



Dermal injuries caused by purse seine capture result in lasting physiological disturbances in coho salmon

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ABSTRACT

Fish vitality can be measured by classifying reflex impairments (i.e., a visual impression of the ability to respond to induced stimuli) and visible injuries. These metrics can predict survival probability following release from fisheries, and monitoring physiological disturbances following capture can help understand mechanisms of mortality. To test the hypothesis that severity of injury and reflex impairment influences the time course of physiological recovery, coho salmon (*Oncorhynchus kisutch*) were held for up to 84-h following capture by purse seine. We classified reflex impairments and visible dermal injuries, and through repeated blood sampling, assessed metrics indicative of stress, exhaustion, and osmoregulatory disturbances. Reflex-impairments and blood lactate levels suggested fish were exhausted upon capture but recovered after 48 h. Conversely, fish with dermal injuries showed disruptions to ion homeostasis that were greater in more severely injured fish and increased over time. While reflex impairments may predict short term post-release mortality, the prolonged physiological disturbances caused by dermal injuries are likely to be responsible for delayed mortality; our results suggest that disruptions to ion homeostasis is a possible mechanism of post-release mortality.

1. Introduction

When fish escape or are discarded from fisheries, the stress and exhaustion they experience can be severe (Davis, 2002). Upon perception of stress, an adaptive neuroendocrine response circulates stress hormones and mobilizes energy reserves (Barton 2002). Depending on the extent of exhaustion (Kieffer, 2000) or asphyxiation (via crowding or direct air exposure; Cook et al., 2015), blood and intracellular acidosis (respiratory and metabolic acidosis) can develop that can be lethal, or require an extended and energetically costly recovery period. Quantifying physiological disturbance post-capture can elucidate the magnitude of trauma, and potentially reveal mechanisms of mortality. Additionally, capture severity can be estimated with vitality metrics, defined herein as a visual impression of survival potential (Davis, 2010).

The most common vitality metric is reflex impairment, an assessment of the presence of involuntary responsiveness to induced stimuli. Reflex impairment is a reliable predictor of post-release mortality

among various fish species and capture methods (Barkley and Cadrin, 2012; Davis, 2007; Davis and Ottmar, 2006; Humborstad et al., 2009), including coho salmon (*Oncorhynchus kisutch*) released from purse seines (Cook et al., 2018a; Raby et al., 2015). The other common vitality metric is visible injury, likewise important to survival (Baker et al., 2013; Baker and Schindler, 2009; Butcher et al., 2010; Cook et al., 2018a; Marçalo et al., 2010; Suuronen et al., 1996). The integument in fish is metabolically active, adapts rapidly to changes in the external environment, and provides a defensive barrier (Mateus et al., 2017). Injuries may cause stress, compromise osmoregulatory function (Olsen et al., 2012; Zydlewski et al., 2010), and provide a pathway for infectious pathogens (Udomkusonsri and Noga, 2005).

Reflex impairment therefore integrates physiological and neurological trauma, while classifications of injury consider external physical damage. Post-release mortality is best predicted by combining both reflex impairment and visible injury (Benoît et al., 2012; Meeremans et al., 2017; Uhlmann et al., 2016). A current knowledge gap is how physiological recovery varies given distinct levels of injury and

Abbreviations: DFO, Fisheries and Oceans Canada; RM-LME, Repeated measures linear mixed effects model; LDA, Linear discriminant analysis

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impairment. Such information may explain how vitality assessments predict survival, while potentially identifying physiological mechanisms underlying post-release mortality.

In British Columbia (BC), Canada, Pacific salmon (*Oncorhynchus* spp.) fisheries encounter a mix of populations and species. They are managed to harvest abundant species while limiting harm to those of conservation concern, achieved in part through species-specific mandatory release (Fisheries and Oceans Canada [DFO], 2001). Coho salmon are among the least abundant species of Pacific salmon, and include a threatened population (Interior Fraser River population; COSEWIC, 2002) that must be released when caught incidentally (DFO, 2017). In a simulated fishery, coho salmon were collected for on-board holding studies, and a concurrent telemetry study quantified rates of post-release mortality (Cook et al., 2018a). Upon capture, coho salmon were assessed for visible dermal injury and reflex impairment. Blood samples were drawn immediately and after different durations of captivity (up to 84 h) to assess physiological recovery. We tested the hypothesis that severity of dermal injuries and reflex impairment influenced the time course of physiological recovery upon release. We predicted that physiological recovery would be prolonged among fish suffering greater injury and impairment upon capture relative to uninjured and unimpaired fish.

2. Methods

Protocols were in accordance with the Canadian Council of Animal Care guidelines and approved by University of British Columbia's institutional Animal Care and Use Program (Protocol #A12-0250). Research took place in the Strait of Juan de Fuca off the west coast of Vancouver Island, BC, from August 30 to September 8, 2013 aboard a commercial purse seine vessel [specifications in Cook et al., 2018a]. Fishing occurred as in a true commercial fishery, except that the vessel was modified to rapidly release pink salmon (*O. gorbuscha*) and other bycatch while retaining coho salmon. Fish were transferred from the pursed net to a sorting table using an industry-standard brailer, a large dip net operated with a hydraulic winch. A total of 43 sets were conducted. Time to brail the fish from the net (i.e., brailing time) was variable, ranging from 13 to 44 min (median = 23 min). Capture times were primarily dependent on catch size (i.e., set size), which ranged from 45 to 3500 fish (median = 508.3). Coho salmon to be held were transferred from the sorting table to adjacent plastic tanks (61 cm deep × 109 cm × 119 cm) with flow-through seawater; all other fish were immediately released overboard. Several fish were processed from each set resulting in a range of times between being landing and initial sampling (median = 30.0, range = 2.2 to 105.1 min). This time was considered in subsequent analyses.

Study fish were processed in a padded, V-shaped trough continuously supplied with seawater. First, reflex impairment was assessed: five reflexes were scored as unimpaired (0) or impaired (1) and the final index was calculated as the proportion of reflexes impaired. Five reflexes were assessed as present or absent: (1) Tail grab: does the fish attempt escape when the handler grabs the tail?; (2) Body flex: does the fish struggle when held out of water with both hands wrapped around the body?; (3) Head complex: does the fish exhibit a regular pattern of ventilation when momentarily held out of water?; (4) Vestibular-ocular response: when turned on its side, does the fish's eye roll to maintain level pitch, tracking the handler?; and (5) Orientation: when the fish is placed upside-down in the holding tote, does it right itself within 3s? Further descriptions are provided in Raby et al. (2012), a validation study for river-migrating coho salmon. Following reflex assessments, dermal injury was classified visually by scoring the presence and severity of three observations: (1) net marks (i.e., dark contusion lines with focused rings of scale loss; present = 1, absent = 0); (2) scale loss (as a percentage of the entire dermal area missing scales: > 50% = 2, < 50% = 1, < 5% = 0; Fig. 1); and (3) other injuries (e.g., torn operculum, severely split fins, predator wounds; present = 1,



Fig. 1. Representation of scale loss categories. Scale loss was classified as the percentage of the entire dermal surface missing scales: > 50% (top), < 50% (middle), < 5% (bottom). A final injury score included scale loss scores as well as classification of the presence of net marks or other external injuries as either present or absent.

absent = 0). As with reflex impairment, injury scores were combined as a proportion (i.e., total score/highest possible score). After the vitality assessment, approximately 1.5 mL of blood was collected by caudal puncture using 3 mL lithium-heparinized vacutainers (B.D. Vacutainer, Franklin Lakes, NJ) with 21-gauge, 3.8 cm needles while the fish was supine in the sampling trough. Fork length (FL) was recorded to the nearest 0.5 cm using a ruler affixed within the sampling trough. The entire assessment and sampling procedure took < 2 min per fish. No anesthesia was used.

The analysis included blood samples taken from 60 fish retained for captive observation and 70 fish selected at random among 220 tagged and released in the concurrent survival study (Cook et al., 2018a). Blood samples collected upon capture from both groups are herein referred to as time zero (T_0) samples.

The on-board holding pen (1.8 m wide × 1.4 m long × 3 m deep) consisted of an aluminum frame lined with 70-mm diamond soft nylon mesh. The pen held up to 30 fish and fit within a larger below-deck tank. A pump provided a continuous supply of seawater, and oxygen was bubbled through a diffusion plate as needed to maintain saturation between 85 and 115%. The tank was kept covered during the entire holding period. Fishing operations were able to continue while fish were below deck. Two back-to-back iterations of the holding experiment each lasted for 84 h. Environmental conditions (e.g., water and air temperatures, sea conditions) were similar across the two holding experiments. Water temperatures ranged from 8.5 °C to 10.6 °C (mean = 9.1 °C) during the first holding period and 8.8 °C to 10.3 °C (mean = 9.4 °C) during the second. In-tank water temperatures were consistent with those recorded overboard. Pens were checked at least every 6 h and any moribund (i.e., loss of equilibrium and unresponsive but still ventilating) or dead fish were removed. Sub-samples of fish were removed at approximately 48-, 72-, and 84-h post-capture (Table 1). A second blood sample was taken from removed fish using identical methodology as T_0 samples, after which fish were released.

No survival analyses or assessments of fate were executed. A comprehensive survival analysis was conducted from fish telemetry-tagged and released (see Cook et al., 2018a), which provides a more realistic estimation of mortality for this population. Survival was also assessed in a similar 24-h holding study in Raby et al. (2015).

Table 1

Sample sizes of fish used in holding studies. Two samples were taken from each fish: once at capture (T_0) and then subsets of fish were removed for a second sampling, after which they were released. Samples taken at random from 70 fish included in a larger telemetry study were included in T_0 analyses.

Sampling period	Live removals	Morbund removals	Remaining	Telemetry-tagged
T_0 : Capture	NA	NA	60	70
T_2 : 48-hrs	16	10	34	NA
T_2 : 72-hrs	16	4	14	NA
T_3 : 84-hrs	13	1	0	NA

2.1. Blood chemistry analyses

Vacutainers containing whole blood were stored on ice for < 3 h. Processing involved centrifugation for 5 min at 10,000g (Compact II Centrifuge, Clay Adams, Parsippany, NJ). Plasma was flash-frozen in liquid nitrogen and stored at -80°C until processing. Plasma cortisol concentrations were measured in duplicate using 96-well ELISA kits (Neogen Corp., Lexington, Ky.) using suitable dilutions, and a 240PC plate reader (Spectramax Molecular Devices, Sunnyvale, CA.). Plasma lactate and glucose concentrations were measured to assess exhaustion and as a secondary stress indicator, respectively, using a YSI 2300 Stat Plus lactate/glucose analyzer (Yellow Springs Instruments). Samples were analyzed for osmoregulatory status [chloride (Haake Buchler digital chloridometer), sodium and potassium (Cole-Palmer, model 410 single-channel flame photometer), and osmolality (Advanced Instruments 3320 freezing-point osmometer)]. Detailed methods are provided in Farrell et al. (2001).

2.2. Data analyses

Highly correlated blood chemistry metrics were reduced with a factor analysis. The appropriate number of factors to extract was determined through observation of a screeplot and a parallel analysis. Kaiser-Meyer-Olkin (KMO) values identified problematic variables (i.e. $\text{KMO} < 0.6$; Child, 2006). A factor loading cut-off of 0.5 was applied for selection of variables included in the final factor extraction. Assumptions of normality and homogeneity of variance were tested in all models and unless otherwise specified, $\alpha = 0.05$ was used for significance testing. All analyses were conducted in R Studio (R R Core Team, 2018).

Variability in blood physiology upon capture among vitality classifications: A two-way MANOVA assessed overall differences among ordinal injury and reflex impairment scores at capture (T_0) in terms of FL and blood chemistry. Linear discriminant analyses (LDA) evaluated how well these variables supported vitality categories. Because LDAs provide no significance testing, univariate ANOVAs (Bonferroni correction; $\alpha = 0.0125$) tested overall significance for each dependent variable, and determined which variables contributed most to observed dispersion.

Effects of injury and impairment on blood physiology over time: Repeated measures linear mixed effects (RM-LME) models evaluated effects of injury and impairment on blood chemistry as a function of time. Models included fixed effects of time, injury score, and impairment score, and the interactions of injury and impairment scores with time. Each category of injury and impairment was compared to uninjured and unimpaired fish, respectively. Models were first tested to optimize random effects structure as per Zuur (2009). Collinearity among predictors was tested with variance inflation factors (cut-off of 2.0; Zuur, 2009).

Data suitability assessments: Combining T_0 samples from holding and telemetry studies was justified given a non-significant MANOVA comparing variables between the two groups. Neither time held prior to sampling, handling time, nor set size differed significantly among injury

or impairment categories (ANOVA, p -values > 0.5). As expected, T_0 plasma lactate, cortisol, and osmolality concentrations increased with time held in on-board tanks while awaiting sampling. However, considerable multicollinearity resulted when models were run with time-corrected variables (i.e., residuals of linear relationship between holding time and each physiological parameter). Therefore, raw physiological variables were used (data in Supplemental Data Table S1).

3. Results

Scale loss (Fig. 1) was the most common injury observed: 19% of fish had a scale loss score of 1 (5–50% of body surface; $n = 25$) and 16% had a scale loss score of 2 (most severe; > 50% body surface; $n = 21$). The remainder ($n = 65$) suffered < 5% scale loss. Net marks were present in 23% of fish and 14% of fish had other external injuries. The only reflexes impaired were body flex ($n = 74$), tail grab ($n = 53$), and orientation ($n = 3$). For both injury and impairment, four ordinal categories resulted from scoring (injury: 0, 0.25, 0.5, 0.75; impairment: 0, 0.2, 0.4, 0.6), whereby higher scores indicate greater injury/impairment. To simplify interpretation of results for both metrics, these scores are herein referred to as “uninjured/unimpaired”, and “minor”, “moderate”, and “severe” injury or impairment, respectively. Total mortality over time in holding studies was 25%; episodic mortality is presented in Table 1.

Factor analysis diagnostics suggested extraction of a single factor. After applying the loading cut-off, the factor was loaded positively with plasma chloride, osmolality, and sodium (Table 2) and explained 73.4% of variance among these variables (eigenvalue = 2.22). This factor, referred to herein as the ion homeostasis score, was used in subsequent analyses.

3.1. Relationships between vitality metrics and blood physiology upon capture

At capture, there were significant differences in measured fish characteristics (FL, ion homeostasis score, and plasma potassium, glucose, cortisol and lactate) among injury and reflex impairment scores (MANOVA; injury: Pillai = 0.41, $F_{3,120} = 3.13$, $p < 0.0001$; impairment: Pillai = 0.39, $F_{3,120} = 2.88$, $p < 0.0001$). The first linear discriminant (LD1) from both LDA models accounted for a high percentage of dispersion in the dataset among categories (87.8% for injury and 84.4% for impairment). Distributions of LD1 differed substantially among all injury categories (Fig. 2). For reflex impairment, large divergences occurred in LD1 between the severe and unimpaired categories. However, distributions were very similar for minor and moderate categories, suggesting no differences between these two levels of reflex impairment (Fig. 3). Univariate ANOVAs revealed a significant effect of injury and impairment on ion homeostasis scores (injury: $F_{3,121} = 9.79$, $p < 0.001$; impairment: $F_{3,121} = 6.45$, $p < 0.001$) and a

Table 2

A Factor Analysis (FA) reduced correlated plasma physiological variabilities to a single metric for use in subsequent analysis. The initial FA, including all variables with Kaiser-Meyer-Olkin (KMO) values exceeding our cut-off of 0.6 showed low communality (h^2) among several included variables. Using a pre-determined loading cut-off of 0.5, a final FA extracted a single factor, positively loaded with the variables of chloride, osmolality, and sodium.

Plasma variable	Initial FA		Final FA	
	Factor loadings	h^2	Factor loadings	h^2
Chloride	0.79	0.62	0.784	0.62
Cortisol	0.33	0.11	–	–
Glucose	0.16	0.03	–	–
Osmolality	0.89	0.79	0.904	0.82
Potassium	–0.28	0.08	–	–
Sodium	0.90	0.81	0.892	0.80

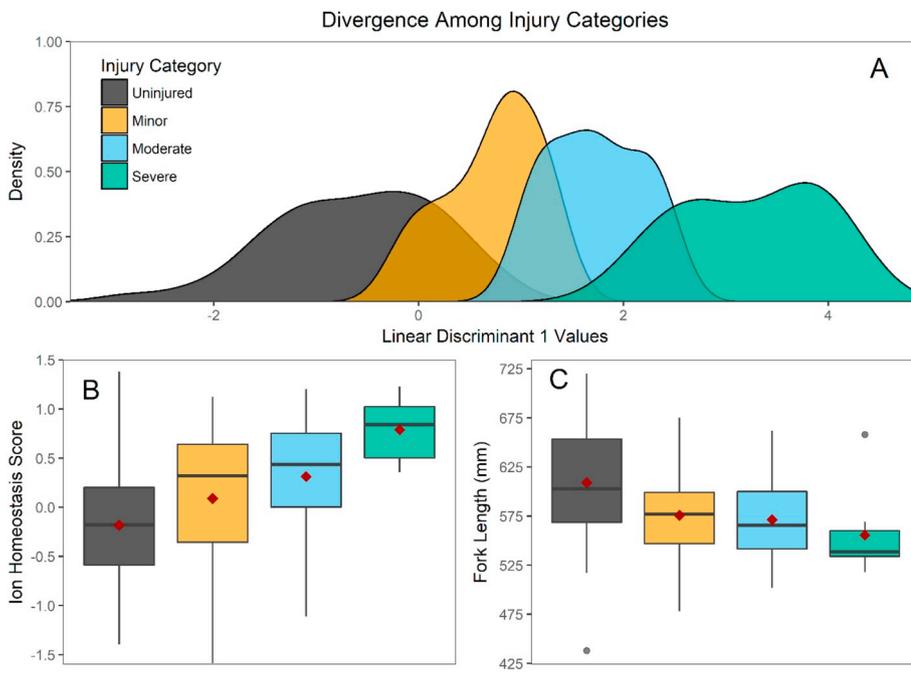


Fig. 2. Differences in fish characteristics among injury categories as determined by a Linear Discriminant Analysis. Shown as a density plot (A), distributions of Linear Discriminant 1 (LD1) reveal the degree of separation. Box plots show distributions of variables driving the observed separation, as identified by univariate ANOVAs. These include: Ion homeostasis score (i.e., a factor with loadings of osmolality, sodium, and chloride; B) and fork length (C). Box plots show median (line within box), 25th percentile (lower edge of box), 75th percentile (upper edge of box), and $1.5 \times$ interquartile range (ends of whiskers). Data points outside this range are represented by circles. Red diamond shows mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significant effect of impairment, but not injury, on plasma lactate (injury: $F_{3,121} = 3.82$, $p = 0.29$; impairment: $F_{3,121} = 15.13$, $p < 0.001$). There were significant differences in FL with injury, but not impairment scores (injury: $F_{3,121} = 7.04$, $p < 0.001$; impairment: $F_{3,121} = 1.11$, $p = 0.34$). Specifically, ion homeostasis values were higher in more injured fish, more severe injuries tended to occur in smaller individuals (Fig. 2), and both plasma lactate and the ion homeostasis score increased with greater reflex impairment (Fig. 3). Other measures of blood physiology did not differ significantly among injury or impairment scores (p -values > 0.0125).

3.2. Effects of injury and impairment on blood physiology over time

Given results of LDA models, mid-range impairment scores were combined into a single category (i.e., minor/moderate) for RM-LME

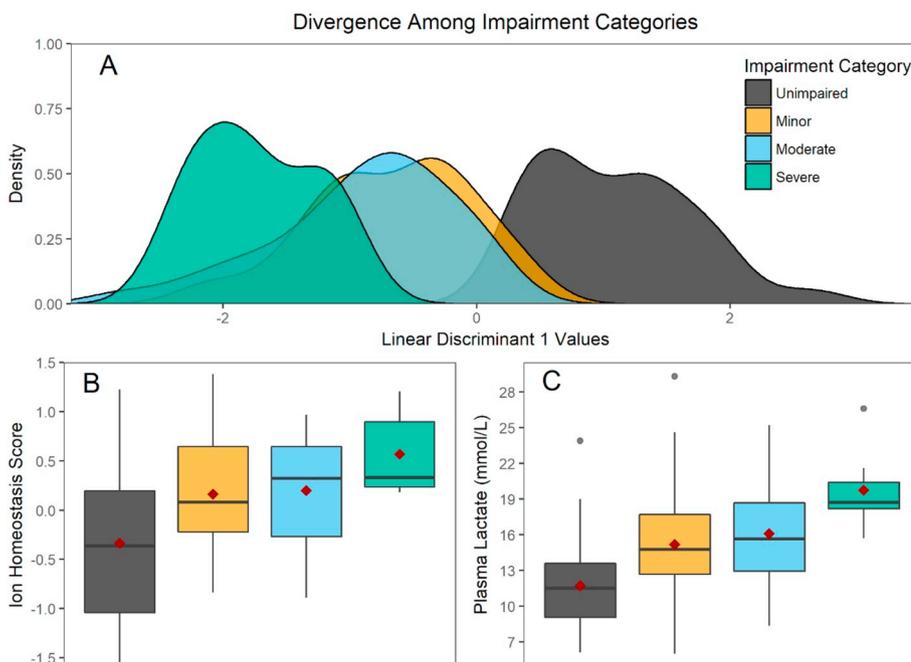


Fig. 3. Differences in fish characteristics among reflex impairment categories as determined by a Linear Discriminant Analysis. Shown as a density plot (A), distributions of Linear Discriminant (LD1) reveal the degree of separation considering measured fish characteristics. Box plots show distributions of variables driving the observed separation, as identified by univariate ANOVAs. These include: Ion homeostasis score (i.e., a factor with loadings of osmolality, sodium, and chloride; B) and plasma lactate (C). Box plots show median (line within box), 25th percentile (lower edge of box), 75th percentile (upper edge of box), and $1.5 \times$ interquartile range (ends of whiskers). Data points outside this range are represented by circles. Red diamond shows mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

models. The optimal random structure was identified as a random slope and random intercept for all models (compared to base model; Ion homeostasis score: $\Delta AIC = 37.05$, $X^2_{(\Delta df=13,16)} = 43.05$, $p < 0.0001$, lactate: $\Delta AIC = 1.09$, $X^2_{(\Delta df=13,16)} = 7.96$, $p = 0.047$, potassium: $\Delta AIC = 4.20$, $X^2_{(\Delta df=13,16)} = 10.20$, $p = 0.017$; cortisol: $\Delta AIC = 24.43$, $X^2_{(\Delta df=13,16)} = 30.43$, $p < 0.0001$), except for the glucose model (not improved with random structure; $AIC_{\text{base model}} = 751.4$; $AIC_{\text{random effects}} = 752.0$). The simple model was thus retained for plasma glucose.

Injury and reflex impairment had the greatest effects on ion homeostasis scores and plasma lactate over time. Ion homeostasis scores differed significantly among all injury and impairment categories when compared to uninjured/unimpaired fish (i.e., significant main effects for all condition classifications; Table 3), being higher among fish in poorer condition. There was also a significant interaction between injury and time in the form of an effect on ion homeostasis scores

Table 3

Results from repeated measures linear mixed effects models assessing how condition categories of injury (inj.) and impairment (imp.) influence plasma physiology over time. Models included fixed effects of time, injury, and impairment and their interactions (*) with time. Significance ($\alpha = 0.05$; significant values in bold) is the result of comparisons between each category to uninjured and unimpaired fish. Injury and impairment categories included minor, moderate (mod.), and severe. For impairment, minor and moderate categories were combined given results of a linear discriminant analyses showing no differences among measured parameters. Optimal random structure was identified as a random slope and random intercept (i.e., individual over sampling period) for all models except the glucose model, for which fit was not improved with random effects.

Predictor	Ion homeostasis score		Measured plasma parameter							
			Lactate		Potassium		Glucose		Cortisol	
	Value \pm SE	P-Value	Value \pm SE	P-Value	Value \pm SE	P-Value	Value \pm SE	P-Value	Value \pm SE	P-Value
	(DF = 125,28)		(DF = 125,28)		(DF = 125,28)		(DF = 125,28)		(DF = 125,28)	
Intercept	-0.51 \pm 0.11	< 0.001	11.2 \pm 0.68	< 0.0001	5.54 \pm 0.40	< 0.0001	6.42 \pm 0.29	< 0.0001	228.34 \pm 20.92	< 0.0001
Minor inj.	0.15 \pm 0.15	0.04	0.55 \pm 0.89	0.51	-0.59 \pm 0.51	0.21	-0.53 \pm 0.38	0.20	28.70 \pm 27.21	0.29
Mod. inj.	0.39 \pm 0.16	0.03	-0.78 \pm 0.97	0.53	-1.54 \pm 0.55	< 0.01	-0.88 \pm 0.41	0.06	6.24 \pm 29.70	0.83
Severe inj.	1.30 \pm 0.25	< 0.0001	4.74 \pm 1.51	0.10	-1.88 \pm 0.86	0.02	1.27 \pm 0.64	0.05	16.92 \pm 46.23	0.72
Minor/mod. Imp.	0.47 \pm 0.13	< 0.001	4.45 \pm 0.76	< 0.0001	-0.75 \pm 0.44	0.06	0.99 \pm 0.33	< 0.01	-8.36 \pm 23.51	0.72
Severe imp.	0.88 \pm 0.27	< 0.01	8.43 \pm 1.66	< 0.0001	-1.59 \pm 0.94	0.09	-0.14 \pm 0.71	0.83	-22.02 \pm 50.92	0.67
Time (t)	0.00 \pm 0.01	0.92	-0.12 \pm 0.01	< 0.0001	-0.03 \pm 0.01	< 0.001	-0.01 \pm 0.01	0.29	0.63972 \pm 1.00	0.53
Minor inj. * t	0.02 \pm 0.01	0.03	-0.01 \pm 0.02	0.41	-0.01 \pm 0.01	0.66	0.06 \pm 0.01	< 0.0001	4.07 \pm 1.41	0.87
Mod. inj. * t	0.03 \pm 0.01	0.03	-0.01 \pm 0.03	0.76	-0.02 \pm 0.02	0.15	0.00 \pm 0.02	0.91	3.21 \pm 2.40	0.30
Severe inj. * t	0.03 \pm 0.02	0.09	-0.00 \pm 0.06	0.82	-0.03 \pm 0.03	0.49	-0.01 \pm 0.04	0.52	6.07 \pm 2.82	0.01
Minor/mod. imp. * t	-0.01 \pm 0.01	0.08	-0.06 \pm 0.02	< 0.01	0.02 \pm 0.01	0.11	0.01 \pm 0.01	0.83	-0.20 \pm 1.20	0.19
Severe imp. * t	-0.04 \pm 0.02	0.14	-0.28 \pm 0.07	< 0.001	0.52 \pm 0.03	0.09	0.52 \pm 0.04	0.13	-4.69 \pm 4.43	0.05

for the minor and moderate categories; scores increased over time in these fish relative to the uninjured group, which showed stable ion homeostasis scores over time (Fig. 4).

Impairment and its interaction with time were highly significant predictors of plasma lactate, and there was also a main effect of time (Table 3). Plasma lactate concentrations recovered (decreased) over time regardless of initial level of reflex impairment (Fig. 5). That is, lactate concentrations increased with level of impairment at T_0 (values of 11.7 ± 0.5 , 15.9 ± 0.5 , and 19.7 ± 1.3 mmol/L for unimpaired, minor/moderate impairment, and severe impairment, respectively), but recovered to ~ 2 mmol/L among groups at all other time periods. Therefore, the significant interactions are reflective of the greater recovery required for fish with greater initial reflex impairment (and higher initial blood lactate levels). There were no significant effects of injury, nor its interaction with time on plasma lactate concentrations (Table 3).

The only significant effect in the cortisol model was the interaction of severe injury and time (Table 3). Plasma cortisol increased over time in severely injured fish but stayed consistent in uninjured fish (i.e., ranged from 222.9 ± 14.7 at T_0 to 279.8 ± 64.2 at T_3 ; Fig. 4).

4. Discussion

The fishing methods employed emulated realistic commercial purse seine practices, and resulted in dermal injuries, reflex impairment, and physiological disturbances. Results support the hypothesis that severity of dermal injuries and reflex impairment influence the time course of physiological recovery in the days following capture. Dermal injuries caused disruption to ion homeostasis, both immediately and over an extended period of days. Both reflex impairment and time were associated with blood lactate such that concentrations were higher among impaired fish relative to unimpaired fish upon capture but recovered among all groups by 48 h.

This study provides further evidence that vitality assessments provide a robust means to evaluate trauma among fish intended for release. The lack of recovery of ion homeostasis over time given dermal injury suggests a possible mechanism by which injuries may predict delayed mortality. Conversely, reflex impairments may be more important to immediate and short-term survival (e.g., given an inability to escape predation upon release; Ryer, 2002).

A further important result was that smaller fish tended to experience more severe dermal injury, which conforms with findings from other species and fisheries (Broadhurst et al., 2006). For example, Raby et al. (2015) found smaller adult coho captured by purse seine to be more susceptible to dermal injury. Additionally, size-dependent rates of mortality have been observed in herring (*Clupea Linnaeus*) and sardine (*Sardina pilchardus*) bycatch in purse seines (Marçalo et al., 2010; Olsen et al., 2012; Tenningen et al., 2012), cases in which dermal injuries and scale loss also predicted mortality. Body size was not a focus of this research, though differences in fork length could be attributed to sex, population, or both, factors that we were unable to control for but that may influence injury severity (Cook et al., 2018a; Cook et al., 2018b).

4.1. Physiology of injury and impairment: osmoregulatory control, stress, and exhaustion

Of all blood parameters measured, lactate and the ion homeostasis score showed the largest differences among vitality scores. During anaerobic exhaustive exercise, lactate accumulates in white muscle and a portion leaks into the blood stream. The osmotic pressure created by intracellular metabolic acidosis causes water to move into white muscle cells, increasing plasma ion concentrations and osmolality (Kieffer, 2000; Milligan, 1996). The finding of elevated plasma lactate and ion concentrations associated with increased reflex impairment agrees with previous research (McArley and Herbert, 2014; Raby et al., 2013; Raby et al., 2015).

Plasma lactate concentrations recovered among all groups by 48 h; the significant interactions between injury classifications simply reflect the greater recovery required among more exhausted fish. However, ion concentrations recovered by 48 h in reflex impaired fish, whereas they did not in injured fish. If osmotic perturbations in injured fish were due to stress and exhaustion alone, recovery would be expected by 48 h (normal concentrations are typically restored in coho salmon following exhaustive swimming in seawater within 24-h; Farrell et al., 2001). Osmoregulatory dysfunction following dermal injury could also be attributed to leakage of body water (Butcher et al., 2010; Olsen et al., 2012; Raby et al., 2015; Suuronen et al., 1995; Zydlewski et al., 2010). In fish, both the integument and mucus layer play a role in ion regulation (Marshall and Bellamy, 2010; Shephard, 1994), and their removal reduces the efficiency of the skin's barrier function (Mateus et al., 2017).

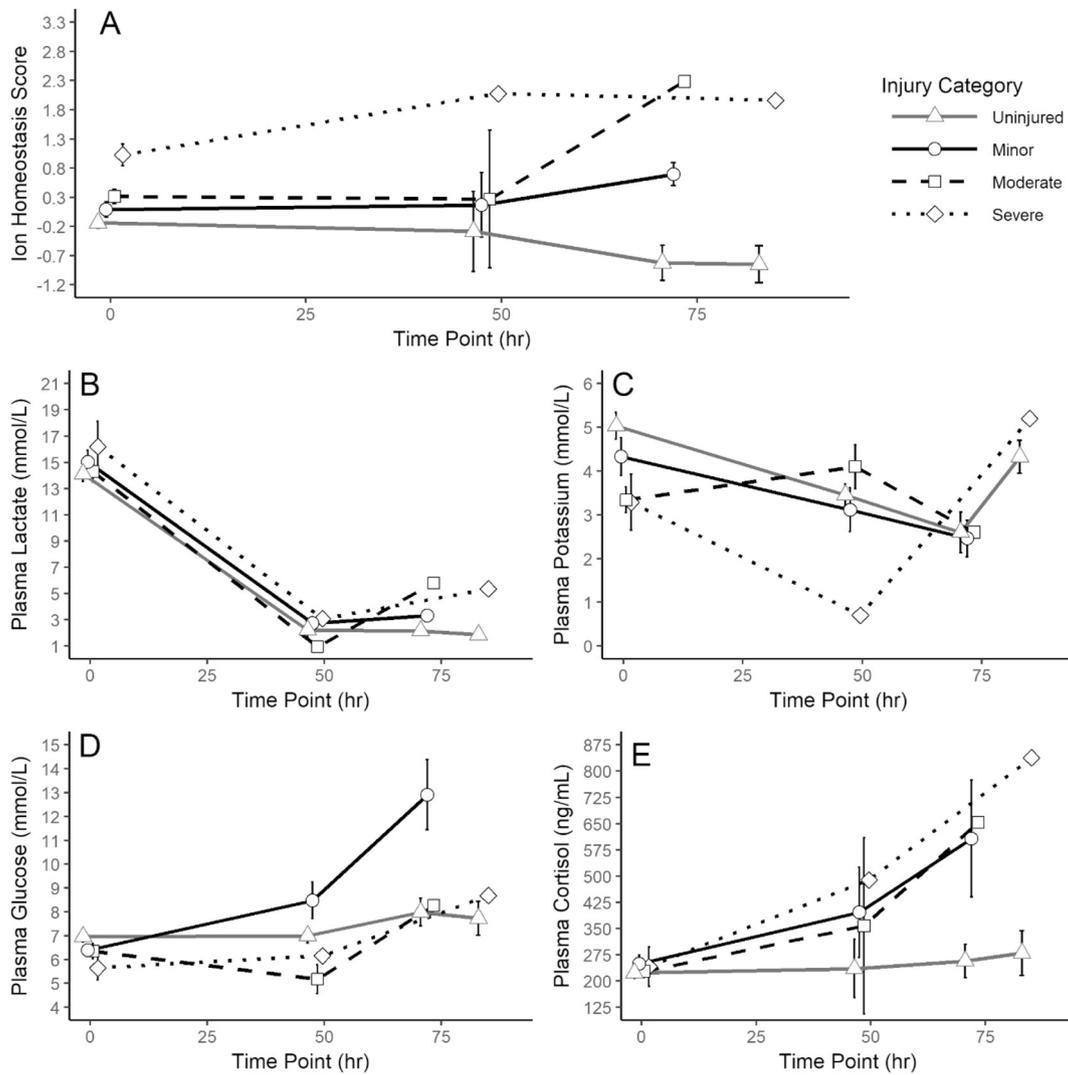


Fig. 4. Averages and standard errors of measured plasma parameters at various times by injury classification. Coho salmon (*Oncorhynchus kisutch*) captured by purse seine were blood sampled upon capture (T_0) and subsets were removed and re-sampled after 48, 72, or 84 h of holding in captivity. Measured parameters include and ion homeostasis score (i.e., a factor with loadings of osmolality, sodium, and chloride; A), lactate (B), potassium (C), glucose (D), and cortisol (E) are shown. Where there are no error bars, there was only one fish in the group.

Chronic stress can also influence osmoregulation (Davis, 2006), has been associated with ulcerations in captive fish (Udomkusonsri and Noga, 2005), and can suppress local immune responses, impairing wound healing (Mateus et al., 2017). Following experimental de-scaling of herring, Olsen et al. (2012) observed a massive cortisol response in scaled fish relative to untreated controls and suggested the proximate cause of mortality was a secondary stress response. Although plasma cortisol did increase over time among injured fish relative to uninjured fish, the only significant effect in the cortisol model was an interaction between injury and time in the severely injured group, which also had the smallest sample size. Adult Pacific salmon present unique challenges when interpreting changes in plasma cortisol. Baseline cortisol concentrations are exceptionally variable in returning adults (Baker and Vynne, 2014; Cook et al., 2014), increasing to “stressed” levels naturally with migratory challenges (Flores et al., 2012). The raw cortisol values measured in injured fish were not exceptionally high for adult Pacific salmon. Average cortisol concentrations from samples taken after T_0 for all fish classified as having some injury were $506 \pm 78 \text{ ng mL}^{-1}$ (range: 46–987 ng mL^{-1}), well below those recorded for severely exhausted coho (i.e., $1271 \pm 75.4 \text{ ng mL}^{-1}$; Farrell et al., 2001). Among sockeye salmon (*O. nerka*) caught at river-entry and exposed to an air exposure treatment, plasma cortisol

concentrations were $857 \pm 36 \text{ ng mL}^{-1}$ in a stressed state, with baseline concentrations recorded up to 470 ng mL^{-1} (mean = 66 ng mL^{-1} ; Cook et al., 2014). The highly variable nature of cortisol in adult Pacific salmon coupled with low sample sizes does limit abilities to draw clear evidence regarding the role of cortisol in this study.

4.2. Implications for Pacific salmon fisheries

We observed a failure of ion concentrations to return to baseline for up to 84 h in fish with dermal injuries. Although fish may recover faster in the wild than in captivity, this finding may have population-level implications. The consequences of capture-induced injury for released fish is largely determined by severity in relation to individual tolerances. Considering potential population-level impacts requires understanding the factors influencing susceptibility to injury. For example, some species are more vulnerable to injury (e.g., fish with deciduous scales; Suuronen et al., 1996; Butcher et al., 2010) and even within a species, diverse factors may contribute injury severity. Specific to Pacific salmon, injuries are more likely to progress following capture in females than males (Cook et al., 2018b), and there is anecdotal evidence that coho salmon are particularly sensitive relative to other species. Here, we observed increased injury in smaller fish, a finding

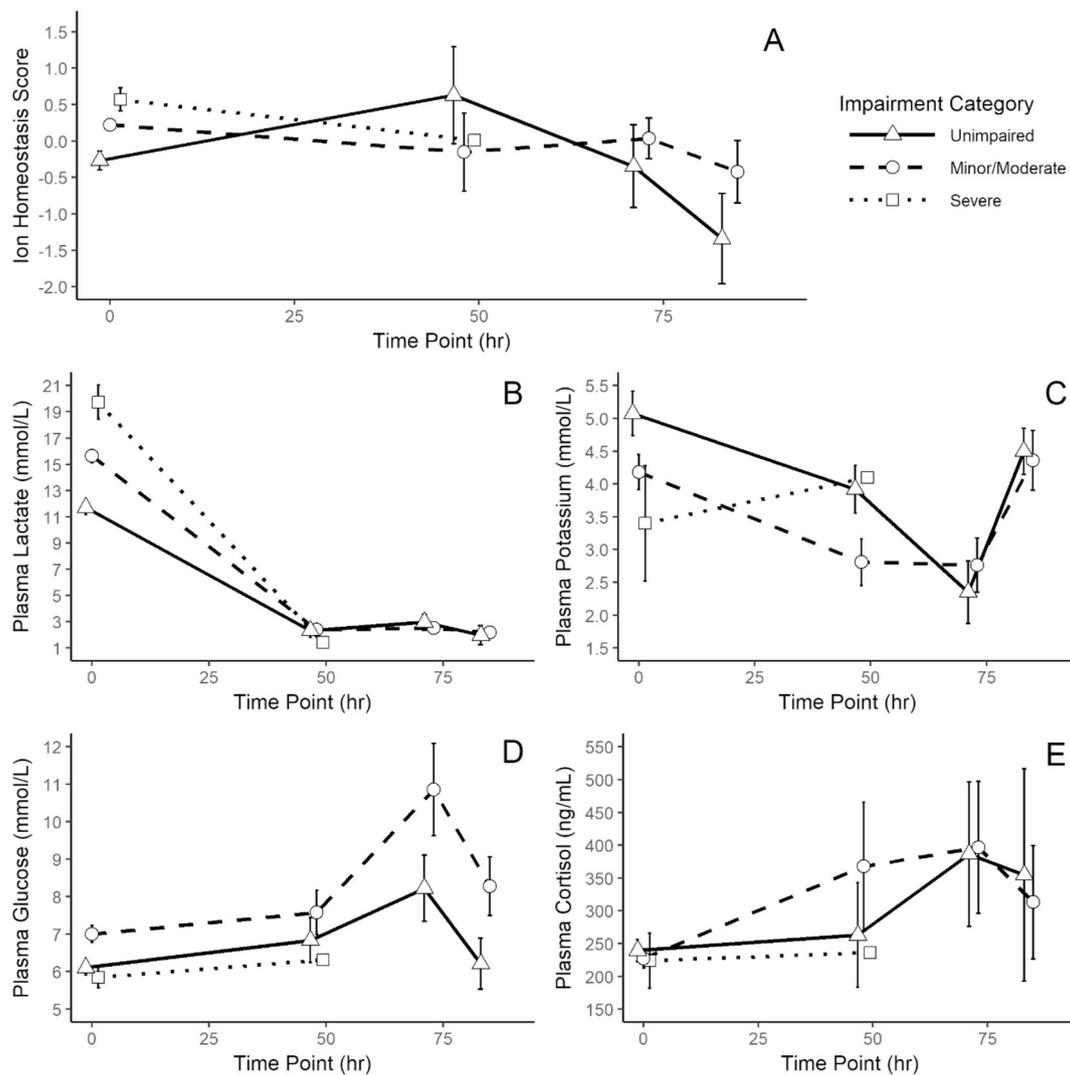


Fig. 5. Averages and standard errors of measured plasma parameters at various times by impairment classification. Coho salmon (*Oncorhynchus kisutch*) captured by purse seine were blood sampled upon capture (T_0) and subsets were removed and re-sampled after 48, 72, or 84 h of holding in captivity. Measured parameters include ion homeostasis score (i.e., a factor with loadings of osmolality, sodium, and chloride; A), lactate (B), potassium (C), glucose (D), and cortisol (E) are shown. Where there are no error bars, there was only one fish in the group.

potentially attributed to population-specific differences (Cook et al., 2018a; Raby et al., 2015).

Perhaps most importantly though is that return migrant Pacific salmon are more vulnerable to scale loss during their ocean silver (i.e., not reproductively mature) phase (Cook et al., 2018b). Therefore, the timing with which salmon are caught in coastal waters and the potential for dermal injuries to impair osmoregulatory capacity is significant. Adult Pacific salmon remodel their osmoregulatory physiology in preparation for freshwater entry, a process initiated long before river-entry (Shrimpton et al., 2005). Any latent effects of dermal injuries on osmoregulatory capacity during this period, likely a time of high natural mortality (Cooke et al., 2006), could substantially impact migration success.

4.3. Conclusions and considerations

It is unknown how results would differ in a natural setting. Confinement stress is a known limitation to holding studies (Rogers et al., 2014), and holding conditions may have exacerbated dermal injuries (though a soft, knotless nylon mesh was used in pens). Holding fish for a longer duration would have also been informative, which was not possible given the cost and logistical constraints of at-sea fishing

charters. Following external injury, an inflammatory response is typically observed for 3–4 days, after which wound areas decrease (Ceballos-Francisco et al., 2017). Therefore, the time course of our study only encompassed this inflammatory period and was not long enough to document healing processes. For example, Zydlewski et al. (2010) only observed osmoregulatory deficiencies in descaled Atlantic salmon (*Salmo salar*) smolts in seawater relative to controls for 3 days post-injury.

Although not investigated here, the potential role of bacterial and fungal infections also warrants recognition. Following a simulated gillnet encounter, Teffer et al. (2017) observed diminished immune responses, increased productivity of infectious disease agents, and osmoregulatory failure in sockeye salmon that died prematurely. In the present study, it is unknown how infectious agents may have influenced the finding of increased osmoregulatory dysfunction among injured fish.

Despite these unknown sources of variation, results provide strong support for the notion that post-release mortality among fish with dermal injuries is due to a loss of osmoregulatory control. However, with few severely injured fish surviving to later sample periods, we cannot rule out that dermal injuries may lead to lethal levels of stress, as suggested by Olsen et al. (2012). Further, reflex-impairments and

blood lactate levels indicated that fish were exhausted upon capture. Although these fish recovered in captivity, a fish released to the wild in this state would be unlikely to evade predation. Results add to the evidence that vitality metrics can effectively reflect capture severity. Additionally, we surmise that while reflex impairments may predict immediate or short-term mortality, injury severity, having long-term physiological consequences, is perhaps more predictive of delayed mortality.

Conflict of interest

Authors have no conflicts of interest to declare.

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Contributions

KVC, SGH, SJC, GDR conceived of the research proposal. DAP oversaw blood physiology assays. Fieldwork conducted by KVC and SMD. Primary writing and interpretation of results conducted by KVC, SMD, and GDR.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbpa.2018.09.026>.

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