

X-RAY INVESTIGATION

Among common diagnostic imaging techniques for marine turtles, certainly X-rays allow useful and complete investigation. It's possible to investigate the digestive tract, the respiratory system, the skeleton, the carapace and plastron. Radiographic exams are performed in dorsal-ventral projection with vertical x-ray beam; cranial-caudal and lateral-lateral projections with horizontal x-ray beams. The combination of the three projections always provides a reliable result. Table 1 details the most suitable radiographic projections in order to have a good view of the organs to investigate.

The placement of the animal is not particularly difficult, and generally no containment is required to limit the patient; during the exam it may be useful to cover the turtle's eyes with a cloth (ideal a dark sock) or to wrap the head with a soft elastic bandage.

In order to perform a dorsal-ventral projection, the patient should be positioned ventrally on the radiographic table or with the plastron in contact with the radiographic cassette (figure 1). For small and medium turtles, it is possible to analyse the whole body in a single radiogram (figure 2), and eventually to repeat the radiogram focusing the x-ray beam on the anatomic region of interest. For large subjects, the exam must be performed by combining several images (figure 3).

For lateral-lateral and cranial-caudal projections, the x-ray beam is oriented parallel to the horizontal plan of the animal lying on a radiolucent support (as a plastic box or a wood block). The diameter of the surface on which the animal is placed should not exceed the width of the plastron. In this way limbs and head will be not supported and once the animal is relaxed, they will be lowered, allowing radiographic images of intracoelomic structures without any overlaps.

For lateral projections, the radiographic cassette is maintained parallel to the median plan of the animal and adhering to the outer inframarginal plaques of the carapace (figure 4).

For cranial-caudal projections, the radiographic cassette is maintained parallel to the transverse plan of the animal and adherent to the supracaudal plates (figure 5). In this projection, the thickness of the anatomical structures on the x-ray path and their distance from the x-ray film can produce blurred images and stains. Therefore special attention is required to correctly place the patient under the x-ray beam and the x-ray projection.

Different positions of the animal under x-ray beam can be used to verify the presence of liquid into the coelomic cavity. In tortoises, it is common to place the patient in an upright and vertical rest or in horizontal and lateral one, but in sea turtles it is important to avoid

these positions to reduce stress for the animal and because in large animals this could be highly dangerous. The volume of the liquid can be estimated by lying the animal on the plastron and tilting the support plan of 25-30 degrees (figure 6a -6b).

For the radiographic study of the skull, standard exams are dorsal-ventral projections with vertical x-ray beam and lateral-lateral projections with horizontal x-ray beam. The latter often present some technical problems due to the difficulty of extending the patient's head and neck. Setting the radiographic cassette on the lateral surface of the skull can also be challenging. In order to improve discrimination between the complex skeletal structures of the skull, it may be useful to perform the lateral radiographs keeping the animal's mouth opened with the use of a radiolucent and not traumatic material (such as plastic, foam rubber, cork, etc., see figure 7).

The investigation can be improved with additional oblique left and right dorsal-ventral projections at 30-45 degrees (figure 8a - 8b). Limbs are investigated mainly by dorsal-ventral projections. Posterior-anterior projections are possible for front flippers only, but they are technically difficult.

Before starting a radiographic examination, it is important to remove all ectoparasites (such as barnacles) from the surface of the animal, because their strong radiodensity can overlap structures and make difficult the interpretation (figure 9).

Digestive tract

Only the dorsal-ventral projection provides a good visualization of the digestive tract as it makes possible to distinguish the different districts. It is impossible to identify different anatomical structures in the other two projections, due to the complete overlap of the digestive tract on the other organs of the coelomic cavity and the lack of adipose perivisceral tissue with similar density. The most frequent diseases of sea turtles digestive tract are: intestinal constipation, intestinal bloating, accidental ingestion of lines, hooks or other foreign bodies, partial or total occlusion for inflammatory processes, abscesses or neoplasms: all can be investigated with X-ray examination.

Intestinal constipation can be identified by the accumulation and stagnation of food material; the density correlates to its nature and time of permanence in the intestinal lumen. The constipation may affect more or less extended tracts of the small and large

intestine, and it is generally characterized by an over-distension of the intestinal loops caused by food material, in the absence of accumulation or production of gas (figure 10). Gastro intestinal bloating can cause flotation syndrome, and it can be related to bacterial infections, metabolic and dietary imbalances and debilitated conditions due to intestinal lesions by lines or hooks. It is characterized by distension of the bowel loops due to fermentation gases, and in more severe forms the phenomenon can even reach the stomach. Radiographic images show clear and evident loops of the intestinal walls caused by the presence of gas. In cases of more severe diseases of the digestive tract, over-distended intestinal loops are also visible in the lateral-lateral projections (figure 11a- 11b). The high radiodensity of metals makes radiographic identification of hooks particularly easy (figures 12 and 13); in this case lateral-lateral projections are useful to determine the exact position of the hook. Both orthogonal projections allow a better assessment of hook position and orientation. This also helps to plan the surgical approach for extraction, especially when hooks pierce the intracoelomic oesophagus close to important anatomical structures (such as bronchi, nerves and the brachiocephalic trunk, see figure 14a - 14b). In case of hook localization in the oesophagus it is very important to perform X-ray dorsal-ventral projections, paying attention to properly straighten the head and neck of the animal: if radiograms are performed with the animal 's neck and head retracted, the anatomic connections between oesophagus and trachea may become distorted and the hook can incorrectly appear to be in a deeper position (figures 15a- 15b).

The partial or total occlusion of the intestinal tube is not rare in sea turtles, as they ingest various types of foreign bodies into the sea (bags and other plastic objects, lines, ropes, pieces of fishing nets, etc.). Sometimes, the relative density of ingested foreign material allows identification of obstructed bowel loops by X-ray (figure 16a- 16b). In many cases, however, diagnosis requires an exam by anterograde contrastography with barium sulphate or iodinated non-ionic media. But in sea turtles this survey is quite difficult, due to their anatomical and physiological characteristics. Although the digestive tract of turtles (as other reptiles) is relatively short compared to mammals, the average time of intestinal transit can be very long. Studies on healthy animals report transit times ingestion that varying from 6-8 days up to 4 weeks (figure 17). Since the intestinal transit may slow considerably in animals that are fed regularly or not in good health conditions, diagnostic confirmation of partial or total occlusion by contrastography requires an unacceptable period of time. In addition, it's important to consider the difficulty administering the contrast medium in sea turtles. If the contrast medium is introduced into the lumen of the

oesophagus it would be dispersed due to the impossibility of spontaneous passage in the stomach. The gastro-oesophageal sphincter is very strong, requiring introduction of the contrast medium directly into the stomach by a probe: this involves the same challenges as force-feeding, such as significant stress for animals and the need to provide an anaesthesia.

The retrograde contrast studies allow quick evaluation only to the colon and the distal and middle part of the small intestine. Execution always involves the animal's deep sedation or anaesthesia to introduce a flexible catheter from the cloaca into the distal colon to administrate the contrast medium (figure 18). This procedure can be difficult as the contrast medium can be introduced accidentally in the urodeo; to avoid such problems, it is always better to perform this survey with iodinated water-soluble non-ionic surfactants contrast media.

Considering these difficulties, ultrasound exams should be preferred to contrastographic investigation when bowel obstruction, radiolucent foreign bodies or intracoelomatic pathological mass are suspected.

Respiratory tract

Lung analysis is best performed with cranial-caudal and lateral horizontal projections. Lateral projection radiographs should be made from both sides, especially for larger animals, because the image of the opposite lung may be not very detailed. For cranial-caudal projections the x-ray beam should be focused on the nuchal plate of the plastron coincident with the middle sagittal and horizontal plans, in order to obtain a good symmetrical image of both middle portions of the coelomic cavity and a clear distinction between lungs and other ventral viscera (see figure 19). If the animal position relative to the x-ray beam and radiographic cassette is incorrect, the images may appear altered with the risk of showing as pathological what is normal (figures 20 and 21).

In turtles, as in all reptiles, the lung anatomical structures have a uniformly crosslinked parenchyma, interspersed with thin bands of connective tissue. This results in a homogeneously radiolucent radiographic image, without possibility to identify the alveolar, interstitial, bronchial and pleural patterns, which are typical in mammals (figure 22).

When the lung parenchyma is covered by other intracoelomic organs, a dorsal-ventral projection guarantees a good view of large pulmonary vessels and the trachea, bronchi and lung area (figure 23).

X-ray is the best tool for diagnosis of lung inflammatory diseases and evaluation of their evolution during therapy. Pneumonia shows more or less marked clouds and haze, localized to restricted portions of parenchyma or spread to one or both lungs (figures 24-25-26).

Carapace and plastron skeletal system

Unlike tortoises, sea turtles rarely suffer from metabolic and degenerative bone diseases. Therefore radiographic exams of carapace and skeletal system are typically used to study traumatic injuries.

Carapace and skull fractures are frequently related to accidental impacts with boats or trauma caused by fishing tools. For a better view of skull lesions, investigations are done in dorsal-ventral, lateral-lateral and lateral oblique right and left projections, highlighting deeper skeletal lesions connected with the central nervous system, eyes, oral cavity and upper respiratory tract (figure 27).

All three orthogonal projections are important to evaluate carapace and plastron lesions. Horizontal projections allow estimation of the depth of the lesions and the possible involvement of the spine (figure 28). Vertical and ventral-vertical projections provide easy evaluation of the extension of lesions and the degree of displacement of fractures of the underlying bony plates (figure 29).

Skeletal flipper lesions are mostly caused by the constriction of lines tangled around fins or by the turtle's movements when caught in a fishing net. The bone most commonly fractured is the humerus. However, it is not uncommon to discover bone lesions in the femur, radius and ulna (figure 30).

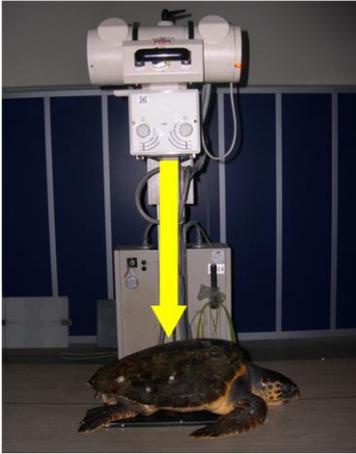


Figure 1 – Positioning the patient to perform radiographic exam in dorsal-ventral projection with vertical x-ray beam.



Figura 2 – Generic radiogram of a small turtle by dorsal-ventral projection with vertical X-ray beam.

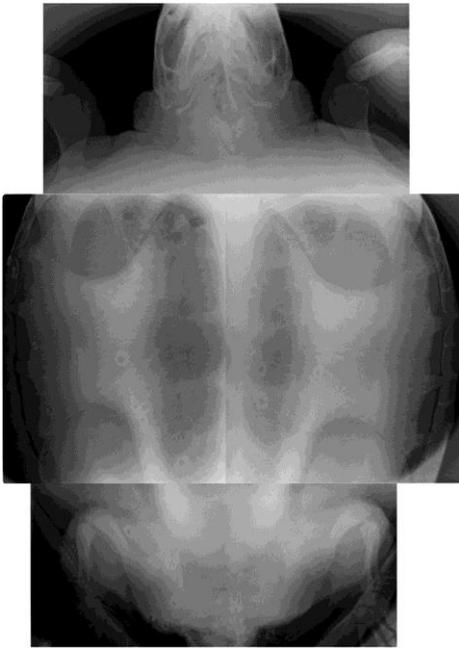


Figure 3 – Generic radiographic study of a large turtle by dorsal-ventral projection with vertical x-ray beam, obtained by the combination of 4 radiograms.



Figure 4 – Positioning the patient to perform radiographic exam in lateral projection with horizontal x-ray beam.



Figure 5 – Positioning the patient to perform radiographic exam in cranial-caudal projection with horizontal x-ray beam.



Figures 6a-6b – Radiographic exam by lateral projection, performed with the animal at an angle of 25-30°. X-ray image makes evident a considerable volume of liquid inside the coelomic cavity.



Figure 7 –Correct positioning of the patient to perform the skull radiographic exam in lateral-lateral projection. If the patient's mouth is maintained open by a radiolucent material, the visualization of front splanchnocranium bones appear better distinguished.



Figures 8a-8b – Positioning of X-ray for the oblique dorsal-ventral projections for left and right skull exam.

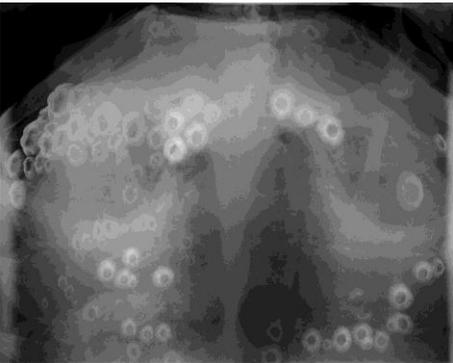


Figure 9 – The radiograph shows several radiopaque ring-shaped formations, typical of the presence of barnacles on the animal's surface, their overlap makes difficult to evaluate the skeletal and intracoelomic structures.

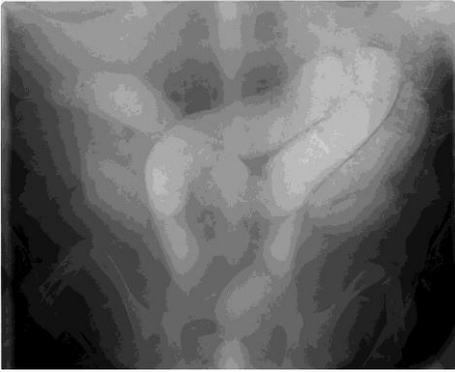
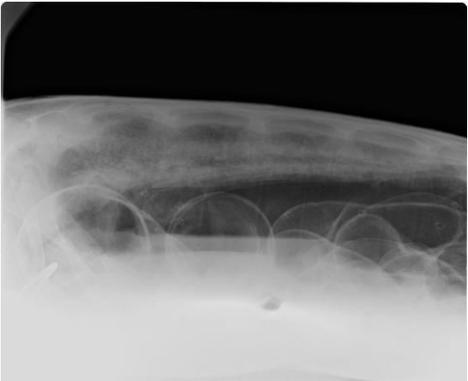


Figure 10 –Constipation of colon and the last part of ileum by fragments of shells ingested.



Figures 11a-11b – Diffused bloating gastrointestinal tract associated with the presence of a hook in the esophagus.

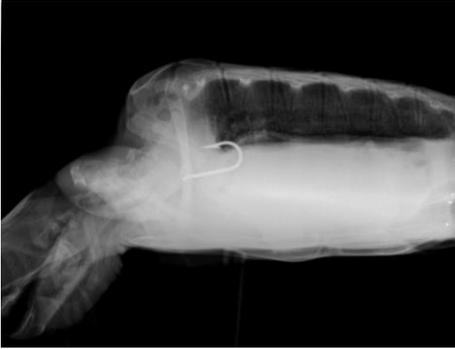


Figure 12 –X-ray dorsal-ventral projection showing a hook into the oesophagus wall.



Figure 13 –X-ray dorsal-ventral projection showing a hook in the intestinal lumen.





Figures 14a-14b –Dorsal-ventral (a) and lateral (b) projection: comparing the two images it's possible to determine the hook point is very close to the right bronchus.

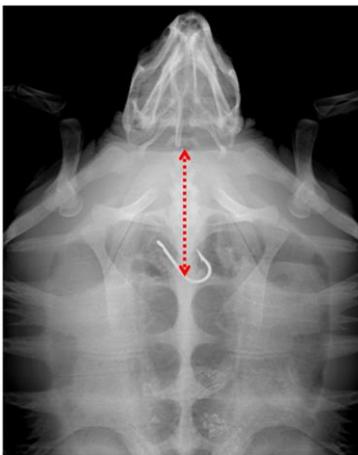


Figure 15a-15b – If head and neck are not properly stretched (a) the hook appears to be in a very deep position, behind the pectoral girdle. The exam in the correct position (b) allows to assess the real position of the hook , located in the terminal part of the cervical oesophagus.

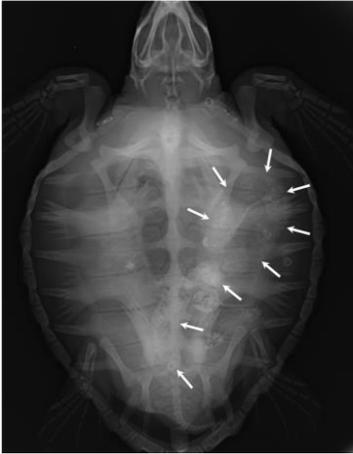


Figure 16a-16b –Bowel obstruction of a large portion between duodenum and ileum (white arrows outline the profile of the loop bowel) caused by ingestion of plastic debris and lines with algae and other food.



Figure 17 – Radiographic exam performed after 15 days of the anterograde administration of iodinated, water soluble, non-ionic surfactant contrast material in a subject with normal gastrointestinal function: the contrast medium is still passing into the small intestine and the colon has not yet committed.



Figure 18 –Retrograde contrastographic exam in a suspected intestinal obstruction and plication caused by a line. The black arrows points the catheter placed in the rectum to introduce the iodine contrast medium, the white arrows indicates the point of intestinal obstruction caused by the traction of the line.



Figure 19 –Lung X-ray image in the cranial-caudal projection, positioning correctly the animal.

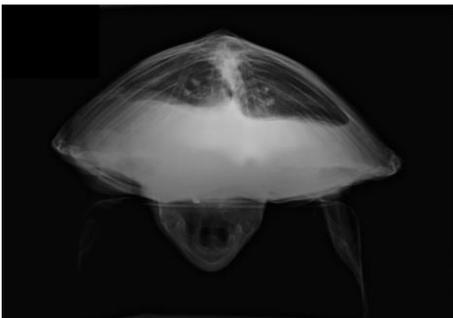


Figure 20 –In this X-ray the right lung appears shifted dorsally and smaller than the left one. The picture does not indicate a pathological situation: it is the result of an incorrect positioning of the animal, where the axial support appears rotated laterally relative to the x-ray beam.

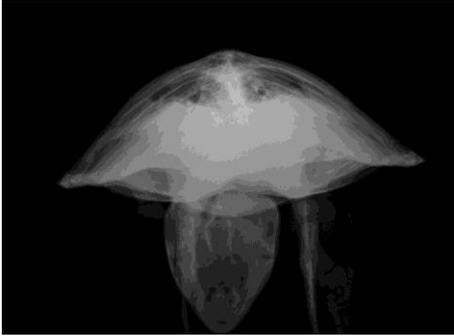


Figure 21 –In this image the lung appears shorter and the parenchyma denser than in normal conditions. This is the result of the anomalous inclination of the x-ray beam to the transverse support of the animal.

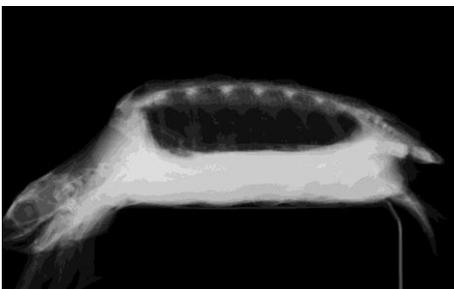


Figure 22 –Lung X-ray in lateral projection in a healthy patient. It clearly shows a normal lung parenchyma, homogeneously radiolucent.

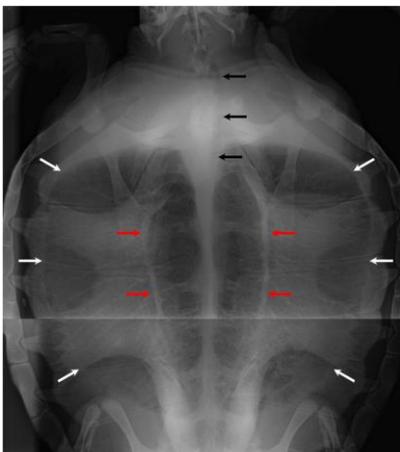
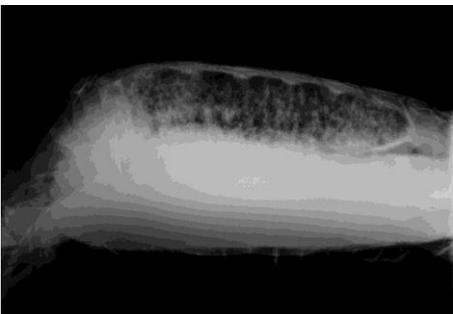


Figure 23 –Lungs and upper respiratory tract X-ray in dorsal-ventral projection. It's easy to distinguish the course of trachea and bronchi (black arrows), large pulmonary vessels (red arrows) and the profile of the lung areas (white arrows).

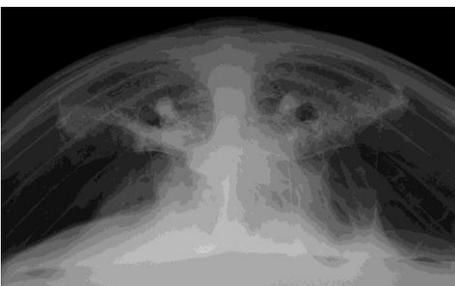
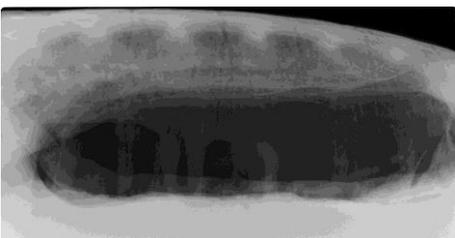


Figures 24a-24b-24c –Lateral, cranial-caudal (horizontal beam) and dorsal-ventral (vertical beam) radiographs illustrating apparent thickening of the peribronchial lung parenchyma (white arrows) during a pneumonia.

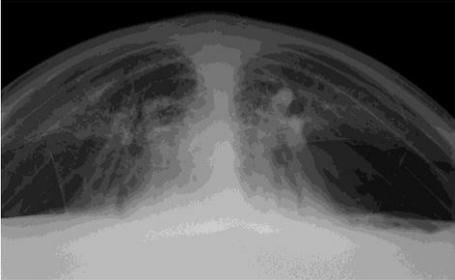
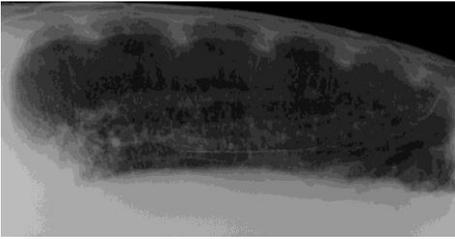




Figures 25a-25b-25c – Lateral, craniocaudal (horizontal beam) and dorsoventral (vertical beam) radiographs illustrating total severe opacification of the lungs caused by bacterial pneumonia. Figure 25a-25b-25c - Lateral, cranial-caudal (horizontal beam) and dorsal-ventral (vertical beam) radiographs illustrating a total opacification of lungs caused by bacterial pneumonia.



Figures 26a-26b –These radiographs in lateral and cranial-caudal (horizontal beam) projection show a rare case of pneumonia caused by the parasite *Hapalotrema loossi*, characterized by intense opacification of the parenchyma of the dorsal portion of both lungs, focused around large pulmonary vessels.

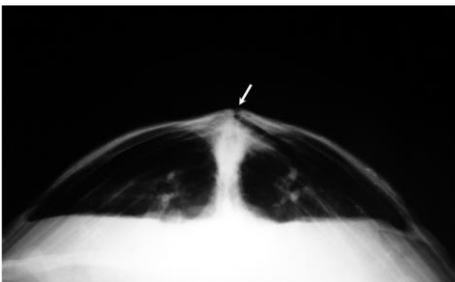


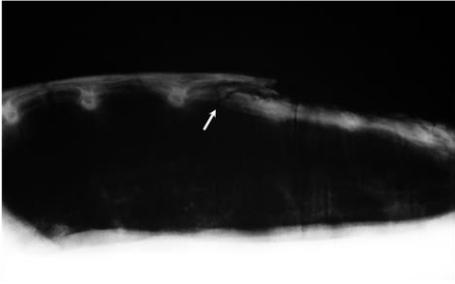
Figures 26c-26d –Radiographic images of the previous case after 3 months of therapy show the almost total resolution of pneumonia.





Figures 27a-27b-27c-27d – (a) Deep lesions of the skull affecting the parietal bones, postorbital, frontal and prefrontal. An x-ray projection back in the ventral (b) shows numerous fractures in the postorbital bones, jugali and jaw. In the oblique right and left lateral projections (c) (d), fractures of the pterygoid and palatine bones appear evident.

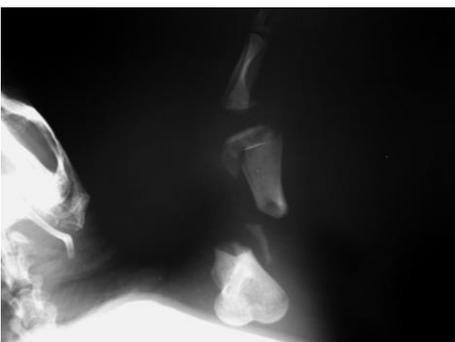




Figures 28a-28b-28c – (a) Severe carapace damage caused by impact with a boat. The radiographic exam in craniocaudal (b) projection shows a deep fracture of the second and third vertebral shield close to neural bones. The radiograph in lateral view (c) projection shows the involvement of the spine.



Figure 29 –X-rays in dorsal-ventral projection shows numerous fractures of vertebral and lateral plaques.



Figures 30a-30b –An x-ray projection in dorsal-ventral and anterior-posterior projections of the right front flipper: it clearly shows a complete fracture of the shaft of the humerus associated with a detachment of the distal physis.