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Comparison Between Effects of Four Crystalloid Solutions on Acid-Base and Electrolyte Abnormalities in Stranded Juvenile Loggerhead Sea Turtles (*Caretta caretta*)

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Dehydration, electrolyte abnormalities, and acid-base alterations are common findings in stranded sea turtles. Fluid therapy is essential for reestablishment of homeostasis. The aim of this study was to compare the efficacy and effects on acid-base and electrolyte status of four different crystalloids (0.9% NaCl solution, 0.9% NaCl and lactated Ringer's solutions 1:1 ratio, Plasmalyte, and Jarchow's solution) in 63 stranded juvenile loggerhead turtles (*Caretta caretta*). Crystalloid fluids were administered intracoelomically on the day of admission for a duration of three consecutive days at a rate of 20 mL/kg/day through the inguinal fossa. Blood samples were collected at three timepoints: on admission, 24 h after discontinuing fluid therapy and prior to release. Samples were analyzed using a portable electronic blood analyzer for pH, pCO_2, pO_2, bicarbonate, lactate, sodium, potassium, chloride, glucose, and BUN concentration. Thirty-four loggerhead turtles (53.9%) had some type of acid-base alteration at the time of admission. The combination of 0.9% NaCl and lactated Ringer's solutions resulted in the highest percentage of improved/resolved acid-base and electrolyte abnormalities (33.4 % more animals with normal acid-base status compared to the admission time) compared to Jarchow's solution, which was the least effective (15.8% decrease in the number of animals with normal acid-base status compared to the admission time). This study constitutes the second controlled study of fluid therapy in sea turtles, and corroborates the recommendation made previously on the use of 0.9% NaCl + lactated Ringer solution to resolve mild to moderate acid-base alterations in juvenile loggerhead turtles. In addition, convalescent acid-base, electrolyte and plasma biochemical reference intervals are also provided as a standard profile for sea turtle rehabilitation centers.

Keywords: *Caretta caretta*, fluid therapy, homeostasis, loggerhead, sea turtle

INTRODUCTION

The loggerhead turtle (*Caretta caretta*) is an endangered species commonly found on the Canary Islands, because this location is in their migration routes. Stranding of loggerhead turtles is common for the Canary Islands (1), and diseases and causes of mortality and/or stranding among loggerhead turtles stranded in their coasts have been described (2–4). Causes of anthropogenic origin are the most frequent (72%), including entanglement in fishing nets and/or plastics (51%), ingestion of hooks and monofilament lines (12%), boat strikes (5%), crude oil (3%), and ingestion of plastics (1%) (4). Dehydration, electrolyte abnormalities, and alterations of acid-base homeostasis are common findings in many of these stranded animals, as evidenced the retrospective study carried out on 66 stranded juvenile loggerhead turtles, in which it was observed that acid-base disorders were present in 86.36% of turtles at the time of admission to our rehabilitation facility (1).

Most studies on metabolic and respiratory alterations in sea turtles have been focused on cold-stunned Kemp's ridley turtles (*Lepidochelys kempii*) (5–7); hyperkalemia, low venous pH, low venous pO_2, low bicarbonate concentration, and an elevated venous pCO_2 usually carry a poor prognosis in these turtles (6, 7). In a study on captured loggerhead turtles, pound nets and trawls caused alterations in blood gas, acid-base, and lactate status, though alterations were greater in trawl captured turtles (8). It has also been reported that severe lesions in the salt glands can cause stranding and/or death of loggerhead turtles due to high plasma concentrations of sodium and chloride (9).

Studies on acid-base and electrolyte status of stranded loggerhead turtles are scarce (1, 8, 10, 11). The effects of several crystalloid fluids on the acid-base and plasma biochemical values in stranded juvenile loggerhead turtles in the Canary Islands were reported (12), being the only controlled study published in reptiles. Authors used four different crystalloid fluids: 0.9% NaCl solution, 5% dextrose and 0.9% NaCl solutions 1:1 ratio, 0.9% NaCl and lactated Ringer's solutions 1:1 ratio, and lactated Ringer's solution. The highest percentage of acid-base recovery and electrolyte balance was detected in loggerhead turtles treated with mixed saline-lactated Ringer's solution followed by 0.9% NaCl solution, lactated Ringer's solution, and 5% dextrose and 0.9% NaCl solutions 1:1 ratio (12).

Expanding on our previous work, the aim of this new study was to compare the efficacy and effects on acid-base and electrolyte status of different crystalloid fluids in stranded juvenile loggerhead turtles. The due to their poor performance in improving acid-base and electrolyte abnormalities, the lactated Ringer's solution and dextrose-saline solutions from the previous study were replaced with Plasmalyte (a commercial isotonic crystalloid fluid buffered with gluconate and acetate) (13) and Jarchow's solution, containing 5% dextrose, lactated Ringer's solution and 0.9% NaCl solution in a 1:1:1 ratio (14, 15). Our hypothesis was that Plasmalyte could present a high efficacy, while Jarchow's solution, given its composition, could present similar results to the mixed dextrose-saline solution (12).

MATERIALS AND METHODS

Animals

A total of 63 loggerhead turtles that stranded between April 2017 and September 2021 and were admitted to the Tafira Wildlife Rehabilitation Center or the Fuerteventura Sea Turtle Rehabilitation and Conservation Center were included in this study. No visualization of the gonads was performed. Median (lower quartile, upper quartile; range) straight carapace length (SCL) of the turtles included in this study was 38 (31, 47; 19–56) cm, and weight was 8.8 (6.3, 16.9; 1.1–46.6) kg. Based on SCL, all specimens were identified as juvenile (16). The causes of stranding were entanglement in derelict fishing gear and/or plastics (*n* = 42; 66.6%), ingestion of hooks and monofilament lines (*n* = 6; 9.5%), traumatic injuries caused by boat strikes (*n* = 4; 6.3%), buoyancy disorders (*n* = 2; 3.2%), malnutrition (*n* = 1; 1.6%), and unidentified causes (*n* = 8; 12.7%). Median (lower quartile, upper quartile; range) length of stay in the rehabilitation centers was 81 (53, 137; 18–210) days.

Fluid Administration

Four groups were established depending on the crystalloid fluid administered. Group 0.9% NaCl solution (*n* = 13); group 0.9% NaCl + lactated Ringer's solutions 1:1 ratio (*n* = 12); group Plasmalyte (Baxter, Deerfield, IL) (*n* = 10); group Jarchow's solution (*n* = 15). An untreated control group (*n* = 13) was also included. Each time a loggerhead turtle arrived at the rehabilitation center, it was assigned to one of the five groups (including the control group) in a cyclic order, regardless of disease severity. Crystalloid fluids were administered intracoelomically on the day of admission for a duration of three consecutive days at a rate of 20 mL/kg/day through the inguinal fossa (17–19). When necessary, other surgical and/or medical procedures were performed. Turtles were transferred to individual outdoor pools with continuous flow of filtered sea water. Data were collected daily, including clinical status, physical activity, food ingestion, cloacal temperature, and weight (19, 20).

Venous blood (0.2 ml) was obtained from the cervical sinus using a nonheparinized syringe. Samples were immediately analyzed using a portable electronic blood analyzer (i-STAT, Heska, Loveland, CO) and CG4+ cartridges (Heska, Loveland, CO) for pH, pCO_2, pO_2, bicarbonate, and lactate concentration, and EC8+ cartridges (Heska, Loveland, CO) for concentrations of sodium, potassium, chloride, glucose, and BUN. All turtles were sampled at three timepoints: (a) on admission, prior to any treatment for evaluating the type of acid-base or biochemical disorder, (b) 24 h after the last fluid therapy dose, and (c) prior to release only if they had not received any drug 7 days prior. The same time intervals were used in the control group. Each turtle was released when the veterinary services of each rehabilitation center evaluated it as clinically healthy (1, 12). The reference intervals for convalescent acid-base and electrolyte values (measured at the third timepoint) were used to assess severity of the abnormalities at admission (first timepoint) and after fluid therapy (second timepoint). A turtle was considered to have a normal acid-base status if all pH, pCO_2

and bicarbonate concentration were within the ranges of the obtained reference intervals.

Because the portable analyzer i-STAT performs the blood tests at 37°C, cloacal temperature of each turtle was recorded with a digital thermometer (Digi-Sense Thermocouple T, Cole-Parmer Instrument Co, Vernon Hills, IL) and several equations more adequate for sea turtles than the algorithms of the i-STAT were used to correct these parameters. The pH, pCO_2, and pO_2 were corrected for the cloacal temperature using the following equations (21) (where $\Delta T = 37°C$—cloacal temperature):

$$\text{Corrected pH} = \text{pH (at } 37°C) + 0.014\,\Delta T$$
$$\text{Corrected } pCO_2 = pCO_2 \text{ (at } 37°C)\, 10^{-0.019\,\Delta T}$$
$$\text{Corrected } pO_2 = pO_2 \text{ (at } 37°C)\, 10^{-0.0058\,\Delta T}$$

Corrected HCO_3^- concentrations were calculated using the Henderson-Hasselbalch equation. The CO_2 solubility coefficient (αCO_2) and pK were calculated using specific equations for sea turtles (22).

Anion gap (mmol/l) and osmolality (mOsm/kg) were calculated using the following equations (23):

$$
\begin{aligned}
\text{Anion gap} = {} & \text{(sodium concentration} \\
& + \text{ potassium concentration)} \\
& - \text{(chloride concentration} \\
& + \text{ corrected } HCO_3^- \text{ concentration)}. \\
\text{Osmolality} = {} & 2\text{(sodium concentration} \\
& + \text{ potassium concentration)} \\
& + \text{(glucose concentration/18)} \\
& + \text{(BUN concentration/2.8)}.
\end{aligned}
$$

Statistical Analysis

The statistical analysis was performed using the R statistical environment version 4.1.2 (R Development Core Team, Vienna, Austria). Variables with normal distribution were described as mean and standard deviation. Not normally distributed variables were described as median and quartiles. For every treatment (group), differences between variable values through the study (i.e., at admission, after fluid therapy, and prior to release) were assessed by using repeated measures ANOVA for normally distributed and repeated measures Friedman test form not normally distributed variables. Those cases in which there were significant differences between measures, *post-hoc* tests were carried out using pairwise *t*-tests for normal variables, and pairwise Wilcoxon tests for not normally distributed variables. In both cases, *P*-values were adjusted with Bonferroni method. For comparing baseline characteristics of the loggerhead turtles in the different treatment groups upon admission, and also the effects of the different treatments after fluid therapy, analysis of variance was used for normally distributed variables, and Kruskal-Wallis test for not normally distributed variables. When significant differences were observed, *post-hoc* comparisons were made using Tukey tests for normally distributed variables, and Conover tests for not normally distributed variables. Values of $P < 0.05$ were considered statistically significant.

Reference intervals for convalescent loggerhead turtles were obtained from the pre-release values of convalescent turtles. Each variable was assessed using histograms, boxplots and normal probability plots. Normality was tested by using the Shapiro-Wilk test, and Box Cox transformations were performed on variables that were not normally distributed. For variables that after transformation did not conform to a Gaussian distribution, non-parametric method was used for reference interval estimation. In addition to visual examination of boxplots and histograms, outliers were identified using the Horn outlier detection algorithm (24) and were manually removed from reference interval estimation. The 95% reference intervals were estimated using the robust method following AVSCP reference intervals guidelines (2.5–97.5 percentiles) (25). Associated 90% confidence intervals (CIs) for the limits of each interval were computed by bootstrap methods following same guidelines. All computations were made by using the R package Reference Intervals.

RESULTS

Thirty-four turtles (53.9%) had some type of mild to moderate acid-base alteration at the time of admission. Of these, thirteen (20.6%) had metabolic and respiratory acidosis, and seven turtles (11.1%) had respiratory acidosis or metabolic acidosis. Of those with metabolic and respiratory acidosis, four turtles had high anion gap values, and the other nine had normal anion gap values with hyperchloremic acidosis. There were no statistically significant differences between treatment groups at baseline.

Fourteen turtles died or were euthanized during their stay in the rehabilitation center after receiving fluid therapy, so that pre-release values could not be obtained; therefore, the reference intervals were calculated based on 49 released turtles. Neither euthanasia nor death during stay in the rehabilitation facilities were overrepresented in any group. Nine additional turtles that had died or had been euthanized between admission and complete fluid therapy, and therefore the biochemical blood values could not be obtained, were excluded from the study.

The group treated with 0.9% NaCl + lactated Ringer's solution showed the highest percentage of resolution of acid-base abnormalities (33.4% compared to admission) followed by the group receiving 0.9% NaCl (28.8% compared to admission). In comparison in the control group, only 9.8% of the turtles showed resolution of the acid-base abnormalities (**Figure 1**). When Plasmalyte and Jarchow's solutions were used, a 10% and 15.8% decrease in the number of animals with normal acid-base status compared to the admission time were observed, respectively. It was observed an increase in the number of turtles with metabolic alkalosis in the Plasmalyte group.

Values of pH, blood gas and plasma biochemical parameters at the three timepoints (on admission, 24 h after fluid therapy, and prior to release) for treated groups and control group are shown in **Tables 1**, **2**, respectively.

There were several statistically significant differences between admission values and those values obtained after therapy and just prior to release. Initial pH values were significantly lower ($P = 0.0017$) than post-therapy values in the group treated
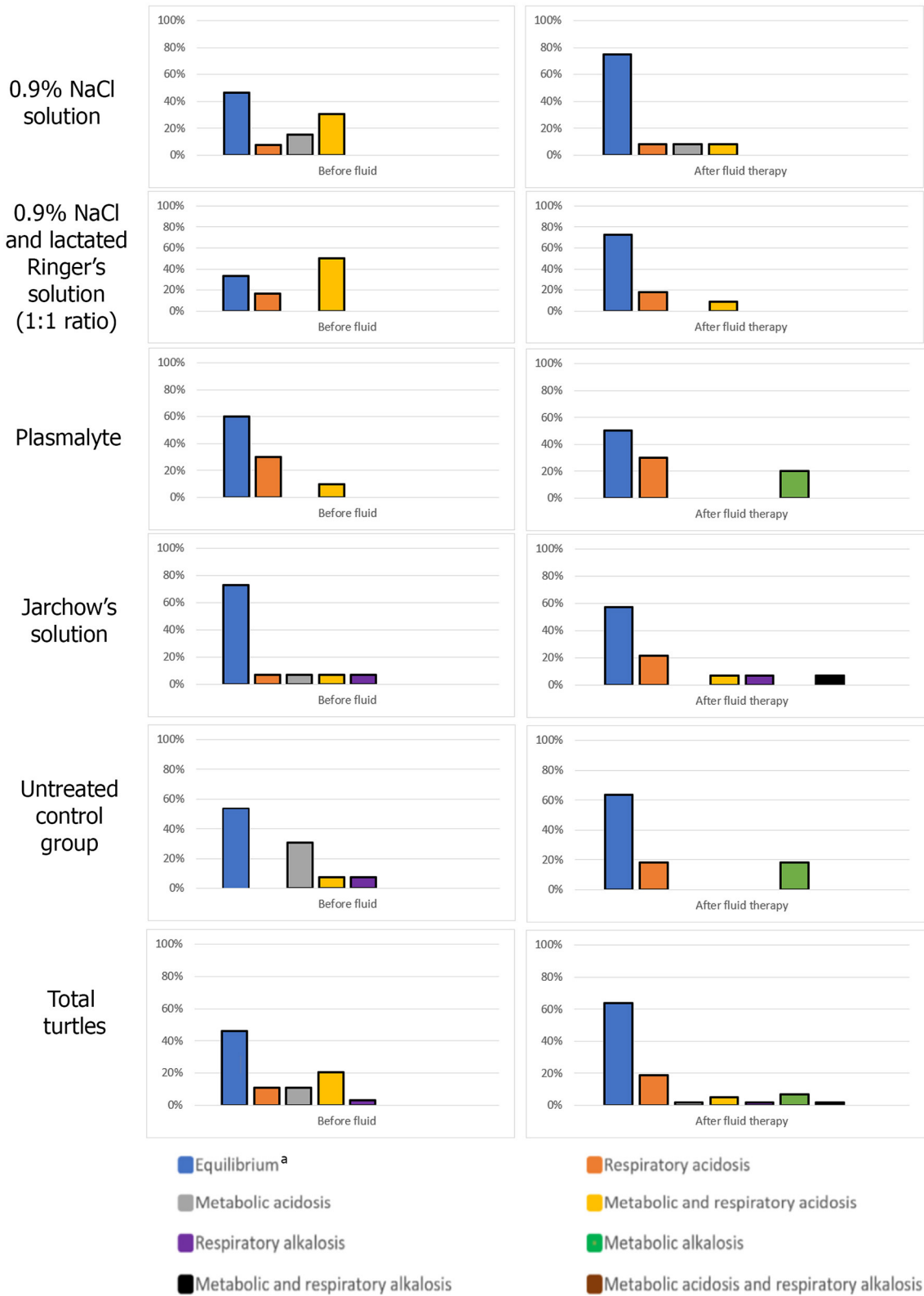
FIGURE 1 | Acid-base status upon admission and after fluid therapy for each loggerhead turtle group. [a]All pH, pCO_2 and bicarbonate concentration within the ranges of the obtained reference intervals.

TABLE 1 | Median (and quartiles), and mean and standard deviation of pH, blood gas and plasma biochemical parameters during times of sampling of the four treatment groups.

	0.9% NaCl			0.9% NaCl + lactated Ringer solutions (1:1 ratio)			Plasmalyte			Jarchow's solution		
	Admission	After fluid therapy	Prior to release	Admission	After fluid therapy	Prior to release	Admission	After fluid therapy	Prior to release	Admission	After fluid therapy	Prior to release
Not normally distributed												
pH	7.57 (7.49, 7.61)	7.62 (7.55, 7.64)	7.6 (7.55, 7.62)	**7.48**[a] (7.45, 7.57)	**7.57**[b] (7.54, 7.61)	**7.66**[c] (7.61, 7.68)	7.51 (7.5, 7.54)	7.62 (7.59, 7.72)	7.56 (7.55, 7.6)	7.61 (7.5, 7.65)	7.65 (7.61, 7.68)	7.59 (7.56, 7.61)
pO₂ (mmHg)	68.63 (63.89, 76.52)	74.6 (67.98, 81.67)	72.58 (61.99, 77.99)	83.3 (74.16, 145.23)	89.7 9 (72.5, 119.44)	72 (68.32, 77.17)	73.26 (59.55, 84.53)	67.19 (60.77, 88.87)	75.93 (61.7, 84.56)	83.46 (72.3, 124.93)	80 (72.87, 109.9)	75.46 (67.6, 96.98)
Lactate (mmol/L)	4.79 (1.87, 8.51)	0.85 (0.37, 1.11)	0.75 (0.52, 1.16)	3.42 (2.29, 5.51)	1.11 (0.61, 1.76)	0.98 (0.4, 1.65)	**4.4**[a] (2.54, 6.96)	0.71 (0.48, 1.74)	**0.9**[c] (0.7, 1.98)	2.72 (1.88, 4.17)	1.35 (1, 1.9)	**1.06**[c] (0.45, 1.4)
Sodium (mmol/L)	150 (148, 154)	149 (146.5, 151)	151.5 (149.25, 153)	**149**[a] (147, 151.25)	**146**[b] (145, 147.5)	151 (147.5, 154)	150.5 (146.25, 152)	149 (147, 150.75)	153.5 (150, 154)	150 (148, 152)	**149**[b] (146.25, 151)	**152.5**[c] (151, 156)
Potassium (mmol/L)	**3.5**[a] (2.7, 3.8)	**3.1**[b] (2.55, 3.1)	3.3 (3.2, 3.63)	3.5 (2.95, 3.87)	**3.4**[b] (2.9, 3.8)	**2.95**[c] (2.73, 3.15)	**3.85**[a] (3.3, 4.07)	2.95 (2.4, 3.15)	**3.25**[c] (2.82, 3.6)	3.2 (2.65, 3.6)	2.7 (2.5, 3.2)	2.8 (2.38, 3.6)
Anion Gap (mmol/L)	**7.33**[a] (5.39, 9.78)	4.38 (1.2, 7.78)	**3.55**[c] (0.91, 4.68)	**5.45**[a] (3.31, 11.5)	0.85 (-0.12, 2.58)	3.18 (0.89, 6.08)	3.89 (-0.9, 5.98)	−0.39 (-3.3, 6.92)	**7.55**[c] (5.99, 7.71)	5.69 (3.25, 6.64)	1.82 (0.32, 7.89)	6.2 (3.99, 9.06)
Glucose (mg/dL)	102 (87, 115)	100 (90, 129)	112.5 (107.25, 120)	110.5 (83.75, 137.5)	86 (77, 133)	107.5 (92.5, 114.5)	94 (67.25, 105.5)	113 (87.75, 124.5)	98 (85.75, 105)	135 (100.5, 157)	124 (104, 176)	119.5 (113.5, 122.5)
BUN (mg/dL)[d]	55 (34, 90)	**58**[b] (48.5, 71)	**140**[c] (140, 140)	**50.5**[a] (39.5, 60.25)	**68**[b] (55, 72)	**140**[c] (139, 140)	**71.5**[a] (57.25, 105)	**79.5**[b] (66.25, 98.25)	**138**[c] (133, 140)	**63**[a] (48.5, 86)	**71**[b] (50, 112)	**140**[c] (111.75, 140)
Normally distributed												
pCO₂ (mmHg)	27.96 ± 5.44	29.23 ± 4.61	29.96 ± 4.65	28.51 ± 5.57	30.91 ± 7.39	28.45 ± 4.85	35.18 ± 8.18	34.18 ± 8.67	29.83 ± 3.64	28.42 ± 6.08	30.62 ± 9.7	30.71 ± 4.78
HCO₃ (mmol/L)	**34.88 ± 11.79**[a]	38.24 ± 6.2	**38.1 ± 3.01**[c]	**31.84 ± 8.91**[a]	39.11 ± 12.15	**41.3 ± 5.55**[c]	**40.06 ± 10.99**[a]	**50.89 ± 9.5**[b]	37.49 ± 5.57	**37.66 ± 7.26**[a]	**44.25 ± 8.4**[b]	40.01 ± 4.78
Chloride (mmol/L)	**113.92 ± 5.63**[a]	**107.73 ± 6.89**[b]	114.1 ± 3.98	**113.42 ± 8.12**[a]	107.45 ± 7.57	109.6 ± 6.48	**107.8 ± 3.94**[a]	**99.7 ± 4.67**[b]	111.17 ± 6.74	**110.6 ± 5.63**[a]	**104.08 ± 6.44**[b]	109.67 ± 6.33
Osmolality (mOsmol/kg)[d]	338.09 ± 20.54	**333.04 ± 11.64**[b]	**365.58 ± 7.6**[c]	328.05 ± 8.71	**324.61 ± 14.18**[b]	**360.65 ± 9.87**[c]	336.54 ± 26.77	**339.48 ± 14.97**[b]	**364.58 ± 7.85**[c]	338.3 ± 14	**337.62 ± 19.29**[b]	**361.55 ± 13**[c]

[a]Statistically significant differences between admission and after fluid therapy values within each group; [b]Statistically significant differences between after fluid therapy values and prior to release values within each group; [c]Statistically significant differences between admission and prior to release values within each group; [d]Osmolality and BUN values obtained prior to release were underestimated because BUN values exceeded in many turtles the analytical range of the analyzer (140 mg/dL). Bold values indicate with statistically significant differences.

TABLE 2 | Median (and quartiles), and mean and standard deviation of pH, blood gas and plasma biochemical parameters during times of sampling of untreated turtles (control group).

	Admission	Control day	Prior to release
Not normally distributed			
pH	7.65	7.68	7.69
	(7.51, 7.7)	(7.65, 7.71)	(7.58, 7.73)
pO₂ (mmHg)	109.09	90.12	82.16
	(92.19, 126.31)	(74.14, 135.2)	(75.81, 104.18)
Lactate (mmol/L)	**4.36**[a]	**1.93**[b]	**1.09**[c]
	(2.8, 7.01)	(0.84, 2.17)	(0.47, 1.42)
Sodium (mmol/L)	149	150	151
	(147, 150)	(147.5, 152)	(149.25, 156)
Potassium (mmol/L)	**3.7**[a]	**3.2**[b]	2.75
	(3.4, 3.7)	(2.8, 3.3)	(2.52, 3.18)
Anion Gap (mmol/L)	**7.33**[a]	−0.59	5.13
	(6, 10.37)	(-2.53, 1.11)	(4.56, 5.66)
Glucose (mg/dL)	99	98	107
	(82, 110)	(90, 117)	(99, 123.25)
BUN (mg/dL)[d]	**59**[a]	**108**[b]	**140**[c]
	(47, 93)	(71.5, 120.5)	(140, 140)
Normally distributed			
pCO₂ (mmHg)	**23.79 ± 5.86**[a]	29.37 ± 4.65	26.20 ± 4.46
HCO₃ (mmol/L)	**32.57 ± 8.13**[a]	**47.34 ± 8.83**[b]	**40.29 ± 5.72**[c]
Chloride (mmol/L)	**113.31 ± 5.23**[a]	**104.82 ± 6.87**[b]	110.2 ± 6.97
Osmolality (mOsmol/kg)[d]	**336.79 ± 17.79**[a]	**345.19 ± 17.3**[b]	**365.09 ± 12.07**[c]

[a]*Statistically significant differences between admission and control day values;* [b]*Statistically significant differences between control day values and prior to release values;* [c]*Statistically significant differences between admission and prior to release values.* [d]*Osmolality and BUN values obtained prior to release were underestimated because BUN values exceeded in many turtles the analytical range of the analyzer (140 mg/dL). Bold values indicate statistically significant differences.*

with 0.9% NaCl + lactated Ringer's solution. Bicarbonate concentrations were significantly higher after fluid therapy compared to the admission concentrations in all treated groups ($P < 0.0247$), and were significantly higher prior to release compared to the admission concentrations in groups 0.9% NaCl solution ($P = 0.0247$) and 0.9% NaCl + lactated Ringer's solutions ($P = 0.0082$). Post-therapy lactate concentrations were lower than those obtained on admission, but only statistically significant ($P = 0.011$) in the group treated with Plasmalyte. Post-therapy sodium concentrations were lower than initial concentrations in all treated groups, but these differences were statistically significant ($P = 0.02$) only in the group treated with 0.9% NaCl + lactated Ringer's solution. Post-therapy chloride concentrations were significantly lower than those obtained on admission in all groups ($P < 0.025$). Potassium concentrations were lower after fluid therapy compared to the admission concentrations in all treated groups, but only statistically significant in the groups treated with 0.9% NaCl ($P = 0.0498$) and Plasmalyte ($P = 0.03$). Although differences in glucose concentrations after treatment compared to initial concentrations were not significant in any group, post-therapy hyperglycemia was observed in 69.2% of the turtles treated with Jarchow's solution.

Reference intervals and 90% confidence intervals for portable blood gas analyzer (i-STAT) parameters in convalescent loggerhead sea turtles are presented in **Table 3**.

DISCUSSION

Over half of stranded loggerhead turtles (53.9%) exhibited some type of mild to moderate acid-base alteration at the time of admission, which is consistent with that reported in previous studies on juvenile loggerhead turtles stranded in the Canary Islands (1, 12). Other studies, particularly on cold-stunned turtles showed more extreme acid-base derangements (6, 11). Metabolic acidosis is very common in stranded sea turtles, being associated with an increased production of acid or loss of base (1); potential causes of acid gain include entanglement in fishing nets, involuntary submergence, shock, and renal disorders (1). Among the causes of loss of base are digestive disorders (e.g., diarrhea), and renal disorders (26). Respiratory acidosis occurs due to CO_2 retention, as seen with hypoventilation, upper or lower airway obstruction (such as bronchopneumonia) or hypoxia (27).

The choice of the appropriate fluid to administer to the turtle should be made according to the condition of each individual (18), but these analytical determinations are not always possible, especially in rehabilitation centers with limited material and human resources. Crystalloid fluids containing gluconate, acetate and lactate contribute to increasing alkalinity thus counteracting acidosis (28). In the present study, the crystalloid solutions with the worst results in the 2015 study (lactated Ringer's solution and dextrose-saline solutions) were replaced by two different solutions: Plasmalyte and Jarchow's solution. The solutions with the best results in the 2015 study (mixed saline-lactated Ringer's solution, and saline solution) were maintained in order to corroborate the results of the previous study, and also comply with the guidelines of the Ethical Committee for Animal Experimentation. Because the study involved clinical actions on animals with special protection, it was necessary to guarantee, in addition to investigating possible treatments, the highest possible survival rate. As in the previous study (12), the greatest increase in the number of animals with normal acid-base status compared to the admission time after 3 days of therapy was observed when the mixed saline-lactated Ringer's solution was used. Although several authors suggested that lactated crystalloids may exacerbate hyperlactatemia in reptiles (29, 30), concerns that its use may be harmful to reptiles have not been validated (13). Lactate is rapidly converted to bicarbonate in the liver, and its use is recommended unless there is concern for hepatic dysfunction (31).

Treatment with Jarchow's solution presented the worst results restoring the acid-base balance, as was observed in the previous study (12) when a similar solution containing 1:1 5% dextrose + 0.9% NaCl was administered. Administering this dextrose solution to a hypovolemic patient would not only fail to expand the intravascular space and restore acid-base balance, but would also lead to extravasation, edema, altered oxygen transportation to the tissues and therefore potentially exacerbate acid-base abnormalities. Furthermore, similarly to

TABLE 3 | Reference intervals and 90% confidence intervals for portable blood gas analyzer (i-STAT) parameters in convalescent loggerhead sea turtles.

	Median (range)	Lower limit (90%CI)	Upper limit (90% CI)
pH	7.60 (7.51–7.77)	7.47 (7.49–7.44)	7.76 (7.80–7.72)
pCO$_2$ (mmHg)	29.38 (17.29–42.52)	21.68 (19.84–23.38)	37.03 (35.72–38.49)
pO$_2$ (mmHg)	75.91 (50.93–198.07)	51.72 (55.00–48.43)	129.83 (153.61–113.26)
HCO$_3$ (mmol/L)	39.52 (29.99–51.64)	29.32 (27.20–31.17)	49.47 (47.32–51.61)
Lactate (mmol/L)	0.89 (0.30–6.72)	0.23 (0.28–0.18)	7.34 (13.13–4.53)
Sodium (mmol/L)	151.50 (143.00–160.00)	144.29 (142.67–145.67)	159.68 (158.21–161.34)
Potassium (mmol/L	3.00 (2.10–4.10)	1.99 (1.80–2.18)	4.07 (3.89–4.27)
Chloride (mmol/L)	110.50 (98.00–122.00)	98.35 (95.91–100.58)	123.39 (121.02–126.12)
Anion Gap (mmol/L)	4.70 (−8.24 to 14.07)	−1.37 (−2.85 to −0.03)	11.42 (10.19–12.79)
Glucose (mg/dL)	110.00 (70.00–152.00)	77.33 (69.23–85.30)	143.84 (137.17–150.10)
Osmolality (mOsmol/kg)	364.58 (333.91–381.27)	341.5-378.22	345.62-383.7

the study in 2015 (12), the highest post-therapy glucose levels were observed when Jarchow's solution was administered, due to its dextrose content. Recently, administration of Jarchow's solution for rehydration of experimentally dehydrated bearded dragons (*Pogona vitticeps*) resulted in severe hyperglycemia and significant reductions in plasma osmolarity and sodium and phosphorus concentrations (32).

Results after Plasmalyte treatment were unexpected. Plasmalyte is commonly used in reptile medicine (32), especially as its buffers (gluconate and acetate) are not liver dependent due to their pharmacokinetics (30). Gluconate produces bicarbonate in peripheral tissues and muscles, but excessive administration of this compound may lead to metabolic alkalosis and hypokalemia (30). When alkalosis occurs, there is a gastrointestinal and renal compensatory hydrogen loss, which also produces electrolyte loss that may lead to hypokalemia (33). Considering that most of the turtles in this study had mild to moderate acid-base abnormalities, Plasmalyte might have overcorrected those. Therefore, Plasmalyte might be only appropriate to correct severe acid-base abnormalities and it should be reserved for those cases.

The present study had several limitations when evaluating clinical fluid therapy for loggerhead turtles. Although its use is widespread in rehabilitation centers around the world, the portable electronic blood analyzer i-STAT is not validated for use in reptiles. In addition, hepatic, pancreatic, and complete renal panels were not performed in this study. It would have been useful to know how possible disorders of these organs could influence the response to fluid therapy. And finally, there were several additional factors (cause of stranding and associated diseases, medical treatments received, and length of stay in the rehabilitation facilities) that could have influenced the results of our study.

In conclusion, up to 53.9% of the stranded loggerhead turtles of this study presented a mild to moderate acid-base alteration. The highest percentage of acid-base and electrolyte balance after 3 days of therapy was observed when the mixed saline-lactated Ringer's solution was used, which corroborates a previous study in which other solutions were administered (12). Furthermore, establishment of convalescent acid-base, electrolyte and plasma biochemical reference intervals could be useful for rehabilitation centers caring for stranded loggerhead turtles.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethical Committee for Animal Experimentation (CEEA-ULPGC) .

AUTHOR CONTRIBUTIONS

AI and JO: conceptualization, methodology, investigation, data curation, and write-up (original draft). ÁS: statistical analysis. AC, PC, and AS-P: methodology and data curation. All authors contributed to the article and approved the submitted version.

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