Seasonal variations in the physiological stress response to discrete bouts of aerial exposure in the little skate, *Leucoraja erinacea*

Angela M. Cicia a,⁎, Lela S. Schlenker b, James A. Sulikowski a, John W. Mandelman c

a University of New England, 11 Hills Beach Rd, Biddeford, ME 04005, USA
b Dauphin Island Sea Lab, 101 Bienville Blvd, Dauphin Island, AL 36528, USA
c New England Aquarium, Central Wharf, Boston, MA 02110, USA

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A B S T R A C T

Aerial exposure and acute thermal stress have been shown to elicit profound physiological disruptions in obligate water-breathing teleosts. However, no study has investigated these responses in an elasmobranch. To address this, venous blood samples were collected and evaluated from little skates (*Leucoraja erinacea*) subjected to discrete aerial exposure durations (0, 15, and 50 min) coupled with differing abrupt thermal changes (gradient between seawater and air; winter: ΔT = −3°C; summer: ΔT = +9°C) in two distinct laboratory studies. In general, blood acid–base properties (e.g. decline in pH; elevation in PCO₂) and select metabolites (elevated whole-blood lactate) and electrolytes (elevated plasma K⁺) were significantly disrupted by aerial exposure, and were most disturbed after skates were exposed to air for 50 min. However, the magnitude of the blood acid–base perturbations, metabolic contribution to the resulting blood acidosis, elevations to ionic and metabolic parameters, and delayed mortality were more extreme during the summer study, suggesting that acute thermal stress exacerbates the physiological impairments associated with aerial exposure in little skates. Conversely, a reduced thermal gradient (from seawater to air) may attenuate the magnitude of metabolic and ionic perturbations, resulting in a high physiological threshold for coping with extended aerial exposure.

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1. Introduction

Exposure to air can lead to the collapse of the gill lamellae and functionally inhibits gas exchange in fishes (Ferguson and Tufts, 1992). As such, sustained bouts of aerial exposure following commercial or recreational fishing capture have been shown to elicit marked physiological changes in obligate water-breathing teleosts. These include the induction of extracellular acidosis (Ferguson and Tufts, 1992), the accumulation of metabolites in the blood (Arends et al., 1999; Davis and Schreck, 2005; Suski et al., 2007), cardiovascular alterations (Cooke et al., 2003) and osmotic/ionic disruptions (Waring et al., 1996; Milston et al., 2006; Hur et al., 2007). Moreover, the effects of aerial exposure appear to be additive, whereby lengthy exposure durations result in more severe physiological disruptions (Davis and Schreck, 2005), increased immediate and delayed mortality following fishing capture and release (Davis and Parker, 2004; Gingerich et al., 2007) and longer recovery periods (Suski et al., 2007).

In addition to aerial exposure, teleosts captured during demersal fishing operations may also experience abrupt changes in ambient temperature when transitioning from water to air during gear retrieval (Olla et al., 1998). Acute thermal stress (e.g. from elevated air temperature (Tair, relative to water), which varies by season and environment/geography, has been shown to exacerbate physiological alterations and mortality associated with extended periods of aerial exposure (10–60 min) in teleosts (Davis and Parker, 2004; Davis and Schreck, 2005). For example, Olla et al. (1998) found mortality rates were higher in teleosts subjected to 15 min of aerial exposure during the summer and early fall, when the thermal gradient between the bottom and surface seawater exceeded 10 °C. Although the aforementioned stressors represent two critical components of capture and have been suggested to exaggerate physiological alterations and associated mortality in teleosts (Ferguson and Tufts, 1992; Suski et al., 2007; Trushenski et al., 2010), no study to date has considered the effects of seasonally elevated Tair on aerial exposure in an elasmobranch. Moreover, the physiological responses to aerial exposure have received only brief attention in sharks (Cliff and Thurman, 1984; Frick et al., 2010a), with no studies examining this in batoids (skates and rays), which are routinely captured, exposed to air, and discarded as non-targeted bycatch in ground fishing operations.
worldwide (Dulvy et al., 2000). In the northwest (NW) Atlantic, the little skate (Leucoraja erinacea) is almost exclusively discarded following commercial capture because of its small size and low market value. Thus, the objective of the current study was to independently assess the acute physiological effects and recovery from two discrete aerial exposure increments in the little skate, during distinct seasons (i.e. winter and summer). Additionally, the extent of influence temperature change had on the magnitude of the blood chemical responses to aerial exposure was also qualitatively compared.

2. Materials and methods

2.1. Animal acquisition and holding

To allow for the largest possible disparity in seasonal temperature elements in which to evaluate the physiological responses to aerial exposure, two independent studies were conducted and will be referred to hereafter as the “winter” (February) and “summer” (August) studies. Accordingly, mature little skates (48–56 cm total length; TL) were captured during discrete collection efforts (February and August, 2010) by short duration (~20 min) otter trawls in coastal waters (depth ranging 53–67 m) off New Hampshire (USA). Fishing gear consisted of a standard otter trawl constructed of stretch diamond mesh (net body mesh size: 16.5 cm; codend mesh size: 15.2 cm), with 7+ Bison doors, and a modified sweep (11 m) and head rope (18.5 m). Following capture in both studies, skates were immediately placed in a large live well and a modified sweep to maintain near bottom seawater temperatures, minimizing the thermal stress exposure prior to the actual experiment. Skates were then transported (~50 min) to the University of New England’s (UNE) Marine Science Center (MSC) in an insulated container (1 x 1 x 1 m) equipped with aeration. Once at the laboratory, each skate was measured (TL), sexed, marked with a Floy spaghetti tag (Floy Tag & Mfg. Inc., Seattle, WA, USA) and housed in a 3.7 x 1.5 x 1.2 m oval tank with an open, flow-through seawater system (turnover rate of 38 L min⁻¹) delivering unfiltered ambient seawater from the Saco Bay. Skates in both the winter and summer studies were acclimated to laboratory conditions for 10 days and fed Atlantic herring (Clupea harengus) daily ad libitum, until two days prior to the experiments. During acclimation, salinity ranged between 27–30 ppt (winter and summer studies), with distinct seasonal variations in tank water temperature (T_W) (Table 1).

2.2. Air exposure experiments and phlebotomy

To determine the physiological effects of acute aerial exposure in both studies, distinct groups of skates were randomly assigned to one of three aerial exposure treatments (control (~1 min), 15, or 50 min), representing typical “on deck time” experienced while the catch is sorted and processed. Although the following protocol was consistent between the winter and summer studies T_W, T_A and the corresponding thermal gradient (ΔT; difference between T_W and T_A within a given sub-study) differed by season (Table 1).

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Water temperature (°C)</th>
<th>Air temperature (°C)</th>
<th>Thermal gradient (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>4</td>
<td>1</td>
<td>−3</td>
</tr>
<tr>
<td>Summer</td>
<td>18</td>
<td>27</td>
<td>+9</td>
</tr>
</tbody>
</table>

For both experiments, control (winter: n=9; summer: n=6) animals were netted and brieﬂy (~1 min) exposed to air while blood samples were collected. Alternative subsets of skates were also netted and immediately placed outdoors (winter experiment: T_A = 1°C; ΔT = −3°C; summer experiment: T_A = 27°C; ΔT = +9°C) on wet (cooled with ambient seawater) plastic carts, exposed to the sun (no shade), on a semi-enclosed patio for either 15 (winter: n=11; summer: n=7) or 50 (winter: n=11; summer: n=6) min. At the conclusion of each exposure period, ventilation rate (spiral beats min⁻¹) was recorded while skates were in air and −2 mL of venous blood was drawn immediately (~<1 min) from the caudal vein, using chilled heparinized 23 gauge needles with a 3 mL plastic syringe. The physiological disturbances elicited by this phlebotomy method have previously been shown to be minimal in an elasmobranch (e.g. Cooper and Morris, 1998).

Following the completion of experiments, skates were immediately returned to the holding tank and delayed (short-term) mortality was monitored for the subsequent five days. At the conclusion of this period, blood (~2 mL) was redrawn (according to the previously described protocol for control skates) from all surviving skates to assess the extent of blood biochemical recovery.

2.3. Blood processing and analytical methods

Immediately following blood collection (<30 s), ~95 μL of whole-blood was deposited into a CO4+ cartridge and processed in an i-STAT portable clinical analyzer (i-STAT PCA; Abbott Laboratories, Abbott Park, IL, USA), to determine lactate (mmol L⁻¹), and non-temperature corrected (raw) blood pH, and PCO₂ (mmHg). Although the i-STAT PCA also reports PO₂; an accurate assessment/interpretation of this parameter from venous blood has been shown to be problematic in elasmobranchs, and therefore was excluded from further analysis. Furthermore, because the i-STAT PCA is temperature sensitive, the surrounding T_A was maintained between 20–23 °C using small space heaters during blood processing in both studies. Micro-hematocrit tubes were filled in duplicate with 0.10 mL of whole-blood and centrifuged at 3000 rpm for one min to determine hematocrit (Hct). The remaining whole-blood was centrifuged at 3500 rpm for five min, and the supernatant was then removed and stored for ~30 °C for subsequent analysis of the plasma fraction of each blood sample. Utilizing a modified protocol from Mandelman and Farrington (2007), plasma samples were thawed and diluted (1:1) for assay on a Stat Critical Care Xpress (CCX) blood chemistry analyzer (Nova Biomedical, Waltham, MA, USA) to quantify plasma electrolyte (Na⁺, Cl⁻, K⁺, Ca²⁺, Mg²⁺; mmol L⁻¹) and metabolite (glucose; mg dL⁻¹) concentrations.

2.4. Temperature conversions and calculations

The i-STAT PCA is intended for the diagnostic analysis of mammalian species at 37 °C. When examining the blood of a ectotherm, post-hoc temperature corrections of raw (i-STAT) pH and PCO₂ values are necessary to better approximate in vivo values. To address the broad range of seawater and air temperatures skates were exposed to in the present study, elasmobranch specific temperature conversions for pH and PCO₂ from Mandelman and Skomal (2009) were utilized, where M and TC refer to measured and corrected values, respectively, and temperature (T) equates to estimated body temperature (T_B) of each skate at the time of blood draw.

\[ pH_{IC} = pH_M - 0.011(T - 37) \]  \hspace{1cm} (1)

\[ PCO_{2IC} = PCO_{2M} \left( 10^{-0.0186T} \right) \]  \hspace{1cm} (2)

Estimates of T (and thus T_B) in both the winter and summer studies were based on the T_W (in control skates) and the T_A (in the 15
and 50 min aerial exposure groups) (see Table 1 for exact temperatures). In the current study, although internal $T_w$ was not measured directly in aerial exposed skates, it was assumed that internal temperatures equilibrated to the ambient $T_a$. It should be noted that Eqs. (1) and (2) have been found to underestimate the full extent of the needed correction factor to convert raw i-STAT values to 25 °C (Gallagher et al., 2010). Although the conversions were still utilized to standardize the broad range of sample temperatures in the current study, the absolute in vivo accuracy of the corrected values should be interpreted with caution.

To estimate and compare the metabolic contribution to the blood acidosis between the control and the 15 and 50 min experimental groups respectively, mean whole-blood metabolic acid load ($\Delta H^+ m_{WB}$) was calculated for each aerial exposure interval following a modified protocol from Milligan and Wood (1986):

$$\Delta H^+ m_{WB} = \left[\text{HCO}_3^- - \left[\text{HCO}_3^-\right] - \beta (PH_{IC1} - PH_{IC2}) \right]$$  \hspace{1cm} (3)

Where $\text{HCO}_3^-$ and $\text{HCO}_3^-$ values represent mean values (within respective treatment groups), $\beta$ represents the non-bicarbonate buffer capacity reported in Gilmour et al. (2002) and Richards et al. (2003) (−7.13 mmol pH unit $^{-1}$ L$^{-1}$). Values for $\text{HCO}_3^-$ and $\text{HCO}_3^-$ were initially calculated using the Henderson-Hasselbalch equation from $\text{PCO}_2$ and $\text{HCO}_3^-$, with values for the $\text{pK}'$ and $\text{CO}_2$ values for the nearest validated temperature point in Boutilier et al. (1984) were utilized.

### 2.5. Statistical analyses

Due to distinct groups of research animals and seasonal variations in acquisition/acclimation conditions, blood biochemical parameters were not statistically compared between winter and summer studies, but rather qualitatively compared a posteriori. Prior to analyses within respective studies, the following transformations were performed to satisfy assumptions of normality and/or homoscedasticity: logarithmic (winter: ventilation rates), exponential (winter: pH, Na$^+$, Cl$^-$; summer: Cl$^-$) or square root (winter: Mg$^{2+}$, glucose; summer: Na$^+$). The immediate effect of aerial exposure treatments (control, 15, and 50 min) on individual physiological parameters were independently analyzed for the winter and summer studies via one-way analysis of variances (ANOVA) followed by a Tukey's HSD pairwise post-hoc tests. In cases where failed assumptions could not be satisfied by transformations (winter: lactate; and summer: ventilation rates), a Kruskal–Wallis and Dunn's post-hoc test were utilized.

Preliminary ANOVAs of recovery bleed data revealed that blood values (for respective parameters) were similar among the surviving skates ($P>0.05$) regardless of initial aerial exposure duration in the winter study (in the summer study, the low number of surviving skates ($n=3$) at the five day point precluded any statistical analysis and recovery data was not reported). In the surviving control animals, all initial blood chemical values for each parameter were compared to those following the five day monitoring period to assess recovery (winter study) using a student's paired $t$-test. By deductive inference, immediate control values could thus function as a reference point for the resolution (or lack thereof) of all blood biochemical parameters in surviving skates during the winter study. Therefore, because no statistical difference ($P>0.05$) was observed in the recovery blood values in all surviving skates (regardless of the initial treatment) and control recovery values were statistically similar to initial values ($P>0.05$), all recovery data was pooled in the winter study. For $\text{pH}$ and $\text{PCO}_2$, analyses were performed on temperature-corrected rather than raw data. In all cases, data is presented as means ± SEMs of non-transformed data. All analyses were performed using SYSTAT 11 (Systat Software Inc., Chicago, IL, USA) and statistical significance was accepted at $P<0.05$.

### 3. Results

#### 3.1. Skate mortality

Skate mortality rates evident at the end of the five day recovery period were as follows: winter (0%, 18% and 27% for the control, 15 and 50 min group, respectively); and summer (37%, 86% and 100% for the control, 15 and 50 min group, respectively).

#### 3.2. Winter study

##### 3.2.1. Immediate and delayed influence of increased aerial exposure on ventilation rate and blood acid–base properties

There was no significant effect of acute aerial exposure on the observed ventilation rate in skates (one-way ANOVA, $P>0.05$, Fig. 1a). However, aerial exposure did significantly influence venous $\text{pH}_T$ (one-way ANOVA, $F_{2,28}=35.92$, $P<0.001$) and $\text{PCO}_2$ (one-way ANOVA, $F_{2,27}=6.27$, $P<0.01$). Specifically, blood $\text{pH}_T$ became progressively more depressed at each increasing aerial exposure increment, declining ~0.25 pH units in skates exposed to air for 50 min relative to control values (Fig. 1b). Although $\text{PCO}_2$ values following 15 min of aerial exposure did not differ significantly from control values, skates exposed for 50 min exhibited a 27% increase from control values, and were significantly higher than either subsequent treatment group (Fig. 1c). Ventilation rate, $\text{pH}_T$, and $\text{PCO}_2$ were similar among surviving skates after five days, regardless of original aerial exposure duration (one-way ANOVAs, $P>0.05$) (Fig. 1 a–c). Finally, the aforementioned parameters in control skates were similar between the initial (experimental) blood draw and those obtained after the five day recovery period (paired $t$-test, $P>0.05$).

##### 3.2.2. Immediate and delayed influence of increased aerial exposure on plasma metabolite concentrations

Although aerial exposure did not elicit any observable differences in plasma glucose concentrations (one-way ANOVA, $P>0.05$, Fig. 2a), there was a significant effect on whole-blood lactate (Kruskal–Wallis one-way ANOVA, $H_{3}=18.11$, $P<0.001$), and mean values were significantly higher at each increasing aerial exposure increment (Fig. 2b). As with blood acid–base properties previously described, mean lactate and glucose values in skates alive after the five day recovery period were similar, independent of initial aerial exposure duration (one-way ANOVA, $P>0.05$) (Fig. 2a–b). In control skates, values of both parameters remained similar from the initial to the recovery blood draw (paired $t$-test, $P>0.05$) (Fig. 2a–b).

##### 3.2.3. Immediate and delayed influence of increased aerial exposure on plasma electrolytes

Aerial exposure duration elicited no observed response in Hct or Na$^+$, Cl$^-$, Ca$^{2+}$ and Mg$^{2+}$ concentrations (one-way ANOVA, $P>0.05$, Fig. 3a, b, c, d and e); however, there was a significant effect on plasma K$^+$ (one-way ANOVA, $F_{2,22}=4.376$, $P<0.05$) concentrations. In skates exposed to air for 15 min, plasma K$^+$ was two-fold higher relative to control skates and which was similar to those in the most prolonged (50 min) exposure group (Fig. 3f). After the five day recovery period, Hct and all measured electrolytes were similar in surviving skates regardless of the original aerial exposure duration (one-way ANOVA, $P>0.05$) (Fig. 3a–f). In addition, electrolyte values and Hct in control skates were similar between the initial (experimental) blood draw and those obtained after the five day recovery period (paired $t$-test, $P>0.05$) (Fig. 3a–f).
3.3. Summer study

3.3.1. Immediate and delayed influence of increased aerial exposure on ventilation rate and blood acid–base properties

In contrast to findings in the winter study, aerial exposure had a significant effect on ventilation rate (Kruskal–Wallis one-way ANOVA, $H_3 = 15.60, P<0.001$). Similar to the winter study, there was an effect on blood pH$_{TC}$ (one-way ANOVA, $F_{2,14} = 153.28, P<0.001$) and PCO$_{2TC}$ (one-way ANOVA, $F_{2,14} = 28.12, P<0.001$). Specifically, the mean ventilation rate in skates exposed to 15 min of air was significantly lower (67%) than controls, but was not different than the rate observed in skates exposed to air for 50 min (Fig. 4a). Blood pH$_{TC}$ became progressively more depressed at each increasing aerial exposure increment, declining ~0.8 pH units in skates exposed for 50 min relative to control values (Fig. 4b). Inversely, PCO$_{2TC}$ (Fig. 4c) was significantly higher at each aerial exposure duration and values were 260% higher than control values after the 50 min treatment.

3.3.2. Immediate and delayed influence of increased aerial exposure on plasma metabolite concentrations

Unlike the winter study, aerial exposure had a significant effect on plasma glucose (one-way ANOVA, $F_{2,13} = 11.45; P<0.001$) concentrations. In skates subjected to 15 min of aerial exposure, glucose concentrations remained similar to control skates, however, values were significantly elevated after 50 min in air (Fig. 5a). Similarly to the winter study, whole-blood lactate was affected by acute aerial exposure (one-way ANOVA, $F_{2,13} = 55.85; P<0.001$), with a significantly higher mean concentration at each increasing exposure increment (Fig. 5b).

3.3.3. Immediate and delayed influence of increased aerial exposure on hematocrit and plasma electrolytes

Although aerial exposure duration did not elicit any observable changes in Hct (one-way ANOVA, $P>0.05$, Fig. 6a), there was, in contrast to the winter study, a significant effect on all measured electrolytes (one-way ANOVAs, Na$^+$: $F_{2,14} = 5.43, P<0.05$; Cl$^-$: $F_{2,14} = 4.92, P<0.05$; Ca$^{2+}$: $F_{2,14} = 8.75, P<0.01$; Mg$^{2+}$: $F_{2,14} = 8.32, P<0.01$; K$^+$: $F_{2,14} = 58.22, P<0.001$). Mean concentrations of plasma Na$^+$ (Fig. 6b), Cl$^-$ (Fig. 6c), Ca$^{2+}$ (Fig. 6d), Mg$^{2+}$ (Fig. 6e), and K$^+$ progressively more depressed at each increasing aerial exposure increment, declining ~0.8 pH units in skates exposed for to air for 50 min relative to control values (Fig. 4b). Inversely, PCO$_{2TC}$ (Fig. 4c) was significantly higher at each aerial exposure duration and values were 260% higher than control values after the 50 min treatment.

![Fig. 1](image1.png) Mean (±SEM) (a) ventilation rate, and whole-blood (b) pH$_{TC}$ and (c) PCO$_{2TC}$ in the little skate (Leucoraja erinacea) immediately (●) after subjection to 0 (control), 15 and 50 min of aerial exposure in the winter study ($\Delta T = -3 \, ^\circ C$). Dissimilar letters denote statistically significant pairwise differences among aerial exposure treatment groups ($P<0.05$); (○) represents a composite mean (±SEM) from all animals alive after the five day recovery period ($P<0.05$). Samples sizes are indicated above each treatment group in parentheses.

![Fig. 2](image2.png) Mean (±SEM) (a) plasma glucose and (b) whole-blood lactate in the little skate (Leucoraja erinacea) immediately (●) after subjection to 0 (control), 15 and 50 min of aerial exposure in the winter study ($\Delta T = -3 \, ^\circ C$). Dissimilar letters denote statistically significant pairwise differences among aerial exposure treatment groups ($P<0.05$); (○) represents a composite mean (±SEM) from all animals alive after the five day recovery period ($P<0.05$). Sample sizes are indicated above each treatment group in parentheses.
(Fig. 6f) in the 15 min exposure group were all significantly higher than control values. With the exception of K⁺, which was further heightened after 50 min (122% higher than control values), all remaining electrolyte values plateaued and no differences were exhibited between the 15 and 50 min aerial exposure groups.

3.4. Immediate influence of increased aerial exposure on mean whole-blood metabolic proton load

In the winter and summer studies, the metabolic contribution to the observed blood acidosis increased with aerial exposure duration, although the magnitude of these contributions were greater in the summer study. In the winter study, the mean $\Delta H^+_{\text{WB}}$ relative to control values was 1.77 and 3.95 mmol L$^{-1}$ in the 15 and 50 min exposure groups, respectively. In the summer study, mean $\Delta H^+_{\text{WB}}$ relative to control values was 5.08 and 8.00 mmol L$^{-1}$ for 15 and 50 min exposure groups, respectively.

4. Discussion

This study represents the first investigation to consider the impacts of seasonally elevated $T_a$ on the physiological alterations elicited by aerial exposure in an elasmobranch. In general, results in the present study are consistent with similar investigations in teleosts, which suggest that the physiological effects of these stressors are potentially lethal (Davis and Schreck, 2005; Milston et al., 2006). For example, ventilation rates in the summer study significantly declined in skates subjected to (ultimately lethal) bouts in air.
Although no studies have directly assessed ventilation rates in teleosts or elasmobranchs during aerial exposure, Gingerich et al. (2007) suggested that markedly depressed ventilation rates (observed after submergence) in blue gill (Lepomis macrochirus) following aerial exposure may be indicative of subsequent (delayed) mortality. It is clear that the combined effects of extended aerial exposure and acute thermal shock (summer) were more physiologically compromising in skates than aerial exposure alone (winter).

In almost all fishes, the majority of gas exchange occurs across the secondary lamellae, which are structurally supported by hydrostatic pressure (Boutilier, 1990). When exposed to air, the lamellae collapse, rendering the gills non-functional and inhibiting gas exchange (Ferguson and Tufts, 1992). More specifically, aerial exposure in the present study compromised the ability to offload CO$_2$ at the gills, yielding a hypercapnic state (i.e. acutely elevated PCO$_{2TC}$) and concurrent (presumed respiratory) acidosis in the blood of aerially exposed skates in the winter (after 50 min) and summer (after 15 and 50 min) studies. Similarly, Ferguson and Tufts (1992) reported an almost complete inhibition of gas exchange across the gills and a more extreme alteration in blood acid–base status (increase in PCO$_2$ and decline in pH) when exhaustively exercised rainbow trout (Onocorhynchus mykiss) were also exposed to air for 60 s. The presumed respiratory component was only a partial contributor to the blood acidosis observed in the present study. The estimated metabolic contribution (e.g. net influx of metabolic protons into the blood) increased with each aerial exposure increment in both the winter and summer studies, indicating a mixed metabolic/respiratory origin, which has previously been reported in elasmobranchs subjected to capture and transport stress (e.g. Manire et al., 2001; Frick et al., 2011). Comparatively, the more extreme metabolic acid load in both aerial treatments during the summer study is likely a reflection of the added temperature elements (e.g. higher T$_W$, T$_A$, and a larger gradient between the two). It is well established that in ectotherms, metabolic rate varies directly with environmental temperature (Q$_{10}$ effect) (e.g. Cooke et al., 2003). For example, standard metabolic rate in the bat ray (Myliobatis californica) was approximately twofold higher following a 6 °C (8 to 14 °C) increase in ambient T$_W$ (Hopkins and Cech, 1994). In the current study, ambient T$_W$ was 14 °C higher during the summer experiment, indicating that even before aerial exposure experiments commenced, the standard environmental conditions would expectedly have a marked influence on metabolic rate.

metabolic rate of these skates were potentially elevated in comparison to the winter specimens. Moreover, summer specimens were subjected to higher $T_A$ and a more acute thermal shock when removed from seawater (+9 °C in summer versus −3 °C in winter). Thus, the combination of elevated metabolic rates, enhanced energy demands and more acute thermal stress likely stimulated a greater reliance on anaerobic metabolism, which resulted in an elevated efflux of protons to the blood, contributing to the more pronounced blood acidosis in both aerial exposure groups during the summer study.

The shift to anaerobic metabolism in aerially exposed little skates was also evident by an increase in whole-blood lactate (e.g. Holeton and Heisler, 1983; Milligan and Wood, 1986; Wood, 1991). Similar to teleosts, elevations in whole-blood lactate were directly proportional to the duration of aerial exposure, suggesting an increasing contribution of anaerobic functioning to satisfy energy demands (Davis and Schreck, 2005; Suski et al., 2007). However, the peak expression after 50 min in air during the winter (1.29 mmol L$^{-1}$) and summer (5.36 mmol L$^{-1}$) were substantially lower than lactate concentrations reported in other elasmobranchs (>20 mmol L$^{-1}$) subjected to exhaustive exercise associated with capture and handling (Cliff and Thurman, 1984; Hoffmayer and Parsons, 2001; Manire et al., 2001; Mandelman and Farrington, 2007; Frick et al., 2010b). Previous research suggests that low whole-blood lactate levels may be a characteristic of relatively inactive, sedentary fish species (Milligan and Farrell, 1986; Milligan and Wood, 1987) and may be applicable to skates, which possess a similar lifestyle (Hove and Moss, 1997). In addition, low whole-blood lactate levels in little skates may also be attributed to a reduced diffusion rate from white muscle-to-blood allowing enhanced rates of glycogen

Fig. 6. Mean (± SEM) (a) hematocrit and plasma (b) Na$^+$, (c) Cl$^-$, (d) Ca$^{2+}$, (e) Mg$^{2+}$, and (f) K$^+$ in the little skate (L. Erinacea) immediately (●) after subjection to 0 (control), 15, and 50 min of aerial exposure in the summer study ($\Delta T = +9$ °C; salinity = 30 ppt). Dissimilar letters denote statistically significant pairwise differences among aerial exposure treatment groups ($P<0.05$). Samples sizes are indicated above each treatment group in parentheses.

resynthesized in situ, which has been observed in other sedentary teleost species (Turner et al., 1983; Milligan and Wood, 1987).

Elevated plasma glucose is also commonly observed in elasmobranchs subjected to capture related stressors (e.g. Wells et al., 1986; Frick et al., 2010a). However, a hyperglycemic response was only observed in little skates subjected to 50 min of aerial exposure in the summer study (80% increase relative to control values). Moreover, the magnitude of this rise was substantially less than in the dusky smoothhound shark (Mustelus canis) exposed to air for 80 s (142%) (see reference in Cliff and Thurman, 1984) and lingcod (Ophiodon elongatus) (233%) subjected to a similar duration (45 min) in air as the skates (50 min) in the present study (Milston et al., 2006). The absence of a glucose response during the winter and the moderate response during summer could again be functions of the sedentary lifestyle and low basal metabolic rate in the little skate, factors previously contributing to low glucose expression in benthic teleosts (Vijayan and Moon, 1994; Waring et al., 1996). In addition, a temperature-induced depression in metabolic rate inhibited glucose mobilization in striped bass (Morone saxatilis) following confinement stress and may also have contributed to the absence of a glucose response in little skates during the winter study (Davis and Parker, 1990).

Monovalent (Na⁺ and Cl⁻) ion concentrations were also significantly elevated relative to control values following the 15 and 50 min exposure groups during the summer study, with the magnitude of the observed elevations similar to those reported in aerially exposed teleosts (Waring et al., 1996; Arends et al., 1999; Milston et al., 2006; Suski et al., 2007). In these studies, increases in Na⁺ and Cl⁻ were attributed to increased gill permeability (e.g. increased influx from environment). However, this was not plausible in the present study because aerially exposed little skates were no longer surrounded by a hypertonic medium to promote an influx of these ions. Hoffmayer and Parsons (2001) attributed stress induced increases in plasma osmolarity in angled Atlantic sharpnose sharks (Rhizoprionodon terraenovae) to a net shift of water out of the vascular compartment in response to a rise in intracellular lactate or an increased sodium influx. However, in the current study, the observed elevations in Na⁺ and Cl⁻ in the little skate during the summer study occurred in the absence of any alterations in hematocrit, detracting from the notion that a vascular water shift was the causative factor. A more detailed assessment of fluid volume changes and ion regulatory mechanisms associated with aerial exposure are needed before any further conclusions are drawn.

In the summer experiment, divalent (Ca²⁺ and Mg²⁺) ion concentrations rose significantly in skates exposed to both 15 and 50 min of aerial exposure. An elevation in divalent ion concentrations have been previously attributed to a capture induced decline in pH in juvenile dusky sharks (Carcharhinus obscurus) (Cliff and Thurman, 1984) and has been suggested to offset cardiac damage caused by blood acidosis in pelagic teleosts and elasmobranchs (Cliff and Thurman, 1984; Wells et al., 1986; Mandelman and Farrington, 2007). Furthermore, peak Ca²⁺ concentrations observed in the summer study coincided with maximal disturbances in acid–base parameters (lowest pH and highest PCO₂), inferring that this counterbalance might have also occurred in the little skate. In contrast, the less extreme decline in blood pH observed in the winter study, may potentially explain the absence of any significant rise of Ca²⁺ and Mg²⁺ after 15 or 50 min of aerial exposure.

Interestingly, K⁺ was the only electrolyte to be significantly impacted by aerial exposure in both the summer and winter studies. Hyperkalemia has been observed in both marine teleosts (Milston et al., 2006) and elasmobranchs (Frick et al., 2010a) subject to various bouts of aerial exposure. Previous research has attributed hyperkalemia in stressed elasmobranchs to an increased eflux of K⁺ from damaged muscle cells due to intracellular acidosis (Cliff and Thurman, 1984; Frick et al., 2010b). In addition, Martini (1974; see reference for Cliff and Thurman, 1984) reported plasma K⁺ concentrations >7 mmol L⁻¹ induced myocardial dysfunction in stressed spiny dogfish (Squalus acanthias). In the winter study, K⁺ concentrations (>5.5 mmol L⁻¹) remained well below this reported threshold in both aerial exposure groups, which may partially account for the high observed post-exposure survivability. In contrast, this threshold was exceeded (8.5 mmol L⁻¹) in skates exposed to air for 50 min in the summer study, which may be a causative factor in the 100% delayed mortality (short-term) within this group. Furthermore, a continued elevation in plasma K⁺ concentrations (and eventual mortality) has been shown to occur following the cessation of capture related stressors in the gummy shark (Mustelus antarcticus) (Frick et al., 2010b). It is therefore plausible that a continued (unaccounted) rise (post-exposure) in K⁺ in the 15 min (summer) exposure group (5.80 mmol L⁻¹) may have been causative in the high mortality rate (86%) also observed in this exposure group.

4.1. Implications on mortality, management considerations, and conclusions

Previous research on teleosts has indicated that extended periods of aerial exposure following capture or exhaustive exercise can increase subsequent mortality (e.g. Ferguson and Tufts, 1992; Parker et al., 2003; Davis and Schreck, 2005; Suski et al., 2007). For example, exposure to air for 45, 60, and 75 min corresponded with mortality rates of 0%, 33% and 100%, respectively, in adult lingcod following simulated trawl capture (Davis and Olla, 2002). In the current study, mortality rates in little skates also increased according to aerial exposure duration in general. Despite marked alterations in blood biochemistry, skate mortality rates in the winter study were far exceeded by those in the summer study. Thus, low ambient temperatures and the absence of an acute thermal change when transitioning from seawater to air may attenuate the magnitude of the associated metabolic and ionic perturbations, resulting in a high physiological threshold for aerial exposure, and increased survivability in the little skate. Conversely, the dramatically more disturbed blood chemical alterations and higher post-exposure mortality observed following aerial exposure in the summer study indicates that acute thermal shock (e.g. elevated ΔT) may be the prominent factor in predicting mortality induced by aerial exposure in little skates, a conclusion consistent with previous research on teleosts (Olla et al., 1998; Davis and Parker, 2004; Davis and Schreck, 2005). Interestingly, elevated mortality was also observed in control skates (37%) during the summer study, despite minimal aerial exposure (<1 min) and identical handling/processing methodologies as those utilized in the winter study. This increase in mortality (e.g. in control animals), may suggest that elevated Tₜ during the summer study compromise the physiological tolerance of little skates to cope with minor handling stressors and this notion should be examined further.

Since aerial exposure in the present study was examined in isolation and without the preceding capture and handling rigors that would ordinarily occur under actual conditions, physiological disturbances under all treatments should also be viewed as conservative. Thus, for the little skate, which like other skate species, is frequently captured as non-target bycatch in NW Atlantic demersal fisheries across multiple seasons, capture and aerial exposure during warmer months (when temperature gradients from seawater to air are most extreme) may substantially inflate subsequent mortality. Consequently, more stringent fishing/handling protocols may be required during the summer to reduce immediate and post-release mortality. Furthermore, the results of the present study emphasize a need to address the physiological impacts of other capture stressors (e.g. tow duration, tow weight, exhaustive exercise) on a seasonal basis to provide more accurate estimates of bycatch mortality, which are critical to the successful management of this and other rajid species. Future studies should also probe deeper into the physiological mechanisms and resultant alterations incited when extended aerial
exposure is preceded by other (capture related) stressors, in little skates as well as other commonly captured elasmobranchs.

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