Journal of Comparative Physiology B
https://doi.org/10.1007/s00360-025-01629-w

ORIGINAL PAPER

Osmorespiratory compromise in an elasmobranch: oxygen consumption, ventilation and nitrogen metabolism in dogfish sharks (*Squalus suckleyi*) exposed to hypoxia in different salinities

Marina Giacomin[1,2,4] · **Patricia M. Schulte**[1] · **Chris M. Wood**[1,2,3]

Received: 19 February 2025 / Revised: 22 June 2025 / Accepted: 15 July 2025

Abstract

Fish face a functional trade-off at the gills between minimizing ion movement and maximizing oxygen uptake – the osmorespiratory compromise, but the extent of this trade-off remains poorly understood in elasmobranchs. Using the Pacific dogfish shark, we assessed the impacts of progressive hypoxia in animals acclimated to 25, 30 and 36 ppt for 4 days at 12 °C. Plasma osmolality increased with water osmolality at 36 ppt (osmoconformation) and decreased at 25 ppt. Plasma urea decreased at 25 ppt, though to a lesser extent than plasma Cl^-, while plasma urea increased to a greater extent than plasma Cl^- at 36 ppt. In normoxia, oxygen consumption rate (MO_2) was elevated by 60% at 36 ppt, and ventilatory index (frequency x amplitude) was elevated by 70%, reflecting increases in both components of ventilation, but these parameters remained unchanged in sharks exposed to 25 ppt. During progressive hypoxia, MO_2 and ventilation exhibited different patterns at the three salinities, but in all three, MO_2 fell linearly below a water PO_2 of ~80 Torr (10.7 kPa), indicating oxyconformation. Under hypoxia (45 to 5 Torr; 6.0 to 0.7 kPa) MO_2 was the same at all salinities, while ventilatory amplitude was elevated at both 25 and 30 ppt. At 36 ppt, frequency decreased during hypoxia. Ventilatory index increased during hypoxia only at 30 ppt and not at the other salinities. From these data it is clear that dogfish sharks face an osmorespiratory compromise balancing the needs for urea retention against those of O_2 uptake.

Keywords Urea, chloride · Osmoconformation · Oxyconformation · Ventilatory index

Introduction

The gills of fishes must balance the needs to maintain water and ionic homeostasis with those of oxygen uptake (MO_2) and respiratory gas exchange, processes which are dependent on their intrinsic morphological and functional characteristics that influence diffusional processes, such as large surface area and short diffusion distance. This functional trade-off is known as the osmorespiratory compromise (Randall et al. 1972; Gonzalez and McDonald 1992; reviewed by Gilmour and Perry 2018; Wood and Eom 2021). Despite efforts in advancing our understanding of the osmorespiratory compromise in elasmobranchs (Zimmer and Wood 2014; Giacomin et al. 2017, 2022), this group remains largely overlooked in comparison to teleosts.

Elasmobranchs are particularly interesting with respect to the osmorespiratory compromise because in contrast to teleosts, they are ureotelic osmo-conformers. The major osmolyte in their blood plasma is the nitrogenous waste

Communicated by: Bernd Pelster.

✉ Marina Giacomin
marina@coregeo.ca

Patricia M. Schulte
pschulte@zoology.ubc.ca

Chris M. Wood
woodcm@zoology.ubc.ca

[1] Department of Zoology, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada

[2] Bamfield Marine Sciences Centre, Bamfield, BC, Canada

[3] Department of Biology, McMaster University, Hamilton, ON, Canada

[4] Core Geoscience Services Inc., Whitehorse, YT, Canada

Published online: 30 September 2025

product urea, which is actively retained at gills, kidney, rectal gland, and gut for osmoregulation (reviewed by Wright and Wood 2016). This results in an internal osmolality slightly hyperosmotic to the environment, which in turn promotes the osmotic influx of water, largely eliminating the need for drinking (Smith 1931, 1936). When environmental salinity decreases, elasmobranchs allow their internal urea concentrations to decrease, and the opposite occurs when environmental salinity increases, thus facilitating osmo-conformation (reviewed by Ballantyne and Fraser 2013; Wright and Wood 2016). The strategy has considerable metabolic costs, because the conversion of ammonia into urea through the ornithine-urea cycle (OUC) occurs at the expense of 5 moles of ATP per mole of urea (2 mol of urea-N) produced (Kirschner 1993; Ballantyne 1997).

One of the physiological challenges that elicits the osmorespiratory compromise is environmental hypoxia, a phenomenon of both localized and global proportions (Ficke et al. 2007; Díaz and Breitburg, 2011). Regional hypoxic areas have increased greatly in the past decades (Breitburg et al. 2018) and threaten estuarine and coastal environments in more than 500 different areas globally (Intergovernmental Oceanographic Commission 2018).

Many species of elasmobranchs have evolved the ability to withstand periods of hypoxia but tolerance varies greatly. Broadly, hypoxia tolerance is associated with either an ability to down-regulate metabolic rate or a capacity to maintain MO_2 relatively stable independent of lowered water PO_2, also known as oxygen regulation. Many physiological adjustments can underlie this capacity, including changes in ventilation (known as the hypoxic ventilatory response), cardiac output, increase in gill functional surface area (the number of lamellae that are perfused), improvements in blood O_2 carrying capacity, Hb-O_2 affinity and others (Dejours 1981; Farrell and Richards 2009).

Hypoxia exposure in elasmobranchs may lead to a variety of cardiovascular and hematological responses in addition to metabolic and acid-base disturbances (Butler and Taylor 1975; Piiper et al. 1977; Butler et al. 1979; Speers-Roesch et al. 2012). Perry and Gilmour (1996) reported marked increases in ventilation frequency as well as volume amplitude (measured indirectly through pressure changes of the spiracle cavity) when Pacific spiny dogfish (*Squalus suckleyi*) were acutely exposed to hypoxia. These findings were corroborated by Acharya-Patel et al. (2018) on the same species, where the animals were exposed more slowly to hypoxia. On the other hand, De Boeck et al. (2024), again working on *S. suckleyi*, recorded only very modest increases in frequency and amplitude (with frequency dominating) when hypoxia was induced over a similar time frame. While there seems to be a general consensus that both ventilatory components (frequency and amplitude) respond to hypoxia, their relative contributions to the hypoxic ventilatory response in elasmobranchs are unclear.

Zimmer and Wood (2014) found that the spiny dogfish acutely exposed to moderate and severe hypoxia showed a significant reduction in arterial blood oxygen, which was readily recovered upon return to normoxia. These findings were associated with marked increases in urea and ammonia losses to the water during hypoxic exposure. The authors suggested that the physiological mechanisms that enhance MO_2 in hypoxia could have caused an impairment of the branchial urea retention mechanisms (Zimmer and Wood 2014).

The Pacific spiny dogfish (*S. suckleyi*) and its close congener *Squalus acanthias* are known to be moderately euryhaline, commonly using estuaries and river mouths as foraging and breeding grounds (McMillan and Morse 1999), where they can experience large fluctuations in environmental salinity, temperature and dissolved oxygen. While low salinities are common due to freshwater runoff, there is also increasing evidence of elevated salinities in inshore waters (Esbaugh 2024). Richards (2004) surveyed oceanic fishery data, reporting routine occurrence of *S. suckleyi* between 29 and 33.5 ppt salinity. However, in the lab, this species survives up to 39 ppt for at least 24 h (Deck et al. 2016), and down to 21 ppt (e.g. Guffey and Goss 2014; Deck et al. 2016; Weinrauch et al. 2020) for at least several days. Wright and Wood (2016) classified them as "dilution tolerators".

To our knowledge, no study to date has looked at how elasmobranchs may respond to the dual osmorespiratory challenges of salinity change and hypoxia, such as they might encounter when entering estuaries. Our goal was to investigate if exposure to different salinities (within an environmentally relevant range) would affect the rate of O_2 consumption in dogfish sharks and in turn alter their response to progressive hypoxia. We hypothesized that due to the costs associated with synthesizing and actively retaining urea at the gills, kidney, rectal gland, and gut, pre-exposure to 25 ppt would lessen the metabolic burden of ureotelism and result in a lower O_2 consumption, since plasma urea concentrations are known to be lower at lower salinities (Deck et al. 2016; Guffey and Goss 2014; Weinrauch et al. 2020). Conversely, we predicted that at higher salinities, maintaining an elevated plasma-to-water urea gradient (high plasma urea) would be reflected in a higher rate of O_2 consumption. In parallel, we also hypothesized that during progressive hypoxia, in view of these altered osmoregulatory costs, disturbances in O_2 consumption and ventilation would be reduced at lower salinity and elevated at higher salinity. Additionally, we wanted to further our understanding of the hypoxic ventilatory response in sharks, by individually measuring the two components of ventilation, frequency

and amplitude (representing stroke volume). We predicted that changes in amplitude would make the larger contribution, because with respect to efficiency, there are energetic advantages to varying stroke volume rather than frequency when pumping water, which is a medium of high density and viscosity (Shelton et al. 1986; Perry and Wood 1989).

Elasmobranchs rely partly on plasma urea, and partly on plasma ions to regulate plasma osmolality (Ballantyne and Fraser 2013; Wright and Wood 2016). In view of the high cost of urea synthesis and retention outlined above, and the elevation of urea and ammonia loss rates observed in dogfish exposed to hypoxia at 30 ppt (Zimmer and Wood 2014) we predicted differential regulation of plasma urea and Cl^- levels. Specifically, we posited that greater decreases in plasma urea than in plasma Cl^- concentration would occur at lowered salinity, while greater increases in plasma Cl^- than in urea concentration would occur at elevated salinity, especially given the energetic challenges of hypoxia.

To address these goals, we simultaneously measured ventilatory parameters, O_2 consumption rate, and urea and ammonia excretion rates in Pacific spiny dogfish while sharks were progressively depleting the water O_2, in a closed-system respirometry set-up. These experiments were performed in dogfish that had been pre-exposed to different salinities [25, 30 (acclimation salinity) and 36 ppt] for 96 h, and were conducted in the salinity to which the fish had been pre-exposed. Lastly, at the end of the respirometry trials, a blood sample was collected for measurements of plasma osmolytes and to examine the effects of salinity on the shark plasma composition.

Materials and methods

Animal collection and acclimation

Dogfish sharks (*Squalus suckleyi*) were collected by angling in Barkley Sound, a fjord-type estuary, near Bamfield Marine Science Centre (BMSC), Bamfield, British Columbia, Canada. Animals were collected under Department of Fisheries and Oceans (DFO) collection permit XR 32 2016. Fish were held at BMSC for approximately 3 weeks in a large (150 m^3) circular indoor concrete tank, with flowing filtered natural seawater (~12 °C, 30 ppt salinity), constant aeration (dissolved oxygen kept at >130 Torr) and photoperiod at 10 h dim light:14 h dark. Sharks were fed twice a week to satiation with commercially purchased frozen hake (*Merluccius productus*). Feeding was stopped at least 48 h prior to any experimentation. All experimental procedures were approved by joint animal care committees at BMSC and the University of British Columbia (AUP A14-0251), following the guidelines outlined by the Canada Council of Animal Care (CCAC).

Experimental design

Acclimated dogfish sharks were acutely exposed to three different salinities for 96 h in total, and at the end of this period, they were exposed to progressive hypoxia under the same conditions and MO_2 was measured whilst recording ventilation rate, urea and ammonia excretion rates in a stop-flow respirometry apparatus. The fish were allowed to deplete the oxygen inside of the respirometer at their own pace, and water samples were collected at the start and end of the respirometry trial. Detailed description of the experiments is provided below.

Dogfish sharks were transferred to circular 1500-L tanks, at either 25, 30 or 36 ppt ($n=6$ per salinity), where they remained for 72 h. Average weight for the fish exposed to the different salinities were as follows (in kg): 2.12 ± 0.04 (25 ppt), 2.21 ± 0.05 (30 ppt) and 2.02 ± 0.15 (35 ppt) and not significantly different from one another. 30 ppt was chosen as a "control" salinity as it was the average salinity measured at the acclimation tank, which contained water pumped from the ocean in the vicinity of BMSC. Lower salinity (25 ppt) was achieved by diluting filtered sea water with dechlorinated tap water, and higher salinity (36 ppt) was achieved by adding Instant Ocean™ (Spectrum Brands, Blacksburg, VA, USA) sea salt to filtered sea water. Salinity and temperature were monitored using a WTW ProfiLine Cond 3310 conductivity meter (WTW, Weilheim, Germany). A 50% water change was performed at 48 h. Temperature (12–13 °C) in the tanks was maintained stable by a cooling system that consisted of meters of coils submerged at the bottom of the tanks, in which cold water was constantly running. No mortality and/or change in behaviour was observed for any of the treatments throughout the duration of the salinity exposure.

At 72 h, sharks were removed from the circular tanks, lightly anesthetized (MS-222, Syndel Labs., Parksville, BC, Canada; 0.6 g/L neutralized to pH 7.8 with 5 M NaOH) and placed on an operating table with water flowing through the gill slits constantly. A short piece of PE160 cannula (BD, Intramedic, Franklin Lakes, NJ, USA) with one flared end was inserted through the second gill slit on the right side (with the assistance of a 18G needle), secured in place by a single stitch made using suture silk (3−0, Perma-Hand Silk, Ethicon, Somerville, NJ, USA). A longer (~20 cm) piece of PE90 cannula, again flared, was inserted through the PE160 and both cannulae were glued together using acrylic adhesive (Vetbond, 3 M, Saint Paul, MN, USA). This whole procedure was performed under 5 min. As soon as the glue had set, fish were transferred to individual 40-L wooden boxes

(coated with polyurethane inside) where they remained for an additional 24 h for recovery from the procedure, resulting in a total of 96 h of acute salinity exposure prior to the onset of progressive hypoxia. Each box was constantly aerated through tubing anchored around the entire inner perimeter of the box. The boxes were placed in a 200-L wet Table (2 boxes per table), containing water at the acclimation salinity, and a recirculating pump delivered water from the wet table to the box, and overflow from the box collected in the wet table. These "dogfish boxes" are the same as those used in previous studies with dogfish sharks (Zimmer and Wood 2014; Giacomin et al. 2017, 2022). Temperature in the boxes was maintained by adding ice blocks made out of seawater at the appropriate salinity to the wet table.

At the end of the 24-h recovery period, MO_2 and ventilation rate measurements were performed for each fish. For the latter, the previously-fitted catheter was bridged to a connecting PE50 tubing using a blunt-ended #22-gauge needle. The water-filled PE50 cannula was connected to a pressure transducer (DPT-100, Utah Medical Products, Midvale, UT, USA), calibrated against a column of water. The pressure amplitude (cm H_2O) and breathing frequency (breaths/sec) were recorded throughout the respirometry trial. Without disturbing the fish, aeration was interrupted, water flow to the box was stopped, and the volume in the box was set to 35 L by briefly removing a rubber stopper located at the bottom of the box. The box was sealed with a tightly fitting floating lid to prevent O_2 diffusion from the atmosphere to the water, and PO_2 measurements were taken every 10 min through a sampling port in the lid, using a WTW Oxi 3205 oxygen meter and probe. Water circulation inside of the box was ensured by using a small recirculating pump (Repti Flo 200, Exo Terra, Mansfield, MA., USA) previously placed inside the box. The fish were allowed to consume the oxygen in the box at their own pace, while ventilation rate was continually recorded. The experiment was stopped once PO_2 in the box reached 5 Torr (170–200 min duration). Water samples (5 mL) were taken at the start of the experiment (when the box was sealed) and at the end (immediately before aeration was reintroduced to the box), and immediately frozen at – 20 °C for later analyses of ammonia and urea concentrations. The fish were quickly removed to a water bath with anesthetic, where a blood sample (2 mL) was collected via caudal puncture into a heparinized syringe, and the opercular catheter was removed. Blood samples were immediately spun (10,000 x *g*, 2 min), plasma was separated, flash frozen in liquid N_2 and kept at -70 °C until analysis. The fish were returned to the fully aerated boxes served with flow-through seawater, allowed to recover overnight, then returned to the wild.

Analytical techniques and calculations

For all the calculations in this study, 1 g of fish was assumed to be equivalent to 1 mL. The weight of the fish was subtracted from the wooden box volume to achieve the real experimental volume.

Oxygen consumption rate (MO_2) was calculated for every PO_2 interval using the following equation:

$$MO_2 = \frac{[(\Delta\, PO_2\, x\, \alpha\, O_2)\ x\, V]}{(W\, x\, T)} \quad (1)$$

where ΔPO_2 is the difference between PO_2 (Torr) at the start and at the end of the 10 min interval. αO_2 is the O_2 solubility coefficient (µmol/Torr/L) obtained from Boutilier et al. (1984) for 12 °C at 25, 30 or 36 ppt. *V* is the corrected volume of the box (L), *W* is body weight (kg) and *T* is the measurement period (h). For graphing purposes, MO_2 data were plotted at successive 10-Torr intervals.

Ventilation frequency (breaths/sec) and amplitude (cm H_2O/breath) were measured using a pressure transducer, which was calibrated against a 2-cm water column. An amplifier (LCA-RTC, Transducer Techniques, Temecula, CA, USA) was utilized to amplify the analog signal, which was then digitalized by a PowerLab Data Integrity system (ADInstruments, Colorado Springs, CO, USA), and visualized and analyzed using LabChart v. 7.0 (ADInstruments). The ventilatory index (cm H_2O/sec) was calculated by multiplying ventilation frequency and amplitude. Ventilation data were also plotted at successive 10-Torr intervals.

Net flux rates (µmol-N/kg/h) of ammonia-N and urea-N (note: 2 N-atoms per urea molecule versus 1 N-atom per ammonia molecule) were calculated using the following equations:

$$Ammonia_{net\ flux} = \frac{[(Amm_{(i)} - Amm_{(f)})\ x\, V]}{(W\, x\, T)} \quad (2)$$

$$Urea - N_{net\ flux} = \left\{ \frac{[(Urea - N_{(i)} - Urea - N_{(f)})\ x\, V]}{(W\, x\, T)} \right\} x\, 2 \quad (3)$$

where Amm(f) and Urea-N(f) are the final water ammonia (µmol/L) and urea-N (µmol/L) concentrations; Amm(i) and Urea-N(i) are the initial water ammonia (µmol/L) and urea-N (µmol/L) concentrations; *V* is volume (L), *W* is weight (kg) and *T* is the duration of the measurement period (h). Concentrations of ammonia (µmol/L) in the water were measured colorimetrically following the method outlined by Verdouw et al. (1978). Urea-N concentration in water (µmol-N/L) and plasma samples (mmol-N/L) were measured using the colorimetric method described by Rahmatullah and Boyde (1980). Plasma chloride concentration

(mmol/L) was measured using a chloridometer (Radiometer CMT10, Copenhagen, Denmark) and plasma osmolality (mOsm/kg) was measured using a vapor pressure osmometer (Wescor 5100 C, Logan, UT, USA), both calibrated with manufacturers' standards.

Data analysis

All data are shown as means ± 1 SEM. Data normality and homoscedasticity were checked before all parametric statistical analyses. Appropriate transformations (logarithmic, square root) were used in the few cases where data failed these tests. As a repeated measures design was used within each salinity group, simple pairwise comparisons between mean values (MO_2 and ventilatory parameters) in hypoxia *versus* normoxia were made using Student's two-tailed paired t-test. This was also used to compare the concentrations of osmolytes in plasma to their respective concentrations in seawater sampled simultaneously from the same box. Mean oxygen consumption and ventilatory metrics at selected PO_2 ranges (normoxic: 155 to 95 Torr; hypoxic: 45 to 5 Torr) were compared among salinities with a one-way ANOVA, and treatment differences were identified through a Tukey's post-hoc test. An ANCOVA was used to test if the slopes of linear regressions fitted to the MO_2 vs. PO_2 data were significantly different from each other. To further analyze these data, two methods were used to quantify the relationship between MO_2 and PO_2 during progressive hypoxia. The first was to calculate the traditional critical PO_2 (P_{crit}) at which MO_2 becomes dependent on environmental PO_2. At least 13 different methods have been proposed to provide the best estimate of P_{crit} (cf. Wood 2018; Marshall et al. 2013; Rogers et al. 2016). We used one of the simplest, the "greatest difference" approach, as described by Mueller and Seymour (2011). The other was to calculate the Regulation Index (RI) as introduced and again described by Mueller and Seymour (2011). We have employed both indices in the past (Giacomin et al. 2019a, b; 2020). Mean values were considered statistically different when $p < 0.05$. All statistical analyses and graphing were performed in GraphPad Prism, version 8.0.

Results

Oxygen consumption rate (MO_2) decreased with decreasing PO_2 in dogfish sharks exposed to all three salinities (Fig. 1A). On average, dogfish exposed to 25 ppt took 167.83 ± 14.33 min to consume the oxygen inside of the respirometer and reach 5 Torr, while dogfish exposed to 30 ppt took 185.83 ± 8.27 min and dogfish exposed to 36 ppt took 161.33 ± 11.36 min. However, these durations were not significantly different from one another. The shapes of the relationships varied amongst the different salinity acclimation groups (Fig. 1A). At 30 ppt, MO_2 increased during progressive hypoxia until 85 Torr was reached, after which a linear decline occurred. A 36 ppt, there was an initial increase, then a decline followed by stability between 115 and 85 Torr, followed by a linear decline thereafter. At 25 ppt, MO_2 was more or less stable down to 85 − 75 Torr, with a linear decline thereafter. Thus, at all three salinities, oxyconformation occurred below a threshold of 75–85 Torr. Linear regression lines were fitted through the MO_2 data from 85 to 5 Torr (PO_2), where MO_2 decreased proportionally to the decrease in water PO_2 (Fig. 1D). An ANCOVA was used to statistically compare the slopes of the linear regressions. At 36 ppt, there was a significantly higher slope (26.85 µmol O_2/kg/h/Torr) in comparison to 30 and 25 ppt exposed fish (19.39 and 15.67 µmol O_2/kg/h/Torr respectively) ($p = 0.0117$). Calculation of P_{crit} values on individual fish revealed no significant differences among the three salinities, but the mean values (83–97 Torr) were all relatively high (Table 1). However, regulation index (RI) values calculated on individual fish were significantly greater ($p = 0.0009$) in both the 30 ppt (RI = 0.96) and 36 ppt (RI = 0.66) treatments than in the 25 ppt treatment (RI = 0.35) (Table 1).

In normoxia (155 to 95 Torr), mean MO_2 was significantly higher by about 60% in fish exposed to 36 ppt (about 2600 µmol O_2/kg/h) in comparison to 30 ppt (1600 µmol O_2/kg/h) (Fig. 1B). At 25 ppt, MO_2 was about 1900 µmol O_2/kg/h, not significantly different from the value at 30 ppt (Fig. 1B). Mean MO_2 values during the hypoxic period (45 to 5 Torr) were not significantly different among the three exposure salinities, and for all, they were much lower than during normoxia (Fig. 1B, C).

Figure 2 (A-C) depicts the variation in ventilation parameters (A: frequency (breaths/sec); B: amplitude (cm H_2O/breath); C: ventilatory index (cm H_2O/sec)) at the three different salinities with decreasing PO_2. Panel D shows the O_2 extraction efficiency (µmol O_2/kg/cm H_2O), which was calculated as the ratio between MO_2 (Fig. 1A) and ventilatory index (Fig. 2C). Figure 3 summarizes the mean values in normoxia and hypoxia at the three salinities. Supplementary Fig S1 compares the changes in MO_2 with those in ventilatory index at the same PO_2.

With respect to salinity, under normoxia (155 to 95 Torr), ventilation frequency was significantly higher in fish exposed to 36 ppt (0.79 breaths/sec) in comparison to those at 30 ppt and 25 ppt (Figs. 2A and 3A) (0.66 and 0.61 breaths/sec for 25 and 30 ppt respectively), while during the hypoxic period (45 to 5 Torr) ventilation frequency did not differ among the three exposure salinities (Fig. 3B). Ventilation amplitude under normoxia was significantly higher in 36 ppt-exposed

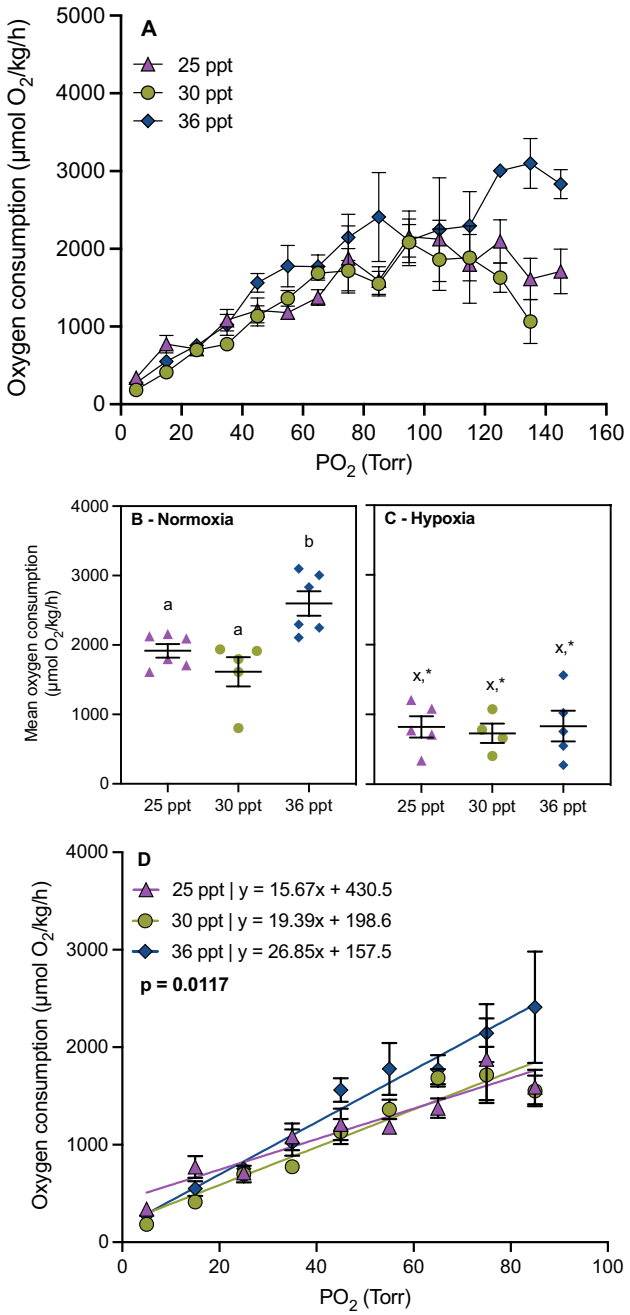

Fig. 1 Oxygen consumption rate. **A** The effect of water PO_2 (Torr) on oxygen consumption rate (MO_2, µmol O_2/kg/h) in dogfish sharks (*S. suckleyi*) exposed to 25 (purple triangles), 30 (green circles) and 36 (blue diamonds) ppt. MO_2 data are plotted in 10-Torr intervals. Symbols connected by a line are means ± SEM (n=6 for 25 and 36 ppt and n=7 for 30 ppt). Mean MO_2 calculated over the **B** normoxic PO_2 range (155 to 95 Torr) and **C** hypoxic PO_2 range (45 to 5 Torr). On panels **B** and **C**, middle lines are means ± SEM (symbols represent individual data points (n-number)). Data sharing same lower-case letters within panels B and C are not significantly different from one another. Panel B (Normoxia): p=0.0024; F=9.525; DFn=2 and DFd=14. Panel **C** (Hypoxia): p=0.0924; F=0.0924; DFn=2 and DFd=11. Asterisks indicate significant differences between MO_2 at the same salinity. Normoxia vs. Hypoxia t-test results: p=0.0135 (25 ppt); p=0.0139 (30 ppt) and p=0.0004 (36 ppt). **D** Comparison of the relationship between PO_2 and oxygen consumption between 85 to 5 Torr by linear regression. Slopes were compared through an ANCOVA (p=0.0117; F=4.588; DFn=2 and DFd=146), revealing a significantly greater slope at 36 ppt than at the other two salinities where the slopes were not different from one another

fish (2.77 cm H_2O/breath) compared to those at 30 ppt and 25 ppt where it was about 1.95 cm H_2O/breath (Figs. 2B and 3C). Under severe hypoxia (45 to 5 Torr), ventilation amplitude in 36 ppt-exposed fish was 2.92 cm H_2O/breath, significantly higher than at 25 and 30 ppt where it was about 2.45 cm H_2O/breath (Fig. 3D). Similar to the other two parameters, ventilatory index, the product of frequency and amplitude, was significantly higher in 36 ppt exposed fish (2.20 cm H_2O/sec) in normoxia, in comparison to 25 and 30 ppt fish (about 1.35 cm H_2O/sec; Fig. 3E). This elevation in ventilatory index (Figs. 2C and 3F) of about 70% corresponded well with the 60% higher MO_2 in dogfish at 36 ppt under normoxia (Fig. 1B) as illustrated in Supplementary Fig. SI). The ventilatory index under hypoxia was significantly lower in 25 ppt fish (1.35 cm H_2O/sec) and did not differ between 30 ppt and 36 ppt (1.76 and 2.15 cm H_2O/sec) (Fig. 3F). Oxygen extraction efficiency was stable through the normoxic PO_2 range (155 to 95 Torr) and decreased progressively with decreasing PO_2 (Fig. 2D).

With respect to specific ventilatory responses to hypoxia, any increases were achieved by elevations in amplitude rather than frequency. Indeed, at 36 ppt ventilatory frequency was significantly lower than in the same fish during normoxia (Fig. 3A, B). However, at 30 ppt and 25 ppt, hypoxia had no significant effect on frequency. In contrast, fish significantly increased their ventilatory amplitude during hypoxia at both 30 ppt and 25 ppt with a non-significant rise at 36 ppt (Fig. 3C, D). Ventilatory index exhibited a significant increase during hypoxia only at 30 ppt (Fig. 3E, F; Supplementary Fig. 1B).

Ammonia-N and urea-N net flux rates were measured during the exposure to progressive reductions in water PO_2 (Fig. 4A, B). Ammonia-N net flux rates were 31, 11, and 38 µmol/kg/h in 25, 30 and 36 ppt exposed fish respectively, not statistically different from each other (Fig. 4A). Urea-N net flux rate in 25 ppt fish was higher (1083 µmol/kg/h) than at the other salinities (Fig. 4B). 30 ppt exposed fish had a urea-N net flux rate of 559 µmol/kg/h (Fig. 4B) and that was not different from 36 ppt exposed fish (507 µmol/kg/h).

Plasma urea concentration (Fig. 5) increased with increasing salinity, from 308 mmol/L at 25 ppt to 390 mmol/L at 36 ppt. Plasma urea values were all different from one another (Fig. 5).

Figure 6 shows the values for plasma osmolality (A, B and C) and plasma chloride (D, E and F), plotted alongside the measured values in the water at the respective salinity. Plasma osmolality was 849 mOsm/kg at 25 ppt, 936 mOsm/

Table 1 P_{crit} and regulation index (RI) values calculated for individual dogfish sharks within each salinity group

	Acclimation Salinity		
	25 ppt	30 ppt	36 ppt
P_{crit} (Torr)	82.5 ± 10.4^{A} (6)	97.0 ± 2.2^{A} (7)	96.0 ± 5.5^{A} (6)
Regulation Index (RI)	0.34 ± 0.04^{A} (6)	0.97 ± 0.15^{B} (7)	0.66 ± 0.08^{B} (6)

P_{crit} was calculated by the "greatest difference" method. See Mueller and Seymour (2011) for calculation details. Within a parameter, means sharing the same upper-case letter are not significantly different. Data are means ± sem (n)

kg at 30 ppt and 1095 mOsm/kg at 36 ppt, all significantly different from one another (Fig. 6A, B and C). Water osmolality values ranged from 737 mOsm/kg at 25 ppt to 1086 mOsm/kg at 36 ppt. At 25 ppt, plasma osmolality was significantly higher by 15% than water osmolality, while there were no significant differences between plasma and water osmolality at 30 and 36 ppt. At 25 ppt, plasma chloride was 233 mmol/L, and that was significantly lower than plasma chloride at 30 ppt (268 mmol/L) and 36 ppt (289 mmol/L) (Fig. 6D, E and F). Water chloride values ranged from 412 mmol/L at 25 ppt to 580 mmol/L at 36 ppt. At 25 ppt, plasma chloride was 43% lower than water chloride, while at 30 and 36 ppt, plasma chloride was approximately 50% lower than water chloride (Fig. 6D, E and F). On both relative and absolute bases, the variations with decreased salinity in plasma urea concentrations (down by 23 mmol L^{-1}, or 9% at 25 ppt) were smaller than those in plasma Cl^{-} concentrations (down by 35 mmol L^{-1}, or 13% at 25 ppt). Conversely, the variations with increased salinity in plasma urea concentrations (up by 55 mmol L^{-1}, or 16% at 36 ppt)

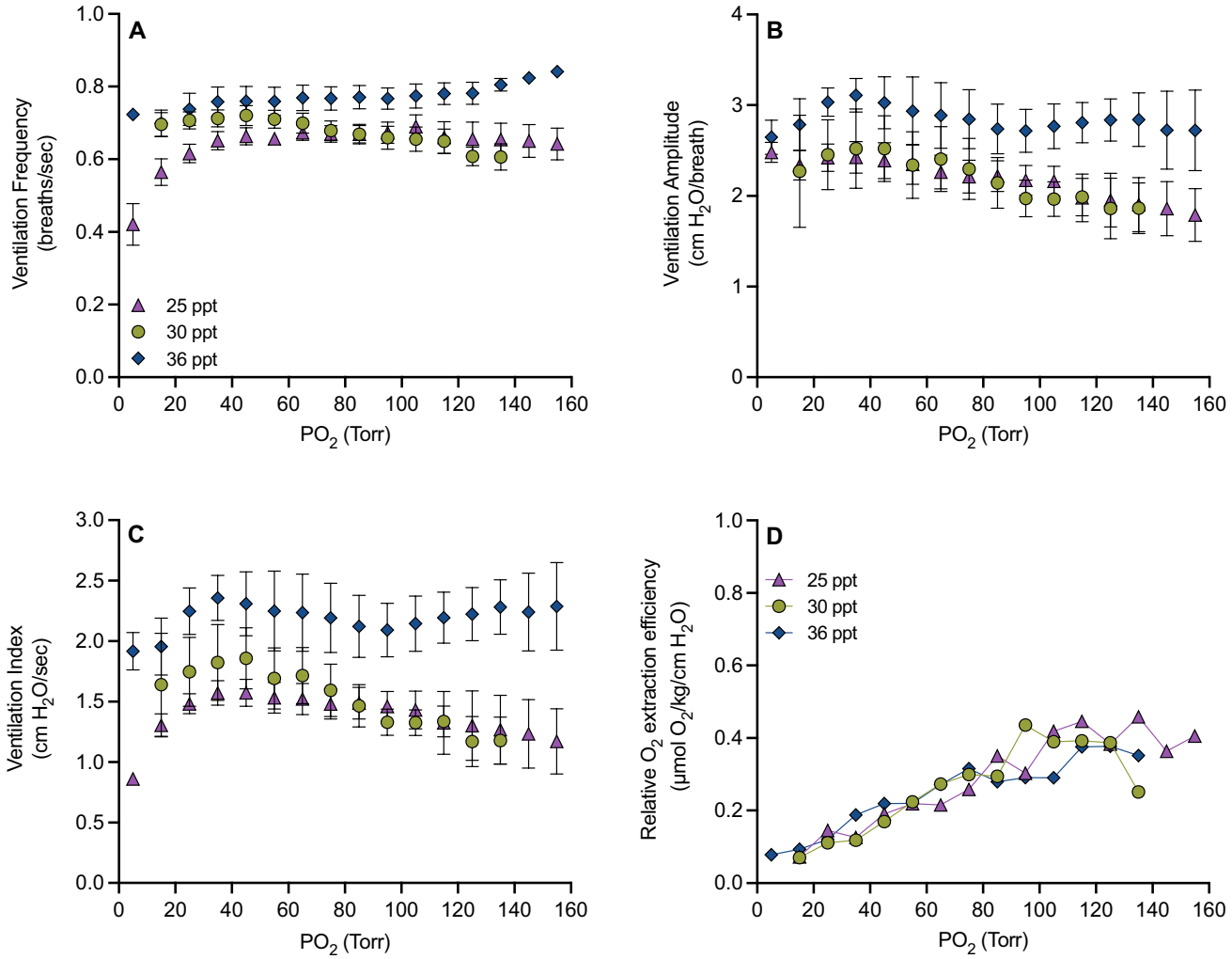

Fig. 2 Ventilation parameters and calculated O_2 extraction efficiency. The effect of water PO_2 (Torr) on **A** ventilation frequency (breaths/sec), **B** ventilation amplitude (cm H_2O/breath), **C** ventilatory index (cm H_2O/sec) and **D** relative O_2 extraction efficiency in dogfish sharks (*S. suckleyi*) exposed to 25 (purple triangles), 30 (green circles) and 36 (blue diamonds) ppt. All ventilation data are plotted in 10 Torr intervals. Symbols are means ± SEM ($n = 6$ for 25 and 36 ppt and $n = 7$ for 30 ppt). Data shown in panel **D** were calculated from the mean values in Figs. 1A and 2C, therefore no error bars are shown

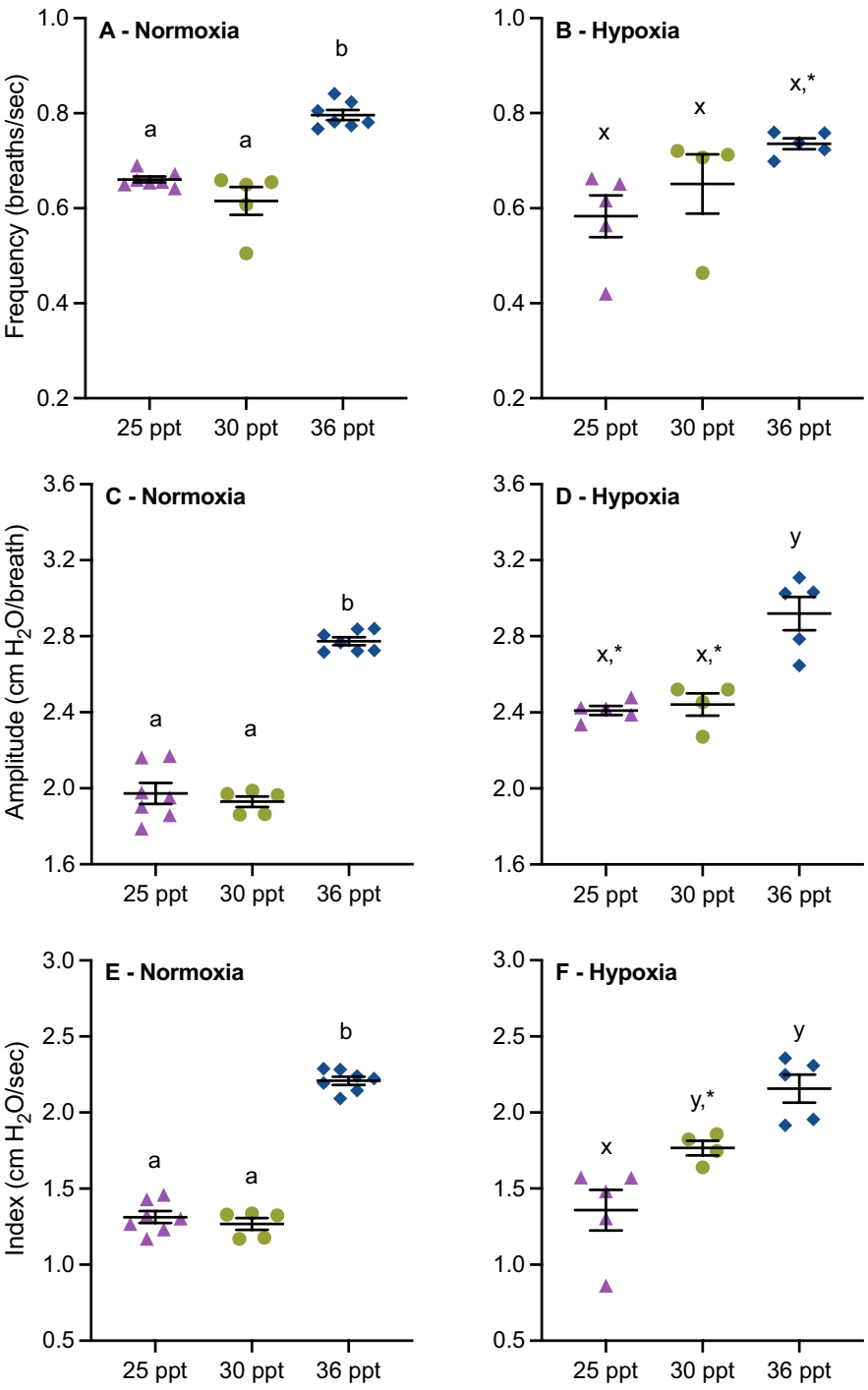

Fig. 3 Mean ventilation rate in normoxia and hypoxia. **A**, **B** Ventilation frequency (breaths/sec), **C**, **D** ventilation amplitude (cm H_2O/breath) and **E**, **F** ventilatory index (cm H_2O/sec) in dogfish sharks (*S. suckleyi*) exposed to 25 (purple triangles), 30 (green circles) and 36 (blue diamonds) ppt. Mean ventilatory parameters were calculated over the (A, C and E) normoxic PO_2 range (155 to 95 Torr) and **B**, **D** and **F** hypoxic PO_2 range (45 to 5 Torr). Data sharing same lower-case letters within each panel are not significantly different from one another. One-way ANOVA results: frequency (A – Normoxia: $p<0.0001$; F = 38.67; DFn = 2 and DFd = 16; B – Hypoxia: $p=0.0612$; F = 3.640; DFn = 2 and DFd = 11), amplitude (C – Normoxia: p = < 0.0001; F = 148.2; DFn = 2 and DFd = 16; D – Hypoxia: $p=0.0002$; F = 21.26; DFn = 2 and DFd = 11) and index (E – Normoxia: p = < 0.0001; F = 238.1; DFn = 2 and DFd = 16; F – Hypoxia: $p=0.0006$; F = 15.78 DFn = 2 and DFd = 11). Asterisks indicate significant differences between normoxic and hypoxic ventilation parameters at the same salinity. Normoxia vs. Hypoxia t-test results: frequency: $p=0.2114$ (25 ppt); $p=0.7644$ (30 ppt) and $p=0.0001$ (36 ppt); amplitude: $p=0.0002$ (25 ppt); $p=0.0087$ (30 ppt) and $p=0.2662$ (36 ppt); index: $p=0.5604$ (25 ppt); $p=0.0107$ (30 ppt) and $p=0.3261$ (36 ppt). On all panels lines are means ± SEM; symbols represent individual data points

were much greater than those in plasma Cl^- concentrations (up by 21 mmol L^{-1}, or 8% at 36 ppt).

Discussion

Our first goal was to investigate if exposure to environmentally relevant salinities would elicit changes in the metabolic rate of a moderately euryhaline elasmobranch, the Pacific spiny dogfish (*S. suckleyi*). Elasmobranchs are ureotelic osmoconformers that actively synthesize and retain urea at the gills, rectal gland, gut, and kidney; all likely involve a high metabolic cost (Kirschner, 1973; Wright and Wood 2016). As higher plasma and tissue urea levels are required to maintain osmotic homeostasis at higher salinities, we predicted that MO_2 would be higher at 36 ppt and lower at 25 ppt relative to 30 ppt. Indeed, plasma urea concentration was significantly elevated at 36 ppt and significantly depressed

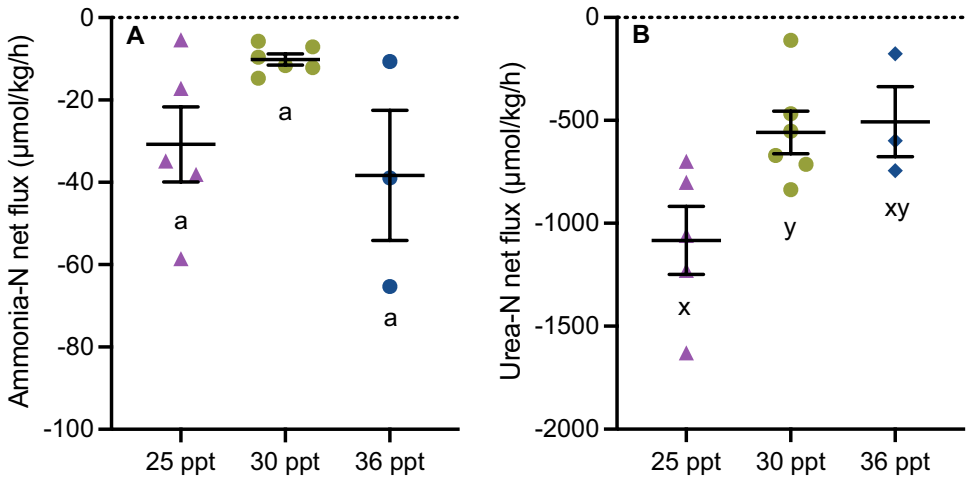

Fig. 4 Osmolyte fluxes in normoxia and hypoxia. **A** Ammonia net flux rate (µmol/kg/h) and **B** Urea-N net flux rate (µmol/kg/h) in dogfish sharks (*S. suckleyi*) exposed to 25 (purple triangles), 30 (green circles) and 36 (blue diamonds) ppt. Osmolyte fluxes were calculated over the entire PO_2 range (155 to 5 Torr). Data sharing same lower-case letters are not statistically different from one another. One-way ANOVA results: Ammonia (A) $p=0.0709$; $F=3.399$; $DFn=2$ and $DFd=11$; Urea (B) $p=0.0285$; $F=5.002$; $DFn=2$ and $DFd=11$). Horizontal lines are means ± SEM; symbols represent individual data points

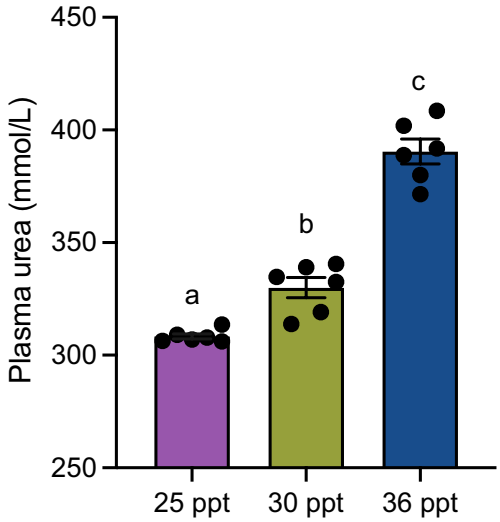

Fig. 5 Plasma urea concentration. Urea concentration (mmol/L) in the plasma of dogfish sharks (*S. suckleyi*) exposed to 25 (purple bars), 30 (green bars) and 36 (blue bars) ppt. Plasma samples were collected at the end of the respirometry trial. Different lower-case letters indicate significant differences among salinity groups. One-way ANOVA results: $p<0.0001$; $F=103.8$; $DFn=2$; $DFd=15$. Data are means ± SEM; symbols represent individual data points ($n=6$)

at 25 ppt (Fig. 5), in accord with the findings of Deck et al. (2016) and Guffey and Goss (2014) in the same species. However, our prediction with respect to metabolic rate was only partially confirmed. MO_2 was significantly elevated by about 60% at 36 ppt under normoxia in accord with our prediction, but was not depressed at 25 ppt (Fig. 1B). The latter contrasts with Guffey and Goss (2014), who reported an approximate 20% drop in MO_2 in *S. suckleyi* exposed to 21 ppt. The explanation for this difference may lie in the more severe hyposalinity (21 ppt *versus* 25 ppt) and the accompanying 2.5-fold greater decrease in plasma urea concentration and 1.5-fold higher urea loss rates reported by Guffey and Goss (2014) relative to the present study (Figs. 4B and 5). Note, however, that urea-N loss rates were measured over a single flux period of progressive hypoxia in the present study, so may not be directly comparable to those reported by Guffey and Goss (2014). An additional note of caution is that we cannot be sure that the sharks were fully acclimated to the elevated or reduced salinity conditions after 96 h of exposure. Nevertheless, 96 h is an environmentally relevant duration for sharks entering hypersaline lagoons or hyposaline estuaries for feeding forays. Overall, our data support the conclusion that metabolic costs of urea-based osmoregulation are substantial, especially when salinity is elevated.

Ventilation is thought to be an energetically demanding process in fishes (Cameron and Cech 1970; Perry et al. 2009) owing to the high viscosity and low oxygen capacitance of the water (Dejours 1988), accounting for 10–20% of the resting metabolic rate in teleost fishes (Wood 2018). Our second goal was to test the prediction that disturbances in O_2 consumption and ventilation during hypoxia would be reduced at lower salinity and elevated at higher salinity. Our actual results were more nuanced. Although there were no significant differences in P_{crit} (Table 1), the patterns of MO_2 response to progressive hypoxia differed significantly among salinities, as captured in the ANCOVA (Fig. 1D) and RI analyses (Table 1). At the 30 ppt, the pattern resembled the type B response of Wood (2018), where MO_2 first increased (yielding the high RI) and then decreased in a linear fashion (oxyconformation) as hypoxia became more intense (Fig. 1A). At 36 ppt, the pattern was midway

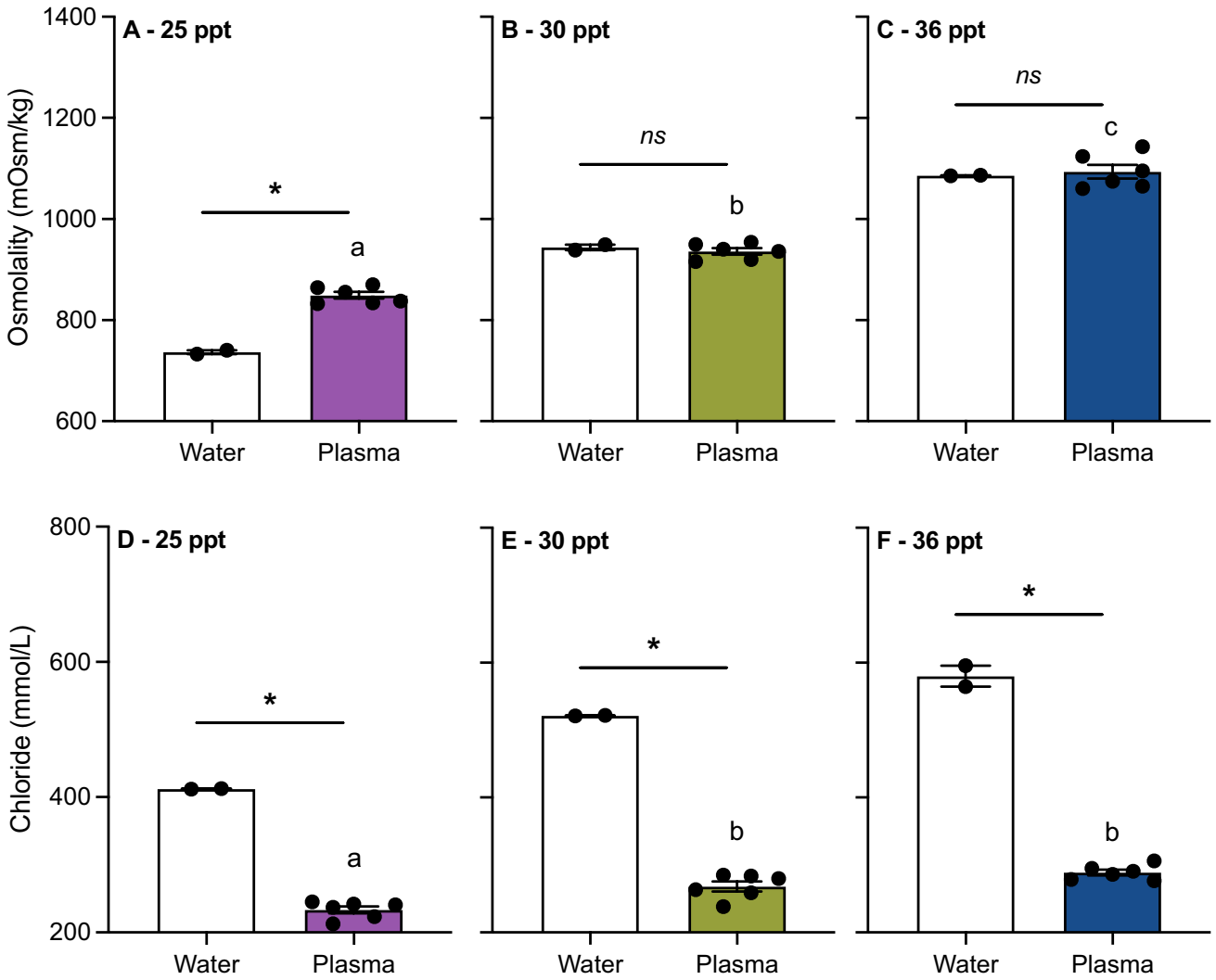

Fig. 6 Plasma and water osmolality and chloride concentrations. **A**, **B**, **C** Water (white bars) and plasma (filled bars) osmolality (mOsm/kg) and **D**, **E**, **F** water (white bars) and plasma (filled bars) chloride concentration (mmol/L) in dogfish sharks (*S. suckleyi*) exposed to 25 (purple bars), 30 (green bars) and 36 (blue bars) ppt. Plasma samples were collected at the end of the respirometry trial. Bars sharing the same lower-case letters are not significantly different. One-way ANOVA results: Osmolality $p<0.0001$; $F=168.3$; $DFn=2$ and $DFd=15$; Chloride $p<0.0001$; $F=22.68$; $DFn=2$ and $DFd=1$). Data are means ± SEM; symbols represent individual data points ($n=6$). Plasma osmolality and chloride at each exposure salinity were compared to their respective concentrations in water through a two-tailed, unpaired t-test. Asterisks indicate significant differences, while *ns* means not significantly different. Water vs. plasma t-test results: osmolality: $p<0.0001$ (25 ppt); $p=0.5345$ (30 ppt) and $p=0.7728$ (36 ppt); chloride: $p<0.0001$ (25 ppt); $p<0.0001$ (30 ppt) and $p<0.0001$ (36 ppt)

between type B and type D responses of Wood (2018) with a small increase and two slope changes prior to oxyconformation (Fig. 1A), yielding an intermediate RI. The slope of the oxyconformation line at 36 ppt, which started at a higher MO_2, was higher than at the two lower salinities (Fig. 1D). Only at 25 ppt, did the data fit the classic P_{crit} pattern proposed by Fry (1947) with relatively constant MO_2 until the slope changed abruptly to oxyconformation, yielding a low RI (Fig. 1A). A simple interpretation is that at 30 ppt, the dogfish exerted extra work in an attempt to maintain MO_2 during progressive hypoxia; part of this work would be in increased ventilation. In this regard, ventilatory index increased significantly only at 30 ppt (Fig. 3E, F). At 36 ppt, the dogfish were already doing a great deal of extra work (greater urea synthesis and retention, greater ventilation), as reflected in the 60% greater MO_2 (Fig. 1B) and 70% greater ventilatory index under normoxia (Fig. 3E) so they had little scope for doing more work to cope with hypoxia. The ventilatory index did not increase (Fig. 3E, F) though ventilation may have become more efficient, as outlined below. At 25 ppt, although MO_2 (Fig. 1B) and ventilatory index (Fig. 3E) were not lower than at 30 ppt during normoxia, the ventilation index was lower during hypoxia than at the other

two salinities (Fig. 3F), so ventilatory costs were likely also lower.

Our third goal was to evaluate our prediction, based on energetic considerations (Shelton et al. 1986; Perry and Wood 1989), that increases in ventilatory amplitude would make a larger contribution than increases in frequency during hypoxia. Our results directly support this prediction. Frequency did not increase significantly at either 25 ppt or 30 ppt, and actually decreased significantly at 36 ppt during hypoxia (Figs. 2A and 3A and B). In contrast, amplitude increased significantly at 25 ppt and 30 ppt, and non-significantly at 36 ppt. At this high salinity where ventilation was already greatly elevated under normoxia, the greater reliance on amplitude than on frequency may have been a strategy for increasing efficiency while maintaining high ventilation.

Unfortunately, this clear result is not apparent in all studies. Both Perry and Gilmour (1996) and Acharya-Patel et al. (2018), although using different time courses, reported increases in both frequency and amplitude in response to hypoxia in *S. suckleyi* at 30 ppt. De Boeck et al. (2024), is the only previous study to combine measurements of MO_2 and ventilation during hypoxia in this species (also performed at 30 ppt). These authors reported considerable inter-animal variability, but their overall results were in broad agreement with the present results. In both studies, oxyconformation predominated below about 80 Torr (Fig. 1A, D), and the ventilation index increased during hypoxia (Figs. 2C and 3C; Supplementary Fig. S1B), peaking at about 45 Torr. However, a marked difference was that in De Boeck et al. (2024), this was achieved by a modest increase in ventilatory frequency with only a non-significant rise in amplitude. The reason(s) for these discrepancies are unknown. Perhaps the degree of instrumentation, and therefore the extent of stress in the animals is an important factor. Future hypoxia investigations on free swimming *S. suckleyi* with minimal instrumentation, such as the early visual study of Metcalfe and Butler (1984), updated with modern technology, may prove informative in this matter.

Our final goal was to test our hypothesis, based on energetic grounds, that greater decreases in plasma urea than in plasma Cl^- concentration would occur at lowered salinity, while greater increases in plasma Cl^- than in urea concentration would occur at elevated salinity. In fact, exactly the opposite occurred. Increases of plasma urea dominated over increases in plasma Cl^- at 36 ppt while decreases in plasma Cl^- dominated over decreases in plasma urea concentrations at 25 ppt (Figs. 5 and 6). These observations agreed with those of Deck et al. (2016) from short term exposures of *S. suckleyi* to more extreme increases and decreases in salinity, but not with the report of Duffey and Goss (2014) where urea loss predominated over Cl^- loss during 4.5-day at 21 ppt. Indeed, while there is little prior information on responses to hypersalinity, the literature in general shows that "dilution tolerators" tend to conserve ions to a greater extent than urea during exposure to hyposalinity (reviewed by Ballantyne and Fraser 2013; Wright and Wood 2016). The exceptions in *S. suckleyi* in the present study and in Deck et al. (2016) may relate to more modest salinity challenges and shorter exposure times respectively. Alternately or additionally, they may relate to trade-offs between Cl^- and urea in the regulation of blood oxygenation, as both factors are known to affect blood O_2 affinity in elasmobranchs (reviewed by Morrison et al. 2016).

To conclude, although marine elasmobranchs are often considered to be osmoconformers, there is accumulating evidence that they exhibit the osmorespiratory compromise. Much of the compromise seems to be focussed on balancing the needs for urea retention against those for O_2 uptake. This is based on challenges of *S. suckleyi* with hypoxia (Zimmer and Wood 2014) temperature (Giacomin et al. 2017), exercise (Giacomin et al. 2017), and the present study with both hypoxia and salinity. While our focus in the present study has been on the gills, we cannot eliminate the possibility that the osmorespiratory compromise in elasmobranchs may, in part, also be due to energetic trade-offs at other important sites of urea-N retention in such as gut, kidney, and rectal gland (reviewed by Wright and Wood 2016) as well as with the biosynthetic costs of urea-N production (Kirschner 1993). These possibilities should be explored in future studies. Relative to the extensive knowledge base on the osmorespiratory compromise in teleosts (e.g. Randall et al. 1972; Gonzalez and McDonald 1992; Gilmour and Perry 2018; Wood and Eom 2021), we have a lot left to learn in elasmobranchs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00360-025-01629-w.

Acknowledgements Supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants to CMW (RGPIN-2023-03714, RGPIN-2017-03843 and RGPIN/473–2012) and PMS (RGPIN-2017-04613) and a Canada Research Chair (CRC-2021-00040) to PMS). M.G. was supported by a 4-year graduate fellowship from the University of British Columbia. We thank Dr. Eric Clelland (Bamfield Marine Sciences Centre research coordinator at the time) and the BMSC research and animal care staff for excellent support. The authors thank Chelsea Greer for invaluable assistance with ventilation data analysis and Dr. Junho Eom for providing training on shark surgical procedures and ventilation data collection. Dr. Renata Giacomin is acknowledged for her support with statistical analysis for the revised manuscript.

References

Acharya-Patel N, Deck CA, Milsom WK (2018) Cardiorespiratory interactions in the Pacific spiny dogfish, *Squalus suckleyi*. J Exp Biol 221:jeb183830

Ballantyne JS (1997) Jaws: the inside story—the metabolism of elasmobranch fishes. Comp Biochem Physiol B 118:703–742

Ballantyne JS, Fraser DI (2013) Euryhaline elasmobranchs. In: McCormick SD, Farrell AP, Brauner CJ (eds) Fish physiology. Euryhalinity, Academic Press, San Diego, pp 125–198

Boutilier RG, Heming TA, Iwama GK (1984) Appendix: physicochemical parameters for use in fish respiratory physiology. In: Hoar WS, Randall DJ (eds) Fish physiology. Gills. Academic, Orlando, pp 403–430

Breitburg D, Levin LA, Oschlies A, Grégoire M, Chavez FP, Conley DJ, Garçon V, Gilbert D, Gutiérrez D, Isensee K, Jacinto GS, Limburg KE, Montes I, Naqvi SWA, Pitcher GC, Rabalais NN, Roman MR, Rose KA, Seibel BA, Telszewski M, Yasuhara M, Zhang J (2018) Declining oxygen in the global ocean and coastal waters. Science 359:eaam7240

Butler PJ, Taylor EW (1975) The effect of progressive hypoxia on respiration in the dogfish (*Scyliorhinus canicula*) at different seasonal temperatures. J Exp Biol 63:117–130

Butler PJ, Taylor EW, Davidson W (1979) The effect of long term, moderate hypoxia on acid-base balance, plasma catecholamines and possible anaerobic end products in the unrestrained dogfish *Scyliorhinus canicula*. J Comp Physiol 132:297–303

Cameron JN, Cech JJ (1970) Notes on the energy cost of gill ventilation in teleosts. Comp Biochem Physiol 34:447–455. https://doi.org/10.1016/0010-406X(70)90184-2

De Boeck G, Lardon I, Eyckmans M, Vu TN, Laukens K, Dommisse R, Wood CM (2024) Spiny dogfish, *Squalus suckleyi*, shows a good tolerance for hypoxia but need long recovery times. Consv Physiol 12(1). https://doi.org/10.1093/conphys/coae054

Deck CA, Bockus AB, Seibel BA, Walsh PJ (2016) Effects of short-term hyper- and hypo-osmotic exposure on the osmoregulatory strategy of unfed North Pacific spiny dogfish (*Squalus suckleyi*). Comp Biochem Physiol A 193:29–35

Dejours P (1981) Principles of comparative respiratory physiology. Elsevier, Amsterdam

Dejours P (1988) Respiration in water and air: adaptation-regulation-evolution. Elsevier, Amsterdam

Diáz R, Breitburg DL (2011) The expanding hypoxic environment. In: Farrell AP (ed) Encyclopedia of fish physiology. Academic, San Diego, pp 1746–1750

Esbaugh AJ (2024) Physiological responses of Euryhaline marine fish to naturally-occurring hypersalinity. Comp Biochem Physiol A :111768

Farrell AP, Richards JG (2009) An integrative synthesis of the responses of fish to hypoxia. In: Richards JG, Farrell AP, Brauner CJ (eds) Hypoxia. Fish physiology. Elsevier Academic, San Diego, pp 448–503

Ficke AD, Myrick CA, Hansen LJ (2007) Potential impacts of global climate change on freshwater fisheries. Rev Fish Biol Fish 17:581–613. https://doi.org/10.1007/s11160-007-9059-5

Fry FEJ (1947) Effects of the environment on animal activity. Publications of the Ontario fisheries research laboratory. Biological Series, University of Toronto, Toronto

Giacomin M, Schulte PM, Wood CM (2017) Differential effects of temperature on oxygen consumption and branchial fluxes of urea, ammonia, and water in the dogfish shark (*Squalus acanthias suckleyi*). Physiol Biochem Zool 90:694296

Giacomin M, Dal Pont G, Eom J, Schulte PM, Wood CM (2019a) The effects of salinity and hypoxia exposure on oxygen consumption, ventilation, diffusive water exchange and ionoregulation in the Pacific hagfish (*Eptatretus stoutii*). Comp Biochem Physiol A 232:47–59

Giacomin M, Bryant HJ, Val AL, Schulte PM, Wood CM (2019b) The osmorespiratory compromise: physiological responses and tolerance to hypoxia are affected by salinity acclimation in the Euryhaline Atlantic killifish (*Fundulus heteroclitus*). J Exp Biol 222:jeb206599. https://doi.org/10.1242/jeb.206599

Giacomin M, Onukwufor JO, Schulte PM, Wood CM (2020) Ionoregulatory aspects of the hypoxia-induced osmorespiratory compromise in the Euryhaline Atlantic killifish (*Fundulus heteroclitus*): the effects of salinity. J Exp Biol 223:jeb216309

Giacomin M, Schulte PM, Wood CM (2022) Osmorespiratory compromise in an elasmobranch: oxygen consumption, ventilation and nitrogen metabolism during recovery from exhaustive exercise in dogfish sharks (*Squalus suckleyi*). J Comp Physiol B 192:647–657

Gilmour KM, Perry SF (2018) Conflict and compromise: using reversible remodeling to manage competing physiological demands at the fish gill. Physiology 33:412–422

Gonzalez RJ, McDonald DG (1992) The relationship between oxygen consumption and ion loss in a freshwater fish. J Exp Biol 163:317–332

Guffey SC, Goss CG (2014) Time course of the acute response of the North Pacific spiny dogfish shark (*Squalus suckleyi*) to low salinity. Comp Biochem Physiol A 171:9–15

Intergovernmental Oceanographic Commission (2018) The ocean is losing its breath: declining oxygen in the world's ocean and coastal waters. Breitburg D, Gregoire M, Isensee K, (eds) IOC Technical Series No. 137 (IOC/2018/TS/137)

Kirschner LB (1993) The energetics of osmotic regulation in ureotelic and hypoosmotic fishes. J Exp Zool 267:19–26

Marshall DJ, Bode M, White CR (2013) Estimating physiological tolerances—a comparison of traditional approaches to nonlinear regression techniques. J Exp Biol 216:2176–2182

McMillan D, Morse W (1999) Essential fish habitat source document: spiny dogfish, *Squalus acanthias*, life history and habitat characteristics, vol 150. NOAA Technical Memorandum NMFS-NE-, pp 1–28

Metcalfe JD, Butler PJ (1984) Changes in activity and ventilation in response to hypoxia in unrestrained, unoperated dogfish (*Scyliorhinus canicula L*). J Exp Biol 108:411–418

Morrison PR, Gilmour KM, Brauner CJ (2016) Oxygen and carbon dioxide transport in elasmobranchs. In: Shadwick RE, Farrell AP, Brauner CJ (eds) Fish physiology. Physiology of elasmobranch fishes: internal processes. Academic, San Diego, pp 127–219

Mueller CA, Seymour RS (2011) The regulation index: a new method for assessing the relationship between oxygen consumption and environmental oxygen. Physiol Biochem Zool 84:522–532

Perry SF, Gilmour KM (1996) Consequences of catecholamine release on ventilation and blood oxygen transport during hypoxia and hypercapnia in an elasmobranch (*Squalus acanthias*) and a teleost (*Oncorhynchus mykiss*). J Exp Biol 199:2015–2118

Perry SF, Wood CM (1989) Control and coordination of gas transfer in fishes. Can J Zool 67:2961–2970

Perry SF, Jonz MG, Gilmour KM (2009) Oxygen sensing and the hypoxic ventilatory response. In: Richards JG, Farrell AP, Brauner CJ (eds) Hypoxia. Fish physiology. Elsevier Academic, San Diego, pp 193–253

Piiper J, Meyer M, Worth H, Willmer H (1977) Respiration and circulation during swimming activity in the dogfish *Scyliorhinus stellaris*. Respir Physiol 30:221–239

Rahmatullah M, Boyde TRC (1980) Improvements in the determination of Urea using diacetyl monoxime; methods with and without deproteinisation. Clin Chim Acta 107:3–9

Randall DJ, Baumgarten D, Malyusz M (1972) The relationship between gas and ion transfer across the gills of fishes. Comp Biochem Physiol A 41:629–637

Richards JM (2004) Distribution and life history of the spiny dogfish (*Squalus acanthias*) off the Oregon and Washington coasts. M.Sc. Thesis. Oregon State University (2004)

Rogers NJ, Urbina MA, Reardon EE, McKenzie DJ, Wilson RW (2016) A new analysis of hypoxia tolerance in fishes using a database of critical oxygen level (P_{crit}). Conserv Physiol 4:cow012

Shelton G, Jones DR, Milsom WK (1986) Control of breathing in ectothermic vertebrates. In: Fishman AP, Cherniak NS, Widdicombe JG, Geiger SR (eds) Handbook of physiology. The respiratory system. Control of breathing, vol 2. American Physiological Society, Bethesda, pp 857–909

Smith H (1931) The absorption and excretion of water and salts by the elasmobranch fishes. II marine elasmobranchs. Am J Physiol 98:296–310

Smith H (1936) The retention and physiological role of Urea in the elasmobranchii. Biol Rev 11:48–82

Speers-Roesch B, Richards JG, Brauner CJ, Farrell AP, Hickey AJR, Wang YS, Renshaw GMC (2012) Hypoxia tolerance in elasmobranchs. I. Critical oxygen tension as a measure of blood oxygen transport during hypoxia exposure. J Exp Biol 215:93–102

Verdouw H, van Echteld CJA, Dekkers EMJ (1978) Ammonia determination based on indophenol formation with sodium salicylate. Water Res 12:399–402

Weinrauch AM, Folkerts EJ, Blewett TA, Bucking C, Anderson WG (2020) Impacts of low salinity exposure and antibiotic application on gut transport activity in the Pacific spiny dogfish, *Squalus acanthias suckleyi*. J Comp Physiol B 190(5):535–545

Wood CM (2018) The fallacy of the P_{crit} are there more useful alternatives? J Exp Biol 221:jeb163717

Wood CM, Eom J (2021) The osmorespiratory compromise in the fish gill. Comp Biochem Physiol A 254:110895

Wright PA, Wood CM (2016) Regulation of ions, acid-base, and nitrogenous wastes in elasmobranchs. In: Shadwick RE, Farrell AP, Brauner CJ (eds) Fish physiology. Physiology of elasmobranch fishes: internal processes. Academic, San Diego, pp 279–345

Zimmer AM, Wood CM (2014) Exposure to acute severe hypoxia leads to increased Urea loss and disruptions in acid-base and ionoregulatory balance in dogfish sharks (*Squalus acanthias*). Physiol Biochem Zool 87:623–639