

# Gas Embolism Assessment in Sea Turtles: Predictors of Mortality for the Deadliest Pathology related to Trawling

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## Research Article

**Keywords:** Sea turtles, gas embolism, mortality, neurological deficits, significant acidosis, prognostication.

**Posted Date:** April 13th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-403204/v1>

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## Abstract

Sea turtles that are entrapped in static and towed nets may develop gas embolism which can lead to severe organ injury and death. Physical and physiologic factors associated with gas-embolism and predictors of mortality were analysed from 482 bycaught loggerheads. We found 204 turtles affected by gas-embolism and significant positive correlations between the presence of gas-embolism and duration, depth of trawl and turtle size and temperature and between mortality and neurological deficits, significant acidosis and involvement of > 12 cardiovascular sites and the left atrium and sinus venosus-right atrium. 164 turtles were released to the sea. Results of this study may be useful in clinical evaluation, prognostication, and treatment for turtles affected by gas-embolism, but bycatch reduction must become a priority for major international organizations.

## Introduction

Gas-embolism (GE) is the presence of gas within the cardiovascular system, which can result in a variety of systemic effects depending on the amount of gas and its anatomic distribution. In humans, GE can occur as a result of trauma, underwater diving, or as a complication of surgical and diagnostic procedures. Gas enters the circulation when a communication between a gas source and the vascular system exists together with a pressure gradient that favors gas translocation<sup>1</sup>. Breath-holding diving vertebrates have been considered to be protected against GE through anatomical, physiological, and behavioural adaptations<sup>2,3,4,5,6,7,8</sup>.

Fisheries interactions are the most serious conservation risk for threatened sea turtle populations<sup>9</sup>. Bycatch is a well-documented, worldwide problem resulting in considerable mortality of non-targeted species<sup>9,10,11</sup>. A minimum of 132,000 sea turtle captures are estimated to occur annually in the Mediterranean by bottom trawlers, longlines and set nets, resulting in a minimum of 44,000 deaths, the majority owing to small-scale fisheries<sup>12</sup>. In particular, more than 52,000 turtles are caught yearly along the Italian coasts and about 10,000 of these die. Trawl nets are the gears involving the highest bycatch probability, and this is a special concern in the Adriatic Sea, whose shallow waters are favorable for trawling and are also foraging areas rich in benthic communities for sea turtles<sup>13</sup>. According to several studies<sup>14,15</sup>, direct mortality due to trawling is correlated with trawl duration, presumably due to prolonged apnoea. Until recently, death has been attributed to the effects of hypoxia and water aspiration associated with forced submergence or to sequelae of traumatic injuries<sup>16</sup>. Delayed post-release mortality has been suspected to be high<sup>13</sup>.

In 2014, a study of bycaught loggerhead sea turtles (*Caretta caretta*) entrapped at depth in trawls and gillnets demonstrated that sea turtles developed gas embolism (GE)<sup>17</sup>. Since that time, GE has been documented in four additional sea turtle species<sup>18,19</sup>. It has been hypothesized that entrapped, submerged turtles develop DCS due to exertional activity (i.e., attempting to escape from the net), and associated catecholamine-induced sympathetic induction and parasympathetic inhibition. These effects are believed to disrupt the normal, protective vagal diving reflex that minimizes blood flow through air-filled pressurized lungs during diving<sup>17</sup>. Lactic acidosis and evidence of decreased renal function and cellular injury have been reported in GE-affected turtles<sup>20</sup>. To date, reported mortality rates due to GE in bycaught sea turtles range from 30–53%<sup>17,20,21</sup>. In the only study to assess survival of immediately released turtles affected by GE<sup>20</sup>, 12 of 28 (43%) animals died on-board prior to release, and 3 of 15 (20%) active turtles released with satellite tags died within 6 days.

The objective of the present prospective study was to examine risk factors associated with GE in a large cohort of bycaught loggerhead turtles, to describe the physical and physiologic status of the turtles, and to evaluate the diagnostic performance of various parameters as predictors of mortality.

## Results

### Clinical and radiographic findings and outcome

Four hundred eighty-two turtles were admitted to the hospital after accidental trawl capture and were evaluated even if the animal did not show clinical signs of disease or trauma. The duration from surfacing (time of capture on fishing boat) until the animal arrived at the STC ranged from 5 to 7 hours.

Turtles were mainly incidentally captured (bycaught) in trawls in the winter months, with the highest incidence in December and January. Out of 482 turtles, 308 (64%) were juveniles. Of 482 trawled turtles, 204 (42.4%) showed radiographic signs of systemic GE of variable severity (Graph 1).

Trawl depth ranged from 11 to 144 m (median of depth 46,8 m) with a duration of fishing net submergence (soak time) ranging from 2–5 hours (3.5 hours median). The range of withdrawal time (ascent time) of fishing gear was from 13 minute (for a depth of 20 m) up to a maximum of 20 minutes (for a depth of 140 m). The withdrawal time of the nets was a parameter that cannot be statistically evaluated because it is correlated only to the fishing depth. There is therefore no difference between the animals with GE and those unaffected.

In Table 1 summarized the comparison of fishing and clinical data between loggerheads with and without GE.

Table 1

Comparison of fishing and clinical data between loggerheads turtles that were accidentally captured during trawling that developed GE (n: 204) and those that did not develop GE (n :278). Parameters with p < 0.05 were significantly different between the two groups.

	Turtles with GE (n = 204)		Turtles without GE (n = 278)		p-value
	median	interquartile range	median	interquartile range	
Trawl Duration (hr)	4	3 to 4	3	3 to 4	0.001
Trawl Depth (m)	49.5	36 to 63.9	37	32 to 54	< 0.0001
Curved Carapace Length CCL (cm)	64	60 to 69	62	56.5 to 68	0.0096
Curved Carapace Width CCW (cm)	58.25	54.75 to 63	57	51.5 to 62.5	0.024
Weight (kg)	31	24.8 to 38.8	28.25	21 to 36.8	0.0278
Turtle temperature (°C)	12.5	10.9 to 14.2	13.3	11.6 to 16.6	< 0.0001
	<b>n</b>	<b>%</b>	<b>n</b>	<b>%</b>	
Male	10	4.9%	16	5.8%	0.2506
Female	55	27.0%	93	33.5%	
Immature	139	68.1%	169	60.8%	

On arrival, 165 (81%) of the turtles with GE presented with good body condition and normal fat stores, 19/204 (9%) were slightly thinner while 20/204 (10%) were very thin. Of the 204 turtles 195 (95%) showed peripheral edema and 31/204 (15%) prolapse of the cloaca (Fig. 1). The mean respiratory rate of turtles with radiographic signs of systemic GE was 0.4 breaths/min. At physical examination 143/183 (77 %) of these turtles were alert, active, and exhibited normal behaviour, 29/183 (16%) were slightly depressed (moderate response to manipulations) and 12/183 (7%) were lethargic/comatose (no response to manipulations) or developed progressive neurological symptoms, including limb paresis or loss of nociception. 10/12 turtles of those that were lethargic/comatose died within 24 hours of arrival. Seven of 184 (4%) turtles showed retraction of the hind limbs under the carapace (Fig. 2A). Three turtles had blood coming from the oral cavity arising from the larynx and died within 8 hours of arrival (Fig. 2B). Among turtles diagnosed with GE, there was a significant correlation between mortality and neurological deficit, comatose state, hind limb retraction under the carapace and lower heart rate (Table S1). Blood coming from oral cavity resulted statistically related to mortality (p = 0,00698, Fisher's exact test). There were no significant correlations between mortality and trawl duration, depth, animal size, sex, weight, body temperature, respiratory rate, presence of peripheral edema and PCV.

We found 14 CAS were gas could be detected. Gas distribution within the cardiovascular system was assigned to one or more of these 14 anatomic sites (Table 2) (Fig. 3, 4, 5).

Table 2

Anatomic sites of gas distribution as seen radiographically in 204 loggerhead turtles affected by GE. Abbreviation: H: hepatic vessels; EI: external iliac vessels, postcaval vein; MC: margino-costal vessels; RP: renal portal vessels AV: abdominal vein; PV: pulmonary vessels; AT: transverse abdominal vein; G: gastric vein; PrC.: precaval vein; bct: brachiocephalic trunk; SM: superior mesenteric artery; sv/ra: sinus venosus / right atrium; LA: left atrium.

Anatomic site	H	EI	PsC	MC	RP	AV	PV	AT	G	PrC.	bct	SM	sv/ra	LA
Number of turtles and percentage of turtles affected at each site	204 (100%)	192 (94.1%)	187 (91.6%)	184 (90.2%)	182 (89.2%)	164 (80.4)	149 (73%)	140 (68.6%)	137 (67.15%)	132 (64.7%)	118 (57.8%)	110 (53.9%)	71 (34.8%)	50 (24)

GE involved at least 2 CAS (median 10, IQR 7–13). Among the 40 turtles that died the median number of VA was 14 (IQR 13–14), while among the 164 survivors the median number of CAS was 9 (IQR 7–11) and the difference was significant (KW = 70.877, p < 0.0001). The logistic regression, with death/no death as outcome and number of CAS as continuous independent variable, resulted in a significant OR of 5.91 (95% CI 3.13–11.19). Applying the ROC curve analysis, it was found that over the threshold of 12 VA there is a high probability of death (AUC = 0.98, SE = 0.0052, p < 0.0001).

A logistic regression was applied also to evaluate which CAS was a higher risk of death. Hepatic vessels were deleted both from univariable and multivariable models because all turtles had involvement of that CAS. A very high risk of death was found for GE in the left atrium (OR = 40.241, 95% CI 14.8–108.9, p < 0.0001), pulmonary vessels (OR = 17.2, 95% CI 2.29–128.81, p = 0.0056), sinus venosus / right atrium (OR = 38.47, 95% CI 11.2–132.24, p < 0.0001), abdominal vein (OR = 10.97, 95% CI 1.46–82.61, p = 0.0201), transverse abdominal vein (OR = 23.37, 95% CI 2.99–137.13, p = 0.0025), and precaval vein (OR = 26.62, 95% CI 3.56–198.72, p = 0.0014).

On radiographic examination 65 (32%) of the 204 animals with radiographic signs of systemic GE also showed signs of drowning (Fig. 5). Of the 65 subjects with signs of drowning, 31 (48%) died while 34 (52%) survived. By contrast, of the 278 turtles that did not show radiographic signs of GE, 8 (3%) showed radiographic signs of drowning without any death. In GE-affected turtles drowning signs emerged as a statistically significant factor increasing risk of death in univariable analysis (OR = 13.19, 95% CI 5.55–31.34,  $p = 0.0001$ ). However, the multivariable model with stepwise selection left in the final model only left atrium (OR = 22.49, 95% CI 5.81–87.18,  $p < 0.0001$ ) and sinus venosus / right atrium (OR = 24.32, 95% CI 7.95–74.45,  $p < 0.0001$ ). No other radiographic variables were independently and significantly associated with death risk.

### Blood analysis results

In some cases, during the venous sampling, abundant presence of gas mixed with blood was observed (Fig. S1). PCV ranged from 22 to 44% (mean  $33 \pm 5.56$ ) and was slightly above normal ranges in about one third of all the turtles with GE analyzed, without any statistical correlations with severity or mortality. Blood gas, hematologic and chemistry analytes of clinical relevance were analyzed in 51 of 184 loggerheads with GE. WBC was within normal range. Creatine kinase (42,130 U/L to 813 mean 1,902 U/L) was elevated for all the turtles tested<sup>22</sup>. Phosphorus was higher in turtles that died (median 10.8 mmol/L) than those that survived (median 6.6 mmol/L) ( $p < 0.0001$ ). No other biochemical analytes were statistically related to mortality.

Results of blood gases analysis in dead and surviving turtles and ROC curve analysis of blood gases biomarker are shown in Tables 3 and 4. The analyte with the highest AUC was pH (AUC = 0.923, 95% CI 0.85–0.99), and the risk of death increased if  $pH < 7.26$ .

A multivariable Cox regression was performed with the following independent variables: pH ( $< 7.26 / > 7.26$ ),  $pCO_2$  ( $> 33 / < 33$  mmHg), BE ( $< -11 / > -11$  mmol/L), Potassium ( $> 3.2 / < 3.2$  mmol/L),  $tCO_2$  ( $< 23.4 / > 23.4$  mmol/L),  $HCO_3^-$  ( $< 24.2 / > 24.2$  mmol/L),  $SO_2$  ( $< 60 / > 60$  %). Following stepwise regression, only pH was retained in the model as an independent risk factor for death and this means that other factors, significant in the univariable model, could not be considered as factors that affect independently the risk of death.

Table 3  
Mean of the blood gas results in dead and surviving GE-affected turtles \*( $p = 0.05$ )

	pH*	PCO2(mmolHg)*	HCO3 (mmol/l)	tCO2 (mmol/l)*	PO2 (mmolHg)	SO2 (%)	O2 Ct (vol%)	Na+ (mmol/l)	K+ (mmol/l)*	Cl- (mmol/l)
Turtles died n :13	7,17	44,1	37,1	24,77	32,30	84,5	14,55	151,45	4,26	114,0
Turtles Survived n :38	7,44	32,23	25,81	28,16	38,024	85,846	20,208	154,42	3,27	115,05

Table 4  
Results of the analysis of the ROC curve of each blood gases biomarker with area under the curve (AUC), its 95% confidence interval, the threshold (cut off), and adjusted p value, as mortality predictors

Blood analyte	Cut off	AUC	95% CI AUC	adjusted p-value
pH	$\leq 7,26$	0,923	0,850 to 0,997	0,0006
PCO2	<b>&gt; 33</b>	0,863	0,759 to 0,967	0,0006
K	$> 3,2$	0,772	0,632 to 0,912	0,0006
tCO2	$\leq 23,4$	0,749	0,595 to 0,902	0,0051
SO2	$\leq 60$	0,739	0,530 to 0,947	0,0602
HCO3	$\leq 24,2$	0,726	0,534 to 0,918	0,0592
PO2	$\leq 21$	0,696	0,503 to 0,890	0,0986
Na	<b><math>\leq 152</math></b>	0,672	0,484 to 0,861	0,1388
O2Ct	<b><math>\leq 12,1</math></b>	0,603	0,402 to 0,803	0,4139
Cl	$\leq 112$	0,575	0,368 to 0,781	0,5428

### Postmortem examination results

Necropsy was completed for 33 turtles. Gas macrobubbles were found in most arterial and venous vessels of all main organs and in major vessels. In these cases, vessels were completely filled with gas even for some centimeters along the length of the vase with side-by-side serial macrobubbles showing evident vascular obstruction. (Fig. S2) In severe cases in correspondence of the lumen of the precaval vein there was no presence of blood which was completely replaced by gas. (Fig. S3 A and B)

In 12 cases the heart appeared very dilated by intracardiac gas and 20–30 ml of gas were aspirated from each atrium. (Fig. S3 C and D)

## Discussion

Delayed mortality of bycaught turtles due to sequelae of near-drowning, metabolic disturbance, and gas embolism upon release<sup>17</sup> is suspected to be high. To date, relatively few studies with small sample sizes have been conducted on GE in trawled sea turtles<sup>17,20,21</sup>. The present study provides the largest data set to date for sea turtles affected by GE, including a diverse collection of variables and clinical data. Approximately 20% (40/204) of GE-affected turtles in this study died, while 80% recovered and were returned to the sea in 1-13 days without specific therapy. About half (21/204) of the GE-affected animals arrived already dead at the STC while the other half (19/204) died during hospitalization within 48-72 hours post-capture, although in severe cases, they died in the first 6 to 8 hours. In previous studies on lower numbers of turtles accidentally caught by trawls and gillnet, the mortality caused by GE was between 41 and 30%<sup>17,21</sup>. These turtles died during hospitalization in a time comparable to that observed in our study. In a recent study<sup>20</sup> 12 of 28 (43%) animals died on-board fishing vessels and 3 of 15 (20%) turtles released with satellite tags died within 6 days. About 90% turtles with GE alive upon arrival at STC recovered from the disease in a time ranging from 1 and 13 days without any supportive drug therapy. They were only *dry-docked* or kept in tanks with low water level in a warm and quiet room. Animals were discharged only when complete resolution of clinical and radiographic signs and normal blood gases were assessed.

According to several studies<sup>14,15</sup>, direct mortality due to trawling depends on tow duration and hence to the submergence time. We found a statistically significant correlation between presence of GE and duration and depth of fishing nets, size, weight and body temperature of the turtles. Intense loggerhead turtle interactions with trawl nets have previously been described in Adriatic Sea<sup>23,24,25,26</sup>. This area is characterized by shallow waters (<100 m) and rich benthic communities where turtles in the demersal stage spend the winter<sup>13,23,24,25,26,27</sup>. Mediterranean loggerhead sea turtles increase time of submergence and rest on the bottom during the coldest periods of the year<sup>23,27</sup>. Bottom trawling mostly interferes with the demersal stage of the loggerheads that are more likely to assemble in shallow water in order to feed on abundant benthic and epibenthic prey<sup>24,25</sup>. Turtles in the present study were trawled mostly in the winter with juveniles comprising over 60% of the bycaught animals. After April in the southern Adriatic Sea, turtles accidentally caught by trawling are almost never observed. The highest rate of bycatch in Valencian coast of Spain occurred between November and March, when most GE cases were encountered<sup>17</sup>.

The majority of the observed turtles (80%) presented with good body condition and normal fat stores. We found peripheral edema in 95% of the turtles and prolapse of the cloaca in 15%, with edema of the cloacal mucosa presumably due to mechanical obstruction of vasculature by bubbles. These anomalies took 3-7 days to resolve. In humans with GE, bubbles may cause platelet and leucocyte aggregation, cytokine release, and activation of the complement, kinin, fibrinolytic and coagulation cascades. The subsequent acute inflammatory response results in increased capillary permeability, edema and haemoconcentration<sup>28</sup>.

Respiratory rates did not differ significantly between turtle that survived (median 0.4, range 0.2–0.6 breaths/min) and those that died (median 0.4, range 0.2–0.5 breaths/min), though respiration was slower in all the GE turtles than reported respiratory rates of loggerhead sea turtles after 30 min on-board recovery following capture by trawl net (trawl T0, median 5.2, range 1.0–10 breaths/min). Respiratory rates were similar to captive swimming unrestrained subadult loggerheads at 22–25 °C (0.34 breaths per min)<sup>29</sup>.

Almost all the turtles (10/12) in a lethargic/comatose state and with progressive neurological symptoms and the three turtles with blood coming from the larynx and out the oral cavity died within 8-24 hours. We found a statistically significant correlation between mortality and neurological deficit, comatose state, hind limb retraction under the carapace, and lower heart rate. Seven turtles (4%) with GE showed retraction of the hind limbs under the carapace. We must consider that the hind limb retraction under the carapace is also a frequent sign of severe stress in sea turtles and it is considered a normal protective rear flipper clasp reflex<sup>30</sup>.

We did not find a correlation between mortality and duration and depth of fishing nets, animal size, sex, weight, body temperature, respiratory rate and peripheral edema. Unlike what has been reported recently<sup>20</sup> where PCV was significantly lower in turtles that died from GE, we found no statistically significant PCV alteration in the turtles that died. However, in about one third turtles analyzed (71/204) we found that PCV was slightly above normal ranges, without any statistical correlations with mortality. In humans, severe GE can be accompanied by hemoconcentration resulting from increased vascular permeability mediated by endothelial damage.

On radiographic examination, 65/204 (32%) animals with radiographic signs of systemic GE also showed signs of drowning and 48% of these turtles died. Conversely, of the 278 trawled turtles without GE, only 8 (3%) showed radiographic signs of drowning and none of these died. Very interesting is that in the multivariable analysis drowning is not a risk factor for death but the only risk factors are the number of the CAS and the presence of GE in the left atrium and sinus venosus/right atrium. According to these data it can be deduced that trawled sea turtles rarely undergo drowning if not also suffering from GE and that mortality is closely associated with the presence of drowning contextually to GE. Specifically, we can therefore hypothesize that the animals probably first undergo severe GE involving the heart and only then drown. In a recent study, GE entails a worse prognosis if it occurs concurrently with water aspiration<sup>20</sup>. In our opinion therefore trawling turtles essentially die for severe undiagnosed GE and water aspiration associated with forced submergence is not the leading cause of death.

In the present study radiographic examination proved to be an excellent diagnostic tool to evaluate GE in sea turtles. Full-body radiographs in the DV projection is easy to perform and allows effective diagnostic evaluation because it is possible to observe all the defined CAS affected by the accumulation of gas. The DV projection is helpful for identifying pathological radiopacity of the lungs as in drowning, but the cranial-caudal and LL projections were more useful to view the lungs without the overlap of celomic soft tissues. The LL projection is useful for the evaluation of the renal CAS (external iliac and renal portal vessels), which however are evident also in the dorsal-ventral projection, and severe accumulation of gas in the heart. As with the liver, the presence of

gas in the external iliac and renal portal vessels is not significant for the prognosis because gas in these vessels is present in almost all GE cases (94% and 90% respectively).

Compared to ultrasound, the radiographic examination allows a better over-view of all the CAS with less stress for the animal. In our experience, because 12/14 CAS was a statistically significant risk factor associated with mortality, it is important to be able to observe all the CAS through the radiographic examination. The advantage of ultrasound is that sea turtles can be examined on-board fishing vessels, but in animals larger than 30 cm length (CCL), many areas, such as the heart or major vessels, cannot be examined<sup>20</sup>. In the present study high risk of mortality was identified just for these CAS (left and right atrium, pulmonary vessels, sinus venosus, and major vessels) and the median CCL of the turtles was 64 cm. Therefore, in most cases radiographic examination is essential for a complete evaluation of the lungs and the CAS and then to define the severity of the disease. Ultrasonography for animals larger than 30 cm in length is useful as a screening to evaluate if they suffer from GE because the renal vessels are involved in more than 90% of the cases and are always easily identifiable<sup>17,20</sup>. During the radiographic follow-up of the turtles with GE we observed that gas in the renal vessels is also the last to disappear, so ultrasonography can be useful to monitor resolution of GE.

One of the goals of the study reported here was to document venous blood gas and acid-base values for loggerhead turtles with GE 5-7 hours after trawling and to investigate differences in these values between turtles that survived and those that died. In turtles with GE the blood circulation is dramatically slowed or even stopped due to the gas emboli disseminated along the cardiovascular system, and peripheral tissue O<sub>2</sub> extraction should be considerable.

In the present study pH, pCO<sub>2</sub>, tCO<sub>2</sub>, HCO<sub>3</sub>, SO<sub>2</sub> and potassium concentration were found to be relevant in the clinical assessment of turtles affected by GE. However, the multivariate analysis showed that only pH was an independent risk factor for death. Turtles that died had significant acidosis compared with turtles that survived.

Considering the high amount of gas present in the cardiovascular system of turtles affected by GE, pathologically this disease could be compared to the massive systemic air macroembolism of humans with a rare but catastrophic and largely underdiagnosed, undertreated, and underreported disease<sup>31</sup>. The brain and heart are the end organs most vulnerable to these ischemic events, which can lead to irreversible sequela or death. The air emboli cause pathological changes by two mechanisms: a reduction in perfusion distal to the obstruction and an inflammatory response<sup>32</sup>. These processes lead to vasogenic edema and worsen ischemia of the end organs<sup>33</sup>. Hyperbaric oxygen (HBO) is the main therapy for massive gas embolism from any cause because may not only decrease the size of air bubbles but also provide an adequate supply of oxygen to the ischemic tissue but such a treatment can be performed only with specialized equipment<sup>34</sup>. There is no evidence that treatment with corticosteroids, anticoagulants, or lidocaine is related to a beneficial outcome in massive gas embolism<sup>32</sup>.

It is well known that in sea turtle during diving, apnea a right-to-left-shunt occurs. Besides intracardiac shunts, pulmonary-to-systemic shunting has been described between the pulmonary arteries and veins suggesting control of perfusion at several levels<sup>35,36</sup>. Recently multiple physiological artero-venous anastomoses were detected between pulmonary arteries and veins on high resolution Multidetector Computed Tomography images<sup>37,38</sup>.

With systemic GE in turtles, the gas is present throughout the venous and arterial circulation because the gas may pass from the heart or arteriovenous anastomoses from the venous to the arterial compartment. It is plausible that the significant amount of gas found throughout the venous and arterial circulation and in the heart causes a slowing or even an arrest of the blood circulation. Nevertheless, "only" 20% of the turtles in the present study died and many animals with severe disease spontaneously eliminated the gas bubbles and recovered.

Freshwater turtles survive weeks of anoxia at low temperatures without suffering from oxidative damage upon reoxygenation. A recent study showed that ATP/ADP pools and low succinate accumulation likely protect turtle hearts from anoxia/ reoxygenation injury<sup>39</sup>. Sea turtles continue to survive long after there is a total absence of oxygen in blood and lung<sup>40</sup>. Loggerheads also maintained electrical activity of the heart for over 1 hour of total anoxia<sup>41</sup> in very sharp contrast to mammals. Turtle brain ATP levels are maintained for at least 2 hours of anoxia<sup>42</sup> while depletion occurs within minutes in mammals.

During extended dives all tissues may become anaerobic and large changes in blood pH and pCO<sub>2</sub> are tolerated<sup>43</sup>. The major adaptation that allows turtles to endure total anoxia for many hours is the special ability of the brain to function in the absence of O<sub>2</sub>.

Loggerhead turtles can rest on the ocean bottom for over 7 hours in cold temperatures<sup>27,44</sup> but if turtles are struggling in nets, heart rate will increase from vigorous activity, which will result in rapid oxygen depletion. Higher heart rate will increase pulmonary blood flow, which may contribute to increased nitrogen absorption, potentially resulting in the development of gas emboli<sup>45</sup>. However, the exact etiopathogenetic mechanism of this systemic macroembolism in sea turtles has not yet been clarified. In the present study the range of withdrawal time of fishing gear was from 13 minute/depth of 20 m up to a maximum of 20 minutes/depth of 140 m and we cannot ascribe that this rate of ascent definitely causes decompression sickness in sea turtles. Moreover, this ascent rate did not differ between animals that developed GE and those that did not.

The turtles with GE, those that arrived alive at the STC and were then *dry-docked* or kept in tanks with low water level in a room at approximately 25-30 °C without any pharmacological treatment had a mortality of about 10%. Theoretically, gas embolism in turtles must be treated in a hyperbaric chamber as soon as possible but studies on large numbers of animals must be carried out to understand if it is promptly effective on turtles with very severe GE, destined to die within a few hours.

There has been still limited study and with small sample size of turtles for post-release mortality of marine turtles following capture by trawl fisheries<sup>20,46,47</sup>. Parga et al. (2020) described that on 15 animals that survived and were released with sPAT tags three (20%) died during the first 6 days and the outcome of a fourth animal was unknown. The immediate release of turtles into the sea can only be an option if there is no rescue center available to refer the turtles to. On

board the fishermen cannot stage the disease and understand its severity. From the statistical data of our study only turtles in a comatose state are obviously serious, but turtles without pathognomonic clinical signs can also be severe and RX staging is always required. All the animals in our study underwent radiographic checks every 48 hours with progressive improvement of the gas inside the vessels and clinical improvement. In no animal have we seen a worsening in quantity of gas in the following days.

In light of the results achieved in this research, it is strongly recommended to transport the turtles as soon as possible to rehabilitation or rescue centers, rather than releasing them directly into the sea, even after a resting phase; in fact, turtles that apparently appear to be in good condition may actually have a GE in progress. Waiting to understand how to stem the phenomenon during fishing, collaboration with the fishermen is essential because it is important that the sea turtles are immediately transported to the rescue centers in order to be housed in the tanks in case of GE so as to control and to reduce their mortality.

The adoption of conservation actions has become a strategic issue in the Mediterranean, where the commercial fishing appears to be the main driver of mortality for marine turtles<sup>12,26,48</sup>. In bottom trawling the most effective solution to reduce GE phenomena is to limit the residence time of turtles inside the net, once incidentally captured. This can be achieved by introducing technological improvements in fishing gear, the so-called Bycatch Reducer Devices (BRDs) as the Turtle Excluder Device (TED) that seems to be the most effective. In the last 6 years, the effect of a new prototype of TED, a flexible grid, on the catching efficiency and performance of commercial bottom trawl has been tested in several bottom trawlers in the Adriatic Sea<sup>49,50</sup>. Easy storage and handling compared with previous devices tested in this area<sup>51</sup> make the flexible TED a practical and valuable solution to reduce turtle bycatch in coastal Mediterranean demersal multispecies fisheries. Because of their effectiveness, which has mainly been demonstrated in prawn trawl fisheries, TEDs have become mandatory in several countries, out of the Mediterranean. Unfortunately, despite BRDs being an increasingly urgent need, they are not accepted in any Mediterranean country, because conservation aims are often hampered by competing political and economic factors<sup>52</sup>.

In conclusion, besides to applying the BRDs and outreach programs, the measures to reduce the impact of bycatch consist of raising fishers' awareness and in training them in the best practices in sea turtle recovery after capture. We deem that the conservation of sea turtles is mainly a technical and political challenge, because all these measures require significant investments but are capable of improving the conservation prospects of these endangered species<sup>16</sup>. Such efforts would so be designed to reach the UN Sustainable Development Goals of 2030 Agenda, particularly the targets of Goal 14<sup>53</sup>, and in the context of the European Union Green Deal and the recent Recovery and Resilience Facility<sup>54</sup>.

Currently, the synergy between fishermen, rescue and rehabilitation centers is important to reduce mortality from systemic GE in sea turtle, because 80% of all the affected animals will have a good chance of survive and be released, if kept in shallow water or dry for at most 2 weeks needed to recover from systemic macroembolism.

## Methods

Between January and April 2016 and October 2016 and February 2017, loggerhead sea turtles incidentally captured (bycaught) in trawls along the Italian coast of South Adriatic Sea were referred to the Sea Turtles Clinic (STC) at the Department of Veterinary Medicine in Bari (Italy) for veterinary evaluation after having been taken to the local Adriatic Sea turtle rescue centre (World Wildlife Fund, WWF, Molfetta). Depth and duration of trawling, withdrawal time of fishing gear (ascent rate), and any symptoms noted on board of the fishing boats were obtained from information provided by fisherman at the time of turtle admission to the STC.

### Clinical and radiographic evaluation

Immediately upon admission, physical examinations were performed of each turtle, including curved carapace length, curved carapace width, and weight, as well as core body temperature that was measured via a temperature probe inserted ~10 cm into the cloaca.

Full-body radiographs in dorsal-ventral (DV vertical beam) (Eurocolumbus, AEX 125Kv/ 300Ma) and cranial-caudal (Cr-Cd horizontal beam) and lateral-lateral (LL horizontal beam) (Diagnostic X-RAY unit, Orange 1060 HF Ultra Plus) projections were performed. All radiographs were assessed independently by three experienced sea turtles veterinarian (A. D. B., D.F. and C. V.) to evaluate for signs of drowning, characterized by interstitial and peribronchial thickening, and gas embolism within the cardiovascular system. In all turtles with radiographic signs of GE, we evaluated and listed the cardiovascular sites (CAS) where the gas was well detectable.

After radiographic examination, in turtles with signs of GE, respiratory rate (assessed visually), heart rate (assessed by ECG), PCV, and neurological and sensory deficits were recorded. Mentation was assessed as alert, depressed and comatose<sup>55</sup>. Additional clinical findings that were recorded included peripheral edema such as neck or palpebral edema, cloacal prolapse and the eventual presence or absence of particular postures such as retraction of the hind limbs under the carapace.

### Blood analysis

Between January and February 2017 blood samples for hematologic and biochemical analysis were collected from loggerheads affected by GE. Venous blood samples were collected anaerobically from the external jugular vein into a heparinized 2,5 ml syringe and processed immediately. Whole blood was used for determination of PCV, leucocyte count (WBC), and blood gas and electrolyte analysis. Plasma was then harvested from the remaining sample after centrifugation. PCV was measured after centrifugation of blood at 13,000 X g for 5 minutes in hematocrit tubes. WBC was performed manually by use of a disposable pipette system<sup>56</sup>. Blood gas and electrolyte analysis included pH, pCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, pO<sub>2</sub>, sO<sub>2</sub>, anion gap, tCO<sub>2</sub>, sodium, potassium and chloride (IDEXX VetStat Electrolyte and Blood Gas Analyzer, IDEXX VetStat Respiratory/Blood Gases, IDEXX Cassette Respiratory/Blood gases, IDEXX laboratories, Inc). Plasma biochemical values were measured by use of an automated clinical chemistry analyzer (Beckman Coulter, AU680). Blood gases and pH were corrected

for the patient's body temperature: values for pH, pCO<sub>2</sub>, and pO<sub>2</sub> were corrected for the patient's body temperature and ionized calcium was corrected for pH using published equations<sup>57</sup>. Bicarbonate concentration was calculated using the Henderson–Hasselbalch equation, temperature-corrected pH and pCO<sub>2</sub>.

## Treatment

Turtles with radiographic evidence of GE were kept in padded boxes out of the water ("*dry-docked*") or in tanks with low water level in a room at approximately 25-30 °C until clinical improvement. GE-affected turtles did not receive any pharmacological treatment. The animals were clinically monitored daily, while blood analysis and radiographic examination were performed every 48 hours until complete resolution of clinical or radiographic evidence of GE, or until the eventual death.

## Postmortem examination

Necropsies were performed within 12 hours after retrieval from fishing gear or within 3-4 hours following death at STC. Systematic sea turtle necropsy procedures were followed<sup>58</sup>. The carcass was positioned in dorsal recumbency and carefully removed the plastron, minimizing artifactual gas infiltration by traction of tissues and during sectioning of blood vessels. We first observed the heart in situ then we opened the pericardial sac to inspect the heart and when it was full of gas, we aspirated it with a 20 ml syringe. The major blood vessels and the entire coelomic cavity to evaluate organs for emboli and lesions were subsequently inspected. The last organs examined were the respiratory system, where we checked for froth and fluid, the kidneys and genital tracts.

## Statistical analysis

Qualitative variables were summarised as counts and percentages and comparison between independent groups were performed by chi-square or Fisher exact test as appropriate. Quantitative variables were described as median and interquartile range (IQR) because they did not approach Gaussian distribution (verified by Shapiro-Wilk test) and comparison between independent groups were performed by non-parametric tests (Kruskal-Wallis or U-Mann-Whitney as appropriate for independent samples). Radiographic signs of GE and drowning were tabulated to determine the number of affected anatomic sites, and to determine which sites were correlated with mortality. To evaluate the effect of each cardiovascular anatomic sites (CAS) for death, a logistic regression analysis was performed with the death event as dependent variable and the presence of GE in each CAS as independent variable. The univariate regression was carried out for each CAS and multivariate regression was performed with all CAS included in the model. A stepwise selection was performed to find the best fit. In the multivariable analysis, drowning signs were entered as another explanatory variable. The odds ratio and its 95% confidence interval are reported.

A ROC curve analysis was performed to evaluate the number of CAS involved that relate to a death event, and the area under the curve and its 95% confidence interval, and the threshold values of the number of CAS were determined. Once the cut-off value was determined, the number of CAS was classified in two ways: over and below the cut-off, so a logistic model was applied to quantify risk of death related to number of CAS, and through the calculation of odds ratio (OR) and its confidence interval.

A ROC curve analysis was performed to evaluate the diagnostic performance of quantitative variables of blood analytes, determining the area under the curve (AUC), its 95% confidence interval, cut-off values and their sensitivity, specificity. To evaluate the risk of death related to blood gas and electrolytes variables, classified as over/ below the cut –off, a Cox model was used with time to death as outcome. A univariable and a stepwise multivariable Cox model were performed, reporting hazard ratio (HR) and its confidence interval. A p-value <0.05 was considered as statistically significant. If appropriate for multiple testing procedure, the adjustment of p-value was performed with the FDR (false discovery rate) approach. Analyses were performed with SAS 9.4 and Medcalc 16.2.1.

## Declarations

### Competing interests:

Authors declare that they have no competing interests.

### Ethical statement:

Animal care was applied within institutional guidelines. This study was approved by the ethical committee of the Veterinary Medicine Department of Bari (CESA DiMeV Bari). Clinical information generated for this study was derived from the regular veterinary procedures provided in order to establish an appropriate diagnosis and prognosis in sea turtles. All activities related to veterinary evaluation of bycaught turtles in this study were conducted to provide appropriate care and maximize survivorship. No procedures were conducted solely for research purposes.

### Funding:

This research received no external funding

### Author contributions:

Conceptualization: DF, ADB

Methodology: DF, ADB, SC, CV, PT

Investigation: DF, CV, SC, SP, FC, ADB

Visualization: PT, SC, CV, SP, FC

Funding acquisition: not applicable

Project administration: DF, ADB

Supervision: DF, ADB

Writing – original draft: DF, PT, AL

Writing – review & editing: DF, SC

## Acknowledgements

The authors would like to thank Professor Craig Harms and Doctor Charles Innis for the revision of the manuscript and for their valuable suggestions. We thank Pasquale Salvemini that helped bring and care for the animals at the rehabilitation facility at WWF Molfetta Rescue Center. We are grateful for the fishermen that collaborated in this study.

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## Figures



A

B

C

Figure 1

Clinical findings in turtles with GE. (A) Eyelid and conjunctival edema and external foam coming from ramphotheca and nostrils in a Loggerhead turtle (*Caretta caretta*) with GE and drowning; (B) prominent edema of the eyelid and neck; (C) prolapse of the cloaca in *Caretta caretta*

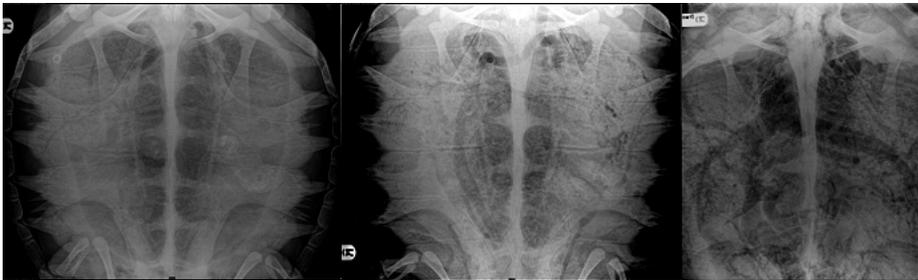


A

B

Figure 2

(A) Hind limbs flexed under the carapace in a turtle with GE; (B) Turtle with blood coming from the oral cavity arising from the larynx that died 6 hours after arriving at the STC (11 hours after trawling)



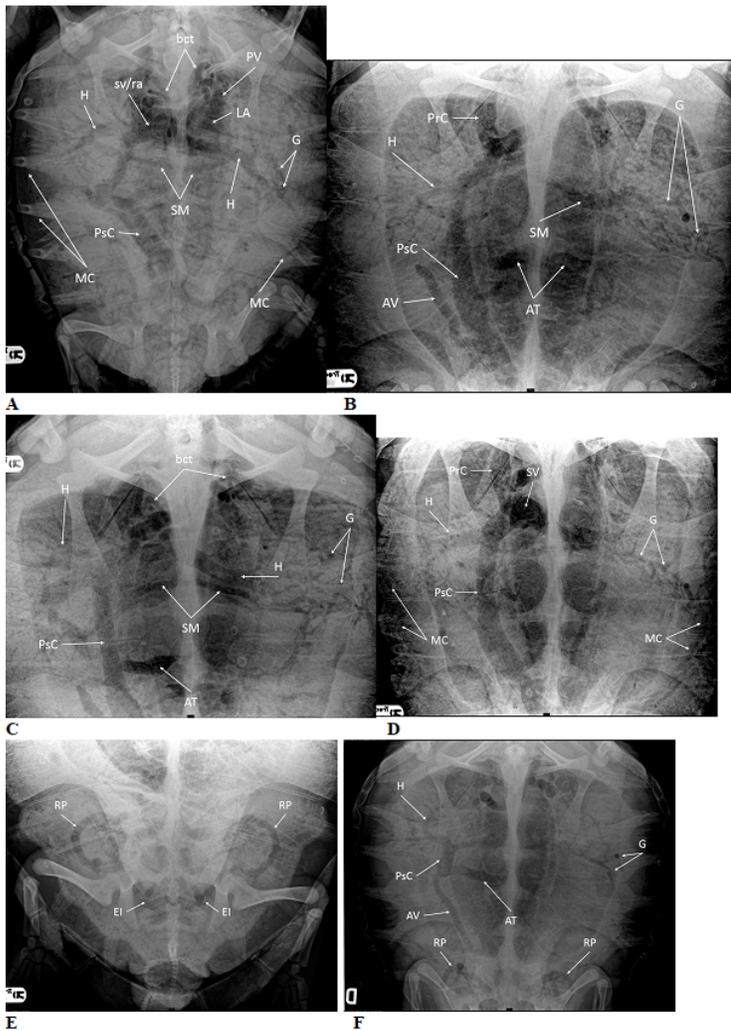
1A

1B

1C

Figure 3

Dorsoventral radiograph of 3 Loggerhead turtles (*Caretta caretta*) with GE of mild (A), medium (B) and severe (C) radiographic degree



**Figure 4**

(A) Dorsoventral radiograph of a Loggerhead turtle (*Caretta caretta*) with severe GE. Gas is evident in the sinus venosus/right atrium (sv/ra), left atrium (LA), pulmonary vessels (PV), vessels of the brachiocephalic trunk (bct), hepatic vessels (H), gastric vessels (G), postcava vein (PsC), superior mesenteric artery (SM) and marginocostal vessels (MC); (B and C) Dorsoventral radiograph of two Loggerhead turtles (*Caretta caretta*) with GE of medium to severe degree. Precava (PrC) and postcava vein (PsC), vessels of the brachiocephalic trunk (bct), hepatic vessels (H), gastric vessels (G), superior mesenteric artery (SM), abdominal vein (AV) and transverse abdominal vein (AT); (D) Dorsoventral radiograph of a Loggerhead turtle (*Caretta caretta*) with severe GE. Gas fills the sinus venosus (SV), left atrium (LA), precava (PrC) and postcava vein (PsC), hepatic vessels (H), gastric vessels (G) and marginocostal vessels (MC); (E) Dorsoventral radiograph of a Loggerhead turtle (*Caretta caretta*) with GE of mild degree. Gas is present in renal portal vessels (RP) and external iliac vessels (EI); (F) Dorsoventral radiograph of a Loggerhead turtle (*Caretta caretta*) with GE of medium degree. Gas is present in postcava vein (PsC), hepatic vessels (H), gastric vessels (G), abdominal vein (AV), transverse abdominal vein (AT) and renal portal vessels (RP).

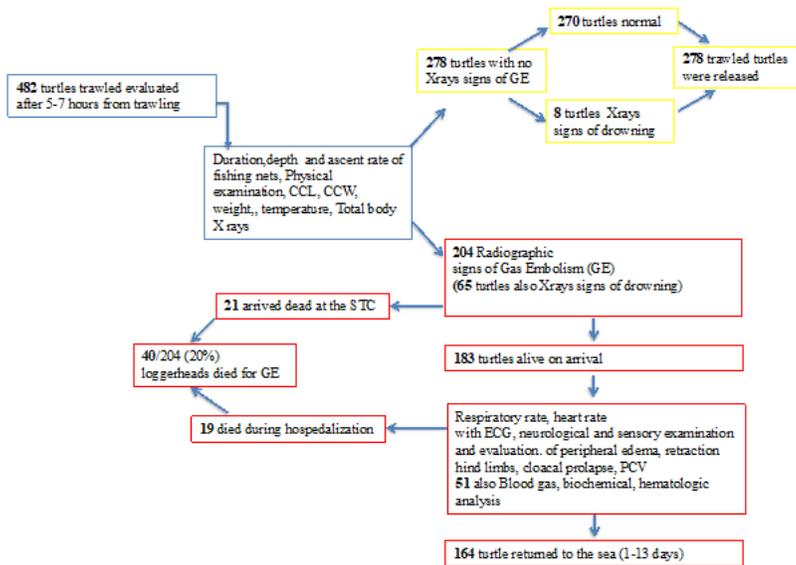


Figure 5

Graph 1. Flowchart of the outcome and clinical findings for 482 loggerhead turtles after being accidentally captured by trawling.

## Supplementary Files

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