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Short Communication

Analysis of epibiont data in relation with the Debilitated Turtle Syndrome of sea turtles in *Chelonia mydas* and *Lepidochelys olivacea* from Concepción coast, Chile

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ABSTRACT. Epibionts on the surface of the skin and shell of a specimen of *Chelonia mydas* and three *Lepidochelys olivacea* found floating on the coast of Concepción, Chile, between June 2010 and December 2012, were analyzed. These epibionts were analyzed during the clinical inspection and the tissue changes related to its settlement, with filamentous algae around, were observed. Subsequently, the epibionts were identified by morphological observation. The knowledge about theses epibionts in Chile is reviewed and the potential occurrence of Debilitated Turtle Syndrome (DTS) in these turtles is discussed. The presence of sea turtles in the Chilean coast is considered a casual event, so there is little information on this issue. We propose it is necessary to carry out more studies on the association between turtles and epibionts because their identification, colonizing reptiles' surface may give relevant information to a better understanding of different diseases, including DTS, that affect these marine reptiles and facilitates the development of strategies intended to recover their populations.

Keywords: *Lepidochelys olivacea*, *Chelonia mydas*, sea turtles, epibionts, Concepción coast, Chile.

Análisis de los datos de epibiontes en relación con el Síndrome de Debilitamiento de Tortugas marinas en *Lepidochelys olivacea* y *Chelonia mydas* de la costa de Concepción, Chile

RESUMEN. Se presenta el hallazgo de epibiontes en la superficie de la piel y caparazón de un ejemplar de *Chelonia mydas* y tres de *Lepidochelys olivacea* encontrados flotando frente a la costa de Concepción, Chile, entre junio de 2010 y diciembre de 2012. Los epibiontes se visualizaron clínicamente y se observaron alteraciones tisulares relativas a su asentamiento junto con algas filamentosas a su alrededor. Los epibiontes se identificaron por observación morfológica. Se presenta una revisión respecto del conocimiento en Chile de los organismos encontrados y se discute la potencial ocurrencia del Síndrome de Debilitamiento de Tortugas (SDT) en los caparazones analizados. La presencia de tortugas marinas en el litoral chileno es un evento ocasional y existe escasa información respecto de esta problemática. Se recomienda realizar estudios sobre la asociación entre las tortugas y epibiontes porque su identificación, colonizando la superficie de estos reptiles, puede aportar datos relevantes para una mejor comprensión de las enfermedades que afectan a las tortugas marinas, incluyendo el SDT, y facilita el desarrollo de estrategias destinadas a recuperar sus poblaciones.

Palabras clave: *Lepidochelys olivacea*, *Chelonia mydas*, tortugas marinas, epibiontes, costa de Concepción, Chile.

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Among the seven sea turtles species existing worldwide, four of them have been recorded in Chilean waters: Green turtles (*Chelonia mydas* Linnaeus, 1758), Loggerhead turtles (*Caretta caretta*, Linnaeus 1758), Leatherback turtles (*Dermochelys coriacea* Vandelli, 1761) and Olive Ridley turtles (*Lepidochelys olivacea* Eschcholtz, 1829). Although few turtles are documented in northern Chile, their movements into Chilean waters is considered passive and likely attributable to oceanic currents, meteorological events and atmospheric changes due to El Niño Oscillation events (Ibarra-Vidal & Ortiz, 1990). On the other hand, in certain seasons, when the littoral waters of central to southern Chile are suitable, their pelagic habits may allow them to migrate and temporarily occupy habitats beyond their typical southernmost range in northern Chile reaching the waters of the present study area (Azócar *et al.*, 2011).

Sea turtles are an appropriate substrate for the attachment of a great variety of algae and invertebrate larvae present in the water column. This attachment results in associations of the commensal, opportunistic and mutualism type with organisms such as cirripeds, algae, bryozoans, cnidarians, polychaetes and amphipods among others (Frick & Pfaller, 2013). Because these associations occur only when the distribution of these species and their hosts overlap, these organisms can be important biological markers of the movement patterns and geographical distribution of sea turtles, as well as indicators of host turtle activity and migration routes (Eckert & Eckert, 1988).

From June 2010 to December 2012, one specimen of *Chelonia mydas* and three of *Lepidochelys olivacea* were found at four different localities along the central-south coast of Chile, particularly in the littoral habitats of the Concepción coast (36°46'22''S-37°23'52''S). The four turtles were collected by the National Fishery Service of Chile and they were sent to the Veterinary Hospital of San Sebastian University, Concepcion, Chile. Species were identified on the basis of morphological characteristics, as defined by Márquez (1990), and sex, maturity status, length and weight for each animal were also recorded (Table 1). The health condition of each animal was determined through clinical examinations all of which showed that the four turtles in question were clinically unhealthy (inattentive to surrounding stimuli, general weakness, plastron weakness and lesions of diverse degrees, notably ocular ulcerations and scars in the skin and carapace). Haematology and clinical biochemistry tests were perfomed and obtained values from the four turtles showed alterations when compared with normal reference values (Anderson *et al.*, 2011; Santoro & Meneses, 2014). Consequently, condition of the four animals determined their hospitalization and emergency clinical procedures were applied. After a careful external examination (carapace, plastron, flippers, skin, head and neck, cloaca and tail) epibiotic organisms and algae were removed using tweezers and a spatula, fixed in 10% buffered formalin and finally placed in 70% ethyl alcohol for further analysis. Epibionts and algae were sent to the Laboratory of Parasitology, Faculty of Biological Sciences, University of Concepcion, Concepción. Chile where, using light microscopy and bibliographic references (Hoffmann & Santelices, 1997; Young, 1999), specimens were identified to the lowest possible taxon.

The taxonomic identification of epibionts and algae found on the four turtles is detailed in Figure 1 and Table 1. In the case of epibionts found on *C. mydas*, they were two specimens of *Chelonibia testudinaria* located on the second central scute and second lateral scute, as two masses firmly adhered to the surface and with abundant filamentous algae of the genus *Ulva* (Case study 1). One *L. olivacea* turtle, whose clinical condition was considered as the most serious, displayed the highest density of epibiont aggregations and died shortly after collection (Case study 3). On *post-mortem* exami-nations, this turtle had emaciation and abundance signs of secondary infections. The remaining three turtles recovered satisfactorily and where set free along the coast of northern Chile. The epibionts of *L. olivacea* were typically represented by masses of filamentous algae and sand. Goose barnacles *Lepas anatifera* were observed from all three *L. olivacea* at various locations on the skin, including both front flippers on one animal (Case study 2), on the left front flipper of the second (Case study 3) and on the right back flipper of the third turtle (Case study 4). Additionally, two *L. olivacea* hosted numerous hydrozoans growing in association with the macroalgae *Ulva* on several carapace scutes where sandy areas had accrued (Case study 2 and 3). The most intensively colonized animal also showed the presence of algae of genera *Polysiphonia* and *Rhodymenia* (Case study 3).

In Chilean waters, the presence of epibionts in *C. mydas* is recorded in only five specimens at the northern coast, revealing the presence, on the shells, of gastropod mollusks and crustacean belonging to the families Talitridae, Aoridae and Gammaridae (López *et al*., 2007). Although *C. testudinaria* has already been reported in Chilean waters (Bettini & Ross, 2002), this is the first report of this organism attached to a *C. mydas* in Chile. *C. testudinaria*, an obligate commensal of motile marine animals with a cosmopolitan distribution, is frequently found on the shell and plastron of turtles but they also can be found on the head, flippers and skin (Frick & Ross, 2001). Three species of cirri-

Table 1. Sea turtle species, location rescue, reproductive and biometrical characteristics, epibionts and algae found of four sea turtles from south-central Chile (2010-2012).

Case report sea turtle species Date/Location	Reproductive and biometrical characteristics	Epibionts and algae (number of specimens/locations)
Case 1 *Chelonia mydas* 06/21/2010 Itata Beach (36°37'0"S, 72°57'00"W)	Adult, male Weight: 48 Kg Lenght: Head: 34 cm; tail: 27 cm Carapace: lenght:78 cm width: 74 cm	- *Chelonibia testudinaria* (2/carapace) - *Ulva* sp. (carapace)
Case 2 *Lepidochelys olivacea* 08/12/2011 Penco Beach (36°43'0"S, 72°58'00"W)	Adult, male Weight: 32 Kg Lenght: Head: 25 cm; tail: 24 cm Carapace: lenght:68 cm width: 70 cm	- Hydrozoa (55/carapace) - *Lepas anatifera* (10/anterior flippers) - *Ulva* sp. (carapace)
Case 3 *Lepidochelys olivacea* 05/30/2012 Tubul Beach (37°13'44"S, 73°26'36"W)	Adult, female Weight: 45 Kg Lenght: Head: 25 cm; tail: 20 cm Carapace: lenght: 67 cm width: 71 cm	- Hydrozoa (17/carapace) - *Lepas anatifera* (3/left anterior flipper) -*Ulva* sp., *Polysiphonia* sp.,*Rhodymenia* sp. (carapace)
Case 4 *Lepidochelys olivacea* 12/11/2012 Talcahuano Beach (36°43'0"S; 73°07'00"W)	Adult, female Weight: 35 Kg Lenght: Head: 22 cm; tail: 18 cm Carapace: lenght:67 cm width: 70 cm	- *Lepas anatifera* (8/right back flipper)

peds and one decapod has been reported on *L. olivacea* turtles captured along the central to southernmost portions of Chile (Retamal & Hermosilla, 1969; Brito, 2007). Meanwhile, *Lepas anatifera*, is an opportunist pelagic barnacle common in all oceans, including Chilean waters (Hinojosa *et al.*, 2006). It is frequently observed on the carapace region and skin of turtles of different species that spend a great deal of time at the water's surface –including healthy turtles. This barnacle has been detected on the carapaces of two specimens of *L. olivacea* along the central-south coast of Chile (Miranda & Moreno, 2002). Their occurrence on the tips of the front and rear flippers of turtles, however, is generally indicative of limited flipper activity by host turtles, and could indicate relatively inactive turtles (M.G. Frick, *pers. comm.*). Although, hydrozoans specimens found on *L. olivacea* have not been identified, their presence associated to barnacles, such as *Lepas anatifera* and algae would be frequent due to their necessity to occupy the surface of these animals to settle, feed and reproduce themselves. With respect to the algae observed on the turtles examined here, their presence on the surface of the epibiotic barnacles we observed is not surprising as all are common and often lead to the recruitment of other epibiotic forms onto host turtles (Scharer, 2003). In the present case, however, the density and size of some of the algal species encountered herein suggests that a potential decrease in the activity of the host turtle is likely the responsible for the observed growth. Algae of genera *Ulva*, *Polysiphonia* and *Rhodymenia* are common in the Chilean coast (Hoffmann & Santelices, 1997).

The observation of specimens of sea turtles into the waters of central-southern Chile is unusual and, in accordance with our experience, it is the result of an event implying some damage or health deterioration of the animal. While meteorological events may be involved, we think that the debilitated state of the turtles of this study likely represent turtles that were cold-stunned, then got sick, and then were passively carried into more southerly waters. Cold-stunning symp-

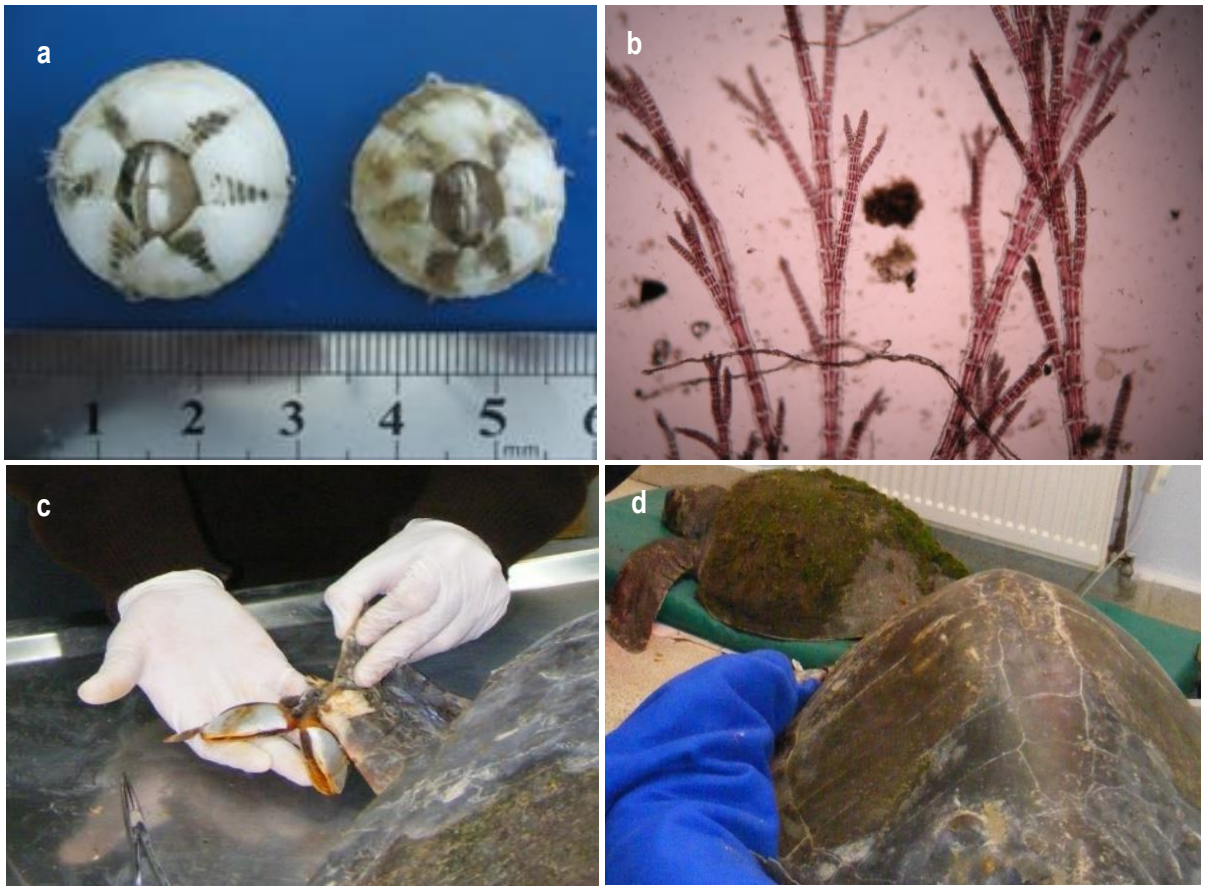

Figure 1. Macroscopic and microscopic features of epibionts and algae observed on sea stranded turtles at the coast south central Chile (2010-2012). a) *Chelonibia testudinaria* extracted from *C. mydas* (2010), b) *Polysiphonia* sp. from *L. olivacea,* 100x (2012), c) two specimens of *Lepas anatifera* (back-lateral location; left anterior flipper of *C. mydas* (2012), d) Turtle's carapace (*L. olivacea*) densely colonized by epibionts and algae.

toms, such as debilitation, lethargic movement, scarce mobility in head and flippers and feeble attempts to dive, may be present when sea turtles are exposed to rapidly cooling water temperatures or are exposes to water temperatures of less than 15°C (Milton & Lutz, 2003) as it is the case of waters of central-southern Chile (Sobarzo, 1994). Moreover, we believe that the four turtles suffering Debilitated Turtle Syndrome (DTS), based on the clinical features described above and the results of haematology and clinical biochemistry tests. Turtles exhibiting symptoms of DTS are characteristically emaciated, anemic, hypoglycaemic, and are often covered by barnacles when they strand (Sloan *et al*., 2014). Consistent with this, it was remarkable that at least two of the four turtles (case study 2 and 3) of this study showed low plasmatic concentration of total protein, albumin, cholesterol and glucose, which could be attributed to poor nutrition or altered carbohydrate metabolism by liver damage. In addition, the same turtles showed high levels of uric acid and urea nitrogen, elements which are considered as the best indicators of liver damage, because both are end products of protein metabolism. Therefore, these values may reflect poor nutrition, protein loss due to diarrhea or nephropathy, chronic infection, parasitism, immune deficiency related to their state of weakness, or a combination of all these causes (Deem *et al*., 2009). This condition (DTS) may have favoured the epibiont colonization. The state of debilitation of turtles were suffering DTS results in a sustained period of reduced activity, stationary floating and decreased grooming, which likely facilitates algae and invertebrate larvae attachment (Day *et al*., 2010). Current information indicates that debilitated turtles are immunosuppressed or lethargic prior to barnacle colonization and that limited mobility by the host likely facilitates rapid and prolific colonization of barnacles (Frick & Pfaller, 2013). Likewise, the type of epibiont growth and the effects associated reported here would represent secondary agents in the decline in the health of these turtles.

We conclude it is necessary to realize the studies on the association between turtles and epibionts because their identification, colonizing reptiles' surface may give relevant information to a better understanding of different diseases that affect marine turtles and facilitates the development of strategies intended to recover their populations.

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