtaposed scales of the coronal dentate type (Fig. 4a) as well as guard hairs with structural damage, including loss of cuticular scales, perforations, incrustations, and loss of margins (Fig. 4b). The structure of the secondary hairs was markedly more preserved (Fig. 5). The level of damage of the cuticular ultrastructure of fur seals with mild to moderate alopecic patches varied, with some individual hairs revealing well-conserved structures and uniform scales, and others showing cuticular scales with no tip and diffuse organization, lack of scales, perforations, and mild loss of margins (Fig. 6). In two of the four GFS that had no evidence of alopecia, at least one of the guard hairs analyzed in each sample had mild damage, observed as ultrastructural changes in the integrity of the scales (Fig. 6b). In contrast, samples collected from GFS with extended diffuse alopecic patches, most of the guard hairs showed severe damage, consisting of an absence of cuticular scales, evident perforations, incrustations, and marked loss of margins that fractured the hair transversally (Fig. 7). The incrustations appeared as crystal-like material that did not reveal any similarity to fungal, parasitic, or bacterial structures (Fig. 8). We found no evidence of ultrastructural damage in the underfur (secondary hair) collected from alopecic individuals.

The X-ray dispersion analysis (EDX) analysis used to further examine the guard hairs with severe ultrastructural damage revealed that the incrustations were composed of sodium (Na), calcium (Ca), and chloride (Cl) (Fig. 9).

Discussion

Free-ranging GFS juveniles in the San Benito Archipelago are susceptible to develop alopecia, as has been recorded for GFS yearlings in poor body condition stranded along the California coast during the GFS UME of 2015–2021 (McCue et al. 2021). That UME coincided with a period of anomalously

Fig. 3. Microscopy of Guadalupe fur seal (*Arctocephalus philippii townsendii*) secondary hair treated with potassium hydroxide. (a) Cuticular structure showing preserved toothed scales; (b) secondary hair without scales, with breakage and irregular edges at the margin. Scale bar: 50 μ m. Magnification 40X.

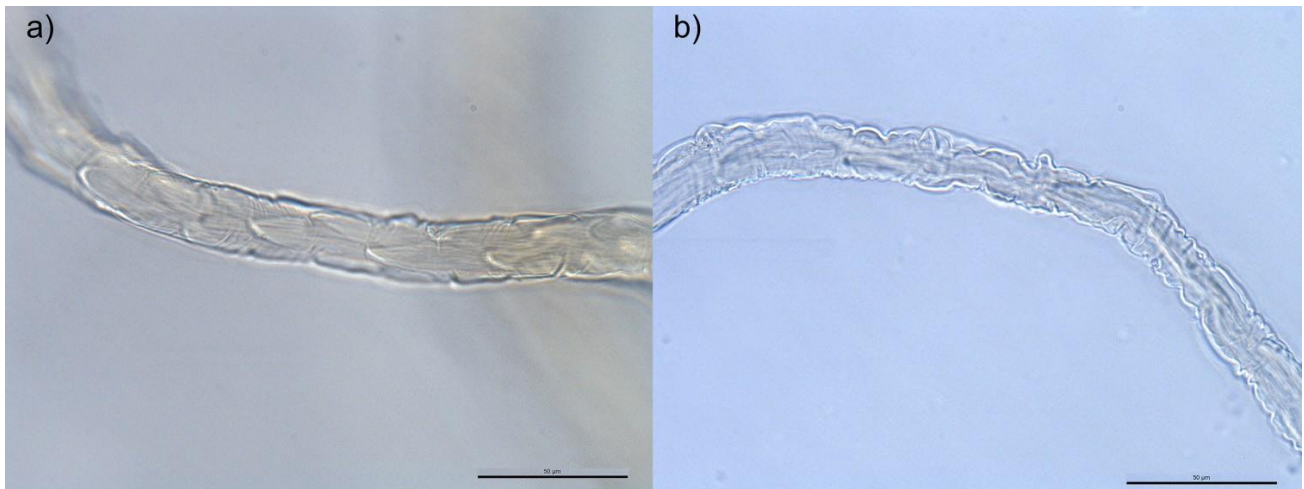
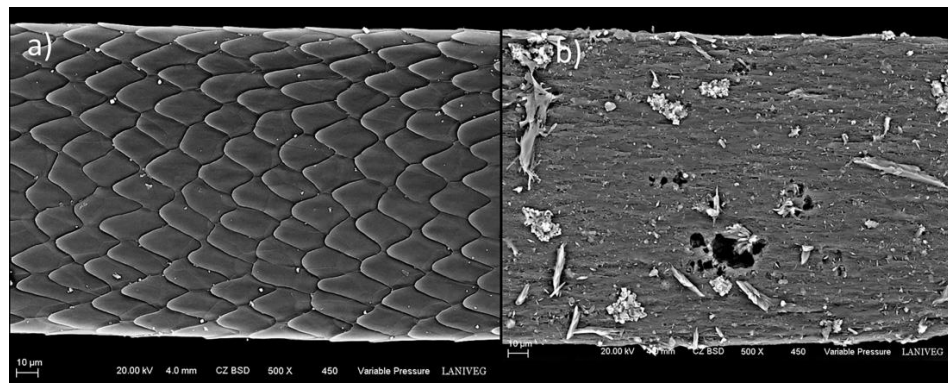


Fig. 4. Scanning electron micrographs of (a) well-conserved cuticular scales in guard hair from Guadalupe fur seal (*Arctocephalus philippii townsendii*). (b) Marked loss of cuticular scales, perforations, incrustations, and loss of margin in guard hair. Scale bar: 10 μ m. Magnification 500X.



high SSTs (2.5 °C) due to “The Blob” or North Pacific heatwave (2014–2016) and an El Niño event (2015–2016) in the Northeast Pacific Ocean (Bond et al. 2015; Tseng et al. 2017) which led to marked biological changes in the marine ecosystem of the Northeast Pacific (Cavole et al. 2016). Here, we examined the fur of free-ranging GFS juvenile males in good body condition, but with alopecia and compared the structure of their fur to that of GFS juvenile males in good body condition without alopecia.

During our field surveys we did not find evidence of alopecia in GFS pups or in sexually mature individuals, only in juvenile males. This is contrary to reports of alopecia in polar bears and otariid species, such as the Australian fur seal (Lynch et al. 2011, 2012; Bowen et al. 2015). However, it is important to note that in the study site, around 84% of the fur seals are juveniles (Elorriaga-Verplancken et al. 2016; Norris and Elorriaga-Verplancken 2019), making it more likely to have observed alopecia in this age group.

Studies of alopecia in captive pinnipeds have reported the presence of ectoparasites associated with the condition (Dailey and Nutting 1980; Reisfeld et al. 2015). However, stress associated with captivity is known to influence both ectoparasitic infections (Gelling et al. 2012) and alopecia (Novak et al. 2017). Free-living pinnipeds commonly harbor ectoparasites in their fur and skin, but these do not tend to cause alopecia or to be related to disease unless the host’s immune response is suboptimal (Ferrer et al. 2014; Leonardi et al. 2021; Foley et al. 2023). We found no evidence of lice, mites, or any other ectoparasites in the skin underlying the fur that was collected, nor did we find evidence of any ectoparasite attached to the collected fur.

Also, the samples did not reveal evidence of dermatophyte fungi, which can be associated with alopecia as they colonize keratinized tissues (hair and skin), and can cause inflammation, subsequent dermal lesions, and hair loss (Guillot et al. 1998). Bacteria and viruses can also be associated with hair loss in pinnipeds when they cause epidermal lesions and

Fig. 5. Scanning electron micrographs of the ultrastructure of a secondary hair with long dentate scales from Guadalupe fur seal (*Arctocephalus philippii townsendii*). Right panel shows a magnified (250X) image of the dentate scales. Scale bar: 20 μ m.

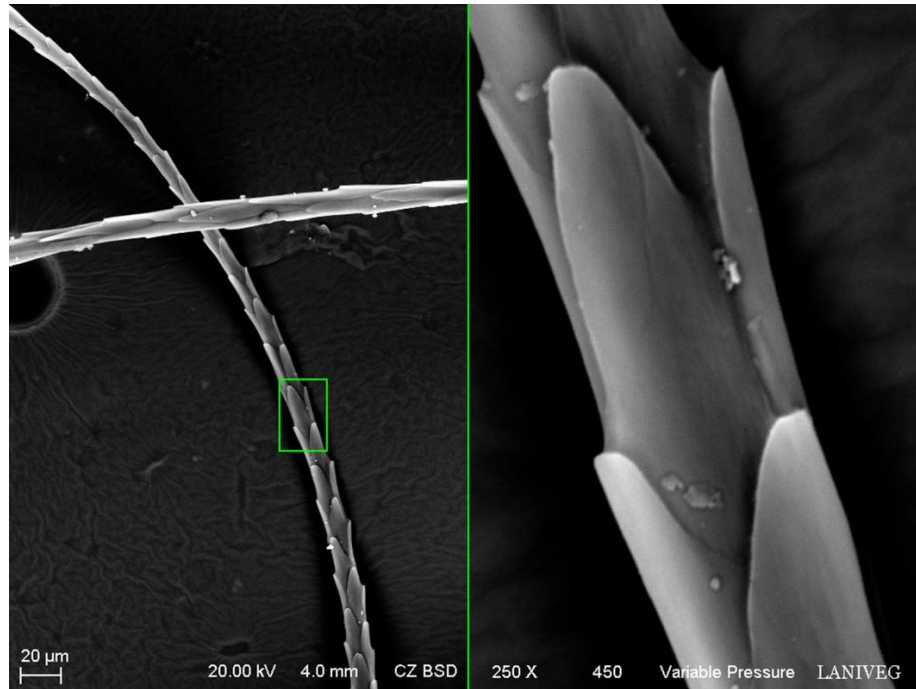
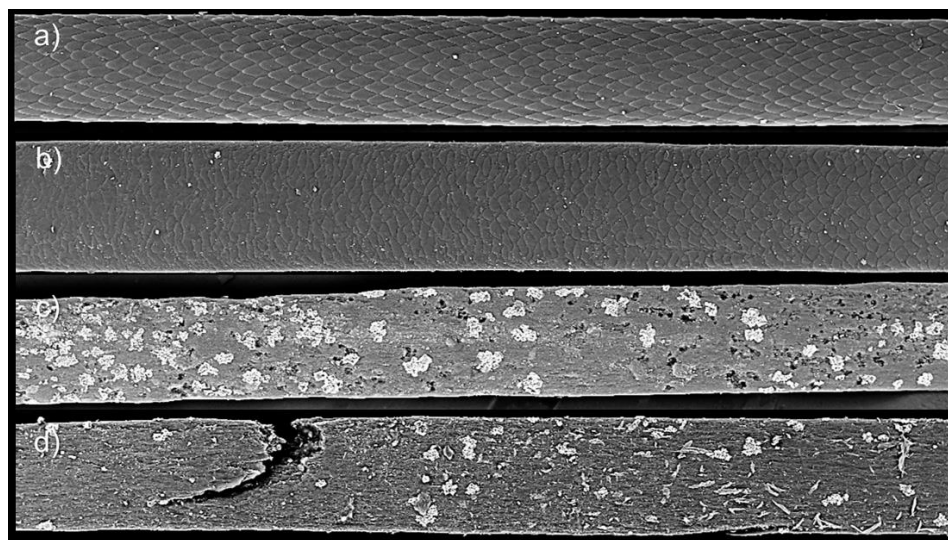


Fig. 6. Ultrastructural microscopy of guard hairs from Guadalupe fur seal (*Arctocephalus philippii townsendii*) showed different levels of loss of structural integrity. (a) Guard hair with uniform scales and normal structure. (b) Guard hair with mild loss of definition in scales. (c) Guard hair with some perforations and encrusted foreign material. (d) Severe damage to guard hair, with many perforations, encrusted material, and ruptured shaft.



inflammation that can involve hair follicles (Hadlow et al. 1980; Stack et al. 1993). However, the skin under the alopecic patches appeared healthy, with no macroscopic evidence of lesions (specifically, we observed no erythema, no ulcerations, and no vesicles). Due to permit restrictions, we were unable to collect skin biopsies for histological examination, so we cannot discard the possibility that the skin beneath the alopecic patches had microscopic alterations.

While based on the methods we used it is not possible to rule out an infectious agent as the primary or contributory factor of alopecia in the juvenile GFS, it appears unlikely that a pathogenic or opportunistic virus or bacteria was present in the skin or hair follicles of these animals.

Structural alterations of the hair were observed primarily in the guard fur (primary hairs), and only minimal damage was observed in the underfur (secondary hairs). Both

Fig. 7. Scanning electron microscopy images of guard hair from Guadalupe fur seal (*Arctocephalus philippii townsendii*). Left panel shows the transversal fracture of the hair. Right panel shows a magnified (100X) image the fractured area. Scale bar: 100 µm.

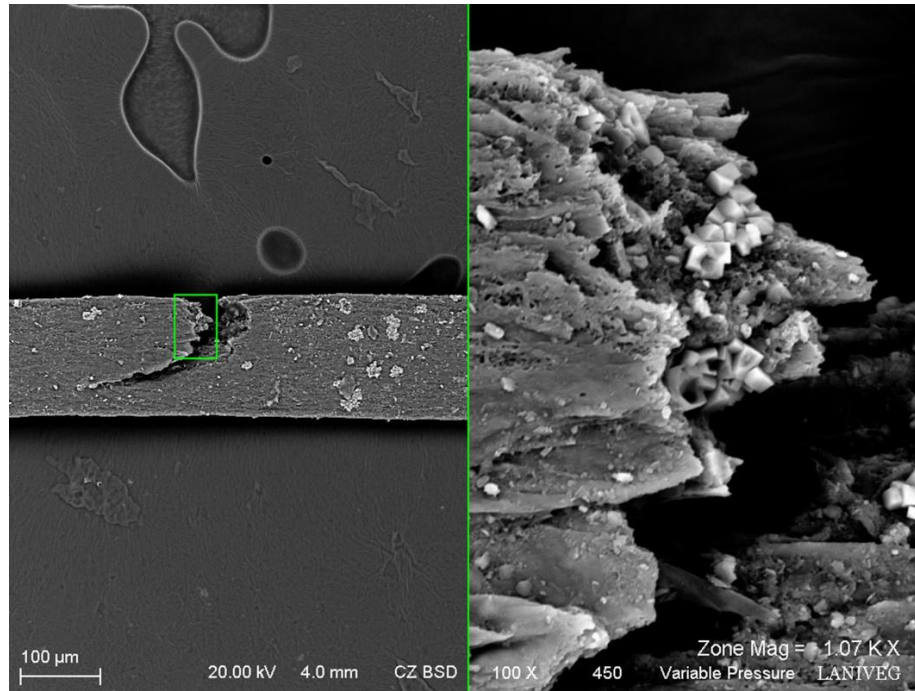
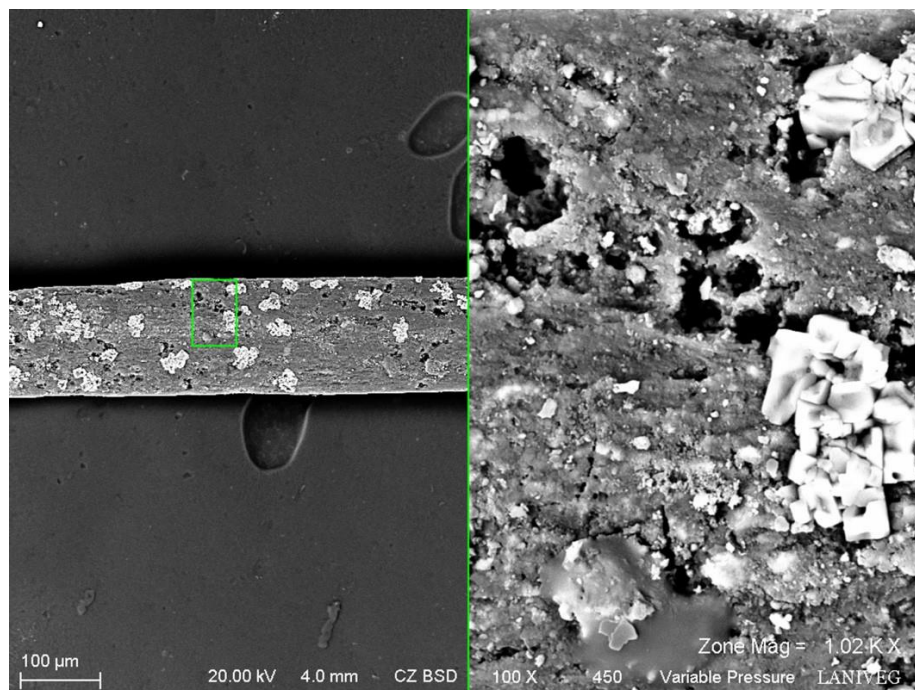


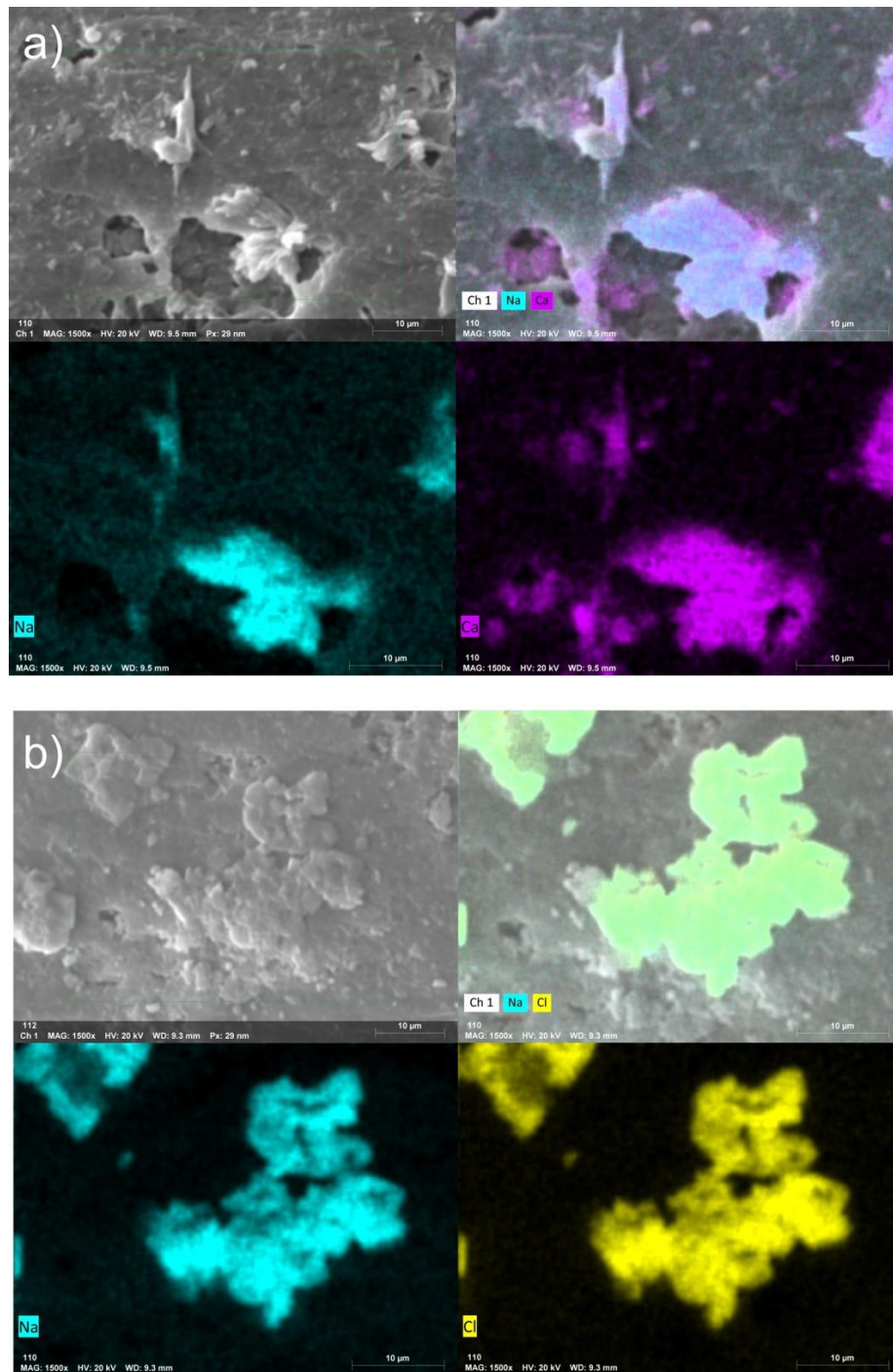
Fig. 8. Scanning electron micrograph of perforations and encrusted particles found in Guadalupe fur seal (*Arctocephalus philippii townsendii*) guard hairs. Right panel shows a magnified (100X) image of the encrusted particles. Scale bar: 100 µm.



types of otariid fur vary in thickness, resistance, and composition. Specifically, secondary fur lacks a medullary composition, making guard hair intrinsically more resistant (Scheffer 1964). When healthy, fur seal guard hair is coarse and resistant to physical abrasion due to its ultrastructure, which renders them virtually unscathed even when undergoing di-

gestion or carcass decomposition (Barros 2018). It is possible that damaged guard fur still offers protection to the underfur, and only when the damage is extreme, will secondary hairs start to exhibit signs of damage. Additionally, the irregular alopecic patches could be due to natural asynchrony with the anagenic (i.e., growth), catagenic (i.e., rest), and telogenic (i.e.,

Fig. 9. EDX analysis of the elemental composition of the encrusted material found with the perforations in guard hair from Guadalupe fur seal (*Arctocephalus philippii townsendii*). Particle composition was sodium (Na; marked as blue), calcium (Ca; marked as purple) and chloride (Cl; marked as yellow). (a) Electron microscopy (left) showing the presence of Na and Ca (right); the bottom row shows each element separately. (b) Electron microscopy (left) showing the presence of Na and Cl (rows); the bottom row shows each element separately.



loss) phases of the follicular cycles of neighboring hair follicles (Favarato and Conceição 2008). These phases do not occur synchronously throughout the coat, but rather in waves or patches, as if in mosaics (Chase and Eaton 1959; Miller et al. 2012). Normally, growth succession patterns lead to a uniform fur coat, but at times a particular follicular stage can last longer in one area than in others (Feltz and Fay 1966), a

phenomenon that leads to patchy alopecia and mostly occurs in sick, pregnant, or semi-starved animals, who cannot start a new follicular cycle due to lack of nutritional or energetic resources (Chase and Eaton 1959). However, there was no indication that the juvenile males sampled were starving or ill, based on the examination performed by a trained veterinarian during animal handling.

When striving to understand causes of alopecia, the duration of the follicular cycles also needs to be considered. This is because during the growth phase, hair is especially sensitive to stressors (Restrepo 2010). As guard fur has a longer growth phase than secondary fur (Chase and Eaton 1959), and will complete its cycle over many months, it is possible that the quicker turnover of secondary fur leads to the comparatively lesser damage that we observed in our study. The length of the follicular cycle of guard fur is also important to consider when interpreting our results, given that the observed damage could have begun months before, following exposure to a stressor or stressors that compromised the integrity and strength of the fur.

The shape and the resistance of hair strands is determined by the structure of fibrous proteins such as alpha-keratin, whose amino acids include cysteine monomers (Popescu and Höcker 2007) that form disulfide bridges where their sulfhydryl groups join. If cysteine is reduced, the disulfide bridges are broken, and the hair becomes brittle due to structural changes (Nelson and Cox 2017). In humans, hair structure can also be altered when exposed to extremely high (up to 235 °C) temperatures, such as those of heat-styling tools (Gamez-Garcia 2011; Bloch et al. 2019; Yang et al. 2020; Essendoubi et al. 2022), particularly when there is a concurrent zinc deficiency (Pigatto et al. 2020). Hair structure alterations can also occur after exposure to chemicals such as thallium, arsenic, selenium, and mercury (Yu et al. 2018), and in some pinniped species, by a mixture of persistent organic pollutants (Beckmen et al. 1997; Taylor et al. 2018). While we can reasonably dismiss direct exposure to extremely high temperatures as the cause of alopecia of the GFS from San Benito Archipelago, exposure to chemical pollutants cannot be dismissed as a possible leading or contributory cause of hair damage. Future analyses could aim to address this possibility.

Given that GFS are known to be vulnerable to climatic anomalies that decrease availability of their preferred prey and force them to consume different items, often of lower nutritional value (Elorriaga-Verplancken et al. 2016; Amador-Capitanachi et al. 2020; Gálvez et al. 2020, 2023), it is reasonable to propose that the switch in prey could affect GFS cysteine synthesis and, thus, decrease fur strength, leading to alopecic patches. Cysteine biosynthesis depends on the availability of methionine, an essential amino acid that is solely obtained from eating food items with high protein content (Elango 2020). So, it is possible that the dietary protein intake of the GFS juveniles has been suboptimal, leading to a sustained alteration of biochemical pathways necessary to synthesize cysteine (Miniaci et al. 2016) or reducing the normal growth cycle of the fur coat due to nutritional restrictions (Ling 1970). At this point this is speculation, and further studies could aim to address this possibility.

It is important to highlight the fact that disease is rarely due to a single factor but rather, to the additive effects of different factors, and alopecia is not an exception. Evidence of this was reported by Bowen et al. (2015) in a transcriptome-based study of alopecia in polar bears. They found that bears with alopecia not only overexpressed genes involved in antigen presentation and processing, lymphocyte activation, innate immune responses to pathogens, and inflammation rela-

tive to those unaffected, but also overexpressed genes related to DNA damage responses, methylation, catabolism, and the regulation of fatty acid biosynthesis (Bowen et al. 2015). The authors proposed that the alopecia was caused by various interacting determinants, including dietary switches due to environmental changes, that affected the polar bears. We propose that, similarly, the alopecia of GFS from the San Benito Archipelago is related to multiple determinant factors.

We were able to determine that the crystal-like material found within the rugose surface of the damaged hair shaft was granules of sodium and chloride. The most parsimonious explanation is that these are sea salt granules that became lodged in the hair shaft perforations and were not simply adhered to the wet fur. This is because all samples had been rinsed prior to analysis, and we did not find any evidence of these granules in the fur samples collected from animals with no alopecia. If the salt granules are lodged in the perforations, they could further increase damage to the shaft and increase the risk of strand breakage. Interestingly, we also detected calcium in the severely damaged guard hair shafts, which cannot be explained as being part of the dried sea salt granules. Perifollicular deposits of calcium have been described in some dog breeds during aging (Seaman and Chang 1984), but the GFS examined herein were all juveniles. Furthermore, we detected the calcium within the perforations of the guard fur, and not surrounding the follicles. It is plausible that the calcium detected in the damaged guard fur of the GFS is a trace element from the blood that became incorporated into the keratin matrix of the hair during the growth of the hair shaft (Cone 1996), given that calcium cations are known to bind strongly to melanin granules (Kempson et al. 2003). If so, keratin-bound calcium could be another indication of metabolic changes in the alopecic GFS, as has been reported for humans (Lee et al. 2016).

If the alopecia syndrome of GFS reflects metabolic alterations due to nutritional deficiencies that are, in turn, related to abnormally high SST events, then the prevalence of alopecia should exhibit temporal variations, as happens with other climate-related diseases (Altizer et al. 2006). Future long-term studies that consider nutritional, toxicological and immune assays of GFS with and without alopecia, and fatty acid profiling of their prey would help to ascertain this possibility. Also, yearly examination of isotopic signatures of the hair of GFS could provide evidence of climate-related prey shifts, and comparisons of isotopic signatures between alopecic and non-alopecic GFS could help understand dietary differences that might contribute to the condition. It is also, of course, possible that the observed alopecia is related to an as yet unidentified systemic condition that is sensitive to environmental factors (Lynch et al. 2012; Atwood et al. 2015).

If alopecia syndrome of GFS is related to dietary-induced metabolic changes secondary to climatic anomalies, consequences are likely to be far deeper than fur. This has already been shown for another pinniped species, the California sea lion, *Zalophus californianus* (Lesson, 1828), that inhabits the same geographic area. Namely, California sea lion pups born during the 2015 high SST year had reduced inflammatory responses and antibody levels (Banuet-Martínez et al. 2017) as well as red blood cell membrane alterations (Flores-Morán et

al. 2017), suggesting that the physiological impact of climatic anomalies is wide. Furthermore, suboptimal nutrition driven by changes in prey availability will lower the amounts of resources available to the GFS. As loss of fur would demand more energetic resources to maintain thermal homeostasis (Hind and Gurney 1997), other costly physiological processes would compete for resources (Husak et al. 2021; Ohmer et al. 2021), leading to trade-offs, as per the resource allocation theory (Glazier 2008).

Even if GFS alopecia is found to be independent of high SST, it is likely to be a problematic condition for the species. The alopecic patches were observed in the ventral chest and abdomen of the GFS studied (and, less frequently, in other areas of the body). The skin of the ventral chest and abdomen is relevant to heat dissipation given its high vascularization and numerous arteriovenous anastomoses (Khamas et al. 2012). Alopecia would expectedly increase exposure of the underlying skin to the environment and hinder the GFS's ability to thermoregulate (Mauck et al. 2000), particularly as the cuticular scales, critical for maintaining the fur coat waterproof and providing insulation during and after immersions (Liwanag et al. 2012) were found to be affected. This could potentially impact the foraging behavior and diving patterns of the GFS.

In conclusion, ours is the first description and microscopic examination of alopecia in free-ranging GFS in Mexican waters. We aimed to describe the syndrome and to help elucidate its probable contributory factors. We found evidence that leads us to propose that the syndrome could be secondary to metabolic alterations related to ecological factors. Given that the frequency of climatic anomalies in the north-eastern Pacific appears to be increasing in the past decades (Bond et al. 2015; Joh and Di Lorenzo 2017), and that xenobiotics that impact metabolism and immune function of marine mammals (Gulland et al. 2020) continue to degrade coastal and insular marine habitats, it is not surprising that novel health conditions emerge in wild populations. These conditions might be particularly relevant for species that are in the process of recovery, like the GFS (Lotze et al. 2011). Regardless of the precise cause of alopecia in GFS juveniles, various ecological factors are likely to contribute synergistically to increase the risk of damage to their fur. If these factors persist, it is plausible that the GFS population health could be negatively affected.

Acknowledgements

We thank the Abalone Fishermen Cooperative (Cooperativa Nacional de Abuloneros, S.C.) for their logistical support during fieldwork. Luis A. Soto-García, Cecilia Barragán-Vargas, Marina Banuet-Martínez, Fabiola Guerrero de la Rosa, and Hiram Rosales Nanduca provided invaluable help with the Guadalupe fur seal captures.

Article information

History dates

Received: 8 October 2024

Accepted: 10 January 2025

Version of record online: 24 September 2025

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Data availability

All data underlying this article are available within the article.

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Competing interests

The authors declare there are no competing interests.

Funding information

Fieldwork was partly funded under a CONACYT Research Grant (A1-S-16417) awarded to K.A.W for research on pinipeds.

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