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Final Report

Project Title: Evaluation of population-specific behavioural impairment and mortality in Pacific salmon incidentally captured in marine commercial purse seine fisheries

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Project Location: DFO Statistical Area 3

Abstract

In the commercial Pacific salmon fisheries of BC, only those species with sufficient abundance to support exploitation are harvested, and there is a mandatory release of all other non-target salmonids captured. The survival of these released species is a key component of assessing the impacts of seine fisheries and developing harvest limits. However, there is poor understanding of post-release mortality in general, as well as the factors underlying this mortality. Using the 2016 Pacific salmon purse seine fishery in Canadian DFO Statistical Area 3 as a model system, we evaluated how variable handling and sorting practices influence survival and condition following release, and how these relationships change throughout the season. The research was conducted on chum and sockeye salmon, both species commonly released in Area 3 fisheries. Short term survival and condition was evaluated in chum (n=169), a species of conservation concern on the North Coast, after a 5-day holding period and at multiple time periods throughout the fishing season. To provide a more accurate estimate of post-release mortality, Nass River Sockeye (n=396) were used a model species to evaluate relative differences in fate to freshwater among capture treatments in a radio telemetry study. Results of this study can advise the optimization of handling and release practices, inform release mortality estimates assigned to purse seine fisheries, and help elucidate when fish may be more vulnerable to the effects of capture/release.

Contents

Introduction

The occurrence of bycatch in commercial fisheries has been widely recognized as a substantial threat to biodiversity (Davies *et al.*, 2009) and has become a forefront issue in fisheries management (Gilman, 2011). In the commercial Pacific salmon fisheries of BC, selective fishing has been in place since 1998 where species or stocks of concern are released, allowing harvest only of those with sufficient abundance to support exploitation (Department of Fisheries and Oceans, 2001). Harvest rates are therefore allotted, in part, given estimates of post-release mortality for non-target salmon species. The survival of released salmon is a key component of assessing the impacts of seine fisheries and developing harvest limits. However, there is poor understanding of post-release mortality in general and the factors underlying this mortality.

A captured fish is exposed to multiple separate acute stressors. Fish inevitably suffer some form of physical injury (*i.e.* from minor mucus and scale loss to large wounds), will fight to exhaustion, and must cope with rapid environmental changes and oxygen deprivation through either direct time in air or exposure to hypoxic conditions. Air exposure is arguably one of the most severe forms of acute stress that a fish can be exposed to (Ferguson and Tufts, 1992; Cook *et al.*, 2015). Not surprisingly, many studies have found that reducing the time a fish spends on deck is the strongest measure to promote post-release survival (e.g., Neilson *et al.*, 1989; Davis, 2002; Parker *et al.*, 2003; Benoît *et al.*, 2010). The stress and injuries resulting from a fisheries interaction may also contribute indirectly to mortality through changes in behaviour. Typically quantified using reflex impairment tests, behavioural responses reflect both internal and external conditions, providing a neurological integration of both physiological stress and physical damage (Davis, 2010). Increasing behavioural impairment corresponds to a greater departure from homeostasis, from which recovery becomes less likely. Certainly, there is now substantial evidence that behavioural impairment in Pacific salmon released from fisheries is associated with increased mortality *(Cook et al., In Press*; Raby *et al.*, 2013, 2015a) and therefore is an effective means to evaluate the relative effect of different capture scenarios.

If handling and release practices can be identified that beget good survival, their implementation would reduce harm to non-target salmon across the entire fleet. Adoption of consistent and appropriate release practices will additionally appease public concerns, increase efficiency of harvest, potentially allow additional harvest opportunities, and reduce impacts on stocks of concern. Moreover, sociological human dimensions research conducted this past year by our group has identified that commercial fishers would be more willing to modify handling practices if the rationale for doing so was supported by scientific results (Watson *et al., unpublished data*). Using the 2016 Pacific salmon purse seine fishery in Canadian Statistical Area 3 as a model system, we evaluated how variable handling and sorting practices influence survival and condition following release, and how these relationships change throughout the season. The research was conducted on chum and sockeye salmon, both species commonly released in Area 3 fisheries.

Research support provided by the Northern Endowment therefore supported two separate but concurrent studies conducted aboard a purse seine vessel chartered to operate an experimental fishery that mimicked actual commercial operations. In one study, short term survival and condition was evaluated in chum after a 5-day holding period, at multiple time periods throughout the fishing season. While such holding studies may elucidate short-term relative survival, and can provide insight into the mechanisms of impairment and mortality, telemetry is required to provide accurate estimates of post-release mortality. In a telemetry study fish are not exposed to the confinement stress associated with holding and experience realistic predation pressures. However, to evaluate post-release mortality using telemetry, population structure and migration route must be known to ensure released fish are detected by deployed arrays. Nass River Sockeye were therefore used a model species to evaluate relative differences in fate to freshwater among capture treatments. Injury and impairment was evaluated in nearly 400 sockeye that were tagged with radio transmitters prior to release. A small non-lethal gill tissue sample was also collected to measure genomic responses to capture and pathogen loads. Post-release survival was monitored throughout their migration up the Nass River and when tagged individuals were re-captured by Nisga'a Fisheries technicians, another non-lethal gill sample was collected to evaluate progression of condition and pathogen loads.

The results inform the optimization of handling and release practices and accuracy of bycatch mortality estimates assigned to purse seine fisheries. Additionally, results help elucidate when fish may be more vulnerable to the effects of capture/release, how this may differ throughout the season, and provide a better understanding the basic genomic responses to capture stress and how they may change over time. This research builds upon a cooperative and proactive approach initiated in 2015 by the Marine Conservation Caucus (MCC) and Canfisco to collaborate with an independent body (UBC) to study chum bycatch in Area 6. Such a collaborative and integrative initiative is evidence that industry prioritizes the management of fisheries according to measures of sustainability and in accordance with conservation goals. A collaborative project of this nature in which principal investigators and academics regularly engage with stakeholders, including commercial fishers, has the potential to make real changes in the way fish are handled prior to release.

Methods

This research builds onto a study completed in 2015 on chum salmon in Area 6 where although a large pink salmon fishery was expected, the fishery did not open and abundances were low. As a result, we were forced to take a more experimental approach in 2015 under conditions not representative of an actual fishery. In 2016, Area 3 was chosen because the fishery is more predictable and catches are relatively consistent year to year. Treatments used resulted from findings of the Area 6 study.

Chartered fisheries that simulated commercial operations were conducted from Jul-22 to Aug-11, 2016. Upon capture, as per industry standards, fish were brought from the pursed net into the boat using a brailer and deposited on a sorting table. Once on the vessel, handling of fish varied by designated treatment.

Treatments

In both studies, variable handling and sorting methods were employed to replicate different levels of capture severity. All studies included controls that were dip-netted directly from the set (i.e. not brailed onto sorting table). Set size, a variable that could not be controlled, was estimated by the captain of the vessel.

Chum holding study: How the net is handled was kept as consistent as possible and all fish were held for 30-45 mins prior to brailing, a time chosen to mimic holding durations characteristics of a large set. Air exposure, timed as soon as fish were first removed from the net, was modified according to three treatments: moderate (1-2 minutes), severe (2.5-3.5 minutes) and very severe (4-5 minutes). The maturation state of the fish was classified as silver, silver-bright, or mature. Silver fish showed no colouration except for faint vertical stripes and their scales were loose. Silver-bright fish were beginning to show colouration (i.e. vertical stripes clearly present and maybe 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.

Sockeye tagging study: As with the chum study, how the net was handled was kept as consistent as possible. Sockeye to be tagged were exposed to varying times in the net (short, <10min or long >30 mins) and exposed to varying times on deck (i.e. air exposure time) – either < 1 min or ~2mins. Air exposure durations were reduced compared to those used in the chum treatments given an expectation of increased mortality in fish released to the wild relative to fish held in net pens. Maturation of the fish was noted, though did not vary considerably as with the chum, and if possible, sex was determined.

Several fish were processed from each set. Upon completion of treatment, fish were held in recovery totes until they could be processed. Inevitably, fish were in the recovery totes for variable amounts of time. This holding time was recorded and will be controlled for in final analyses as required.

Condition monitoring

Immediately after treatment, fish were removed individually from the recovery totes and transferred to a V-shaped trough with flow-through sea water. Impairment and injury were immediately quantified. Impairment was measured first, using a standardized approach of observing the presence or absence of reflexes (as in Davis 2010). A set of reflexes consistent in minimally stressed individuals are first established (see Raby *et al.*, 2012 for validation study in coho salmon) and then these reflexes are evaluated immediately following capture. The final impairment score is the proportion of total number reflexes tested tested as absent. The outcome is a graduated response variable that is robust among a range of fishery-induced stressors (Davis, 2010). The magnitude of injury was estimated via a semi-quantitative scoring system that classified observations of injury as per Table 1; scores encompass both sides of the body. Each observation was then scaled to a value between 0 and 1 to ensure equal weighting and summed, producing a continuous injury variable.

Table 1: Injury scoring system used for both sockeye and chum captured by purse seine in Area 3.

Observation	**Value**	**Description**
Scale Loss	Percent in increments of 10	Any area where scales are missing
Skin Loss	Percent in increments of 10	Includes wound surface area and areas of exposed flesh. Does not include fins.
Wound Depth	0 (no wound); 1 (scales missing, skin visible); 2 (skin missing, flesh/muscle visible); 3 (chunks of muscle missing); 4 (organs/ bones/cartilage visible)	An estimate of wound (i.e. places where skin loss occurs) severity, if present.

Fin Damage	0-7, representing a count of the number of rayed fins damaged	Fin damage includes splits, tears, or wounds. Score does not encompass severity of damage
Fin Severity	0 (no fin damage); 1 (minor nicks and splits); 2 (wounds, small chunks missing); 3 (large wounds/exposed bone)	Categorization of the most damaged fin (i.e. severity of damage on one fin only).

Sample collection

In both studies, gill tissue samples were collected for genomic analyses and adipose fin clips were collected for genetic stock identification.

Tissue biopsies and genomic analyses: Tissue biopsies analyzed with high throughput genomic bioassay methods provide an additional means to assess physiological impairment through changes in gene expression. High-throughput techniques can simultaneously quantify expression of target genes (e.g. stress and immune genes) and screen for the presence of potentially damaging pathogens. Pathogen development becomes increasingly important in the days following release because the injury and stress sustained during capture may cause immunosuppression and accelerate pathogen development (Miller *et al.*, 2014).

A small (<2 mm) piece of gill tissue was collected from all sockeye prior to release and from all chum before and after the 5-day holding period for genomic analyses. The tissue was stored in RNA later until analyses. Samples collected from sockeye were analysed in March 2017 at the Pacific Biological Station (DFO, Nanaimo). Detailed methods of genomic analyses can be found in Teffer *et al.*, (2017). In short, the RNA was extracted and normalization prior to making copy DNA. Using copy DNA, primers targeting specific gene sequences of interest were used in a qPCR reaction to amplify and concentrate desired genetic material. High-throughput microfluidic real-time quantitative qPCR was then used to quantify relative expression of target genes and loads of target pathogens. Samples collected from the chum holding studies have not yet been run or analyzed, and those from the sockeye study have been run with analyses forthcoming.

Genetic stock ID: Adipose fin clips were collected from all study fish. Sockeye salmon not detected on or proximal to spawning grounds were identified to conservation unit using microsatellite analyses (as in Beacham *et al.*, 2004). Current genetic analytical methods are not of sufficient precision to identify chum populations to conservation unit but adipose fin clips were nonetheless collected and are stored for potential future analyses.

Tagging

Sockeye: Radio telemetry was used because receiver sites are present on the Nass that had been used previously with success (LGL limited, *personal communication*). Sockeye were tagged gastrically, whereby the tag was inserted through the mouth using a small plunger and the antenna protrudes from the mouth, a common technique in return migrant Pacific salmon. Gastric tagging is rapid (~15s) and does not require sedation. Sockeye were also tagged externally with a floy anchor tag with an individual ID and phone number so that if captured, the fish could be reported. After tagging fish were released overboard and were detected by deployed receiver stations as they migrated upstream (see ‘Telemetry tracking infrastructure’)

Chum: Fish retained for holding studies were all tagged with a uniquely numbered spaghetti tag that was threaded through the dorsal musculature just posterior to the dorsal fin and tied with a double reef knot. After processing, fish were placed back into recovery totes for transport to net pens.

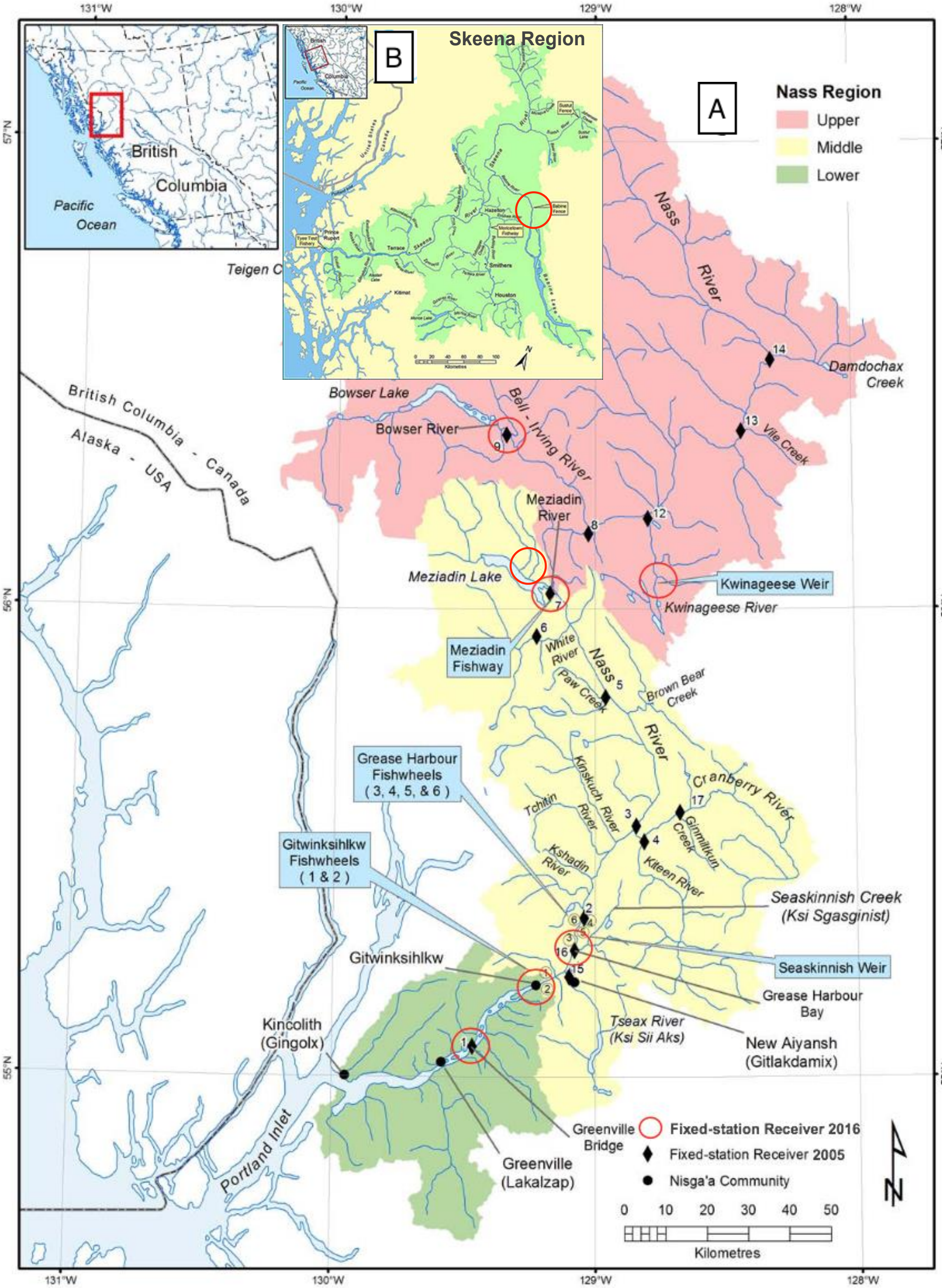
Fish transport and holding (chum)

Study fish were transported to pens in industry-standard recovery totes at densities that did not exceed 6 fish/tote. Every attempt was made to minimize transport time, but it ultimately varied with fishing location from 20 minutes to 45 minutes. Study fish were held for 5 days in floating net pens (4.6 x 1.5 x 1.5m) that were affixed to an anchored punt from which all subsequent processing was completed. Net pens were made of an aluminum frame that was fully surrounded by 100-mm bunt mesh. A hatch opened at the top of the pens to retrieve the fish. Densities did not exceed 40 fish/pen.

Telemetry tracking infrastructure (sockeye)

Eight fixed solar-powered telemetry stations were set up on alongside river mainstems and at spawning areas in the Nass (n=7) and Skeena (n=1) regions (see Map 1). Fixed-station plus mobile tracking (by foot, boat, and helicopter) continued until mid-October. The fishwheels and fishway shown on the Nass River in Map 1 are where Nisga'a fisheries technicians were able to obtain additional non-lethal gill samples from intercepted tagged sockeye.

Map 1. Approximate geographic locations of the eight fixed biotelemetry receiver stations (red circles) used to monitor radio-tagged sockeye on their spawning migrations in the (A) Nass and (B) Skeena watersheds in July-October 2016. First Nations (Nisga'a) communities (black circles) and fixed biotelemetry receiver stations from a previous Nisga'a-led salmon tracking study (2005; black diamonds) are also shown.



Summary of Findings

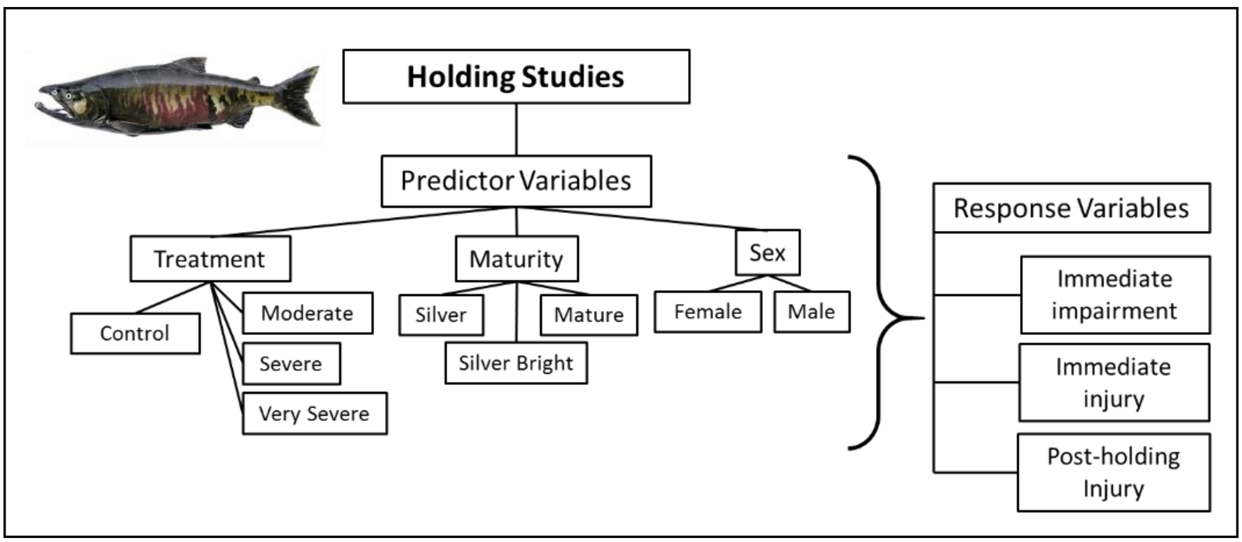
Analyses conducted to date for both studies are ongoing and therefore the results presented herein are preliminary. Data will be presented at conferences and submitted for publication in peer-reviewed literature with lead authors K.V. Cook (chum) and A.J. Reid (sockeye).

Chum holding study

A total of 169 chum were held for 5 days. There was very minimal mortality, with a total of 6 fish dying throughout the holding period (3.5% mortality) and no effect of treatment on observed mortality. We caution that this mortality rate is not representative of that expected in an actual commercial fishery given the transport time in revival tanks and holding time in a 'safe' environment (i.e. net pens) away from predators. Condition parameters (injury, impairment) were used in analyses as proxies of mortality.

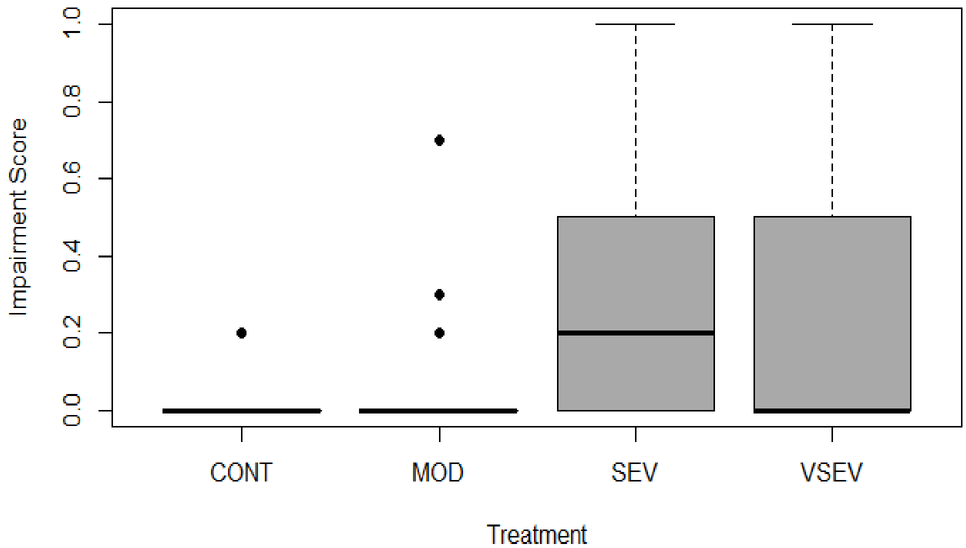
As a preliminary analysis, 3 factorial ANOVA models were run, one for each response variable (Figure 1; immediate impairment, immediate injury, and post-holding injury) that included predictor variables of treatment, maturity, sex (as in Figure 1) and their interactions.

Figure 1: Flow chart of predictor and responses variables resulting from chum holding studies conducted with Area 3 purse seine fishery. As a preliminary examination of the data, each response variable was considered in an individual factorial ANOVA model.



Impairment: There was a significant effect of treatment on impairment, but no effect of sex or maturity. Impairment was higher in the severe and very severe treatments and little impairment was observed in moderate and control groups (Figure 2).

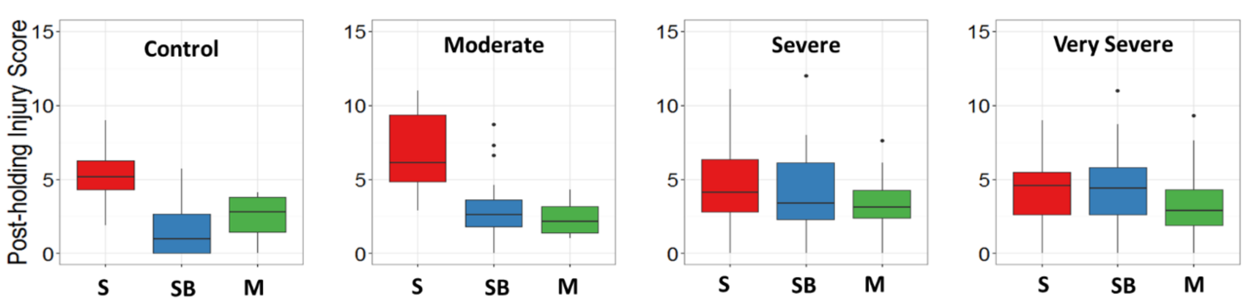
Figure 2: Differences in impairment among treatment groups for chum salmon exposed to varying capture severity. Control fish were dip-netted out of the seine. All other treatments were held in the net for ~30 minutes but air exposure duration varied (details in Methods section).



Initial injury: Neither treatment nor maturity influenced initial injury but there was an effect of sex, with females sustaining significantly more injury than males.

Post-holding injury: There was a significant effect of maturity, sex, and the interaction of maturity and treatment on post-holding injury. The sex effect was the same as with initial injury, whereby females were more susceptible to injuries. With respect to the main effect of maturity, post-holding injuries were significantly elevated in 'silver' fish compared to both fish categorized as silver bright and mature. However, the significant maturity and treatment interaction suggests that this relationship differs with the severity of treatment. As seen in Figure 3, fish categorized as being 'silver' had elevated post-holding injury scores than 'silver-bright' and 'mature' fish in the control and moderate capture categories but not the severe or very severe capture categories. There was also greater variability in injury scores in silver bright and mature fish in the severe and very severe capture treatments.

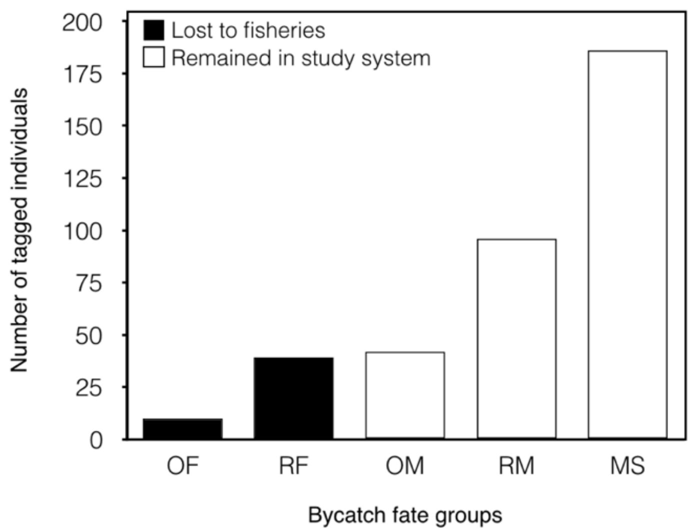
Figure 3: Results of a significant treatment*maturity interaction resulting from a factorial ANOVA with a response variable of post-holding injury score. The relationship between post-holding injury and maturity differed by treatment.



Sockeye telemetry study

A total of 396 sockeye were radio-tagged and released. Males (n=197, $\bar{x}$=60.7 cm) were significantly larger than females (n=199, $\bar{x}$=57.9 cm), and females had significantly higher injury scores than males. As seen in Figure 4, 49 individuals were removed by fisheries: 10 in ocean fisheries (2.5%) and 39 in river fisheries (9.8%). Of the remaining fish in the system (n=347), 42 were never detected in-river (assumed 'ocean mortalities'; 12.1%), 119 were detected in-river but not in spawning areas ('river mortalities'; 34.3%), and 186 were found in spawning areas ('migration survivors'; 53.6%). Detection efficiency was high across sites (generally >97%), except for the ocean-most Nass site (77%) likely due to tidal influence.

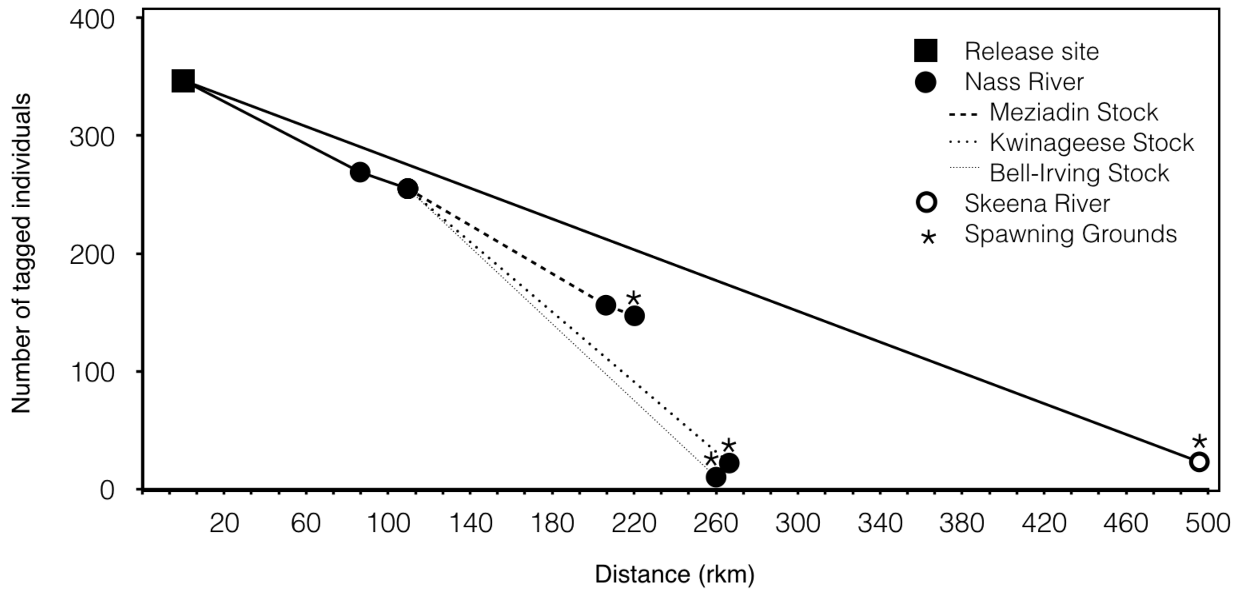
Figure 4. The fate of radio-tagged sockeye released from purse seine fisheries. The number of tagged individuals is presented per fate group: OF=ocean fisheries; RF=river fisheries; OM=ocean mortalities; RM=river mortalities; and MS=migration survivors.



We caution that these results are based on positive tag detections in monitored areas, which included the mainstem and all major spawning areas in the Nass, as well as the largest sockeye spawning area in the Skeena. Fish that went undetected because they belonged to other populations outside of monitored areas may currently be categorized as 'ocean mortalities' or 'river mortalities'. However, this will be corrected when the results from the genetic stock identification are included into data exploration and analyses (forthcoming).

As shown in Figure 5, 179 sockeye reached spawning grounds in the Nass River (51.6%) and 23 sockeye reached spawning areas in the Skeena River (6.6%). As a preliminary analysis, multinomial logistic regression models were run to examine the effect of treatment type, immediate impairment, immediate injury, sex, and their interactions on the fate of released sockeye. Using the Akaike Information Criterion, the most parsimonious and best explanatory model solely included immediate injury as a predictor of fate. Analysis of variance revealed that treatment type was a significant predictor of immediate impairment, but treatment type was not predictive of fate outcome. Further analyses are forthcoming to include data on pathogen presence and load, as well as genetic stock indentification. Stock is being identified for all fish not tracked to (or proximal to) natal spawning grounds (n=128).

Figure 5. Survivorship of radio-tagged sockeye released from purse seine fisheries. The number of tagged individuals is presented over migration distance in river kilometers, at the location of each fixed biotelemetry receiver station (one station is excluded because of poor detection efficiency lifely due to tidal influence). Note: tagged individuals lost to fisheries (see Figure 4) are excluded here and from all statistical analyses.



Pathogen presence and load are being assessed in a subset of sampled fish, including 30 individuals per fate group with near-equal representation of capture treatment types; priority for inclusion in assessments was given to fish from which multiple tissue samples were obtained. Across this subset of fish, 15 different pathogenic microbes were detected including bacterial species (*e.g. Candidatus Branchiomonas cysticola*), myxyzoans (*e.g. Parvicapsula pseudobrnachichola*), viruses (*e.g. Piscine reovirus*), and parasitic ciliates (*e.g. Ichthyopthirius multifiliis*). No further analyses have been conducted but information on the potential effect of these pathogens described in (Miller *et al.*, 2014; Bass *et al.*, 2017; Teffer *et al.*, 2017).

Discussion of Preliminary Results

Although data analysis is ongoing, results provide insight into aspects of handling and release that may influence survival outcomes for chum salmon and inform mortality estimtes for sockeye released from Area 3 fisheries. We provide a summary of preliminary findings by topic.

Mortality

Results from the chum holding studies revealed very low mortality (3.5%), indicating that chum are either fairly resilient to capture stressors or, more likely, that holding studies are not an effective means to estimate capture-induced mortality. Our study chum were exposed to an unintentional recovery treatment during transport - logistic constraints unfortunately necessitated that in our study the fishing vessel activities and holding pens could not be in close proximity which enabled some 'recovery' during transport. There is evidence to suggest that these recovery boxes used to transport fish do have the capacity to revive severely impaired fish (Farrell *et al.*, 2001). Further, chum were held in net pens where there was no predator exposure. Although the magnitude of post-release predation is rarely quantified (Raby *et al.*, 2014), anecdotal evidence suggest it occurs in these fisheries and it has been observed in other commercial fisheries (Gadomski *et al.*, 1994; Ryer, 2002; Weise and Harvey, 2005). However, chum from the severe and very severe treatments did have initial impairment scores suggesting significant impairment. In a coho capture/release study where impairment levels were related to post release mortality, the authors found that a combination of severe of impairment and scale loss scores in coho was associated with 75 ± 9 % enroute post release mortality over approximately 4 days (Cook *et al.*, *In Press*).

The mortality resulting from the sockeye telemetry study is perhaps the most representative estimate available for this fishery. Set sizes were consistent with those commonly observed during commercial openings in Area 3 and fish were, to the best of our abilities, handled as they would have been in an actual fishery. Additionally, detection efficiencies up receiver arrays were high, reducing the amout of error surrounding our estimtes. Our preliminary estimate of short-term mortality (i.e. prior to river-entry) is 12.1% and in-river mortality is estimated at 34.3%. In total, survival to spawning was 53.6%. Within these preliminary estimates however it is important to consider that results of genetic stock ID are not yet available and appropriate modeling has yet to be conducted to assess the confidence intervals of these estimates.

There was no treatment effect on mortality in the sockeye telemetry study, suggesting the difference between <1 and 2 minutes of air exposure may be negligible. This is a promising result as with the proper infrastructure (i.e. a sorting table and chute or other similar means to rapidly release incidentally caught species), it is unlikely that salmon captured in the purse seine fishery would be on deck for over 2 minutes.

Injury

Injury was the measured variable most predictive of fate in the sockeye study, cooroboating findings from other recent research from our group (Cook *et al., In Review*, A.L.Bass, *Unpublished data*) and others (Baker and Schindler, 2009; Nguyen *et al.*, 2014; Uhlmann *et al.*, 2016), suggesting that any means to reduce the injuries sustained during capture will improve the probability of a successful release. The injury score included in analyses was comprosied of several individual metrics (e.g. fin damage, scale loss, skin loss) and future analyses will attempt to decompose which aspects of injury are most predictive of fate.

Impairment

Impairment was not predictive of fate in the telemetry study but as with the chum study, was increased in the more severe treatments. Fish released in an impaired state typically show reduced survival (Davis and Ottmar, 2006; Davis, 2007; Raby *et al.*, 2012; Uhlmann *et al.*, 2016). Our failure to observe an effect of impairment on survival could be because of the magnitude of impairment observed (i.e. few sockeye showed very severe impairment). As in the chum study, sockeye were held in revival totes prior to tagging, as only one fish could be tagged at a time. Therefore, study fish spent a variable amount of time in the recovery tote awaiting tagging; this temporal variation has not yet been controlled for in analyses and may have an effect on impairment results.

In the chum study, the large observed increase in impairment between the moderate and severe treatmeants but yet no difference between the severe and very severe treatments is interesting. The moderate treatment involved 1-2 minutes of air exposure while the severe and very severe treatments >2.5 minutes of air exposure, suggesting that 2 minutes of air exposure may be a threshold, after which recovery probability decreases substantially. This assertion is supported by the lack of treatment effect in the sockeye study where air exposure durations were kept below 2 minutes.

<u>Maturity</u>

The chum holding studies revealed an effect of maturity whereby less mature fish are more susceptible to capture-induced injury. Returning Pacific salmon undergo dramatic physiological and physical changes throughout the migration and it is thought that this maturation may confer a certain resiliency to capture stressors (Raby *et al.*, 2013, 2015b), though this hasn't been assessed previously in marine-capture fish. As Pacific salmon mature, their skin thickens and scales are absorbed, reducing or eliminating the scale loss that normally occurs during capture. Scale loss can be susbtantal in non-mature, ocean-caught silver fish, increasing the negative effects of dermal injuries. For eacmple, damage to the epithelial layer due to capture can increase pathogenic infection in fish (Svendsen and Bogwald, 1997; Van West, 2006), potentially leading to delayed mortality (Svendsen and Bogwald, 1997; Howe and Stehly, 1998; Davis, 2002).

The significant interaction effect between maturity and treatment observed though indicates that the relationship is not as simple as silver fish being more susceptible to injuries. In the severe and very severe treatments, injury scores did not differ among maturity categories with silver bright and mature fish showing greater variability in post-holding injury scores. Therefore, with increased time on the deck (e.g. >2.5 minutes), even mature fish can sustain high capture-induced injuries.

Acknowledgments

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