

**I. Project Title:** “Juvenile Bycatch and survival assessment of spiny dogfish (*Squalias acanthias*) in a Western Atlantic trawl fishery”

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**Organization:** New England Aquarium

**Grant Number:** NA03NMF4270126

**Award Period:** July 31, 2003 to December 31, 2005

**Date:** April 15, 2006

**Amount of Grant:** *Federal* \$169,580 *Match* \$28,147 *Total* \$197,727

## II. Abstract

Blood chemistry and mortality data provided a comprehensive picture of the physiological stress response and resiliency in *Squalus acanthias*. Blood acid-base balance was significantly affected by trawl capture; short-term mortality was low; and female dogfish were more resilient to trawling stress. The depressed blood pH values indicated that physiological stress had been profound. Although blood chemistry was markedly affected by trawl capture and transport, dogfish exhibited corrected values and negligible mortality following 30 days in captivity. In addition to acid-base balance, recovery of the following disturbances elicited by trawl capture and transport were demonstrated following captivity (Appendix B): heightened electrolyte concentrations (Appendices B & C), increased metabolic products such as lactate (Appendices B & C) and additional hematological disturbances (i.e. - hematocrit elevations; Appendices B & C). These findings indicated that if not fatally injured outright in the net, dogfish were capable of withstanding and recovering from the physiological perturbations due to capture. Despite being housed in fairly confined sea pens, surviving trawl-caught dogfish managed to resolve these perturbations. The similarities found between trawl and hook-and-line (used as a control) mortality was surprising but was still below the discard rate currently postulated for trawled dogfish in the fishery. Considering that dogfish are active and migratory raises the issue of whether observed mortality in sea pens or the captive environment can be confidently applied to actual conditions. Although it is not possible to quantify these differences, any mortality occurring in the study was presumed equal to or less than under actual conditions. Future work should address the resiliency of dogfish and other commonly captured (and discarded) elasmobranchs in both the field and captive environments. In particular, the impacts of differential capture-methods and the correlations between physical trauma and physiological status are recommended. For trawling specifically, additional work should address the effects of greater fluctuations in tow-times, tow-weights, air-exposure (in the lab and the field) and physical and behavioral indices as primary study parameters.

### III. Executive Summary

The work encompassed three primary areas: short-term mortality of dogfish; trawling, transport and captivity stress and mortality in dogfish; the physiological impacts of trawl capture in dogfish.

#### **Short-term mortality of dogfish**

To assess the short-term post-release mortality of otter-trawl captured spiny dogfish (*Squalus acanthias*), individuals landed by 45-60 minute trawls or by less intensive hook-and-line (control) were held in experimental pens deployed *in-situ* for 72-hour trials. Although mortality was similar between the two capture methods (28.7% for trawl vs. 23.6% for hook-and-line), mortality in trawled males (37%) exceeded that in hook-and-line caught males (25.0%). A greater percentage of trawl-caught males died compared to females (38.0% vs. 21.7%) but were on average (78.7 +/- (0.47) cm) larger than males (75 +/- (0.54) cm). The sizes of live and dead dogfish were similar (within each gender) for trawl but differed for hook-and-line caught males (mean (+/- SEM) live/dead animal size = 76.67 (0.44) cm/73.92 (0.75) cm). Collectively, the weight and duration of a trawl could positively predict for dogfish mortality in an associated pen trial; weight was the more dominant predictor. The vastly lower vascular pH values obtained from trawled dogfish alludes to the more strenuous nature of that capture method. The comparable 72-hour mortality (28.7% trawl; 23.6% hook-and-line) associated with both capture methods indicates a potential influence of the pens themselves. It is therefore postulated that assuming unexceptional tow-weights, handling procedures and periods of air-exposure, trawled dogfish mortality is comparable or lower than the observed 28.7%, when discarded under actual conditions- a level below that currently estimated in the Northwest Atlantic otter trawl fishery.

#### **Trawling, transport and captivity stress and mortality in dogfish**

To assess the physiological responses and associated mortality in spiny dogfish following otter trawl capture, 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<sub>2</sub>, pCO<sub>2</sub>), serum Ca<sup>++</sup> and Cl<sup>-</sup>, and hematocrit were maximally disrupted at T1 but progressively resolved to baseline values by T3. Concentrations of whole-blood lactate, plasma total protein, additional sera electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>++</sup>) and BUN (urea) were maximally compromised at T2 but also recovered by T3. In contrast, serum glucose levels remained elevated at all three sampling intervals. Although blood parameters were substantially altered, dogfish mortality was low (2/34; 5.9%) suggesting a high degree of resiliency to compounded stressors associated with capture, transport and captivity.

#### **The physiological impacts of trawl capture in dogfish**

To investigate the physiological responses to otter-trawling capture in spiny dogfish, blood values from trawled individuals were evaluated against those landed by rapid hook-and-line. Significant effects of trawling were reflected in all parameters excluding log plasma protein. Blood gas parameters (pO<sub>2</sub> (45% decrease); pCO<sub>2</sub> (82% increase)) and the lactate anion (125% increase) were most perturbed relative to changes in other parameters. The concentrations of sera monovalent electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>) and glucose were significantly elevated by trawling but not to the magnitude seen in other studies on capture stress. Hematocrit significantly rose while BUN (urea) levels were significantly reduced by trawl capture. Lactate concentrations and hematocrit in trawled male dogfish were significantly higher than in females indicating a more pronounced stress response for that gender. Overall, trawl capture elicited considerable blood chemistry disturbances relative to normal physiology in spiny dogfish.

## IV. Purpose

### IV A. Detailed description of problem

Spiny dogfish (*Squalus acanthias*) populations historically have been heavily targeted in the Northwest Atlantic commercial fisheries. Between 1962 until 1989 only 54,000 million tons of dogfish were landed whereas between the years 1990 and 1997 landings totaled 154,000 million tons (Rago et al., 1998). Between 1989 and 2001, the biomass of mature females, the larger of the genders, was reduced by more than 50% (NMFS 2001). Targeted removal of females led to the ensuing low levels of recruitment, which developed into record lows in the late 1990's. In 1998, spiny dogfish were designated overfished by the New England Fishery Service Council. Increased landings, the diminishing mature female population and limited recruitment led to a *status of concern* for the spiny dogfish stock. A management plan was designed to decrease fishing mortality, to allow for recovery of the stock and to protect biodiversity and ecosystem structure and function. Stock assessments derived from Spring and Fall ground fish trawl surveys set the yearly catch limits. Restrictions also called for an avoidance of large females.

An issue of major importance in the Western Atlantic commercial fishing industry is the fitness of discarded bycatch. This applies to both members of non-targeted fisheries, and to immature members of the targeted species from a particular directed fishery. As a result of the trauma attributable to capture and handling, survival is often diminished for fish returned to the sea and has major implications for management of individual species.

Of significant consequence for example, are current regulations that relate to size limits, quotas, and net sizes in the case of otter trawling. In order to assess the effectiveness of such regulations and the impacts on resulting discards, the rates of both short-term and long-term survival of the potential discards must be assessed. Since every species is different in terms of resiliency and ability to recover, species-specific analysis is imperative. While a majority of the stress and survival work has been conducted on teleosts and more specifically salmonids, little has been achieved on the subclass *elasmobranchii*. This is surprising for many reasons, including the facts that other aspects of elasmobranch physiology have been so tediously examined, they possess many unique anatomical and physiological characteristics, and that many of their life history traits render them particularly susceptible to rapid depletion. The latter is of critical importance to fishery related issues since many coastal shark populations have been recently threatened because their reproduction has been overwhelmed by harvesting rates. Therefore, it is essential to re-evaluate fishery-related regulations regarding elasmobranchs in order to attain their respective maximum sustainable yields.

We successfully conducted an extensive stress and survival study on spiny dogfish. The spiny dogfish evolved into a directed fishery, primarily because of the decline of more traditional groundfish resources (NMFS, 2002). Despite regulations relegating the species to an indirect fishery in May of 2000, heavy "Spurdog" landings persisted as bycatch in the form of juveniles and non-targeted landings in other directed fisheries. This study characterized the relative physiological condition of the species' discards following trawling stress and associated survivability.

The correlation between spiny dogfish survivability (short and long-term) and the associated changes in specific blood parameters, more fully characterized the physiological

determinants for induced spiny dogfish mortality (also creating a starting point for similar work with other species). Finally, this work examined overall elasmobranch physiology under commercial fishing-induced stress, an area that warrants immediate attention to improve management protocols.

#### **IV B. Objectives of the project.**

To assess survivorship of trawl-caught discards as a means of designing more efficient management of the species since current post-release mortality estimates of discarded spiny dogfish bycatch are mostly unstudied.

To quantify the degree of stress induced by the otter trawling method of capture relative to both hook and line and sampling of acclimated captive individuals through the analysis of stress parameters in the blood.

To conduct the first survivability study on trawled spiny dogfish that includes blood chemistry analyses.

To investigate the physiological changes that occur in elasmobranchs while being captured by commercial fishing gear.

In addition to the traditional short-term survivorship analysis, to investigate the long-term survivorship (by keeping experimental individuals captive for longer durations) following trawl-induced stress, handling, and deck time (air exposure), an area that has been absent in previous survival work.

#### **V. Approach**

*A. Detailed description of the work that was performed can be found in the manuscripts located in appendices A-C.*

*B. Project Management:*

Dr. Farrington, the principal investigator, supervised all of the aspects of this work. Dr. Farrington planned for the logistical support of the cruises, obtained all of the necessary permits/approvals for the work, scheduled/trained technical personnel, collected data, performed biochemical analyses and submitted the requisite technical interim reports.

Dr. John Mandeman was especially involved in the fulfillment of the responsibilities outlined in this grant. He was principally involved with the collection of blood samples, performed the biochemical analyses, created the databases for their statistical analysis and performed these analyses. He was the principal author for writing the peer reviewed papers from the information obtained in this investigation.

The Office of Sponsored Programs of the New England Aquarium prepared the quarterly financial reports.

#### **VI. Findings**

*VI A. Actual accomplishments and findings are described in detail in the manuscripts found in*

*appendices A-C.*

**VI B. Significant problems:** intended fieldwork in the June (2004) was aborted due to a lack of dogfish in the chosen experimental areas. Work resumed in July (2004) and no additional delays were required.

**VI C. Description of need, if any, for additional work:** no additional funding is required to complete the study objectives.

## **VII. Evaluation**

### **VII A. Describe the extent to which the project goals and objectives were attained.**

This description should address the following:

*1. Were the goals and objectives attained? How? If not, why?*

The objectives to generate spiny dogfish trawl discard mortality estimates were successful for both short- and long-term trials; the physiological responses to trawling stress in dogfish were characterized relative to presumed basal physiology.

*2. Were modifications made to the goals and objectives? If so, explain.*

- Day trips were used in lieu of long cruises for data collection. This provided greater methodological flexibility and did not at all impact the stated objectives of the proposal.
- The decision was made to assess the survivability and physiological stress in dogfish of all sizes instead of solely those below 80 cm. When the dogfish fishery is non-directed, trawl capture implications are vital for the entire population.

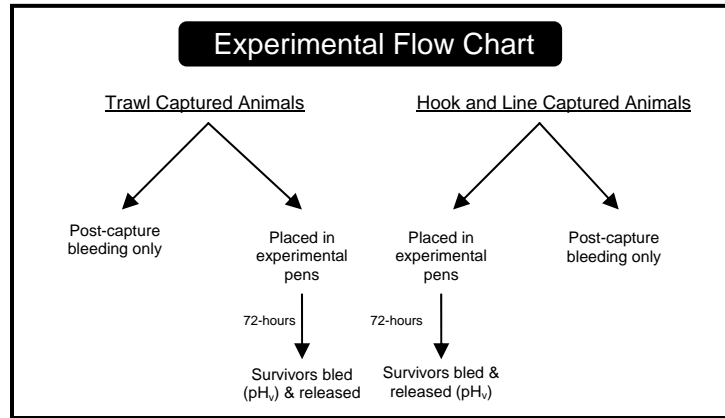
### **VII B. Dissemination of Project results:**

Explain, in detail, how the project results have been, and will be, disseminated:

- Appendix A will be submitted to the Journal of Fisheries Research.
- Appendix B has been submitted to the ICES Journal of Marine Science.
- Appendix C will be submitted to the Environmental Biology of Fishes.
- Portions of this work have/will be presented in the following forums/conferences:
  - Joint Meeting of Ichthyologists and Herpetologists (American Elasmobranch Society portion) (Manaus, Brazil- June/July 2003).
  - University of Rhode Island Graduate School of Oceanography Spring Seminar Series (Narragansett, RI- May 2005).
  - NEFSC NOAA/NMFS Spring Seminar Series (Woods Hole, MA- May 2005)
  - First International Symposium of the Biology of Spiny Dogfish (Seattle, WA- June 2005).
  - The Biology of Fishes Conference (Symposium on the Physiological and Behavioral Impacts of Capture) (St. Johns, Newfoundland- July 2006).

# Appendix A

# Experimental protocol for Appendix A.





## POST-RELEASE MORTALITY IN A NORTHWEST ATLANTIC OTTER-TRAWL FISHERY

### **Abstract**

To assess the short-term post-release mortality of otter-trawl captured spiny dogfish (*Squalus acanthias*), individuals landed by 45-60 minute trawls or by less intensive hook-and-line (control) were held in experimental pens deployed *in-situ* for 72-hour trials. Although mortality was similar between the two capture methods (28.7% for trawl vs. 23.6% for hook-and-line), mortality in trawled males (37%) exceeded that in hook-and-line caught males (25%). A greater percentage of trawl-caught males died compared to females (38% vs. 21.7%) but females were on average (78.7 +/- (0.47) cm) larger than males (75 +/- (0.54) cm). The sizes of live and dead dogfish were similar (within each gender) for trawl but differed for hook-and-line caught males (mean (+/- SEM) live/dead animal size = 76.67 (0.44) cm/73.92 (0.75) cm). The collective weight and duration of a trawl could positively predict for dogfish mortality in an associated pen trial (weight was the more dominant predictor). The vastly lower vascular pH values obtained from trawled dogfish alludes to the more strenuous nature of that capture method. The comparable 72-hour mortality (28.7% trawl; 23.6% hook-and-line) associated with both capture methods indicates a potential influence of the pens themselves. It is therefore postulated that assuming unexceptional tow-weights, handling procedures and periods of air-exposure, trawled dogfish mortality is comparable or lower than the observed 28.7%, when discarded under actual conditions; a level below that currently estimated in the Northwest Atlantic otter trawl fishery.

### **Introduction**

The spiny dogfish (*Squalus acanthias*) is a coastal squaloid elasmobranch found in temperate waters worldwide (Compagno 1984). Like many elasmobranchs, dogfish possess K-selected life history attributes such as slow growth, low fecundity, a long life span and a close concordance between recruitment and maternal stocks (Nammack et al. 1985; Hoenig and Gruber 1990; Stevens et al. 2000; ASMFC 2002). The species also displays an especially prolonged maternal gestational period (18-22 months) (NEFSC 2003). Inherently larger than males, females are also preferentially selected in directed fisheries. Increased Northwest Atlantic dogfish landings between 1998 and 2003 reportedly contributed to a 75% reduction in mature female dogfish stocks and an associated lack of recruitment spanning 1997 to 2003 (ASMFC 2002; NEFSC 2003). The rapidity and magnitude of these declines highlight the difficulty in sustaining Northwest Atlantic populations during periods of elevated fishing pressure.

The effects of capture and ultimate fate of discarded bycatch are pivotal issues affecting fish populations and associated management. The nonselective nature of many commercial fisheries necessitates the return of countless numbers of non-targeted animals and excess or juvenile members of targeted species to the sea. Depending upon the degree of mortality during capture and post-release, the magnitude of incidental landings on different species can have significant effects on a population. Due to the variabilities in biology among species, capture methods, fishing conditions and additional factors that influence mortality, the effective estimation of post-release mortality is a complex process (Davis 2002), and is complicated by the limited number of appropriate *in situ* methodologies for obtaining such data. Due to the utility of, and demand for, data assessing the short-term mortality of individual fish species however, many field-oriented studies have used either on-deck

holding pens (Neilson et al. 1989; Kaimmer and Trumble 1998; Laptikhovsky 2004; Reville et al. 2005) the laboratory/captive environment (Chopin et al. 1996; Davis et al. 2001; Davis and Olla 2001; Davis and Olla 2002), and sea pens (Carr et al. 1995; Milliken et al. 1999; Farrington and Carr 2003; Farrington et al. 2003; Chisholm 2003) to establish post-release or escapee (Chopin and Arimoto 1995) survivability estimates for individual species associated with their most common capture-modes.

If evaluated in isolation, post-capture blood physiology can effectively aid in ascertaining the degree of imposed stress on a particular species. However, if physiological analyses are coupled with mortality investigation, predicting the ultimate likelihood of recovery and aiding in the estimation of discard mortality rates becomes possible. Assuming the eventual growth of a robust database linked to capture-induced mortality rates, physiological analyses may ultimately help provide an estimation of intrinsic species-specific discard survivability across specific capture methods. Although blood physiology has been used extensively to assess responses to capture in teleosts, less work has focused on elasmobranchs (Cliff and Thurman 1984; Wells and Davie 1985; Wells et al. 1986; Hoffmayer and Parsons 2001; Manire et al. 2001). Although this group has been described as particularly sensitive to capture-stress (Wells et al. 1986), a field-based study has yet to simultaneously address discard mortality and physiology in one of its species.

Due to the high discard rates of incidentally landed dogfish in the Northwest Atlantic otter-trawl and gillnet fisheries, the species' estimated post-release bycatch mortality is of great interest to fisheries management. The need for estimates of discard mortality was highlighted in the species' Interstate Fishery Management Plan (FMP): mortality estimates can influence dogfish stock assessments, help shape the necessary direction of related policy, and reveal potentially worthwhile changes in fishing practices and technologies (ASMFC 2002). Studies examining dogfish discard mortality would help determine the potential for recovery of Northwest Atlantic dogfish populations during periods of curtailed directed fishing. At present, fishery-models account for a 50% dogfish bycatch discard mortality rate following otter-trawl capture (NEFSC 2003), however, an early study found a 24.9% 48-hour (h) discard mortality rate for trawled dogfish (Chisholm 2003). The goal for the present study was to expand the post-release period by holding trawled dogfish in pens for 72 h. In that it has been shown to correlate most extensively with additional secondary blood parameters in juvenile dusky sharks (*Carcharhinus obscurus*) (Cliff and Thurman 1984) and the spiny dogfish (personal observation), vascular pH (pH<sub>v</sub>) was concurrently assessed as a proxy for physiological status at each juncture of the study (post-capture and in survivors, post- 72 h). To identify near steady-state blood levels and whether pens influenced mortality and physiology, dogfish caught by an abbreviated and less intensive capture method (hook-and-line) were also held for 72 h and subjected to identical blood sampling protocols as were trawled dogfish.

## **Methods**

All fieldwork was conducted aboard Fishing Vessel (FV) Joanne A III (Chatham, MA). The trawling, mortality assessment, and blood physiology was conducted at the "Tuna Grounds" southeast of Chatham Lighthouse and additional areas southeast of Chatham Inlet (Fig. 1). To prevent seasonal biases, eliminate air and water temperature as study variables, and ensure adequate spiny dogfish catch numbers, fishing trips were run in the summer months between June and September (2004) when the species is typically most abundant in our

experimental area. Fishing was carried out in depths ranging from 46.0-73.0 meters (m) on cobble and sand bottom, with surface water temperatures 13-16 °C. Depths and locations were recorded for every fishing set and pen trial. Within each capture method, the dogfish landings were randomly placed into one of two groups: those to be blood sampled or those to be placed in experimental holding pens designed to monitor 72 h post-release survivability.

#### Otter-trawl fishing

Efforts were made to adhere to routine practices aboard the vessel. Trawling was conducted using a Danish bottom trawl with an 18 mm mesh-size, 305 meshes in the head-rope and foot-rope collectively (fishing circle), 4.6 m legs and a hard-bottom sweep to avoid boulders on the substratum.

*General routine:* The mean (+/- SEM) depth for trawls associated with the blood and mortality work was 61 (1.1 m). In order to try and reflect typical commercial conditions without subjecting individuals to extreme periods of air-exposure, treatment of dogfish persisted for ~20 minutes following haul-back and dumping of the catch. Because the study aimed to comparatively assess trawling impacts and the collective resiliency of dogfish as opposed to impacts on specific animals, individual dogfish were not exposed to all of the experimental phases nor were they tagged as was done with Atlantic halibut (*Hippoglossus hippoglossus*) in Neilson et al. (1989). Instead, animals were arbitrarily assigned for either blood sampling or 72 h housing in sea pens to mortality. The gender and size (tip of snout to extended tip of hypochordal caudal lobe) was determined for every dogfish captured. As spiny dogfish tend to congregate by size and upon reaching adulthood, by gender (ASMFC 2002), many catches were disproportionately segregated according to those factors. Although duration-of-tow varied from 30-95 minutes, the majority lasted 45-60 minutes and mean (+/- SEM) fell at 46.31 (5.79) minutes. A total of 26 trawls with estimated tow-weights ranging between 90.0 and 900.0 kg were conducted in order to fulfill intended blood physiology and an adequate number of pen trials.

#### Hook-and-line fishing

To establish a basis of comparison with trawl-captured dogfish in the present study, a sample of minimally stressed individuals was obtained by hand-line. Due to opportunistic feeding displayed by dogfish and general ease of capture, this method enabled the landing of individuals within three minutes of hook deployment. A short makeshift longline was used to hang five squid-baited standard circle hooks per set. Once each set was retrieved by hand, dogfish were immediately de-hooked, bled, or placed in the temporary holding bins if awaiting subsequent pen-deployment. Although each dogfish experienced laceration from being hooked, bleeding subsided rapidly as evidenced by the lack of blood in the seawater holding tanks after roughly one minute. Due to the abundance of male spiny dogfish during this phase of the study, the sample of hook-and-line-captured females was scant (n = 3) and gender differences were not considered during analyses.

#### Post-capture vascular acidemia/alkalemia

To avoid inducing additional stress (to that experienced during the capture) in the individuals to be monitored for mortality in pens, post-capture blood samples were alternatively obtained from surrogate dogfish randomly taken from the same trawls. These individuals were then discarded. During the 20-minute sampling periods, multiple phlebotomists and

relatively few dogfish sampled per tow (or per group of hook-and-line-caught individuals) prevented wide variations in the length of time dogfish sat on deck. Therefore, blood sampled individuals were exposed to air for uniform periods. Approximately 1.0 ml blood samples were obtained by caudal-veni puncture using Fisher non-heparinized 18-20 gauge 0.04 m stainless steel syringe needles fitted to 5 ml plastic (Beckman Dickinson) syringes. This is a rapid and relatively non-invasive method of blood collection in elasmobranchs that limits the disturbance of the animal (Cooper and Morris 1998). Upon obtaining a sample, needles were immediately plugged with cork (to slow diffusive gas loss) and 20.0  $\mu$ l deposited into individual (CG4+) cartridges for Heska portable clinical analyzer (ISTAT) on-site calculations within 10 seconds of being drawn. Temperature adjusted values were generated by the ISTAT following approximately three minutes of processing. Slight value discrepancies might have existed as a function of whether obtaining venous, mixed venous or arterial blood as is the case in humans (Relman 1986). However, the magnitudes of pH differences were not likely significant enough to discernably effect results. Because inter-tow variations in mean  $\text{pH}_v$  were not significant (one-way analysis of variance (ANOVA:  $F_{8,28} = 0.5$ ,  $P > 0.8$ ), post-trawl values from the multiple tows were pooled for analyses. A total of 35 and 15 blood samples were analyzed following trawl and post hook-and-line capture respectively.

#### Pen design and construction

Holding pens were modified from Chisholm's (2003) prototype. To prevent sensory overload for the spiny dogfish, metal was completely excluded from the design. Each of the eight pens measured 3.7 x 1.2 x 1.2 m and was comprised of two 1.2 x 1.2 m sections book-ending the rectangular form (Fig. 2). A 1.2 x 1.2 m permanently fixed middle section and eight removable 1.8 m PVC sections enabled pen-compression and easier storage and transport. This enabled on-deck expansion while tows were in progress. Pens were custom-made with 25.0 mm mesh-sized netting tailored to encase the frame (Nylon Net Company, Memphis, TN). To simplify deposition and removal of dogfish while reducing escape potential (during the 72 h trials), a specialized Velcro opening was included in the netting for each pen. The frames were constructed with 25.0 mm schedule-40 PVC with holes drilled along the lengths of each section and joint to ensure negative buoyancy. The pens were maneuvered in the water column using bridles secured to whale-safe swivels connected to a main line.

#### Experimental pen work (72-hour survivability)

In order to investigate the short-term post-capture implications on the catch, landed dogfish were randomly placed in experimental pens. Similar to those that were blood sampled, dogfish placed in the pens were subjected to essentially the same period of deck-time/air exposure. Individuals to be assessed for mortality were transferred into a pen awaiting release on the stern *en masse* or temporarily into on-deck [ambient seawater fed] holding tanks if a short steam to a pen-deployment site was required. Each pen was hydraulically deployed, anchored to the substrate with either a 36.3 kg railroad tie or an 18.1 kg mushroom anchor, left *in-situ* for 72 h, and retrieved. In addition to mapping the location of each, pens were also marked with a surface buoy and highflyer with radar reflector. Pens were situated at a mean (+/- SEM) depth strata of 60.0 (0.9) m and because these levels did not vary significantly between trials, stratum was not analyzed as a study variable. Seventeen total pen trials were conducted. To maximize sample sizes while minimizing crowding, the

targeted dogfish quota was set at 16 animals per pen according to Chisholm's methods (2003).

*Trawl:* A total of 185 dogfish (106 females, 79 males) across twelve pen trials were analyzed for mortality. Individuals ranged in size between 61.0 and 93.0 cm. In order to correlate aspects of a particular tow to the 72 h mortality, each pen was comprised of dogfish from a single tow. Because no significant differences in mortality were found between pen trials (chi-square:  $X^2_{11} = 8.82$ ,  $P > 0.5$ ), mortality data across pens was pooled.

*Hook-and-line:* A total of 55 dogfish (52 males, 3 females) ranging in size from 70-85 cm were monitored across four pen trials. The negligible sample of females prohibited gender analyses. The difference in percent-mortality among the pen trials was not significant (chi-square:  $X^2_3 = 2.07$ ,  $P > 0.5$ ) allowing data from the four trials to be pooled.

#### Post-72-hour vascular acidemia/alkalemia

To assess the changes in  $pH_v$  experienced during the 72 h trial, each surviving dogfish was blood sampled. The protocol was identical to that used for the initial post-capture bleeding methodology. Because no significant  $pH_v$  differences were found in dogfish across multiple pen trials for either capture mode (one-way ANOVAs, trawl:  $F_{11,31} = 0.46$ ,  $P > 0.9$ ; hook and line:  $F_{3,9} = 1.2$ ,  $P > 0.3$ ), mean vascular pH data was pooled for subsequent analyses. A total of 32 and 10 arterial dogfish blood samples were analyzed post-72 h for trawl and hook-and-line pens, respectively.

#### **Statistical analyses**

*Mortality:* Chi-square analysis was used to assess differences in mortality between capture-methods, between capture methods (males only), and between genders (trawl only). Independent-samples  $t$  tests were used to assess differences in overall mean body-size (cm) between male and female dogfish (trawl only). Once it was determined that size might have been contributing to the significant differences observed between male and female mortality (trawl), independent-samples  $t$  tests were also utilized to compare mean-live versus mean-dead animal size within each gender (trawl) and males only (hook-and-line). To test whether reproductive maturity affected mortality, differences in mortality between females smaller than 80.0 cm and females larger than 80.0 cm were also assessed using chi-square analysis. Since dogfish in a given pen were linked to a particular tow, multiple regression analysis was utilized to assess whether tow-weight and tow-length could predict for percent-mortality of dogfish within associated pens (collinearity between –length and –weight was confirmed to be absent: Pearson pairwise correlation = 0.39,  $P > 0.2$ ). As a single tow (95 minutes) exceeded the 99% confidence limit (63.99 minutes) for the tow-duration distribution, it was considered an exception and not included in the multiple regression analysis. A linear regression was also used to determine whether the length of a tow could predict for weight of its catch.

*Vascular acidemia/alkalemia:* To assess potential pen- and tow-effects on the dogfish in a given pen, mean live animal  $pH_v$  was regressed against mortality in that pen. Effects of dogfish-gender (factor) on  $pH_v$  values following trawl capture at a given capture-time (factor) (post-capture and post-72 h dogfish) were absent (two-way analysis of covariance (ANCOVA) capture-time\*gender:  $F_{1,60} = 0.24$ ,  $P > 0.6$ ) while accounting for dogfish-size (covariate). Gender was therefore excluded as a variable in subsequent tests applied to both capture

methods. Because it was not possible to stabilize the variance in  $\text{pH}_v$  among groups ( $P < 0.05$ ), a Welch-ANOVA test for heterogeneous variances was utilized. A two-way (full-factorial) ANCOVA with dogfish-size (cm) the covariate was then used to evaluate  $\text{pH}_v$  changes across all four bleeding regimes (both post-capture and post-72 h for trawl and hook-and-line respectively). Tukey HSD testing was used to compare the multiple pairs of least squares  $\text{pH}_v$  means. All analyses were performed using JMP 4.04 Software (SAS Institute, SAS Campus Drive, Cary, NC 27513).

## **Results**

There was no immediate post-capture mortality for trawl and hook-and-line-caught dogfish.

### Experimental pen mortality (otter-trawl)

Without accounting for gender or size, the pen trials revealed a 28.7% 72 h mortality rate (Fig. 3). Many of the surviving dogfish lacked vigor and/or possessed noticeably abraded rostrums. Overall mortality of male dogfish was significantly greater (38% vs. 21.7%) than that of females ( $X^2_1 = 4.18$ ,  $P < 0.05$ ) (Table 1). However, mean female body-length was greater than males in the study (independent-samples  $t$  test:  $t_{177} = 5.152$ ,  $P < 0.0001$ ). This implies that greater female body-sizes may have been driving their comparatively lower levels of mortality. When further assessed however, differences in the sizes between live and dead dogfish were not significant within either male (independent-samples  $t$  test:  $t_{75} = 0.35$ ,  $P > 0.7$ ) or female (independent-samples  $t$  test:  $t_{100} = 0.73$ ,  $P > 0.4$ ) (Table 1). Females exceeding 80 cm also displayed similar mortality rates to those below that threshold (chi-square:  $X^2_1 = 0.13$ ,  $P > 0.7$ ). Multiple regression analysis revealed that weight- (Std. beta = 0.61, stronger predictor) and length of a tow (Std. beta = 0.26, weaker predictor) could collectively predict (model:  $R^2 = 0.65$ ,  $F_{2,10} = 8.36$ ,  $P < 0.01$ ) dogfish percent-mortality in an associated pen trial (Fig. 4A and B). The duration of a tow was not a significant predictor of its catch-weight ( $R^2 = 0.15$ ,  $F_{1,10} = 1.57$ ,  $P > 0.2$ ).

### Experimental pen mortality (hook & line-control)

The 72 h post hook-and-line percent-mortality (23.6) did not differ significantly from that found in otter-trawl (28.7) (chi-square;  $X^2_{15} = 1.19$ ,  $P > 0.1$ ) (Fig. 3). However, the mortality rate solely for trawl-caught males exceeded that for hook-and-line caught males (37% vs. 23.6%) (Table 1). However, this difference was not statistically significant ( $X^2_1 = 1.84$ ,  $P > 0.1$ ). Mean body-length of live males was significantly greater than that of dead males (independent-samples  $t$  test:  $t_{50} = 3.15$ ,  $P < 0.003$ ) implying a potential size-effect on mortality (Table 1).

### Post-capture and post-72-hour vascular acidemia/alkalemia

When accounting for the size of dogfish (covariate), significant differences in  $\text{pH}_v$  values existed between capture modes (independent of time) and due to the depressed post-trawl values, between times (independent of capture mode) (Fig. 5 and Table 2). However, the significant interaction between capture-method and time (Table 2) is indicative of the recovery in  $\text{pH}_v$  within trawl-caught dogfish following 72 h pen trials, such that differences between trawl and hook-and-line are not independent of time (Fig. 5). While accounting for dogfish-size, pairwise comparisons revealed that the post-capture  $\text{pH}_v$  of trawled dogfish differed from  $\text{pH}_v$  at all three of the other sampling times (Fig. 5) while comparisons among the other three sampling times revealed similarities: the  $\text{pH}_v$  of hook-and-line and trawl caught dogfish were statistically similar to each other and to post-capture (hook-and-line

only) levels following 72 h pen trials in the full model (Fig. 5). This implies physiological maintenance (for dogfish initially landed by hook- and-line) and recovery (for dogfish initially landed by trawl) while housed in pens. When assessing trawl and hook-and-line pens collectively, surviving dogfish mean  $pH_v$  was not a predictor of 72 h percent-mortality in a given pen trial ( $P > 0.5$ ) (Fig 2.6).

## **Discussion**

Although sea pens have been heavily utilized in mortality investigation at sea (e.g. Suuronen et al. 1996; Milliken et al. 1999), it is very difficult to distinguish whether capture or pen-related stressors are the greater contributors of mortality. The strongest indicator that the pens affected mortality in the present study was the similar rates found between trawl and the hook-and-line caught dogfish despite the seemingly benign nature of the latter (see below). Within pens, factors enhancing mortality were predicted to be equal or greater in magnitude than those inhibiting it. Despite anticipated pen influences, it was therefore believed that post-trawl discard mortality in the pens would serve as a liberal estimate of mortality under actual conditions.

The post-release mortality of the present study (28.7%) is similar to that found in Chisholm's 2003 study (24.9%); both figures are lower than the 50% currently estimated in the fishery (NEFSC 2003). However, the observed gender specific differences (38% male vs. 21.7% female) would support variability in trawl-induced mortality if predominantly capturing one gender over the other. The limited discard mortality studies involving elasmobranchs captured by mobile-gear have reported mostly low but slightly mixed degrees of mortality. A fairly significant (~40%) number of individuals from eight ray species expired immediately after being captured by trawl (Laptikhovsky 2004). Conversely, Revill et al. (2005) reported only a 2% 48 h mortality rate for lesser-spotted dogfish (*Scyliorhinus canicula*) following capture by beam trawling. Rodriguez-Cabello et al. (2001) reported 22% mortality for the same species in the short aftermath of otter-trawl capture, an exceptionally low rate given the prolonged tow-times (3-6 h) used in this study. Although these studies used on-deck holding pens and similar capture modes between them, results should be compared with caution as major differences existed regarding methods and post-capture durations, such as sort-times and mortality monitoring periods.

Although tow-weight and length could positively predict for percent-mortality within an associated pen, tow-weight was a relatively stronger predictor of mortality (Fig. 4A). Longer tows have been associated with greater catch mortality in several teleosts such as Pacific halibut (*Hippoglossus stenopolis*) (Richards et al. 1995). As it was not intended as a primary study variable, tow-times were not widely diverse and relative to tow-weight, the comparatively weaker predictor of dogfish mortality. Although, longer tows should intuitively induce greater harm, the weaker relationship is partly attributable to the small range in tow-times used. Further, as displayed by the inability for duration to predict for tow-weight (Fig. 4B), longer tows do not necessarily equate to greater catch weights. When conducting mortality work in the field, many interacting variables (i.e. – the length of dogfish residence times in the mouth of the net or as suggested by Neilson et al. (1989), the uncertainty related to when capture is actually occurring during the course of the tow) contribute to the effects and complicate the ability to isolate individual factors like tow-

length. There is no path to ensure that dogfish are captured in early minutes and may be exposed to more stress if caught in the beginning of a 20-minute tow as opposed to the late stages of a 60 minute tow. This is supported by the variable mortality observed in pens linked with tows of similar durations (i.e. - two tows that persisted for the median time (45 minutes) resulted in very different mortalities of 6.7% and 42.9%). Despite the potential pen-effects, tow-weight was still a positive predictor of mortality (Fig. 4A). Neilson et al. (1989) also cited a positive correlation between halibut mortality and dogfish biomass in a given tow. Inter-tow dogfish biomass didn't vary enough in the present study to assess the extent of physical trauma versus mortality. However, under true conditions, this factor certainly drives greater conspecific and alternate species mortalities in exceptionally packed dogfish tows. Because a heavily packed net will presumably elicit greater damage over time, an effective way to mitigate discard mortality might be to quickly abort a tow if a large skate or dogfish load is discernible.

As blood-sampling is stressful in itself, obtaining samples from 'undisturbed controls' is inherently difficult in vertebrates. It is currently held that dogfish discard mortality following hook-gear capture is 25%, comparatively less than for trawl (50%) or gillnet (75%) capture (NEFSC 2003). Because individuals were exposed to brief hook-times and minimal air-exposure, this capture method was viewed as the most effective means of capturing minimally "stressed" dogfish. The extreme vigor displayed in these dogfish, rapid capture by hook-and-line was presumed to have imposed relatively minimal stress. Indeed, the comparatively high vascular pH values and extreme vigor displayed by dogfish landed by this method attest to its minimal impact. On that basis, post hook-and-line mortality was therefore expected to be less than both the 28.7% found for trawl and the 25% estimate currently in place for hook-caught (i.e. - longline) dogfish discard mortality in the wild (NEFSC 2003). However, mortality rates were similar across all three scenarios. The comparable levels associated with the two capture methods in the present study support the notion that mortality was affected by the pens.

Although the mortality rate of trawled males exceeded that of females (38% vs. 21.7%), the comparatively larger overall size of female dogfish in the study may have been responsible for their resiliency. However, there were no differences in the sizes of live and dead animals (within each gender). Mortality was also similar between females at or exceeding 80.0 cm (considered mature) and those below that threshold. These findings indicate that gender rather than size played a more prominent role in dictating mortality in trawled dogfish. It is therefore conceivable that females captured by hook-and-line would have a lower mortality than the 25% found in males. Preliminary (non-significant) results from this study also indicate that trawled males died at a greater rate (37% vs. 25%) than hook-and-line caught males. The comparable mortality between the two capture methods might have resulted from hook-and-line caught dogfish being predominantly male in the study. By this logic, greater numbers of hook-and-line caught females would have translated into significantly less overall mortality linked with that capture method. Conversely however, size was negatively correlated with mortality in hook-and-line individuals. Along with the fact that the two capture methods are vastly different, it is therefore uncertain whether greater female resiliency involving trawl capture applies to hook-and-line capture.

Minimal work has addressed the differences in fishing induced mortality as a function of gender or size in elasmobranchs. Chisholm (2003) found greater size but not gender to



decrease discard mortality in trawled dogfish. Smaller size has also been shown to enhance mortality in several trawled teleosts monitored in sea pens. These include lingcod (*Ophiodon elongatus*) (Davis and Olla 2002), haddock (*Melanogrammus aeglefinus*) (Sangster et al. 1996), Pacific halibut (Richards et al. 1995), Atlantic herring (*Clupea harengus*) (Suuronen et al. 1996), and Atlantic cod (*Gadus morhua*) (Milliken et al. 1999). It is conceivable that larger animals are better able to cope with the rigors of the trawl and pen confinement, but the small variability in dogfish size likely prevented the observation of this result in the present study. As it has never been documented in an elasmobranch, the significantly lower female mortality exhibited is intriguing and if true in nature, would hold major fisheries implications. However, the negative correlation between size and mortality that has repeatedly been cited in teleosts suggests that it would be premature to dismiss this as factor in mortality of trawled dogfish.

The lack of females captured prohibited gender analyses within hook-and-line. However, male survivors were larger than the male fatalities. Accounting only for the capture process, it is feasible that larger dogfish with inherently greater blood volumes might have been more able to cope with the blood loss associated with hook capture. However, since dogfish (and elasmobranchs in general) tend to clot extremely rapidly (personal observation), observed mortality was more likely a function of the pen influences. Moreover, as the difference in size between live and dead male dogfish was only on the order of 3.0-4.0 cm, statistically significant differences may lack relevance from an ecological and fisheries management perspective.

Relative to values from hook-and-lined individuals, blood pH in trawled dogfish was greatly depressed in the present study. This was a likely consequence of both anaerobic and aerobic exhaustion as well as ventilatory inhibition experienced in the trawl net and during on-deck air-exposure. Anoxia and exhaustion from sustained swimming and net entrapment respectively are among the many stressors that have been linked to capture by towed gear in fishes (Davis 2002). These factors, which induce lactic acidosis and hypercapnia, drive synergistic decreases in extracellular pH through metabolic ( $[H^+]$  elevation and  $[HCO_3^-]$  depression) and respiratory ( $pCO_2$  elevation) acidosis (Heisler 1988). Despite the likelihood that the specific moment of dogfish capture varied among individuals and tows, post-capture  $pH_v$  was similarly depressed across dogfish. This suggests that  $pH_v$  became depressed relatively rapidly. While immediately discernable drops in blood pH have been reported in exhaustively exercised (Piiper et al. 1972) and confined (Cliff and Thurman 1984) elasmobranchs of comparable stature to spiny dogfish, delayed responses have also been reported in response to hypoxia (Butler et al. 1979) and sustained periods on a hook (Hoffmayer and Parsons 2001). Compensatory alkalinity resulting from an enhanced ventilation volume was cited as a possible explanation for these delays. Regardless, the magnitude of  $pH_v$  declines found presently in trawled dogfish was greater than that in other studies, indicating that trawling stress was especially acute in this species.

The comparatively elevated post-capture  $pH_v$  in hook-and-line caught dogfish likely reflected minimally stressed levels. This is supported by the similar  $pH_v$  levels attained in dogfish subsequent to 30 days in captivity (chapter three). As  $pH_v$  increased substantially in trawled dogfish that survived the 72 h trials, individuals were able to correct capture-induced disruptions in blood acid-base balance despite being held in pens. This indicates a strong ability to recover and further supports the stressful nature of trawl capture relative to being

housed in pens. However, it also suggests that dogfish are capable of tolerating large shifts in blood pH. In fishes, potentially lethal shifts in intracellular pH are avoided at the expense of more extreme changes in extracellular levels (Milligan and Wood 1986). The low mortality associated with dogfish that had exhibited such depressions in  $pH_v$  indicates that dogfish absorbed a majority of the stress in recoverable downward shifts in blood pH. This has also been observed in trawled dogfish that resolved  $pH_v$  disturbances during transport and protracted captivity (chapter three).

Despite observed pen fatalities,  $pH_v$  in surviving dogfish caught by both hook-and-lined and trawled dogfish was in a mostly resolved state. Dogfish that did not succumb were seemingly able to recover. Coupled with the fact that surviving dogfish  $pH_v$  did not predict for mortality in associated pens (Fig. 6), pen fatalities appear to have been of random nature. Due to the low mortality and marked recovery in  $pH_v$  levels observed in survivors, vascular pH does not appear to be a reliable predictor of mortality. A lack of correlation between blood values and mortality has also been reported in the sablefish, *Anoplopoma fimbria* (Davis et al. 2001).

The 72 h post-capture period utilized here is comparable to the 96 h said to be adequate for monitoring short-term mortality in trawl caught animals (Wassenberg and Hill 1993). Although this window likely accounted for short-term fatalities, the fate of dogfish beyond 72 h remains uncertain. Delayed sub-lethal effects may enhance discard mortality (see Davis 2002). These factors include: greater vulnerability to predation (Davis and Olla 2001), delayed infection (Neilson et al. 1989) and decreased immune performance (Lupes et al. 2006). Reproductive implications are also assumed but very difficult to document or track. Physical maladies from capture may not be immediately consequential or apparent. Integumentary lesions in dogfish produced by interactions with gear and/or the catch have failed to visually materialize until days post-capture (personal observation). Whether or not they are conspicuous immediately, such lesions might instigate secondary infection and unaccounted mortality. Similarly, cryptic internal injuries may eventually cause mortality in periods beyond those monitored in the study. Mortality beyond 72 h has been reported in several post-capture studies on teleosts (e.g. Suuronen et al. 1996; Kaimmer and Trumble 1998).

Other factors relate to the consistent (controlled and uncontrolled) fishing regiment employed in the study versus what might occur during actual conditions. Importantly, the mortality observed presently did not account for prolonged deck-times or aggressive handling. Due to species-specific intolerances to air-exposure, increased time-on-deck has been linked to greater teleost discard mortality in several studies (see Davis 2002). Present conclusions also assume moderately packed trawls and 45-60 minute tow-times and did not account for the impacts of immensely large dogfish catches that occasionally occur in the commercial trawl industry. Because elasmobranchs possess cartilaginous skeletons and lack rib cages, their internal viscera and musculature is vulnerable when physically compacted (Smith et al. 2004). In extreme cases, physical trauma likely supercedes physiological disturbance as the primary source of immediate and delayed mortality. These factors can greatly influence the fate of discarded individuals and should be considered when assessing post-release discard mortality in dogfish as well as alternative species.

A final source of unaccounted mortality involves the study's inability to account for cod-end escapees. Although smaller individuals were likely held in the trawl net for short durations, the study has demonstrated that tow-weight appears to take precedence over tow-length. Juveniles are less robust and significantly more vulnerable in a trawl net. The low post-capture mortality observed may not apply to dogfish measuring less than 60.0 cm.

*Conclusion:*

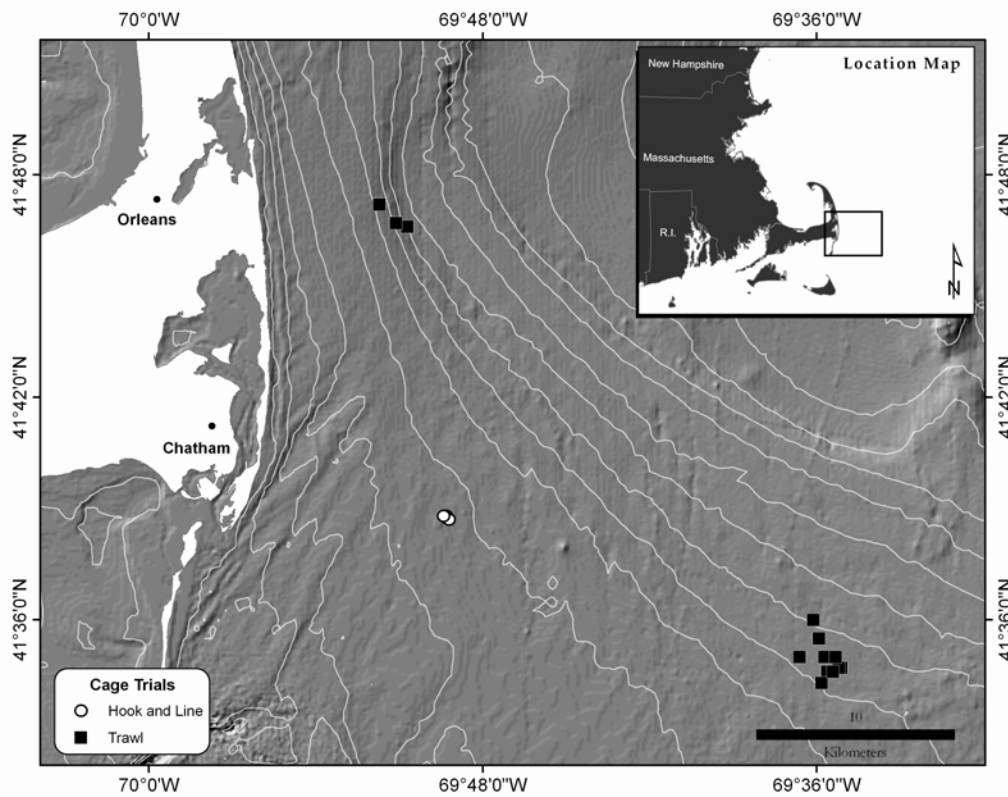
Discard mortality in dogfish fell below the 50% presently estimated following trawl capture in the wild. It can be concluded from the relatively low percentage of dying animals following the 72 h period and the acid-base recovery demonstrated by survivors that spiny dogfish are able to survive at a high percentage when faced with routine trawl-capture stress. Additional work should address the effects of greater fluctuations in tow-times, tow-weights, seasonality, (air and seawater) temperatures, air-exposure (in the lab and the field), capture modes (i.e. – gillnetting) and physical and behavioral indices as primary study parameters. The apparent influence of gender on dogfish mortality also requires additional investigation under more controlled settings. For studies seeking to assess mortality through the use of holding pens at sea, the integration of a camera system is highly recommended in order to document occurrences potentially influencing mortality or physiology. As dogfish have demonstrated the ability to quickly acclimate to round seawater holding tanks (personal observation), the utilization of round as opposed to more narrow rectangular holding pens are also endorsed if monitoring motile species post-capture.

<b>Factor</b>	<b>Male- Trawl</b>	<b>Female-Trawl</b>
Size (overall)	75 (.54) <sup>a</sup>	78.7 (.47) <sup>b</sup>
%-Mortality	38 <sup>a</sup>	21.7 <sup>b</sup>
Size (live)	75.2 (1.1)	78.9 (.59)
Size (dead)	74.9 (.7)	78 (.57)
	<b>Male- Hook &amp; Line</b>	
Size (overall)	76.01 (.57)	N/A
%-Mortality	23.6	N/A
Size (live)	76.67 (.44) <sup>*</sup>	N/A
Size (dead)	73.92 (.75) <sup>*</sup>	N/A

**Table 1.** The size (Mean (+/- SEM), gender and mortality differences both within and between capture methods. %-survival represents pooled pen-trial data for each gender within a particular capture-mode. Superscript (\*) in horizontally adjacent cells represent significant differences (P < 0.05) within gender. Different superscript letters in vertically adjacent cells represent significant (P < 0.05) differences between genders.

<i>Effect test</i>	<i>df</i>	<i>Mean Square</i>	<i>F</i>	<i>P</i>
Capture Mode	1, 76	0.3	30.3	< <b>0.0001</b>
Time	1, 76	0.17	17.44	< <b>0.0001</b>
Dogfish size	1, 76	0.01	1.42	> 0.2
Capture Mode*Time	1, 76	0.32	32.37	< <b>0.0001</b>
Animal Size*Capture Mode	1, 76	0.02	1.2	> 0.1
Time*Animal Size	1, 76	0.004	0.38	> 0.5
Time*Animal Size*Capture Mode	1, 76	0.007	0.72	> 0.3

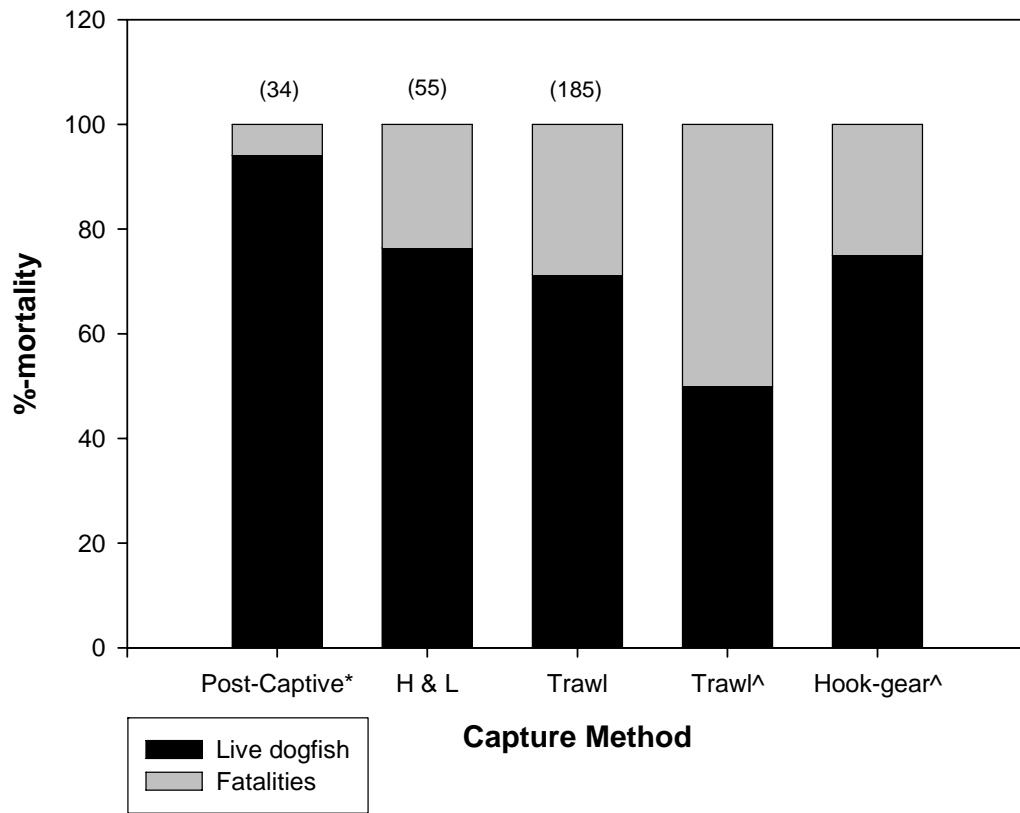
**Table 2.** Two-way (full-factorial) ANCOVA table describing the effects of time (post-capture and post-72h pen) and capture-method (trawl and hook-and-line) on vascular pH while accounting for dogfish-size (covariate). See Figure 2.5 for results of multiple comparisons between means (Tukey HSD).



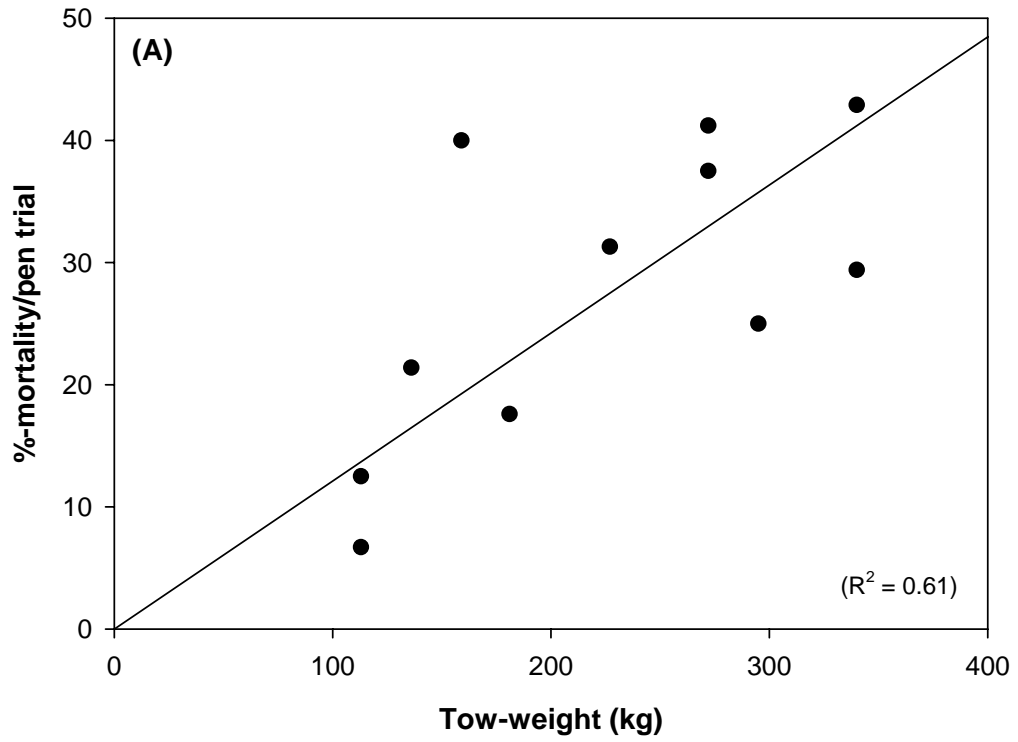
**Fig. 1.** Map of the experimental region. The clustering of trials was not a function of the experimental design and had no impact on mortality.



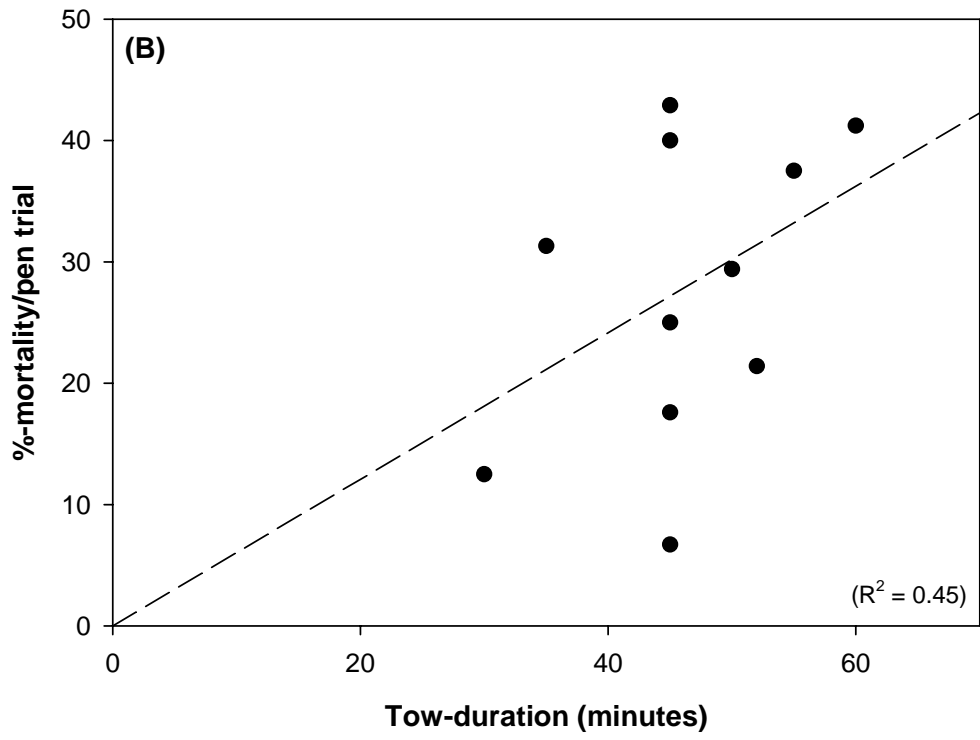
**Fig. 2.** Photograph (unmodified but reduced to page scale) of an experimental pen just after construction. Measurements are 3.7 x 1.2 x 1.2 m.



**Fig. 3.** Plot of percent-mortality for post-captive (\* = Chapter three); post-72h hook-and-line and trawl and estimated discard mortality rates for trawl and hook-gear (^ = Northeast Fisheries Science Center (NEFSC) 2003).

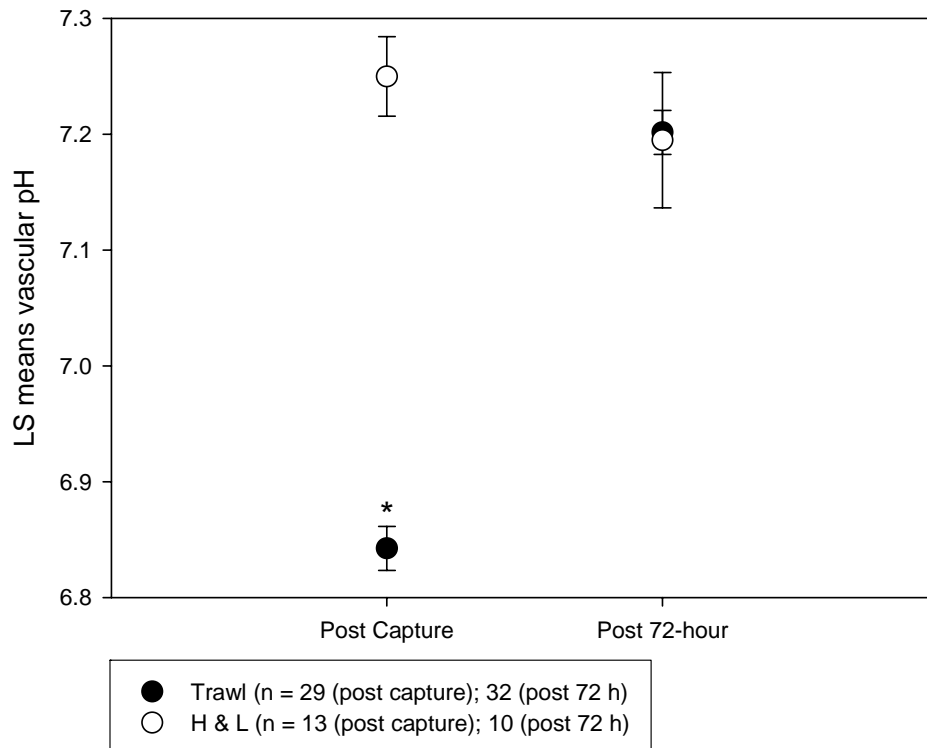


**Fig. 4A.** Tow-weight as a predictor of percent-(72h) mortality in associated pens following trawl capture. See Figure 2.4B. caption on pg. 53 for more details.

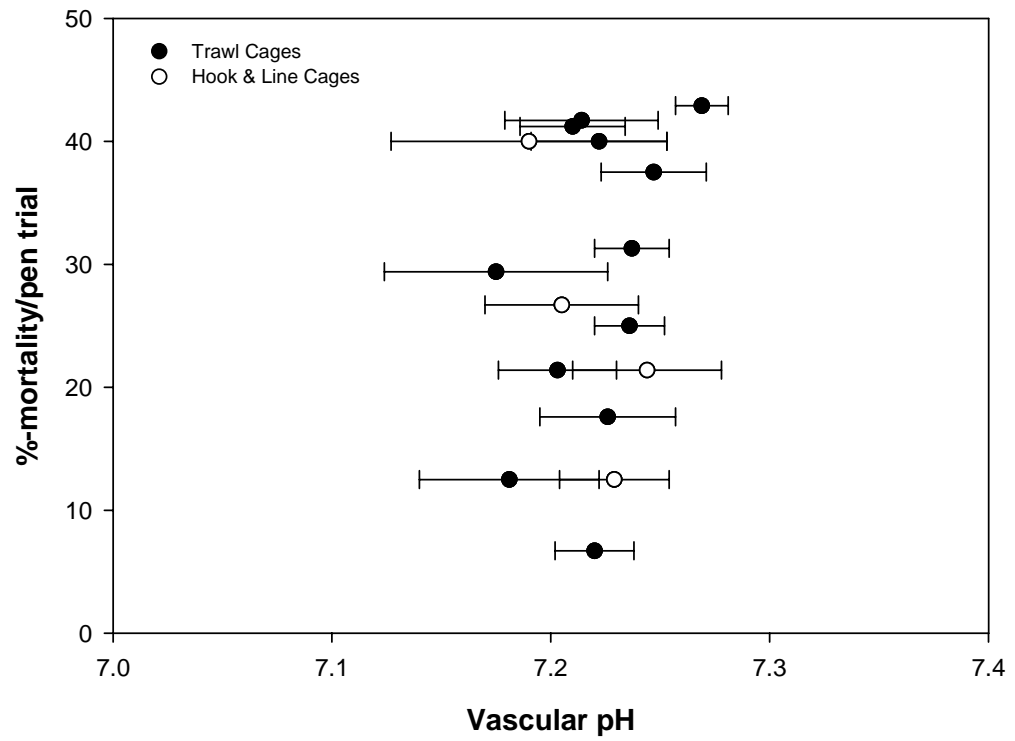


**Fig. 4B.** Tow-duration as a predictor of percent-(72h) survival in associated pens following trawl capture. For 4A & B, multiple regression ( $R^2$ ) = 0.65. These two factors could collectively predict for mortality. See text for additional multiple regression data.





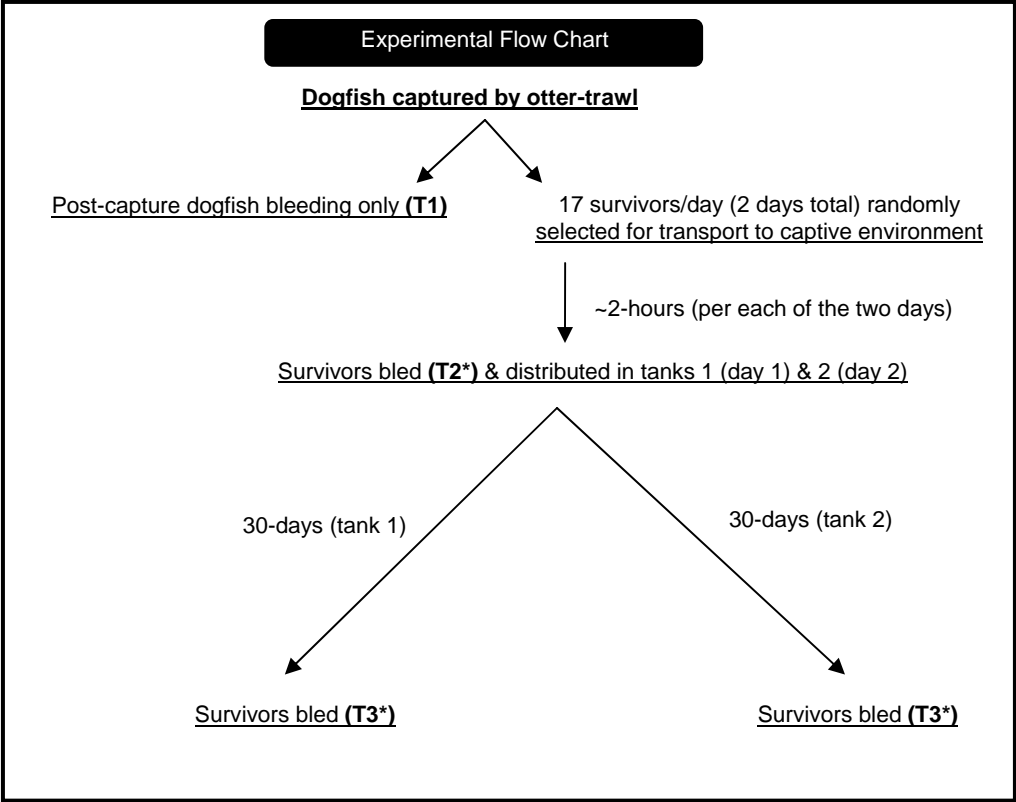
**Fig. 5.** Least squares mean ( $\pm$  SEM) vascular pH for both capture modes (trawl and hook and line) across both sampling intervals (post-capture and post-72 hour (pen)) when accounting for dogfish-size (covariate) in the full model (see Table 2). (\*) represents significant differences with remaining data points ( $< 0.05$ ; Tukey HSD) which were similar among each other.



**Fig. 6.** Mean vascular pH ( $\pm$  SEM) of surviving dogfish as a predictor of mortality within pen trials ( $R^2 = 0.03$ ).

## **Appendix B**

Experimental protocol for Appendix B (\* = same animals).



# THE PHYSIOLOGICAL STATUS AND MORTALITY ASSOCIATED WITH OTTER-TRAWL CAPTURE, TRANSPORT AND CAPTIVITY

## **Abstract**

To assess the physiological responses and associated mortality in spiny dogfish (*Squalus acanthias*) following otter trawl capture, blood samples were obtained at three sampling intervals; capture (T1) transport (T2); and captivity (T3). The results indicate that marked differences existed in blood physiology at each sampling interval. Acid-base parameters (vascular pH, pO<sub>2</sub>, pCO<sub>2</sub>), serum Ca<sup>++</sup> and Cl<sup>-</sup>, and hematocrit were maximally disrupted at T1 but progressively resolved to baseline values by T3. Concentrations of whole-blood lactate, plasma total protein, additional sera electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>++</sup>) and BUN (urea) were maximally compromised at T2 but recovered by T3. In contrast, serum glucose levels remained elevated at all three sampling intervals. Although blood parameters were substantially altered, dogfish mortality was low (2/34; 5.9%) suggesting a high degree of resiliency to compounded stressors associated with capture, transport and captivity.

## **Introduction**

The spiny dogfish (*Squalus acanthias*) is a coastal squaloid shark with a range extending from Labrador to Florida in the Northwest Atlantic (Sosebee 2000). Like most elasmobranchs, this species exhibits K-selected life history characteristics, which include slow growth, maturity late in life and low fecundity (Nammack et al. 1985; ASMFC 2002). In response to heightened fishing pressure in the Northwest Atlantic, mature female stocks have reportedly declined by 75% between 1998 and 2003 precipitating an associated 1997-2003 absence in recruitment (ASMFC 2002; NEFSC 2003). Low trip limits and limited commercial value have also led to consistently high amounts of dogfish discarded in the Northwest Atlantic commercial fishing industry. Thus, post-release mortality holds major implications regarding the species' stocks and associated management. For example, such data can provide insight regarding a species' capability to recover following a particular form of capture- important when assessing how populations are affected if heavily landed and discarded as bycatch.

Although many studies have investigated the individual and/or collective physiological responses to capture/handling, transport and confinement stress in salmonids and additional teleosts (see Barton et al. 2003; Sulikowski and Howell 2003), fewer have done so with elasmobranchs (Cliff and Thurman 1984; Torres et al. 1986; Denton et al. 1987; Smith 1992). Moreover, no investigation to date has addressed the physiological resiliency of dogfish or additional elasmobranchs related to the rigors of catch and release and no study has documented the post-capture physiological plight of an elasmobranch landed by mobile-fishing gear. In order to gain a greater understanding of dogfish resiliency and physiological stress responses, a sample of trawl-captured dogfish were transported, held captive for 30-days, and assessed for physiological status and mortality following the completion of each study phase.

## **Methods**

### **Animal collection**

Otter-trawling was conducted southeast of Chatham Inlet (41°38"O'North x 69°48"O'West), aboard Fishing Vessel (FV) Joanne A III (Chatham, MA). Depths ranged from 50-65 meters (m) on cobble and sand bottom, with 16.0-17.0 °C surface water temperatures. In

accordance with routine vessel practices, fishing utilized a Danish bottom trawl with an 18.0 mm mesh-size, 305 meshes in the head-rope and foot-rope collectively (fishing circle), a hard-bottom sweep to avoid boulders on the substratum, and 4.6 m legs. All dogfish in the study ranged between 69-87 cm (with the majority 72-84 cm) and were primarily female (85%). Dogfish caught simultaneously across two days (September, 2004) and six 45-minute moderately packed (~270-300 kg) otter-trawls were randomly selected for one of the following two treatments described independently as follows:

### **A) Post-capture physiology (T1)**

To enable the determination of blood values while minimizing the number of required samplings from the transported individuals, surrogate dogfish (n = 33) randomly selected from the same tows were bled to gain physiological indices of post-trawl status (Time (T) 1) and to develop a comparative basis for blood physiology exhibited following subsequent bleeding regimes. Dogfish were bled and assessed for morphometric data and gender during 10-minutes maximal post-capture intervals on-deck. Multiple phlebotomists enabled sampling to ensue while exposing animals to similar periods of air-exposure.

### **B) Dogfish to undergo transport and captivity**

Concurrently, dogfish (n = 34) were randomly selected for transport and placed in one of two on-deck square holding pens measuring as follows: 1) 1.2 m x 0.9 m and 1.4 m deep with a ~700 l seawater capacity; 2) measured 1.85 m x 1.1 m and 0.9 m deep with a ~1000 l seawater capacity. Once the targeted number (n = 17) of dogfish was obtained each day, the one-hour return steam to port ensued. During this segment, the tanks were continuously flushed with ambient surface seawater (~16.0-17.0°C) through the Vessel's deck-hose. The dissolved oxygen saturation % (DO) ranged between 90% and 104%.

### **Transport component and T2**

Flexible vinyl stretchers were used to carry dogfish in a horizontal position from the on-deck holding tanks to the awaiting truck as documented by Smith et al. (2004). The deck-hose was used to fill the truck tank. Unusually high harbor water temperatures resulted in a comparatively elevated tank temperature on day two (18.4- 21.0°C). Land transport to the captive facility was conducted using a New England Aquarium (NEAq) Fishes Department (Boston, MA) transport truck containing a 2.0 m diameter, 1.1 m deep, circular tank with a seawater capacity of ~2650 l. By manipulating delivery of pure-O<sub>2</sub> to the seawater via air stones, DO levels of ~105%- 115% were maintained in accordance with previously successful short-distance transport of the sandtiger shark, *Carcharias* or *Odontaspis Taurus* (Holly Martel-Bourbon- personal communication). Throughout the two-hour period between dogfish deposit and removal from the truck tanks at the captive facility, DO and temperature were recorded every 10 minutes using a portable O<sub>2</sub>-probe. NH<sub>3</sub> levels were negligible during both transport days.

Upon arrival at the Marine Biological Laboratory (MBL) (Woods Hole, MA) and prior to tank placement on both days, each dogfish was blood sampled (T2) and tagged using T-bar vinyl anchor tags (Floy Tag, Inc. - 4616 Union Bay Place NE, Seattle, Washington USA 98105). This represented post-transport values and would enable the tracking of physiological changes and fatalities in specific dogfish over the 30 days. Each of the two transport days corresponded with its own captive holding tank for dogfish.

### **Captive component and T3**

Dogfish were held for 30 days in circular holding tanks (3.0 m diameter/0.9 m deep and 3.7 m diameter/0.8 m deep respectively) in the Marine Resources Center (MRC). 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  $\sim 13$ - $14^{\circ}\text{C}$ . Water quality monitoring was conducted by the facility and tanks possessed negligible ( $\sim 0$  PPM)  $\text{NH}_3$ -, nitrite- and nitrate-levels throughout. The feeding regimen was maintained at 2.0 l of squid/capelin per tank  $\sim$ three times a week. Although the species possesses relatively depressed metabolic rates (Brett and Blackburn 1978), dogfish began feeding on day 1. During the thirty days, the investigators conducted weekly checks to monitor status, behavior and potential fatalities. At the 30-day endpoint, surviving dogfish were blood sampled for a final time (T3).

### **Blood sampling and processing**

Blood samples ( $\sim 5.0$  ml) were obtained by caudal veni-puncture, a method described as relatively non-stressful for elasmobranchs (Cooper and Morris 1998), utilizing methods documented in chapters one and two. Briefly, bleeding was conducted using Fisher non-heparinized 18-20 gauge 0.04 m stainless steel syringe needles fitted to 5.0 ml plastic (Beckman Dickinson) syringes. Upon drawing a sample, needles were immediately plugged with cork (to slow diffusive gas loss) until reaching the ISTAT portable clinical analyzers (within 10 seconds) where it was removed. Twenty  $\mu\text{l}$  of whole-blood was then deposited into specialized (CG4+) cartridge from which the ISTAT calculated vascular pH ( $\text{pH}_v$ ) and the partial pressures of oxygen ( $\text{pO}_2$  (mm x  $\text{Hg}^{-1}$ )) and carbon dioxide ( $\text{pCO}_2$  (mm x  $\text{Hg}^{-1}$ )).

The remaining blood sample (slightly  $< 5.0$  ml) was then evenly distributed between three types of vacutainer tubes ( $\sim 1.6$  ml blood per tube): 1) lithium-heparin coated (“green-top”); 2) dried EDTA-coated (“purple-top”); and 3) non-heparinized (“red-top”). Micro-hematocrit tubes were filled in triplicate with whole-blood via capillary-action from (1), packed with hematocrit and stored on ice (Biron and Benfey 1994) for on-site analysis of whole-blood hematocrit (see assay description below). To deproteinate blood from (2), 0.5 ml of whole-blood was added to 1.0 ml of ice cold 8% perchloric acid. To ensure complete digestion and protein denaturation, this solution was kept on ice for  $\sim 45$  minutes. The (remainder of 1) and the (perchloric acid solution in 2) were then stored on ice while (3) was left to clot at room temperature (60 minutes) until appropriately processed to obtain the following: plasma (from 1); perchloric acid extracts of the haemolysed whole-blood (from 2) and serum (from 3). Protocols for processing and later analyses of these fractions are described in detail below. All references to individual blood parameters in the text correspond to their [concentrations] in those respective mediums.

### ***Anti-coagulated whole-blood***

To determine hematocrit, the micro-hematocrit tubes were removed from ice within one-hour and spun in triplicate at 8,000 x gravity (g) for three minutes. Percent-values were determined (in triplicate) against the scale of a standard microhematocrit reader.

### ***UV Spectrophotometry***

Total soluble protein concentration was obtained from plasma. The “green-top” tubes containing approximately 1.25 ml of whole-blood were spun at 1,400 x g for five minutes.

The supernatant (~ 0.75 ml of plasma) was aliquoted into Fisher cryovial tubes and transported in liquid nitrogen to -80.0°C freezers until assay. Preserved samples were processed in triplicate using a BCA protein assay reagent kit (23225) (*Pierce Biotechnology Inc.*, Rockford, IL) and run in triplicate at wavelength of 562 nanometers (nm) on a *Molecular Devices* (Emax Precision) *Microplate Reader* to obtain optical densities (OD). 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 known standard (standard curve).

The lactate anion concentrations were obtained from perchloric acid extracts of the haemolysed whole-blood (will be designated as whole-blood). The, "purple-tops" were centrifuged at 3000 x g for ten minutes to pellet cellular debris and protein. The supernatant (~ 1.0 ml of whole-blood) was then aliquoted and preserved adhering to same approaches described and plasma. Processing for the assay was conducted enzymatically (*Sigma Diagnostics*, Procedure No. 826-UV, St. Louis, MO) and run in triplicate at wavelength of 340 nm (vis with blue filter) on a *Beckman Coulter* DU-640C UV Spectrophotometer 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).

### ***NOVA blood chemistry analyzer***

Electrolytes ( $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ), glucose and BUN (urea) concentrations were obtained from serum. Clotted blood from red-top tubes was centrifuged at 3,500 x g for five minutes. The supernatant (~ 1.0 ml of serum) was then aliquoted, transported, and preserved by the same methods as used for plasma. For assay, samples were thawed to room temperature and to determine values for electrolytes/glucose and BUN (urea), 50% (sterile-grade de-ionized water) and 10% (0.9 % saline) serum dilutions respectively were run in duplicate on a *Stat Profile Critical Care Xpress (CCX)* blood chemistry analyzer (Nova Biomedical, Waltham, MA).

### **Statistical tests and procedures**

#### *Statistical analyses*

Blood value means among the study's six tows similar (one-way ANOVAs,  $P > 0.1$  for each blood parameter). Blood physiology in dogfish was 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 the study's three sampling moments relative to all blood parameters. Differences and comparisons of means across all three bleeding sessions were assessed using univariate ANOVAs and Tukey pairwise HSD testing for each blood parameter. As individuals bled at T2 and T3 represented repeated measures, paired-samples *t* tests were also conducted for each blood parameter to assess changes occurring over the 30-days. T2 blood values from subsequently deceased dogfish were included in T2 analyses but excluded from paired-samples *t* tests. Linear regression analyses were used to determine whether dogfish-size could predict for the values of blood parameters most extensively correlated with additional parameters ( $\text{pH}_v$ , the lactate anion,



and Na<sup>+</sup>) at the specific sampling times. All analyses were performed using JMP 4.04 Software (*SAS Institute*, Cary, NC). Values are presented as mean ± SEM.

## **Results**

### **Mortality, behavior, and physical appearance**

Only two dogfish fatalities occurred during the entirety of the study (2/34; 5.9% mortality). The remaining 32 dogfish were successfully sampled at T3.

### **Temporal differences in physiological parameters**

Dogfish physiology differed across the three bleeding regimes when accounting for all blood parameters (One-way MANOVA: Wilks' lambda = 0.01,  $F_{26,94} = 28.11$ ;  $P < 0.0001$ ) and as a function of individual blood parameter differences (univariate ANOVAs): protein ( $P = 0.01$ ); pO<sub>2</sub> ( $P = 0.005$ ); hematocrit ( $P = 0.002$ ); all electrolytes, BUN (urea), pCO<sub>2</sub>, pH, lactate, glucose ( $P < 0.0001$ ) differences for the remaining blood parameters.

However, individual blood parameters did not necessarily vary (Tukey  $< 0.05$ ) regarding each pairwise (sampling regime) comparison: vascular pH ( $P < 0.05$  for each set of sampling pairs) was maximally depressed post-capture (T1) and progressively increased to peak levels following captivity (T3) (Fig. 1A). Inversely, pCO<sub>2</sub> ( $P < 0.05$  for each set of sampling pairs) peaked at T1 and progressively sank to its lowest values at T3 (Fig. 1B). Lactate ( $P < 0.05$  for each set of sampling pairs) was maximally elevated following transport (T2) and lowest by T3 (Fig. 1C). pO<sub>2</sub> ( $P < 0.05$  for T2 vs. T3) was similarly depressed at T1 and T2 but highest by T3 (Fig. 1D). Concentrations of K<sup>+</sup> (Fig. 2A) and Na<sup>+</sup> (Fig. 2B) ( $P < 0.05$  for each set of sampling pairs) reached maximal levels at T2 and declined to their lowest by T3. Concentrations of Cl<sup>-</sup> ( $P < 0.05$  for each set of sampling pairs) peaked at T1 and decreased to lowest levels at T3 (Fig. 2C). Ca<sup>++</sup> concentrations ( $P < 0.05$  for T1 vs. T2; T1 vs. T3) peaked at T1 but decreased to stabilize across T2 and T3 (Fig. 3A). Mg<sup>++</sup> concentrations ( $P < 0.05$  for each set of sampling pairs) reached maximal levels at T2 decreasing to their lowest by T3 (Fig. 3B). Inversely, BUN (urea) concentrations ( $P < 0.05$  for each set of sampling pairs) were at their lowest at T2 but peaked at T3 (Fig. 4A). Glucose concentrations ( $P < 0.05$  for T1 vs. T3; T2 vs. T3) were comparably low at T1 and T2 but increased to peak levels at T3 (Fig. 4B). Reaching their maximum at T1, hematocrit levels ( $P < 0.05$  for T1 vs. T3) progressively fell to their lowest at T3 (Fig. 5A). Protein concentrations ( $P < 0.05$  for T2 vs. T3) were similar at T1 and T3 but maximally elevated at T2 (Fig. 5B).

Paired-samples *t tests* revealed that each parameter (minus Ca<sup>++</sup>,  $p > 0.1$ ) changed significantly during the 30 days in captivity ( $P < 0.001$  between T2 and T3). The results and nature of relationships mirrored those found by the Tukey comparisons (see Figures) with the lone exception of hematocrit where the decrease during captivity (T1 vs. T2) was found to be significant ( $P < 0.01$ ) using the paired test but non-significant ( $P > 0.05$ ) by Tukey HSD. Finally, when dogfish's size was regressed against an associated value (pH<sub>v</sub>, lactate, and Na<sup>+</sup>) at all three bleeding times ( $R^2 < 0.08$ ,  $P > 0.2$  for all nine combinations), no significant relationships were detected.

## **Discussion**

Despite being subjected to otter-trawling capture, transport and captivity, dogfish in the present study exhibited a very low rate of mortality (5.9%). This value was well below those

described by Chisholm (24.9%; 2003) and the 28.7% (chapter two) in dogfish following trawl capture and short-term housing in sea pens.

In the present study, blood parameter values (minus glucose) following captivity (T3) mirrored those obtained in “minimally stressed” control hook-and-line caught dogfish (chapters two and four). Despite being 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. On that basis, the (T1) physiology of dogfish was markedly disturbed by trawl capture and relative to certain parameters, continued to be so during transport (T2). The general disturbance in blood parameters was similarly found in a trawled and transported teleost up to five days post-capture (Bourne 1986).

The inhibition of ventilation when tightly confined in the trawl or on-deck has been described as a potential cause of anoxia and death in fishes (Davis 2002). Although not yet studied directly, the consequences of branchial restriction presumably leads to forced hypoventilation and serious disturbances to acid-base balance. Time on deck has also been shown to interrupt normal ventilation in teleosts (Ferguson and Tufts 1992). In the present study, Trawling incited an upward spike in  $p\text{CO}_2$  and massive drop in  $\text{pH}_v$  relative to resting (T3) values in dogfish. These responses were presumably a combined function of net constriction, exhaustive activity and brief periods on deck following capture. Inversely related  $p\text{CO}_2$  increases and blood  $\text{pH}$  decreases have also been reported in other elasmobranchs (Püper et al. 1972; Holeton and Heisler 1983; Cliff and Thurman 1984) as well as teleosts (e.g. Wood et al. 1977; Wood et al. 1983; Schwalme and Mackay 1985a and b; Milligan and Wood 1986; Ferguson and Tufts 1992), subsequent to exhaustive activity and/or capture-stress. However, the magnitude of vascular  $\text{pH}$  difference between highly stressed (T1) and resting dogfish (T3) was more extreme than in the previous studies, an indication that trawling stress profoundly affects this species. It is well established that respiratory disruptions exacerbate metabolic acidosis in exhaustively exercised fishes (Wood 1991). Judging by the elevated lactate and  $p\text{CO}_2$  levels at T1, drops in dogfish  $\text{pH}_v$  were a synergistic consequence of metabolic ( $[\text{H}^+]$  elevation and  $[\text{HCO}_3^-]$  depression) and respiratory ( $p\text{CO}_2$  elevation) acidosis (see Heisler 1988). Despite these shifts,  $p\text{O}_2$  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 down dogfish levels to the extent (T2) where they were significantly different than T3

In the current study electrolyte levels were profoundly impacted by trawl capture and transport. In comparison to T3 levels, concentrations of all five electrolytes were elevated by trawl-capture. This mirrors what occurred following trawl capture in a teleost (Bourne 1986). However, the fact that disruptions in  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Mg}^{++}$  were further aggravated during the transport but still able to recover following captivity indicates that either electrolyte imbalances induced by trawling alone were not particularly extreme, or that dogfish are fully capable of withstanding increases of this magnitude. Exposure to stress profoundly effects salt and water balance in marine fishes (Wells et al. 1986). For example,  $\text{Na}^+$  and  $\text{Cl}^-$  elevations presently observed in stressed dogfish have also been reported in pelagic elasmobranchs (Wells et al. 1986) and teleosts (Fletcher 1975; Wood et al. 1983; Haux et al. 1985; Arends et al. 1999) responding to angling stress but did not change significantly in juvenile dusky sharks following hook-capture and transport (Cliff and

Thurman 1984), when compared to minimally stressed individuals in three gillnet-captured sharks (Manire et al. 2001), and in additional teleosts (Soivio and Oikari 1976; Beggs et al. 1980).

Steady-state dogfish hematocrit levels have ranged from 16.4% (“normal” on the day following surgery in unfed dogfish) (DeRoos et al. 1985); 18.7% (“control” in the lesser spotted dogfish) (Torres et al. 1986); and ~ 20% in hook-and-line caught dogfish (chapter four). In the present study, the T1 hematocrit values of dogfish were significantly greater than those at T3. Studies suggest that erythrocytic swelling (hemoconcentration) may have driven the trawling-induced hematocrit increases observed in the present study. For example, elevations have been reported in both pelagic elasmobranchs and teleosts captured by hook-gear (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 hemoconcentration as the instigator of elevated hematocrits in the present study.

Relative to levels following captivity (T3) in the present study, BUN (urea) levels were negatively affected by capture (and declined further during transport). Although the gills of elasmobranchs are normally highly impermeable to urea loss (Wood et al. 1995), Evans and Kormanic (1985) reported significant urea losses in spiny dogfish pups when exposed to handling, anesthesia, and weighing stresses. Such losses were attributed to increases in urea-permeability and gill epithelial surface areas in response to stress. Such mechanisms might have presently occurred within adult dogfish subjected to trawling and transport stress. Although eventually resolved, the magnitude of BUN (urea) losses in (T1) dogfish further indicates that osmotic balance was heavily compromised by trawl capture.

Although physiological status following capture was established, continued changes observed following transport could have been due to residual or delayed effects from the initial capture. Thus, perturbations in T2 values were considered functions of the capture exacerbated by the transport. This excluded hematocrit,  $Ca^{++}$ ,  $Cl^-$ ,  $pH_v$  and  $pCO_2$  which managed to partially resolve during the transport. The beginning resolutions of  $pH_v$  and  $pCO_2$  were also found to occur following initial perturbations in juvenile dusky sharks remaining confined (Cliff and Thurman 1984). The partial resolution of certain parameter levels in (T2) dogfish just after confined transport indicates that normal ventilation was comparatively more impeded during the trawl experience.

Although the lactate levels of dogfish in the present study were only moderate following trawl capture, they had climbed significantly upon transport completion. The protection of intracellular acid-base balance at the initial expense of extracellular pH has been well described in fishes (see review by 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 (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. For spiny dogfish in the current study, the blood acidemia and moderate lactate levels at T1 implied the onset of lactic acidosis during the trawl capture (along with the respiratory acidemia) but due to the delayed lactate diffusive rates as seen in the previous studies, peak levels were not apparent until T2.

Dogfish  $K^+$  concentrations initially heightened by trawling increased even further during transport. Elevated  $K^+$  concentrations have also been reported in other elasmobranchs subjected to angling stress (Cliff and Thurman 1984; Wells et al. 1986; Manire et al. 2001). In these studies, heightened extracellular levels were attributed to increased efflux (“leakage”) from the intracellular compartment of muscle cells due to intracellular acidemia. The elevated blood  $K^+$  and lactate anion elevations in dogfish suggest that intracellular acidemia occurred to some degree as a result of the capture here. However, the extremely depressed post-trawl  $pH_v$  values insinuate that potentially lethal changes to the intracellular acid-base balance were 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. Thus, if marked blood acidemia was potentially fatal to dogfish in the current study, a greater mortality would have been observed.

The elevated post-trawl levels of  $Ca^{++}$  and  $Mg^{++}$  and the continued heightening of  $Mg^{++}$  during dogfish transport may have been attributable to the capture-induced acidemia. This has been previously referenced in relation to a stressed elasmobranch as it is the case in mammals (Cliff and Thurman 1984). Interestingly, an elevated  $Ca^{++}$  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). Although blood acidemia was pronounced in dogfish, maximal  $Ca^{++}$  concentrations ( $\sim 2.6 \text{ mmol} \times \text{l}^{-1}$  post-trawl) were on the order of two times below those in the previous study and it is not certain whether such levels could substantially affect acidemia. However, the magnitude of  $Mg^{++}$  change induced by the capture in dogfish is similar to that found in captured and transported juvenile dusky sharks where 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 increases in  $Mg^{++}$  as well as  $K^+$  could have participated in the lethargy and apparent tetany exhibited during transport. The in-transport resolution of baseline dogfish  $Ca^{++}$  concentrations was far swifter than in a trawled teleost where it took up to 72 hours to correct perturbations in divalent ions (Bourne 1986).

Similar to the results found in captured, hyperactive and confined juvenile dusky sharks (*Carcharhinus obscurus*) (Cliff and Thurman 1984), dogfish  $pO_2$  values were highly variable following capture and transport. Although decreases observed between T1 and T2 in this parameter were not significant in the current study, the dogfish managed to partially resolve the biologically low  $pH_v$  and  $pCO_2$  levels during the confined transport period. This was similar to resolutions of blood acidemia following 72-hour pen trials in trawled dogfish (chapter two), a confined setting of somewhat parallel nature. Depressions in hematocrit via hemodilution have reportedly occurred in response to extreme confinement stress in lesser spotted dogfish (Torres et al. 1986).

As indicated by the resemblances of T3  $pH_v$  and blood gas values to those found upon rapid hook-and-line capture (chapters two and four), dogfish managed to resolve acid-base disruptions during the course of the 30-days in captivity. Judging by the partial resolutions during the transport and the rapid rate (within 14-24 hours) in which other elasmobranchs have been shown to recover following rigorous activity (Piiper et al. 1972; Holeton and Heisler 1983; Cliff and Thurman 1984), routine acid-base status was likely restored early-on

during the 30-day captive period. The fact that dogfish were already correcting disruptions during the transport (~ 1-3 hours post-capture) alludes to an even swifter recovery than found in those other studies and in commonly studied teleosts like the rainbow trout (Milligan 1996). As vigorous activity was unapparent, environmental O<sub>2</sub> was not a limiting factor and ventilation presumably returned to normal, lactate anion values were also negligible at T3.

Concentrations Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> also returned to assumed steady-state during the captivity. This resembled the captive recovery of monovalent ions after initial post-capture/transport elevations in a teleost (Bourne 1986). Although dogfish K<sup>+</sup> levels (Fig. 2A) approached the reported threshold for myocardial disruption (7.0 mmol x l<sup>-1</sup>) following the cumulative stress of capture and transport (Cliff and Thurman 1984; Wells et al. 1986), these levels recovered to “resting” levels by T3. Interestingly, the directionality of K<sup>+</sup> shifts was inversely related to that of glucose shifts in the present study. Depletions in blood glucose have been cited as one of the potential causes of increasing K<sup>+</sup> in elasmobranchs (Manire et al. 2001). In a teleost, K<sup>+</sup> has been shown to be significantly higher in dying animals than in survivors following exhaustive activity (Wood et al. 1983). For those survivors, K<sup>+</sup> concentrations returned to baseline levels within 12 hours of activity. Post-transport (T2) K<sup>+</sup> concentrations for the two dogfish that subsequently expired in the present study were similar to those of survivors.

In the current study, glucose values were low following capture and transport relative to those after 30 days in captivity. This was the only blood parameter in which post-captivity levels failed to mirror those found in hook-and-line caught dogfish (chapter four). The onset of hyperglycemia in response to exhaustive exercise, air exposure; capture/transport, induction through injection (of catecholamines) or handling, and/or confinement stress has been cited 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. Wells et al. 1986; Torres et al. 1986; Hoffmayer and Parsons 2001). In contrast, hypoglycemia 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 hypoglycemia in response to trawling and transport in the present study. However, T3 concentrations were approximately 40% higher than found in hook-and-lined dogfish (chapter four), 20% higher than concentrations found at T1 and similar to those at T2. Relative to baseline hook-and-line values, this indicates a hyperglycemic response to trawling and subsequent recovery during transport. 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 require feeding every two weeks (DeRoos et al. 1985). Thus, 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 (Hoffmayer and Parsons 2001).

Despite the progressive drops in BUN (urea) observed at T1 and T2, levels analogous to those from hook-and-line caught dogfish (chapter four), the concentration of this solute had returned following 30-days in captivity. Although the magnitude of the initial declines appeared great, they were rectified and significant shifts did not appear deleterious to the

sharks. However, as it the primary organic osmolyte in marine elasmobranchs, the effects of stress on blood urea concentrations and implications of resulting fluctuations are areas warranting greater investigation.

*Conclusion:*

Trawling and transport induced marked physiological changes in the blood of spiny dogfish. Despite this, mortality levels in the study remained negligible and post trawling recovery of blood acid-base disturbances appeared to begin prior to completion of transport. Results also indicate that any delays in the recovery of other physiological parameters (i.e. – 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 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 resiliency of dogfish and other commonly captured (and discarded) elasmobranchs in both the field and captive environments. In particular, investigation into differential capture-methods, the effects of gender and size-class on resiliency as well as correlations between physical trauma and physiological status, are recommended. Continued investigation into the fates of dogfish and additional elasmobranchs discarded as bycatch is also needed across the most relevant fisheries.

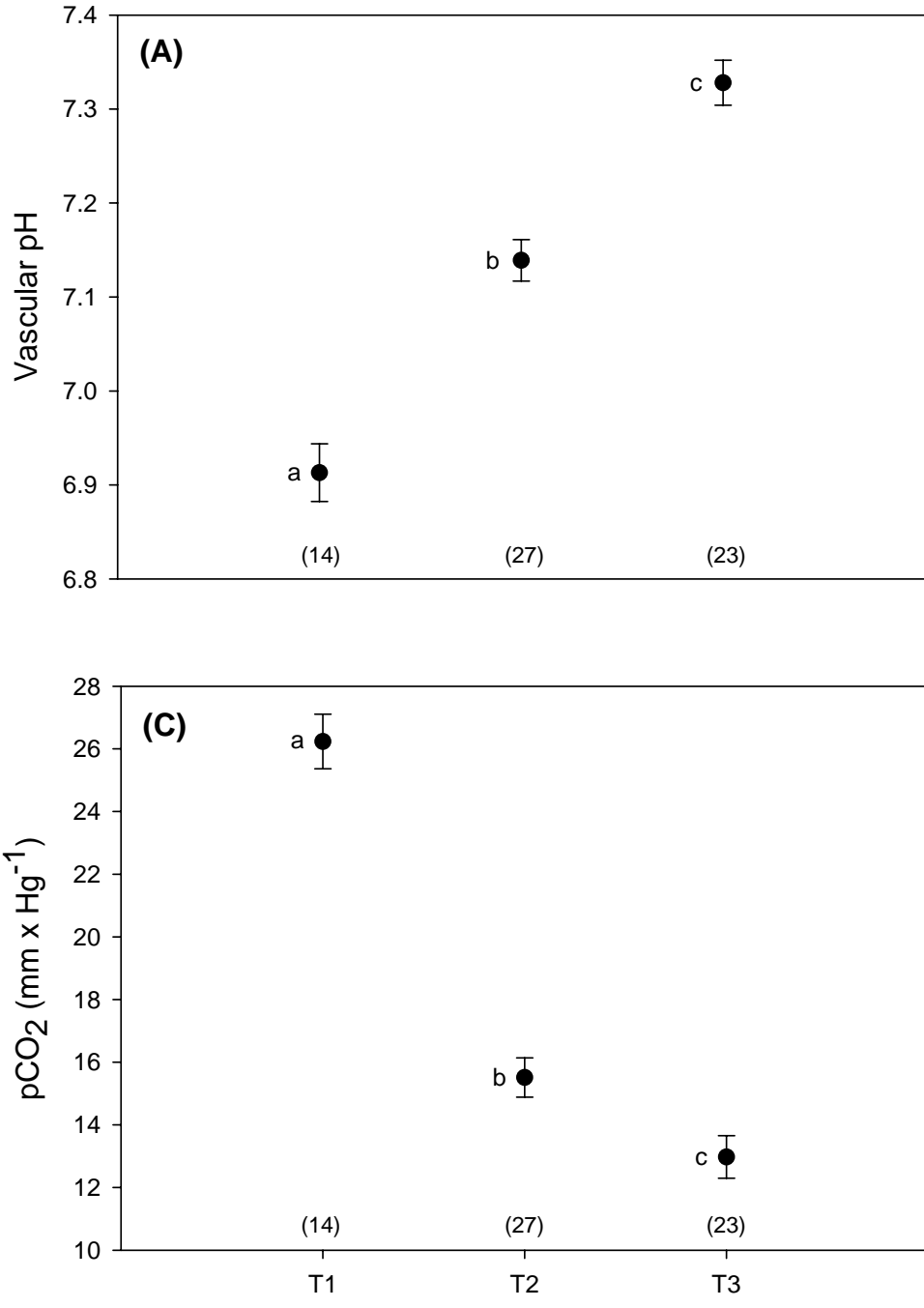
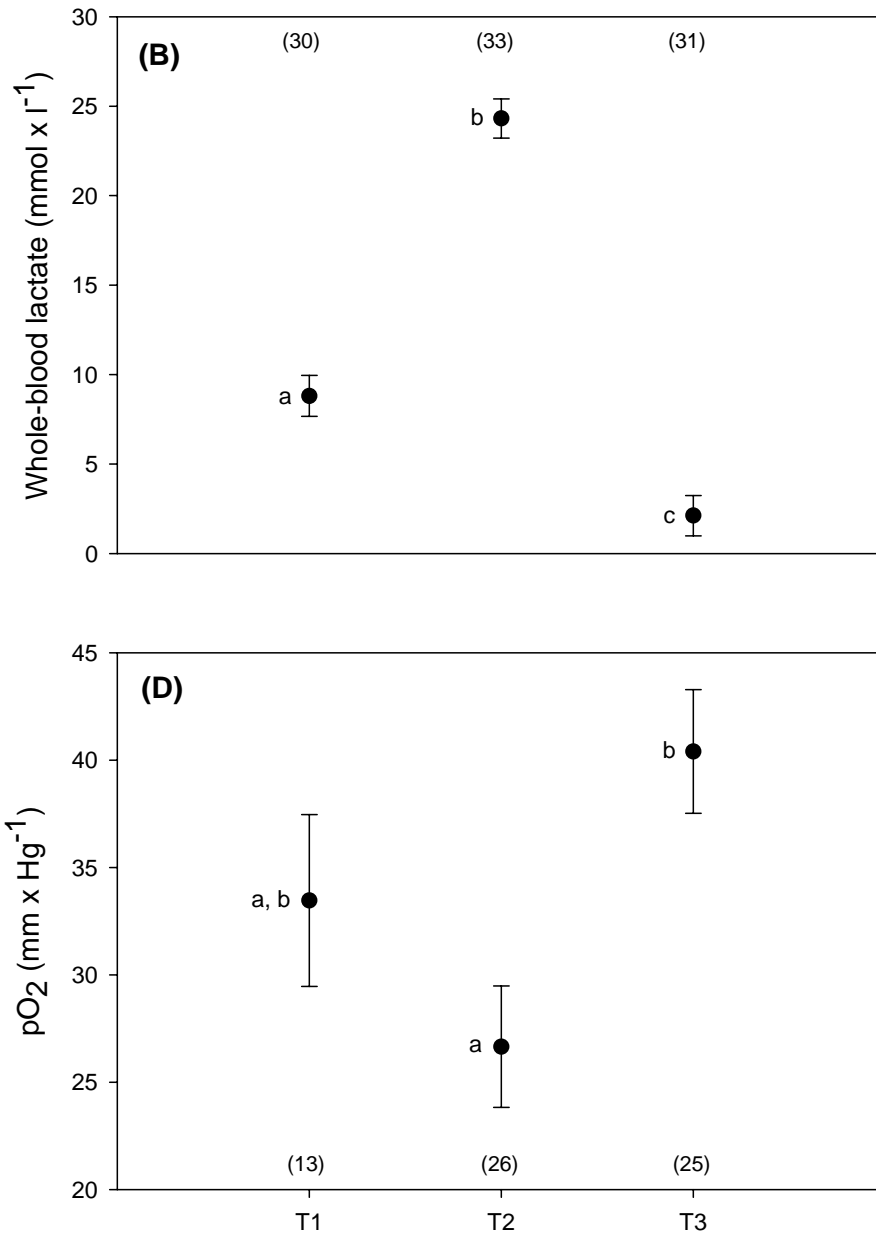
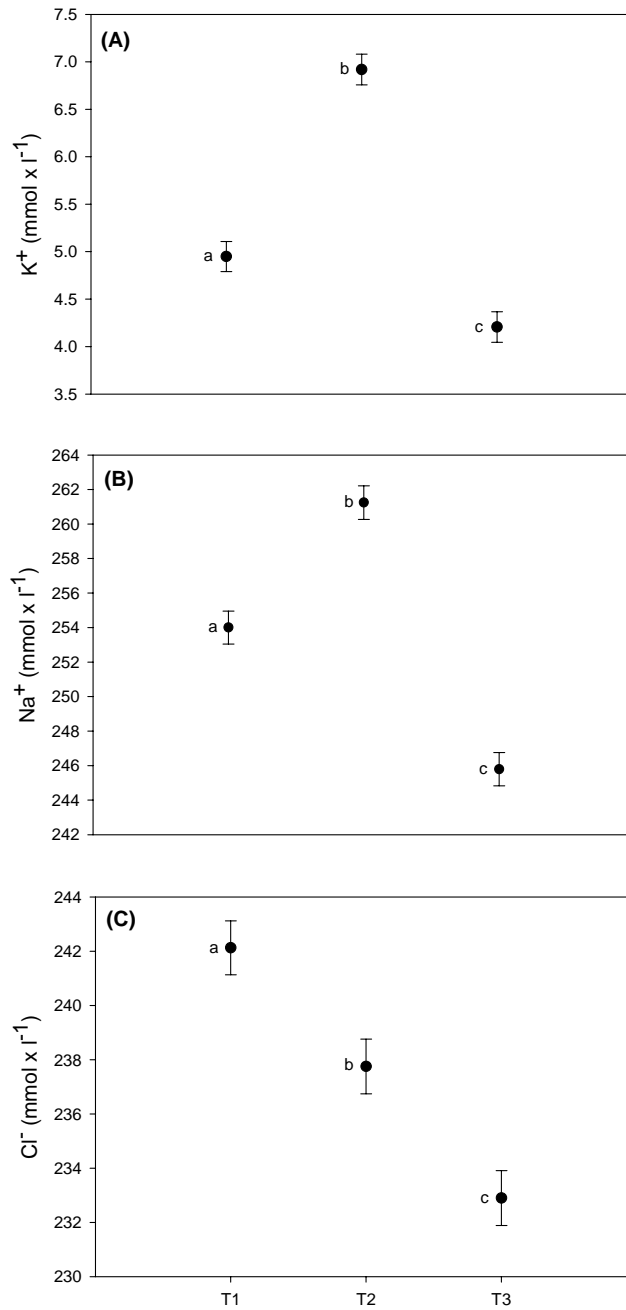


Fig. 1. A) vascular pH & C) pCO<sub>2</sub>. See Figure 1B. & D. caption for details.

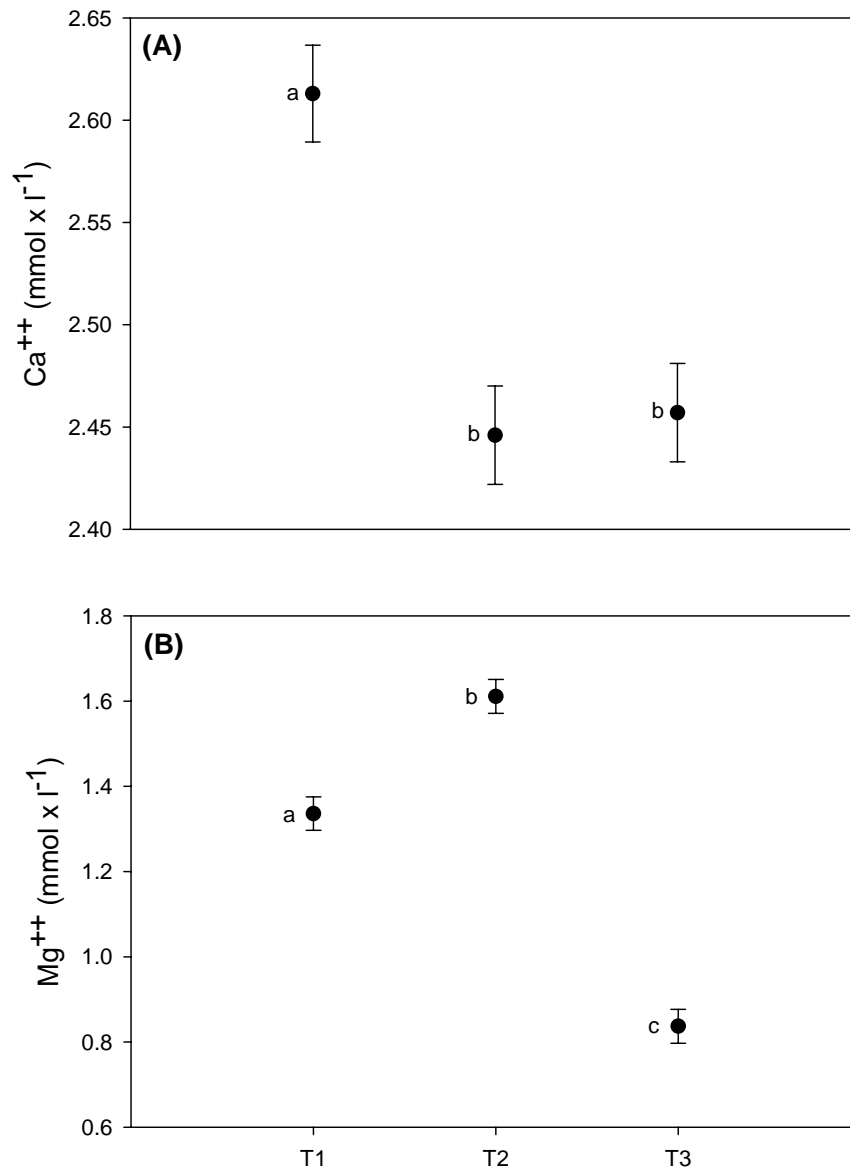


**Fig. 1. B)** lactate anion & **D)** pO<sub>2</sub>. For A-D, mean (+/- SEM) blood parameter values at T1 (post-capture, 45-minutes), T2 (post-transport, 2-hours) and T3 (post-captivity, 30-days). Different lowercase letters represent significant differences (P < 0.05). Sample-sizes in parentheses.

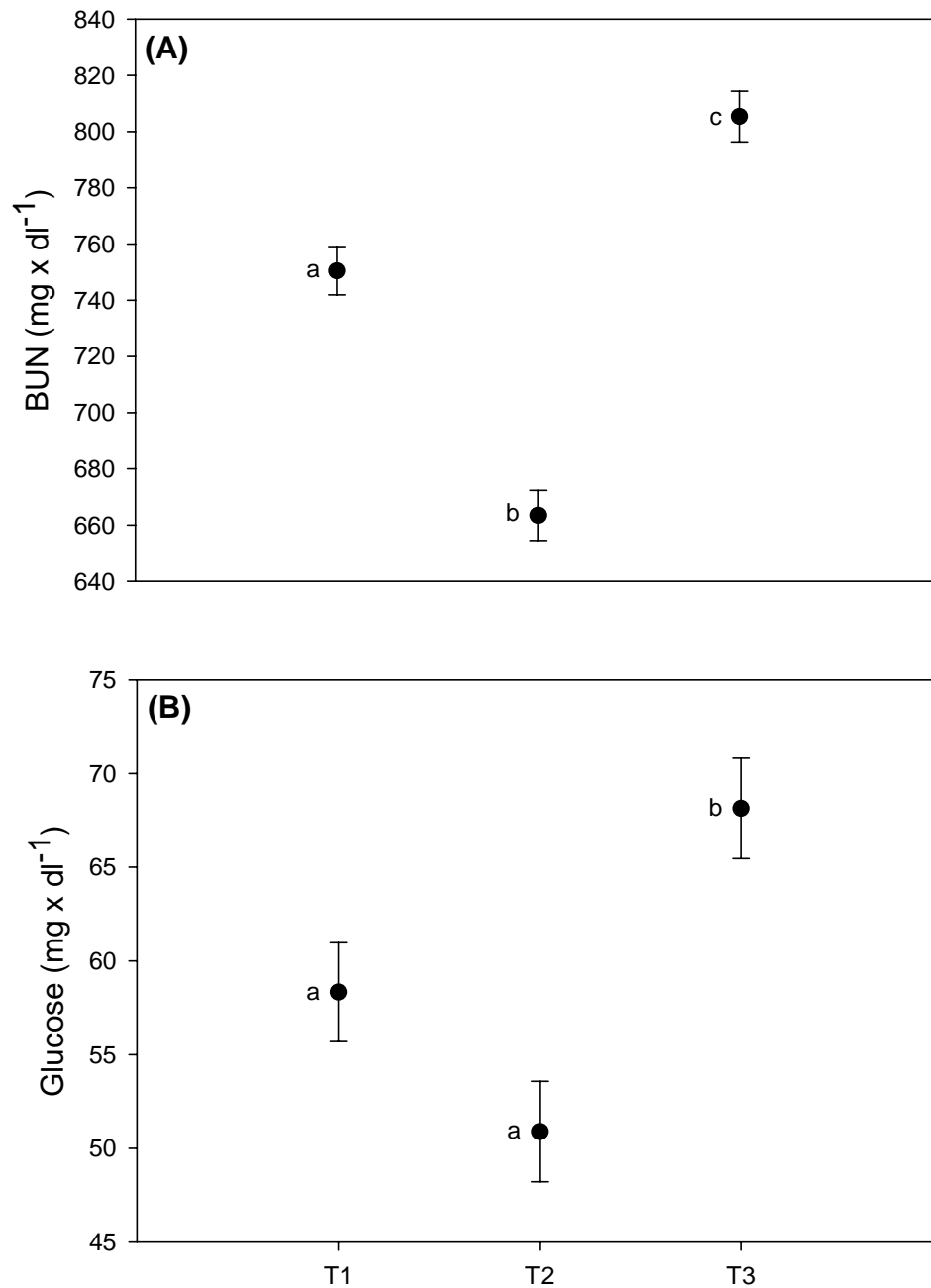




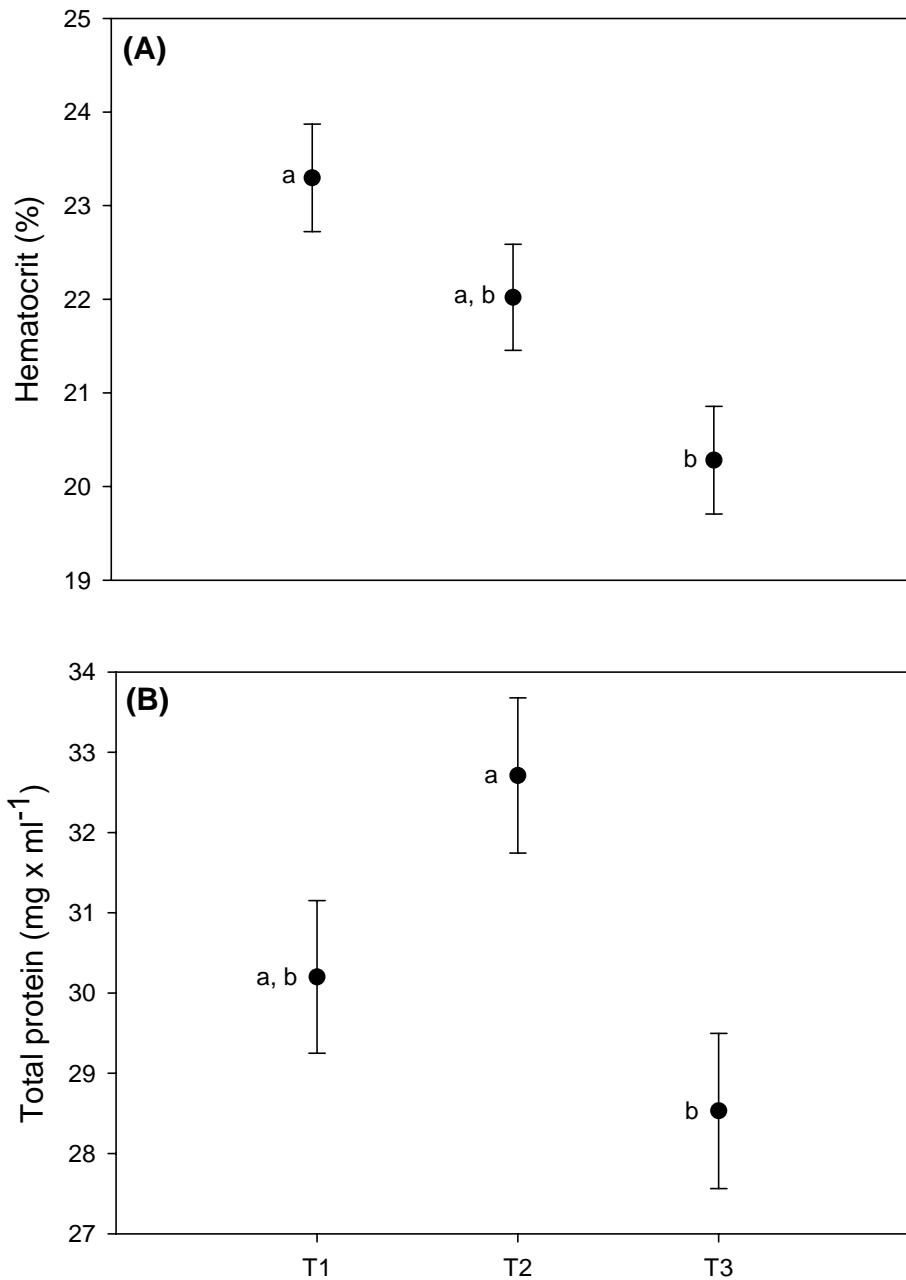
**Fig. 2.** **A)** K<sup>+</sup>, **B)** Na<sup>+</sup> & **C)** Cl<sup>-</sup>. Mean (+/- SEM) blood parameter values at T1 (post-capture, 45-minutes), T2 (post-transport, 2-hours) and T3 (post-captivity, 30-days). Dogfish sample-sizes: T1 (n = 30), T2 (n = 29), T3 (n = 29). Different lowercase letters represent significant differences (P < 0.05).



**Fig. 3.** A) Ca<sup>++</sup> & B) Mg<sup>++</sup>. Mean (+/- SEM) blood parameter values at T1 (post-capture, 45-minutes), T2 (post-transport, 2-hours) and T3 (post-captivity, 30-days). Dogfish sample-sizes: T1 (n = 30), T2 (n = 29), T3 (n = 29). Different lowercase letters represent significant differences (P < 0.05).



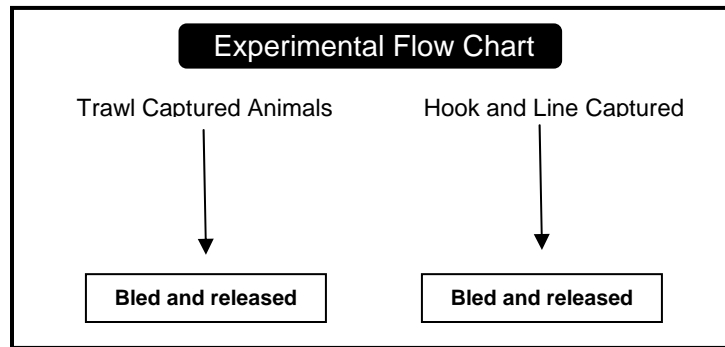
**Fig. 4.** A) BUN (urea) & B) glucose. Mean (+/- SEM) blood parameter values at T1 (post-capture, 45-minutes), T2 (post-transport, 2-hours) and T3 (post-captivity, 30-days). Dogfish sample-sizes: T1 (n = 31), T2 (n = 29), T3 (n = 29). Different lowercase letters represent significant differences (P < 0.05).



**Fig. 5.** A) hematocrit & B) total protein. Mean (+/- SEM) blood parameter values at T1 (post-capture, 45-minutes), T2 (post-transport, 2-hours) and T3 (post-captivity, 30-days). Dogfish sample-sizes: T1 (n = 32), T2 (n = 33), T3 (n = 32). Different lowercase letters represent significant differences (P < 0.05).

## Appendix C

## Experimental protocol for Appendix C.



## BLOOD CHEMISTRY ALTERATIONS INDUCED BY OTTER-TRAWL CAPTURE

### Abstract

To investigate the physiological responses to otter-trawling capture in spiny dogfish (*Squalus acanthias*), blood values from trawled individuals were evaluated against those landed by rapid hook-and-line. Significant effects of trawling were reflected in all parameters excluding log plasma protein. Blood gas parameters ( $pO_2$  (45% decrease);  $pCO_2$  (82% increase)) and the lactate anion (125% increase) were most perturbed relative to changes in other parameters. The concentrations of sera monovalent electrolytes ( $Na^+$ ,  $K^+$ ,  $Cl^-$ ) and glucose were significantly elevated by trawling but not to the magnitude seen in other studies on capture stress. Hematocrit significantly rose while BUN (urea) levels were significantly reduced by trawl capture. Lactate concentrations and hematocrit in trawled male dogfish were significantly higher than in females indicating a more pronounced stress response for that gender. Overall, trawl capture elicited considerable blood chemistry disturbances relative to normal physiology in spiny dogfish.

### Introduction

As the condition and ultimate fate of discarded bycatch greatly affect fisheries management and ecosystem dynamics, the consequences of capture stress on teleosts (e.g. Wood et al. 1983; Haux et al. 1985; Lowe and Wells 1996; Chopin et al. 1996; Barton et al. 2003) and elasmobranchs (e.g. Cliff and Thurman 1984; Hoffmayer and Parsons 2001; Manire et al. 2001) have been extensively investigated. However, the majority of these studies have addressed angling and fixed-gear capture. Far fewer have dealt with capture by mobile gear which subjects the catch to acute stressors which include and/or incite hyperactivity, extreme net confinement and barotraumas (Davis 2002). Included in the many consequences presumably caused by these predicaments are physical traumas, exhaustion and exposure to hypoxia/anoxia. Although marked physiological responses to simulated towing augmented by additional capture-related stressors have been found in teleosts (Olla et al. 1997; Olla et al. 1998; Davis et al. 2001; Davis and Olla 2002), studies addressing the effects of towing on feral species in the field environment are scant (Bourne 1986).

Although several investigations have attempted to gauge discard mortality in elasmobranchs landed by towed-gear (Chisholm 2003; Laptikhovskiy 2004; Revill et al. 2005), no study has documented its effects on a representative species' blood chemistry. In general, elasmobranchs appear particularly sensitive to capture-stress (Wells et al. 1986) and have exhibited considerable physiological alterations when subjected to capture-related stressors (Cliff and Thurman 1984; Wells et al. 1985; Torres et al. 1986; Wells et al. 1986; Hoffmayer and Parsons 2001; Manire et al. 2001).

The spiny dogfish (*Squalus acanthias*) is a squaloid elasmobranch ranging from Labrador to Florida in the western North Atlantic (Sosebee 2000). Incidental dogfish landings in the Northwest Atlantic otter-trawl fishery remain plentiful despite reported compromises in its unit stock during recent years (NEFSC 2003). Based upon comparatively depressed vascular pH levels, initial indications support a substantial stress response in trawled dogfish relative to those hook-and-line captured (chapter two). However, dogfish have also exhibited low short- (Chisholm 2003; chapter two) and long-term (chapter three) mortality rates subsequent to otter-trawling capture and the physiological implications of this landing

method remain mostly unstudied in this species. Such data will shed light on how profoundly trawl capture affects dogfish physiology and augment the current knowledge pertaining to its resiliency.

To address this, selected physiological parameters in dogfish captured by (45-60 minute) otter-trawls and a rapid and relatively less vigorous (1-2 minute) hook-and-line method were compared. In presuming the latter to have induced only minimal stress, blood value differences between capture methods could be interpreted as *changes* precipitated by trawl-capture.

## **Methods**

All field-work took place aboard the commercial trawler *Joanne A. III* east of Chatham, MA (USA- (41°36'0"North x 69°36'0"West to 41°48'0"North x 69°48'0"West). Otter-trawling and hook-and-line fishing were conducted during discrete periods in the summer and fall seasons when spiny dogfish were abundant in experimental regions. All fishing was carried out in depths ranging from 45.0-80.0 meters (m) on cobble and sand bottom. Surface water temperatures ranged between 13-16 °C. Protocols for both dogfish capture methods are described in detail as follows:

### **Otter-trawling**

Trawling was conducted using a Danish bottom trawl with an 18.0 mm mesh-size, 305 meshes collectively in the head-rope and foot-rope, 4.6 m legs and a hard-bottom sweep to avoid boulders on the substratum. Tows were kept at 45-60 minutes on a similar basis as described by Bourne (1986). To reflect deck-time that occurs in fishing operations, post-trawl blood sampling was conducted during 20 minute post-capture periods with generally similar increments of air-exposure (for individual dogfish) made possible by multiple phlebotomists. As spiny dogfish school according to size and when mature, by gender (Sosebee 2000), catches were often constituted by a majority of dogfish from a single size-class and/or gender. Animals undergoing blood sampling were caught across 19 trawls with estimated catch weights ranging from 100.0 to 850.0 kg. Following phlebotomy, gender and morphometric data was ascertained and each dogfish was released. A total of 96 female and 87 male dogfish were sampled in the study.

### **Hook-and-line capture**

A short makeshift longline was used to hang five squid-baited standard circle hooks at a given time. Dogfish were landed within two minutes of deploying this mini-set. Unlike trawling, dogfish were de-hooked and bled immediately upon retrieval. Although individuals experienced lacerations from hooking, clotting appeared to ensue rapidly. This was interpreted by the disappearance of blood in the seawater flushed holding tanks containing additional individuals just captured by hook-and-line (personal observation). A total of 70 male and 7 female dogfish landed by this capture method were sampled in the present study.

### **Blood sampling and processing.**

Blood samples (~5.0 ml) were obtained by caudal veni-puncture, a method described as relatively non-stressful for elasmobranchs (Cooper and Morris 1998), using Fisher non-heparinized 18-20 gauge 0.04 m stainless steel syringe needles fitted to 5.0 ml plastic (Beckman Dickinson) syringes. In addition to the 183 trawled and 77 hooked dogfish, certain individuals (n = 29 (trawl); 13 (hook-and-line)) were also randomly assessed for



blood-gas partial pressures. Following sampling in these individuals, needles were immediately plugged with cork in order to slow diffusive gas loss (Bob Cooper, personal communication). Upon reaching the ISTAT portable clinical analyzers within ten seconds of tapping, the cork was removed and 20.0  $\mu\text{l}$  of whole-blood deposited into specialized (CG4+) cartridges (*Heska Corporation*, Loveland, CO). The device then calculated temperature adjusted single values for partial pressures of oxygen ( $\text{pO}_2$  ( $\text{mm} \times \text{Hg}^{-1}$ )) and carbon dioxide ( $\text{pCO}_2$  ( $\text{mm} \times \text{Hg}^{-1}$ )) on-site. For the remainder ( $4.5 \text{ ml} \leq X \text{ ml} \leq 5.0$ ) of the samples run on the ISTAT and for all other samples that were not analyzed for blood gases, treatment ensued as follows:

Individual blood samples were allocated across three varieties of vacutainer tubes ( $\sim 1.6$  ml blood per tube): 1) dried lithium-heparin coated (“green-top”); 2) dried EDTA-coated (“purple-top”); and 3) non-heparinized (“red-top”). Micro-hematocrit tubes were then filled in triplicate with whole-blood from (1) via capillary-action, packed with hematocrit and stored on ice for under an hour (Biron and Benfey 1994) for on-site analysis of whole-blood hematocrit (see below). To deproteinate blood, 0.5 ml of whole-blood from (2) was added to 1.0 ml of ice cold 8% perchloric acid. To ensure complete digestion and protein denaturation, this solution was kept on ice for  $\sim 45$  minutes. The remainder of (1) and the perchloric acid solution in (2) were then stored on ice while (3) was left to clot at room temperature (60 minutes) until processed for the following: plasma (from 1); perchloric acid extracts of the haemolysed whole-blood (from 2) and serum (from 3). With the exceptions of hematocrit and blood gases, references to individual blood parameters in the text correspond to their concentrations in mediums described.

### ***Readings for hematocrit***

Whole-blood hematocrit (%) was determined by spinning anticoagulated whole-blood (in micro-hematocrit tubes) in triplicate at 8,000 x gravity (g) for three minutes. Percent-values were determined against the scale of a standard microhematocrit reader.

### ***UV Spectrophotometry***

Total plasma soluble protein concentration ( $\text{mg} \times \text{ml}^{-1}$ ) was obtained by spinning “green-top” tubes (1.25 ml of anticoagulated whole-blood) at 1,400 x g for five minutes. The supernatant ( $\sim 0.75$  ml of plasma) was aliquoted into Fisher cryovial tubes and transported in liquid nitrogen and preserved in  $-80^\circ\text{C}$  freezers until assay. Samples were processed in triplicate utilizing a BCA protein assay reagent kit (Procedure No. 23225, *Pierce Biotechnology Inc.*, Rockford, IL) and assayed spectrophotometrically in triplicate (wavelength of 562 nanometers) (nm) on a *Molecular Devices* (Emax Precision) *Microplate Reader* to obtain optical densities (OD). Final values were derived 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).

To determine concentrations ( $\text{mmol} \times \text{l}^{-1}$ ) of the lactate anion from perchloric acid extracts of the haemolysed whole-blood (will be designated as whole-blood), “purple-tops” were centrifuged at 3000 x g for ten minutes to pellet cellular debris and protein. The supernatant ( $\sim 1.0$  ml of whole-blood) was aliquoted and preserved adhering to same methods described for plasma. The assay was processed enzymatically (Procedure No. 826-UV, *Sigma Diagnostics*, St. Louis, MO) and conducted spectrophotometrically in triplicate (wavelength of 340 nm- vis with blue filter) on a *Beckman Coulter* DU-640C UV Spectrophotometer to

acquire ODs. Values were derived 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).

### ***NOVA blood chemistry analyzer***

Sera monovalent electrolytes ( $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$ ) ( $\text{mmol} \times \text{l}^{-1}$ ), glucose and BUN (urea) concentrations ( $\text{mg} \times \text{dl}^{-1}$ ) were obtained by centrifuging clotted blood from red-top tubes at  $3,500 \times g$  for five minutes. The supernatant ( $\sim 1.0$  ml of serum) was then aliquoted, transported and preserved adhering to the same methods as for plasma. Prior to the assay, samples were thawed to room temperature. To obtain concentrations of electrolytes and BUN (urea), 50% (sterile-grade de-ionized water) and 10% serum dilutions (0.9 % saline) respectively were run in duplicate on a *Stat Profile Critical Care Xpress (CCX)* blood chemistry analyzer (Nova Biomedical, Waltham, MA).

### **Statistical analyses**

Once it was confirmed that statistical similarities existed across the multiple tows conducted ( $n = 19$ ) using one-way analyses of variance (ANOVAs), post-trawl values were pooled for each blood parameter. Differences between post-trawl and post-hook-and-line physiology were collectively (minus blood gas sub-set) assessed using a multiple analysis of covariance (MANCOVA) accounting for dogfish-size (cm) (covariate). Differences were also assessed independently (including blood gases) using one-way analyses of covariance (ANCOVAs) accounting for dogfish-size (covariate). Values for lactate, protein,  $\text{Cl}^-$ , and  $\text{pO}_2$  were log transformed in order to stabilize variances prior to subsequent analyses.

The effects of gender on physiology were assessed (one-way ANCOVAs (covariate = dogfish-size)) within trawl for selected parameters (lactate anion, hematocrit, protein) most highly correlated with additional parameters. Negligible samples of hook-and-lined females prohibited gender comparisons between capture methods.

Percent (mean) value differences between capture methods were derived for each parameter by the following:  $((\text{mean trawl value}) - (\text{mean hook-and-line value})) \times (\text{mean hook-and-line value})^{-1}$ . For log-transformed parameters, percent change was elucidated for non-transformed means. Values are presented as least squares (LS) means ( $\pm$  SEM). All analyses were performed using JMP 4.04 Software (*SAS Institute*, Cary, NC). Using a conservative interpretation of the data, results were reported as significant according to  $\alpha = 0.001$ , adjusting for heteroscedastic variances.

### **Results**

Graphic representations reflect non-transformed data. A multivariate difference in dogfish blood chemistry existed between trawl and hook-and-line capture (one-way MANCOVA, Wilks' lamda = 0.29,  $F_{24, 722.78} = 15.84$ ,  $P < 0.0001$ ).

#### **Sera electrolytes**

Post-trawl sera monovalent ion ( $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{K}^+$ ) concentrations were each significantly greater than post-hook-and-line (One-way ANCOVA capture-method effects-tests:  $\text{Na}^+$  ( $F_{1, 256} = 149.01$ ,  $P < 0.0001$ );  $\text{Cl}^-$  ( $F_{1, 256} = 203.19$ ,  $P < 0.0001$ ) (Fig. 1A) and  $\text{K}^+$  ( $F_{1, 256} = 154.47$ ,  $P < 0.0001$ ) (Fig. 1B)). Concentrations of  $\text{K}^+$  displayed larger percent differences

(26.5%) between capture modes than either  $\text{Na}^+$  (4.0%) or  $\text{Cl}^-$  (4.0% for non-transformed data) (Table 1).

### **Metabolites**

Serum BUN (urea) and glucose were significantly lower (-7%) and higher (16%), respectively, following trawl capture versus hook-and-line (one-way ANCOVA capture-method effects-tests: glucose ( $F_{1,256} = 28.98$ ,  $P < 0.0001$ ); BUN ( $F_{1,256} = 159.47$ ,  $P < 0.0001$ ) (Fig. 2). Log lactate concentrations were significantly greater (125% for non-transformed data) following trawl than hook-and-line capture (one-way ANCOVA capture-method effects-test:  $F_{1,256} = 228.75$ ,  $P < 0.0001$ ) (Fig. 3A).

### **Whole-blood hematocrit and plasma total protein**

Whole-blood hematocrit (%) following trawl capture was significantly greater (13% rise alludes to magnitude of difference and not a literal shift in hematocrit) than hook-and-line (one-way ANCOVA capture-method effects-tests:  $F_{1,256} = 40.8$ ,  $P < 0.0001$ ) (Fig. 4A). Log plasma protein (7% greater post-trawl for non-transformed mean) was the only parameter found to be similar between the two capture methods (one-way ANCOVA capture-method effects-tests:  $F_{1,256} = 7.4$ ,  $P = 0.007$ ) (Fig. 5).

### **Blood gas parameters**

Post-trawl  $\text{pCO}_2$  levels were significantly greater (82%) than post-hook-and-line ((one-way ANCOVA capture-method effects-test:  $F_{1,38} = 24.9$ ,  $P < 0.0001$ ) (Fig. 6). Log  $\text{pO}_2$  was significantly lower (-45% for non-transformed data) following trawl than hook-and-line capture (one-way ANCOVA capture-method effects-test:  $F_{1,38} = 17.17$ ,  $P = 0.0002$ ) (Fig. 6).

### **Gender effects**

Trawled male dogfish possessed significantly greater lactate concentrations (One-way ANCOVA gender effects test:  $F_{1,179} = 11.66$ ,  $P < 0.001$ ) and hematocrits (One-way ANCOVA gender effects test:  $F_{1,179} = 5.56$ ,  $P < 0.01$ ) than trawled females (Fig. 7). Log plasma protein was similar between trawled males and females (One-way ANCOVA gender effects test:  $F_{1,179} = 1.52$ ,  $P > 0.2$ ).

### **Discussion**

The difficulty in gaining baseline physiological values often discourages field-oriented stress investigation on larger fishes and has previously mandated the use of smaller non-conspecifics as surrogate controls (Wells et al. 1986). In the present work, rapid capture by hook-and-line was anticipated as an effective means to gain minimally-stressed blood parameter values in spiny dogfish. Indeed, values ultimately mirrored those obtained from dogfish following 30 days in the captive environment (chapter three) and landed by abbreviated longline sets (chapter one). These similarities and the discernable vigor displayed by the hooked dogfish support that minimal stress was imposed.

Conversely, the blood parameters of trawled individuals were markedly altered relative to hook-and-line values. Although limited studies have assessed the physiological impacts of mobile-gear capture with which to draw comparison, extensive work has dealt with responses to stressors that can be associated with this capture type (Davis 2002). These include responses to extreme confinement/crowding (e.g. Lockwood et al. 1983; Swift 1983; Torres et al. 1986; Arends et al. 1999) and exhaustive exercise (e.g. Piiper et al. 1972;

Holeton and Heisler 1983; Wood et al. 1983; Kieffer et al. 1994; Milligan 1996; Wilkie et al. 1997). It is clear from these studies that cardiovascular and respiratory function, osmotic and acid-base balance, and other aspects of metabolic performance can be severely disrupted in response to stressors akin to those experienced by fishes landed by mobile-gear.

In the present study, monovalent ion concentrations were significantly elevated by trawl capture. Similar elevations have also been reported in teleosts captured by towed-gear in simulated (Bourne 1986) and field (Davis et al. 2001) environments. This conflicts with Opdyke et al. (1982) who did not observe a significant rise in  $\text{Na}^+$  in the aftermath of exhaustive exercise in spiny dogfish. However, this might be explained by comparatively brief (3-minutes) duration of strenuous activity in that study. Exhaustive exercise in marine teleosts has repeatedly been shown to induce major ionic/water balance imbalances in response to water shifts from the extra- to intracellular compartments (eg. Wood et al. 1983; Piiper et al. 1972; see Wood 1991). Presumably through a concentrating effect on ionic constituents, erythrocytes (hemoconcentration) and additional analytes in the blood elevations have also been cited in elasmobranchs (Wells et al. 1986) and teleosts (e.g. Fletcher 1975; Haux et al. 1985; Thomas et al. 1987; Arends et al. 1999). Interestingly, in studies that have assessed steady as opposed to exhaustive exercise in fishes, electrolytes levels have not markedly shifted (Wood 1991). This suggests that the degree of discernable ionic increases might scale with degree of intensity as opposed to the duration of a particular stressor (Thomas et al. 1987; Wood 1991).

The trawl-induced  $\text{K}^+$  elevation (27%) presently observed exceeded the magnitude in other electrolytes (~4%) (Table 1). Significant elevations in plasma  $\text{K}^+$  have also been found in spiny dogfish exhaustively exercised for only three minutes (Opdyke et al. 1982). In dogfish, changes in the concentration of this parameter appear particularly sensitive to capture-related stressors.  $\text{K}^+$  climbs are likely a function of leakage from the intracellular compartment as has been suggested for other elasmobranchs responding to capture-stress (Cliff and Thurman 1984; Wells et al. 1986; Manire et al. 2001).

In the present study, lactate levels climbed significantly during trawl capture. Lactic acidosis is the end product of vertebrate anaerobic metabolism. Although primarily driven by the reliance on glycolysis for burst energy, it can also be spurred by anoxia/hypoxia (Heisler 1988). Both instigated burst activity and inhibited ventilation are invariably induced by mobile-gear capture (Davis 2002). Blood lactate climbs have also been shown to occur following simulated towing in sablefish (*Anoplopoma fimbria*) (Davis et al. 2001) and many alternatively stressed (i.e. – exhaustively exercised) teleosts (e.g. Wood et al. 1983; Wells et al. 1986; Ferguson and Tufts 1992; Wilkie et al. 1997) and elasmobranchs (Piiper et al. 1972; Holeton and Heisler 1983; Wells and Davie 1985; Cliff and Thurman 1984; Manire et al. 2001). In the present study, lactate concentrations rose ~125% during trawl capture relative to the presumed normality reflected by hook-and-line values (Table 1). Despite its gravity, this difference likely represents only the initial extent of lactic acid build-up that occurred during the trawls. Dogfish previously captured by trawl-gear displayed ~300% increases (from ~8.0 to 24.0  $\text{mmol} \times \text{l}^{-1}$ ) in whole-blood lactate between the trawl-landing and arrival two hours later at a holding facility (chapter three). The majority of this climb was likely a delayed artifact of the initial trawl capture. Intracellular acid-base balance is protected at the initial expense of vascular pH via the more rapid diffusion of  $\text{H}^+$  over the lactate anion from white muscle to the vascular compartment (see reviews by Heisler 1988; Wood 1991). Peak

blood loads therefore only become discernable 1-4 hours following the removal of source of metabolic acidosis (Milligan and Wood 1986). For these reasons, lactate does not accurately reflect the extent of lactic acid build-up at a given time (Wood et al. 1983). Conversely, the diffusive delays increase the efficacy that hook-and-lined dogfish values reflected baseline in this parameter.

The blood glucose levels in dogfish increased significantly (16%) during trawl capture (Table 1). This is in contrast with the absence of glucose changes in response to simulated towing in sablefish (*Anoplopoma fimbria*) (Davis et al. 2001). However, many studies have documented hyperglycemic responses to exhaustive exercise and additional capture-stressors in teleosts (e.g. Black 1958; Fletcher 1975; Schwalm and Mackay 1985a; Pagnotta and Milligan 1991) and elasmobranchs (Cliff and Thurman 1984; Wells et al. 1986; Hoffmayer and Parsons 2001). Collectively, these studies cited the presence of circulating catecholamines, corticosteroids and gluconeogenesis as the stress-induced stimulators of the hyperglycemia. Although glucose levels of extreme polarity may portend mortality (Cliff and Thurman 1984; Manire et al. 2001), the (16%) upward shifts in trawled dogfish were fairly unremarkable relative to bi-directional shifts in other studies. For example, Bourne (1986) witnessed a 4-fold increase in the glucose levels of trawled and transported plaice (*Pleuronectes platessa*). Elasmobranch species of comparable size experienced a 40% (Hoffmayer and Parsons 2001) and 77% (Cliff and Thurman 1984) decline during stressful events of similar duration to the trawling herein. Further, resting and stressed glucose values tend to vary widely by species and individual. Manire et al. (2001) found a large diversity in blood glucose concentrations among gill-net caught elasmobranchs and despite observed hypoglycemia in certain species, exceptionally high glucose levels in dying bonnethead sharks (*Sphyrna tiburo*). Despite capture and transport induced hyperglycemia, Cliff and Thurman (1984) found extremely low glucose levels in dying dusky sharks. Trawling stress did not elicit changes in glucose to the same extent as in other studies or to the same degree as additional dogfish stress parameters.

Similar to other osmolytes, blood urea nitrogen (BUN) concentrations in trawled dogfish were significantly altered (reduced) relative to hook-and-line levels. Stress induced decreases in BUN (urea) have only been previously reported in spiny dogfish pups (Evans and Kormanik 1985). Stress may compromise what is normally great impermeability to urea losses at the branchial-seawater interface (Evans and Kormanik 1985). As a function of maintaining overall osmotic balance, urea losses could deliberately serve to off-set the stress-induced increases in other ions. This is supported by inverse shifts between monovalent ions and BUN previously found in dogfish (chapter three). Those individuals displayed even greater BUN (urea) losses when trawling stress was exacerbated by transport. Judging by the resolved BUN (urea) values following 30-days in captivity in that instance, dogfish appear able to correct even greater imbalances than observed in the present study. Interestingly, as urea gains are enhanced by sustenance (Haywood 1973), resolution of presumed baseline levels could have been due to increased food-intake. In natural environments, trawl-induced BUN (urea) losses may be more difficult to overcome if foraging capabilities are compromised in the aftermath of release.

Plasma protein was the lone blood parameter similar in dogfish landed by both capture methods. In previous trials with dogfish, significant protein elevations only occurred after trawling stress was compounded with transport (chapter three). Manire et al. (2001) also

observed a lack of change across three species of elasmobranchs exposed to stress following gill-net capture. The absence of change incited by trawling differs from other studies where comparable stressors have induced protein concentration changes in both teleosts (Wood et al. 1983) and elasmobranchs (Rasmussen and Rasmussen 1967). Stress-induced increases in total blood protein are likely initiated by plasma water losses (Wood et al. 1983) augmented by the sequestering of additional proteins/enzymes. Thus, the absence of more pronounced changes in this parameter is surprising considering the concomitant increases in blood electrolytes whose concentrations are also linked to water balance.

Following trawl capture, hematocrit levels were (13%) higher than in hook-and-lined dogfish. Post-trawl values were variable with more pronounced polycythemia (28-31%) exhibited in certain individuals. Hematocrit elevations have also been documented in smaller elasmobranchs subjected to confinement (Torres et al. 1986) and pelagics exposed to capture and additional stressors (Wells and Davie 1985; Wells et al. 1986). Stressed teleosts have also exhibited stress-induced increases in hematocrit (Fletcher 1975; Mazeaud et al. 1977; Lowe and Wells 1996; Frisch and Anderson 2000). Hematocrit levels failed to change in elasmobranchs similar to dogfish in size and behavior following hooking (Hoffmayer and Parsons 2001) and gillnetting (Manire et al. 2001) along with subsequent stressors in both cases. Spiny dogfish do not sequester additional erythrocytes from the spleen in response to sympathetic stimulation and circulating catecholamines (Opdyke and Opdyke 1971). Thus, increases observed in the present study were likely produced by hemodilution or hemoconcentration relative to previously circulating erythrocytes.

### **Blood gases**

The blood gas levels of trawled dogfish were especially disturbed relative to hook-and-lined dogfish. Net entrapment or constriction with other animals in the cod-end of a trawl presumably leads to compromised ventilation and potential suffocation in fishes (Davis 2002). Forced hypoventilation in the trawl net augmented by exhaustive exercise and deck-time likely explain dogfish becoming hypercapnic in the present study. The trawl-induced pCO<sub>2</sub> increases (82%) also inversely correlate with the vascular pH depressions found during previous trawls in dogfish (chapter two). Consequential drops in blood pH from increased pCO<sub>2</sub> have been reported in teleosts (Wood et al. 1983; Schwalm and Mackay 1985b; Milligan and Wood 1986; Ferguson and Tufts 1992), and elasmobranchs (Piiper et al. 1972; Holeyton and Heisler 1983; Cliff and Thurman 1984) following exhaustive activity and/or capture-stress. The respiratory distress also fueled marked decreases (45%) in dogfish pO<sub>2</sub>. Declines (28%) in blood oxygen tension have also been observed in exhaustively exercised rainbow trout (*Oncorhynchus mykiss*) (Ferguson and Tufts 1992). However, comparatively greater drops (81%) ensued after those animals were subsequently exposed to air. This would support the notion that a majority of the decreased dogfish pO<sub>2</sub> in the present study resulted from hypoxia in the trawl net and/or on-deck. A lack of pO<sub>2</sub> change was found in captured and transported juvenile dusky sharks (*Carcharhinus obscurus*) (Cliff and Thurman 1984). Therefore, this parameter may not always reflect stress in an elasmobranch or, trawling might have been comparatively more strenuous treatment in the present study.

### **Gender effects**

In the present study, trawl caught male dogfish exhibited higher lactate concentrations and hematocrits than did females. Considering that male dogfish have also died at a higher rate in short-term mortality trials following trawl capture (chapter two), these findings are

intriguing. Although the inherently larger size of female relative to male dogfish might be an expected contributor to these differences, size was not found to affect either mortality (chapter two) or other findings in the current study. Differences in the physiological stress response on the basis of gender have yet to be studied in elasmobranchs or teleosts. Although implications from the extent of differences found here are uncertain, their extent was below the magnitude of those between the capture methods themselves. Regardless, any gender-based disparities in resiliency could weigh heavily in the fishery and need to be studied further.

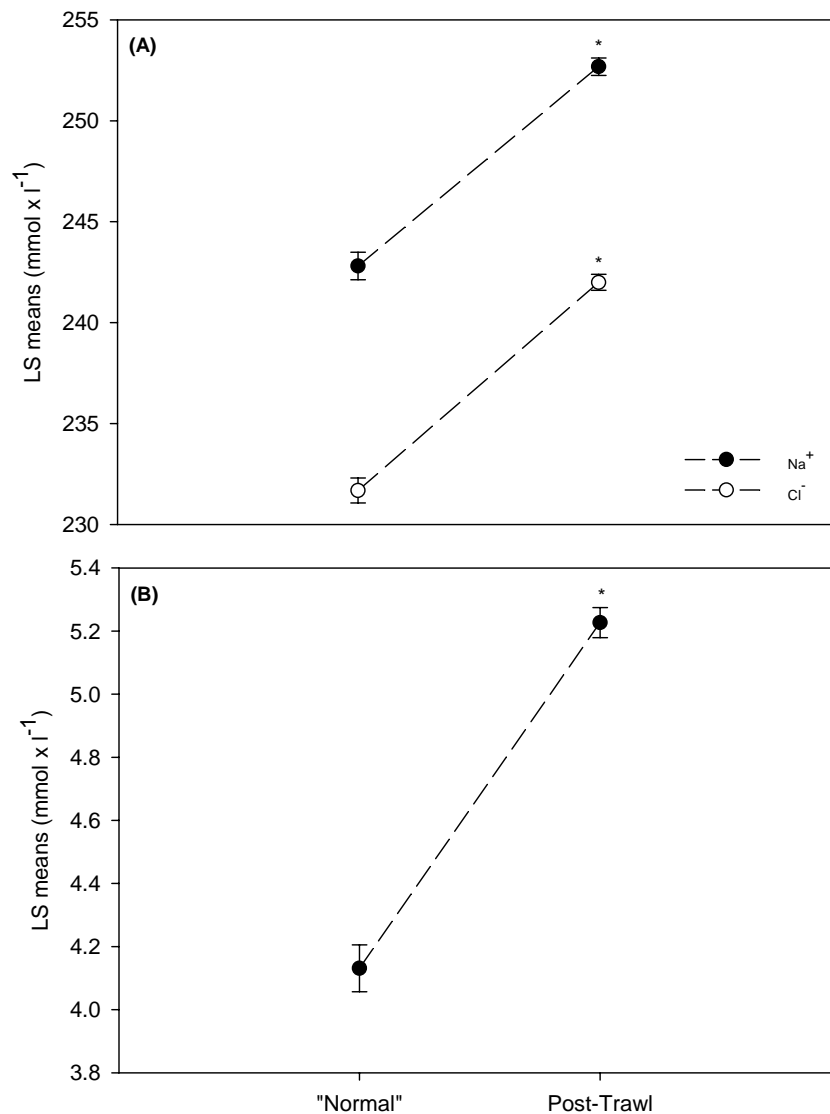
## **Conclusions**

Based upon the perturbations in blood values relative to presumed baseline levels in hooked individuals, the physiological implications of trawl capture are considerable in dogfish. Independent of consequence, it is clear that values of certain blood parameters exhibited comparatively greater shifts than others in response to the type of stressors encountered in the trawl net and on-deck. In particular, the study parameters ( $p\text{CO}_2$ ,  $p\text{O}_2$  and lactate) most invariably linked to acid-base balance, experienced marked alterations in response to being trawled. Factoring in the concomitant blood pH drops previously observed in trawled dogfish (chapter two), compromises in normal ventilation and the provocation of exhausting activity appear to be predominant physiological stressors associated with trawl capture. Future work should address physiological differences in dogfish and other species commonly landed by mobile gear as a function of tow-duration and –weight. Investigators should also strive to correlate incidences of physical trauma and physiological changes that are imposed during trawl capture. Finally, further short- and long-term post-capture mortality work in dogfish and other commonly captured elasmobranchs is encouraged across multiple gear-types. In such, the initially intriguing results alluding to variable gender resiliencies can be addressed.

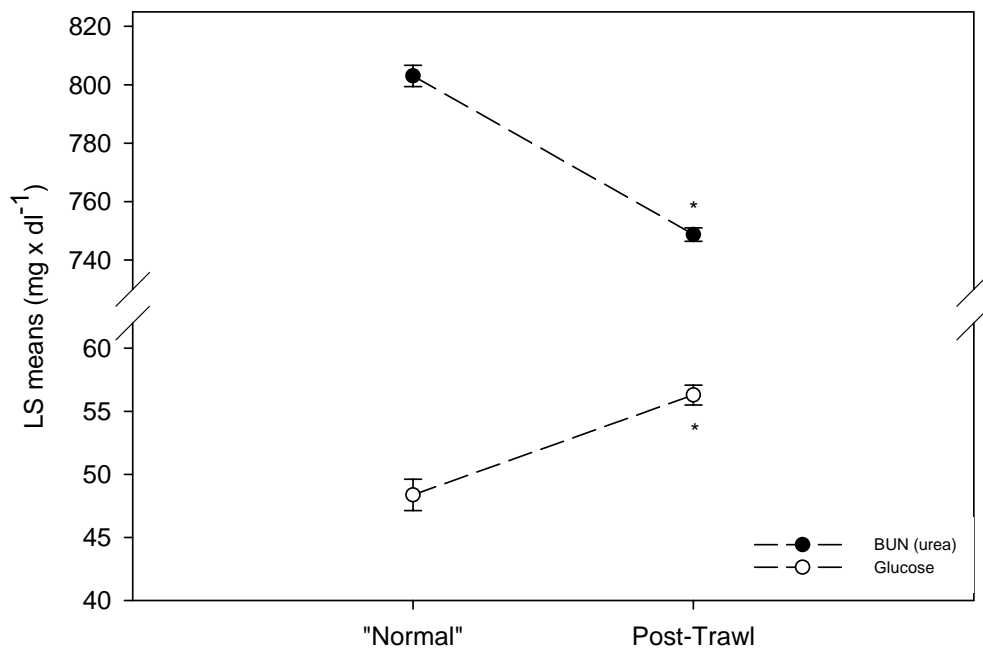
Parameter	Post H & L	Post Trawl	%-difference
<b>Na<sup>+</sup> (mmol x l<sup>-1</sup>)</b>	242.80 (0.68)	252.68 (0.44)	<b>4.07</b>
<b>Cl<sup>-</sup> (mmol x l<sup>-1</sup>)</b>	231.68 (0.62)	241.99 (0.40)	<b>4.45</b>
<b>K<sup>+</sup> (mmol x l<sup>-1</sup>)</b>	4.13 (0.07)	5.23 (0.05)	<b>26.53</b>
<b>Hematocrit (%)</b>	19.96 (0.34)	22.51 (0.22)	<b>12.78</b>
<b>Lactate anion (mmol x l<sup>-1</sup>)</b>	2.95 (0.26)	6.64 (0.17)	<b>125.3</b>
<b>Glucose (mg x dl<sup>-1</sup>)</b>	48.37 (1.24)	56.29 (0.79)	<b>16.37</b>
<b>BUN (mg x dl<sup>-1</sup>)</b>	803.03 (3.62)	748.76 (2.31)	<b>(-) 6.76</b>
Total protein (mg x ml <sup>-1</sup> )	28.07 (0.54)	29.99 (0.35)	6.84
<b>pO<sub>2</sub> (mm x Hg<sup>-1</sup>)</b>	54.59 (4.42)	29.81 (2.40)	<b>(-) 45.38</b>
<b>pCO<sub>2</sub> (mm x Hg<sup>-1</sup>)</b>	11.93 (1.61)	21.72 (0.87)	<b>82.06</b>

**Table 1.** Non (dogfish-size) adjusted means (+/- SEM) and percent differences for hook-and-line versus trawl-caught animals. Bolded parameters/percent-differences reflect those possessing significant differences between capture methods both with and without accounting for size.

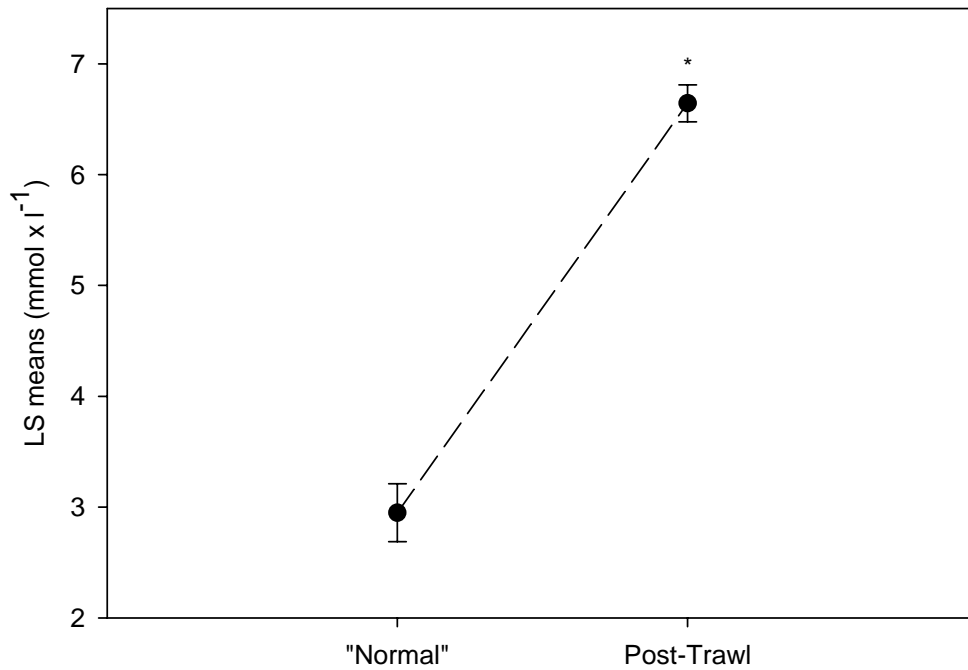




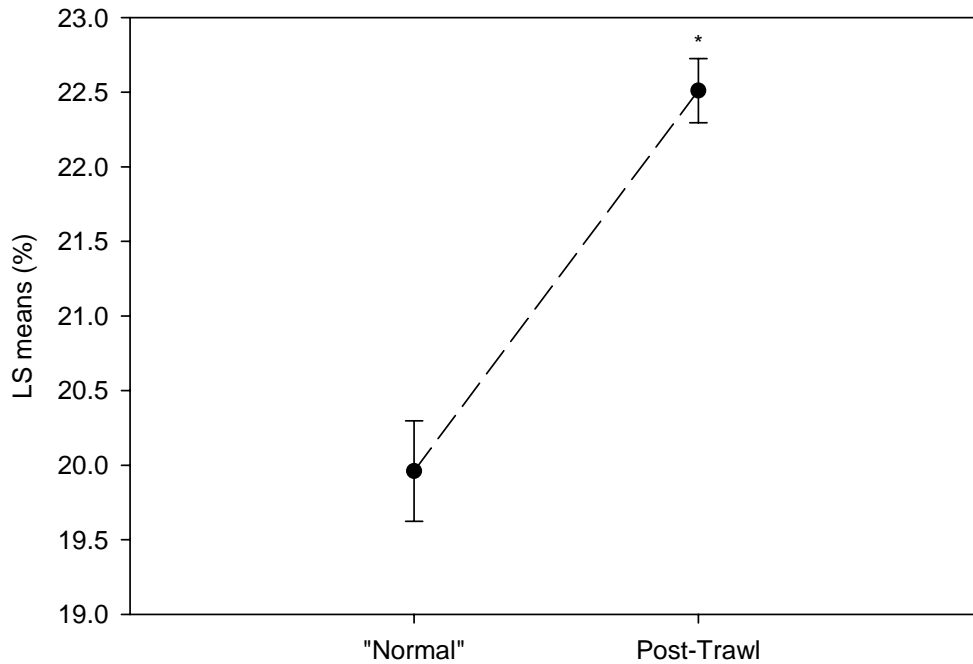
**Fig. 1.** Post-capture least squares means (+/- SEM): serum A) Na<sup>+</sup>, Cl<sup>-</sup> and B) K<sup>+</sup> in "normal" (hook and line caught) versus trawled dogfish (while accounting for dogfish-size). Asterisks denote significant differences between "normal" and trawled values (P < 0.01- for Cl<sup>-</sup>, applies to both transformed and non-transformed data). Dashed lines signify that the differences are based upon the presumed "normality" represented by hook-and-line values and do not imply temporal changes. (n = 183 (trawl); 77 (hook-and-line)).



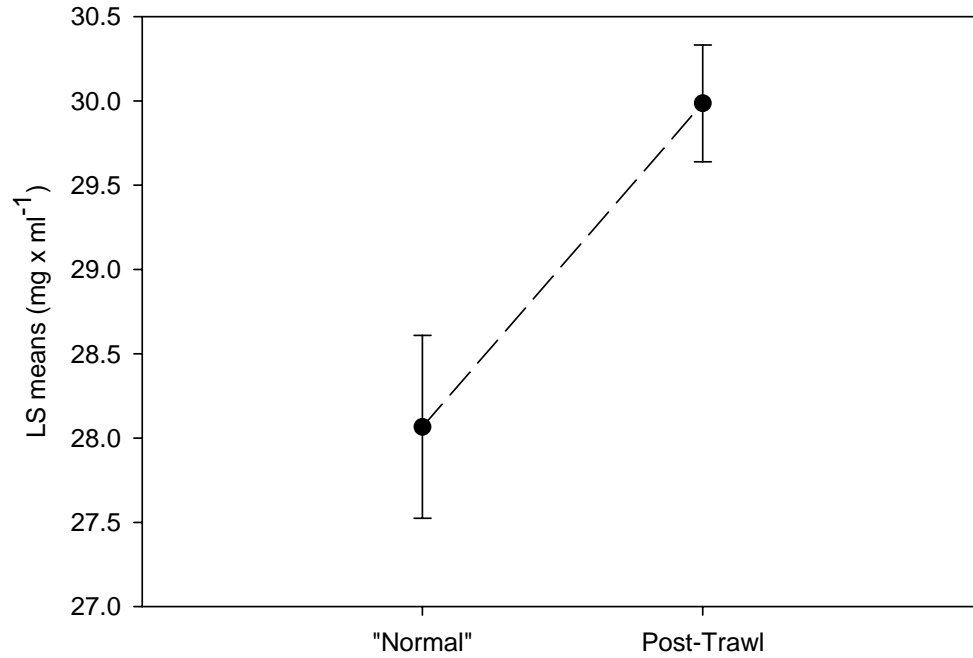
**Fig. 2.** Least squares serum BUN (urea) and glucose means (+/- SEM) in “normal” (hook and line caught) versus trawled dogfish (while accounting for dogfish-size). Asterisks denote significant differences between “normal” and trawled values ( $P < 0.01$ ). Dashed lines signify that the differences are based upon the presumed “normality” represented by hook-and-line values and do not imply temporal changes. ( $n = 183$  (trawl); 77 (hook-and-line)).



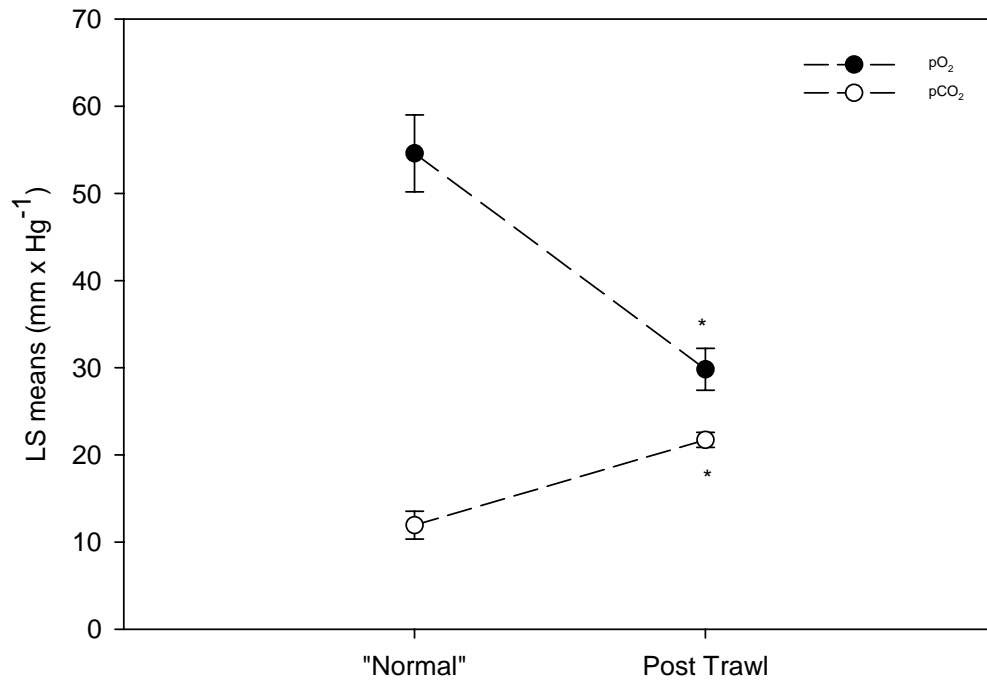
**Fig. 3.** Least squares whole-blood lactate means (+/- SEM) in “normal” (hook and line caught) versus trawled dogfish (while accounting for dogfish-size). Asterisks denote significant differences between “normal” and trawled values ( $P < 0.01$ - applies to both transformed and non-transformed data). Dashed lines signify that the differences are based upon the presumed “normality” represented by hook-and-line values and do not imply temporal changes. ( $n = 183$  (trawl);  $77$  (hook-and-line)).



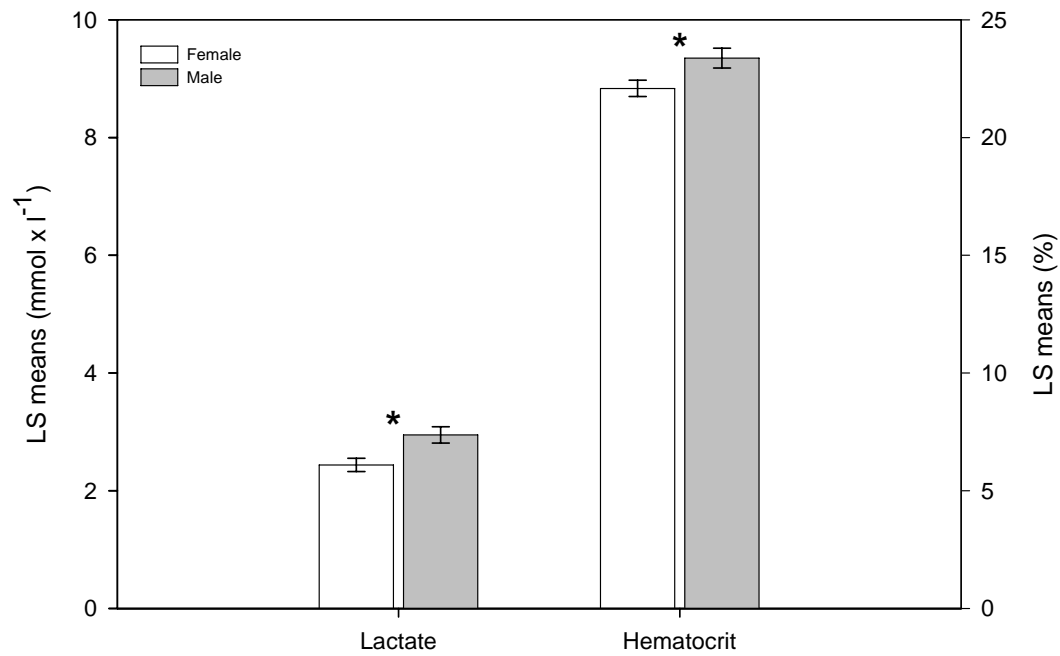
**Fig. 4.** Least squares whole-blood hemtocrit means (+/- SEM) in “normal” (hook and line caught) versus trawled dogfish (while accounting for dogfish-size). Asterisks denote significant differences between “normal” and trawled values ( $P < 0.01$ ). Dashed lines signify that the differences are based upon the presumed “normality” represented by hook-and-line values and do not imply temporal changes. ( $n = 183$  (trawl); 77 (hook-and-line)).



**Fig. 5.** Least squares plasma total soluble protein means ( $\pm$  SEM) in “normal” (hook and line caught) versus trawled dogfish (while accounting for dogfish-size). Means for both transformed and non-transformed data were similar ( $P > 0.01$ ). Dashed lines signify that the differences are based upon the presumed “normality” represented by hook-and-line values and do not imply temporal changes. ( $n = 183$  (trawl);  $77$  (hook-and-line)).



**Fig. 6.** Least squares vascular pCO<sub>2</sub> and pO<sub>2</sub> means (+/- SEM) in “normal” (hook and line caught) versus trawled dogfish (while accounting for dogfish-size). Asterisks denote significant differences between “normal” and trawled values ( $P < 0.01$ ). Dashed lines signify that the differences are based upon the presumed “normality” represented by hook-and-line values and do not imply temporal changes. ( $n = 29$  (trawl); 13 (hook-and-line)).



**Fig. 7.** Least squares whole-blood lactate and hematocrit means (+/- SEM) in male versus female dogfish (while accounting for dogfish-size) captured by otter-trawl. Asterisks denote significant genders difference relative to that blood parameter ( $P < 0.01$ ). (n = 96 (female); 87 (male)).

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