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SKIN DISORDERS OF COASTAL DOLPHINS AT AÑIHUÉ RESERVE, CHILEAN PATAGONIA: A MATTER OF CONCERN

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ABSTRACT

Epidemiological characteristics and progression of skin disorders were documented in Peale's (*Lagenorhynchus australis*) and Chilean (*Cephalorhynchus eutropia*) dolphins resident in Añihué Reserve (43.8041ºS; 72.9786ºW) in Chile's Aysén Region. Since 2004, sea-pen based salmon farms began to surround the Reserve. We analyzed 5,734 frames in a graphic SQLite database obtained during systematic inshore monitoring of cetaceans in 2010-2013, comprising 115 photo-identified *L. australis* and several unidentified *C. eutropia*. In 2013, the prevalence of skin disorders peaked in *L. australis* at 81.7 %, an increase of 30.2 % versus 2011. Morbidity is unknown. Of six cutaneous conditions, 1-4 affected both species: (1) tattoo skin disease with 39.1 % prevalence in *L. australis*; (2) "pale skin patches", highly prevalent (74.8 %) in *L. australis* and characterized by opaque to translucent patches that expanded rapidly, then generally resolved within 10 days and occasionally recurred; (3) "focal skin diseases" were clusters of lesions of variable severity that affected all age categories with a 13.9 % prevalence in *L. australis* and that led to deep cutaneous ulcers in a *C. eutropia*; (4) rare "skin lineal anomalies" (4 *L. australis*, 1 *C. eutropia*) of variable-length, dark, sometimes reticulated, that may persist for years; (5) a "necrotizing tissue mass" that grew rapidly in one *L. australis* over a preexisting injury for 40 days before scarring; (6) "ulcerative dermatitis (UDD)", a condition with hypodermal involvement seen in a cluster of 7 *L. australis*. Except for TSD, aetiologies of skin disorders remain unknown, however water pollution associated with the expanding salmon farm industry is suspected to have contributed to their emergence.

Key words: skin diseases, emerging skin anomalies, dolphins, eutrophication, pollution, public health, aquaculture, Patagonia.

RESUMEN

Enfermedades cutáneas en delfines costeros en la Reserva Añihué, Patagonia Chilena: un motivo de preocupación. Se documentaron características epidemiológicas y desórdenes en la piel de delfín austral (*Lagenorhynchus australis*) y delfín chileno (*Cephalorhynchus eutropia*) residentes en Reserva Añihué (43,8041ºS; 72,9786ºO), Región de Aysén, Chile. Desde 2004, granjas para engorda de salmones basadas en jaulas flotantes, comenzaron a rodear a la Reserva. Analizamos 5.734 cuadros en una base de datos gráfica SQLite, incluyendo 115 individuos foto-identificados de *L. australis* y varios *C. eutropia* no identificados, que fueron obtenidos durante observaciones costeras sistemáticas de cetáceos entre 2010 y 2013. La prevalencia de desórdenes en la piel de *L. australis*, alcanzó un máximo en 2013 de 81,7 %, un aumento del 30,2 % respecto a 2011. Se desconoce la morbilidad. De seis condiciones cutáneas, 1-4 afectaron a ambas especies: (1) enfermedad del tatuaje con 39,1 % de prevalencia en *L. australis*; (2) "manchas pálidas", altamente prevalentes en *L. australis* (74,8 %) y caracterizadas por manchas opacas hasta translúcidas de rápida expansión, resueltas generalmente dentro de 10 días y con reaparición ocasional; (3) "dolencias cutáneas focales" fueron grupos de lesiones de severidad variable afectando todas las categorías de edad (13,9 % de prevalencia en *L. australis*) y ocasionando profundas ulceraciones en un *C. eutropia*; (4) escasas "anomalías lineales" (4 *L. australis*, 1 *C. eutropia*) de largo variable, oscuras, en ocasiones reticuladas y que pueden persistir por años; (5) en un *L. australis* creció rápidamente una "masa de tejido necrotizante" sobre una lesión preexistente por 40 días antes de cicatrizar; (6) en un grupo de 7 *L. australis* fue registrada una "dermatitis ulcerativa" con compromiso hipodérmico. La etiología de los desórdenes de piel, salvo por los "tatuajes", es desconocida si bien se sospecha han emergido con la contribución de la contaminación de las aguas relacionada con la expansión de las granjas para salmones.

Palabras clave: enfermedades de piel, anomalías emergentes de piel, delfines, eutrofización, salud pública, acuicultura, Patagonia

INTRODUCTION

Cetaceans are considered sentinel species for the oceans and human health (Bossart 2010). Coastal odontocetes are of special interest as indicators for the presence in the environment of heavy metals, persistent organic contaminants (POPs) and biological pollutants of anthropogenic origin (Holden 1978; Borrell 1993; Kannan *et al*. 1993, 2005; Colborn and Smolen 1996; Smyth *et al.* 2000; Jepson *et al*. 2005; Stewart *et al.* 2014), providing valuable information for diverse stakeholders including fisheries, tourism and public health management. However, assessing health dynamics can be challenging in these highly mobile marine mammals. Recent photographic studies carried out in inshore common bottlenose dolphins (*Tursiops truncatus*), Indo-Pacific bottlenose dolphins (*Tursiops aduncus*), Guiana dolphins (*Sotalia guianensis*) and Irrawaddy dolphins (*Orcaella brevirostris*) have provided valuable insights into the macroscopic and epidemiological characteristics and evolution of several skin disorders (Wilson *et al.* 1997, 1999; Van Bressem *et al.* 2003, 2007, 2009a, 2012, 2014; Murdoch *et al.* 2008; Daura-Jorge and Simoes-Lopez 2011). Tattoo skin disease (TSD), lobomycosis and lobomycosis-like disease (LLD) are now relatively well-documented cutaneous syndromes in small cetaceans from South America (de Vries and Laarman, 1973; Simões-Lopes *et al*. 1993; Van Bressem *et al*. 1993, 2007, 2009a,b; Van Bressem and Van Waerebeek 1996; Moreno *et al*. 2008; Siciliano *et al*. 2008; Daura-Jorge and Simões-Lopes, 2011). The epidemiological pattern of TSD is a likely indicator of cetacean population health. Besides, LLD and nodular skin disease may represent possible indicators of a compromised marine environment (Reif *et al*. 2009; Van Bressem *et al.* 2009b). Other cutaneous conditions descriptively referred to as "green-brown plaques" (GBP), "orange patches" (OPA), "cutaneous nodules" (NOD), "pale dermatitis" (PAD) and "expansive annular lesions" (EAL) are emerging in several inshore and estuarine populations, possibly because their habitats are increasingly contaminated (Van Bressem *et al.* 2007; in press). With the exceptions of TSD and lobomycosis the aetiology of these disorders has not been determined as the diagnosis was based only on macroscopical evaluation of high-resolution images taken for photo-identification (PI) purposes (Van Bressem *et al.* 2007; in press).

Chilean Patagonia is a vast and complex geographic area, accessible to large-vessel shipping mainly through maritime pathways not directly related to the small scale environment of narrow fjords and bays which provide an important habitat used by coastal dolphin and porpoise populations (Sanino and Yáñez 2012). Since 2006, Nomads of the Seas (NOTS), a Chilean high-end tourism company, has operated during the southern summer season in remote Patagonian fjords with mother-ship M/V *Atmosphere*, carrying small boats, kayaks and a large Zodiac Hurricane 920OB R.I.B. This provided the first author (GPS) as on-board naturalist the opportunity to access and observe wildlife in remote areas in the Los Lagos and Aysén Regions, including Palena Bay and the shores of Añihué Reserve. In 2006-07 only two salmon farms, of the originally installed in 2004, were present in this area, namely at the northern limit of Aysén Region (Región Aysén del General Carlos Ibáñez del Campo). When cruising in northern Patagonia, NOTS selected the coasts of Añihué Reserve and Refugio Channel for tourism purposes precisely because of the absence of salmon farms, since most of Chiloé, Guaitecas Archipelago and the most northern sites were already saturated by the salmon industry. Schools of dolphins were regularly sighted, allowing weekly whale-watching activities. However, since 2008 the remaining pristine areas were reached by the salmon industry that installed farms of variable sizes in naturally protected bays and sounds at the northern access of Refugio Channel, inside the channel, and several at the southern section on the same sites (44.0176ºS, 73.1251ºW) where one of us (GPS) regularly encountered dolphin communities before 2008. This experience allowed us to directly assess the impacts of sea pen based salmon farms on the coastal cetacean populations. From boat-based work, G.P. Sanino observed (unpublished data) that the behavior of local dolphins became more evasive, that the number of groups sighted per unit of effort and mean group size decreased and that abnormal skin marks were increasingly present. Systematic shore-based observer effort, started at Añihué Reserve in 2010, confirmed these observations. Skin conditions reached an unprecedented incidence of 51.5% in the Peale's dolphin (*Lagenorhynchus australis*) in January-December 2011 (Sanino and Yáñez 2012; Sanino, unpublished data). In Añihué Reserve *L. australis* showed high site fidelity during all seasons and an unexplained extreme demographic asymmetry (1.47 % of calves, 7.35 % juveniles and 91.18 % of adults) (Sanino and Yáñez 2012).

In this paper we present the results of an extensive study on cutaneous disorders in *L. australis* using graphic data (2010-2013), complemented with initial findings on a similar effort in Chilean dolphin (*Cephalorhynchus eutropia*) and some of the most recent material of Añihué Reserve. We discuss the importance of our findings in the context of an increasingly chemically and biologically polluted, eutrophied aquatic environment synchronous with, and apparently related to, the boom of sea pen salmon farming (Buschmann *et al.* 1996; Sullivan-Sealey and Bustamante, 1999; Cabello 2006; Buschmann *et al.* 2012).

MATERIALS AND METHODS

Study area

The study area comprises the shore of the 2.1 kilometer long sandy "Tonina Beach" in Añihué Reserve (RA; 43.8041ºS; 72.9786ºW), a private coastal reserve located on the continental coast of Cisnes Commune, at the northernmost limit of Aysén in the northern part of Chilean Patagonia. Two sites, R1 and R2, were prepared for observation profiting from their natural elevations and close distance to the water (see Sanino and Yáñez 2012).

Physical oceanography

Since 2012, the Helmholtz Center for Polar and Marine Research of the Alfred Wegener Institute, in partnership with Añihué Reserve, has been studying the marine ecology and physical oceanography of the reserve. We used CTD data from several stations in the area from 2012 to characterize the salinity levels (measured in Practical Salinity Units) of the environment of coastal cetaceans (Laudien *et al.* 2012).

Cetacean data collection

Graphic data were collected both by trained volunteers, mainly during the summer of 2011, and assistant observers trained for maintaining monitoring efforts from 9:00 to 13:00 h and from 13:00 to 17:00 h every day except Sundays, including under precipitations and with a Beaufort sea state of 4 or less, measured with a weather station Advantage Pro2 (Davis) installed on one of the cabins in front of the beach.

Cetaceans, primarily Peale's and Chilean dolphins, were sighted using binoculars (7x50; 20-100x70; and 12x42). Effort, as effective sighting time, was recorded with HandBase (a customizable data-collector application) installed in an android smartphone or directly on paper. Photos were taken with SLR cameras with telephoto lenses. A Nikon D300 SLR camera with a VR 400 millimeters lens was initially used but later replaced by a Nikon D5100 with a 55-300 mm lens. Additional cameras were tested with the support of the volunteers' personal equipment, including: Canon S2-IS, Canon EOS Rebel XS, Nikon D3000 and Nikon D3100. Occasionally, video and photography data (SONY HDR-FX7), including underwater video (GoPro Hero), were collected from the boat belonging to the Reserve, M/V *Añihué*; a 7 meters long polyethylene plastic boat with two Yamaha 100 HP 4-stroke engines and a fiberglass closed cabin.

The material produced from underwater filmography did not provide enough detail for a full-body assessment of skin diseases and accounted for just a few re-identified individuals. Therefore, the anatomical distribution of skin conditions was determined only from the visible body surface (VBS), the parts of the body that were visible to the observer, or could be photographed, from a boat or from shore (Van Bressem *et al*. 2009b).

Data processing and management protocols

Our method consisted of a set of protocols for step-by-step data management, from data recording to its final catalog, known as DVideo-ID developed by Sanino and Yáñez (2001a) and later adapted for inshore efforts (Sanino and Yáñez 2012). Selected frames were classified with tags corresponding to natural marks ranging from nicks, dorsal fin shape, teeth marks, scars, to marks attributed to skin diseases, among others, organized by anatomical section and side. Our main application for graphic tagging, cataloguing and filtering was Digikam a KDE Linux-based software distributed under the GNU General Public License V2. Behind its user graphic interface, Digikam is a SQLite database that can be associated through a sqliteODBC connector with LibreOffice.base to perform queries on the information that Digikam's interface may not allow.

The protocols for vessel approaches to dolphins, frame production, selection and classification, individual coding, individual tag summary, search and comparison process, and re-identification process were described in detail in Sanino and Yáñez (2001a, 2012). However, for further focusing on skin lesions attributed to pathologies, we included some modifications.

Modifications to DVideo-ID method

Filtering power of the photographic database, for re-identification tests, diminished with time due to the abundance of frames for each individual album and to the growth of the entire database. The risk was ending with searches presenting more filtered frames with a specific set of selected tags than the total number of individuals, thereby loosing all efficiency when filtering the catalog to compare the candidate frame with an intended small fraction of the material managed by the catalog. To mitigate this effect, when dealing with large datasets, we included an arbitrary tool for scoring the quality or importance of a frame within an individual album by using the "stars" tool of Digikam. Moreover, natural marks change with time (*e.g.* the addition of a nick in the dorsal fin) and therefore only the recent main features of an individual should be searched during the filtering process. The Digikam photo quality stars were used to select, among an individual album, only those frames that included recent features and with the best quality as to minimize redundancy.

Since several characters were noticed specifically on the saddle, *i.e.* the *dorsum* directly behind the dorsal fin, we added this new tag to the anatomic sections of the body as well as tags according to the pathomorphism of skin marks attributable to skin diseases (SD), having discarded other possible origins (*i.e.* trauma, malformation, individual pigmentation pattern or artifacts). A dedicated tag, "ID Profile", was added to help select the images created with the image editor "The Gimp" to be used as individual tag summary. When filtering, this allowed to reduce the entire catalog to a single file per individual. Añihué Reserve as well as the water bodies next to it were divided into distinctive geographic zones and added as tags as a simple georeference. Finally, classification was done using 77 possible tags (for reference see Table 1 in Sanino and Yáñez 2012).

We included in the protocol the deletion of frames when new photos provided better quality of the same features, leaving only one photo as voucher of the sighting. This process greatly reduced the size of the database.

Skin diseases

Macroscopic skin anomalies were described on the basis of their color, form, relief, relative size and type of external limit (discrete, smooth, irregular or lobulated). Their anatomic location and evolution over time were recorded following the slightly modified protocol from Sanino and Yáñez (2012): the fins (dorsal, pectoral, flukes); the head (snout tip to anterior insertion of flippers); thorax (between head and the anterior insertion of dorsal fin); lumbar (section behind thorax delimited posteriad by the anus, recognizable laterally by a ventral inflection point) and the peduncle (tail stock limited by the anterior margin of the flukes). When skin lesions were noted while in the field we recorded any unusual behavior of the affected dolphin.

When possible, prevalence of a specific skin conditions was inferred as the proportion of individuals with evidence of having contracted that condition regardless of the lesion status (active or in regression) over the number of photo-identified *L. australis* (N= 115). However, considering the difficulties to diagnose remnant TSD lesions in this species, due to its irregular pigmentation, the records of this disease were in practice based mainly on active TSD lesions. Significance of differences in prevalence rates were tested with two-tailed Z-tests for two population proportions.

Size time-dynamic of SD lesions

Since most of the individuals of *L. australis* were photo-identified and re-sighted during the study period, we attempted to assess lesion evolution over time. For this purpose we used only ID frames of excellent photographic quality and minimum perspective from similar shooting angle and incidental light angle. When individuals where re-sighted with resolved SD lesions, we considered that the disease had cured and calculated the probable maximum duration time of that lesion as the time interval between the first lesion observation and the 'resolved lesion' sighting.

To assess the size time-dynamic of skin lesions, two measurements were taken on each ID frame: (s) maximum size of a cutaneous mark and (S) additional measurement between two easily re-identifiable points on the individual (*i.e.* the base of the dorsal fin), describing a line of similar angle orientation to "s" but larger whenever it was possible to diminish the effect of the measured error. We used the *light table* feature of Digikam (on an OpenSuSE 13.1 based custom made PC), in full screen mode, connected to a 23" LED screen (Samsung SyncMaster PX2370 - Full HD1080). Both measurements were directly taken in millimeters over the image without changing the amplification (zoom level). To minimize the error due to image perspective and differences in the distance to the subjects between the images, we used the lesion size (s) relative to the additional measurement (S) as a ratio (s/S) in a time (Tx) measured in days. Therefore the relative size variation of the skin lesion on any date after the first day it was recorded (T0), resulted from comparing their respective "s/S" indexes. The proportional average daily variation rate between recorded events (*i.e.* between T1 and T0), was obtained as follows:

$$\text{Daily variation rate between T0 and T1} = \left(\frac{s1/S1}{s0/S0} - 1\right) \Big/ (T1 - T0)$$

For categorical definitions of the lesion sizes, we considered the eye of the individual and its dorsal fin as visual references: "small-sized" corresponding to affected areas smaller than half of the eye's diameter; "medium-sized" lesions with a diameter lower than twice the eye's diameter; "large-sized" up to half of the base length of the dorsal fin; and bigger lesions as "very large-sized".

RESULTS

Physical oceanography

In 2012, 20 CTD casts were realized in the area of Palena Bay and the Pitipalena Fjord. Salinity levels, were recorded for several sites of the study area (Figure 1). The sites Las Hermanas Islands (a), Los Patos Estuary (b), the mouth of Palena River (c) and Tonina Beach (d and e) are directly exposed to the Gulf of Corcovado, while the other sites were CTD casts distributed over the inner water bodies including Pitipalena (g) (Figure 1).

While the salinity was expected to increase with depth, all the stations were distributed in two distinct groups, differentiated by only *ca*. 3 units and with little increase in waters deeper than 10 m, regardless of proximity to oceanic water. In contrast, salinity increased dramatically (about 15) with depth in shallow waters less than 10 m. Surprisingly, nearshore waters at Tonina Beach in Añihué Reserve facing the ocean, where most dolphin observations occurred, had as low salinity as the sheltered shore of Pitipalena Fjord or even Brazo Pillán (Figure 1).

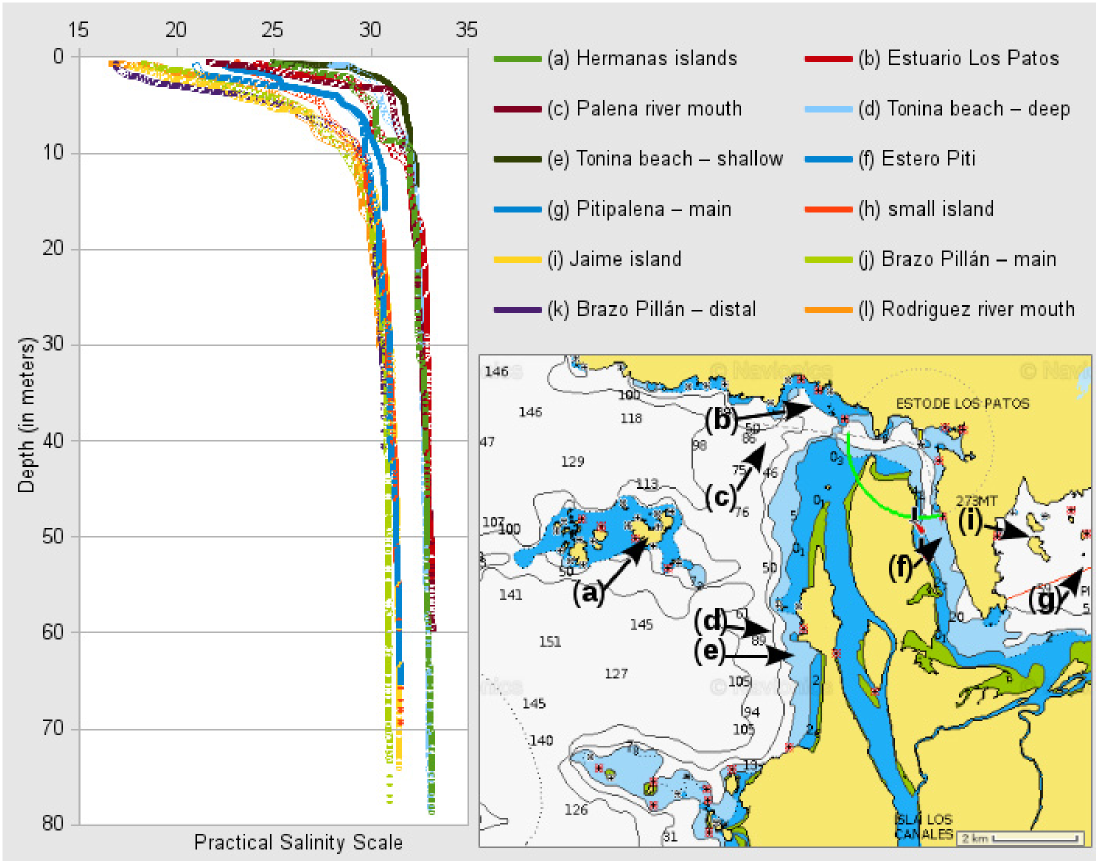

FIGURE 1. Depth related salinity values retrieved from CTD casts distributed along Palena Bay and adjacent water bodies in February 2012; (d) and (e) in front of Tonina Beach in Añihué Reserve.

Coastal dolphins of Añihué Reserve were sighted year-round during their daylight foraging along the coastline in waters not deeper than six meters which, based on the salinity values, correspond to an almost fresh water environment due to freshwater run-off from the nearby Palena River, with the steepest gradient in salinity within the first few meters. Data collected from diving computers showed a water temperature range between 12.5 and 13.5 ºC.

Collected data

Since *L. australis* was the most frequently sighted cetacean in the study area, its individual identification catalog, validated between January 2010 and June 2013, was more developed than for the other sympatric species. We focused mainly on this dataset requiring accurate re-identifications in the analysis of prevalences and indexes. Individual identification codes in this paper refer to *L. australis*. This material was complemented with preliminary data for a similar effort in *C. eutropia* in order to verify species-specific conditions, as well as with highly relevant, information for *L. australis* in 2014.

The selected ID frames included 5446 images corresponding to 115 identified *L. australis* of which 82.6 % were re-sighted. Skin marks that could not be attributed to traumas, malformations or individual variation in pigmentation were allocated on the basis of their pathomorphisms to the following categories: tattoo skin disease (TSD), pale skin patch (PSP), focal skin disease (FSD), skin lineal abnormalities (SLA), necrotizing tissue mass (NTM) and ulcerative dermatitis (UDD).

Skin disease prevalence and anatomic distribution

Among the 115 individually identified *L. australis* from Tonina Beach, Añihué Reserve, 94 (81.7 %) had skin lesions and 70 (60.9 %) presented lesions in more than one body region. The prevalence of skin disorders for major sections of the body, as defined in Methods, are provided in Table 1.

Tattoo skin disease

The lesions, referred to as "tattoos" and caused by poxvirus infection, were dark or light gray, had distinct rounded borders and a characteristic stippled pattern (Geraci *et al*. 1979; Flom and Houk, 1979;Van Bressem *et al*. 1993). They seemed mostly flat but, in some cases, appeared slightly depressed. Tattoos often occurred in clusters (agminate) and, in a few cases, fused into larger lesions.

TABLE 1. Distribution of skin disease lesions over the visible body surface of *L. australis* in Añihué Reserve.

	Head	Thorax	Dorsal fin	Lumbar	Peduncle
# affected individuals	24	55	67	69	16
% of total individuals	20.9 %	47.8 %	58.3 %	60.0 %	13.9 %
# frames	117	282	554	451	78

Lagenorhynchus australis

TSD was observed in 45 of 115 (39.1 %) *L. australis*. TSD lesions were most frequently seen on the head (around the eyes), the flanks (lateral of and behind the dorsal fin) and on the dorsal fin (Figure 2), recognizing that the *ventrum* was rarely visible. The lesions were large and numerous on the head and around the eyes, medium-sized on the thoracic and lumbar body sections plus the peduncle and single or small clusters on the dorsal fin. After a variable time, including years, TSD lesions tended to acquire a lighter tone but, for the duration of this study, we did not record a complete disappearance on any individual.

New lesions often appeared near older ones but were also observed to develop on healthy skin. Small and medium-sized tattoos were identifiable only in high-quality images. Generally it was easier to diagnose these lesions on the white/light parts of the body and more difficult on the darker parts. The general behavior of infected individuals did not differ from healthy members of the pod. The left eye of one of the TSD affected dolphins (RA1_56) was completely opaque (white). The relationship between these two conditions, if any, is unknown.

Cephalorhynchus eutropia

In *C. eutropia*, TSD lesions were mainly detected on the *dorsum*, flanks, dorsal fin and head, especially around the eyes. At least six specimens were infected, but no prevalence estimate was available. Affected individuals tended to be seen alone or in small groups rather than in large schools.

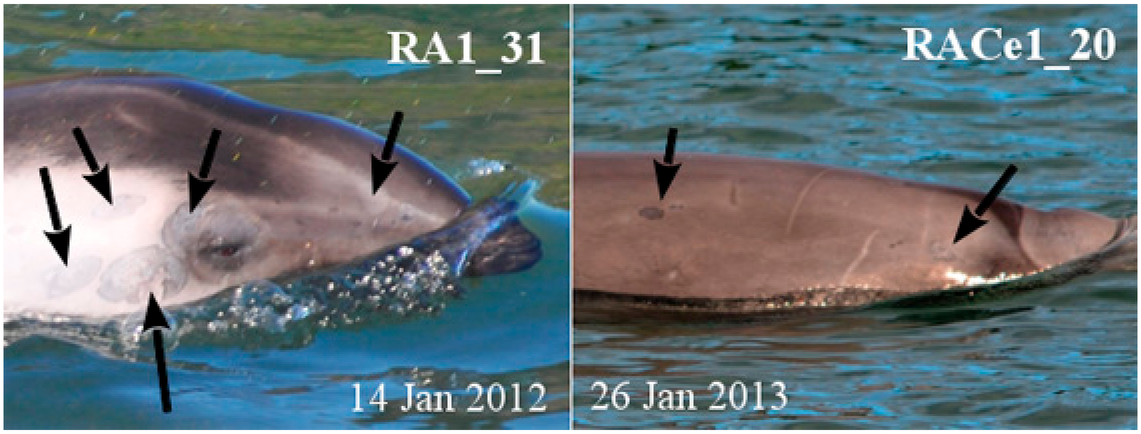

FIGURE 2. Tattoo skin disease (TSD) lesions (black arrows) in *L. australis* RA1_31 (a), and *C. eutropia* RACe1_20 (b) from Añihué Reserve, Chile.

FIGURE 3. Unidentified mother-calf Chilean dolphins, *C. eutropia*, with confirmed TSD lesion in the mother (left) and probable TSD lesions in the calf (right) in Añihué Reserve, Chile.

Epidemiology and size time-dynamic of TSD

Adults and juveniles of *L. australis* were affected but no calves. In *C. eutropia*, a calf had lesions evoking tattoos but the image quality was not good enough to allow a definitive diagnosis. The accompanying adult, presumably the mother, also showed tattoo-like skin lesions (Figure 3).

Evolution of the lesions in adult *L. australis* was variable (Figure 4). In two individuals TSD lesions showed slight or no changes in size over time. For instance, in RA1_41 the tattoos did not vary in size or color during 12 days. After almost a year, the tattoos in RA1_36 only presented a lighter tone. However, in three other adults the size of the lesions increased over time and the accumulated growth of some tattoos relative to their first sighting was measurable (Figure 4).

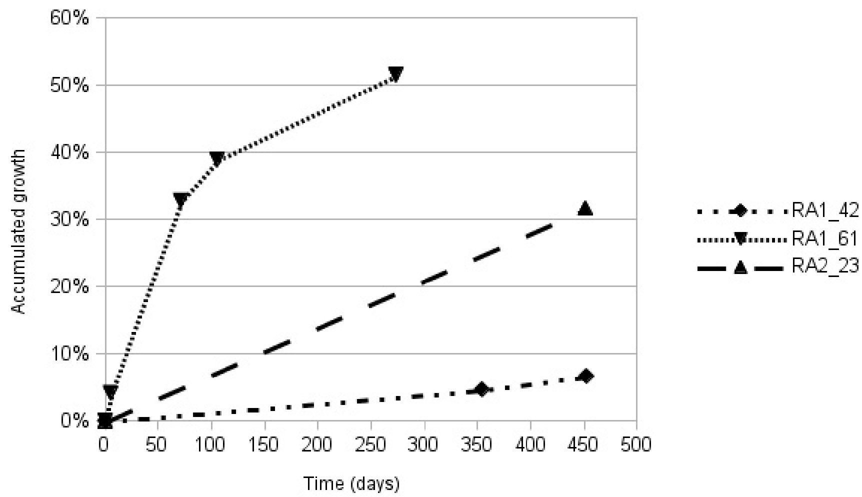

FIGURE 4. Accumulated growth of tattoo skin disease lesions, relative to their first sight in three *L. australis* in Añihué Reserve, Chilean Patagonia.

Most of the TSD lesions that could be followed over time grew centrifugally from a small center of infection producing the classic round or oval ringed shape. The mean daily rate of growth, estimated for each time lapse between re-sightings showed differences between individuals and at the individual level over time, but was always below 0.90 %. RA1_42 reached at 354 days the same daily rate (0.17 %) as RA2_23 at 451 days. Both individuals, RA1_42 and RA1_61, with at least three lesion monitoring events showed a decreasing growth rate with time. However, lesions in RA1_61 and RA1_42, after 273 and 452 days respectively, still did not fully halt expanding (Figure 5).

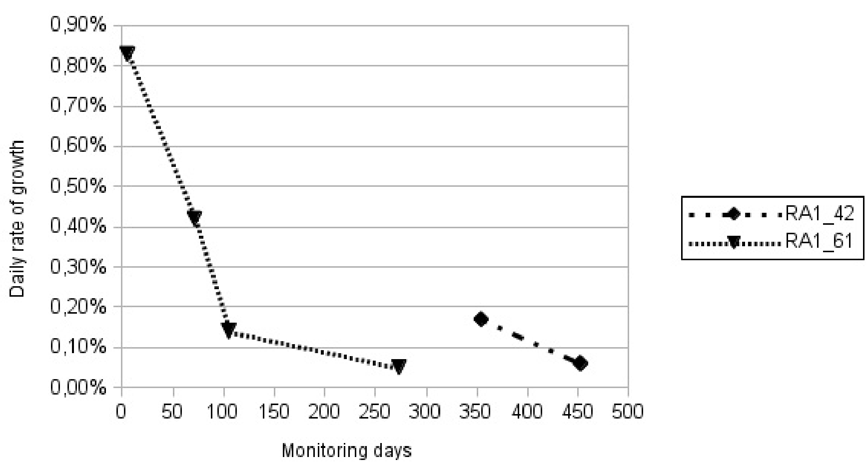

FIGURE 5. Daily rate of growth of tattoo skin disease lesions measured on four time lapses in RA1_61 and two in RA1_42, *L. australis* individuals in Añihué Reserve, Chilean Patagonia.

L. australis RA2_11 presented tattoos near its right eye among other body locations. After a year some lesions that had not obviously grown appeared lighter while those that had increased in size maintained their dark tone. A hemicircular and previously long, narrow thin tattoo with no noticeable growth in length had however significantly increased in width (Figure 6). At close inspection of Figure 6b, the hemicircular line comprises in fact a series of tiny individual tattoos linked in a string-like arrangement. We admonish precaution when evaluating apparently linear lesions from a distance, as TSD can be involved.

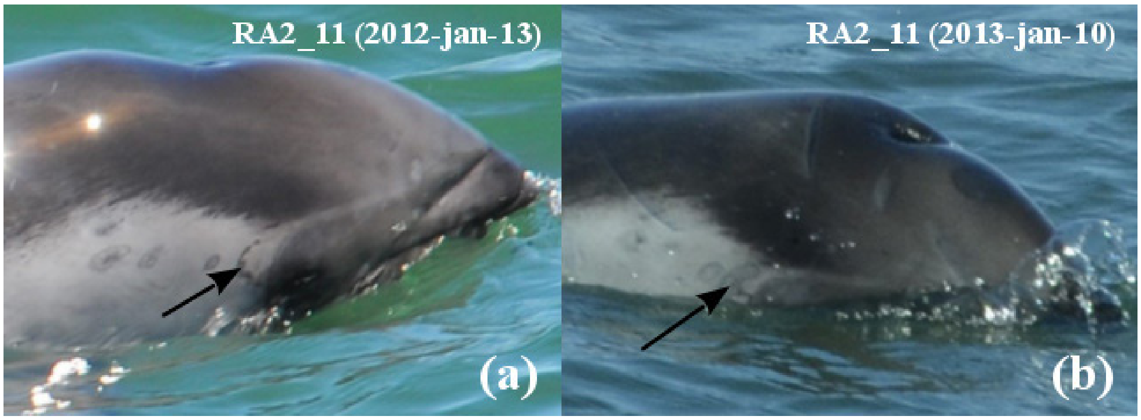

FIGURE 6. Examples of tattoo skin disease in *L. australis* RA2_11, presenting growth in lesion width over a one year period from 13 January 2012 (a) to 10 January 2013 (b).

Pale skin patches

General characteristics

A disorder of unknown aetiology we refer to as pale skin patch syndrome (PSP) was observed in *L. australis* and *C. eutropia*. It was characterized by areas of opaque to translucent skin that seemed to have completely or partially lost their normal pigmentation and had acquired a light gray or whitish coloration. The patches had variable shapes and rounded, distinctive borders (Figure 7). They ranged in size from some square centimeters to an area covering over a quarter of the visible body surface. Though PSP seem flat on most images, they may also appear slightly raised (Figure 7b).

FIGURE 7. (a) Two pale skin patches (PSP) covering a large area of the dorsum in *L. australis* RA1_35; (b) slightly raised PSP (white arrow) on the dorsal fin of *L. australis* RA1_20.

PSP were easy to recognize from shore with binoculars or from boats by direct, close observation. However, under certain incident sunlight angles they went almost unnoticed in some images though they were clearly detected in others belonging to the same surfacing event (*i.e.* RA1_36, RA1_33, RA1_41). Thus, PSP prevalence can be underestimated if this characteristic is not taken into account. In contrast to TSD, PSP was difficult to locate in the lighter body areas of *L. australis*. PSP may develop independently of other skin lesions. However, in six *L. australis* and three *C. eutropia*, the patches were associated with recent skin injuries, mostly tooth rakes (Figure 8).

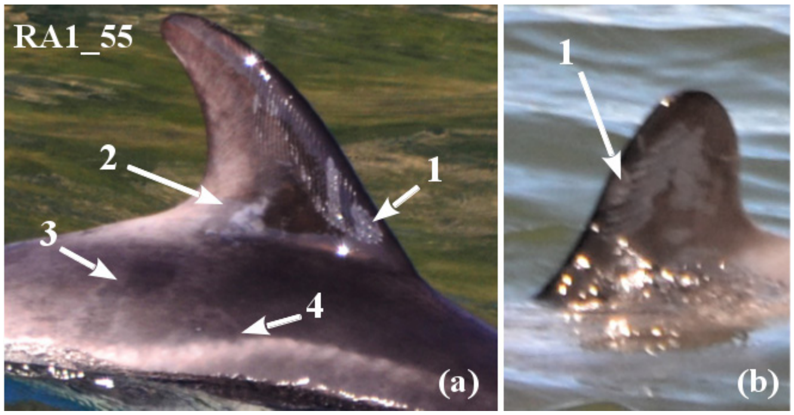

FIGURE 8. (a) Early stage of PSP associated with fresh tooth rakes in L. australis RA1_55 and (b) *C. eutropia* at Añihué Reserve. Arrows indicate the location of PSP and their sequence relative to development stages.

Size time-dynamics of PSP

We detected three stages in the progression of PSP. In the first stage, the marks are opaque, and pale, may have darker borders and a complex shape. The inner core is homogeneous and expands quickly in various directions for about three to four days. In the second stage, PSP stop expanding and the core becomes partially translucent, heterogeneous in small patches and fades from inside-out. In the third and last stage the core darkens while the borders acquire a lighter tone. The patches fade into their original state as they resolve without leaving any permanent marks (Figures 8 and 9). The last stage seems to be as short as the first one. For example in RA2_23 PSP patches had completely disappeared three days after they had last been recorded.

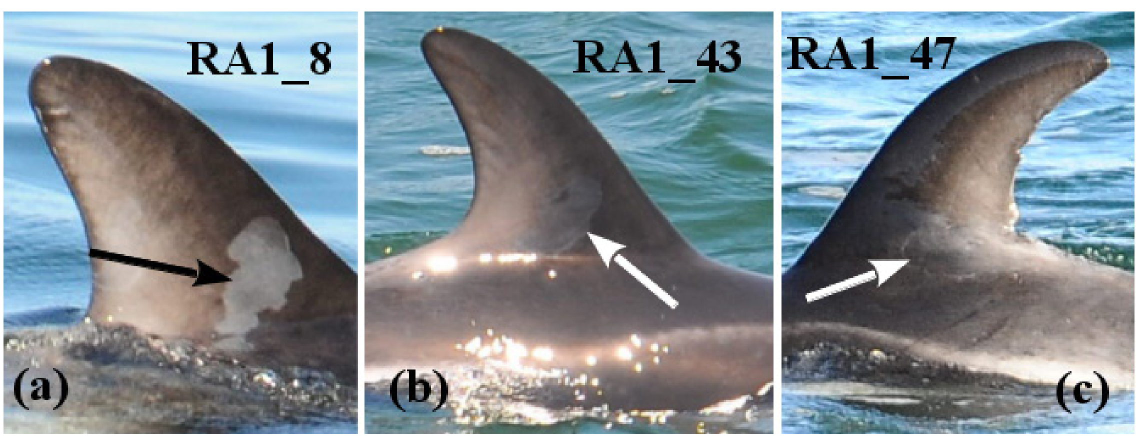

FIGURE 9. Stages of PSP in three *L. australis* at Añihué Reserve: (a) first or expansive stage with homogeneous, opaque aspect, (b) second stage, more translucent but still mostly homogeneous core and (c) third or resolving stage with translucent heterogeneous core and light border.

Generally most PSP cases resolved within 10 days after first detection. However, those located along the longitudinal line below either side of the dorsal fin (basidorsal) tended to persist for a longer time, up to two months in the second stage. Perhaps due to this longer span, the basidorsal area was the most frequent location for PSP stage 2. Table 2 shows the approximate duration for 22 cases of PSP in 13 *L. australis*, the stage at the first record of the event and their location relative to the basidorsal area. Due to the short duration of PSP syndrome (days) it was not possible to assess its daily growth rate.

TABLE 2. Approximate duration of pale skin patches, their development stage at first sight and relative location to the basidorsal area, for 13 *L. australis* ranging in waters of Añihué Reserve

ID code	Approximate duration	Stage	Basidorsal
RA1_27	1) N/A (several months before resighted)	1	no
	2) < 5 days	1	no
	3) N/A (several months before resighted)	2	yes
	4) Almost 2 months	2	yes
RA1_32	1) About 9 days	1	no
	2) < 4 days	1	no
RA1_36	1) 3 months	2	yes
RA1_41	1) 19 days	2	no
	2) 2 days	2	no
	3) 66 days	2	yes
RA1_43	1) N/A (several months before resighted)	1	no
	2) < 50 days	2	no
	3) < 7 days	2	no
RA1_47	1) N/A (several months before resighted)	3	yes
RA1_51	1) < 13 days	1	no
RA1_58	1) < 7 days	2	no
RA2_14	1) < 7 days	3	yes
RA2_20	1) < 2 months	1	no
RA2_23	1) < 3 days	3	no
	2) one month	2	yes
RA3_4	1) < 6 days	1	no
RA3_5	1) < 7 days	1	no

Epidemiology of PSP

Among the 115 photo-identified *L. australis* in Añihué Reserve, 86 (74.8 %) individuals presented at least one event of PSP. Prevalence was highly variable between pods and differences remained stable over time. For instance, we found the same pods with most of their members affected by large PSP during this study. PSP was documented on all individuals independent of their sex or age class. Thus, neonate RA5_1 had its back and flank covered with PSP (see Figure 10).

Recurrence of PSP was identified. After clearing initial PSP patches on its dorsal fin, RA1_32 was resighted with new patches at different stages of development on its flanks eight days later. The dolphin had again cleared the condition after four days. Subsequently, it was seen with stage 2 PSP 17 days later. Direct observation revealed several individuals presenting multiple events of PSP over time, simultaneously on

FIGURE 10. Contrast enhanced photography of neonate *L. australis* RA5_1b with PSP on most of its dorso-lumbar section in Añihué Reserve.

several body areas (RA1_35 among others), and even including cases with superimposed layers of PSP in different (RA1_44) and similar (RA1_33, RA2_14) developmental stages (see Figure 9).

Focal skin diseases

Skin disorders of unknown aetiology characterized by clusters of highly distinct, smallish, round or oval lesions, not attributable to injuries or scars from traumatic source, were classified as focal skin diseases (FSD). In general, the size of FSD lesions was inversely related to their abundance. When occurring in large numbers, the lesions tended to be small-sized and were lighter than the normal skin. Lesion sizes relative to the images were too small to allow to estimate the size time-dynamics of FSD. A light, a dark and an ulcerative pathomorphism (defined below) were recognized during the analysis of FSD. It was unclear whether their aetiology was related.

FSD prevalence

FSD was recorded in 16 of 115 (13.9%) *L. australis*. Some pods presented more affected individuals than others. The most common FSD pathomorphism is illustrated by Figure 11. FSD affected *L. australis* and *C. eutropia* regardless of their sex and age. Young individuals tended to present smaller numbers of lesions and these were more spatially dispersed than in adults. Occurrence of FSD tended to be shared between pod members swimming in association.

Light FSD

Light FSD was characterized by several light gray, small rounded flat marks, distributed in an almost uniform pattern on the *dorsum* and flanks. Light FSD was documented only in Peale's dolphins. In some cases small lesions seemed to have fused to form medium-sized FSD (Figure 11). Density of light FSD greatly varied from few lesions over a wide area to hundreds on the flanks and back (Figure 12).

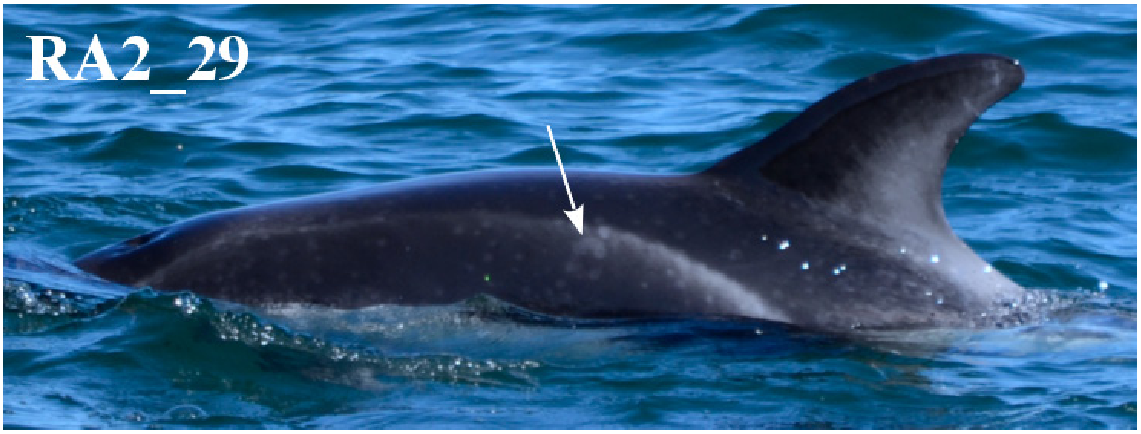

FIGURE 11. Small and medium-sized light FSD lesions (arrow), in *L. australis* RA2_29.

Two FSD affected Peale's dolphins completely cleared the skin disorder after 98 days (RA2_14) and 137 days (RA1_1), suggesting that light FSD is a self-limiting disease that resolves without trace. In both cases the general behavior, including diving pattern, was not visually affected.

FIGURE 12. Numerous light FSD lesions in *L. australis* RA1_1.

Dark FSD

Dark FSD was characterized by numerous small or medium-sized dark grey, round or oval skin lesions that may present "pinholes" in their core and may fuse, occurring on all parts of the visible body (Figure 13). It affected both species.

Though affected individuals did not exhibit noticeable behavioral nor diving pattern alterations, this disease was associated with ocular anomalies in calf *L. australis* RA1_52b that presented many dark FSD around the left eye (Figure 14). In general these lesions resolved within a year leaving indented scars of normal skin color in Peale's and Chilean dolphins at least for the time period of this study. These scars were useful for long-term individual-identification purposes.

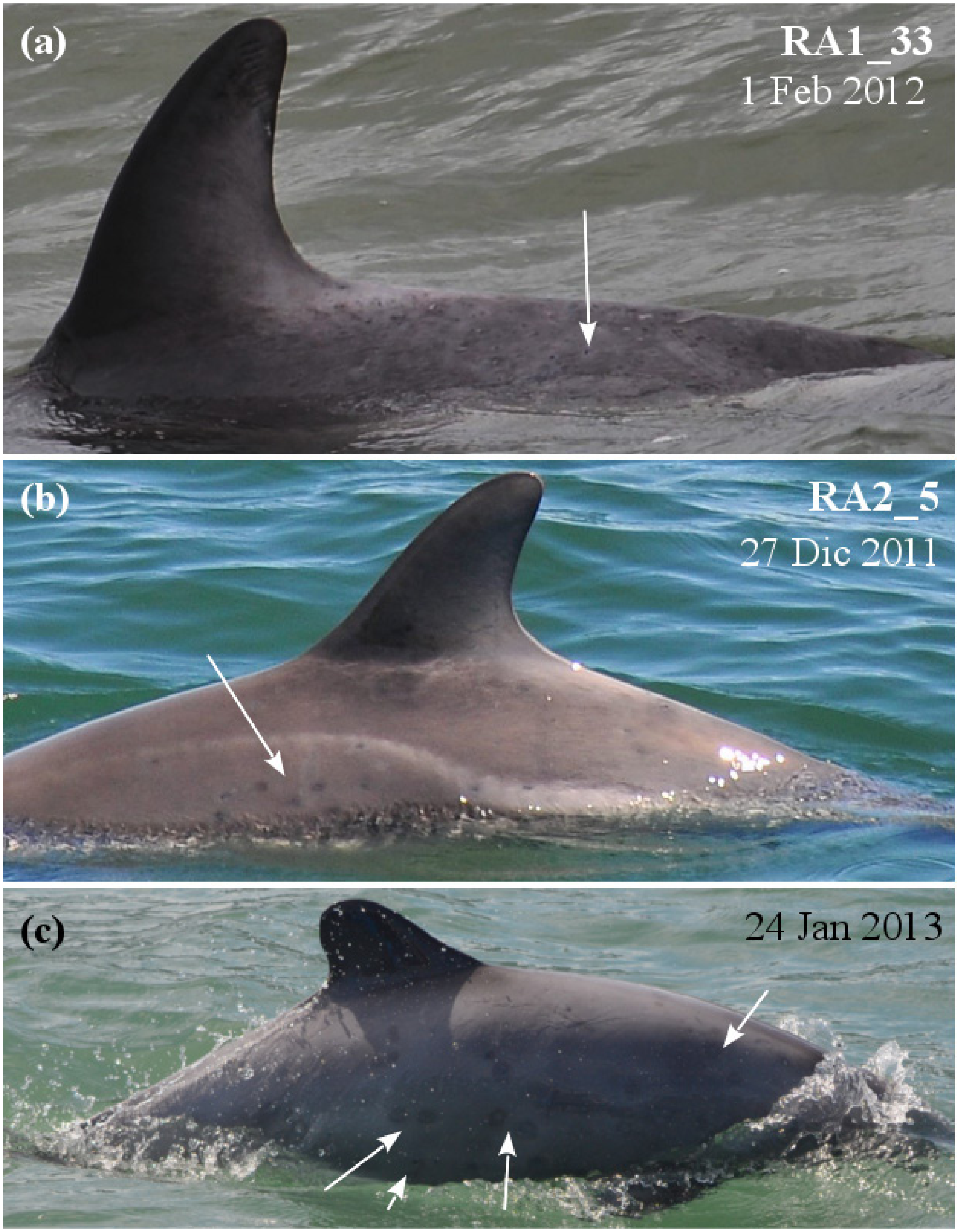

FIGURE 13. (a) Small-size dark focal skin disease lesions (FSD) as "pinholes" (arrow) in *L. australis* RA1_33; (b) cluster of medium-size dark FSD lesions (arrow) in *L. australis* RA2_5 and (c) numerous medium-size dark FSD lesions in an unidentified *C. eutropia*.

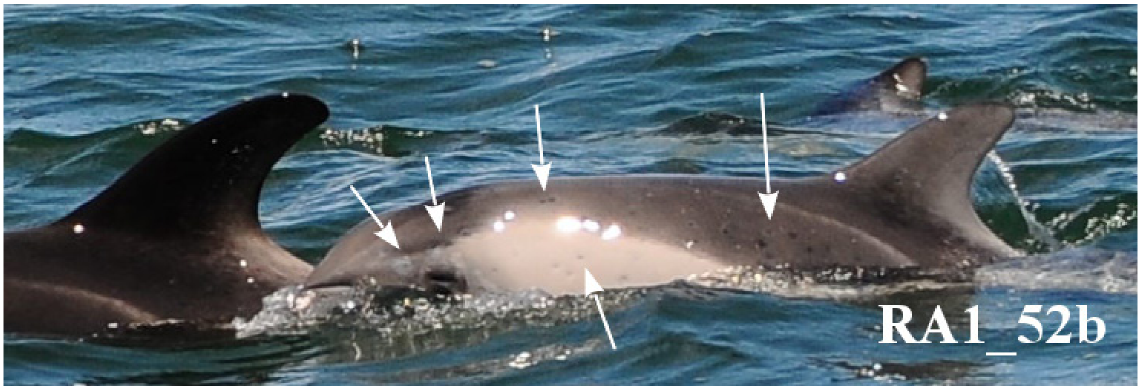

FIGURE 14. Calf *L. australis* RA1_52b affected by FSD lesions in Añihué Reserve.

Ulcerative FSD

On 25 January 2013, a mature *C. eutropia* individual with a severe skin condition was sighted alone, swimming with difficulty close to the shore in Añihué Reserve. The dolphin had rounded dark marks of variable sizes (small to very large) circumscribed by a light-colored outline. The small marks resembled dark FSD but several of the larger lesions were ulcerated. The dorsal fin was almost covered by a massive annular-shaped cutaneous lesion (Figure 15).

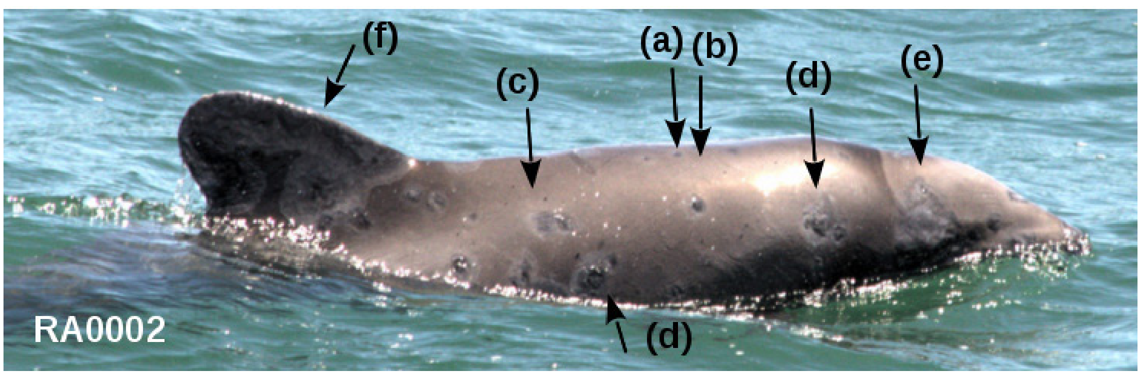

FIGURE 15. Free-ranging *C. eutropia* RA0002 in Añihué Reserve, presenting several small (a), medium (b, c), large (d, e), and very-large fused dark skin ulcers (f).

A male Chilean dolphin was found dead on 5 February 2013 on the shore of Tonina Beach and was re-identified as RA0002 by the type and pattern of some of its skin lesions. Most of the body surface, including the head, flanks, abdomen, peduncle and dorsal fin, presented skin ulcers of variable sizes. The largest were located on the peduncle, thoracic flanks and dorsal fin. Large and medium-sized ulcers seemed composed of the fusion of smaller lesions. Skin loss and tissue destruction exposed deep layers of the dermis (Figure 16). This is the sole case of this pathomorphism we have recorded during this study. The immediate cause of death was attributed to septicaemia following perforation of the main stomach, possibly induced by the high load of anisakid nematodes.

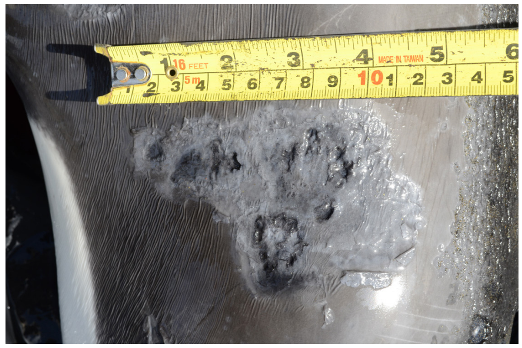

FIGURE 16. Close-up view of a large ulcerated lesion, *ca*. 11 cm in diameter, in the stranded freshly dead *C. eutropia* RA0002.

Skin lineal anomalies

Skin lineal anomalies (SLA) were characterized by dark lines of variable-length, occasionally interconnected, in an intricate reticulated pattern and with borders that were either diffuse or well-delimited (Figures 17 and 18, respectively). Of unknown aetiology, SLA occurred in both *L. australis* and *C. eutropia*. These highly unusual marks did not seem to be associated with any known traumas and did not change in size, or pattern, in up to three years. Their hue lost some contrast with the normal skin in the most recent graphic material. SLA were present mainly on the dorsum and head, and were more extensive in *L. australis* than in *C. eutropia*. The disorder was recorded in 4 (3.48 %) of 115 photo-identified *L. australis* and in one *C. eutropia*. A typical example is illustrated in Figure 17, where RA1_22 also seems to have some deformity of the left hemimandible. The individual was sighted several times over three years and always had these marks. Striking reticulated marks on RA2_17 were not directly associated with any known skin pathology, and did not change over time.

FIGURE 17. SLA with diffuse borders, in *L. australis* RA1_22 from Añihué Reserve.

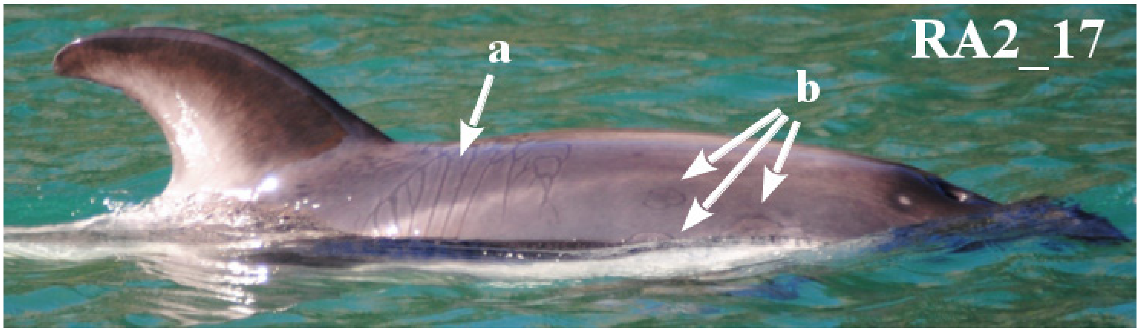

FIGURE 18. SLA on *L. australis* in Añihué Reserve: (a) indicates reticulated lineal marks of unknown aetiology and (b) tattoo skin disease lesions.

Necrotizing tissue mass

Over the course of this study a necrotizing tissue mass (NTM) developed in RA1_40, one of the longest monitored *L. australis* from Añihué Reserve, sighted since July 2011. TSD lesions and tooth rakes by conspecifics were documented in this dolphin since 26 December 2011. The largest bite injury observed on the left side of the dorsal fin's trailing edge in July 2011, mostly healed into a dark scar that progressively became lighter in September 2012. However, the lower end of the wound persisted as a small notch that did not fully heal and repeatedly re-opened. On 13 January 2014, a small swollen mass appeared close to the notch (Figure 19a). Thirty days later it morphed into a necrotizing tissue mass, yellowish with erythematous areas on its periphery, growing and expanding towards both sides of the fin (Figure 19b and c). From a distant resighting on 21 February, although blurry, the mass could still be detected, affecting a larger area with lesser volume and some damage to the lower trailing edge of the fin (Figure 19d).

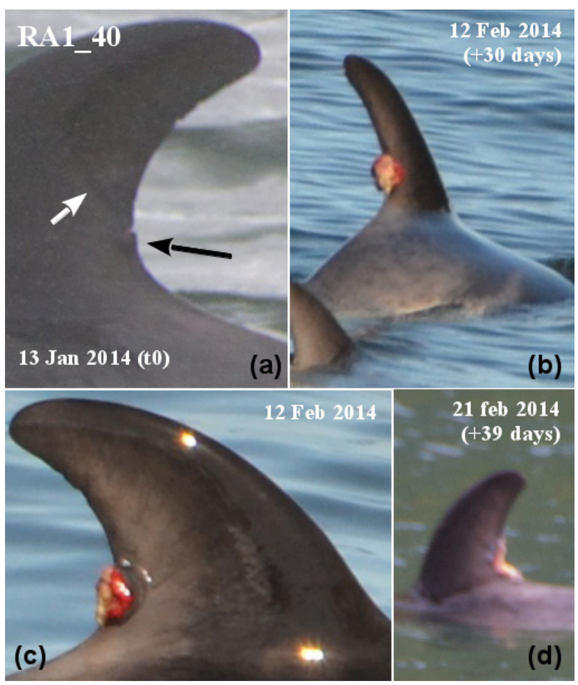

FIGURE 19. Development process of a NTM over a previous injury on the dorsal fin trailing edge of L. australis RA1_40, in Añihué Reserve; (a) local inflammation on small notch; (b, c) NTM developed on the same spot 30 days later; and (d) further extended affected area with possible loss of necrotizing tissue nine days later.

To assess the size time-dynamics of this lesion we measured its total height and the dorsal fin's height. The mass grew quickly, however presented a proportionally decreasing daily growth rate between the second and third time lapses (Table 3).

TABLE 3. Size time-dynamics of a necrotizing tissue mass on dorsal fin of *L. australis* RA1_40 at Añihué Reserve, based on the relative size comparison over time from images taken during three sightings in 2014.

RA1_40 with NTM	T0	T1	T2
Relative measure (s/S)	0.0333	0.2778	0.4933
Sighting record date	13 Jan.	12 Feb.	21 Feb
Time duration relative T0 (days)	0	30	39
Accumulated variation relative T0	0.00 %	733.33 %	1380.00 %
Daily variation rate between events	0.00 %	24.44 %	8.62 %

Ulcerative dermatitis

Since 12 April 2014, several *L. australis* individuals have been sighted with severe skin sores attributed to a shared pathology of unknown aetiology we here refer to as ulcerative dermatitis (UDD). The condition was characterized by single or paired, circular or oval, erythematous ulcers of medium to large size, exposing the hypodermal *stratum* (blubber) or, on the dorsal fin, the subcutaneous fibrous tissue. The ulcers may present a whitish, narrow raised edge of inflamed peripheral tissue. UDD was located on the leading edge of the dorsal fin, thoracic flank and dorsally on the peduncle (Figure 20). It is unclear whether or not UDD is aetiologically related to the pathomorphism we labeled necrotizing tissue mass.

FIGURE 20: Severe cases of ulcerative dermatitis in *L. australis* from Añihué Reserve, exposing areas of subcutaneous fibrous tissue on the dorsal fin of RA1_4 (a, b) and blubber on the tailstock of NN147 (c).

UDD was detected contemporaneously with an intense cold weather front, during which the number of affected individuals grew quickly from one to seven within three weeks. Due to the harsh weather conditions observers produced only limited graphic material, allowing to re-identify only two individuals during the episode (RA1_4 and RA2_26). When the storm dissipated and monitoring conditions improved, only one new case of UDD was documented in a two weeks period.

DISCUSSION

This contribution represents the first dedicated assessment of skin disorders from systematically monitored (photo-identified) coastal dolphin communities in Chilean Patagonia. These waters comprise a suitable area for the study of cetacean health at population level through the assessment of skin anomalies in view of its complex geography and distinct anthropic factors. The high diversity of habitats in this wide geographic area offers an ideal environment for small coastal cetacean populations with high site-fidelity (Sanino and Yáñez, 2012).

During this study most known *L. australis* individuals ranging nearshore of Añihué Reserve were seen with one or more skin conditions. PSP and TSD were most commonly observed in *L. australis* and *C. eutropia*, despite for the latter a populational assessment has not been finalized. Skin diseases and anomalies occurred on all body sections, but not surprisingly, most lesions seen were those exposed during surfacing. To diagnose the aetiology of these syndromes, analysis of fresh samples is obviously needed. While dedicated biopsy sampling may be feasible using modified bow or crossbow systems from shore or boat, concerns exist that unknown pathogens might be inoculated into deeper tissue layers of the sampled individual. Also, intense, acute stress such as from boat chases and targeting could cause widespread ischemic injury to tissues and negatively affect an already health-compromised individual (Bearzi 2000; Cowan and Curry 2008). Instead we recommend a precautionary approach and urge local authorities to coordinate efforts in reporting events of stranded dolphins for their immediate sampling by trained personnel.

Prevalence and morbidity

Compared to previous data (Sanino and Yáñez 2012) for a limited area of Palena Bay between 2010 and 2013, present results show a 30.2 % increase in skin disorders in the *L. australis* community occurring year-round in Añihué Reserve, currently reaching 81.7% prevalence. Evidently, any morbidity at such a rate is a matter of grave concern. An increase in morbidity leading to reduced fitness or increased mortality in Chile's coastal delphinids would add to the unresolved threat posed by long-term anthopogenic mortality caused by bycatch and illegal takes, documented for decades over the entire Chilean coast (Aguayo 1975; Cárdenas *et al*. 1987; Guerra 1984; Guerra *et al*. 1987; Lescrauwaet 1989; Lescrauwaet and Gibbons 1994; Reyes and Oporto 1994; Sanino and Yáñez 2000, 2001b; Sanino *et al*. 2007; Sielfeld *et al.* 1977; Sielfeld 1983; Torres *et al*.1979, 1990; Van Waerebeek and Guerra 1986; Van Waerebeek *et al*. 1994, 1999).

Only two cases of stranded dead dolphins, one *L. australis* and one *C. eutropia*, were recorded in the area and for neither the direct cause of death was attributed to skin diseases, though the *C. eutropia* had extensive ulcerative FSD. Besides, 20 *L. australis* (17.4 % of the studied individuals) have not been re-sighted in more than a year and some may have died, therefore morbidity is unknown. The prevalence of skin conditions is so high that even by slightly reducing individual fitness they may have the potential of impacting significantly at the community and even population level. Skin diseases, such as FSD and PSP also affected *L. australis* calves, sometimes extensively (Figures 3, 10 and 14). Their impact on calf survival and general health should be further explored, especially as calving is very low in *L. australis* from Añihué Reserve (two calves for 115 individuals over 3.5 years; Sanino and Yáñez 2012; Sanino unpublished data).

Tattoo skin disease

TSD had been previously reported to affect *C. eutropia* and *L. australis* in the Guaitecas Archipelago (43.9110°S; 73.6995°W) in 2007 and *C. eutropia* in Reñihué estuary 2002-2004 (Van Bressem *et al.* 2009a), which is consistent with direct observations since 2006 from NOTS along the straits and continental fjords, including Reñihué, in Los Lagos Region (Sanino, unpublished data). During this study TSD was observed in both dolphin species from Añihué Reserve in the period 2010-2013, confirming the presence of this disease along the continental shores of the northern limit of Aysén region. Altogether these data indicate that TSD has been circulating in small cetaceans from Chilean Patagonia for at least 10 years. Prevalence level of TSD in *L. australis* (39.1 %, N= 115) was higher than the prevalence level observed in *L. australis* (27.6 %, N= 29) from Guaitecas Archipelago in 2007, but not significantly so (Z-score 1.152; p= 0.25). It differed significantly however from TSD prevalence in *C. eutropia* (17.4 %, N= 23; Z-score 1.99; p= 0.047) from Guaitecas Archipelago in 2007 (Van Bressem *et al*. 2009a), was significantly higher (Z-score = 2.482; p= 0.013) than in *T. truncatus* from Portugal's Sado Estuary in 1996-1997 (15.6 %, N= 32) while not statistically different from the higher prevalence in 1994-1995 (21.9 %, N= 32) and was much higher (Z-score = 9.42; p< 0.001) than the prevalence in *T. truncatus* from the Strait of Gibraltar (4.5 %, N= 334, Jiménez-Torres *et al*. 2013). Interestingly the higher prevalence levels were seen in communities that were living in a contaminated environment (Sanino *et al*. 2008; Van Bressem *et al.* 2003, 2009a). During this study we observed TSD in juveniles and adults, as reported for other species (Van Bressem and Van Waerebeek 1996). TSD lesions were frequently seen around the eyes of *L. australis* and *C. eutropia* (Figure 2a). A similar corporal distribution was observed in Burmeister's porpoises (*Phocoena spinipinnis*) from Peru and Chile and in harbour porpoises (*Phocoena phocoena*) from the British Isles (Van Bressem *et al*. 2009a).

Pale skin patches

From direct observations by the first author and local inhabitants and from graphic material collected during extensive year-round sailing in the region by tourism operator NOTS, PSP seems to be a recent emerging skin condition in Palena Bay dating back to 2006-2007. Albeit the aetiology is unknown, considering the very high prevalence (74.8 %) in *L. australis,* and concurrent occurrence in *C. eutropia* at Añihué Reserve, it is possible that PSP comprises a contagious disease with no seasonality and with unknown long-term effects on these dolphin communities. In some individuals, PSP were associated with tooth rake injuries and TSD lesions which may further suggest an infectious process. Alternatively, PSP may be multicausative and some cases may result from sloughing of epidermis following long-term permanence of dolphins in quasi fresh water habitat (Norris, 1966; Greenwood *et al*. 1974) in the vicinity of the Palena River mouth (Figure 1) or from contacts with chemical irritants used in the salmon farm industry (see below).

PSP were somewhat reminiscent of "cloudy lesions" described by Wilson *et al*. (1997) in the Scottish Firth of Forth *T. truncatus* population, although cloudy lesions were distinctly heterogeneous in hue.

Focal skin diseases

Focal skin diseases referred to one or more, possibly contagious, diseases of unknown aetiology without seasonality and of significantly lower prevalence in *L. australis* (13.9 %) than PSP (Z-score = 9.867; p< 0.001) and TSD (Z-score = 5.040; p< 0.001). Though similar lesions were described in other species, this is the first record in Chilean waters. Herpesviruses were detected by electron microscopy in non-ulcerative FSD lesions sampled in dusky dolphins (*Lagenorhynchus obscurus)* caught off central Peru, in free-ranging and captive beluga whales (*Delphinapterus leucas*) from the St. Lawrence estuary and the Churchill river, Canada and in a *T. truncatus* stranded along the Atlantic coast of the US (Martineau *et al.* 1988, Barr *et al*. 1989; Van Bressem *et al*. 1994; Manire *et al*. 2006). Graphic material suggests that ulcerative FSD may be an advanced form of dark FSD. The severe lesions observed in the stranded dead *C. eutropia* RA0002 may occur only in terminally ill individuals with a highly compromised immune response. Bertulli *et al*. (2012) documented a minke whale off Skjálfandi Bay, Iceland, in June 2009, exhibiting annular marks that resembled ulcerated FSD.

Skin lineal anomalies

Skin lineal anomalies of various appearances were documented in both species and in low numbers (4 *L. australis* and 1 *C. eutropia*). None of the SLA were related to noticeable changes in behavior or general body condition compared to other group members. Size time-dynamics were not conclusive since we did not notice evident changes during this study, other than a reduced contrast of the lesions over time. Present information does not allow us to relate SLA anomalies to any known pathology. Clearly, additional data are needed to better understand clinical characteristics and its aetiology.

Necrotizing tissue mass

L. australis RA1_40 was the sole individual presenting a NTM. For about 40 days of monitoring, the necrotizing mass continued to grow and it almost doubled in size over nine days. The association of NTM with a preexisting injury, may suggest an infectious process. Regardless of the aetiology of NTM, considering the extensive year-round monitoring effort in Añihué Reserve, the high visibility of NTM and the high site-fidelity of the monitored coastal dolphin populations (Sanino and Yáñez 2012), we regard NTM as a newly recognized pathological condition for the study area.

Ulcerative dermatitis

During a stormy weather period of just three weeks in 2014 ulcerative dermatitis disease emerged in seven *L. australis* (Figure 20). The main differences with ulcerative FSD consisted in the much lower number of sores, a raised peripheral border and a greater depth of the lesions with apparent hypodermal involvement. The aetiology of UDD, and its possible relationship with atmospheric and other environmental variables, are yet to be determined. However, similar lesions were reported in a *T. truncatus* stranded in North Carolina in the 1970s (Cusick and Bullock, 1973). They were caused by *Aeromonas hydrophila*, a bacterium that thrives in the aquatic environment and which has been recorded in the proximity of salmon farms in Chile (Mirand and Zemelman 2002).

Lesion on NN147 (Figure 20c), presented some similarities with the “crater-form” lesions observed in two *T. truncatus* of Monterey Bay in California by Riggin and Maldini (2010). However, the density of the lesions, the width of the borders and the previous “velvety” condition differ from UDD as well as the emaciated condition of some individuals.

Environmental stressors in Añihué Reserve

Natural stressors

The two target species at Añihué were sighted most of the time patrolling or foraging within 100 meters from shore, generally just behind the surf break where depth is just a few meters. Observation sites at Tonina Beach are less than two kilometers from the Palena River’s mouth which has an average flow of 700 m^3/s. CTD results showed that the dolphins inhabit, at least most of daylight time, brackish and almost fresh waters with the biggest salinity range (about 15) within the first 10 meters of depth (Figure 1). Fine-scale diurnal movements within the dolphins’ home range are highly relevant in relation to the amplitude of variations in salinity and water temperature, factors that co-define potential exposures to significant physiologic stress but also as a limitation for infestation due to the unsteady conditions. Water temperature ranged between 12.5ºC and 13.5ºC. In several *T. truncatus* populations, low salinity and low water temperature were associated with a higher prevalence and severity of epidermal lesions, possibly because they alter epidermal integrity or cause a more general physiological stress (Wilson e*t al.* 1999). The water temperature observed during our study equaled Wilson *et al*. (1999) lower values, and salinity was even lower, suggesting conditions of increased susceptibility to skin diseases. Intensely cold water may compromise immune response by limiting blood flow in peripheral body areas (Feltz and Fay 1966).

Anthropogenic stressors

Activities of the local inhabitants of Añihué Reserve include fishing, farming and agriculture, though to a small extent. The small port hosts only about 300 inhabitants. Trash is processed inland preventing it from accessing the water bodies and most products are shipped on ferry from Quellón, Chiloé, or by land from Coyhaique. Previous studies have shown that the development of the extensive sea-pen salmon and mussel farming industry in Chilean Patagonia jeopardizes the possibility of assessing coastal wildlife populations in their natural condition and could threaten their long-term survival (Buschmann *et al.* 1996; Sullivan-Sealey and Bustamante, 1999). Even protected areas such as Añihué Reserve are home to an increasing number of salmon farms. Comprised of moored farms and their associated services, this industry is a potential source of chemical, biological and acoustic pollution, eutrophication, vessel collisions, introduction of exotic species and habitat exclusion (Alvial, 1991; Beveridge *et al.* 1994; Claude and Oporto, 2000; Tovar *et al*. 2000; Heinrich, 2006; Ribeiro, 2003; Ribeiro *et al.* 2005, 2007; Soto *et al.* 2001; Tovar *et al.* 2000; Van Waerebeek *et al.* 2007). Barton (1997), concluded that the number of sea-pen farms has grown at such a rate that it has outpaced the capabilities of the authorities to regulate their activities. Since then, policy changes tending to spatially organize the production in clusters ("barrio salmonero"), macro-zones separated by farm-free corridors and salmon density limits for the farms, have represented significant attempts to contain epizootic events (*e.g.* infectious salmon anemia outbreak in 2007) that may contribute to partially mitigate some of the impacts by the world's second largest salmon producer. However, the main problems persist since the sea-pen method, instead of inland pools, was implemented assuming its compatibility with the environment despite the lack of an in-depth environmental impact assessment. The affected area is immense and continues to expand rapidly, with thousands of applications for Aysén Region. Therefore, the potential for efficient law enforcement decreases due to the increasing number of farms and resulting influence over the decision making process, high costs to access remote sites and the large affected area.

The use of antifouling paints by the shipping and salmon farming industries in the Chilean Patagonia is the main anthropic source of heavy metal (mainly copper) introduction to the environment (Bravo *et al*. 2003, 2005). The International Convention on the Control of Harmful Anti-Fouling Systems on Ships (AFS Convention), which entered into force on 17 September 2008, prohibits the use of organotin biocides (*e.g.* TBT) in anti-fouling paints. As a consequence, ships had to either replace or overcoat their existing organotin-based anti-fouling systems (IMO 2009). Bravo *et al.* (2003, 2005) analyzed the increasing use of antifouling paints by the Chilean salmon farming industry from self-reported information, characterized by 9.6 liters per ton of produced salmon, translating in an estimate of 4.7 million liters of paint containing copper oxide leaching into the environment in 2003 alone. Chilean salmon production is expected to reach record levels in 2014, namely over 500,000 tons (Salmonexpert, 2014). The bioavailable portion of copper oxide that is released to the water column and the sediments below the cages, where it accumulates, varies depending on local factors. Diverse wildlife *taxa* have been reported to be affected by this metal pollutant (see Les Burridge *et al*. 2008; 2010). As top food chain level species, coastal cetaceans are most likely to bioaccumulate these toxins with however unknown health effects.

Although not in our study area, a copper and zinc based mesh has been experimented with as a nylon netting substitute in salmon production processes since 2004 (CODELCO 2009). In seeking to develop new copper applications nationally, antifouling paint is replaced by a recyclable metal grid every 5 years (CODELCO 2009). Its impact on the environment is still to be assessed. However, the first author (GPS) is familiar with the model applied in Auchemó Bay, where preliminary results in Palvitad Fjord (43.0213ºS; 72.8069ºW) have demonstrated similar skin disease occurrence as in Palena. While it is possible that the cumulative effect of the long-term application of antifouling and/or continued use in neighbouring salmon farms in Auchemó may be masking its potential benefits, the effectiveness of this technology in relation to cetacean skin diseases requires a dedicated study.

The generalized use of large volumes of wide-spectrum prophylactic antibiotics by the Chilean salmon farming industry has resulted in the emergence of antibiotic resistance and more virulent pathogenic bacterial recombinants despite present regulation prohibiting the prophylactic use of antibiotics (Cabello, 2004, 2006; Buschmann *et al*. 2012). In this context it is possible that the cutaneous lesions that are emerging in *L. australis* and *C. eutropia* at Añihué Reserve are associated with the increased presence of pathogenic antibiotic resistant bacteria in their environment. Marine mammals and birds in the Northwest Atlantic are reservoirs for potentially antibiotic resistant zoonotic pathogens, that may be transmitted to beach-goers, fishermen and wildlife health personnel (Bogomolni *et al.* 2008). A similar situation may occur in Patagonia. This could have serious impact on the health of local communities.

The pyrethroids cypermethrin and deltamethrin are commonly used by the Chilean salmon industry to control frequent infestations of *Caligus rogercresseyi*, so-called sea lice (Crustacea, Copepoda, Caligidae). These persistent organic pesticides are highly toxic to fish, algae and aquatic invertebrates (Mauck and Olsen, 1976; Ural and Saglam 2005; WHO 2009). Though their long-term effects on marine mammals are poorly known, recent data indicate that several pyrethroids, including cypermethrin and deltamethrin, bioaccumulate in franciscana dolphins (*Pontoporia blainvillei*) and are transmitted during gestation and lactation (Alonso *et al*. 2012). Besides, in other mammal species these compounds affect the central nervous system, have significant adverse effects on the reproductive system, cause endocrine disruption, are genotoxic and suspected carcinogens (Jiménez *et al*. 2008; Watts 2014a,b). Pyrethroids are skin and eye irritants in humans (Sheikh 2012) which suggests that they may have played a role in the aetiology of some of the cutaneous disorders observed in dolphins from Añihué Reserve. Worryingly, ocular defects are also increasingly observed in these dolphins (G.P. Sanino, pers. observations). In this context, the precautionary principle is strongly advised.

Management recommendations

Cutaneous diseases in resident or semi-resident coastal cetaceans may serve as bioindicators of the overall health of their habitat. Photographic surveys serve as a non-invasive and relatively low-cost research method as to document epidemiological characteristics and progression of disease within the studied cetacean communities (Wilson *et al.* 1999; Van Bressem *et al.* 2003, 2009b; this paper). Future work should preferentially include tissue collection from stranded specimens for histopathologic and molecular analysis, as biopsy sampling would incur risks of disseminating infections and may be acceptable only as a last resort. Stranded cetacean carcasses can provide relevant biological and pathological information depending on their decomposition degree and should be addressed by qualified personnel. The Chilean National Museum of Natural History (Museo Nacional de Historia Natural) has the expertise and legal mandate to accomplish these tasks (Sanino and Yáñez, 2005).

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