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Experimental capture and handling of chum salmon reveal thresholds in injury, impairment, and physiology: Best practices to improve bycatch survival in a purse seine fishery

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ABSTRACT

Recommendations and regulations regarding handling of non-target fish (i.e. bycatch) are often vague and subjective in commercial fisheries. Identifying how different components of capture influence the condition of discarded fish can help develop specific guidelines and best handling practices. Using an experimental approach, we modified the severity of capture stressors in commercial purse seine fisheries for Pacific salmon and monitored indices of injury and reflex impairment in chum salmon (*Oncorhynchus keta*), a species commonly discarded from these fisheries. Study fish were held for 5 or 10 days. Modeling of changes in injury and impairment sought to disentangle the latent effects of capture stressors and the role of sex and maturity. Thresholds in physiological responses to times (i) pursed in the net and (ii) air exposed on deck were also evaluated. Injury progressed throughout holding, was more extensive in females, and accelerated faster in less mature fish. Both crowding severity and set size (i.e. estimated number of fish caught) increased injury and impairment, effects that were exacerbated with time pursed. Physiological indicators of exhaustion also increased with time pursed and 15 min was identified as an important transition point, potentially representing the temporal limit to anaerobic exercise. The time between 1 and 3 min of air exposure was identified as being important to survival, and after 6 min of air exposure, endogenous energy stores may have become exhausted. Resulting recommendations include keeping nets loose during sorting, releasing fish prior to 15 min of being pursed, and keeping air exposure within the range of 1–2 min, or less. Additionally, females and less mature fish appear to be more susceptible to the injurious effects of capture.

1. Introduction

For non-target fish discarded from fisheries, evaluations of how condition changes under different scenarios can help determine appropriate measures to maximize probability of survival (Benoît et al., 2012). Fish captured in fisheries incur physical injury (from minor mucus and scale loss to large wounds), exhaust themselves fighting against entrapment, and can be subject to rapid environmental changes and oxygen deprivation through exposure to hypoxic conditions or direct exposure to air (Davis, 2002). Air exposure is arguably one of the most severe forms of acute stress that a fish can experience (Cook et al., 2015) and unsurprisingly, several studies have found that reducing the time that non-target fish spend exposed to air has the greatest impact on their survival (e.g. Humborstad et al., 2009; Benoît et al., 2010). The degree of injury sustained during capture is likewise important to survival outcomes (Baker et al., 2013; Meeremans et al., 2017) and, when combined with the stress of capture, can lead to performance-altering behavioural changes (e.g. weakened swimming or predator evasion abilities; Davis, 2002). By quantifying the magnitude of injury sustained in addition to abilities to respond to stimuli (i.e. impairment, typically measured through reflex action testing; Davis, 2007), we can evaluate key aspects of the fishing process that limit the survival of non-

Abbreviations: AIC, Akaike information criterion; DFO, Fisheries and Oceans Canada; POLR, proportional odds linear regression; RM-LME, repeated measures linear mixed effects

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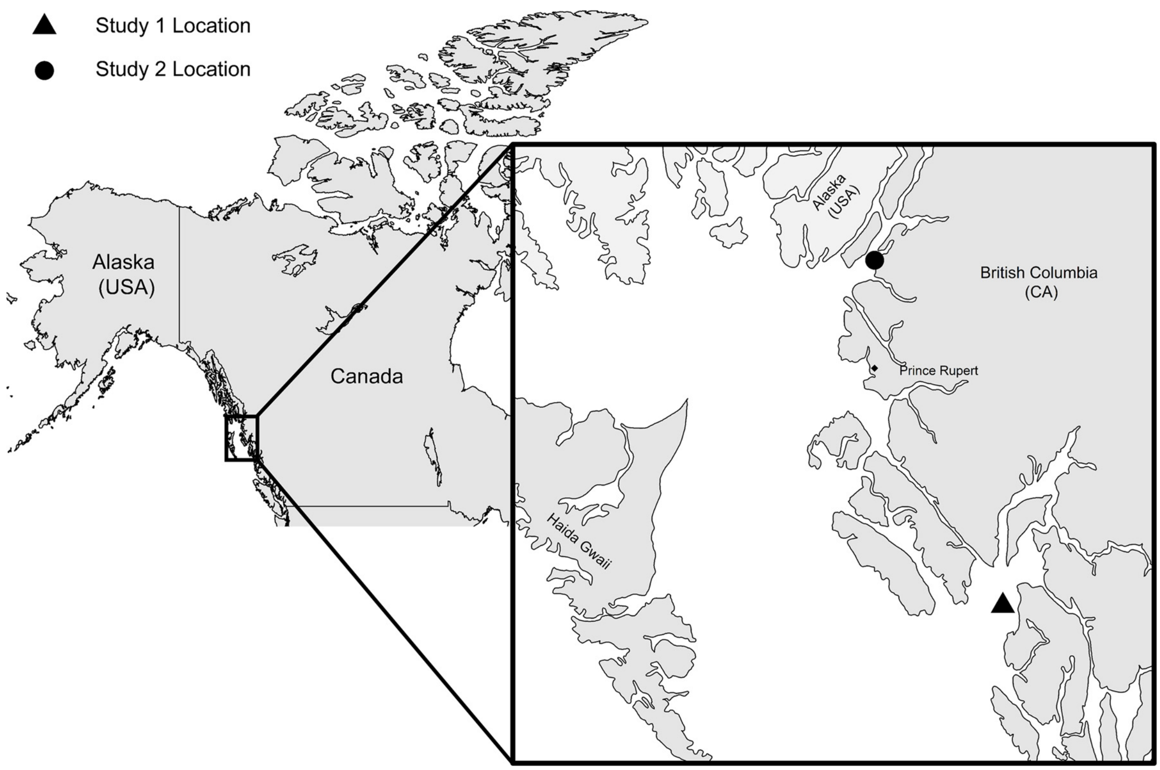

Fig. 1. Map of study locations across two years of research with the purse seine fleet on the northern coast of British Columbia, Canada. The first year of research (Study 1) was conducted on the southern end of the North Coast of BC, and the second year of research (Study 2) near the coastal border with Alaska (USA).

target fish.

Many interacting factors contribute to the magnitude of injury and impairment sustained by non-target fish during capture, especially in commercial operations where capture durations may be protracted. Although many of these factors cannot be changed or controlled, such as the gear encounter itself, others can be avoided or minimized through gear and vessel modifications, or improved fish handling. Specific recommendations regarding aspects of handling are lacking from most commercial fisheries that practice discarding (for exception see Poisson et al., 2014). Subjective recommendations are often given to simply prioritize the return of non-target species to the water by a means that minimizes harm. Although much weight can be placed on human behaviour and willingness to comply, sociological research has revealed greater compliance with suggested best handling practices given clear evidence of their effectiveness (Watson et al., in press; Campbell and Cornwell, 2008).

In British Columbia (BC), Canada, coastal commercial fisheries targeting Pacific salmon (*Oncorhynchus* spp.) capture a mixture of co-migrating species and populations, some that are able to support exploitation and others that are of conservation concern (Shaklee et al., 1999). Those of conservation concern are protected in part by a program of mandatory release. Currently, the conditions of license for fisheries targeting Pacific salmon in Canada indicate that non-target fish are released with 'the least possible harm' [Fisheries and Oceans Canada (DFO), 2017]. However, there remain concerns regarding handling practices in many commercial operations and vague directives leave release practices open to multiple interpretations (Watson et al., in press). There is thus a need for science-based assessments of the specific aspects of the capture process that are most harmful for discarded fish. Moreover, as Pacific salmon fisheries target adults on their homeward migration, captured fish are undergoing dramatic physiological and physical changes in preparation for spawning. The speculation that maturation may confer a certain resiliency to capture stressors also warrants investigation (Raby et al., 2013).

Research was conducted with the purse seine fleet of BC's North Coast. Chum salmon (*O. keta*), commonly discarded from these fisheries, were the focal species. North Coast seine fisheries targeting Pacific salmon are managed as mixed-stock fisheries and encounter broad geographic aggregates of all species. The status of North Coast chum stocks has been a concern in recent years, and therefore commercial fisheries targeting other salmon species typically operate under non-retention provisions for chum (Spilsted and Pestal, 2009). Seine catches are increasingly dominating the proportion of total salmon landings in BC (Haas et al., 2016). Therefore, with large catch volumes relative to other gear types, the number of discards and hence the magnitude of impact to non-target populations, can be high. With few large chum populations in central and northern BC, maintaining abundance and diversity of small populations is critical to maintaining their resiliency (Spilsted and Pestal, 2009).

In an experimental purse seine fishery, we sought to understand the relative effect of various capture stressors on chum salmon bycatch by modifying the severity of standard capture and handling stressors. Through holding studies, we were able to observe latent effects of treatments. Chum salmon have relatively short freshwater migrations. Therefore, the maturity of those encountered in coastal fisheries progresses through the season thereby providing an opportunity to test the effect of maturation status on condition following a fishery interaction. Evaluating injury, reflex impairment, and physiological stress indicators immediately upon capture and observing the progression of injuries in the days following capture, our ultimate objective was to inform best handling practices for salmon incidentally captured in purse seine fisheries.

2. Materials and methods

Data from two years (i.e. 2015 and 2016 fishing seasons) of research with the commercial purse seine fleet of BC are presented. Research was conducted within the northern coastal regions of BC, but locations differed by year. Study 1 was completed in DFO Management Area 6 from Jul-19 to Aug-12, 2015, and Study 2 in DFO Management Area 3 from Jul-22 to Aug-11, 2016 (Fig. 1). Due to low abundances of returning salmon in 2015, the Area 6 fishery did not open, precluding our abilities to realistically simulate a commercial fishery. A more experimental approach (described below) was therefore taken in this first year of research. Area 3 was chosen for the second year for its more predictable fishery and relatively consistent catches. In this second study, we built upon findings from the first with less exploratory treatments. In both years, pink salmon (*O. gorbuscha*) were the target species of the fishery and there was a mandatory release in place for all other species, including chum.

2.1. Fish capture

Experimental fisheries were conducted from chartered purse seine vessels under a scientific research permit operating in-season on days that study areas were closed to commercial fisheries. This approach to simulate fishery operations, used previously with success (Cook et al., 2018; Raby et al., 2015), provides results as representative of the actual fishery as possible. Chartered vessels were modified to rapidly release all pink salmon and other bycatch while retaining chum salmon for further investigation. Modifications simply included having a trap door and chute from the sorting table to facilitate easier release of pink salmon. Fish were captured using a 549 m long and 55 m deep seine net with 100-mm bunt mesh (shown deployed in Fig. 2A). Following industry standards, nets were brought aboard with a drum until just the bag of the net was 'pursed' alongside the boat (Fig. 2B). Fish were then transferred to a sorting table on deck using a brailer (a large dip-net operated with the assistance of a hydraulic winch; Fig. 2C) and sorted (Fig. 2D). Once on the vessel, handling of fish varied by designated treatment (described in Section 2.2). Water temperatures ranged from 12.6 °C to 15.4 °C (Study 1) and 11.2 °C to 15.5 °C (Study 2).

Table 1
Scoring of injury and reflex impairment in chum salmon captured by purse seine (used in models as response variables). Individual injury scores were all scaled to a value between 1 and 2 for equal weighting and summed together. Impairment scores were tabulated as the total number of reflexes impaired.

	Observation	Description of Measurment
Injury	Scale Loss	Percent in increments of 10
	Skin Loss	Percent in increments of 10
	Wound Depth	0 (none); 1 (scale loss); 2 (skin loss, muscle visible); 3 (muscle missing); 4 (organs or bones visible)
	Fin Damage	0–7, representing a count of the number of rayed fins damaged
	Fin Severity	Observation of the most damaged fin: 0 (no damage); 1 (minor nicks or splits); 2 (wounds); 3 (large wounds with exposed bone)
Impairment	Tail grab	Is the fish capable of burst swimming?
	Orientation	Can the fish maintain orientation in water?
	Vestibular ocular	Does the fish's eye track the handler?
	Spontaneous flex	Does the fish fight on a flat surface?
	Restrained flex	Does the fish fight when restrained?
	Ventilation	Does the fish exhibit regular ventilation patterns?

2.2. Handling and sampling

Handling refers to activities occurring from when a fish is under control of the fisher (i.e. once the seine is pursed) to when it is released. Different treatments of net handling and sorting methods were employed to simulate various levels of handling severity. Injury and impairment were measured in response to capture treatments. Injury was estimated via a semi-quantitative scoring system applied to each fish

Fig. 2. Experimental capture was conducted from commercial purse seine vessels and simulated standard operations. Following deployment of the seine (A), the net was brought aboard with a drum until just the bag of the net was 'pursed' alongside the boat (B). Fish were held in the pursed net for a pre-determined amount of time and then transferred to a sorting table on deck using a brailer (i.e. a large dip-net; C) and sorted according to treatment (D).

that classified various observations (Table 1). To eliminate zeros and ensure equal weighting, each observation was scaled to a value between 1 and 2 and summed (i.e. minimum 5 and maximum 10). Impairment was measured using a standardized approach of observing the presence or absence of reflexes that has proven effective in predicting survival in several species of fish, including Pacific salmon (Raby et al., 2012). The final score of impairment represents the total number of reflexes impaired (Table 1). Injury and impairment were quantified immediately following handling and sorting treatments. If several fish were collected at a time from each treatment, fish were held at low densities in totes with continuous flow-through seawater until processing. This holding time while awaiting processing was recorded and did not exceed 30 min. All sampling occurred in a foam-lined trough with flow-through water.

Control fish for holding studies (i.e. minimum handling or sorting time) were removed directly from the pursed net by dipnet. In Study 1, injury and impairment assessments were not conducted on holding controls to reduce handling, but in Study 2, these condition assessments were completed on control fish. Handling treatments differed slightly between studies. In Study 1, we modified aspects of net handling and air exposure duration whereas in Study 2, we kept net handling consistent, modified air exposure duration, and recorded fish characteristics of sex and maturity. Number of fish captured within the net (i.e. set size), a variable that could not be controlled, was estimated by the captain of the vessel for both studies. Testing for physiological thresholds was only conducted in Study 1; a restricted timeline for research in the second year did not allow for this.

2.2.1. Net handling and sorting practices (Study 1)

Handling treatments under study included crowding severity, time pursed (i.e., time fish spent pursed in the net prior to being brought onboard, as in Fig. 2B), and air exposure time (Table 2). For crowding severity, treatments were either crowded or not. In the crowded treatment, the net was pulled up tightly to restrict captured fish near the surface of the water. This practice is colloquially known as 'drying up the set', which some captains believe to facilitate faster brailing. In the uncrowded scenario, the net was left loose, allowing fish to swim in position. Captured fish were held in the pursed net prior to brailing for a range of representative times, herein referred to as 'time pursed'. This procedure simulated the time fish would be held within the net while the catch is sorted and ranged from 4 to 43 min. Air exposure time, ranging between 1 and 12 min, began once fish were first dropped on the sorting table. Consequently, fish classified as having zero air exposure were still subjected to a short duration for brailing (~20 s) but not to any additional air on deck. Although it would be unusual for released fish in these fisheries to be air exposed for more than 5 min under standard protocols, an extended time course was required to detect a threshold in responses. Therefore, the numbers of fish exposed to extreme durations of air (> 6 min) were limited (n = 23; 6% of sample). Experimental fishing occurred around commercial openings, resulting in three distinct capture periods that were categorized as 'early', 'mid', and 'late' (Table 2).

2.2.2. Capture stressors and fish characteristics (Study 2)

Study 2 involved fewer modifications to handling. All fish were held within the net for 30–45 min prior to brailing, a time chosen to simulate sorting times characteristic of a large set during normal fishery operations. Air exposure was modified according to three treatments (1–5 min; Table 2). The maturation state of the fish was visually classified as silver, silver-bright, or mature. Silver fish showed no colouration except for faint vertical stripes and scales were loose. Silver-bright fish were beginning to show colouration (i.e. vertical stripes present and some dark colouration on the back), but scales were still loose. Mature fish were coloured and scales were mostly absorbed or completely absorbed. Sex was also recorded.

2.3. Threshold identification

Physiological thresholds were evaluated for both crowding and air exposure time through the non-lethal collection of blood (n = 132; Study 1 only). These fish were sampled onboard vessels and were released following sampling; they were not included in the Study 1 holding experiments. All blood sampling occurred on a single day, eliminating variable effects of water and air temperature. For the crowding treatment, fish (n = 105) were removed individually from the set with a dipnet, sampled and released. For the air exposure treatment, fish (n = 27) collected from the first brailer of two sets were air exposed on the sorting table prior to blood sampling. Sample sizes were increased for times where a threshold was thought to exist (i.e., between 3 and 6 min) based on prior observations of fish behaviour. Following blood sampling, air exposed fish were placed into totes with flowing seawater for recovery and then released.

Approximately 1.5 mL of blood was collected within 30 s by caudal puncture using 3 mL lithium-heparinized vacutainers (B.D. Vacutainer, Franklin Lakes, NJ) with 21-gauge, 1.5″ needles. Vacutainers containing whole blood were stored on ice for no more than 3 h. Processing involved centrifugation for 5 min at 10,000*g* (Compact II Centrifuge, Clay Adams, Parsippany, NJ) after which plasma was collected and

Table 2
Description of both response and predictor variables resulting from both holding studies and used in statistical models. Data was collected immediately after capture (Day 0), and/or after 5 or 10 days of holding.

Study	Variable Type	Variable	Measurement
1: Air exposure and net handling	Response	Injury	Sum of scored injury observations; measured at Day 0, 5, and 10
		Impairment	Number of reflexes impaired; measured at Day 0
		Lactate, chloride, glucose	Plasma concentrations (mmol/L) measured in response to durations of air exposure and time pursed (fish not used in holding studies)
	Predictor	Set size	Estimated number of fish captured foreach set
		Crowding severity	Binary as either crowded (i.e. 'dried-up'; 1) or not crowded (i.e. kept loose; 0)
		Air exposure	Continuous measurement of time exposed to air (1–12 min)
		Time pursed	Continuous measurement of time pursed in net (4–43 min)
		Capture period	Categorized given capture date: early (July 22–24), mid (July 29–31) or late (August 5–6)
2: Capture severity and fish characteristics	Response	Injury	Sum of scored injury observations; measured at Day 0, 5, and 10
		Impairment	Number of reflexes impaired; measured at Day 0
	Predictor	Capture treatment	Categorical; Air exposure duration (minutes) following 35–45 min net time: 'Moderate' (1–2), 'Severe' (2.5–3.5) or 'Very Severe' (4–5). Control fish were collected by dipnet
		Maturation State	Categorization as silver (least mature), silver bright, or mature (most mature)
		Set size	Estimated number of fish captured for each set
		Sex	Female or male

stored at −80 °C. Plasma was analyzed in the laboratory for chloride (digital chloridometer [Haake Buchler Instruments, New Jersey, USA]), lactate and glucose (2300 Stat Plus analyzer [YSI, Ohio, USA]) using methods described by Farrell et al. (2000). These metrics were chosen as response variables because stress induces energy mobilization (e.g. glucose) and compromises hydromineral balances (Wendelaar Bonga, 1997). If the response becomes anaerobic, waste products (e.g., lactate) accumulate and water moves into the muscle, also influencing ion homeostasis (Kieffer, 2000; Wendelaar Bonga, 1997).

2.4. Fish transport, holding, and repeat sampling

Upon completion of treatment and condition assessments, fish to be used in holding studies were tagged with a uniquely numbered spaghetti tag (Floy, Washington, USA) threaded through the dorsal musculature. Control fish were tagged immediately following removal from the net. Study fish were transported to holding pens in industry-standard recovery totes (0.6 × 0.7 × 1.2 m) with a high velocity continuous flow of seawater. Densities did not exceed 6 fish per tote. Every attempt was made to minimize transport time but due to fishing location, times ranged from 20 to 45 min. Study fish were held for either 5 (n = 236) or 10 (n = 123) days in floating pens (4.6 × 1.5 × 1.5 m) affixed to an anchored structure. Pens were made of an aluminum frame fully surrounded and covered by 100-mm bunt mesh. Densities did not exceed 35 fish/pen and pens were checked daily for mortalities. Fish were removed by dip net at Day 5 (both studies) and Day 10 (Study 1 only) to evaluate progression of injuries. In Study 2, all fish were released after 5 days. In Study 1, fish were returned to the pens after sampling on Day 5 for re-sampling at Day 10, and then released. However, at each timepoint a subset of fish were sacrificed for a disease monitoring program (n = 40 per time point; data not included). With low mortality observed in both studies, fate was not analyzed as a response variable.

2.5. Statistical analyses

Measured variables differed between studies (Table 2), but both sought to identify factors contributing to injury and impairment. We tested for the effect of specific conditions of capture (Study 1) and fish characteristics given treatment severity (Study 2) on immediate reflex impairment and injury severity, both immediately and over time. Repeated measures linear mixed effects (RM-LME) models were employed to assess changes in injury through holding. For reflex impairment, a recent study provides evidence for improved predictive abilities with retention of the ordinal and multinomial nature of the score (Meeremans et al., 2017). Therefore, proportional odds linear regression (POLR) models evaluated the probability of having a given impairment score as a result of specific capture scenarios (both studies) and with air exposure (Study 1), as in Agresti, (2002). Because POLR models produce probabilities on a scale from 0 to 1, impairment scores are presented as number of reflexes impaired rather than the proportion value described in Davis, (2007). Identification of physiological thresholds in response to air exposure and time pursed was tested using spline regression analyses for the response variables of plasma glucose, lactate, and chloride concentrations.

2.5.1. RM-LME models with response of injury

A total of three RM-LME models were run separately by study. Predictor variables differed by study year and Study 1 had three sampling periods, whereas Study 2 only had two sampling periods. In Study 1, the experimental design was such that fish exposed to longer durations of air exposure were not subjected to the severe net handling methodologies due to a priori concerns of high mortality. Therefore, given uneven distribution of treatments in Study 1, separate models identified 1) the effects of air exposure during capture (air models) and 2) the effects of net handling (net models) on injury through time (Day 0, 5, and 10). Data were restricted to fish that were not air exposed for net models. The simplified experimental design of Study 2 allowed all predictors to be explored in one model structure.

Models were first tested to optimize random effects structure and then followed a step-down approach as per Zuur, (2009). Testing for optimal random effects used the restricted maximum likelihood (REML) framework and included all fixed effects and their interactions, and compared models with 1) no random term, 2) a random intercept model by individual, and 3) a random slope and intercept model by individual over sampling period. The optimal structure of random effects was selected by comparing the Akaike information criterion (AIC) estimates; the model with the lowest AIC estimate was compared to the base model with a likelihood ratio test. Variable selection began with the most complex model and followed a stepdown approach with a maximum likelihood (ML) framework. Non-influential cases (low t-value and high *p*-value) were removed and stepdown selection continued until only significant predictors and main effects of significant interactions remained. Selected predictors were included in a final model with a REML framework. Model fit was assessed through observation of residuals and collinearity among selected predictors was tested with variance inflation factors (cut-off of 2.0). Results are reported from the most parsimonious models.

2.5.2. POLR models with response of impairment

As with RM-LME models, separate POLR models were run to assess the effects of air exposure (Study 1), net handling methods (Study 1) and the interaction of fish characteristics and capture severity (Study 2) on the probability of having a given number of reflexes impaired. Data restrictions and predictor variables were identical to those used in RM-LME models. Model procedures, including an AIC-based variable selection method, were conducted as per Faraway, (2016), whereby the final model provided predicted probabilities of being in one category of impairment versus being in the category above it as a function of each significant independent variable.

2.5.3. Spline regression modeling

Spline models were fit with a single knot fixed a priori (i.e. only one breaking point evaluated) at every possible location. AIC selection criteria determined optimal knot location (Molinari et al., 2004). F-tests determined if the selected spline model significantly improved fit from a simple linear regression. When inclusion of a spline did not improve model fit, the data failed to provide a threshold and the linear regression model was retained, if significant. This is a new approach for analyzing changes in blood physiology data over a time course, but one that has been used with success in identifying thresholds in fish body size in tagging studies (Ashton et al., 2017; Cook et al., 2014)

Fish sampled for physiological thresholds in response to air exposure were collected from two different sets to achieve sufficient sample size. The time these fish spent pursed in the net prior to air exposure treatment differed between the two sets given logistics of net handling by the crew. To test for the potentially confounding effect of differences in time pursed between the two sets, analyses of variance (ANOVA) models with main effects of time pursed, air time, and their interaction were conducted for each physiological parameter. We included fish from both sets in the spline regression given a non-significant interaction. Even if there was a main effect of the encounter time prior to air exposure (i.e. time pursed) on physiology, a non-significant interaction would indicate that the relationship between the given physiological parameter and air exposure time did not differ between the two sets.

All fish were collected from a single set to test for physiological thresholds resulting from crowding. Times ranged from 8 to 46 min and analyses were conducted as per air exposure times.

3. Results

Overall estimated set sizes ranged from 30 to over 2000 fish and were more consistent in Study 2 (median = 350; range = 30–1000) than in Study 1 (median = 260; range = 30–2100). Fishing time, encompassing net handling times prior to researchers having access to captured fish (i.e. time to tow, load net onto boat, and purse the net), ranged from 28 to 52 min. Every attempt was made for this time to be as consistent as possible, but fishing procedures were influenced by factors beyond control (e.g. presence of marine mammals, net tangles).

There were 21 total mortalities among the 191 fish held for Study 1 (10.9% overall mortality), 11 of which died immediately following air exposure of greater than 5 min. An additional 132 fish were blood-sampled and released for the physiological threshold component. A total of 169 fish were treated and held for Study 2, of which there were 6 mortalities (3.6% mortality). The median number of reflexes impaired among the 19 delayed mortalities was 3 (range = 0–6). No control fish died in either study.

3.1. Capture conditions and progression of injuries

Results from RM-LME modeling revealed a consistent impact of holding time: injury increased with sampling period in both studies. In study 1, injury scores (mean ± SE) increased from 6.42 ± 0.04 upon capture to 7.36 ± 0.06 and 7.59 ± 0.05 at Day 5 and 10, respectively. Study 2 saw an increase from 5.76 ± 0.06 upon capture to 6.21 ± 0.07 at Day 5. All individual measures of injury increased, but differences were most pronounced in measures of fin damage and fin severity; patterns of injury progression by individual metric are available in Supplementary Fig. S1.

The RM-LME model assessing the impact of net handling methods on injury (Model 1) included the fixed effects of sampling period, time pursed, whether fish were crowded, capture period (early, mid, or late), set size, and all two-way interactions. Optimal random structure was identified as a random slope and random intercept (compared to base model; $\Delta AIC = 74.57$, $X^2_{(\Delta df = 3)} = 80.57$, $p < 0.0001$). The final model included the main effect of sampling period, the interaction of time pursed and set size, and their non-significant main effects (Table 3). After approximately 20 min of being held in the pursed net, injury scores increase with set size, a relationship that does not exist when considering only short holding durations (i.e., < 25 min; Fig. 3).

The RM-LME model assessing the impact of air exposure on injury (Model 2) included the fixed effects of sampling period, air exposure time, set size, and all two-way interactions. As with the previous model, optimal random structure was identified as a random slope and random intercept (compared to base model; $\Delta AIC = 142.13$, $X^2_{(\Delta df = 3)} = 148.13$, $P < 0.0001$). Model reduction methods resulted in small improvements in model fit, and although the final model was significant, the only retained fixed effect was sampling period (Table 3). Air exposure duration had no effect on injury.

The RM-LME model for Study 2 assessing fish characteristics in addition to treatment effects (Model 3) included the fixed effects of sampling period, maturity, capture treatment (i.e. consistent net handling with varying air exposure), sex, set size, and all two-way interactions. Optimal model random structure was identified as random intercept without random slopes (i.e. compared to base model; $\Delta AIC = 113.27$, $X^2_{(\Delta df = -1)} = 115.27$, $P < 0.0001$). The final model included a fixed effect of sex, the interaction of sampling period and maturity category, and their main effects, of which sampling period was significant and maturity category was not (Table 3). Results indicate that injury increased over time and was greater in females (Fig. 4). The significant interaction between sampling period and maturity category suggests injuries accelerate faster in less mature fish. The difference in magnitude of injury between sampling period 1 and 2 is progressively less as maturity increased (Fig. 4).

3.2. Causes of impairment following capture

POLR models, which used the same structures as RM-LME models, revealed a consistent effect of air exposure on impairment. Time pursed, crowding severity, and capture period were also predictive of impairment.

The final POLR model assessing impacts of net handling methods (Model 1) included capture period, the interaction of time pursed and crowding, and associated main effects, which were significant (Table 3). When fish were not crowded during pursing, the probability of obtaining a low impairment score (i.e. one or no reflexes impaired) decreased with time pursed, while the probability of having two or three reflexes impaired increased with time pursed (Fig. 5, left panel). However, when fish were crowded, the predicted probabilities of each impairment score remained consistent with time pursed (Fig. 5, right panel). The main effect of capture period revealed that overall, fewer reflexes were observed impaired in the late compared to the early capture period (Table 3).

With respect to air exposure and capture parameters, the final POLR model included a main effect of air exposure only (Table 3). The model revealed that the probability of having low reflex impairment (i.e., fewer than two reflexes impaired) decreased quickly between 0 and 5 min. Conversely, the probability of having three reflexes impaired peaked at 5 min and then decreased as it became more common to have four or more reflexes impaired (Fig. 6).

When assessing fish characteristics in addition to capture treatments in Study 2, the final POLR model included a main effect of treatment only (Table 3). Treatments were designed to keep fishing conditions (i.e. time pursed, crowding) consistent while assessing the additional effect of air exposure. Average air exposure durations (minutes) by treatment were 1.27 (moderate; range = 1–2), 2.90 (severe; range = 2.5–3), and 4.15 (very severe; range = 4–5). The treatment effect showed no significant differences when comparing the moderate treatment to the control treatment, but significant differences were found between the control treatment and both the severe and very severe treatments (Table 3). Predicted probabilities suggest that 82 ± 10% of control fish will have no reflexes impaired, but this drops to 49 ± 5.8% and 48 ± 12% for the severe and very severe treatments, respectively (see Supplementary Fig. S2 for predicted probabilities by category and treatment).

3.3. Physiological thresholds to air exposure

Inclusion of fish from the two different sets in a single spline regression analysis for each measured plasma variable was justified given a non-significant interaction between time pursed and air exposure for all metrics (lactate: $F_{(1)} = 1.30$, $p = 0.27$; chloride: $F_{(1)} = 1.07$, $p = 0.32$; glucose: $F_{(1)} = 0.48$, $p = 0.50$). Fish were in the first set for 6 min prior to air exposure and for 11 min in the second set. There was a significant main effect of time pursed on both lactate and chloride plasma concentrations (lactate: $F_{(1)} = 17.7$, $p < 0.01$; chloride: $F_{(1)} = 25.1$, $p < 0.001$), where both metrics were higher when pursed for the longer duration. There was no effect of time pursed on glucose concentrations ($F_{(1)} = 0.88$, $p = 0.37$).

Simple linear regressions revealed that the duration of air exposure significantly predicted both lactate and glucose concentrations (Table 4). Lactate concentrations increased with air exposure time and the optimal threshold location was identified at 2 min. Although the model was significant with inclusion of a spline, AIC scores were not improved with the new model and thus the simple linear regression was retained. For glucose, optimal knot location was identified at 6 min. The spline model was significant overall and a significantly better fit than simple linear regression (Table 4). According to the spline model, glucose concentrations remained consistent and had a non-significant relationship with air exposure until 6 min of air exposure, after which concentrations decreased significantly with time (Fig. 7). For chloride, optimal knot location was identified at 4 min. Chloride concentrations first decreased significantly, and then had a positive but non-significant relationship with air exposure after 4 min (Fig. 7). However, although

Table 3
Results from repeated measures linear mixed effects (RM-LME) models and proportional odds linear regression (POLR) models assessing the effects of various capture stressors on repeated measures of injury through holding or on the probability of being assigned a given impairment score (i.e. number of reflexes impaired) upon capture, respectively. Results are only provided for final models but change in Akaike information criterion (ΔAIC) scores along with test statistics from likelihood ratio tests comparing final reduced models with full models. In POLR models, intercepts are provided for each impairment category relative that above it; test statistics are therefore not provided for each intercept and *p*-values are summarized. Sampling period refers to sampling day, of which they were 3 sampling periods (Day 0, 5, and 10) in Models 1 and 2 and 2 sampling periods (Day 0, 5) in Models 3. Maturity categories include silver (S), silver-bright (SB), and mature (M).

Model	ΔAIC	$X^2_{(\Delta df)}$	Retained model parameters		
			Predictors	β ± SE	p-value
RM-LME Model 1: Net handling	8.54	$3.46_{(6)}$	Intercept	6.62 ± 0.18	< 0.0001
			Sample period	0.095 ± 0.0090	< 0.001
			Time pursed* set size	$2.2 \times 10^{-5} \pm 9.9 \times 10^{-6}$	0.016
			Time pursed	0.01 ± 0.006	0.119
			Set Size	0.00 ± 0.00	0.193
RM-LME Model 2: Air exposure	2.78	$5.22_{(4)}$	Intercept	6.48 ± 0.038	< 0.0001
			Sample period	0.12 ± 0.0052	< 0.0001
RM-LME Model 3: Capture severity and fish characteristics	31.16	$20.84_{(26)}$	Intercept	4.22 ± 0.15	< 0.001
			Sex	−0.30 ± 0.11	0.0084
			Sample period × Maturity (SB vs. S)	−0.31 ± 0.095	0.0016
			Sample period × Maturity (M vs. S)	−0.43 ± 0.11	< 0.0001
			Sample period	0.68 ± 0.072	< 0.001
			Maturity (SB vs. S)	0.25 ± 0.19	0.2
			Maturity (M vs. S)	0.29 ± 0.22	0.19
POLR Model 1: Net handling	2.47	$1.53_{(2)}$	Intercept	NA	< 0.05
			Capture period (mid vs. early)	−0.22 ± 0.45	0.062
			Capture period (late vs. early)	−1.81 ± 0.61	0.0029
			Time pursed × crowding severity	−0.092 ± 0.035	0.0091
			Time pursed	0.093 ± 0.028	< 0.001
			Crowding severity	2.45 ± 0.91	0.0073
POLR Model 2: Air exposure	3.22	$0.78_{(2)}$	Intercept	NA	< 0.001*
			Air exposure	8.64 ± 0.057	< 0.0001
POLR Model 3: Capture severity and fish characteristics	22.13	$5.87_{(14)}$	Intercept	NA	< 0.01
			Treatment (mod vs. control)	−1.05 ± 0.82	0.2
			Treatment (severe vs. control)	1.46 ± 0.63	0.02
			Treatment (very severe vs. control)	1.52 ± 0.62	0.014

* All model intercepts significant ($p < 0.001$) except that for 1 vs. 2 reflexes impaired ($p = 0.23$), suggesting that the probability of having one reflex impaired did not differ from the probability of having two impaired.

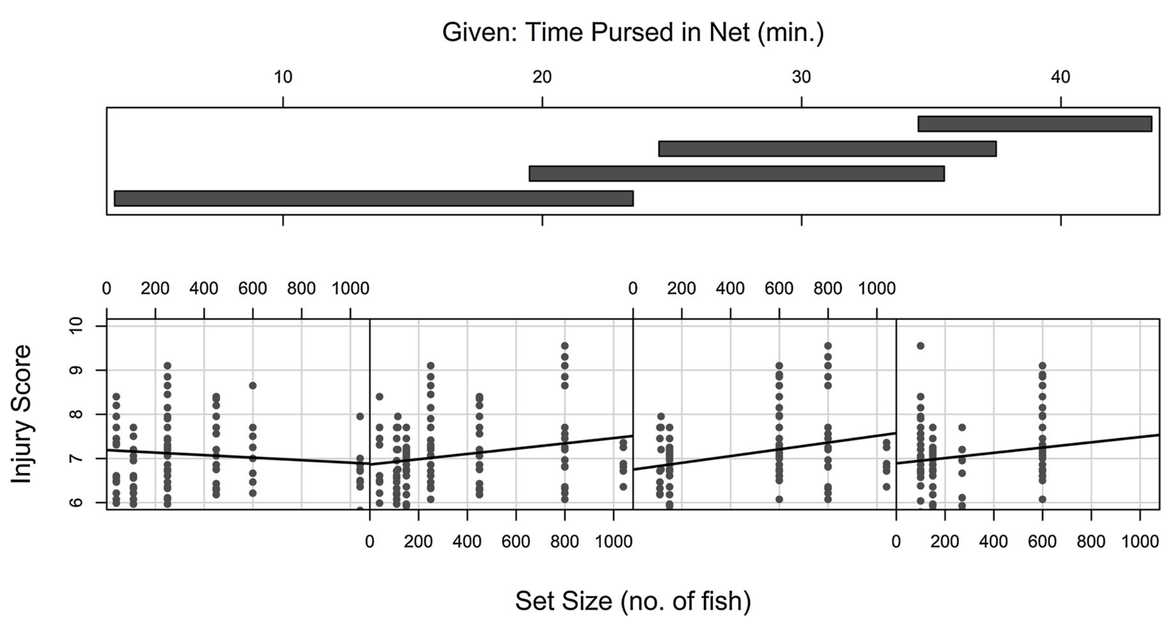

Fig. 3. A conditioning scatter plot shows the significant interaction between set size (i.e. estimated number of fish captured) and time pursed on injury scores (repeated measures linear mixed effects model) among chum salmon captured by purse seine condition on time in the net. If fish are removed quickly, injury scores are consistent regardless of set size, but with longer time in the net, injury increases with set size. The four bars above scatterplots represent the range of net times (minutes) encompassed within each of the four scatterplots below showing relationships between set size and injury scores. The range encompassed within the bars are such that each plot has a similar number of observations.

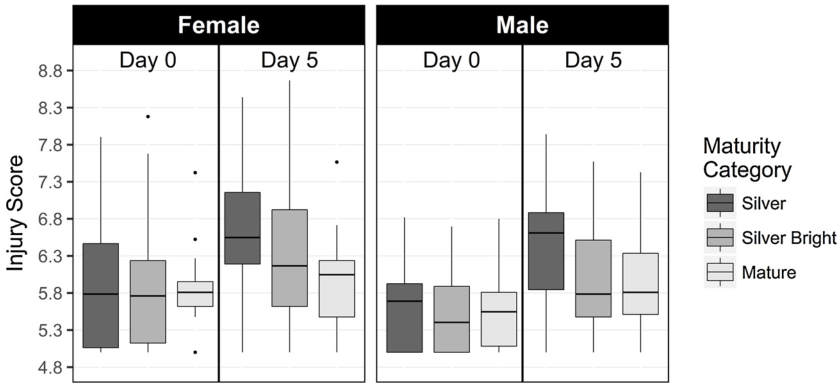

Fig. 4. Following capture by purse seine, injury scores among chum salmon are higher in females compared to males and increase with holding time, but increase faster in less mature (i.e. silver) fish. Center line indicates the median, top and bottom of the box the 25th and 75th percentile, respectively, and the vertical lines of the box indicate the range excluding outliers, which are shows as points. Maturity was not a significant main effect of the model (repeated-measures linear mixed effects).

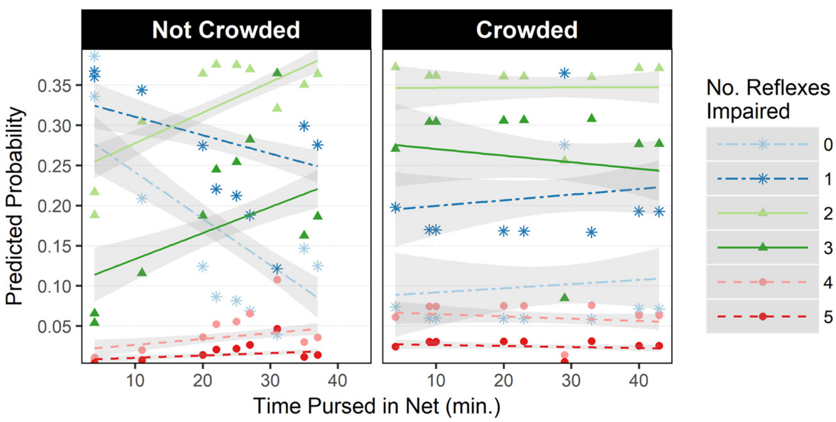

Fig. 5. A significant interaction of crowding and time pursed on the probability of being assigned a given impairment score in a proportional odds linear regression model reveal that when not crowded, the probability of sustaining low impairment scores (blue) decreases with time pursed but the probability of sustaining high impairment scores (green and red) increases with time pursed. These relationships do not exist when fish are crowded as impairment scores are higher, regardless of time held in the net. Shaded areas indicate confidence intervals. Main effects of both crowding and time pursed were also significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

inclusion of the spline improved AIC scores, F-test results were marginally non-significant at $\alpha = 0.05$, suggesting the spline model was not a better fit statistically than the simple regression model (Table 4). Additionally, neither the spline nor the linear model was significant overall.

In air exposure treatments, control fish were available to evaluate the physiological impairment resulting from air exposure alone, above that occurring during capture. These comparisons were only possible for parameters significantly predicted by air exposure (i.e., lactate, glucose). Mean lactate values for non-air exposed fish (6.0 mmol/L) were less than those predicted by the regression line at time zero (6.3 mmol/L; Fig. 7). For glucose, mean values for non-air exposed fish (5.4 mmol/L) crossed the regression line at 1.6 min (Fig. 7).

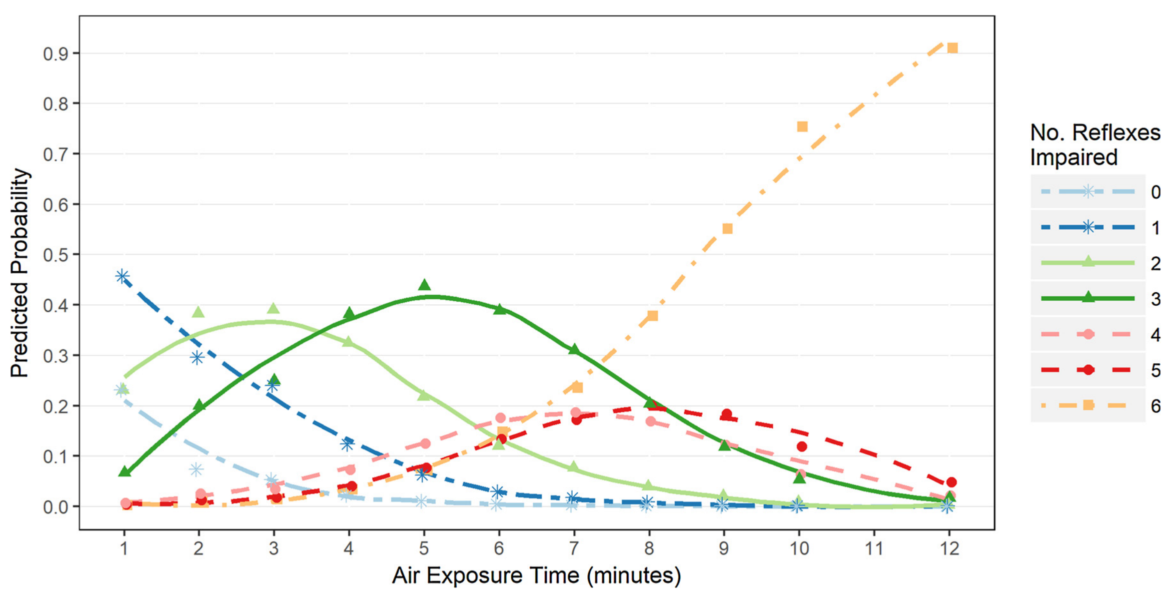

Fig. 6. Results from proportional odds linear regression models show the effect of air exposure on the probability of being assigned a given impairment score (i.e. number of reflexes assessed as being impaired). The probability of having fewer than two reflexes impaired (blue lines) decreases between 0 and 5 min. The probability of having 3 reflexes impaired, typically associated with mortality, peaks at 4 min and then decreases as it becomes more common to show ever greater impairment. Lines fit with LOESS smoothing. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Results from linear and spline regression analyses assessing the impact of both air exposure time and time pursed in the seine on plasma concentrations (mmol/L) of lactate, chloride, and glucose. Asterisks denote significance. Where AIC scores were not improved with addition of the spline, results of spline regression are not included. Within the spline model, *p*-values are provided for the relationship between time and physiological parameter overall and for lines on either side of the knot. Model comparisons reflect if the spline regression model significantly improved fit compared to linear regression.

Explanatory Variable	Response Variable	Linear Regression			Spline Regression						Model Comparison		
		$F_{(df)}$	Adj.R^2	P	Knot	$F_{(df)}$	Adj.R^2	P-values			ΔAIC	$F_{(df)}$	P-value
								Whole Model	Line 1	Line 2			
Air Exposure Time	Lactate	$43.6_{(1,33)}$	0.56	***	2	$23.27_{(2,32)}$	0.57	***	*	***	< 0	NA	NA
	Chloride	$0.3_{(1,33)}$	0.02	0.57	4	$2.2_{(2, 32)}$	0.064	0.13	*	0.95	2.06	$3.9_{(1, 32)}$	0.056
	Glucose	$9.9_{(1, 33)}$	0.21	**	6	$8.5_{(2, 32)}$	0.30	***	0.93	***	3.58	$5.5_{(1, 32)}$	*
Time Pursed	Lactate	$50.9_{(1, 102)}$	0.33	***	15	$31.3_{(2, 101)}$	0.37	***	***	***	6.01	$8.1_{(1, 103)}$	**
	Chloride	$7.0_{(1,100)}$	0.06	**	15	$8.1_{(2,98)}$	0.12	***	**	***	6.55	$8.7_{(1,99)}$	**
	Glucose	$0.4_{(1,102)}$	0.01	0.54	14	$2.3_{(2,101)}$	0.02	0.11	0.05	0.57	2.25	$4.2_{(1,102)}$	*

* Indicates $p < 0.05$.
** Indicates $p < 0.01$.
*** Indicates $p < 0.001$.

3.4. Physiological thresholds to time pursed

Simple linear regressions revealed that time pursed positively predicted both lactate and chloride concentrations. Optimal knot location was identified at 15 min for both parameters (Fig. 7; Table 4). Additionally, models were significant with inclusion of a spline, and the spline model was a significantly better fit than the linear regression. Lactate and chloride concentrations showed similar patterns with the slope of the line being positive on both sides of the knot, but steeper before the knot. In all cases the slopes were significant. Similar to the lactate and chloride spline regressions, optimal knot location in the glucose model was identified at 14 min. However, although inclusion of a spline did improve AIC scores, neither the linear nor spline model were statistically significant overall (Table 4). This suggests that time pursed does not in this case predict plasma glucose.

4. Discussion

Across two years of holding studies, we evaluated the effects of capture and handling practices on injury and impairment in chum salmon discarded from purse seine fisheries. There is now substantial support for the use of indices of injury and impairment as proxies of mortality (Benoît et al., 2010; Davis, 2007; Meeremans et al., 2017; Raby et al., 2012). Therefore, injury and impairment are discussed according to an assumption of being associated with fate. The low short-term mortality observed during our holding studies did not permit the exploration of fate as a response variable.

Injury and impairment were each associated with discrete aspects of capture. Reflex impairment often results from exhaustion and asphyxia – elements that cause stress but do not directly cause injury, and can be highly consequential if discarded fish are unable to establish orientation

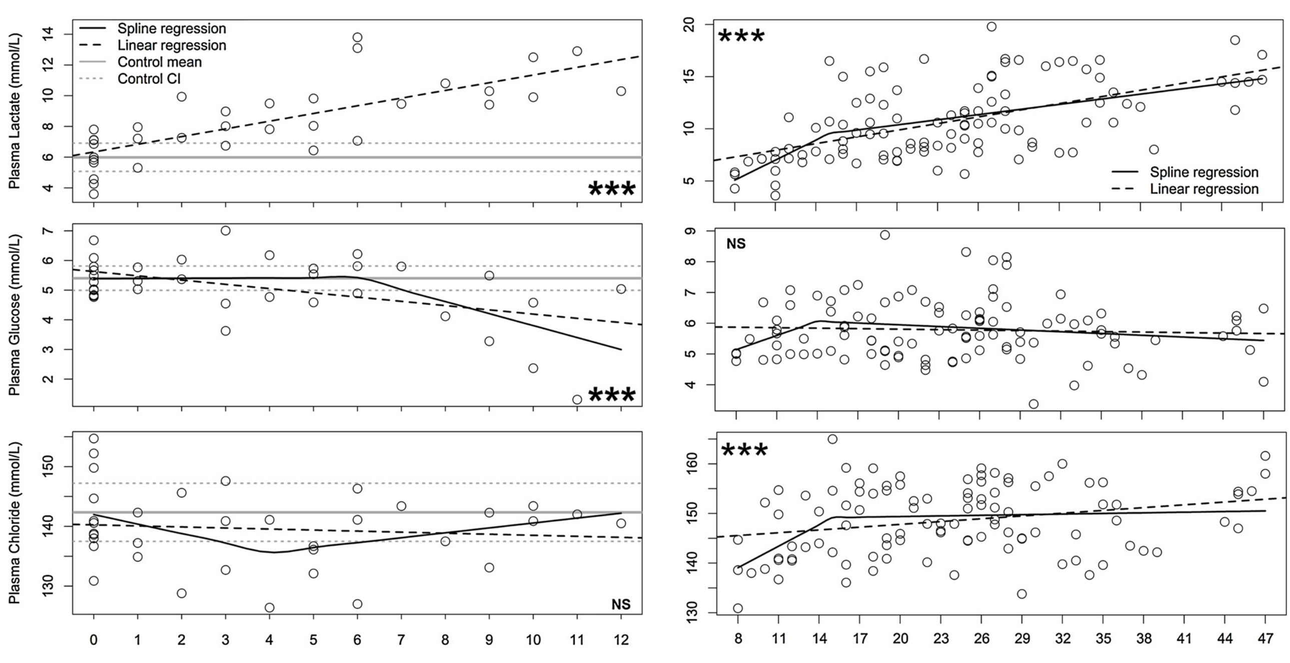

Fig. 7. Linear (dotted black line) and spline regression (solid black line) models evaluating thresholds in physiological indices in response to air exposure and time pursed in a seine. Significance is shown of the spline regression or, if not retained, of the linear regression by asterisks denoting a *p*-value of < 0.0001 or non-significance (NS). Horizontal grey line represents mean concentrations from control fish (i.e. air exposure of '0', those captured but not subjected to any additional air exposure beyond that involved with brailing, ~20 s). Dotted grey lines are confidence intervals around this mean. Control fish were only available for air exposure experiments.

or escape predation (Ryer, 2004). If impairment causes migratory delays (as in Donaldson et al., 2012; Cook et al., 2018), there is also a greater likelihood that fish will be captured again, intensifying physiological responses and potential for mortality with every release. Conversely, injuries occur through encounters with fishing gear or other captured fish, and effects are often delayed, influencing immune function and susceptibility to infection (Baker et al., 2013; Baker and Schindler, 2009). However, the two responses are intertwined and survival is best predicted when the effects from both injury and impairment are considered together (Meeremans et al., 2017).

Capture treatments caused both injury and impairment. Trauma was severe in some cases, such as in the levels of impairment observed following prolonged air exposure. Often when fishery-relevant stressors are applied experimentally to Pacific salmon, impaired reflexes are those attributed to muscular fatigue (Cook et al., 2018; Davis, 2007; Raby et al., 2015, 2012). However, our treatments also induced a failure of reflexes potentially indicative of the brainstem becoming unresponsive (i.e. failure to roll eyes or lift operculum), that require longer recovery times (Kestin et al., 2002). With respect to injuries, although they were minor relative to those typical of other gear types (e.g. gill nets, trawls; Patterson et al., 2017), the progression of injuries that occurred through holding in both studies is notable.

Given the experimental design, we cannot ascertain the degree of injury progression attributable to the stress of captivity or to interactions with holding pens. Though holding and handling likely played a role, there is indication from both field and laboratory research that capture-induced injuries will progress through migration in Pacific salmon (Baker and Schindler, 2009; Teffer et al., 2017). Observations in the days and weeks following capture of tissue necrosis and fungal infections are common (Teffer et al., 2017), and increasingly likely with severe injuries (Baker and Schindler, 2009). With fixed endogenous energy stores that are primarily allocated to maturation and reproduction, the capacity for Pacific salmon to recover from injury during the return migration may be limited. In rainbow trout (*O. mykiss*), genomic signatures indicating wound healing are not present until 1–2 weeks following injury (Schmidt et al., 2016). If this holds true for Pacific salmon, a progression of injuries would be expected following release, making them considerably more susceptible to fungal infections and disease (Van West, 2006; Baker and Schindler, 2009). However, in the present study we are unable to separate the magnitude of injury progression attributed to holding and handling from that attributed to capture and treatments. The potential for the severity of fishery-induced injuries to progress following release warrants further exploration under more natural conditions.

4.1. Set size and net handling

The tightness of the net during the sorting process and the number of fish captured (set size) influenced impairment and, to some degree, injury. These relationships were intensified with handling time. While injury and impairment responded differently to the measured capture parameters, set size (associated with increased injury) and crowding (associated with increased impairment) are both measures of fish density, which has been associated with deteriorating condition among fish released from other commercial fisheries (Huse and Vold, 2010; Raby et al., 2015; Van der Reijden et al., 2017). Crowding can cause oxygen depletion (Raby et al., 2012), and captured fish may display escape responses (Huse and Vold, 2010), behaviour that further reduces oxygen availability and increases likelihood of asphyxiation. The practical implications of these results are that in small sets, impairment can be minimal, and although some degree of harm is potentially unavoidable for fish held in the net during sorting of larger sets, its can be minimized if sets are kept loose.

The probability of sustaining injury due to encounters with the net or other organisms is likely to increase as a function of handling time and catch size (Davis, 2007; Huse and Vold, 2010; Marçalo et al., 2010; Raby et al., 2012). However, the effects of set size and time pursed on injury were not clear; main effects were not significant and set size only emerged as important in Study 1. It is probable that the magnitude of injury sustained is also related to the experience of an individual fish within the net. For example, those in the middle of the net are less likely to incur injury than a fish entangled in the mesh, both of which can occur in a purse seine irrespective of set size. In a similar study, Cook et al. (2018) also failed to see an effect of set size on condition or survival among coho salmon (*O. kisutch*) released from a purse seine fishery and work on sardines (*Sardina pilchardus*) was found to be inconclusive with respect to density effects (Marçalo et al., 2010).

4.2. Physiological effects of restraint

Physiological thresholds are characterized by abrupt changes in the relationship between an external parameter and the physiological response to that parameter (Nickerson et al., 1989). Such thresholds were observed in plasma lactate and chloride concentrations after 15 min of holding. After this turning point, lactate continued to increase, but at a slower rate, whereas chloride concentrations plateaued. In Farrell et al. (2000), coho salmon captured by purse seine and classified by authors as ‘severely exhausted’ had plasma lactate values of 11.5 mmol/L, a value similar to that at the identified spline point. As observed in the spline regression, concentrations increased to over 20 mmol/L during recovery in the Farrell et al. (2000) study. Net confinement was not a severe stressor, and although we would not expect full recovery to occur, fish may have begun recovering from the initial exercise-induced exhaustion.

The time course of capture was initiated once the net was pursed, but the capture event began approximately 30 min prior with initiation of net towing. Each individual fish would have launched a physiological stress response upon first perception of the gear, and likely began making active escape attempts upon perception of confinement (i.e., with pursing). The observed physiological transition may indicate a point of exhaustion where captured fish ceased escape attempts using burst swimming, a type of exercise that can only be maintained for short periods (Kieffer, 2000). That plasma chloride concentrations also shifted after 15 min of being pursed supports this suggestion. Anaerobic exercise typically results in an influx of ions and loss of water in marine fishes (Avella et al., 1991). The rapid increase observed in chloride concentrations could be the result of passive ion influx in the absence of energy available for active ion transport. Slowing of anaerobic activity with cessation of active escape attempts may have initiated a stabilization of ion homeostasis, and we would expect concentrations to remain high while still under stress as well as during recovery upon release (Farrell et al., 2000).

For the glucose models, although the spline regression was a better fit then the linear regression, time pursed did not significantly predict glucose concentrations. Glucose mobilization is induced by stress hormones, which increase rapidly upon initial perception of a stressor (Wendelaar Bonga, 1997). Therefore, the timing of our sampling could have been too delayed to encompass the full glucose response, with results mostly capturing a stabilization period.

Our blood physiology results point to a transition point at 15 min of being held pursed in the net. If indeed this point represents the temporal limit to anaerobic exercise, ideally fish should be released prior to 15 min of being pursed to avoid reaching a point of complete exhaustion, thereby ensuring a greater likelihood of re-establishing themselves and avoiding post-release predation.

4.3. Physiological and physical impairment resulting from air exposure

Impairment consistently increased with air exposure duration. In purse seine fisheries, air exposure occurs during brailing and sorting, the extent of which largely depends on set size and species composition. In the Study 2 holding experiments, significant increases in impairment occurred between the moderate (average of 1.2 min air exposure) and severe (average of 2.9 min air exposure) treatments, suggesting the time between 1 and 3 min is important to survival outcomes. Accordingly, at 2 min of air exposure in the Study 1 holding experiments, a fish was most likely to have one or two reflexes impaired. Previous research with coho salmon released from seines suggests this level of impairment would be sufficient to influence survival: survival was 86% with no reflex impairment but ~60% with 1 or 2 reflexes impaired (K.V. Cook, unpublished data; study described in Cook et al., 2018 and see Raby et al., 2012 for similar in-river survival data). While coho and chum salmon possibly differ in their sensitivities to capture stressors, this provides the best surrogate data in the absence of a reliable impairment/survival relationship for chum.

The length of time a fish can survive air exposure ultimately depends on its capacity for anaerobic energy production to meet metabolic demands (Cook et al., 2015). Upon exhaustion of readily available endogenous energy stores (and hence capacity for anaerobic energy production), the state of metabolic stress reaches fatal levels from which recovery is unlikely (Methling et al., 2017). A physiological threshold to air exposure was observed in plasma glucose. Concentrations were stable until 6 min, likely because our sampling missed initial increases, and then began decreasing. Typically, plasma glucose will increase with hypoxia in fish (e.g. Jorgensen and Mustafa, 1980; Ishibashi et al., 2002). In one case where decreases were observed, Pérez-Jiménez et al. (2012) explained the unexpected results by suggesting that the hypoxic conditions of their study did not sufficiently limit oxygen for study fish to require anaerobic metabolism. However, the hypoglycemia (glucose deficiency in the bloodstream) in our study was accompanied by a continuous increase in plasma lactate, suggesting anaerobiosis was occurring (Farrell et al., 2000). As an important energy substrate, plasma glucose needs to be maintained between approximately 4–7 mmol/L to sustain life (Hruska et al., 2010). The sub-optimal levels present after 6 min indicates an exhaustion of available endogenous energy and an inability to mobilize glucose readily; recovery from such a physiological state would be exceptionally challenging.

These blood physiology results indicate that air exposure should be kept well below 6 min. Corroborating this is that 50% of mortality in Study 1 occurred to fish exposed to over 5 min of air exposure. Fortunately, exceeding this threshold would be unusual when brailing requirements are followed. Impairment data is perhaps most informative for developing air exposure recommendations. In Study 2, impairment scores were low with an average air exposure of 1.2 min, but scores known to be associated with mortality resulted when air exposure exceeded 2.5 min. That glucose concentrations dropped below the average of those fish not exposed to air at 1.5 min also suggests that between 1 and 2 min of air exposure is an important timepoint. This transition is potentially a precursor to the severe hypoglycemia observed later. A caveat of any discussion of air exposure thresholds is that at no point is any air exposure safe among non-air breathing fish (Cook et al., 2015). Other researchers suggest air exposure is safe when no reflex impairment results (e.g. Humborstad et al., 2009). However, even when air exposure was minimized as much as possible (e.g., in the net models), it was most probable that chum would have 2 reflexes impaired. Undoubtedly, the more that air exposure can be reduced, the more likely we are to have higher survival outcomes. There is definite concern for post-release survival when the most probable outcome of a capture scenario is to have two to three reflexes impaired, such as is the case when chum were exposed to over two minutes of air exposure.

4.4. Maturity, sex, and other considerations

In accordance with the finding of injury scores differing by sex, female Pacific salmon can also suffer higher mortality following release from fisheries (Martins et al., 2012), a trend thought to be partially attributable to naturally higher cortisol levels in females having immunosuppressive effects (Jeffries et al., 2012; Teffer et al., 2017). Our injury metric combined injuries sustained both prior to and during capture. Certainly, an immunosuppressed individual would be less likely to heal injuries sustained prior to capture, and a state of poorer condition relative to males would make females more susceptible to new injuries. Injury is not a direct fitness measure, but our results do highlight a potential mechanism for observations of higher mortality rates among females captured and released from fisheries.

Results additionally reveal less mature fish to be more susceptible to the injurious effects of capture. With maturity, there are pronounced changes in body morphology in both sexes, including a reabsorption of scales and a thickening of the skin. Accordingly, our classifications of maturity were based on observations of skin characteristics. To our knowledge, there are no other observations of skin characteristics of mature salmon conferring resiliency to capture stressors. Other researchers have speculated as much (e.g. Raby et al., 2013; Patterson et al., 2017), and Benfey et al. (1989) noted greater scale loss following repeated handling among triploid pink salmon, which do not undergo physiological maturation, than diploid conspecifics that did exhibit skin thickening with maturation. Interestingly, in Study 1, fewer reflexes were impaired in the late compared to early capture period. Because maturity was not quantified in Study 1, this division of time periods was thought to encompass expected differences in maturity as the study progressed. Our data provides no explanation as to why impairment would differ with maturation or capture period. Maturity was not explicitly measured in Study 1 and did not influence impairment in Study 2, and therefore these results may be spurious or explained by another unquantified factor.

There are several factors not considered in this research that are known to contribute to the condition of fish upon release (see Patterson et al., 2017), many of which we do not believe to be particularly relevant to our results. For example, although Pacific salmon are known to be sensitive to small temperature variations (Martins et al., 2012), the water temperatures observed during this research were moderate, stable, and below the range known to be deleterious. Factors such as water quality, air temperature, ocean conditions, and catch composition also deserve recognition (Benoît et al., 2010; Davis, 2002). Across our two study years, environmental conditions were relatively stable and consistent, and therefore it is unlikely these factors affected our findings.

It is also notable that both studies saw low mortality compared to other research on Pacific salmon released from purse seines (e.g. Raby et al., 2015, Cook et al., 2018). This may suggest that chum salmon are quite robust with respect to their abilities to overcome capture stressors. However, because all fish were transported to holding in industry-standard recovery boxes, survival results could be as much indicative of the resiliency of our study species, as it is of the effectiveness of recovery box treatments to facilitate their recovery (as described in Farrell et al., 2000).

5. Conclusions and recommendations

To the extent that a fish's reflex impairment and severity of injury influence eventual survival, our results are relevant for providing recommendations for suggested best practices. First, an important finding was that of increased injury among females and less mature fish. A cautious approach would be to manage fisheries according to expected impacts to females, and to avoid non-target species before they have started to mature. With respect to fishing methods, crowding and set size negatively influenced fish, but the most consistent contributor to injury and impairment was time in the net, with 15 min being identified as an important threshold. While releasing fish prior to this threshold may be possible in small sets, sorting large sets will unavoidably exceed 15 min. Managing fisheries to reduce set sizes would effectively decrease both densities and net times but, with the increased effort required to maintain catch volumes, would incur additional costs to fishers. Other fisheries have successfully implemented catch reductions through increasing incentives, for example, by ensuring that smaller sets can improve product quality and yield higher prices (Hilborn et al., 2005). Such options could be explored, but the most practical recommendation is that fish be sorted as fast as possible and that nets be left loose, reducing crowding. Perhaps the most probable means to improve the condition of released fish in these fisheries is to minimize air exposure. Impairment data provided a strong indication that air exposure within the range of our Study 2 moderate treatment (average = 1.2 min; range = 1–2 min) will minimize impairment. Notwithstanding the recommendation that air exposure be reduced by the greatest extent possible, ensuring fish from each brailer are sorted within one minute of initiation of brailing is realistic, attainable, and tools are available to facilitate this. Taken together, our studies indicate that these improvements in commercial fishing practices could keep incidentally-captured chum salmon (and potentially other salmonids) below the injury, impairment, and physiological tipping points identified herein.

Ethics approval

All research was in accordance with the Canadian Council of Animal Care guidelines and approved by University of British Columbia's institutional Animal Care and Use Program (Application #A15-0205).

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.fishres.2018.04.021.

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