

## Remodelling of skeletal tissues bone and structural specialisations in an elasmosaurid (Sauropterygia: Plesiosauroidea) from the Upper Cretaceous of Patagonia, Argentina

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(Received 3 September 2013; accepted 5 November 2013)

Elasmosauridae were cosmopolitan Late Cretaceous plesiosaurs with conspicuous morphological diversity. Within this group, vertebral morphology is a criterion for estimating relative age in plesiosaur. On the other hand, the microstructure of plesiosaur bone is considered as indicative of ontogenetic stage. However, knowledge about ontogenetic tissue transformation in different elements of the skeleton is poorly known. Resorption and remodelling of skeletal tissues are required for development and growth, mechanical adaptation, repair and mineral homeostasis of the vertebrate skeleton. This contribution analyses different postcranial elements of a Late Cretaceous elasmosaurid from Patagonia. Characterisation of bone microstructure indicates the presence of compact bone inner organisation in an adult derived plesiosaur from the Cretaceous and that the distribution of bone specialisations depicts conspicuous variations within a single skeleton depending on the skeletal element considered. Bone compactness or degree of remodelling in elasmosaurids is not necessarily correlated with the ontogenetic age of the animal or to costal versus pelagic lifestyles. The available data are still scarce, but we propose a topic of discussion: perhaps the degree of remodelling and compactness also may be related to the activity level and increased mechanical load in different skeletal elements.

**Keywords:** bone histology; microanatomy; plesiosaur; Late Cretaceous; Patagonia

### Introduction

Elasmosauridae (Sauropterygia, Plesiosauroidea) were cosmopolitan Late Cretaceous plesiosaurs (Gasparini et al. 2003; Vincent et al. 2011) with conspicuous morphological diversity, including numerous small-headed forms such as *Elasmosaurus* up to aberrant and unusual large-headed forms such as *Aristonectes parvidens* (O’Keefe 2001; Otero et al. 2012; O’Gorman et al. 2013). Histological features of plesiosaurs are known from the study of limb bone (propodials and phalanges), vertebra and rib sections (Kiprijanoff 1881–1883; Biró-Bagóczy and Larraín 1986; Wiffen et al. 1995; Fostowicz-Frelik and Gazdzicki 2001; Salgado et al. 2007; Street and O’Keefe 2010; Liebe and Hurum 2012). Within this group, as well as in other plesiosaurs, vertebral morphology has been used for estimating relative age (e.g. the fusion of the neural arch to the vertebral centrum, Brown 1981). On the other hand, the microstructure of plesiosaur bone is considered as indicative of ontogenetic stage. Traditionally, it had been hypothesised that the pachyostotic *sensu lato* (see Houssaye 2009) skeletons of juvenile plesiosaurs indicated coastal habits, while lightened skeletons of adults indicated pelagic lifestyle (Wiffen et al. 1995; Fostowicz-Frelik and Gazdzicki

2001). In this paper we present evidence for compact bone inner organisation in adult elasmosaurid from the Upper Cretaceous of Patagonia. Wiffen et al. (1995) hypothesised that the condition of plesiosaurian bone tissue changes throughout ontogeny. However, the knowledge we have about ontogenetic tissue transformation in different elements of the skeleton is still scarce. Besides increased and/or decreased bone mass, an interesting aspect to consider is resorption and remodelling of skeletal tissues. These two processes (resorption and remodelling) of the vertebrate skeleton are essential processes for development, growth, repair, mechanical adaptation and mineral homeostasis (Witten and Huysseune 2009). Vertebrate hard tissue structures are never permanent and thus the skeleton is subject to constant reshaping (bone and cartilage), remodelling (bone) and replacement (teeth) (Hall and Witten 2007). Also, different bones from the same individual vary in bone microstructural organisation (Castanet et al. 2000).

In order to analyse the use of the degree of compaction and/or remodelling as a relative indicator of ontogenetic stage, we selected one specimen of an elasmosaurid from the Upper Cretaceous of Patagonia and analysed the microstructural pattern of postcranial elements within a single skeleton.

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## Materials and methods

In order to explore the microstructural pattern of postcranial elements, representing the axial and appendicular skeletons, we selected one specimen of an elasmosaurid plesiosaur (MUC-Pv 92), recovered from Campanian–Maastrichtian levels of the middle member of the Allen Formation surrounding Lake Pellegrini (67°44'W; 38°33'S), Patagonia, Argentina. These levels are characterised by massive or finely laminated claystones and siltstones. The heterolithic structures represent alternating periods of traction and decantation and suggesting tidal flat environment (Gasparini et al. 2007). MUC-Pv 92 has been identified as an *Elasmosauridae* indet. (Gasparini and Salgado 2000) based on anterior cervical vertebrae that are longer than high, joint faces and bilobed lateral crest, three typical characters of Cretaceous elasmosaurids (Brown 1993; Bardet et al. 1999). Based on the degree of fusion between centres and vertebral arches, present in all regions except in the caudal, O'Gorman et al. (2011) considered this specimen as an adult.

Within the material available and authorised we made thin transverse sections of the selected bones: one caudal vertebra (MUC-Ph 26), two indeterminate bones of the pelvic girdle (MUC-Ph 10 and MUC-Ph 28), one distal end of the humerus (MUC-Ph 27) and one rib fragment (MLP-Ph 65). Thin sections were obtained following Chinsamy and Raath (1992) protocol, at Departamento de Geología de la Universidad Nacional de San Luis (Argentina). Some thin sections have been described previously by Salgado et al. (2007) (MUC-Ph 10, MUC-Ph 26, MUC-Ph 27 and MUC-Ph 28). This paper reviews and improved that description, including an examination under polarised light. To facilitate comparisons between sections with clear criteria, compactness index (CI) was calculated by means of the software ImageJ (Abramoff et al. 2004) for all sections as the area occupied by bone multiplied by 100, divided by the total sectional area (Houssaye and Bardet 2012). Images of each thin section were captured by a digital camera and were reassembled in Adobe PhotoShop. Nomenclature and definitions of structures used in this study are derived from Francillont-Vieillot et al. (1990) and de Ricqlès and de Buffrénil (2001).

## Institutional abbreviations

MUC-Pv, vertebrate palaeontology collection of the Museo de la Universidad Nacional del Comahue, Neuquén Province, Argentina; MLP-Ph, palaeohistology collection of the Museo de La Plata, Buenos Aires Province, Argentina; MUC-Ph, palaeohistology collection of the Museo de la Universidad Nacional del Comahue, Neuquén Province, Argentina.

## Results

### Caudal vertebrae sections

#### MUC-Ph 26

Transverse section was obtained from the vertebrae comprising part of the centrum and neural arch. The demarcation between the periosteal cortical and endosteal trabecular regions is not very distinctive. Both areas are made of dense compacted bone tissue. The transverse sections show a compact bone (Figure 1a and b), increasing the degree of porosity to the area between the centrum and neural arch (CI = 84.6%) (Table 1 and Figure 3a). This area is occupied by large internal spaces and these spaces are variably shaped and surrounded by lamellar tissue (Figure 1c). The outermost part of the cortex contains mainly primary and secondary osteons in different stages of formation and a uniform matrix of interstitial tissue. The matrix is rich in osteocyte lacunae with a chaotic arrangement. Osteocyte lacunae have an elliptical shape with poorly developed *canaliculi*. The arrangement of the collagen fibres in the woven component of the matrix does not have a large spatial ordering. Growth marks are not observed.

### Pelvic girdle sections

#### MUC-Ph 10

The cross section shows that the bone is relatively compact, especially in the cortical region (Figure 1f), appearing somewhat spacious internally and in some sectors of the periphery (CI = 69.8%) (Table 1 and Figure 3b). In the medullary area, the tissue shows several different form spaces (resorption areas) surrounded by endosteal lamellar tissue. The degree of vascularisation varies from poor to moderate. In the inner region, the spaces become more developed than in the cortex, forming cancellous bone (Figure 1d and e). The osteocyte lacunae are abundant in the whole sample and in general are randomly arranged. The osteocyte lacunae are variable in shape ranging from elliptical to stellate and have well-defined *canaliculi* but not very numerous. In the cortical region, it is possible to see some secondary osteons and primary osteons in different stages of formation. Growth marks are not observed.

#### MUC-Ph 28

A cross section was made to a flat bone, with approximately parallel surfaces. Internally, the bone has some spaces that do not amount to a genuine, cancellous bone (CI = 87.5%) (Table 1 and Figure 3c). Externally, the bone looks compact (Figure 2a and b), although towards the thickest part of the bone it shows an increased degree of development of resorption areas. In the deepest

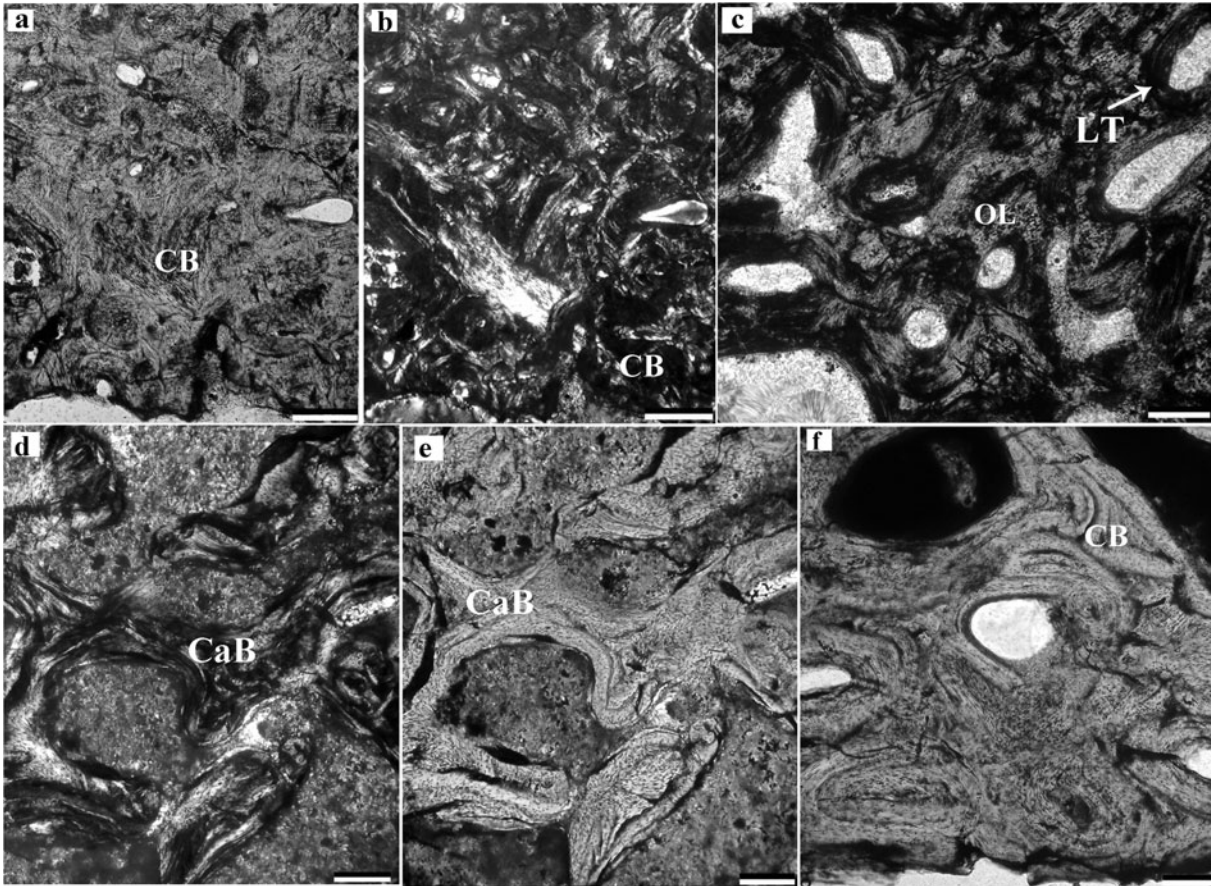


Figure 1. Elmosaurid plesiosaur (MUC-Pv 92). Caudal vertebrae sections (MUC-Ph 26): (a and b) view of cortical region and (c) view of medullary region. Pelvic girdle sections (MUC-Ph 10): (d and e) view of medullary region and (f) view of cortical region. Parts a, e and f were in normal light and b–d were in polarised light. Scale bar: 500  $\mu$ m. *Abbreviations*: CaB, cancellous bone; CB, compact bone; LT, lamellar tissue; OL, osteocytes lacunae.

area of the bone there are large internal spaces, surrounded by lamellar tissue. As in the previous sample, note that the tissue is poorly to moderately vascularised (Figure 2c). The cortical regions show a modest Haversian secondary tissue development. The secondary osteons are so variable. There are two or more generations osteons overlapping side, which shows the intensity of remodelling processes internal. In turn, the primary osteons are in different stages of formation. There are large amounts of osteocytes lacunae and well-developed *canaliculi*. Growth marks are not observed.

Table 1. List of the material analysed, with corresponding indices.

Bones (elements)	Collection number	CI (%)
Rib	MLP-Ph 65	91.7
Pelvic girdle	MUC-Ph 28	87.5
Caudal vertebrae	MUC-Ph 26	84.6
Humerus	MUC-Ph 27	82.9
Pelvic girdle	MUC-Ph 10	69.8

### Humerus sections

#### MUC-Ph 27

Cross section of the humerus below the tuberosity was studied. A cortical and medullar region cannot be differentiated. The medullar cavity is occupied by some internal spaces, these spaces are surrounded by lamellar tissue but not constituting a true cancellous bone or trabecular bone (CI = 82.9%) (Figure 2d and e). The cortex consists of compact bone with some spaces surrounded by lamellar tissue and primary and secondary osteons (Figure 2f) (Table 1 and Figure 3d). Flattened osteocyte lacunae are distributed throughout the sample. These lacunae have good amount of developed *canaliculi*. The primary bone, in the peripheral cortical region show, has large development osteocyte lacunae elliptical or stellate randomly distributed in the matrix and have a great development of radial *canaliculi*. In these same sectors it is possible to observe the presence of Sharpey's fibre groups. No growth marks are evident. The humerus of the specimen studied is more remodelled of the humerus of

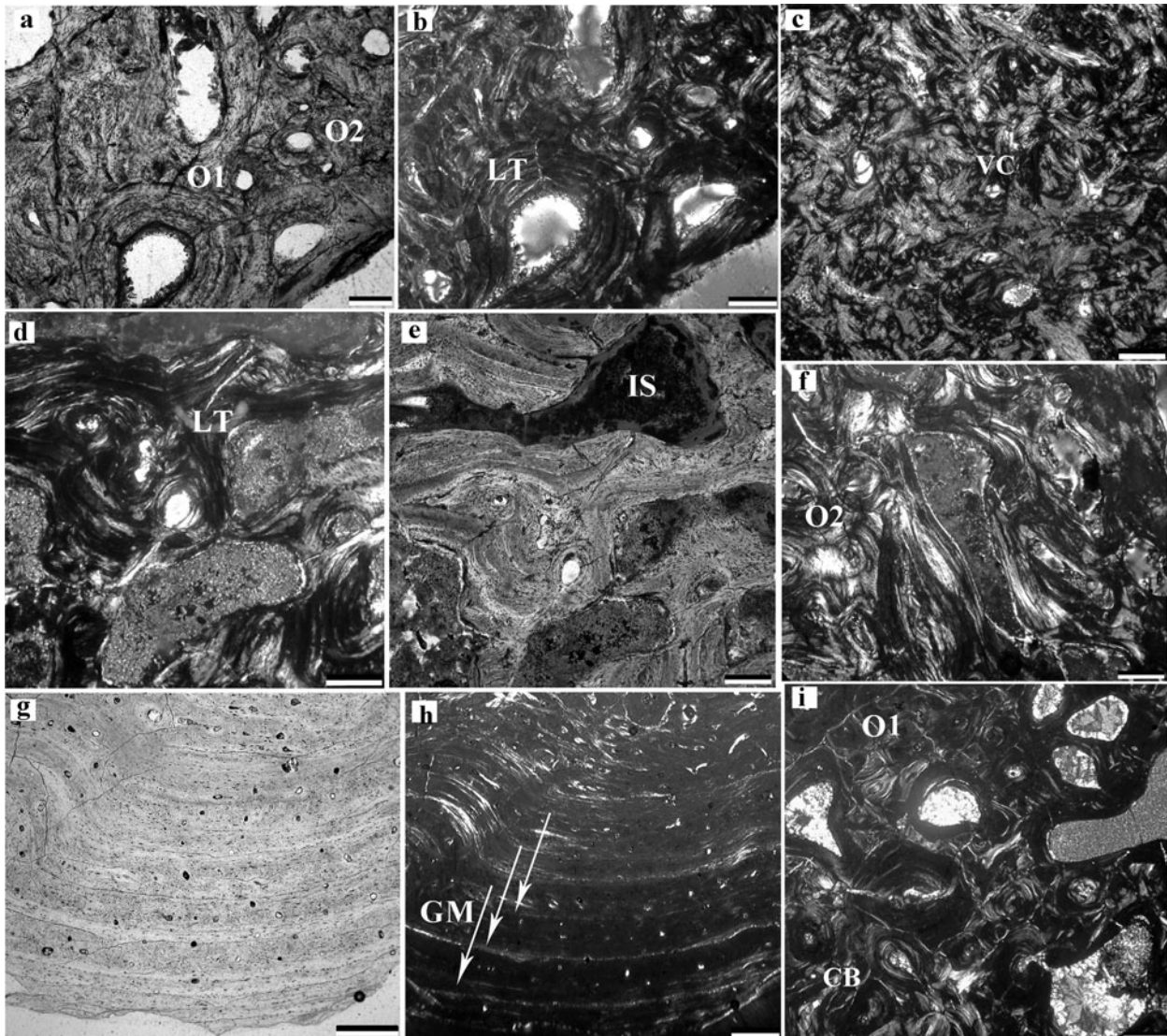


Figure 2. Pelvic girdle (MUC-Ph 28): (a and b) view of cortical region and (c) view of medullary region. Humerus sections (MUC-Ph 27): (d and e) view of medullary region and (f) view of cortical region. Rib sections (MLP-Ph 65): (g and h) view of cortical region; (i) view of medullary region. Parts a, e and g were in normal light and b–d, f, h and i were in polarised light. Scale bar: 500  $\mu$ m. Abbreviations: CB, compact bone; GM, growth marks; IS, internal spaces; LT, lamellar tissue; O1, primary osteons; O2, secondary osteons; VC, vascular channels.

juvenile specimen studied by Liebe and Hurum (2012), in which the canals are mostly primary vascular canals not yet surrounded by lamellar bone, in addition to some primary osteons and no remodelling seems to have taken place.

### Rib sections

#### MLP-Ph 65

The transverse section obtained shows very dense and compact structure (CI = 91.7%) (Table 1 and Figure 3e).

The compact cortex is composed of lamellar bone tissue with osteocyte lacunae, these are variable in shape ranging from elliptical to stellate and possess *canaliculi*, but not very numerous. In cortical region, the growth marks are evident (Figure 2g and h). The section exhibits an internal medullary region with spaces surrounded by endosteal lamellar bone (Figure 2i). Some primary osteons occur in the medullary region, they are surrounded by lamellar bone. This rib is remodelling a grade lower than that observed in *Cryptocleidus* by Houssaye (2013b) in which a rather dense Haversian system occurs in most of the cortex as a result of strong remodelling.

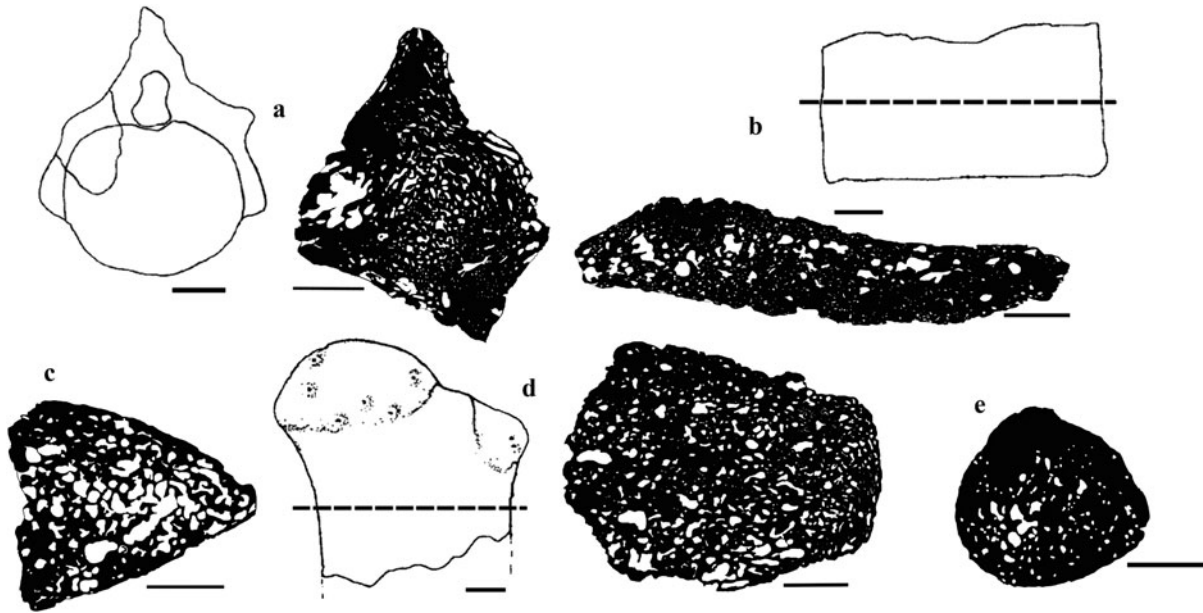


Figure 3. Elasmosaurid plesiosaur (MUC-Pv 92). General view of the transverse section of the (a) caudal vertebrae (MUC-Ph 26), (b) pelvic girdle (MUC-Ph 28), (c) pelvic girdle (MUC-Ph 10), (d) humerus (MUC-Ph 27) and (e) rib (MUC-Ph 65). Scale bar: 1 cm.

### Discussion

Our results confirm the presence of compact bone inner organisation in an adult derived plesiosaur from the Cretaceous and that, within a single skeleton, there are conspicuous differences both in structural specialisations of bone tissues (cancellous vs bone mass increase) and in the degree of remodelling depending on the element selected.

### Structural specialisation

The two main structural specialisations of bone tissues among aquatic tetrapods, which is a trend to compaction of inner structure and increase in bone mass (bone mass increase, for revision see Houssaye 2009, 2013a) versus decrease in bone density and mass due to an extensive porosity (cancellous bone), have been reported in elasmosaurids and pliosaurids (Wiffen et al. 1995; Cruickshank et al. 1996; Fostowicz-Frelik and Gazdzicki 2001). Contrary to what was previously assumed, Houssaye and Bardet (2012) demonstrated that different degrees of bone compaction in the vertebrae and ribs of advanced mosasauroids are not characterised by a diminished and/or increase of bony mass, but there is a restructuring of its microanatomy without losing bone weight. Independently, if the same process occurred in other marine reptiles, bones of plesiosaurus depict significant differences in their bone compactness. In plesiosaurs, distributions of this specialisation had been related to ontogenic differences: while extensive distribution of compact bones was reported in juvenile skeletons, overall cancellous bones are widely

distributed in adult skeletons (Wiffen et al. 1995). Wiffen et al. (1995) and Fostowicz-Frelik and Gazdzicki (2001) suggested that plesiosaurian bone tissues change throughout the life span. These changes through ontogeny were also related with changes in living strategies, while heavy skeleton in juveniles was assumed to diminish speed and rapid manoeuvres, accurate for a shallow coastal lifestyle, adults were long pelagic cruisers. Cruickshank et al. (1996) reported compact rib cage and vertebrae in the Jurassic plesiosaur *Pachycostasaurus dawni*. The holotype, and only known specimen was a juvenile, and they interpreted this type of specialisation as an indicator of slow swimming and benthic feeding habits. Recently, Street and O'Keefe (2010) reported an increase in overall size of the gastralia and a peculiar distribution of 'pachyostosis' in the gastralia in a mature specimen of the cryptocleidoid plesiosaur *Tanectes laramiensis*. In *Tanectes laramiensis*, the distribution of osseous bone tissue of the gastralia was described as a 'sandwich-like conformation' with two layers of hyperplastic cortical bone surrounding osteoporotic-like tissue. These authors conclude that in this case, bone structure was indicative of the environment rather than the ontogenetic stages. Liebe and Hurum (2012) analysed the gross internal structure and microstructure of plesiosaur limb bones, and rejected the view that all plesiosaur bones became more porous through ontogeny.

Our results, as those of *Tanectes laramiensis* (Street and O'Keefe 2010), indicate that the distribution of this histological and microanatomical features (i.e. compact vs cancellous bone) is complex, and that it must be interpreted together with other evidence.

In the elasmosaurid MUC-Pv 92, bone compactness is unevenly distributed through the skeleton according to not only the skeletal element selected but also with the portion of the element analysed (Table 1). Thus, within the same bone (girdle fragment), compactness ranges from 87.5% to 69.8%. A high degree of compactation is observed in the fragment corresponding to the bone region dorso-ventrally compressed (the plate-like portion of the girdle bone) (Figure 3b), while in the region stouter (Figure 3e), the bone is nearly cancellous. These results highlighted the limitations of using compactation parameters alone as a criterion for relative ontogenetic age determinations. On the contrary, differences in bone compactness of the different elements, in particular of the same bone of the pelvic girdle, are better explained in terms of bone architecture. Another point to consider is that the osteology and ossification in other plesiosaur lineages (e.g. Cryptoclididae, *Cryptoclidus*) have variations among the limbs, and ossification can be delayed until the adult stage due to paedomorphosis, thus generating a pattern in the adult which is not consistent with the ossification pattern observed among other anatomical portions of the skeleton (Caldwell 1997). Heavy or light bones in tetrapods secondarily adapted to life in water are often thought to contribute to the buoyancy of the animal as a consequence of adaptation to different ecological niches. Animals that live close to the shore and that do not depend on swimming fast commonly show a local or general increase in skeletal mass, by processes called pachyostosis, osteosclerosis or pachyosteosclerosis. This is known for several groups of amphibians, reptiles, birds and mammals such as the manatee, and is often connected to a heterochronic development by which calcified cartilage persists and remodelling is inhibited (Liebe and Hurum 2012). Bone Plesiosaur (MUCPv-92) should be assigned as osteosclerotic. This specimen is not observed calcified cartilage. The lack of calcified cartilage is likely due to the adult nature of the specimen. Liebe and Hurum (2012) noticed as the amount of calcified cartilage decreases ontogeny.

### **Modelling and remodelling**

Our results also indicate that within single skeleton, different elements depict different degrees of remodelling. Secondary remodelling often results in the production of Haversian bone, in which much of the bone is occupied by secondary osteons (Haversian systems) or interstitial lamellae. The formation of secondary osteons in general weakens bone; and reasons for this widely spread process have been long debated (Currey 2003). Remodelling of the vertebrate skeleton is an essential process for development, growth, repair, mechanical adaptation and mineral homeostasis (Witten and Huysseune 2009). Currey (2003)

analysed the different adaptations of bones, and proposed that some of them were long-term adaptations (e.g. amount of mineralisation), while others short term (e.g. secondary remodelling), although as an overall mechanism was determined over evolutionary time scales, were factors acting during the animal's lifetime. Uneven distribution of remodelling tissue in the skeleton of the elasmosaurid plesiosaur reported here brings an interesting topic of discussion. According to the intensity of remodelling, from major to minor, analysed fragments can be ordered as follows: proximal portion of the humerus, vertebra, pelvic girdle and ribs. This distribution has an approximate coincidence with the degree of compactation, being the humerus and the thick pelvic fragment the lesser compact elements, and plane fragment of the pelvis and rib being more compact and less remodelling. Bone reacts to its mechanical environment where stress usually increases, and stimulates bone growth, and lack of stress absorbs bone and decreases bone density and strength (Lieberman et al. 2003). Bones react to stress, and increase strength, by remodelling dimensions (growth), by increasing bone mineral density or, in the case of trabecular bone also by reshaping the microstructure (Niinimäki et al. 2013). Recently, the reshaping of microstructure has been reported as the process responsible for the peculiar microanatomical features of the advance hydropelvic mosasauroids vertebrae (Houssaye and Bardet 2012).

Maybe the parameters of compaction and remodelling can be an indicator of coastal versus pelagic lifestyles, another possibility is that these parameters may indicate different activity levels in different skeletal elements.

Carpenter et al. (2010) noticed the plesiosaur swimming mode is ultimately unique: no modern animal swims or flies with this kind of active propulsion with four limbs, with an underwater fly with their limbs moving through a figure 8. It seems reasonable to assume that the anterior–posterior limits of movement possible in the plesiosaur girdles mark the maximum anterior and posterior zone for the flippers in a figure 8 cycles. Plesiosaurs are unique among underwater flyers in that there are two sets of 'wings' and the amount of the movement is not the same for both sets. This asymmetrical movement of the flippers is probably made for more stability of the rear part of the body and for maneuvering (Carpenter et al. 2010). The paired flippers of plesiosaurs, located anterior and posterior to the centre of gravity (CG), have dual roles of producing thrust and maneuvering. As Fish (2002) has noted for cetaceans, several features of the plesiosaur's flippers enhance stability: the control surfaces are far from the CG (especially the rear flippers), the CG is anteriorly located, the flippers (control surfaces) are swept down and back thereby shifting the centre of lift posteriorly and reducing drag, and the controlling rear flipper has reduced motion. The rear flippers are best

situated for control because they are far from the CG and can generate large directional moments because of the long lever arm. Furthermore, the rigid body also enhances stability. However, too much stability makes maneuverability difficult (Carpenter et al. 2010). Maneuverability is critical for predators to make rapid turns to catch prey. Plesiosaurs have solved this problem with anhedral flippers (angled negative to the horizontal in neutral position similar to those of cetaceans (Fish 2004)), which allowed controlled yaw and roll in maneuvering. Because the swimmers performed better when they linked themselves together tightly, the robust and often tightly packed gastralia in plesiosaurs may have a functional explanation as a rigid structural element linking the front and rear halves of plesiosaur bodies for the advantage of more efficient swimming. The marked improvement in swimming effectiveness and efficiency that was observed from wrist-flicking of the flipper tips at the top and bottom of each stroke during the experiment may explain the changes seen in the flippers during plesiosaur evolution (Carpenter et al. 2010). This type of locomotion is congruent with the histology pattern found in different bones of a single skeleton. From the different functions attributed to secondary remodelling, the uneven distribution of remodelling in different elements of a single skeleton allows rejecting those linked to the relative ontogenetic age of the specimen. A close relationship between activity levels and increased mechanical load and the remodelling process (e.g. in teleost jaw bones, Witten and Huyseune 2009; mammalian limbs, Lieberman et al. 2003; lumbar region of humans, Niinimäki et al. 2013) has been demonstrated. Not surprisingly, the MUC-Pv 92 skeletal elements with a higher degree of remodelling are those with more activity levels (i.e. motion), like the humerus and caudal vertebrae, and undergoing musculoskeletal stress (stout portion of the pelvic girdle). On the contrary, the portion of the skeleton that was probably less mobile (ribs and plane process of the pelvic girdle) depicts a lesser degree of remodelling.

Our results (i.e. uneven distribution of tissue types among different bones of the same skeleton) agree with previous studies on bone histology of extant birds, which demonstrated that different bones show different growth rates and consequently different types of tissue (Castanet et al. 1996, 2000); and highlight the significance of examining more than single skeletal elements from the same specimen when inferring relative ontogenetic age.

Further studies on broader samples of marine reptiles will be useful to test whether the distribution of specialised bone tissues in elasmosaurid skeleton from Patagonia represents an 'elasmosaurid pattern' or a more generalised pattern; and whether this pattern is constrained by phylogenetic and ecological (functional) factors.

## Conclusions

Characterisation of bone microstructure of the elasmosaurid MUC-Pv 92 confirms the presence of compact bone inner organisation in an adult and indicates that the distribution of bone specialisations presents conspicuous variations within a single skeleton depending on the skeletal element (and also portion of the same element) considered. Bone compactness values ranges from  $\sim 70\%$  (thicker portion of the pelvic girdle) up to  $\sim 92\%$  (rib). Even within a single skeletal element (pelvic girdle), bone compactness increases up to 87.5% in the flat portion of the bone, suggesting that distribution of bone compactness is related to bone architecture. Distribution of remodel bone is also uneven. The rib shows less remodelling than the remaining elements sampled, while humerus depicts a comparatively higher degree of remodelling. We hypothesised that in elasmosaurids, the degree of remodelling in the humerus and thick portion of the pelvis was connected to the level of physical activity (movement), and/or musculoskeletal stress. Our results emphasise the significance of considering these two processes, resorption and remodelling in several elements of the skeleton, for inferring relative ontogenetic age (and/or lifestyles) in extinct forms. Bone compactness or degree of remodelling in elasmosaurids is not necessarily correlated with the ontogenetic age of the animal or to costal versus pelagic lifestyles. The available data are still scarce, but we propose a topic for discussion: perhaps the degree of remodelling and compactness also may be related to the activity level and increased mechanical load in different skeletal elements.

## Acknowledgements

We thank A. Houssaye and an anonymous reviewer for their constructive comments, which considerably improved the quality of this paper.

## Funding

Financial support was given by Consejo Nacional de Investigaciones Científicas y Tecnológicas [grant number PIP 0433]; Agencia Nacional de promoción Científica y Tecnológica [grant number PICT 0261]; Universidad Nacional de La Plata [grant number N607]; Universidad Nacional de Río Negro [grant number 40-A-244] of Argentina.

## Note

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