Use of Positive Pressure Ventilation in Cold-Stunned Sea Turtles: 29 Cases (2008–2014)

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Abstract: Between 2008 and 2014, mechanical positive pressure ventilation (PPV) was used to manage 48 critically ill, cold-stunned sea turtles during their first week of treatment. Twenty-nine turtles had complete records for analysis and no ventilator complications, including 21 Kemp's ridley turtles (*Lepidochelys kempii*), four loggerhead turtles (*Caretta caretta*), and four green turtles (*Chelonia mydas*). Indications for PPV included poor responsiveness, bradypnea or apnea, bradycardia, and respiratory acidosis. Twenty (68.9%) turtles were successfully extubated, seven (24.1%) turtles died naturally during PPV, and two (7.0%) turtles were euthanized during PPV. Of the 20 turtles that were extubated, 11 (55%) survived over 24 h after extubation and were considered successfully weaned from the ventilator, but only four (20%) turtles survived to eventual release. Throughout PPV, improvements in respiratory, cardiovascular, and clinicopathologic status were variable, likely related to the degree of illness at presentation. On average, however, ventilation resulted in expected physical and physiologic improvements between the beginning and end of ventilation, including significant increases in activity, heart rate, venous pH and partial pressure of oxygen and decreases in venous partial pressure of carbon dioxide, potassium, and mortality prediction index score. Results of this study indicate that mechanical PPV is effective in improving the respiratory status of some moribund cold-stunned sea turtles; however, further refinement of the ventilation methods is recommended, and the prognosis remains poor to guarded for the most severely debilitated turtles.

Key Words: turtle, cold-stunned, ventilation, positive pressure ventilation, mechanical ventilation, reptile.

Introduction

Sea turtles are globally endangered species that are often found stranded on beaches due to natural and anthropogenic causes (Deem *et al.*, 2009; Anderson *et al.*, 2011; Stacy *et al.*, 2013). Many stranded turtles are admitted to rehabilitation hospitals for medical care, with the goal of eventually releasing them back to the wild. In the northeastern United States, the predominant cause of strandings is hypothermia or "cold-stunning" that occurs in sea turtles that fail to migrate to warmer waters in late autumn and early winter. Cold-stunned turtles are often affected by dehydration, renal dysfunction, electrolyte imbalance, decreased cardiorespiratory function, pneumonia, and other pathologic processes (Innis *et al.*, 2009a,b, 2014, 2016; Anderson *et al.*, 2011; Keller *et al.*, 2012). Comparisons of venous blood data in surviving and nonsurviving cold-stunned Kemp's ridley turtles (*Lepidochelys kempii*) indicated that severe respiratory and metabolic acidosis were common and that these conditions were associated with mortality (Keller *et al.*, 2012). Mortality prediction indices for cold-stunned turtles indicate that venous blood pH, potassium, partial pressure of carbon dioxide (pCO_2), and partial pressure of oxygen (pO_2) are among the most important analytes for predicting mortality (Stacy *et al.*, 2013).

Treatments adapted from physician-based and domestic animal medicine have been used for successful management of cold-stunned sea turtles (Wyneken *et al.*, 2006). It is common for approximately 70% of live cold-stunned turtles to be successfully treated and released back to the wild (Gerle *et al.*, 2000; Wyneken *et al.*, 2006; Innis *et al.*, 2014). Among turtles that do not survive, the majority of deaths occur within the first several days of hospitalization (Wyneken *et al.*, 2006; Innis *et al.*, 2014). These most severely affected turtles present in a terminal or moribund condition and have high mortality despite aggressive intensive care. Such turtles often have severe acidemia caused by a combination of respiratory acidosis due to hypoventilation and metabolic acidosis due to poor perfusion and anaerobic metabolism (Innis *et al.*, 2007; Cavin *et al.*, 2010; Keller *et al.*, 2012). The primary cause of hypoventilation is bradypnea or apnea and likely results from decreased brainstem respiratory center function due to temperature-induced central nervous system depression, combined with respiratory

muscle fatigue (Moon *et al.*, 1997). In some cases, submersion injury (near drowning) and pneumonia likely contribute to hypoxia (Innis *et al.*, 2009a; Stockman *et al.*, 2013).

In 2008, in an effort to improve the survival outcomes of cold-stunned sea turtles presenting with severe respiratory depression and acidosis, New England Aquarium (NEAQ) implemented mechanical positive pressure ventilation (PPV) for moribund turtles that could be intubated without sedation (Cavin *et al.*, 2010). Case selection was at the discretion of attending clinicians, as described below, and was influenced by caseload and available resources. To our knowledge, aside from descriptions of the use of PPV during general anesthetic procedures (Chittick *et al.*, 2002; Bertelsen *et al.*, 2015), there is no peer-reviewed report on the clinical use of PPV in reptiles.

Retrospective studies of PPV use in cats and dogs have reported positive outcomes in some animals with primary ventilation failure or hypoxemia refractory to conventional therapy (Hopper *et al.*, 2007; Hopper and Powell, 2013; Webster *et al.*, 2013; Trigg *et al.*, 2014). PPV provides ventilation without energy expenditure by the patient, and it permits a more controlled setting to monitor and correct arterial pCO_2 and pO_2 by using varied breathing rates, tidal volumes, inspiratory pressures, and concentrations of inspired oxygen. Several factors have been correlated with PPV patient outcomes in cats and dogs, including signalment and weight, reason for PPV, and PPV settings and intubation duration (Campbell and King, 2000; Beal *et al.*, 2001; Lee *et al.*, 2005; Hopper *et al.*, 2007; Hoareau *et al.*, 2011; Trigg *et al.*, 2014). Such indicators can be used to select patients that are most likely to benefit from PPV and to improve ventilator management (Campbell and King, 2000).

We hypothesized that PPV would provide a means to sustain critically ill turtles while hypothermia, electrolyte abnormalities, dehydration, acidemia, and other pathologic conditions were addressed. The study reported here retrospectively analyzed clinical data from ventilated cold-stunned sea turtles, with the purpose of describing the clinical population that received ventilator support, ventilation procedures, and outcomes. In addition, we wished to determine prognostic variables in ventilated patients to further inform decisions about resource allocation and treatment of stranded sea turtles.

Materials and Methods

Rehabilitation and treatment of turtles at NEAQ was conducted with authorization of the U.S. Department of the Interior Fish and Wildlife Service (permit TE-697823) and the U.S. Department of Commerce National Marine Fisheries Service. Stranded turtles were recovered from beaches of Cape Cod, MA, by volunteers and staff of Massachusetts Audubon Wellfleet Bay Sanctuary and were transported to NEAQ for medical care. Medical assessment and management was conducted using previously described methods (Wyneken *et al.*, 2006; Innis *et al.*, 2007, 2009b, 2014; Stockman *et al.*, 2013). Turtles were evaluated by physical examination and clinicopathologic analysis. Fluid therapy, cardiorespiratory support, and other therapeutic interventions were provided at the discretion of the attending clinicians. Aside from brief supervised swimming trials, turtles were initially maintained individually out of water in padded containers in a climate controlled environment and gradually warmed by daily ambient temperature increases of approximately 3°C (5°F) until the temperature reached 24°C (75°F).

Case selection criteria: Medical records of all live cold-stunned turtles that were mechanically ventilated during the first week of hospitalization at NEAQ from October 2008 through December 2014 were reviewed, representing seven separate autumn stranding periods. Turtles that were classified as dead on arrival but were ventilated during cardiopulmonary resuscitation attempts were excluded from the study. Turtles ventilated solely for general anesthetic purposes were also excluded. Finally, turtles were excluded if medical records contained inadequate data (e.g., unclear duration of ventilation, absence of clinicopathologic data) or if ventilator malfunction occurred during the intended ventilation period.

Preventilation assessment and intubation: Turtles were intubated and ventilated when determined to be moribund and at risk of death by the attending clinician. Uncuffed endotracheal tubes were used, ranging in size from 3 to 10 mm (inner diameter), dependent on the size of the turtle. Indications for PPV often included poor responsiveness, bradypnea (respiratory rate [RR] < 1 breath per 15–20 min) or apnea, bradycardia (heart rate [HR] < 10 beats/min), and evidence of respiratory acidosis (venous pCO_2 > 40 mm Hg, pH < 7.4).

Ventilator types: Turtles were ventilated by either a human pediatric ventilator (Servo-I Ventilator, Maquet Inc., Bridgewater, NJ) or a veterinary anesthesia ventilator that was customized by the manufacturer to deliver low RRs as appropriate for turtles (model 2002IE, Hallowell EMC, Pittsfield, MA). The Maquet ventilator was set in infant patient, synchronized intermittent mechanical ventilation (SIMV) pressure limited mode with pressure support. Positive end expiratory pressure (PEEP) was set at 2 cm H_2O and peak inspiratory pressure (PIP) at 8–10 cm H_2O, while delivering a fractional inspired oxygen concentration (FiO_2) of 21%. A ConchaTherm Neptune heated humidifier (Hudson RCI, Durham, NC) was added to the Maquet ventilator circuit. The Hallowell ventilator delivered FiO_2 of 100%, PIP of 8–10 cm H_2O, and lacked a PEEP control function. The RRs of both ventilators were set at 1–2 breaths/min unless otherwise noted.

Monitoring during ventilation: During PPV, turtles were periodically, but not constantly, monitored by attending clinicians for voluntary movement, reflex responses, HR, and blood gas analysis as described below. Monitoring was variable in frequency because clinicians were often responsible for managing dozens to hundreds of cases concurrently during mass stranding events. Rarely, turtles were left for hours without monitoring (e.g., overnight) as it was deemed better to continue PPV than to allow for hours of apnea. In such cases, the endotracheal tube was not secured in place such that it could be easily expelled if the turtle became responsive.

Weaning: Turtles that demonstrated clinical improvement (e.g., spontaneous movement and ventilation, improved HR, improved blood gas data) during PPV were extubated and received a trial without ventilator support. Turtles that did not receive PPV again and survived for more than 24 h after their final extubation from the ventilator were defined as successfully weaned. If clinical status declined and resumption of PPV was indicated, turtles were reintubated and ventilated again. A period during which the turtle was unsupported by the ventilator between two periods of PPV was defined as a failed weaning (FW) period.

Outcome: Turtles were designated as unsuccessfully weaned if they died or were euthanized while on PPV or within 24 h after final extubation. Two subsets were defined within the "successfully weaned" group: turtles that survived to later release and those that later died during hospitalization (successfully weaned but died more than 24 h after final extubation).

Reviewed parameters: Signalment, weight, and straight carapace length (SCL) at the time of admission were obtained from medical records. Dates of stranding, hospital admission (day 1), start and end of PPV, and date of death or release were recorded. Parameters recorded between day 1 of hospitalization and 2 days after final extubation were assessed, including ventilator settings, body temperature or ambient temperature, HR detected by Doppler or echocardiography, RR, venous blood gas data, a calculated mortality prediction index (MPI) score (Stacy *et al.*, 2013), activity score (described below), and results of radiographs. In addition, results from gross necropsies and histopathologic examinations of deceased turtles were recorded.

Turtles found deceased at first observation in the morning (i.e., died overnight when observers were not present) were assigned a day of death equal to 0.5 + previous hospitalization day. For these cases, the time of death was calculated as the average between the time of the last prior observation and the time that the turtle was found deceased.

Time periods: Physical and clinicopathologic data were retrospectively analyzed at important time periods during treatment. The specific duration and limits of these time periods were selected to maximize inclusion of available data, while still allowing for practical categorization. Admission data were obtained during the initial physical examination on the day of admission to the hospital. Preventilation data were defined as the last data recorded within 6 h preceding initial intubation. Final extubation data were defined as the first data recorded less than 2.5 h after final extubation. The data recorded on day 1 and day 2 after the day of final extubation were designated as postventilation day 1 and day 2 data.

To evaluate temporal improvement with PPV, data obtained during the first 7 h of PPV (<7 h PPV) were analyzed (including FW period and final extubation data). If there were multiple data points available for an individual turtle within this time period, the first data point after initial intubation was used. To assess the clinical status of patients that failed to wean from the ventilator and were reintubated, the last data recorded before reintubation for all FW periods was analyzed.

Venous blood data: Heparinized venous blood samples were anaerobically collected from the jugular vein using previously described methods (Innis *et al.*, 2007). A clinical point-of-care analyzer (Critical Care Express, NOVA Biomedical, Waltham, MA) measured venous blood analytes including pH, pCO_2, pO_2, and whole blood concentrations of potassium (K).

The values for temperature-dependent blood analytes (pH, pCO_2, pO_2) were corrected for Kemp's ridley and loggerhead turtles as described by Keller *et al.* (2012) and for green turtles as described by Anderson *et al.* (2011). Values were calculated for bicarbonate (HCO_3) by using the Henderson–Hasselbalch equation and αCO_2 and pK values derived for sea turtles (Stabenau and Heming, 1993).

Mortality prediction index: Mortality prediction index scores for selected time points were calculated retrospectively for each turtle by using the "MPI5" scoring system for cold-stunned Kemp's ridley turtles (Stacy *et al.*, 2013).

Activity score: Medical record notations about the turtles' ability to swim were retrospectively evaluated to categorize turtles' attitudes by using a novel scoring system, or "activity score" (A-Score). A-score was assigned as follows: 1, unable to swim, or no effort made to swim; 2, lethargic, minimal swimming effort for under 30 min; 3, immediately begins swimming, swimming sustained for over 30 min, but tires over time; and 4, able to remain in a pool all day and the turtle is bright, alert, responsive, and approaching normal swimming, diving, and resting behavior.

Gross necropsy and histopathology: Gross necropsy and histopathology data were recorded when available. Sex was determined grossly or histologically at the time of necropsy because the sex of juvenile turtles cannot be determined externally. Histopathologic examination was conducted at Connecticut Veterinary Medical Diagnostic Laboratory (University of Connecticut, Storrs, CT) or the Marine Animal Disease Laboratory (University of Florida, College of Veterinary Medicine, Gainesville, FL).

Statistical analysis: Statistical analysis was conducted using Real Statistics Using Excel (www.real-statistics.com). Continuous data were analyzed with a t-test if the data were normally distributed, or a Mann-Whitney U-test for data that were not normally distributed, or had unequal variance. Categorical data were analyzed with Fisher's exact test to compensate for small sample sizes. The Wilcoxon signed rank test was used to compare paired data. Statistical significance was assigned at $P \leq 0.05$.

Results

Between 2008 and 2014, there were 1,291 live, cold-stunned Kemp's ridley, loggerhead, and green turtles admitted to the hospital at the NEAQ. Forty-eight turtles required PPV within the first week of hospitalization. Of these turtles, medical records regarding ventilation were complete for 29 turtles. Nineteen turtles were excluded from the study due to inadequate ventilator data (n = 11), ventilator malfunction (n = 2), or dead-on-arrival status (n = 6). The 29 turtles were admitted from November to January. Caseload, signalment, initial morphometric data, admission body temperature, and sex are provided in Table 1. Summary PPV and outcome data are provided in Table 2. Table 3 provides PPV data categorized by absence or presence of FW periods, and Tables 4 and 5 summarize physical examination and clinicopathologic findings at admission and at various time periods during hospitalization.

Twenty (68.9%) turtles that were managed by PPV were later successfully extubated, seven (24.1%) turtles died naturally during PPV, and two (7.0%) turtles were euthanized during PPV. Of the 20 successfully extubated turtles, 11 (55%) turtles were successfully weaned and four (20%) turtles survived to be released to the wild. The number of turtles that were successfully weaned or survived to release did not differ statistically between turtles that began ventilation on day 1 vs. subsequent days (successfully weaned, Fisher's exact test, $P > 0.9$; survived to release, Fisher's exact test, $P > 0.6$), nor between turtles ventilated with the Hallowell vs. Maquet ventilator (successfully weaned, Fisher's exact test, $P > 0.3$; survived to release, Fisher's

Table 1. Total caseload 2008–2014; and admission day, morphometric data, cloacal temperature, and sex determined at necropsy for 29 cold-stunned sea turtles of three species that were managed with mechanical positive pressure ventilation. Data are shown as sample size (n), except for morphometric and temperature data that are shown as mean ± SD [median; range].

	Kemp's ridley	Loggerhead	Green
Total hospitalized turtles	1,104	138	49
Ventilated turtles	21	4	4
Admission day (ventilated turtles)			
Same day as stranding	15	2	4
Day after stranding	6	2	0
Body weight (ventilated turtles) (kg)	2.68 ± 0.65 (2.75; 1.25–3.80)	23.84 ± 6.69 (22.68; 17.55–32.45)	2.59 ± 0.46 (2.70; 1.95–3.00)
SCL (ventilated turtles) (cm)	26.6 ± 2.5 (27.0; 20.6–30.4)	56.0 ± 7.6 (54.6; 48.7–66.0)	27.8 ± 1.9 (28.0; 25.2–29.8)
Temperature °C (°F) (ventilated turtles)	10.4 (50.7) ± 2.4 (4.4) (11.2 (52.1); 5.0 (41.0)–14.8 (58.7))	8.0 (46.4) ± 2.2 (3.9) (7.8 (46.0); 6.1 (42.9)–10.4 (50.7))	10.7 (51.2) ± 3.3 (5.9) (10.7 (51.2); 6.8 (44.3)–14.8 (58.7))
Sex			
Male	2	0	0
Female	8	2	2
Undetermined	11	2	2

SCL = straight carapace length.

exact test, $P > 0.2$). There was no statistical difference in duration of PPV between turtles that were successfully and those that were not successfully weaned (Mann-Whitney *U*-test, $P > 0.2$) or in duration of PPV between turtles that survived to release and those that subsequently died (Mann-Whitney *U*-test, $P > 0.2$). There was no statistical difference in the rate of successful weaning or survival to release of turtles with no FW periods vs. turtles that experienced one or more FW periods (successful weaning, Fisher's exact test; $P > 0.7$; survival to release, Fisher's exact test; $P > 0.6$).

For 13 cases ventilated on day 1, admission data also met the definition of preventilation data and thus could not be used for paired comparisons within this time period. For 16 turtles with separate admission and preventilation data, significant changes in values between those two time points included decreases in venous pH and HCO_3 and increases in venous pCO_2, venous K, and MPI score (Wilcoxon signed rank test, $P < 0.05$).

For 20 turtles with both preventilation and <7 h PPV data, there were significant decreases of venous pCO_2, venous K, venous HCO_3, and MPI5 score, in addition to significant increases in A-score, HR, venous pH, and venous pO_2. For 22 turtles with both preventilation and final extubation data, significant decreases of venous pCO_2, venous K, and MPI5 score were observed, and there were significant increases in A-score, HR, venous pH, and venous pO_2 between these two time points. For 10 turtles that survived to postventilation days 1 and 2, the only significant change in values between those two time points was an increase in RR between postventilation days 1 and 2 (n = 5) (all comparisons, Wilcoxon signed rank test, $P < 0.05$).

During treatment with PPV, therapeutics were administered at the discretion of the attending clinician (Table 6). Within the first week of hospitalization, radiographs were obtained from 12 turtles, including two turtles radiographed 1 day before PPV, three turtles radiographed during PPV, and seven turtles radiographed 1–3 days after PPV. Of these, eight turtles (six Kemp's ridley, two loggerhead) had radiographic evidence of pulmonary disease, and four turtles (one Kemp's ridley, one loggerhead, two green) had radiographically normal lungs.

Gross necropsy was performed for 17 of 25 turtles that died, including 11 turtles that were not successfully weaned from PPV, and six turtles that were successfully weaned but died later during hospitalization. Gross necropsy was performed for 12 Kemp's ridley turtles, three green turtles, and two loggerhead turtles. Histopathologic examination was conducted for one Kemp's ridley turtle and one green turtle that were not successfully weaned and for two Kemp's ridley turtles and one green turtle that were successfully weaned. For 11 turtles that were not successfully weaned, gross necropsy revealed pulmonary abnormalities in five cases, including caseous nodules (n = 3) and excessive colorless-to-serous, clear-to-frothy fluid in the lungs (n = 2). Histopathologic evaluation revealed acute heterophilic pneumonia and pulmonary edema in one green turtle and renal tubular degeneration in one Kemp's ridley turtle. For six turtles that were successfully weaned but died during hospitalization, gross necropsy revealed multifocal caseous nodules within the lungs of five cases, and histopathologic evaluation of three of these cases revealed severe fungal pneumonia. Other significant histopathologic findings included renal tubular degeneration in one Kemp's ridley turtle and renal tubular mineralization with cast formation in one Kemp's ridley turtle.

Table 2. Summary ventilation data for 29 sea turtles managed by mechanical positive pressure ventilation (PPV). Data are reported as number of turtles (n) or mean ± SD [median; range].

Day of initial ventilation	2.0 ± 1.4 (2; 1–6))
Ventilator type	Hallowell (13 Lk, 4 Cc, 1 Cm); Maquet (5 Lk, 3 Cc); Both (2 Lk); NR (1 Lk)
Duration of PPV (h) (including FW periods)	14.04 ± 20.37 (5.1; 0.33–93.33)
Died naturally during PPV	7
Euthanized during PPV	2
Successfully extubated	20
Successfully weaned	11 (8 Lk, 2 Cc, 1 Cm)
Duration of PPV for successfully weaned turtles (h)	9.23 ± 10.68 (2; 0.58–27)
Duration of PPV for unsuccessfully weaned turtles (h)	16.97 ± 24.34 (7.29; 0.33–93.33)
Day of death for unsuccessfully weaned turtles	3.3 ± 1.7 (3; 1.5–7)
Day of death for successfully weaned turtles that later died	12 ± 8.8 (12; 4–29)
Survived to be released	4 (2 Lk, 2 Cc)
Duration of PPV for turtles that survived to be released (h)	5.8 ± 9.12 (1.58; 0.58–19.46)

Lk = *Lepidochelys kempii*; Cc = *Caretta caretta*; Cm = *Chelonia mydas*; NR = not recorded; FW = failed weaning.

Discussion

The turtles described here are typical of the most severely affected cold-stunned turtles that have been described in previous studies (Sadove *et al.*, 1998; Wyneken *et al.*, 2006; Innis *et al.*, 2007, 2009a,b, 2014; Keller *et al.*, 2012; Stockman *et al.*, 2013). Before ventilation, turtles had a high MPI score (Stacy *et al.*, 2013) and evidence of severe respiratory and metabolic acidosis, hypoxemia, and hyperkalemia. Among those turtles that had paired admission and preventilation data, results indicated increasingly severe acidosis. In fact, preventilation values for many venous blood analytes in this study were more severely deranged than those previously associated with mortality for this species (Keller *et al.*, 2012). Preventilation MPI scores of all turtles equaled ≥6, which was previously shown to predict mortality (Stacy *et al.*, 2013). Many turtles included in this study were concurrently affected by undetectable cardiac activity or severe bradycardia and bradypnea or apnea (Table 4). Such conditions are common in cold-stunned sea turtles, making it sometimes challenging to determine whether the patient is alive. Thus, clinicians often rely on other findings (e.g., the presence of intact, even if weak, neurologic reflexes) to diagnose the patient as alive. It is clear that among the nearly 1,300 turtles hospitalized during the study period, ventilated turtles were among those with the poorest prognosis and it is very likely that they would have died without ventilatory support. Therefore, the high mortality rate documented here is not surprising. Nonetheless, it is notable that four endangered turtles that would have otherwise died were successfully rehabilitated and released to the wild.

Ventilation resulted in physiologic improvements that are consistent with those observed in other species (Hopper *et al.*, 2007; Hopper, 2015). Although this may not be surprising and has been demonstrated experimentally in snakes (Bertelsen *et al.*, 2015), this study is the first to document that such improvements do occur during ventilation of clinical reptile patients. These changes included overall improvements in blood pH, pCO_2, pO_2, K, and HR. On average, the MPI score of ventilated turtles decreased over time, indicating that the mortality risk decreased, and the activity score improved over time.

Gross necropsy findings were recorded for the majority of turtles that died, whereas histopathologic evaluation was pursued for only five cases. Due to the large caseload admitted to NEAQ and the existence of an already established pathology database for cold-stunned turtles (Manire *et al.*, 2002; Innis *et al.*, 2009a, 2014; Kennedy *et al.*, 2012; Stockman *et al.*, 2013; NEAQ, unpublished data), postmortem investigations were not pursued for every turtle. In general, pathologic findings were consistent with previous observations of cold-stunned turtles, including a moderate incidence of fungal pneumonia and renal pathology (Manire *et al.*, 2002; Innis *et al.*, 2009a). Because these findings are common in general in cold-stunned turtles, it is difficult to determine what role ventilation may have played in the pathogenesis of these conditions. Based on the limited radiographic findings before ventilation in this study, as well as previously published radiographic data for stranded sea turtles, it is very likely that turtles were affected by pneumonia prior to ventilation (Stockman *et al.*, 2013). However, the presence of severe fungal pneumonia in several of the successfully weaned turtles should prompt consideration that ventilation could be contributing to the development of pneumonia in some cases. In dogs and cats, ventilator-acquired pneumonia is common, but invariably bacterial in origin (Epstein, 2015). In humans, ventilator-acquired pneumonia, including fungal pneumonia, is also common (Azoulay *et al.*, 2006). Based on these observations and previous evidence of fungal and bacterial infections in cold-stunned sea turtles (Innis *et al.*, 2009a, 2014), it is reasonable to assume that ventilated turtles may benefit from prophylactic antibiotic therapy, as was provided to the majority of turtles in this study. Antifungal therapy may also be warranted.

It was hoped that this study might provide information that could aid clinicians in determining the likelihood of success for an individual ventilated patient. However, this study failed to show that any particular changes during ventilation were predictive of outcome. For example, it was the authors' clinical impression that turtles that required shorter duration of ventilation, or turtles that experienced no failed weaning periods may have a better prognosis, but this study failed to demonstrate such differences, perhaps limited by the available sample size. Nonetheless, in light of the PPV duration for turtles that survived to be released, there may be a clinically relevant trend toward a better prognosis for turtles with shorter ventilation times, and this trend could become significant with a larger sample size. At this time, clinicians must continue to use judgment to determine whether to continue or abandon ventilation attempts for an individual turtle. Clinicians based extubation decisions on patient activity, physical examination, and blood

Table 3. Summary ventilation data for sea turtles managed by mechanical positive pressure ventilation (PPV) categorized by absence or presence of failed weaning (FW) periods. Data are reported as number of turtles (n) or mean ± SD [median; range].

Turtles with no FW periods that did not die during PPV	No. of turtles	12
	Duration of PPV (h)	2.55 ± 3.25 (1.62; 0.33–12)
	Successfully weaned	7
	Duration of PPV (h): successfully weaned	1.65 ± 0.83 (1.92; 0.58–3)
	Duration of PPV (h): not successfully weaned	3.80 ± 4.97 (1.32; 0.33–12)
Turtles with FW periods	No. of turtles	12
	No. of FW periods (number of turtles)	1(6); 2(2); 3(4)
	Duration of FW periods (h)	6.91 ± 11.6 (1; 0.08–44)
	Duration of PPV before, between, or after FW periods (h): all turtles	4.77 ± 5.54 (2; 0.25–20.83)
	Duration of PPV before, between, or after FW periods (h): Hallowell ventilator	3.32 ± 4.69 (1.5; 0.25–18.13)
	Duration of PPV before, between, or after FW periods (h): Maquet ventilator	7.16 ± 6.3 (4.95; 0.83–20.83)

FW = failed weaning.

analysis; however, the importance of specific variables for determining the likelihood of successful weaning remains unclear. Even in better studied domestic animals, the outcome of ventilator weaning remains unpredictable and is associated with the severity of disease (Haskins and King, 2004; Clare and Hopper, 2005; Hopper, 2015). Overall, this study confirms that turtles that require ventilation have a generally poor prognosis.

It is acknowledged that many variables likely affected the physiologic status of the ventilated turtles. Due to their endangered status, clinicians intervened to maximize the likelihood of survival, and it would not have been ethical to deprive turtles of care to assess the effects of individual variables. It is clear that increasing ambient temperature, parenteral fluid therapy, and other therapeutics likely contributed to the observed improvements.

The MPI score used in this study was previously validated for Kemp's ridley turtles (Stacy *et al.*, 2013). Among the three models validated in that study, we chose to use the so-called MPI5 score as it was most inclusive of the available blood parameters. Although the MPI4 and MPI6 scores could have also been investigated, it was beyond the scope of this study to assess all three of these models. The activity score used in this study was novel, and it was developed to provide a method to categorize the apparent changes in, for example, turtles' strength, behavior, and swimming ability. Based on results of this study, it seems that this system is effective for capturing such information numerically, and use of this system may be helpful for future studies of sea turtle rehabilitation.

Two types of ventilators were used during the years included in this study, simply as the result of availability. The human pediatric ventilator was loaned to NEAQ for 2 years. The customized veterinary anesthesia ventilator was used at other times. There were no significant differences in rates of successful weaning or survival to release between ventilators, but the data did not allow for robust statistical comparison of the changes in individual physiologic parameters between the two ventilators. In principle, there are likely advantages and disadvantages to each type of ventilator. One major difference between ventilators was the delivery of compressed air vs. 100 % oxygen. For turtles, specifically, it has been suggested that ventilation with 100% oxygen during anesthesia could suppress respiratory drive and contribute to prolonged apnea and right to left shunting, although this has not been confirmed by controlled studies (Moon and Stabenau, 1996). Another potential concern is oxygen toxicity (Jackson, 1985). At this time, the choice of ventilatory gas remains at the discretion of the attending clinician and available resources. Recently, NEAQ has customized the anesthesia ventilator to allow for delivery of compressed air, but there are insufficient data to assess the effect of this change to date.

The ventilator parameters and decisions that were made by clinicians during management of turtles were based on crude estimation, discussion with colleagues, and discussion with ventilator manufacturer representatives. There are many aspects of management that could be worthy of future clinical studies and consideration. For example, there are currently no data to allow clinicians to determine whether sea turtles benefit from commonly used mammalian ventilator strategies such as SIMV, PEEP, and humidification. The optimal airway pressure for turtle ventilation is not known, but 10 cm H_2O is commonly used for turtle anesthetic procedures. Whether cold-stunning or lung pathology affects lung compliance and ideal airway pressure of sea turtles is unknown. It is clear that sea turtle respiratory function is quite different than that of mammals. In general, sea turtles inspire, hold the inspired gas within the lungs during the interventilation period (often while diving), and then rapidly expire and inspire again. This breath-hold pattern could be mimicked mechanically and could be more effective than the traditional mammalian pattern that was used in this study. It is also possible that alternative respiratory rates may be more effective. Given the highly variable respiratory rate of turtles, which depends on factors such as whether they are diving for

Table 4. Serial physical and clinicopathologic data from cold-stunned turtles supported with mechanical positive pressure ventilation (PPV). Data are reported for each time period as mean ± SD [median; range] and sample size {n}.

	Admission	Preventilation	<7 h PPV	Failed weaning period	Final extubation	Postvent day 1	Postvent day 2
A-score[a,b]	1.4 ± 0.7 {29} (1; 1–3)	1.1 ± 0.4 {28} (1; 1–2)	1.6 ± 0.8 {20} (1; 1–3)	1.3 ± 0.6 {16} (1; 1–3)	1.6 ± 0.8 {19} (1; 1–3)	2.3 ± 0.7 {12} (2; 1–3)	2.9 ± 0.9 {10} (3; 2–4)
Cardiorespiratory activity							
Apneic {n}	{15}	{21}	{5}	{5}	{6}	{4}	{0}
Spontaneous breathing {n}	{14}	{7}	{10}	{6}	{10}	{7}	{7}
RR (breaths/min)[c]	1.5 ± 1.9 {13} (0.5; 0.1–7)	0.7 ± 0.7 {7} (0.4; 0.1–2.0)	0.9 ± 0.7 {5} (0.7; 0.3–2.0)	0.7 {1}	0.9 ± 0.8 {4} (0.8; 0.3–2.0)	1.2 ± 1.1 {5} (1.0; 0.4–3.0)	2.1 ± 1.2 {6} (1.8; 1.0–4.0)
Undetected HR {n}	{9}	{9}	{0}	{1}	{0}	{0}	{0}
HR (beats/min)[a,b]	7.5 ± 5.4 {20} (6.0; 1.5–20.0)	7.1 ± 6.4 {19} (4.0; 1.0–24.0)	12.7 ± 3.6 {10} (12.5; 5.0–16.0)	13.8 ± 4.0 {12} (13.0; 6.0–20.0)	13.5 ± 3.7 {10} (15.5; 5.0–16.0)	20.2 ± 6.2 {10} (20.0; 12.0–30.0)	24.3 ± 9.9 {6} (24.5; 10.0–40.0)
pH[a,b,d]	7.35 ± 0.14 {27} (7.35; 7.13–7.65)	7.23 ± 0.14 {25} (7.19; 7.02–7.48)	7.28 ± 0.22 {11} (7.26; 7.00–7.68)	7.20 ± 0.24 {14} (7.24; 6.61–7.62)	7.40 ± 0.27 {10} (7.44; 6.91–7.78)	7.42 ± 0.19 {11} (7.43; 7.08–7.76)	7.48 ± 0.15 {9} (7.48; 7.21–7.66)
pCO_2 (mm Hg)[a,b,d]	39.4 ± 10.7 {25} (36.3; 18.6–65.8)	50.7 ± 13.0 {23} (49.1; 27.2–72.3)	39.3 ± 14.8 {10} (40.0; 14.6–60.1)	41.5 ± 8.6 {12} (40.0; 25.4–54.7)	29.4 ± 14.3 {9} (25.6; 14.6–60.1)	37.9 ± 13.5 {11} (37.3; 16.5–59.2)	34.9 ± 14.1 {9} (39.3; 20.7–59.2)
pO_2 (mm Hg)[a,b]	39.4 ± 26.0 {27} (34.0; 11.6–140.6)	32.3 ± 18.1 {25} (27.8; 11.6–97.1)	73.6 ± 55.1 {11} (65.7; 21.3–226.3)	72.1 ± 43.8 {14} (62.0; 21.3–185.5)	80.6 ± 55.0 {10} (72.1; 26.8–226.3)	70.3 ± 27.4 {10} (65.6; 28.0–125.1)	72.3 ± 22.4 {9} (71.0; 47.2–124.3)
Potassium (mmol/L)[a,b,d]	5.0 ± 1.6 {27} (4.6; 2.7–8.5)	5.8 ± 2.0 {25} (5.5; 3.1–11.2)	4.5 ± 1.6 {11} (3.8; 2.8–7.0)	5.0 ± 1.9 {14} (4.8; 2.2–8.9)	4.3 ± 1.6 {10} (4.0; 2.1–7.0)	4.4 ± 2.1 {11} (3.7; 2.0–8.6)	3.6 ± 1.3 {9} (3.0; 2.4–6.2)
Bicarbonate (mmol/L)[a,d]	31.4 ± 6.0 {25} (30.6; 18.6–42.3)	28.6 ± 6.0 {23} (28.8; 16.1–39.7)	23.7 ± 3.7 {10} (24.0; 16.9–28.4)	24.2 ± 5.4 {12} (25.0; 16.9–32.7)	25.7 ± 6.07 {9} (25.2; 14.4–34.9)	29.8 ± 4.6 {11} (31.7; 19.4–35.3)	30.8 ± 4.8 {9} (30.5; 23.7–40.3)
MPI score[a,b,d]	9.2 ± 4.2 {25} (9; 2–16)	12.1 ± 3.1 {23} (12; 6–16)	7.7 ± 3.6 {10} (7.50; 1–12)	8.6 ± 3.2 {12} (9; 0–12)	5.3 ± 3.2 {9} (5; 1–12)	6.3 ± 2.9 {10} (6.5; 2–12)	4.4 ± 4.1 {9} (3; 1–14)

RR = respiratory rate; HR = heart rate; pCO_2 = particle pressure of carbon dioxide; pO_2 = partial pressure of oxygen; MPI = mortality prediction index.

[a] Significant difference between preventilation and <7 h PPV data.

[b] Significant difference between preventilation and final extubation data.

[c] Significant difference between postventilation (postvent) day 1 and postvent day 2 data.

[d] Significant difference between admission and preventilation data (all comparisons, Wilcoxon signed rank test ($P < 0.05$)).

Table 5. Mean change (± 95% confidence interval [CI]) for paired physiologic and clinicopathologic data for turtles supported with mechanical positive pressure ventilation (PPV). Comparisons shown are between preventilation and <7 h PPV* and between preventilation and final extubation.

	Preventilation to <7 h PPV (n = 20) (mean increase (+)/decrease (–) ± 95% CI)	Pre-ventilation to final extubation (n = 22) (mean increase (+)/decrease (–) ± 95% CI)
A-score[a,b]	+0.4 ± 0.4 (n = 20)	+0.5 ± 0.4 (n = 19)
RR (bpm)	+0.4 ± 0.5 (n = 9)	+0.1 ± 0.7 (n = 10)
HR (bpm)[a,b]	+11.1 ± 2.8 (n = 10)	+12.1 ± 3.1 (n = 9)
pH[a,b]	+0.16 ± 0.11 (n = 9)	+0.27 ± 0.10 (n = 8)
pCO_2 (mm Hg)[a,b]	–20.5 ± 9.7 (n = 9)	–23.9 ± 11.2 (n = 8)
pO_2 (mm Hg)[a,b]	+48.2 ± 41.4 (n = 9)	+57.5 ± 43.9 (n = 8)
Potassium (mmol/L)[a,b]	–0.5 ± 0.5 (n = 9)	–1.0 ± 0.6 (n = 8)
Bicarbonate (mmol/L)[a]	–3.9 ± 2.5 (n = 9)	–1.8 ± 3.4 (n = 8)
MPI score[a,b]	–4.9 ± 2.8 (n = 9)	–6.8 ± 2.3 (n = 8)

* <7 h PPV represents the data obtained during the first 7 h of PPV and includes failed weaning period and final extubation data. If there were multiple data points available for an individual turtle within this time period, the first data point after initial intubation was used.
[a]Significant difference between pre-ventilation and <7 h PPV data.
[b]Significant difference between pre-ventilation and final extubation data (all comparisons, Wilcoxon Signed Rank Test ($P < 0.05$).

extended periods, resting on the water surface, or nesting on land, it is difficult to determine an ideal rate for mechanical ventilation. Healthy, rehabilitated Kemp's ridley turtles at approximately 25°C (77°F) often respire at 3–4 breaths/min while out of water (Hunt *et al.*, 2016). In a recent study that evaluated the efficacy of mechanical ventilation in anesthetized snakes, normocapnia was maintained using a rate of 5 breaths/min (Bertelsen *et al.*, 2015). Clinicians may consider the use of similar rates, slightly higher than that used in most turtles in this study, for future ventilation cases, with close monitoring of blood gas data. Based on comparisons of blood gas data and survival outcome for cold-stunned Kemp's ridley turtles (Keller *et al.*, 2009), as well as data for successfully rehabilitated individuals (Hunt *et al.*, 2016), a target venous pCO_2 of 20–30 mm Hg may be appropriate when turtles' body temperatures are low during the first few days of hospitalization, whereas pCO_2 of 30–40 mm Hg may be acceptable once turtles have achieved a more functional body temperature.

Retrospective analysis of medical records revealed a number of deficiencies in record keeping, and 11 turtles had to be excluded from the study due to inadequate data. For example, it was fairly common for the duration of ventilation to be undocumented, and serial blood gas analysis was not always conducted. It is likely that this reflects resource limitations during acute triage situations, but it is probable that a standardized ventilation protocol and data record would reduce such deficiencies for future cases. The absence of such data limited our ability to perform statistically meaningful comparisons that may have been otherwise informative. For example, we did not have adequate blood data for every defined time period for every patient to allow for thorough meaningful comparisons of all time periods.

The absence of continuous overnight monitoring of ventilator patients was not ideal; however, resources were not available to allow for such care. In general, although clinicians were uncomfortable with unsupervised ventilation, it was determined that it is was better to ventilate the turtle unobserved than to leave it apneic overnight. Precautions were taken to allow the turtle to self-extubate if it became strong enough to do so, including the use of a short uncuffed endotracheal tube that was not affixed to the turtle in any way. Clearly, continual monitoring of ventilator patients is preferred when resources permit.

Table 6. Therapeutic agents used for the management of 29 sea turtles that were also managed by mechanical positive pressure ventilation.

Drug	No. of turtles
Atropine	27
Doxapram	24
Epinephrine	5
Sodium bicarbonate	26
Dextrose	16
Insulin	7
Furosemide	1
Flunixin meglumine	1
Sodium chloride	16
Reptile Ringer's solution	16
Normosol-R (Hospira Inc., Lake Forest, IL)	4
Lactated Ringer's solution	7
Ceftazidime	9
Oxytetracycline	2

The majority of turtles for which sex was determined postmortem were female, but this does not likely reflect a sex-biased ventilation outcome. It is known that the Kemp's ridley sea turtle population has been female biased for some time, but the reason is not clearly defined (Wibbels *et al.*, 2007). Among the species studied here, a relatively high percentage of green turtles were ventilated relative to their overall representation in the caseload. This was not a clinician's species bias, but rather it may indicate that green turtles are less tolerant of severe cold. This phenomenon is worthy of future study.

It is clear that mechanical PPV may not always be a practical option for managing moribund sea turtles. At NEAQ, for example, the use of mechanical PPV is limited to times when caseload and resources allow for the diversion of attention to individual turtles. During some stranding periods, hundreds of turtles may be hospitalized in a single day, and triage principles demand that attention is provided to those turtles with the best prognoses. For many facilities, it may simply be cost prohibitive to purchase a ventilator. Nonetheless, this study indicates that mechanical PPV may be an effective tool for the management of moribund sea turtles.

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