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# Capture-induced stress in deep-water Arctic fish species

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**Abstract** There are significant conservation benefits to studying the stress response of commercially important fish species. Particularly in the Arctic ecosystem, where reducing ice extent has resulted in heightened interest in developing commercial fisheries, there is a need to better understand how fish respond to capture stressors. To that end, blood lactate and glucose of Greenland halibut *Reinhardtius hippoglossoides* and Greenland shark *Somniosus microcephalus* were assessed after capture from fishing gear to evaluate factors that might influence physiological disturbance. Greenland halibut lactate values had a mean of  $1.0 \pm 0.7$  mmol/L and showed a positive relationship with time since capture. Blood glucose concentrations for Greenland halibut had a mean of  $1.8 \pm 0.6$  mmol/L and were positively related to both time since capture and fork length. Greenland shark lactate values varied depending on depth, with individuals captured at 300–600 m displaying significantly lower concentrations ( $1.6 \pm 0.5$  mmol/L) than those caught at depths between 600 and 700 m ( $6.4 \pm 2.4$  mmol/L) and 700–900 m ( $3.7 \pm 1.2$  mmol/L). Glucose values for the Greenland shark had a mean of  $4.9 \pm 1.5$  mmol/L and were positively correlated with body length. Relative to temperate fish species, the metabolite concentrations are low but the inherent challenges with

deep-water fish makes it difficult to obtain baseline (pre-stress) values to quantify the magnitude of stress response. This is the first study to document stress metabolites in these two Arctic species and suggests that there is a body size and depth-related physiological response to capture.

**Keywords** Greenland halibut · Greenland shark · Physiology · Capture stress · Lactate · Glucose

## Introduction

Capture-related stressors alter the internal homeostasis of fish and can potentially result in both lethal and sub-lethal physiological and behavioral impairments post-release (Moyes et al. 2006; Donaldson et al. 2011; Rapp et al. 2012; Gallagher et al. 2014). These impairments are of concern for the sustainable operation of commercial fisheries where bycatch, including both non-target and under-sized target species, are released to reduce the ecological impacts of the fishery (Hall et al. 2000; Davis 2002). Establishing measures of physiological disturbance to assess the risk imposed by fisheries development (see Gallagher et al. 2014) can ultimately improve handling and harvesting practices to reduce the negative effects of capture to released bycatch. Indeed, knowledge of how fish respond to different aspects of the capture and handling processes and the factors that influence those responses has led to meaningful improvements in the sustainability of commercial fisheries (Raby et al. 2015).

The reduction of summer sea-ice and longer ice-free periods in the Arctic Ocean have led to increased interest in expanding commercial fisheries in the north (Christiansen et al. 2014). One of the primary targets is the deep-water Greenland halibut *Reinhardtius hippoglossoides*, a

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circumpolar species inhabiting the benthopelagic zone of much of the Northern Atlantic, Pacific and Arctic Oceans (Bowering and Nedreaas 2000). This species exhibits many of the traits typical of deep-water fish that make them highly vulnerable to overexploitation, including a long life span and slow growth (Treble et al. 2008). In an effort to reduce the risk imposed by commercial fishing of Greenland halibut, current management plans recommend that fish <45 cm should not exceed 15 % of the total catch (DFO 2008a; ICES 2015). However, trawl catches in the Atlantic northwest often exceed the 15 % limit and the rule is not enforced as biological indices suggest the population is healthy (Huse et al. 1999; DFO 2008a, 2013). As the industry expands, there is a greater need to understand the impact that trawl capture can have on Greenland halibut, particularly small, immature undersized fish.

Concurrent with the growth of commercial fisheries for Greenland halibut is the potential for adverse effects on Greenland shark *Somniosus microcephalus* populations, a primary bycatch species in Arctic fisheries regardless of gear type (MacNeil et al. 2012; DFO 2013). Observer coverage and reporting of bycatch in the fixed gear (longline and gillnet) industry is limited; however, catches of Greenland sharks from a single longlining vessel can reach as high as 570 sharks, equating to 8 fish per set (MacNeil et al. 2012). When caught on fixed gear such as longlines, Greenland sharks are often badly entangled, yet currently no studies have directly examined mortality associated with commercial capture for this species. Greenland sharks are apex predators that exhibit slow growth, late maturation and likely low fecundity, and consequently their populations are vulnerable to exploitation which could result in negative impacts to the whole Arctic ecosystem should their numbers decline (Beck and Mansfield 1969; Yano et al. 2007; MacNeil et al. 2012).

The current study aimed to assess the physiological response of Greenland halibut caught in a bottom trawl and Greenland sharks caught on bottom longlines through measuring blood lactate and glucose concentrations. Lactate and glucose are known to increase significantly in stressed fish, as glucose is released for energetic use, and lactate accumulates as a result of strenuous exercise in the absence of sufficient oxygen (Dobson and Hochachka 1987; Girard and Milligan 1992; Milligan 1996; Kieffer 2000). Current knowledge on Arctic species is limited, and as exploitation continues to increase in the face of warming Arctic temperatures, a better understanding of physiological stress indicators in key commercial and bycatch species is needed to help inform fisheries management.

## Methods

All fish were captured aboard the Nunavut research vessel, R. V. Nuliajuk, in September 2013 and 2014 within and around Scott Inlet and Sam Ford Fjord on Baffin Island, Nunavut, Canada (approximately 71°15'N, 70°30'W). The surface water temperature was ~3 °C, with bottom temperatures remaining constant around 1 °C at depths below 450 m as determined by CTD (conductivity, temperature, depth) casts performed on the vessel at the same time as fishing activities.

Greenland halibut were caught in 2013 from a single bottom trawl (Yankee style) at a depth of 700 m (~1463 m of 1.43 cm diameter cable on each drum). The trawl was fished in a straight line at a speed of ~3 knots (2.4–2.8 knots) for 30 min after settling to the bottom, as per standard research trawl procedures. Typical commercial trawls tow for longer than 30 min (anywhere from 1 to 4 h; DFO, personal communication); therefore, these data provide a conservative measure of the physiological response of Greenland halibut to fisheries trawl capture. When the net was brought to the surface, Greenland halibut in good condition were immediately placed in a black plastic bin with a continuous flow of fresh seawater and held until testing. To obtain a blood sample, the caudal peduncle of each fish was severed and blood collected in a plastic vial. A drop of blood was then placed on the testing strip of a Lactate Pro LT-1710 portable analyzer (ARK-RAY Inc., Kyoto, Japan), and an Accu-Chek® compact plus glucose meter (Roche Diagnostics, Basel, Switzerland), following standard protocols (Venn Beecham et al. 2006; Cooke et al. 2008; Stoot et al. 2014). The time from blood collection to analysis was <1 min. The time between testing each fish was ~6 min, leading to a sequential time range of testing from 21 to 188 min following trawl capture.

Greenland sharks were caught on longlines within Scott Inlet and Sam Ford Fjord in both 2013 and 2014. Longlines were soaked for an average of 12 h at varying depths that were binned into three groups of 300–600 m, 600–700 m, or 700–900 m for data analysis. Both the average set time and the depth fished are typical of the longline fishery for Greenland halibut which ranges from 600 to 1000 m depth, and 10- to 12-h set duration (DFO 2008b; DFO, personal communication). The bottom longline consisted of a standard baseline rope (9.2-mm-diameter tarred black sinking line) approximately 735 m long with 50 × 1.5 m steel leader ganglions with size 16 and 17 Tuna circle hooks spaced 5.5 m apart. All hooks were baited with frozen squid and set in the evening, then retrieved in the early morning. Basic morphometric data (total length—cm), sex (presence of claspers to identify males) and blood

withdrawal were undertaken, while the shark was held in tonic immobility next to a small boat (Kessel and Hussey 2015). Processing time of each shark was <20 min. To collect blood samples, a small incision was made with a scalpel blade between the pelvic and caudal fin. A 16-gauge needle attached to a 30-mL non-heparinized syringe was inserted into the incision and approximately 10 mL of blood withdrawn. A drop of whole blood was immediately placed on to the glucose and lactate testing meters described above. In 2014, a Lactate Plus (Nova Biomedical®, Waltham, USA) analyzer was used to replace the Lactate Pro LT-17710, given both meters perform similarly (Tanner et al. 2010). The time from blood withdrawal to testing was <1 min.

All data were assessed for normality using the Wilk–Shapiro test. Multiple Greenland halibut samples fell below the detection limit of the lactate analyzer (minimum detection = 0.8 mmol/L). As a result, the method of median semi-variance (SemiV) was used to apply values (with a range of 0–0.7) to the left-censored data as described in Zoffoli et al. (2013). The variance in the Greenland halibut lactate data could not be corrected by transformation, and therefore a generalized least-squares model with an exponential variance structure was used, which models the heterogeneity in the residuals as a random effect. Both fork length (FL) and time since capture as well as their interaction were used as independent variables. Greenland halibut glucose values were modeled with a multiple linear regression using the same independent variables as lactate (FL and time since capture). A stepwise backward selection was used to determine the final model for both metabolites, removing insignificant terms based on the  $t$  statistic. A linear regression was used to determine the relationship of Greenland shark lactate and glucose with total length (TL); then, a  $t$  test and one-way analysis of variance (ANOVA) was used to test the effect of sex and capture depth, respectively, on both stress metabolites. In the event the ANOVA was significant, a Tamhane post hoc test was used to determine which depth bins varied significantly.

## Results

Twenty-five Greenland halibut ranging in size from 27 to 51 cm FL (20 individuals  $\leq 45$  cm) were sampled from the trawl. Eleven individuals had blood lactate levels below the detectable range of the analyzer; consequently, values used in the following analyses were assigned with the SemiV method (Zoffoli et al. 2013). This resulted in a range of lactate values from 0.2 to 2.8 mmol/L and mean ( $\pm$ SD) of  $1.0 \pm 0.7$  mmol/L. Time since capture was the only significant term in the generalized least-squares model

( $t = 2.83$ ,  $p = 0.0095$ ,  $\delta = 0.78$ ; Table 1; Fig. 1). Glucose values ranged from 1.1 to 3.7 mmol/L with a mean ( $\pm$ SD) of  $1.8 \pm 0.6$  and both time since capture ( $t = 2.11$ ,  $p = 0.05$ ,  $\beta = 0.17$ ) and FL ( $t = 3.09$ ,  $p = 0.005$ ,  $\beta = 0.03$ ) were significant ( $F_{2,22} = 5.20$ ,  $p = 0.01$ ,  $R^2 = 0.32$ , Table 1; Fig. 1) in the regression model.

A total of 46 Greenland Sharks were captured on longlines with a size range of 150–341 cm TL. All sharks appeared in good condition and swam off following release. There were no significant differences for either metabolite (independent samples  $T$  test,  $p > 0.05$ ) between females ( $n = 19$ ) and males ( $n = 27$ ). Lactate ranged between 0.7 and 11.1 mmol/L and was significantly different among depth stratum ( $F_{2,43} = 40.52$ ,  $p < 0.0001$ , Fig. 2) with sharks caught in shallower water (300–600 m depth) having lower values (mean  $\pm$  SD  $1.6 \pm 0.5$ ,  $n = 11$ ) than those caught in deeper water [600–700 m:  $6.4 \pm 2.4$  mmol/L ( $n = 20$ ) and 700–900 m:  $3.7 \pm 1.2$  mmol/L ( $n = 15$ )] based on the Tamhane post hoc test. Lactate did not have a significant relationship with TL. Glucose ranged from 2.6 to 8.1 with a mean ( $\pm$ SD) of  $4.9 \pm 1.5$  mmol/L. Glucose did not vary significantly based on depth stratum ( $p > 0.05$ ), but the regression showed a significant effect of TL ( $F_{44} = 7.50$ ,  $p = 0.0089$ ,  $R^2 = 0.15$ , Fig. 2).

## Discussion

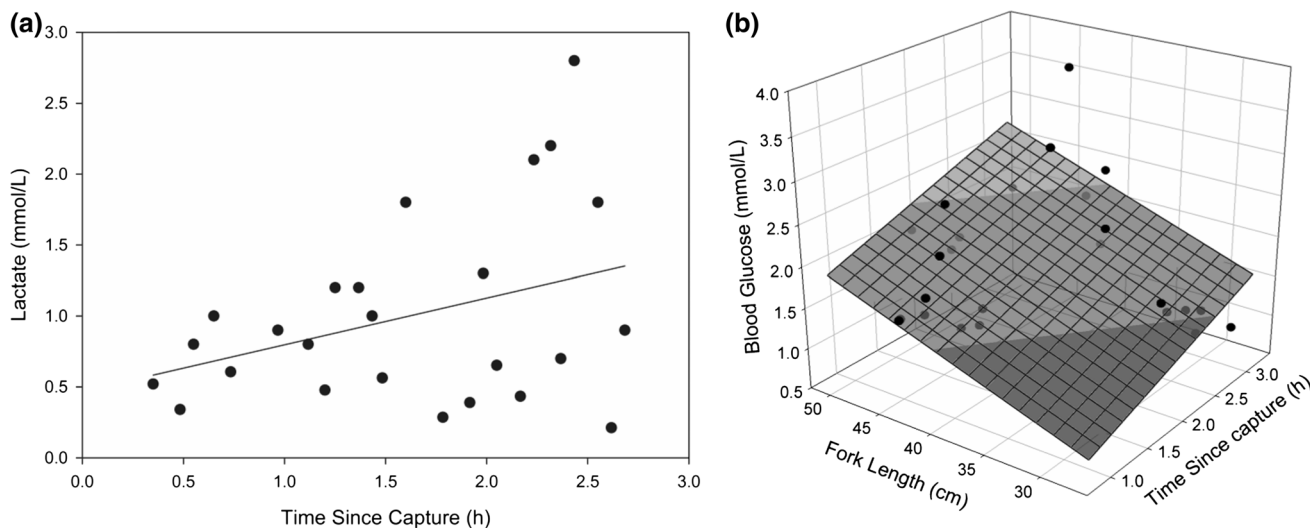
These data are the first to examine lactate and glucose in terms of a stress response to capture in two Arctic fish, the Greenland halibut and the Greenland shark. The lactate values obtained for Greenland halibut ( $1.0 \pm 0.7$  mmol/L) were elevated compared to the resting values of other flatfish such as the winter flounder *Pseudopleuronectes americanus* ( $0.05 \pm 0.04$  mmol/L; Girard and Milligan 1992), European flounder *Platichthys flesus* ( $0.35 \pm 0.01$  mmol/L; Waring et al. 1992) and flathead sole *Hippoglossoides elassodon* ( $0.18 \pm 0.02$  mmol/L; Turner et al. 1983) suggesting an increase caused by capture. Flatfish and other sluggish or benthic species commonly express low lactate values compared to active pelagic species (Waring et al. 1992) even when manually exercised to exhaustion, as lactate produced by muscular anaerobic cellular respiration remains within the muscles to be metabolized as opposed to being released to the blood (Wardle 1978; Girard and Milligan 1992; Dalla Via et al. 1997). For example, winter flounder had a blood lactate concentration of  $1.3 \pm 0.66$  mmol/L (Girard and Milligan 1992) and the common sole *Solea solea* had a value of 3.9 mmol/L (Dalla Via et al. 1997) after forced exercise, while the flathead sole had a value of  $1.6 \pm 0.05$  (Turner et al. 1983) after severe forced exercise that led to the loss

**Table 1** Summary results for the significant statistical tests performed on Greenland halibut *R. hippoglossoides* and Greenland shark *S. microcephalus* stress metabolites (lactate and glucose) after capture in Scott Inlet, Baffin Island

| Stress metabolite                                     | <i>n</i> | FL      | Mean ± SD | Statistical analysis  | Variable   | Parameter estimate | SE    | <i>t</i> value | <i>p</i> value |
|---|----------|---------|-----------|---|------------|--------------------|-------|----------------|----------------|
| Greenland halibut <i>Reinhardtius hippoglossoides</i> |          |         |           |   |            |                    |       |                |                |
| Glucose   | 25       | 27–51   | 1.8 ± 0.6 | Linear regression $R^2 = 0.26$<br>( $F_{2,22} = 5.20, p = 0.01$ ) | Intercept  | -0.79              | 0.42  | -1.89          | 0.072          |
|   |          |         |           |   | Time       | 0.17               | 0.08  | 2.11           | 0.05           |
|   |          |         |           |   | FL         | 0.03               | 0.01  | 3.09           | 0.005          |
| Lactate   | 25       | 27–51   | 1.0 ± 0.7 | Generalized least squares   | Intercept  | 0.42               | 0.12  | 3.02           | 0.006          |
|   |          |         |           |   | Time       | 0.37               | 0.13  | 2.83           | 0.01           |
|   |          |         |           |   | $\delta^a$ | 0.78               |       |                |                |
|   |          |         |           |   |            |                    |       |                |                |
| Stress metabolite                                     | <i>n</i> | TL      | Mean ± SD | Statistical analysis  | Variable   | Parameter estimate | SE    | <i>t</i> value | <i>p</i> value |
| Greenland shark <i>Somniosus microcephalus</i>        |          |         |           |   |            |                    |       |                |                |
| Glucose   | 46       | 150–341 | 4.9 ± 1.5 | Linear regression $R^2 = 0.13$<br>( $F_{1,44} = 7.50, p = 0.01$ ) | Intercept  | 2.10               | 1.04  | 2.01           | 0.05           |
|   |          |         |           |   | TL         | 0.01               | 0.004 | 2.74           | 0.01           |
| Lactate   | 46       | 150–341 | 4.4 ± 2.6 | ANOVA (by depth in meters)  | Depth      | Mean               | SD    | <i>F</i> value | <i>p</i> value |
|   |          |         |           |   | 300–600    | 1.6                | 0.5   | 40.52          | 0.001          |
|   |          |         |           |   | 600–700    | 6.4                | 2.4   |                |                |
|   |          |         |           |   | 700–900    | 3.7                | 1.2   |                |                |

Total sample size (*n*), range of FL and TL (in cm) and the overall mean (±SD) of the given metabolite are listed on the left-hand side of the table. The statistical analyses with significant results are listed with all relevant parameters to the left

<sup>a</sup> Delta ( $\delta$ ) is the estimated parameter for the power of the covariate variance structure used to model the Greenland halibut lactate data

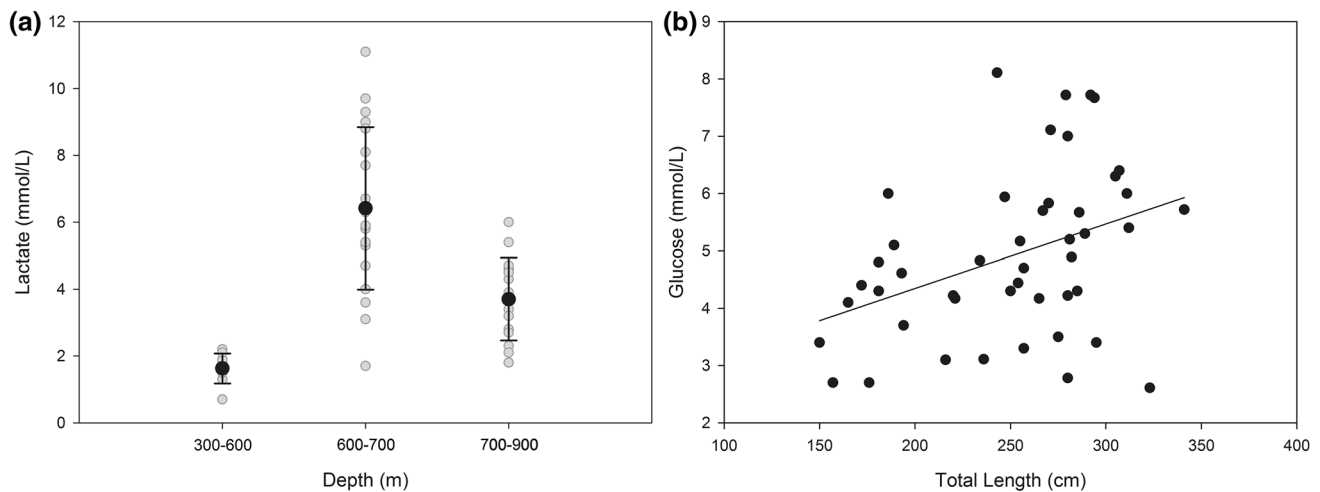


**Fig. 1** The relationship of Greenland halibut *R. hippoglossoides* stress metabolites after capture in a bottom trawl in Scott Inlet, Baffin Island with the variables FL and time since capture as determined by backwards stepwise selection. **a** Generalized least-squares model for Greenland halibut lactate values where time since capture was the only significant variable ( $t = 2.83, p = 0.0095$ , equation:  $y = 0.37$

(time) + 0.42,  $\text{var}(\varepsilon_i) = \sigma^2 \times e^{2(0.78) \times \text{time}_i}$ ). **b** Multiple linear regression for Greenland halibut glucose values with the significant variables time since capture ( $t = 2.11, p = 0.05, \beta = 0.17$ ) and FL [ $t = 3.09, p = 0.005, \beta = 0.03, F_{2,22} = 5.20, p = 0.01, R^2 = 0.32$ , equation:  $y = 0.17(\text{time}) + 0.02(\text{FL}) - 0.77$ ]

of orientation. The Greenland halibut samples in the current study fall within this range from resting to maximally exhausted, with a general increase in lactate with time since capture. Flatfish typically require 2–3 h post-exercise

to reach maximal lactate values (Turner et al. 1983; Dalla Via et al. 1997; Barnett and Pankhurst 1998), and since testing was completed after ~3 h, the linear increase indicates that fish were still responding to the trawl



**Fig. 2** Stress metabolites for Greenland sharks *S. microcephalus* caught on longlines in Scott Inlet, Baffin Island. **a** Greenland shark lactate values from three depth stratum (300–600, 600–700 and 700–900) where sharks captured between 300 and 600 m had significantly lower lactate concentrations than those captured in

deeper water (one-way ANOVA:  $F_{2,43} = 40.52$ ,  $p < 0.0001$ ). *Light gray dots* are the original, untransformed data and the *black dots* are the mean  $\pm$  one standard deviation. **b** Linear regression of Greenland shark blood glucose with TL ( $F_{44} = 7.50$ ,  $p = 0.0089$ ,  $R^2 = 0.15$ ,  $y = 0.01$  (TL) + 2.10)

stressor. Yet the increasing variability in the data from the initial to the final fish tested, modeled by the generalized least-squares method, suggests that some individuals may have begun to recover. Many factors can influence recovery rate, yet body size and temperature (as temperature was constant for all sampled fish), two principal parameters that affect recovery, were not influential in this study. Undefined parameters such as the variation in time of capture in the trawl net, level of fasting, body condition among individuals, or intraspecific variability in stress response could also influence this trend (Kieffer 2000).

The average lactate values obtained for Greenland sharks captured on longlines ( $4.4 \pm 2.6$  mmol/L) were comparable to species such as the tiger shark, *Galeocerdo cuvier* ( $4.9 \pm 6.0$  mmol/L; Marshall et al. 2012) and lemon shark, *Negaprion brevirostris* ( $5.4 \pm 5.5$  mmol/L; Hyatt et al. 2012) caught under similar conditions. Large sharks are difficult to maintain in captivity, and so many studies use smaller species as baseline references which typically express undisturbed lactate values of  $0.42 \pm 0.33$  mmol/L for spiny and smooth dogfish *Squalus acanthias* and *Mustelus canis* (Mandelman and Skomal 2009),  $0.33 \pm 0.04$  mmol/L for Port Jackson sharks *Heterodontus portusjacksoni* and  $0.31 \pm 0.03$  mmol/L for gummy sharks *Mustelus antarcticus* (Frick et al. 2010). Yet sharks express a high degree of variation in their response to capture stress, as species such as the pelagic thresher *Alopias pelagicus* attain average lactate concentrations of  $32.1 \pm 6.0$  mmol/L when captured on a longline similar to this study (Marshall et al. 2012). The variability in lactate concentration of different shark species is likely driven by metabolic scope as it pertains to the species' ability to

respond and recover from the homeostatic disruptions caused by capture (Skomal and Bernal 2010). The only significant predictor of lactate for Greenland sharks in the present study was capture depth, where sharks caught in the shallowest water displayed the lowest concentration (Fig. 2). It is unclear what would cause the increase in lactate concentration with capture depth; however, one possible explanation is the longer time needed to bring the gear up from extreme depths. Further work is needed to understand the increase in lactate concentration with depth for Greenland sharks and determine whether it is also associated with increased risk of mortality (Butcher et al. 2015).

Of the Greenland sharks sampled in 2013, most had acoustic transmitters surgically implanted following blood sampling. Two of these sharks were not detected on a gate of monitors across the entrance of the fjord where tagging took place, suggesting they may have died post-release (Hussey, unpublished data). Interestingly, one of these sharks had the highest recorded lactate concentration of 11.0 mmol/L and the second was caught at the most extreme depth of 890 m (with a blood lactate concentration of 5.4 mmol/L, above the average for all the sharks sampled). Lactate has been cited as the best indicator of mortality in sharks, with moribund values ranging from  $16 \pm 2$  mmol/L in blue sharks, *Prionace glauca* (Hight et al. 2007) to  $34.3 \pm 5.0$  for shortfin mako, *Isurus oxyrinchus* (Marshall et al. 2012). Although data are limited in this study, future work combining stress indicators with post-release telemetry tracking could provide valuable information on the physiological indicators of short- and long-term survival in this species (Donaldson et al. 2008).

Both Greenland halibut and Greenland sharks demonstrated an increase in blood glucose concentration with body size (Figs. 1, 2). A possible explanation for this relationship is that glycogen stores are positively correlated with body size and therefore larger fish have the capacity to release greater amounts of glucose from the liver and white muscle tissues (Ferguson et al. 1993; Kieffer 2000). The resultant physiological effects of this variation in stress response with respect to size are unclear, as smaller fish thus have a lower anaerobic capacity and may reach exhaustion faster than larger individuals; however, they may also recover faster from minor stressors upon release (McDonald et al. 1998; Kieffer 2000; Gingerich and Suski 2012). Concurrently, the lack of a relationship between size and lactate concentration in both species may suggest that not all available glucose is used for anaerobic metabolism as a result of variation in individual activity levels and time of capture. Variation in capture-related mortalities have been reported for different size classes of fish (Davis and Parker 2004; Morgan and Burgess 2007; Milliken et al. 2009; Morgan and Carlson 2010); however, the ability to predict survival based on physiological stress indicators remains uncertain, identifying that further research into physiology and post-release mortality is required (Davis and Schreck 2005; Cooke et al. 2013).

There are a number of inherent challenges with studying the consequences of fisheries interactions on the physiology of wild fish in Polar Regions. Point-of-care devices enable immediate sampling in the field and effectively eliminate the risk of damaging samples during shipping and transportation, but are more limited in terms of detection ranges. Physiology requires context, so information on reference ranges (such as baseline and lethal values) as well as how they vary relative to sex, maturation status, body size and season will need to be considered (Cooke et al. 2013). However, obtaining baseline stress values for these species is difficult given that most fish are captured at great depths and cannot be landed fast enough to measure resting values. An important direction for future work would be to use hook timers and accelerometer sensors on bottom longlines to provide a time of capture and a measure of individual behavior of Greenland sharks when caught. Cameras on bottom trawls for Greenland halibut will also help to establish a response curve for stressor duration. Coupling physiological sampling with survival analysis through the use of telemetry provides an exciting avenue for future research (see Donaldson et al. 2008). Such an approach would provide insights into the mechanistic basis for long-term survival and behavior of these species as well as the risks associated with increased human activities and environmental change in the Arctic ecosystem.

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