

The physiological status and mortality associated with otter-trawl capture, transport, and captivity of an exploited elasmobranch, *Squalus acanthias*

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To assess the physiological responses and associated mortality in spiny dogfish (*Squalus acanthias*) following capture in an otter trawl and exposure to additional conditions, blood samples were obtained subsequent to three sampling intervals: capture (T1), transport (T2), and captivity (T3). The results indicate that marked differences existed in blood chemistry at each sampling interval. Acid–base parameters (vascular pH, pO_2 , pCO_2), serum Ca^{2+} and Cl^- , and haematocrit were maximally disrupted at T1, but progressively resolved to presumed basal values by T3. Concentrations of whole-blood lactate, plasma total protein, additional sera electrolytes (Na^+ , K^+ , Mg^{2+}), and BUN (urea) were maximally compromised at T2, but also recovered by T3. In contrast, serum glucose levels were similar at T1 and T2 but rose to peak levels by T3. Although blood parameters were substantially altered, dogfish mortality was low (2 out of 34; 5.9%), suggesting a strong degree of resilience to compounded stressors associated with capture, transport, and captivity.

Keywords: blood chemistry, captivity, mortality, spiny dogfish, stress, transport, trawl.

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Introduction

The spiny dogfish (*Squalus acanthias*) is a coastal squaloid with a range extending from Labrador to Florida in the western Atlantic (Sosebee, 2000) and a circumboreal global distribution (Nammack *et al.*, 1985). Like most elasmobranchs, dogfish exhibit K-selected life history characteristics, which include slow growth, late maturity, and low fecundity (Nammack *et al.*, 1985; ASMFC, 2002). Female dogfish also display a prolonged (18–22 month) period of gestation and when a directed fishery exists for the species, are selected over males as a function of their larger maximum body sizes at maturity (Sosebee, 2000). Primarily because of these factors, heightened fishing pressure in the western North Atlantic during recent decades led to a reported 75% decline in mature female stocks between 1998 and 2003, and a concomitant scarcity in recruitment (ASMFC, 2002; NEFSC, 2003). Incidental capture of dogfish is also extensive. Low trip limits and limited commercial value have led to the discarding of consistently large quantities of dogfish in western North Atlantic commercial fisheries. Therefore, post-capture condition and discard survivability of dogfish hold major implications regarding stock health and associated management. Augmenting the assessment of capture stress with an investigation of transport and captivity can provide insight regarding a species' capability to recover following a particular form of capture and a variety of additional stressors, an important factor when assessing how resilient populations are when captured and discarded as bycatch.

Although many studies have investigated the physiological responses to capture, handling, transport and confinement

stressors either individually or collectively in teleosts (e.g. Barton *et al.*, 2003; Sulikowski and Howell, 2003), fewer have done so in elasmobranchs (Cliff and Thurman, 1984; Torres *et al.*, 1986; Smith, 1992). Moreover, no investigation to date has addressed the physiological threshold of dogfish related to the rigours of catch and release, and to our knowledge, no study has documented the post-capture physiological implications of mobile-fishing capture in an elasmobranch. In order to gain greater understanding of physiological stress responses and the resilience of dogfish, a sample of trawl-captured dogfish was transported, held captive for 30 d, and assessed for physiological status and mortality following the completion of each study phase.

Material and methods

Animal collection, transport, and holding in tanks

Dogfish were caught in six, 45 min, moderately packed (~270–300 kg) otter trawls during two consecutive days (2 and 3 September 2004) southeast of Chatham Inlet (41°38'N 69°48'W) aboard the commercial fishing vessel "Joanne A III" (Chatham, MA, USA). A 350 hp, semi-high-rise Danish otter trawl containing 302 meshes in the fishing circle and a 15.2 cm mesh was used. The net also possessed 15.0 fathom top and bottom legs and 20.0 fathoms of ground cable. The trawl doors weighed 454 kg. A hard-bottom sweep on the bosom section was utilized to avoid boulders. The depth of trawling ranged between 50 and 65 m on a cobble and sand seabed, with 13.0–14.0°C bottom-water temperature. The total length (TL) of dogfish utilized in the study

ranged from 69 to 87 cm, with the majority at 72–84 cm, and they were primarily female (85%). Once the catch was deposited on deck following each tow, individual dogfish were randomly selected for one of the treatments outlined below.

Dogfish to undergo transport and captivity

Dogfish ($n = 34$ over the 2 d of study) were randomly selected for transport and placed in one of two on-deck, square-holding pens measuring: (i) 1.2 m \times 0.9 m and 1.4 m deep, with an \sim 700 l seawater capacity or (ii) 1.85 m \times 1.1 m and 0.9 m deep, with an \sim 1000 l capacity. Because the dogfish were captured across six tows, the pens never housed more than seven at a time. Once the targeted number ($n = 17$) of dogfish was obtained each day, the ship steamed back to port (1 h). During the steam, the tanks were continuously flushed with ambient surface seawater (\sim 16.0–17.0°C) through the vessel's deck hose. The dissolved oxygen saturation (DO) was maintained in a range between 90 and 104 mg l⁻¹.

Post-capture physiology (T1)

At the same time as individual dogfish were being placed in the on-deck pens, others ($n = 33$) randomly selected from the same tows were bled to gain physiological indices of post-capture status [time T1 post-capture]. Blood samples (\sim 5.0 ml) were obtained by caudal venipuncture, a method described as relatively non-stressful for elasmobranchs (Cooper and Morris, 1998). Each time a catch was hauled and deposited on deck, dogfish were gently picked up, their blood immediately sampled, their TL measured, and then immediately returned to the sea. To ensure that periods of exposure were similar, several phlebotomists worked simultaneously, and the post-capture intervals in which dogfish were placed in pens ranged between 6 and 10 min following the end of a tow.

Transport component (T2)

Land transport to the captive facility was conducted using a New England Aquarium (NEAq) Fishes Department (Boston, MA, USA) transport truck that awaited the return of the vessel. On reaching port on each of the 2 d, harbour water obtained by the deck hose was used to fill the 2650 l capacity of the 2.0 m diameter, 1.1 m deep, circular holding tank on the truck. Dogfish were gently manipulated into flexible vinyl stretchers which had been immersed in the on-deck holding pens, then carried in a horizontal position to the waiting truck according to the methods described in Smith *et al.* (2004). The transfers never exceeded 15 s for any one individual. The trip to the captive facility began once the final dogfish had been placed in the truck's tank. The water temperature was maintained at 16.5°C (\pm 0.7°C) on the first transport day. Unusually high harbour water temperatures resulted in a comparatively elevated tank temperature on the second day (18.4–21.0°C). By manipulating the delivery of pure oxygen to the seawater via air stones, DO levels of \sim 105–115 mg l⁻¹ were maintained in accordance with previously successful short-distance transport of the sandtiger shark, *Carcharias taurus* (H. Martel-Bourbon, pers. comm.). Ammonia levels were negligible during all aspects of the transport phase. The time between the placement and removal of dogfish from the truck tanks was approximately 2 h each day. On arrival at the Marine Biological Laboratory (MBL, Woods Hole, MA, USA), the dogfish were transported from the truck tanks to a particular captive holding tank, using the same methods as previously described,

with each of the two transport days associated with its own captive tank. Prior to tank placement, the blood of the dogfish was sampled [time T2 post-transport] to allow temporal analyses of any physiological changes that might occur over the next 30 d. The dogfish were also tagged to ensure identification in the cases of mortality and subsequent phlebotomy (T-bar vinyl anchor tags; Floy Tag Inc.).

Captive component (T3)

In all, 34 dogfish were held for 30 d at the Marine Resources Centre (MRC), with 17 specimens in each of two circular holding tanks, 3.0 m diameter \times 0.9 m deep and 3.7 m diameter \times 0.8 m deep, respectively. Using a flow-through, treated-water system, seawater was pumped in, filtered through a rapid-rate sand-filter (to \sim 0.03 mm), chilled via titanium plate and frame heat exchangers, and piped into the tanks where seawater was maintained at \sim 13–14°C. Water quality monitoring was conducted by the facility, and the tanks possessed negligible (\sim 0 PPM) levels of ammonia, nitrite, and nitrate throughout. The feeding regime was maintained at 2 l of squid/capelin per tank, three times a week *ad libitum*. Although the species possesses relatively depressed metabolic rates (Brett and Blackburn, 1978), the dogfish began feeding on day 1. During the 30 d, the investigators conducted weekly checks to monitor status, behaviour and potential fatalities. After 30 d, the free-swimming dogfish were rapidly removed from the holding tanks by hand and, within 15 s, blood was sampled for a final time [time T3 post-captivity].

Blood sampling and processing

Phlebotomy was conducted using Fisher non-heparinized, 18–20 gauge, 0.04 m, stainless steel, syringe needles fitted to 5.0 ml plastic syringes (Becton, Dickinson and Co., Franklin Lakes, NJ, USA). On drawing a sample, the needles were immediately plugged with cork (to slow diffusive gas loss), and 20 μ l of whole blood was immediately deposited into a CG4+ cartridge (Heska Corporation, Fort Collins, CO, USA) from which vascular (blood) pH (pH_v) and the partial pressures of oxygen [pO_2 (mmHg⁻¹)] and carbon dioxide [pCO_2 (mmHg⁻¹)] were calculated by i-STAT portable clinical analysers (Heska Corporation) in duplicate. Whole-blood pH and blood gas readings were derived using clinical analysers temperature-calibrated for assessing mammalian rather than ectothermal blood. Thus, caution should be employed if interpreting these data for absolute values.

The remaining blood sample ($4.5 \text{ ml} \leq x \text{ ml} \leq 5.0 \text{ ml}$) was then evenly distributed between three types of vacutainer tube (\sim 1.6 ml blood per tube): (i) lithium heparin coated; (ii) dried EDTA coated, and (iii) non-heparinized. Microhaematocrit tubes were filled in triplicate with whole blood via capillary action from (i), packed with haematocrit, and stored on ice (Biron and Benfey, 1994) for subsequent on-site analysis of whole-blood haematocrit. To deproteinate blood from (ii), 0.5 ml of whole blood was added to 1.0 ml of ice-cold 8% perchloric acid and kept on ice for \sim 45 min to ensure complete digestion and protein denaturation. The remainder of (i) and the perchloric acid solution in (ii) were then stored on ice, whereas (iii) was left to clot at room temperature (60 min), until later processed to obtain the following: plasma from (i), perchloric acid extracts of the haemolysed whole blood from (ii), and serum from (iii). All references to individual blood parameters in the text correspond to their concentrations in those respective media.

Anti-coagulated whole blood

To determine haematocrit, the microhaematocrit tubes were removed from ice within 1 h and spun in triplicate at 8000 g for 3 min. Percentage values were determined (in triplicate) against the scale of a standard microhaematocrit reader.

Spectrophotometry

Total soluble protein concentration was obtained from plasma. The “green-top” tubes (i) containing ~1.25 ml of whole blood were spun at 1400 g for 5 min. The supernatant (~0.75 ml of plasma) was aliquoted into Fisher cryovial tubes and transported in liquid nitrogen to freezers at -80.0°C until assay. Preserved samples were processed in triplicate using a BCA protein assay reagent kit (23225; Pierce Biotechnology Inc., Rockford, IL, USA) and run in triplicate at wavelength of 562 nm on a Molecular Devices (Emax Precision) microplate reader to obtain optical densities (ODs). Final values were obtained by integrating sample ODs into the linear equations derived by the values associated with a concentration range of the kit’s albumin (2.0 mg ml^{-1} solution) standard (standard curve).

The lactate anion concentrations were obtained from perchloric acid extracts of the haemolysed whole blood, which for our purposes was designated as “whole blood”. The “purple top” tubes (ii) were centrifuged at 3000 g for 10 min to pellet cellular debris and protein. The supernatant (~1.0 ml of whole blood) was then aliquoted and preserved, adhering to the same approaches described for plasma. Processing for the assay was conducted enzymatically (Sigma Diagnostics, Procedure No. 826–UV, St Louis, MO, USA) and run in triplicate at a wavelength of 340 nm (with blue filter) on a DU–640C UV Spectrophotometer (Beckman Coulter, Fullerton, CA, USA) to acquire ODs. Values were obtained by integrating sample ODs into the linear equations derived by the values associated with a concentration range of the kit’s known standard (standard curve).

Blood chemistry

Electrolytes (Na^+ , Cl^- , K^+ , Ca^{2+} , Mg^{2+}), glucose, and BUN (urea) concentrations were obtained from serum. Clotted bloods from “red-top” tubes (iii) were centrifuged at 3500 g for 5 min. The supernatant (~1.0 ml of serum) was then aliquoted, transported, and preserved by the same methods as for plasma. For assay, samples were thawed to room temperature, and to determine values for electrolytes, glucose, and BUN (urea), 50% (sterile grade deionized water) and 10% (0.9% saline) serum dilutions, respectively, were run in duplicate on a Stat Profile Critical Care Xpress (CCX) blood chemistry analyser (Nova Biomedical, Waltham, MA, USA).

Statistical analyses

Prior to temporal analyses, it was confirmed that the values among the study’s six tows were similar (one-way ANOVAs, $p > 0.1$ for each blood parameter). Blood physiology in dogfish was also similar between the two transport days (T2; one-way MANOVA, $F_{13,11} = 0.69$; $p > 0.8$) and between the two captive holding tanks (T3; one-way MANOVA, $F_{13,6} = 4.61$; $p > 0.1$). Values were therefore pooled at discrete sampling times among tows (T1), transport days (T2), and holding tanks (T3) prior to temporal statistical analyses.

A one-way MANOVA “between subjects design” was used to examine whether blood physiology fluctuated across T1–T3 relative to all blood parameters. Differences and comparisons of

means across all three bleeding sessions were assessed using univariate ANOVAs for each blood parameter. As individuals bled at T2 and T3 represented repeated measures, paired-sample *t*-tests were also conducted for each blood parameter to assess changes over the 30 d. T2 blood values from subsequently deceased dogfish were included in T2 analyses, but excluded from paired-sample *t*-tests. Linear regression was used to determine whether dogfish size could predict the values of blood parameters most extensively correlated with additional parameters (pH, the lactate anion, and Na^+) at specific sampling times. In cases of heteroscedastic variances or non-normal distributions, Welch ANOVA tests were utilized. Results were reported as significant according to $\alpha = 0.05$. All analyses were performed using JMP 4.04 Software (SAS Institute, Cary, NC, USA). The values are presented as mean \pm s.e.

Results

There were only two dogfish mortalities during the study (2 out of 34; 5.9% mortality). The remaining 32 dogfish were successfully sampled at T3. Both mortalities were during captivity.

The measured physiological parameters displayed marked quantitative variability across the three bleeding regimes (time intervals). Excepting $p\text{O}_2$, acid–base parameters and lactate produced significant differences when comparing each possible combination of time intervals: following capture (T1), vascular pH was maximally depressed ($p < 0.05$) when compared with post-transport (T2) and post-captivity (T3), but had increased by T2 and peaked ($p < 0.05$) at T3 (Figure 1a). The values of $p\text{CO}_2$ peaked ($p < 0.05$) at T1, when contrasted with T2 and T3, but declined progressively ($p < 0.05$) through T2 to reach presumed basal levels ($p < 0.05$) by T3 (Figure 1b). When compared with T1 and T3, lactate concentrations were most elevated ($p < 0.05$) at T2 and most depressed ($p < 0.05$) at T3 (Figure 1c). The values of $p\text{O}_2$ at T1 were similar in individual comparisons with T2 and T3. However, $p\text{O}_2$ values at T3 were maximally elevated ($p < 0.05$) when evaluated against T2 (Figure 1d).

Extracellular monovalent ion concentrations were significantly different when comparing each possible combination of time intervals: Na^+ (Figure 2a) peaked ($p < 0.05$) at T2, but declined ($p < 0.05$) to presumed basal levels by T3. At T1, Cl^- reached maximum concentrations ($p < 0.05$) compared with T2 and T3, but progressively decreased through T2 to presumed basal levels ($p < 0.05$) by T3 (Figure 2b). Concentrations of K^+ were at their maxima ($p < 0.05$) at T2, but declined ($p < 0.05$) to presumed basal levels by T3 (Figure 2c). For divalent ions, Ca^{2+} concentrations were significantly different when comparing two of the three possible combinations of time intervals, and were most elevated ($p < 0.05$) at T1, compared with the static concentrations at T2 and T3 (Figure 3a). Mg^{2+} concentrations were significantly different when comparing each possible combination of time intervals: the ion reached maxima ($p < 0.05$) at T2 when contrasted with T1 and T3, but by T3 had decreased ($p < 0.05$) to its lowest concentrations (Figure 3b).

BUN (urea) concentrations were significantly different when contrasting each possible combination of time intervals, and the parameter was most depressed ($p < 0.05$) following transport (T2), compared with T1 and T3. However, values rose to presumed basal levels ($p < 0.05$) by T3, compared directly with T1 and T2 (Figure 4a). Glucose concentrations were significantly different when comparing two of the possible three combinations

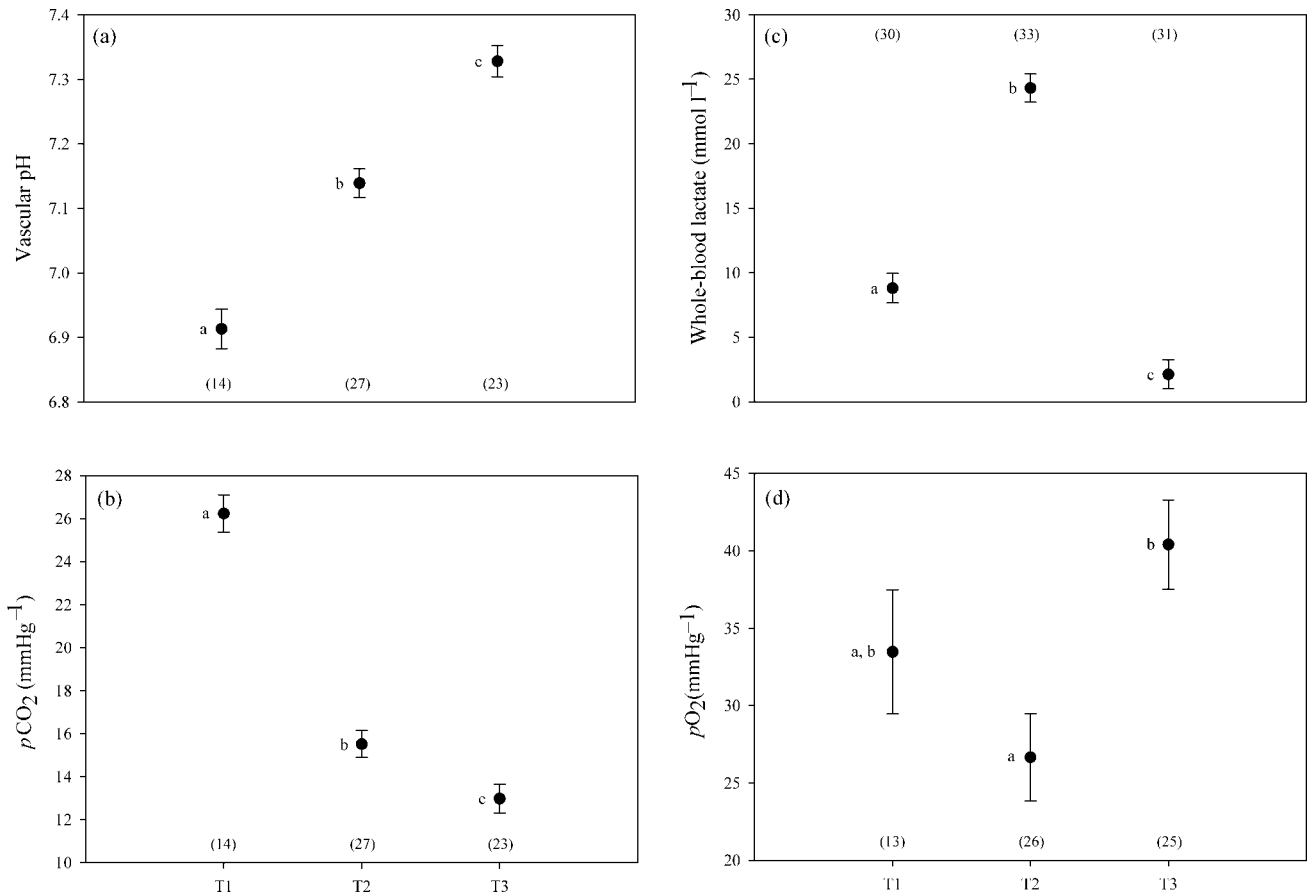


Figure 1. Mean (\pm s.e.) whole-blood parameter values at T1 (post-capture), T2 (post-transport), and T3 (post-captivity). Sample sizes are in parenthesis. Different lower case letters represent significant differences (Tukey HSD, $p < 0.05$).

of time intervals; values were similar between T1 and T2, but increased to peak levels ($p < 0.05$) by T3 (Figure 4b).

Haematocrit values varied significantly when contrasting one of the potential three combinations of time intervals: T1 values were similar to T2 values, but significantly higher ($p < 0.05$) than T3 values, whereas T2 and T3 were similar to each other (Figure 5a). Total protein concentrations were significantly different in a single set of the three possible time interval contrasts: T1 values were similar to those of both T2 and T3, but T2 was maximally elevated ($p < 0.05$) when compared directly with T3 (Figure 5b).

When dogfish size was regressed against an associated value (pH_v , lactate, and Na^+) at all three bleeding times ($r^2 < 0.08$, $p > 0.2$ for all nine combinations), no significant relationships were detected.

Discussion

Despite exposure to otter trawling, transport, and captivity, dogfish in the current study exhibited negligible rates of post-capture (0%), post-transport (0%), and overall (5.9%) mortality. These values were well below the 24.9% reported by Chisholm (2003) and the 28.7% we found ourselves in dogfish subjected to trawl capture and short-term housing (72 h) in sea pens. Although mortality in these latter studies could have been escalated by the pens themselves, dogfish in the present study

demonstrated less mortality, despite exhibiting pronounced physiological changes in response to the capture stress.

Blood parameter values (minus glucose) found in dogfish following captivity (T3) mirrored those obtained in minimally stressed control hook-and-line-caught dogfish (Table 1). Although drawn from dogfish in the captive environment, it is therefore reasonable to designate T3 values as resting when evaluated against those from T1 and T2 in the present study. On this basis, dogfish physiology was markedly disturbed by trawl capture (T1), and in relation to values of certain parameters at T2, further perturbed by transport. The lack of mortality found subsequent to capture and transport implies that dogfish were able to withstand the initial blood chemistry shifts.

Trawling created an upward spike in $p\text{CO}_2$ and a massive drop in pH_v relative to presumed basal (T3) values in dogfish. These responses were presumably a combined function of net constriction, exhaustive activity, and the brief periods on deck following capture. Inversely related $p\text{CO}_2$ increases and blood pH decreases have also been reported for other elasmobranchs (Piiper *et al.*, 1972; Holeyton and Heisler, 1983; Cliff and Thurman, 1984) and teleosts (e.g. Wood *et al.*, 1977, 1983; Schwalme and Mackay, 1985; Milligan and Wood, 1986; Ferguson and Tufts, 1992) subsequent to exhaustive activity or capture stress or a mixture of these two factors. The magnitude of vascular pH difference, however, between highly stressed (post-capture) and presumed resting dogfish (post-captivity) was more extreme than that in the

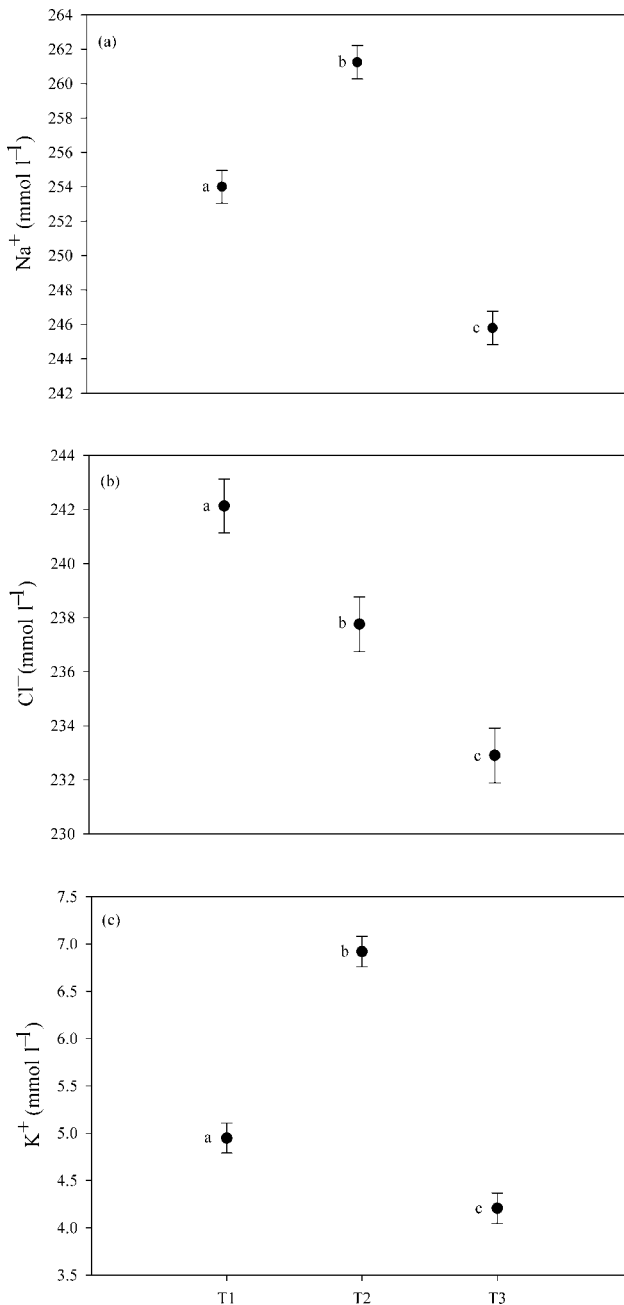


Figure 2. Mean (\pm s.e.) serum values at T1 (post-capture), T2 (post-transport), and T3 (post-captivity). Dogfish sample sizes: (a) Na⁺; (b) Cl⁻; (c) K⁺, T1 ($n = 30$), T2 ($n = 29$), T3 ($n = 29$). Different lower case letters represent significant differences (Tukey HSD, $p < 0.05$).

previous studies, and this is perhaps an indication that trawling stress profoundly affects this species. Although not directly studied, the elevated lactate and $p\text{CO}_2$ levels at T1 and the decreases in dogfish pH_v suggest a synergistic consequence of metabolic ($[\text{H}^+]$ elevation and $[\text{HCO}_3^-]$ depression) and respiratory ($p\text{CO}_2$ elevation) acidosis (Heisler, 1988). Despite these shifts, the $p\text{O}_2$ following capture was similar to that following captivity. In the aftermath of exhaustive exercise in other species, arterial $p\text{O}_2$ has also remained close to normal (Wood, 1991). Apparently, the coupling of capture and transport stress was necessary to drive

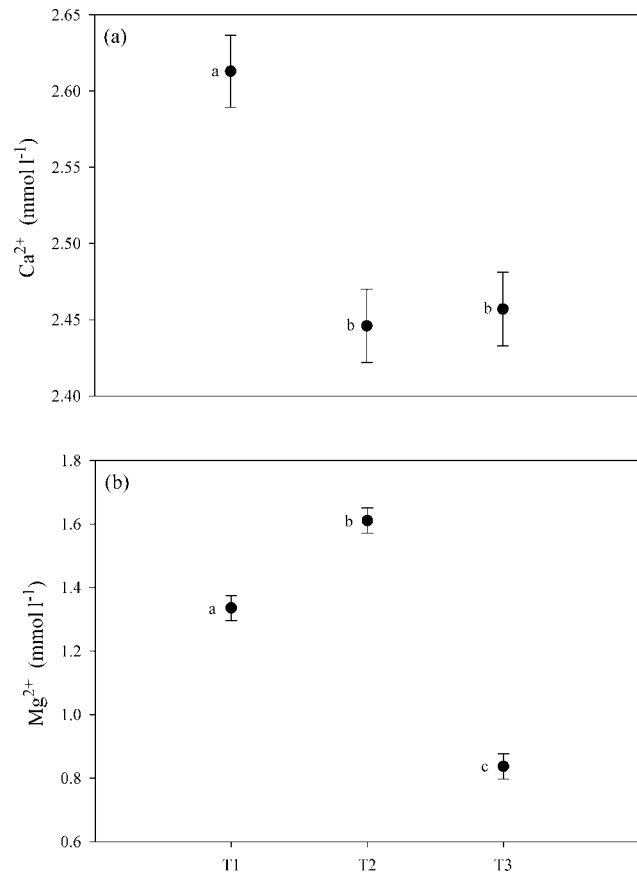


Figure 3. Mean (\pm s.e.) serum values at T1 (post-capture), T2 (post-transport), and T3 (post-captivity). Dogfish sample sizes: (a) Ca²⁺; (b) Mg²⁺, T1 ($n = 30$), T2 ($n = 29$), T3 ($n = 29$). Different lower case letters represent significant differences (Tukey HSD, $p < 0.05$).

down dogfish $p\text{O}_2$ to such an extent (T2) that they were significantly different from presumed basal levels (T3).

Dogfish appeared to resolve acid–base disruptions during the course of the 30 d in captivity, because the pH_v and the other measured blood gas values were analogous to those observed in rapid hook and line capture (Table 1). Moreover, judging by the partial resolutions during the transport and the rapid rate (within 14 and 24 h) at which other elasmobranchs recover following rigorous activity (Piiper *et al.*, 1972; Holeton and Heisler, 1983; Cliff and Thurman, 1984), routine acid–base status seems to have been restored early in the 30 d captive period. The fact that dogfish were already correcting disruptions during the transport ($\sim 1\text{--}3$ h post-capture) points to an even swifter recovery than that found in the studies cited as well as in commonly studied teleosts such as rainbow trout (*Oncorhynchus mykiss*; Milligan, 1996).

Similar to the results found for captured, hyperactive, and confined juvenile dusky sharks (*Carcharhinus obscurus*; Cliff and Thurman, 1984), dogfish $p\text{O}_2$ values were highly variable following capture and transport. Although decreases in $p\text{O}_2$ between T1 and T2 were not significant in the current study, dogfish managed to resolve partially the biologically low pH_v and $p\text{CO}_2$ levels during the confined transport period. This was similar to the resolutions of blood acidaemia demonstrated by trawled dogfish during 72 h, despite being housed in sea pens (Holly Martel-Bourbon, pers. comm.), another confined setting. The ability to

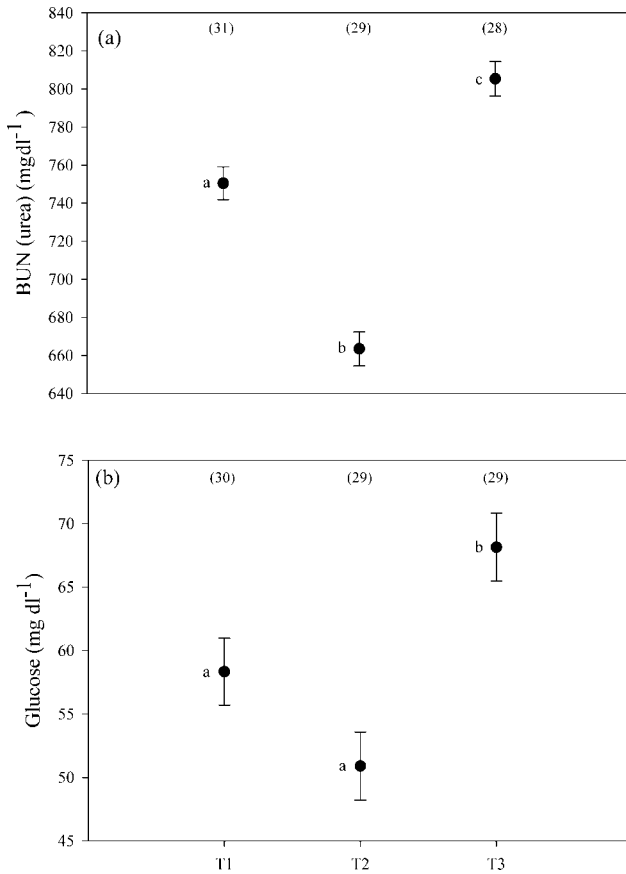


Figure 4. Mean (\pm s.e.) serum values at T1 (post-capture), T2 (post-transport), and T3 (post-captivity). Sample sizes are in parenthesis. Different lower case letters represent significant differences (Tukey HSD, $p < 0.05$).

begin resolving pH_v and pCO_2 while still confined has also been found in confined juvenile dusky sharks (Cliff and Thurman, 1984). These results support the view that it is possible for initial corrections to vascular acid–base imbalances to proceed prior to the removal of acute stressors, as proposed by Heisler (1988).

Although the lactate levels of dogfish in the present study were only moderate following trawl capture, they climbed significantly during transport. The protection of intracellular acid–base balance at the initial expense of extracellular pH has been well described for fish (Claiborne, 1998). For example, the hastened diffusivity of H^+ relative to lactate from white muscle to the vascular system has repeatedly been documented in salmonids (Swift 1983; Wood *et al.*, 1983; Milligan and Wood, 1986; Milligan, 1996; Wilkie *et al.*, 1997) and elasmobranchs (Piiper *et al.*, 1972; Cliff and Thurman, 1984; Heisler, 1988; Hoffmayer and Parsons, 2001), following exhaustive activity or capture stress or both factors taken together. For spiny dogfish in the current study, the blood acidemia and moderate lactate levels following capture imply an onset of lactic as well as respiratory acidosis during trawl capture but, owing to the delayed lactate diffusive rates seen in the previous studies, peak levels were not apparent until after the completion of transport. Following 30 d in captivity, lactate concentrations had declined to presumed baseline levels (Table 1), which also mirrored levels in relatively unstressed bonnethead (*Sphyrna tiburo*; Manire *et al.*, 2001) and juvenile dusky sharks (Cliff and Thurman, 1984).

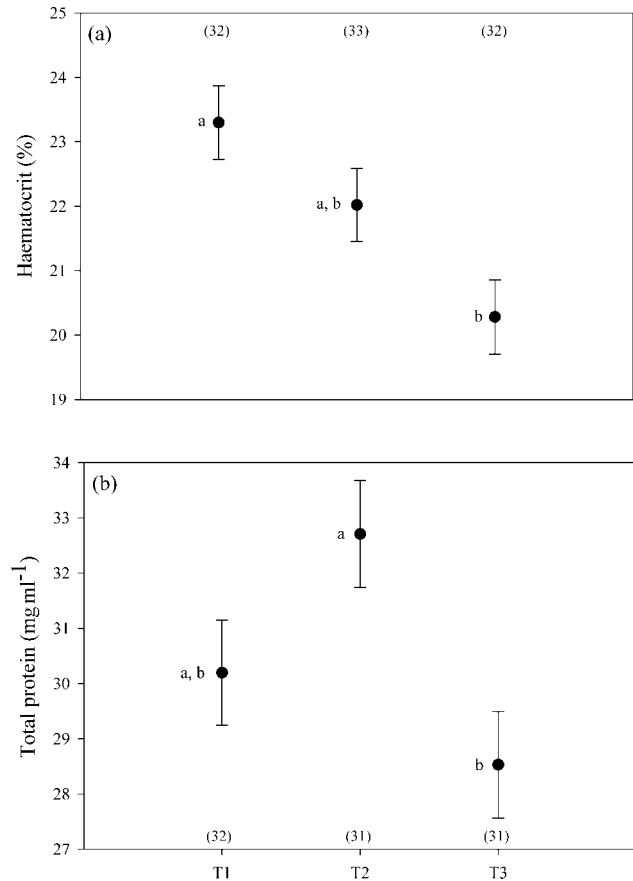


Figure 5. Mean (\pm s.e.) plasma total protein (b) and whole-blood haematocrit (a) values at T1 (post-capture), T2 (post-transport), and T3 (post-captivity). Sample sizes are in parenthesis. Different lower case letters represent significant differences (Tukey HSD, $p < 0.05$).

Table 1. Means (\pm s.e.) comparisons between T3 values and those obtained separately from minimally stressed hook-and-line-caught dogfish.

| Variable | T3 | n | Hook and line | n |
|-------------|---------------------|----|---------------------|----|
| pH_v | 7.33 (0.02) | 23 | 7.37 (0.04) | 7 |
| pCO_2 | 12.97 (0.65) | 23 | 10.8 (1.18) | 7 |
| Lactate | 2.12 (0.99) | 31 | 3.05 (1.05) | 28 |
| pO_2 | 40.4 (2.98) | 25 | 46.86 (5.63) | 7 |
| K^+ | 4.21 (0.14) | 29 | 4.09 (0.15) | 28 |
| Na^+ | 245.79 (0.92) | 29 | 241.71 (0.93) | 28 |
| Cl^- | 232.9 (0.94) | 29 | 231.56 (0.96) | 28 |
| BUN | 805.36 (8.68) | 28 | 800.48 (8.88) | 28 |
| Glucose | 68.14 (2.49) | 29 | 46.75 (2.54) | 28 |
| Haematocrit | 20.28 (0.54) | 32 | 19.64 (0.57) | 28 |
| Protein | 28.53 (0.89) | 31 | 28.27 (0.94) | 28 |

The values were analysed using one-way ANOVAs between intervals T1, T2, and T3. Bold means denote significant differences ($p < 0.05$). Hook-and-line-caught dogfish were sampled within 3 min of hook deployment, and the animals are therefore considered minimally stressed (controls). They were derived in the same way as similar data in the text stemming from different catch scenarios. See figures for T1 and T2 means.

In the current study, electrolyte levels were profoundly impacted by trawl capture and transport. Compared with T3 levels, concentrations of all five electrolytes were elevated by trawl capture, and concentrations of Na^+ , K^+ , and Mg^{2+} were impacted further by transport. Exposure to capture-related stress has previously been shown to impact negatively the salt and water balance in elasmobranchs (Wells *et al.*, 1986) and teleosts (Fletcher, 1975; Wood *et al.*, 1983; Haux *et al.*, 1985; Bourne, 1986; Arends *et al.*, 1999). For monovalent ions specifically, Na^+ and Cl^- elevations have been reported in the mako (*Isurus oxyrinchus*) and blue (*Prionace glauca*) shark in response to angling stress (Wells *et al.*, 1986). Trawl capture elicits significant Na^+ and Cl^- increases in plaice (*Pleuronectes platessa*; Bourne, 1986). The disturbances in Cl^- and Na^+ concentrations observed in the current study returned to an assumed steady state during the 30 d in captivity. This resembled the recovery of the monovalent ionic balance during captivity in response to initial post-capture and transport elevations in plaice (Bourne, 1986).

Dogfish K^+ concentrations initially heightened by trawling were exacerbated by transport. This response was similar to those reported in other elasmobranchs subjected to angling (Cliff and Thurman, 1984; Wells *et al.*, 1986) and gillnetting capture stress (Manire *et al.*, 2001). In those studies, heightened extracellular levels were attributed to increased efflux (“leakage”) from the intracellular compartment of muscle cells attributable to intracellular acidemia. The elevated blood K^+ and lactate anion elevations in dogfish from the present study suggest that intracellular acidemia took place to some degree as a result of trawl capture. However, the extremely depressed post-trawl pH_v values point to potentially lethal changes to the intracellular acid–base balance being deflected to the blood (Boutilier *et al.*, 1986; Milligan and Wood, 1986). In teleosts, Wood *et al.* (1983) concluded that rainbow trout mortality was not attributable to extracellular acidemia following severe exercise. Consequently, if marked blood acidemia was potentially fatal to dogfish in the current study, a greater mortality would have been observed.

Although dogfish K^+ levels (Figure 2a) approached the reported threshold for myocardial disruption (7 mmol l^{-1}) following the cumulative stress of capture and transport (Cliff and Thurman, 1984; Wells *et al.*, 1986), they recovered to baseline levels by the end of captivity (T3). In a teleost, K^+ is significantly higher in dying animals than in survivors following exhaustive activity (Wood *et al.*, 1983). For those survivors, K^+ concentrations returned to baseline levels within 12 h of activity. Post-transport (T2) K^+ concentrations for the two dogfish that subsequently died during the present study were similar to those of survivors. This and the recovered baseline levels in surviving dogfish indicate that even the highest K^+ levels (T2) in the present study were not of sufficient magnitude to induce mortality. Interestingly, the directionality of K^+ shifts was inversely related to that of glucose in the present study. Depletions in blood glucose have been cited as a potential contributor to increasing K^+ concentrations in elasmobranchs (Manire *et al.*, 2001).

The elevated post-trawl levels of Ca^{2+} and Mg^{2+} and the continued heightening of dogfish Mg^{2+} during transport may have been attributable to the capture-induced acidemia. This has previously been cited as a possible explanation for divalent ion increases in stressed dusky sharks and in mammals (Cliff and Thurman, 1984). Interestingly, an elevated Ca^{2+} concentration has also been reported as a possible means to offset cardiac damage caused by acidemia in pelagic teleosts and

elasmobranchs (Wells *et al.*, 1986). This notion is supported in the current study by the fact that maximal disturbances of Ca^{2+} and acid–base parameters (pH_v , pCO_2) occurred in synchrony (T1). The magnitude of the Mg^{2+} change induced by capture in dogfish is similar to that found in captured and transported juvenile dusky sharks, for which changes in the concentration of extracellular divalent cations were reported as a possible disruptor of muscular contraction and neuromuscular nerve transmission (Cliff and Thurman, 1984). The in-transport resolution of baseline dogfish Ca^{2+} concentrations in the present study was swifter than in a trawled plaice, where it took up to 72 h to correct perturbations in divalent ions (Bourne, 1986).

Relative to levels following captivity (T3) in the present study, BUN (urea) concentrations were negatively affected by trawl capture and declined further during transport. Although the gills of elasmobranchs are normally highly impermeable to loss of urea (Wood *et al.*, 1995), Evans and Kormanic (1985) reported significant urea loss in spiny dogfish pups exposed to handling, anaesthesia, and weighing stress. Such losses were attributed to increases in urea permeability and gill epithelial surface areas in response to stress. These mechanisms might have explained the BUN (urea) reductions seen in adult dogfish subjected to trawling and transport stress in the present study. Although ultimately resolved, the magnitude of BUN (urea) losses in (T1) dogfish further indicates that the osmotic balance was heavily compromised by trawl capture. Despite the progressive drops in BUN (urea) observed at T1 and T2, the concentration of this solute had by the end of captivity (T3) returned to levels mirroring those from hook-and-line-caught dogfish (Table 1). Similar to other parameters that were greatly altered, the rectification of presumed baseline BUN (urea) values and the low study mortality imply that shifts were not lethal in magnitude. As urea is the primary organic osmolyte in marine elasmobranchs, the effects of stress on blood urea concentrations and the implications of the resulting fluctuations are areas warranting further investigation.

In the current study, glucose values following capture and transport were low relative to those after 30 d in captivity. This was the only blood parameter in which post-captivity levels failed to mirror those found in hook-and-line-caught dogfish (Table 1). The onset of hyperglycaemia in response to exhaustive exercise, air exposure, capture, transport, induction through injection (of catecholamines), handling, and confinement stress has been mentioned in numerous studies on teleosts (e.g. Arends *et al.*, 1999; Barton, 2000; Frisch and Anderson, 2000; Sulikowski and Howell, 2003) and elasmobranchs (e.g. Torres *et al.*, 1986; Wells *et al.*, 1986; Hoffmayer and Parsons, 2001). In contrast, hypoglycaemia has previously been reported for elasmobranchs following capture and restraint (Manire *et al.*, 2001). If T3 values are considered normal concentrations, this phenomenon could explain the progressive hypoglycaemia in response to trawling and transport in the present study. However, T3 concentration were $\sim 40\%$ higher than those found in presumed baseline hook-and-line-caught dogfish (Table 1) and 20% higher than concentrations found at T1. Relative to hook-and-line values, this indicates a hyperglycaemic response to trawling which was enhanced further during captivity. A possible explanation for heightened post-captive levels involves the degree of maintained sustenance in captivity. In their natural environment, dogfish reportedly possess moderately depressed metabolic characteristics (Brett and Blackburn, 1978) and supposedly feed every 2 weeks (DeRoos *et al.*, 1985). Therefore, the two regimented feedings per week in captivity

exceeded natural sustenance and might have contributed to the heightened T3 glucose concentrations. Pre-capture feeding was cited as a potential instigator of the relatively high glucose concentrations found in captured Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*; Hoffmayer and Parsons, 2001). Additionally, chronically elevated, non-assayed, adrenal parameters could have instigated the captive glucose elevations, with cortisol elevations and increased glucose, that have been demonstrated in teleosts (Barton, 2000).

Steady-state dogfish haematocrit levels have ranged from 16.4% (“normal” on the day following surgery in unfed dogfish; DeRoos *et al.*, 1985) to 18.7% (“control” in the lesser spotted dogfish; Torres *et al.*, 1986) to ~20% in hook-and-line-caught dogfish (Table 1). In this study, the post-capture haematocrit values of dogfish exceeded those found subsequent to captivity. Other work suggests that erythrocytic swelling (haemoconcentration) may have driven the trawling-induced haematocrit increases observed here. For example, elevations have been reported in both pelagic elasmobranchs and teleosts captured by hook (Wells and Davie, 1985; Wells *et al.*, 1986). Further, the spleen of spiny dogfish reportedly failed to release sequestered erythrocytes in response to sympathetic nerve stimulation or circulating catecholamines, as typically observed in mammals (Opdyke and Opdyke, 1971). This would support haemoconcentration as the instigator of increasing haematocrits in our results.

Conclusions

Trawling and transport caused marked physiological changes in the blood of spiny dogfish. Despite this, mortality levels remained low, and post-trawling recovery of blood acid–base disturbances appeared to begin prior to the completion of the transport phase. The results also indicate that any delays in the recovery of other physiological parameters, e.g. electrolyte balance, following capture and transport are corrected in this species after sustained periods in captivity. Judging by the initial rate of recovery from physiological perturbations and the low mortality, it appears that spiny dogfish have an extremely high threshold for the magnitude of trawling and transport stress assessed in this study. Future work should continue to address the resilience of dogfish and other commonly captured and discarded elasmobranchs in both the field and the captive environments. In particular, investigations of the effects of different capture methods, sex, age, and size class on resilience, as well as correlations between physical trauma, reflex actions, and physiological status, are proposed. Continued investigation into the post-release survival of dogfish and other elasmobranchs discarded as bycatch is also needed across many fisheries.

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