Decompression sickness ("the bends") in sea turtles

Short title: The bends in sea turtles

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ABSTRACT

Decompression sickness (DCS), as diagnosed by reversal of symptoms with recompression, has never been reported in aquatic breath-hold diving vertebrates despite the occurrence of tissue gas tensions sufficient for bubble formation and injury in terrestrial animals. Similarly to diving mammals, sea turtles manage gas exchange and decompression through anatomical, physiological and behavioral adaptations. In the former group, DCS-like lesions have been observed on necropsies following behavioral disturbance such as high-powered acoustic sources (e.g. active sonar) and in bycaught animals. In sea turtles, in spite of abundant literature on diving physiology and bycatch interference, this is the first report for DCS-like symptoms and lesions. We diagnose a clinico-pathological condition consistent with DCS in 29 gas embolized loggerhead sea turtles (*Caretta caretta*) from a sample of 67. Fifty-nine were recovered alive and 8 recently dead following bycatch in trawls and gillnets of local fisheries from the east coast of Spain. Gas embolization and distribution in vital organs, was evaluated through conventional radiography, computed tomography and ultrasound. Additionally, positive response following repressurization was clinically observed in 2 live affected turtles. Gas embolism was also evidenced post-mortem in corpses and tissues as described in cetaceans and human divers. Compositional gas analysis of intravascular bubbles was consistent with DCS. Definitive diagnosis of DCS in sea turtles opens a new era for research in sea turtle diving physiology, conservation and bycatch impact mitigation, as well as for comparative studies in other air-breathing marine vertebrates and human diving.

Key words: decompression sickness, the bends, gas bubbles, sea turtles, bycatch, hyperbaric treatment, gas embolism, breath-hold divers.

INTRODUCTION

Decompression sickness (DCS) is a clinical diagnosis encompassing a wide range of manifestations related to formation of gas bubbles within supersaturated tissues after decompression (Francis & Mitchell 2003). In human divers, the effects range from trivial to fatal, and most often involve neurological and musculoskeletal symptoms (Francis & Simon 2003, Vann et al. 2011), including severe pain. In an analysis of 1,070 central nervous system DCS cases, 77% involved the spinal cord (Francis et al. 1988). A wide range of symptoms are caused directly or secondarily by the mechanical, embolic, and biochemical effects of intra- and extravascular bubbles (Vann et al. 2011). Direct effects include the distortion of tissues and vascular obstructions. Secondary effects include endothelial damage, capillary leakage, plasma extravasation, and hemoconcentration (Vann et al. 2011). Definitive diagnosis of DCS is difficult and only confirmed by successful recompression treatment in a hyperbaric chamber (Ferrigno & Lundgren 2003).

Breath-hold diving vertebrates, including marine mammals and sea turtles, classically are considered to be protected against DCS through anatomical, physiological and behavioral adaptations (Berkson 1967, Rothschild & Martin 1987, Burggren 1988, Lutcavage & Lutz 1997, Piantadosi & Thalmann 2004, Fossette et al. 2010, Castellini 2012). However, an acute and systemic gas and fat embolic syndrome similar to DCS in human divers was described in beaked whales that stranded in temporal and spatial association with military exercises involving high-powered sonar (Jepson et al. 2003, Fernandez et al. 2005). Since this first report, there has been accumulating evidence demonstrating the presence of gas bubbles in diving marine mammals (Jepson et al. 2005, Bernaldo de Quirós et al. 2012, Dennison et al. 2012), including dysbaric osteonecrosis (Moore & Early 2004) and gas embolism in bycaught

animals (Moore et al. 2009). Although these findings have challenged our understanding of diving physiology in these species, conclusive clinical data (i.e. diagnosis and therapy) supporting the occurrence of DCS are lacking due to the complexity of working with wild marine mammals.

Sea turtles are among the longest and deepest marine air-breathing diving vertebrates (Byles 1988, Sakamoto et al. 1990, Houghton et al. 2008). They may spend over 90% of time submerged in apnea (Lutcavage & Lutz 1997) and efficiently use oxygen through cardiovascular adjustments, similar to other air-breathing vertebrates (Rothschild & Martin 1987, Burggren 1988, Southwood et al. 1999, Southwood 2013). In addition, osteonecrosis-type lesions, being one of the few long-term lesions observable after certain episodes of DCS, have been described in monosaurs and sea turtle fossils from the Cretaceous Age but are very rarely described in animals younger than the Miocene Age (Rothschild & Martin 1987). This suggests that more recent taxa have evolved physiological and behavioral adaptations to mitigate hyperbaric conditions like DCS.

Bycatch is a well-documented, worldwide problem resulting in considerable mortality of non-targeted species (Lewison et al. 2004a). Over the past decades, there has been a dramatic global decline in sea turtle populations with six of seven species currently categorized as vulnerable, endangered, or critically endangered by the IUCN Red List (IUCN.www.iucnredlist.org (accessed 14 January 2014)). Fishery bycatch is recognized as the greatest threat to their conservation (Wallace et al. 2010) and is considered a moderate or high threat for more than three-fourths of all sea turtle Regional Management Units globally (Wallace et al. 2011, Lewison et al. 2013). Approximately 85,000 sea turtles were reported incidentally captured worldwide from 1990 through 2008, but true total bycatch is estimated to be at least two orders of

magnitude higher (Wallace et al. 2010). Total numbers of global bycaught sea turtles (Lewison et al. 2004b, Hamann et al. 2010, Wallace et al. 2010) and resulting mortality (Lutcavage & Lutz 1997, Epperly et al. 2002, Hamann et al. 2010) remain unclear.

Primary limitations in bycatch estimates are the lack of reliable comprehensive information on total fisheries effort, bycatch in small scale fisheries (Wallace et al. 2010, Casale 2011), and the rate of survivorship of released animals (Chaloupka et al. 2004, Mangel et al. 2011). The rate of survivorship following interaction is considered to be one of the main obstacles to understanding the true impact of fisheries on sea turtle populations (Lewison et al. 2013). Consideration of causes of sea turtle mortality resulting from fisheries interaction largely have focused on the effects of drowning and direct trauma from gear (Poiner & Harris 1996, Gerosa & Casale 1999, Casale 2011, Lewison et al. 2013). The present work describes a previously undescribed condition that can compromise post-release survivorship of incidentally captured sea turtles.

In this study, 67 loggerhead turtles (59 alive, 8 dead) bycaught in trawls and gillnets at depths ranging from 10 to 75m, were evaluated by intensive clinical and pathological examination. Gas embolism (GE) was a consistent finding in a large proportion of live and dead animals. Clinical signs, diagnostic imaging, gross and histological observations and response to recompression and controlled decompression treatment definitively demonstrate that marine air-breathing vertebrates can suffer from DCS. These findings offer a new paradigm to consider in many different aspects of sea turtle research, conservation and management, including basic patho-physiological aspects of diving adaptations, implications on post-capture survivorship estimates, bycatch impact mitigation strategies and devices, clinical treatment of affected turtles, as well as potential additional risks associated with intentional capture of diving turtles.

MATERIAL AND METHODS

Animal acquisition

All sea turtles included in this project were under the authority of the "*Consellería de Infraestructuras, Territorio y Medio Ambiente*" of Valencia Community Regional Government in collaborative official agreement with the Oceanografic Aquarium of the "*Ciudad de las Artes y las Ciencias of Valencia*" for animal rehabilitation and posterior release, and for the postmortem examination of dead individuals.

In 2011, an active campaign involving fishermen from the Valencian coast of Spain was established to collect all (live and dead) sea turtles incidentally captured by gillnets and trawling so that bycaught animals could be medically evaluated. During the period from January 1, 2011 to January 2, 2014, a total of 67 bycaught loggerhead turtles (*Caretta caretta*) were received. Eleven turtles arrived dead and 56 arrived alive. An additional five of 56 live turtles died within 72h. All live animals received comprehensive clinical examination. Examination of all dead turtles included necropsy and histopathology.

For all cases, the date of capture, fishing depth, and sea surface temperature at the originating port were documented (SeaTemperature. www.seatemperature.org (accessed 14 Jan 2014)). Any comments from fishermen related to the condition and behavior of turtles upon capture were also noted.

Clinical diagnosis

All live bycaught turtles were examined within the first 24 hrs (average 12 hrs). Evaluation included routine general veterinary physical and neurological examination, hematology and biochemistry, followed by imaging studies.

Blood was collected from the dorsal cervical sinus with a 5ml syringe and 21G 40mm hypodermic needle (Henry Schein Inc, Melville NY, USA) and transferred to 2ml lithium heparin tubes (Aquisel®, Barcelona, Spain) for immediate analysis (maximum elapsed time of one hour). Analysis included automated hematology with an Abbott Celldyn 3700SL hemocytometer (Abbott Laboratories Illinois, USA), standard manual hematocrit determination and cytological study including manual differential count, and complete biochemistry and electrolyte panel using an Olympus AU400 autoanalyzer (Mishima Olympus CO, LTD, Shizuoka-ken, Japan).

Diagnostic imaging studies included the following:

- Plain radiographic evaluation with a Philips Practix 400 unit (Philips Medical Systems, , Hamburg, Germany,) and a Kodak Direct View Classic CR System (Carestream Health, INC. Rochester, New York, USA) with 35x43cm Kodak cassettes (Kodak PQ Storage Phosphor Screen Regular, and 100 Microns, Carestream Health, INC. Rochester, New York, USA) in dorsal-ventral (DV), cranial-caudal (CC), and lateral-lateral (LL) projections. Focal distances varied between 1-1.5m, using average exposure values between 75-120kV and 7,2-20 mAs depending on projections and animal size. Digital images were processed afterwards through the Kodak Acquisition Software (Onyx-RAD Diagnostic Viewer, Rochester, New York, USA) for better visualization and image interpretation. Some dead bycaught turtles were also radiographed.
- Ultrasonographic general examination was conducted using a General Electric Logiq E Vet ultrasound machine with commercial linear, phase-array, and

microconvex probes (models 12LRS (GE Healthcare, Japan Corporation, Tokyo, Japan), 3S (GE Medical Systems CO, LTD, Jiangsu, China) and 8CRS (GE Medical Systems CO, LTD, Jiangsu, China)), respectively.

- Selected individuals with DCS compatible signs were examined by computed tomography (CT) using a Toshiba Aquilion 16 CT unit (Toshiba Medical Systems, Nasu, Japan). Acquisition parameters through the whole body exploration of the turtle were 5mm slice thickness and 5mm slice interval, with 0.5mm retro-recon acquisition under lung and mediastinal algorithms. Images were post processed with Osirix software version 3.3.1 (Pixmeo, Geneva, Switzerland) and Philips Brilliance Workspace CT software (Koninklijke Philips, Netherlands). A 3D air volume was recreated through volumetric segmented reconstruction (*volume rendering*).

Based on imaging findings upon arrival at the rehabilitation center and/or post mortem examinations, the severity of gas embolism was scored based on total amount of intravascular gas observed and the distribution (Table 1):

-Mild embolism: small amount of gas was only evident at the kidney region on ultrasound and LL radiographic projection.

-Moderate embolism: larger volume of gas was present in kidney region, being clearly evident in ultrasound, LL and also even DV radiographic projections. Other minor vessels in the periphery of the coelom or the liver were also full of gas (gas angiograms) on DV radiographs. On ultrasound, occasional free gas bubbles could be observed in the lumens of major vessels and cardiac chambers (mostly the right atrium).

- Severe embolism: Gas was evident in kidney, liver, major systemic vessels and even cardiac chambers in DV radiographs. Kidney ultrasound images were often

impeded by the large amount of gas present in the area. Abundant bubbles in the blood stream: gas accumulations were present in most cardiac chambers and larger vessels.

Treatment

Individuals without clinical signs and mild embolism detected in imaging studies did not receive any specific supportive treatment on arrival. Individuals that were unresponsive or exhibited neurologic signs, such as stuporous behavior, atonic or single retracted extremities, or reduced sensitivity of the skin as detected by pinching with forceps, received supportive therapy including normal saline solution (FisioVet® saline B. Braun Medical SA, Barcelona, Spain) (10-15ml/kg body weight (bw)) intravenously (IV) and/or subcutaneously (SC). Additional drugs commonly used based on severity of symptoms included: cardiotonics (atropine 0,1mg/kg bw intramuscularly (IM), (Atropine Braun 1mg B. Braun Medical SA, Barcelona, Spain)), respiratory stimulants (doxapram chlorhydrate 5-10mg/kg bw IM (Docatone-V® Fort Dodge Veterinaria SA, Girona, Spain)), analgesics (meloxicam 0,2 mg/kg IM bw (Metacam® Boehringer Ingelheim Vetmedica GmbH, Rhein, Germany), tramadol 5-10mg/kg bw IM (Tramadol Normon, Laboratorios Normon SA, Madrid, Spain)), corticoids (dexamethasone 0,5-1,2mg/kg bw IM, (Fortecortin® 4mg, Merck SL, Madrid, Spain)) and/or supplemental oxygen therapy through an endotracheal tube (Rüsch® Uruguay Ltda., Montevideo, Uruguay), face mask (Kruuse®, Langueskov, Denmark) or commercial critical care unit (Vetario Intensive Care Unit, Brinsea Products Ltd., Sanford, England).

Recompression with hyperbaric oxygen was applied to two clearly lethargic and poorly responsive animals with moderate embolism (one of them with evident paresis and retraction of the hind extremities under the shell). Pressurization was achieved

using a power disconnected regular autoclave (Selecta, Presoclave 30, J.P. Selecta SA, Barcelona, Spain) modified to work as a hyperbaric chamber by means of a connection of a pressurized oxygen cylinder to the draining tube of the autoclave. Animal breathing inside the chamber was stimulated with a previous injection of doxapram chlorhydrate and needle insertion at the acupuncture GV26 point (Litscher 2010). As there were no previous references for reptiles, the most commonly used human recompression-decompression table was applied (Vann et al. 2011). Briefly, an initial pressure of 1.8atm (relative pressure) was applied for 1hr, then decreased to 1atm over the next 30min, stabilized at 1atm for another 3hrs and finally progressively decreased to surface pressure (0atm relative pressure) over 30min. Pure oxygen was used for the entire procedure. Monitoring of the animals inside the chamber was not possible. Recompressed-decompressed individuals were reevaluated through simple radiology, ultrasound and CT (only one case) before and immediately after treatment. Only turtles smaller than 30cm straight-line carapace width were candidates for decompression due to the size of the chamber. Larger individuals were followed clinically for outcome without decompression treatment.

Postmortem examination

Necropsies were performed within 24 hrs after retrieval from fishing gear (except in one case at 36h) or in less than 12hrs following death at the rehabilitation center. Systematic sea turtle necropsy procedures were performed (Flint et al. 2009), with extra caution to minimize artifactual gas infiltration by traction of tissues and during sectioning blood vessels (especially when removing the plastron). Presence of intravascular gas was specifically documented. Samples of skin, muscle, pre-femoral fat, liver, spleen, heart, major vessels, brain, intestine, salt glands, plastron, thyroid

gland, both kidneys, both lungs, both gonads and any gross lesions were routinely collected for histopathology. All tissues were fixed in 10% neutral buffered formalin, processed routinely into paraffin blocks for histopathology and stained with hematoxylin and eosin (H&E). Histopathological examination was conducted in all individuals suspected from DCS. Gas sampling and analysis was performed as previously described (Bernaldo de Quirós et al. 2011) in 13 different samples collected from the same individual approximately 36 hours post mortem.

Ethical statements

Animal care was applied within institutional guidelines. In live animals, clinical information generated for this study was derived from the regular veterinary procedures provided in order to establish an appropriate diagnosis for the application of the best feasible treatment. Hyperbaric oxygen treatment was administered with Governmental and veterinary medical consent and was decided to be necessary based on fatal outcome of similar cases without hyperbaric treatment.

RESULTS

Sea turtle bycatch was higher during months of the year when the water was coldest, particularly from November to March. Regional average monthly water surface temperature ranged from 13.4ºC in February up to 26.3ºC in August (SeaTemperature. www.seatemperature.org (accessed 14 Jan 2014)) (Table1).

Clinical diagnosis, treatment and outcome

Evidence of gas embolism (GE) was found in 6/18 (33.3%) gillnet and 23/49 (46.9%) bottom trawl net bycatch cases (43.3% of all incidental captures) from a depth range between 10-50 m and 25-75 m, respectively. Summary information for different cases is provided in Table 1. The severity of GE was assessed to be mild in 16 cases, moderate in 9 cases and severe in 4 turtles.

According to the fishermen, clinically abnormal turtles exhibited two clearly distinct anomalous behaviors when they surfaced within the fishing gear: comatose or initially hyperactive progressing to stuporous with increasing surface time. Some of the comatose animals showed aspiration of sea water in the respiratory tract as evidenced by an alveolar pattern in radiographs and expelled copious fluid after endotracheal intubation for resuscitation. These animals were diagnosed as drownings and generally responded well to conventional emergency treatment (Norton 2005).

Twenty-one loggerheads arrived at the rehabilitation center alive and were clinically evaluated. All individuals presented with good body condition and normal fat stores. Eight exhibited normal behavior, four were comatose, and nine turtles were hyperactive or developed progressive neurological symptoms, including limb paresis or loss of nociception. The latter group was all caught by trawlers and in some cases terminally displayed rigid pressing of the front flippers against the plastron (Fig. 1a and 1b). These turtles also exhibited an initially increased hematocrit, positive flotation and erratic swimming when returned to water. Without hyperbaric treatment, neurological signs gradually progressed to complete unresponsiveness and death within 72 hrs of capture. Additional animals may have had these signs upon capture and become comatose or died before arrival at the rehabilitation center.

In radiographs, intravascular gas was observed as radiolucency within or distending the heart and vessels (Fig. 1c). The lungs were partially collapsed in severely

affected individuals as evidenced by reduction in field volume and increased radiodensity. In mild cases, latero-lateral projections resulted in the most diagnostic radiographs, providing higher sensitivity than dorso-ventral views for gas visualization within the renal vessels.

Gas bubbles were detected by ultrasound as hyperechoic spots, typically with comet tail artifacts. In all affected individuals, renal ultrasound revealed the presence of gas inside the parenchyma and kidney vessels (Fig. 1d). Cardiac ultrasound demonstrated a much higher prevalence of bubbles in the right atrium compared with the left, similar to the pattern observed in scuba divers (Francis & Simon 2003).

CT imaging techniques were used in 11 cases to confirm the presence and distribution of GE (Fig. 1 and 2a, 2b, 2c). Embolism was confirmed within the kidneys, liver, heart, spleen, and central nervous system (Fig. 2a and 2b). In simple CT slices, gas was revealed inside different regional vessels as hypoattenuated (black) compared to surrounding tissues. As in radiographs, the lungs of severe cases were hyperattenuated (whiter) and expansion reduced due to partial collapse. Notably, midline-sagittal multiplanar reconstructed CT images demonstrated gas within the vertebral canal and central nervous system (Fig. 2c) that was not seen by ultrasound or in radiographs. Gas within or surrounding the nervous system was apparent even in mild cases (Fig. 2c). These findings were observed to be compatible with life even without treatment, although subsequent renal and/or neurological damage or temporal functional impairment could not be discarded.

Five out of 49 (10.2%) bycatch trawl animals were active while presenting moderate to severe GE at the arrival to the rehabilitation center. More animals could have surfaced on board with similar symptoms dying before arrival at the rehab center. All these cases of GE resulted in death within 48-72 h post-capture if not treated with a

hyperbaric protocol while severe cases were generally lethal in the first 6-8 h, thereby reducing the chances for hyperbaric treatment. Two of these animals survived following a hyperbaric oxygen treatment (Table 1). After treatment neurological signs resolved and the sea turtles recovered normal activity. Post-treatment radiographs and CT confirmed the dissipation of most of the intravascular gas and re-expansion of the lungs (Fig. 2d and 2e). After two months under observation, both were considered clinically healthy and were reintroduced into the Mediterranean Sea.

Pathological diagnosis

Complete necropsies were performed on a total of 16 deceased bycaught loggerheads (8 dead on the gear, 3 dead during transport and 5 dead at the rehabilitation center). Gas embolism was found in 13 turtles (81%), which included 8 out of the 11 that arrived dead and the 5 that died following admission. In severe cases, gas was found within the median abdominal, mesenteric, gastric, pancreatic, hepatic and renal veins, as well as within the post cava and other major vessels (Fig. 3). The atria (especially the right atrium) and the *sinus venosus* were distended by gas (Fig. 3). In very severe cases the spleen was gas dilated. Grossly, the kidneys had multifocally extensive red areas consistent with marked congestion. Segmental congestion of the intestinal mucosa was also present. The lungs of some animals were partially collapsed with cranial pulmonary emphysema. Various amounts of fluid within the respiratory tract were evident in some individuals. Other gross findings included coelomic transudate in individuals with severe GE and partially digested contents within the stomach and intestine in most turtles. In moderate cases, GE were not as obvious as observed by imaging and required careful examination. Gas was most visible within mesenteric and renal vessels, as well

as the postcava and sinus venosuson. In one mild case with concurrent radiographic evidence of drowning, GE could not be found macroscopically in any explored tissue.

Histopathological findings included moderate to severe multisystemic congestion with the presence of intravascular gas bubbles in multiple organs including the lung, liver, kidney, and heart (Fig. 3). In addition, perivascular edema and hemorrhages, varying in extent and severity were also present in different tissues. Acute, multifocal, myocardial necrosis with vacuolar degeneration of myocytes, alveolar edema, diffuse microvacuolar hepatocellular degeneration, sinusoidal edema, and intrahepatocyte hyaline globules were frequently evident.

Gas composition analysis in one case confirmed that the main component was nitrogen (75.3 ± 0.9% μmol), followed by carbon dioxide (18.6 ± 2.0% μmol) and oxygen (6.0 ± 1.3% μmol).

DISCUSSION

Differential diagnoses

Alternative differential diagnoses for GE, including traumatic or artifactual intrusion and putrefaction, were ruled out based on clear demonstration of antemortem occurrence in live turtles and absence of any apparent traumatic injuries or surgical procedures. Pulmonary barotrauma could cause arterial air embolism (Vann et al. 2011); however, the physical requirements for barotrauma are not met in bycaught turtles. Turtles are breath hold divers, meaning that the internal pressure in the ediculi (homologous to mammalian alveoli) at the beginning and at the end of the dive would be the same or even lower at the end of the dive due to oxygen consumption. Thus, overexpansion of the lungs is very unlikely. In addition, gas was mainly found in the venous side of the

circulation (as in DCS) instead of in the arterial side. In addition, necropsied turtles were in a good state of preservation and systemic GE was consistent with pathological findings described in DCS in human divers and in stranded beaked whales (Knight 1996, Francis & Simon 2003, Jepson et al. 2003, Fernandez et al. 2005). Also, hydrogen, a putrefaction marker, was not detected in the gas samples collected during necropsy (Bernaldo de Quirós et al. 2013a). Furthermore, decompression-related GE is the only process that is reversed by a hyperbaric treatment (Vann et al. 2011). Dissipation of GE and clinical response fulfill human criteria for medical diagnosis of DCS (Paulev 1965, Vann et al. 2011).

Key facts for the finding of GE in sea turtles

To the best of the authors' knowledge, no report of live or dead wild sea turtles suffering from acute GE has been previously presented. Most of the literature and research done until present, considers this possibility as highly improbable based on different anatomo-physiological adaptations, including relatively small and collapsible lungs (Berkson 1967) and confinement of lung gas to non-respiratory, cartilage-reinforced airways during deep dives (Kooyman 1973, Lutcavage et al. 1989, Lutcavage & Lutz 1997). The metabolic adaptations and physiological mechanisms underlying their diving capacity have been the subject of intense interest for many years, including early studies on forced submergence response in laboratory settings (Berkson 1966) and more recent physiological investigations based on sophisticated remote-monitoring technologies in free-swimming sea turtles (Hochscheid et al. 2007, Southwood 2013).

Berkson (1967) pressurized green turtles to different depths in a hyperbaric chamber demonstrating tolerance to over 100 minutes of forced submergence at 18-

25ºC. Two animals compressed to 18.7atm died several hours after compression (one fast compression and the other in progressive steps) and then fast decompression with numerous gas emboli observed in capillaries of the cervical fascia and right atrium. Death was attributed to gas emboli in the brain after emergence. The study concluded that equilibrium conditions with full nitrogen solubilization were never attained even during a prolonged deep dive (at different depths), providing some kind of underlying protective mechanism, but, in certain extreme circumstances, enough nitrogen could enter the blood to render the green turtle susceptible to gas emboli in the brain and death after emergence. Our findings with wild individuals under field conditions are significantly different. We observed dramatic lesions, with not only bubbles but actually several milliliters of gas in wild animals entrapped at much shallower depths compared to Berkson's studies. The explanation of this disparity remains uncertain, but could be attributed to different factors, including animal species, time of forced submergence, water temperature, movement capabilities when submerged (Berkson's animals in the chamber were fastened to a board with very restricted in movement inside the chamber) and the previous diving profile of exposed individuals. Situations in which wild sea turtles are forcibly submerged due to entrapment in fishing gear suggest that behavioral and physiological responses are drastically different from what has been recorded under controlled laboratory conditions (Berkson 1966, Lutz & Bentley 1985, Lutz & Dunbarcooper 1987, Harms et al. 2003, Stabenau & Vietti 2003, Snoddy et al. 2009, Southwood 2013).

Multiple studies reveal that entanglement in fishing gear has significant effects on the physiology of sea turtles (Lutz & Dunbarcooper 1987, Harms et al. 2003, Stabenau & Vietti 2003, Snoddy et al. 2009, Snoddy & Southwood Williard 2010) but have never described DCS. Various factors may have contributed to the discovery in the current

study, including close collaboration with fishermen allowing access to alive and fresh dead bycaught animals, capacity for intensive medical evaluation following capture, availability of modern imaging technology and familiarity with diving animals and pathology related to GE. In addition, local oceanic conditions and type of fisheries could be unique relative to the circumstances of previous studies.

DCS findings in other marine air-breathing vertebrates: comparative physiology

Similarly to the present description in sea turtles, DCS had not been suspected in marine animals until GE consistent with DCS was described in beaked whales that mass stranded in close temporal and spatial association with military exercises using high-intensity mid-frequency active sonar, as well as in single stranded cetaceans in the UK coast (Jepson et al. 2003, Fernandez et al. 2005, Jepson et al. 2005, Fernández et al. 2013). Over the last decade, there has been an increasing body of evidence showing that marine mammals may suffer from acute and chronic GE, including the description of gas bubbles forming in tissues of fatally bycaught marine mammals trapped in nets at depth and rapidly brought to the surface (Jepson et al. 2003, Moore & Early 2004, Fernandez et al. 2005, Jepson et al. 2005, Moore et al. 2009, Bernaldo de Quirós et al. 2011, Bernaldo de Quirós et al. 2012, Bernaldo de Quirós et al. 2013b). In a recent study of gas composition of bubbles in bycaught dolphins, the authors concluded that nitrogen rich bubbles were formed by off gassing of supersaturated tissues (Bernaldo de Quirós et al. 2013b). These findings provide new evidence of nitrogen accumulation in breath-hold diving taxa despite anatomical and physiological adaptations. However, all marine mammal examples were already dead upon discovery, thus a definitive diagnosis of DCS could not be clinically established. Sea turtles afford a new opportunity for

studying this condition due to their amazing capacity for anoxia tolerance (Berkson 1966, Lutz & Bentley 1985, Lutcavage & Lutz 1997, Southwood 2013) and relative ease of handling, treatment and transport compared to marine mammals.

Hypothetical patho-physiological mechanism

The causal relationship between breath-hold diving in humans and DCS is increasingly being accepted due to the growing number of cases of DCS-like symptoms (Schipke et al. 2006). The pathophysiology of this condition in bycaught sea turtles is unknown.

Turtles have three muscular cardiac chambers, two atria and one ventricle, which allows some intraventricular mixing of systemic and pulmonary blood flow (Shelton & Burggren 1976, Hicks & Wang 1996, Wang et al. 2001). All sea turtles also have vascular adaptations for shunting during diving, including muscular sphincters within the pulmonary arteries and an anastomosis between the left and right aorta (White 1976, Wyneken et al. 2013). Cardiac shunting in sea turtles may confer some advantages under certain physiological conditions, such as diving (Hicks & Wang 1996), but could also risk bypass of gas bubbles from the pulmonary to systemic circulation (Germonpre et al. 1998, Harrah et al. 2008, Vann et al. 2011).

Different studies correlate exercise with breathing frequency, pulmonary blood flow and heart rate in green turtles (Butler et al. 1984, West et al. 1992, Southwood 2013). Exacerbated muscular activity leading to lactic acid built up is induced in free-swimming bycaught turtles, even under very short forced submersion episodes (Lutz & Dunbarcooper 1987, Stabenau et al. 1991, Stabenau & Vietti 2003). Additionally, heart rate and pulmonary blood flow in turtles often increase immediately before breathing

starts, which is suggestive of central mechanisms based on elevated sympathetic tone. This effect could also be induced by catecholamine release during fight-or-flight response resulting from capture (White & Ross 1966, Shelton & Burggren 1976, West et al. 1992, Wang & Hicks 1996, Wang et al. 2001).

We hypothesize that entrapped, submerged turtles develop DCS due to increased activity and catecholamine-induced sympathetic induction/parasympathetic inhibition. These processes disrupt the normal physiological and protective vagal diving reflex that minimizes blood flow through air filled pressurized lungs during diving. This hypothesis is supported by observed disruption of the dive response in struggling green sea turtles that are forcibly submerged (Berkson 1966).

Although speculative, the shunting ability in diving reptiles may not only represent a mechanism of regulating metabolism through modulation of oxygen supply to the tissue (Wang & Hicks 1996, Wang et al. 1997, Wang et al. 2001), but also could minimize nitrogen solubility in blood and subsequent risk of DCS. Sea turtles and sea snakes have the highest shunting capabilities (White 1976, Lillywhite & Donald 1989, Wyneken 2009). If this is the case, the longer the duration of the forced submergence, the higher the amount of nitrogen absorbed. As breath-hold divers, bycaught turtles cannot eliminate all absorbed gas at depth nor in ascent while the gear is retrieved. When the animal is surfaced with the fishing gear, gas bubbles start to form. We also speculate that the spastic retraction of the limbs (Figure 1a) may in part be comparable to the bending of limbs in humans. In our experience, it takes several hours or days for GE to resolve in turtles with even mild embolism.

Potential contributing factors

Environmental conditions, including water temperature and depth and time of immersion, are known to affect risk of DCS in humans and likely are important in sea turtles as well (Germonpre et al. 1998, Harrah et al. 2008, Vann et al. 2011). Tolerance of forced submergence in sea turtles is known to be affected by turtle size, turtle activity, and water temperature (Lutcavage & Lutz 1991, Stabenau et al. 1991).

In the present study, the highest rate of bycatch occurred between November and March, when most GE cases were encountered. When considered by proportion of captured animals with DCS, February, September, and October (average surface temperatures 13.4ºC; 24.5ºC, and 22.0ºC, respectively) were the months with highest occurrence. Hochscheid *et al* (2007) reported that Mediterranean loggerhead sea turtles increase time of submergence and rest on the bottom during the coldest periods of the year. This overwintering behavior could explain the higher trawling capture rates observed during winter in our region. However, the implications of temperature remain unclear from this study due to limited sample size and bias for presentation of cases during colder months.

Lower body temperatures in sea turtles compared to mammals, has been considered a potential protective mechanism against DCS, as body fluids would tolerate a higher pressure of gas dissolved without forming bubbles (Fossette et al. 2010). However, decrease in temperature would also increase nitrogen solubility at depth proportionally, thus increasing the risk of DCS when surfaced compared to mammals. Overwintering behavior could thereby increase the risk of DCS upon capture, especially if the turtle warms up out of water.

Regarding the influence of depth, some animals captured by trawlers fishing at over 60 m depth were full of gas after surfacing while others of similar size, coming from the same waters, same fisheries, same depth and during the same season had no detectable

gas. Possible explanations for this disparity are differences in actual depth of capture (unknown for trawler captures), the length of time submerged, and individual susceptibility to stress. Large depths do not seem to be required for the development of the DCS in sea turtles, as animals entrapped in gill nets as shallow as 10-20m deep presented with moderate or severe GE. One mild case was observed in a turtle bycaught by a vessel fishing at 30 m, although all severe cases of GE in trawlers occurred in turtles bycaught by nets fishing at over 60m depth. Based on these findings, even coastal or shallow fisheries like bottom trawls used to capture shrimp and other coastal fish resulting in high bycatch (Finkbeiner et al. 2011) could induce DCS in sea turtles.

Duration of submergence is another consideration. Berkson (1967) determined that submersion time was not a limiting factor to allow nitrogen saturation during diving, as the nitrogen tensions in blood reached a maximum and then dropped or leveled off well below saturation level. The author suggested that there might be an underlying mechanism for compensation. In contrast, our results suggest that time of submersion is correlated with severity of GE. Animals entrapped in gillnets (generally set at depths as shallow as 10-15m but for an average of 12hrs) tend to show more dramatic embolism than similar animals captured in trawlers in the same waters at a significant deeper depth (25-70m) but with much shorter operating times (2-6hrs).

Potential impacts and future research

The actual contribution of DCS resulting in sea turtle mortality on a global scale is unknown; however, it is notable that our observations originated from interaction with two gear types of foremost concern with regard to sea turtle bycatch. Bycaught sea turtles that are initially active are usually immediately released and are not considered

lethal interactions. Our results show that many turtles could have GE and may subsequently die within hours or days post-release. Mortality following fisheries interaction could be much higher than previously estimated. Accurate data on both immediate and post-release mortality data are crucially important for refining the current mortality estimates used to govern management decisions with far-reaching conservation, economic and social consequences (Southwood 2013).

The cause of death in comatose and dead net caught turtles should be re-evaluated to clarify the percentage of animals dying from DCS instead of drowning or dying from both. Current procedures used aboard fishing vessels to revive comatose turtles, while useful for drowning, are probably ineffective for DCS. Although GE can be detected in the field (e.g. with on-board portable ultrasound) any mitigation measures should focus on prevention and minimization of risk of DCS given that effective treatment is unlikely to be practical under most at-sea conditions.

CONCLUSIONS

The current study demonstrates that bycaught marine turtles can develop and die from DCS. Diagnosis was based on clinical signs, detection of intravascular gas by imaging and necropsy, gas composition analysis, and successful resolution with hyperbaric treatment. To our knowledge, these findings represent the first example of DCS in air-breathing marine vertebrates that fulfill all of these medical diagnostic criteria, providing new clues for the better understanding of the diving response and DCS avoidance in other breath holding diving vertebrates (Piantadosi & Thalmann 2004).

This discovery has significant implications on sea turtle conservation. It would be important in light of the present findings to review regional sea turtle bycatch intervention protocols worldwide after elucidating the real prevalence of the condition based on different fisheries techniques, geographic areas, oceanic conditions, sea turtle species and individual characteristics.

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Table 1. List of bycaught turtles diagnosed with gas embolism, biological, clinical and pathological data.

Gear type	Depth range (m)	CCL range (cm)	Temperature range (ºC)	Clinical classification	GE Diagnosis	Treatment	End result
Gillnet (n=6)	10.5 - 50	30.2 – 41.5	13.4 – 24.5	2 Comatose	1 Mild/Moderate	None	Dead
					1 Mild/Drowned	Medical	
				4 Dead	3 Moderate	None	
					1 Severe		
Trawl (n=23)	30 - 75	28.6 - 74	13.8 - 25	8 Normal	8 Mild	Medical	Reintroduced
				9 Hyperactive /Neurologic	2 Mild	Medical	Reintroduced
					2 Mild/Moderate	Medical	Reintroduced
					3 Moderate	Medical (1)	Dead
						Hyperbaric & Medical (2)	Reintroduced
					1 Moderate/Severe	Medical	Dead
					1 Severe	Medical	Dead
				2 Comatose	2 Mild/Drowned	Medical	Reintroduced
				4 Dead	1 Moderate	None	Dead
					1 Moderate/Severe		
					2 Severe		

Abbreviations: CCL, Curved Carapace Length; Temp, average sea superficial temperature on the month of capture.

FIGURES AND LEGENDS

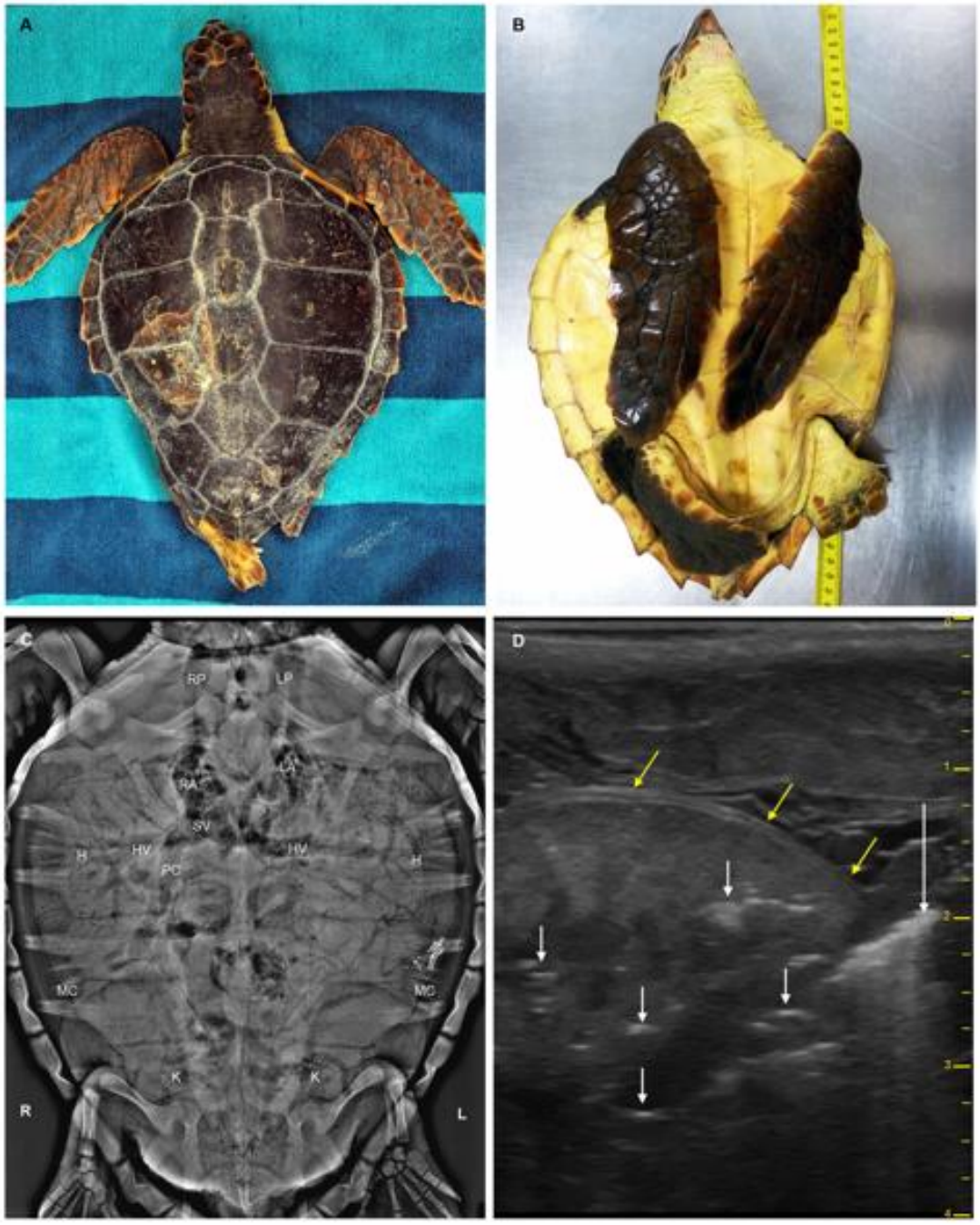

Figure 1. Sea turtles at reception: signs (a and b) and preliminary detection of clinical gas (c and d). (a), Case CcGE21 at arrival. Moderate systemic GE. Note spastic retraction of the hind limbs under the carapace before recompression therapy. These signs resolved immediately after hyperbaric oxygen treatment. (b), External aspect of case CcGE18 with severe systemic GE after a few hours *postmortem*. This animal

arrived alive and did not respond to emergency medical treatment. Note retraction of all four extremities under the body at *rigor mortis*. (c), Dorso-ventral digital radiographic image (technique, 90Kv, 10mAs, 1 m focal distance, right side is to the left of the image) of case CcGE15 with severe systemic GE. Note the lumen delimitation of right and left atrium, *sinus venosus*, and major vessels by the massive presence of intraluminal gas (evidenced as a radiolucent region). Minor vessels are also clearly visualized in the area of projection of the liver and kidneys (gas angiograms). (d), Renal ultrasound of patient CcGE23 with moderate systemic GE. Image obtained with a 12MHz linear probe on the left prefemoral fossa with a ventrolateral-dorsomedial orientation. Note the presence of intraluminal gas in renal major vessels as evidenced by hyperechoic spots and comet tail artifacts (long white arrow). Smaller collections of gas are also clearly visualized disperse inside the kidney parenchyma (short white arrows). Renal margin (yellow arrows). Abbreviations: RP = right precava, LP = left precava, RA = right atrium, SV = *sinus venosus*, HV = hepatic veins, PC = postcava, H = venous hepatic system , MC = marginocostal vein, and K = kidney.

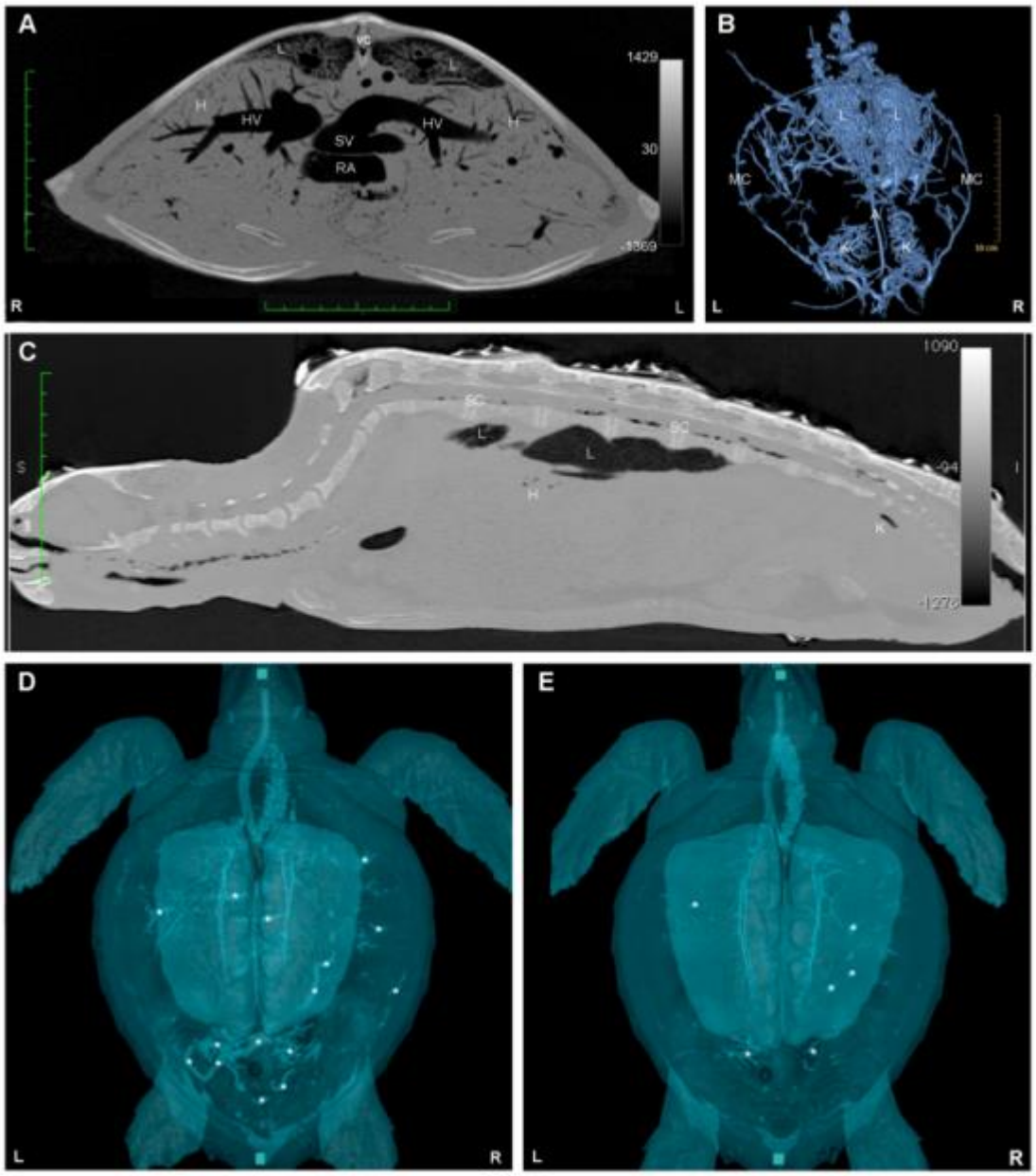

Figure 2. Evidence of GE on computed tomography. (a), Transverse image of mid-cranial coelomic region at the level of the heart of case CcGE15 with severe systemic GE. There is evidence of intraluminal gas (black) inside the heart and major vessels. Gas is also present within the venous hepatic system and vertebral canal. Lungs are hyperattenuated (whiter) due to partial collapse. (b), Dorsal oblique view of 3D volume recreation through volumetric segmented reconstruction (*volume rendering*) from patient CcGE15 with severe systemic GE. Note the presence of gas within the different peripheral and intracoelomic vessels. Lungs contain less gas than normal. The kidneys

are clearly visualized due to the massive presence of intravascular gas in this region. (c), Mid-sagittal image of patient CcGE20 with mild systemic GE. Notice the presence of abnormal gas at the central nervous system, spinal cord and renal and minor hepatic vessels. (d) and (e), Dorsal views of 3D air volume rendering view of total gas volume inside the patient CcGE23 with moderate systemic GE before (d) and after (e) oxygen hyperbaric treatment for recompression. Images were obtained 6 hours apart. All gas in brighter color and intravascular gas pointed with stars. (d), Notice the delineation of hepatic veins and renal vessels by the presence of intraluminal gas before treatment. Lungs expansion is also reduced. (e), Most gas contained in the large vessels has almost disappeared after hyperbaric treatment indicating gas reabsorption while pulmonary expansion is back to normal. Few minor vessels still contain gas in the periphery of the hepatic and renal projection areas. Abbreviations: VC = vertebral canal, L = lung, H = venous hepatic system, HV = hepatic veins, SV = *sinus venosus*, RA = right atrium, MC = marginocostal vein, A = aorta, K = kidney, and SC = spinal cord.

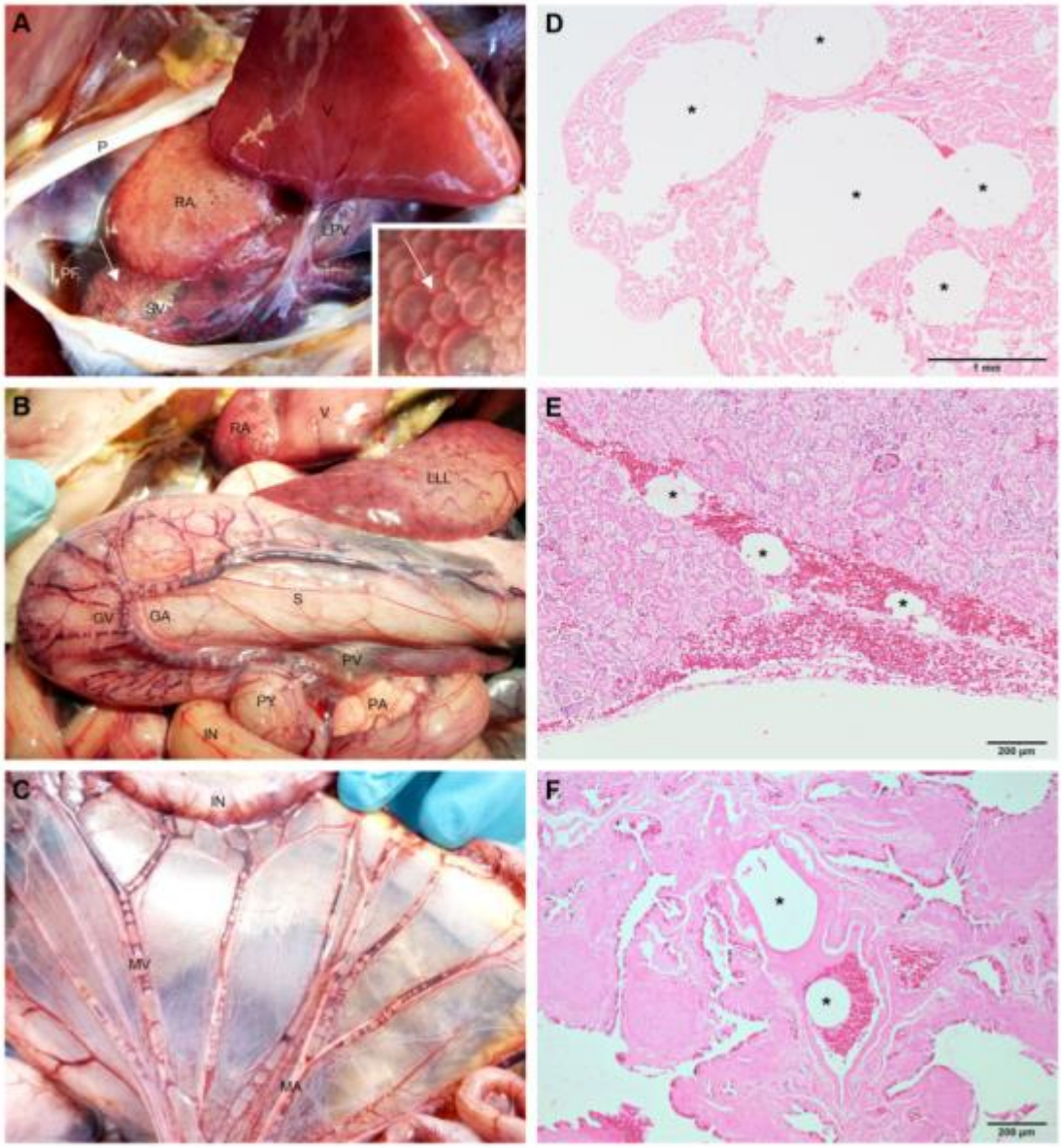

Figure 3. Gross (a-c) and histopathological findings (d-f). (a), Caudo-ventral view of the heart, dorsal surface, of case CcGE18 with mild/moderate systemic GE. The right atrium and sinus venosus (amplified) are diffusely distended with a moderate amount of intracameral, gas bubbles. (b), Left dorso-lateral view of the stomach greater curvature (after being reflected cranially) and liver of case CcGE14 with moderate systemic GE. Note that gastric veins from greater curvature and the pyloric vein are diffusely expanded with variably sized gas bubbles. (c), Small intestine and mesentery of case CcGE14 (moderate systemic GE). Note that mesenteric veins are diffusely expanded

with a large amount of variably sized gas bubbles, coalescing at the mesenteric venous root. (d), Right atrium of case CcGE15 (severe systemic GE). Atrial lumen shows multifocal to coalescing, variably sized, round to oval, fat-negative gas emboli, compressing the adjacent myocardium. H&E; 2x. (e), Kidney of case CcGE18 (mild/moderate GE) Interrenicular veins are multifocally occupied by round to oval, variably sized, fat-negative gas emboli. H&E; 10x. (f), Lung of case CcGE7 (severe systemic GE). Pulmonary veins show intravascular, variably sized, round to oval, fat-negative gas emboli. H&E; 10x. Abbreviations: V = Ventricle, RA and LA = right and left atriums, LPV = left precaval vein, P = pericardium, PF = pericardial fluid, SV = sinus venosus, LLL = liver left lobe, S = stomach, GA = gastric artery, GV = gastric veins, PY = pylorus, PV = pyloric vein, PA = pancreas, IN = intestines, MA and MV = Mesenteric arteries and veins.