Validation of reflex indicators for measuring vitality and predicting the delayed mortality of wild coho salmon bycatch released from fishing gears

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Summary

1. Effective management of fish and wildlife populations benefits from an understanding of the effects of stressors on individual physiology. While physiological knowledge can provide a mechanistic understanding of organismal responses, its applied utility is limited because it cannot easily be used by stakeholders.

2. Reflex action mortality predictors (RAMP) is a method that involves checking for the presence or absence of natural animal reflexes to generate a condition (RAMP) score in response to stressors and to predict fate. The method has previously been validated with fishes in artificial laboratory- and field-based holding studies as a responsive measure of fisheries capture stress and a predictor of delayed mortality, but has not been evaluated in the wild.

3. We used radio telemetry to monitor migration success of 50 endangered coho salmon *Oncorhynchus kisutch* following incidental capture in an aboriginal beach seine fishery in the lower Fraser River (Canada). RAMP was used to measure the condition of fish at release and to predict migration success following capture. Biopsy of an additional 43 coho profiled physiological condition at time of release.

4. Individuals with greater reflex impairment (higher RAMP scores) at release experienced significantly higher rates of migration failure. RAMP scores were also significantly correlated with fishery handling time. Plasma variables showed that captured coho had experienced physiological stress characteristic of exhaustive exercise and hypoxia, with significantly elevated cortisol and lactate values for fish entangled longer in fishing gear.

5. *Synthesis and applications.* This is the first validation of RAMP in a wild setting. Based on our findings, fishers could use the method and make adjustments in fishing behaviour in real-time to improve fish condition and reduce the mortality of bycatch, and conservation practitioners could monitor animal condition and identify problems that deserve management attention. RAMP is an easy, rapid and inexpensive approach to predicting mortality and measuring vitality and performed better than traditional physiological tools that cannot easily be used by stakeholders.

Key-words: conservation physiology, fisheries impacts, freshwater bycatch, incidental mortality, migration, *Oncorhynchus kisutch*, Pacific salmon, radio telemetry, recovery, reflex

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Introduction

Management of animal populations can benefit from an understanding of stressors and their effects on individuals (Cooke & O'Connor 2010), particularly for exploited species, or animals incidentally harmed by human activity. Fisheries bycatch is a common example of human-caused acute stress in wild animals and is regarded as an important conservation issue world-wide in both marine (Hall 1996; Hall, Alverson & Metuzals 2000; Lewison *et al.* 2004) and freshwater (Raby *et al.* 2011) systems. Releasing bycatch alive (i.e. discarding) is commonly employed to conserve populations of incidentally captured animals but relies on the assumption of survival, an assumption that may be false in certain contexts (e.g. Chopin & Arimoto 1995; Campbell *et al.* 2010). Currently, the unintended capture of turtles (Watson *et al.* 2005), seabirds (Gales, Brothers & Reid 1998), mammals (e.g. Read, Drinker & Northridge 2006) and nontarget fishes (Broadhurst, Suuronen & Hulme 2006) is occurring en masse in fisheries and can result in immediate or latent lethal and sublethal outcomes (Davis 2002) that can lead to population declines (Lewison *et al.* 2004).

Most research in the bycatch realm has focused on bycatch rates, but significant bycatch sometimes remains even with the most efficient fishing gear available (Davis 2002; Broadhurst *et al.* 2009). In such cases, researchers have focused on understanding and mitigating the mortality of bycatch (Davis 2002). Research that informs managers on how to reduce the mortality of discards is valuable (e.g. Broadhurst *et al.* 2009), but can be costly as it is commonly achieved by combining measurements of blood physiology with the monitoring of mortality in a contained environment (holding studies, e.g. Davis 2007). In holding studies, animals are exposed to a fishing gear encounter and monitored for mortality in an artificial enclosure over some time period (typically 2–3 days for field studies, weeks for laboratory studies; Gallinat, Ngu & Shively 1997; Davis 2007). The survival estimates derived by monitoring captive animals fail to integrate the conditions and challenges of a wild environment (e.g. predators, environmental heterogeneity and variability in food resources). Applying biotelemetry to bycatch research can provide data on behaviour and survival discards in the wild and thus address the shortcomings of holding experiments. The use of biotelemetry in recreational fisheries (mostly freshwater) has been relatively common, whereas in bycatch research (mostly marine) it has been rare, owing to technological/logistical limitations and prohibitive costs (Donaldson *et al.* 2008). Therefore, studying bycatch in freshwater using telemetry can serve as the basis for advancing an understanding of the fate of discards and for developing predictors of mortality (Raby *et al.* 2011).

Simple measures of animal vitality following capture may predict future survival and hence generate more rapid and inexpensive estimates of bycatch mortality (Davis 2010). Traditional physiological tools (e.g. measuring blood constituents) can be useful (Moyes *et al.* 2006) for predicting delayed mortality but are expensive and require expertise. Reflex action mortality predictors (RAMP) is an easy-to-use and inexpensive field-based assessment tool that measures fish vitality before release and correlates with future survival (Davis 2010). Since its introduction, RAMP has successfully predicted postrelease mortality for fish and shellfish in laboratory- and field-based holding experiments (e.g. Davis 2007; Stoner 2009; Campbell *et al.* 2010). RAMP assessments involve checking for the presence or absence of multiple (e.g. 5) reflexes identified to be consistently present in vigorous individuals. Assessments of reflexes are rapid (< 20 s), and the results are cumulated into a simple index. The appeal of reflexes is that they are intuitive to fishers and are whole-animal indicators of a compromised physiological state (Davis 2010). If validated, RAMP could be used to rapidly generate bycatch mortality estimates for different gears, seasons and fishing techniques – data that can inform the management of and reduction in bycatch mortality and support conservation initiatives.

Wild coho salmon *Oncorhynchus kisutch* Walbaum released from aboriginal beach seine fisheries in the Fraser River, British Columbia (Canada), were used to provide the first comparison of telemetry-based survival estimates with RAMP scores. The interior Fraser River coho salmon population is listed as endangered by the Committee on the Status of Endangered Wildlife in Canada, and therefore, fishers in British Columbia are required to release all wild coho salmon. We assessed whether RAMP scores correlated with delayed mortality and postrelease migration behaviour and compared RAMP scores with plasma physiology to determine
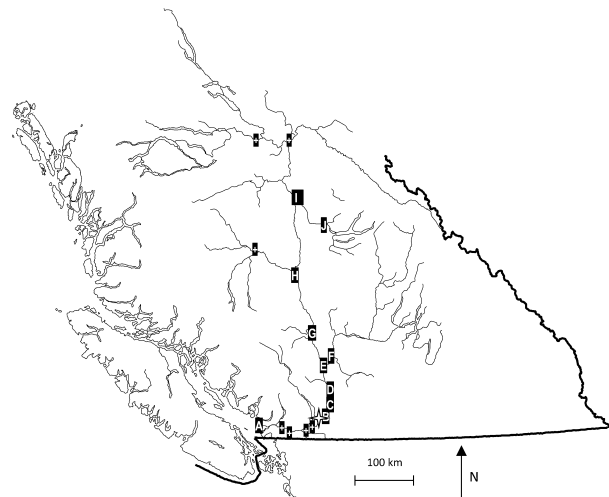
Fig. 1. Map of the Fraser River watershed, British Columbia, Canada, the release site and radio receiver locations. Plus symbols represent receiver locations distributed throughout the Fraser River and its tributaries. The letter A indicates the point of river entry. The star symbol indicates the location of the study/release site. The remaining letter symbols represent receiver locations used for calculating survival and/or migration rate for radio-tagged coho salmon: B (Rosedale), C (Hope), D (Qualark), E (Hell's Gate), F (Thompson River confluence), G (Nicola/Thompson confluence) and H (Seton River confluence). Plus symbols indicate the locations of additional receivers that operated as part of the array.

whether RAMP is a reasonable approach to assessing stress and vitality.

Materials and methods

STUDY SITE AND CAPTURE METHOD

An aboriginal band operating on the lower Fraser River mainstem near Hope, British Columbia, Canada (49°18′32″N, 121°40′03″W; Fig. 1), allowed us to tag or biopsy the coho salmon bycatch in their pink salmon *Oncorhynchus gorbuscha* beach seine fishery, from 21 to 23 September 2009. Fish were captured using a 90 m × 9 m × 5 cm mesh beach seine that was anchored to shore, dragged away from shore, allowed to drift downstream and pulled in using a power boat. Once the net was closed, it was pulled onto the beach by hand until fish were crowded into shallow water (< 0·5 m; see Fig. 2). Once the net was landed, the fishing crew sorted pink salmon into bins, released other species and handed coho salmon to us as they were found. Coho bycatch is normally released directly into the river when encountered in a net. If coho salmon were located in the net while we were occupied processing other fish, they were removed immediately and placed in black hypalon fish bags with mesh ends (1·0 m × 0·2 m) and oriented into flow for holding until they could be processed. Fish were typically exposed to air for 20 s between removal from the seine and placement in a fish bag. For each net set, we recorded the time required to close the net and to pull the net into the beach, as well as the number of fish of each species caught. For each coho salmon, we recorded the amount of time spent in the beached seine net (i.e. fishery handling time) and the duration between being pulled from the net and release by the research team (i.e. researcher handling time). The mean water temperature in the Fraser River during the tagging period was *c*. 16 °C, measured by a permanent temperature probe installed 20 km upstream at Hope (Fig. 1).

TAGGING

Radio telemetry transmitters were deployed in 50 released coho salmon; biopsies were performed on 43 separate individuals. We used established, minimally invasive protocols for gastric tagging of salmon in the Fraser River (see Cooke *et al.* 2005; Donaldson *et al.* 2011). This procedure was carried out while fish were manually held inside flow-through fish bags submerged in flowing river water. The entire tagging procedure lasted < 2 min, no anaesthesia was used, and all protocols were approved by the Carleton University Animal Care committee (for further details see Appendix S1, Supporting information).

PHYSIOLOGICAL SAMPLING

A separate group of 43 coho salmon was biopsied and released using capture methods identical to the telemetry component. For blood sampling, individuals were removed from fish bags and processed in a padded V-shaped trough continually supplied with water (Cooke *et al.* 2005). Two millilitres of blood (< 3% of total blood volume) was collected from each fish using caudal puncture with a 3·8-cm, 21-gauge needle and a heparinized vacutainer (lithium heparin, 3 mL; BD, Franklin Lakes, NJ 07417, USA). For both biopsied and telemetry-tagged fish, a small (0·5 g) adipose fin clip was removed for DNA stock identification, visible injuries were described, and fork length (FL, nearest cm) was measured. The entire biopsy procedure lasted < 3 min and followed Donaldson *et al.* 2011. All fish were released into the river following a reflex assessment.

REFLEX MEASUREMENTS

We used a reflex impairment index modified from the previously developed RAMP method (Davis 2005, 2007). Immediately prior to release, all tagged and biopsied fish were tested for the presence of five reflexes that were consistently present in control, excellent condition fish. Each reflex was assessed categorically (0 = unimpaired, 1 = impaired) in a conservative matter – that is, if the handler had doubt as to whether the reflex was present, it was recorded as being impaired. Reflexes tested were the following: tail grab, body flex, head complex, vestibular-ocular response (VOR) and orientation. Presence of the tail grab response was assessed by the handler attempting to grab the tail of the fish with the fish submerged in water (in a fish bag or holding trough); a positive response was characterized by the fish attempting to burst-swim immediately upon contact. The body flex response was tested by holding the fish out of water using two hands wrapped around the middle of the body. The fish actively attempting to struggle free was characterized as a positive response. Head complex was noted as positive if, when held out of water, the fish exhibited a regular pattern of ventilation (for ∼5 s) observable by watching the opening and closing of the lower jaw. VOR was observed by turning the fish on its side (i.e. on a lengthwise axis) out of water. Positive VOR was characterized by the fish's eye rolling to maintain level pitch, tracking the handler. Finally, upon release, each fish was placed upside-down in the river just below the surface: a positive orientation reflex was noted if the fish righted itself within 3 s. The entire reflex assessment took ≤20 s to complete and was always conducted on fish upon release. If a fish was too vigorous to allow researcher handling and assessment of reflexes, it was assigned an unimpaired status for all reflexes. The reflex actions included in our protocol are thought to

Fig. 2. The aboriginal beach seine being pulled into shore to crowd fish for sorting. Photographer: Sarah McConnachie.

be sufficiently varied that they involve different neurological pathways and/or muscle groups such that there are no redundancies. For example, some of the reflexes are part of the autonomous nervous system (head complex, i.e. respiration), while others clearly are not (tail grab, body flex). Moreover, using this RAMP protocol with Pacific salmon, no two reflexes in the suite of five are consistently present/absent together (see Results; G. Raby, unpublished data). From the reflex results for each fish, we calculated a RAMP score: a simple proportion of the five measured reflexes that were impaired in an individual fish (0 = no reflexes impaired, 1 = all reflexes impaired; Davis 2007).

LABORATORY ASSAYS

Quantification of plasma cortisol, lactate, osmolality, glucose and ions (Cl$^-$, K$^+$ and Na$^+$) followed Farrell *et al.* (2001). Inter-assay variation was <10% for all assays. Population origin for each fish was determined through DNA analysis of flip clips, as described by Beacham *et al.* (2001).

RADIO TRACKING

Radio-tagged coho salmon were tracked using 18 fixed radio telemetry receiver stations (SRX400 series; Lotek Wireless Inc., Newmarket, ON, Canada) with 3- or 4-element Yagi antennas strategically positioned throughout the watershed (Fig. 1). The receiver stations nearest to the release site were 20 km upstream (Hope) and 16 km downstream (Rosedale), respectively. The distances we used between receiver stations were river kilometres rather than straight line distances.

Fish were characterized as en route mortalities if they were not detected at subsequent upstream receiver locations. There was no apparent en route mortality for radio-tagged coho beyond the Thompson River confluence with the Fraser River (130 km upstream of release): all fish detected there were detected at subsequent upstream receivers en route to spawning areas. Therefore, we characterized fish as successful migrants if they were detected by the Thompson River receiver. Previous studies (e.g. Cooke *et al.* 2006) have similarly used arrival at natal subwatersheds to indicate successful migration given the challenges of tracking fish directly to the many potential spawning areas. For statistical analyses, survival to Hope, Qualark and Hell's Gate was also evaluated (see Fig. 1). Migration rates for individual fish were calculated by dividing the distance between two receiver stations by the time between the last detection at the downstream receiver and the first detection at the upstream receiver. If a receiver station failed to detect an individual, that individual was excluded from migration rate calculations for that section of the river. However, in general, the detection efficiency of the receiver stations was quite high (see Appendix S1, Supporting information). Detection efficiency for coho salmon was 100% at both the Hell's Gate and Thompson River receiving stations, the two most important receivers for this study.

STATISTICAL ANALYSES

To evaluate whether RAMP score was associated with delayed mortality (i.e. migration failure), we carried out two tests. First, radio-tracked fish were separated into two groups: successful migrants and unsuccessful migrants (based on criteria outlined above), and the RAMP scores measured at release were compared between these two groups using the nonparametric Mann–Whitney *U*-test. Secondly, we treated RAMP score (0, 0·2, 0·4, 0·6 or 0·8 – no 1·0 scores occurred) as

a categorical predictor variable and used the nonparametric Kruskal–Wallis analysis of variance (ANOVA) to compare the survival rates of fish among those groups. We also wanted to test the power of RAMP as an indicator of fish vitality. Using the assumption that fishery handling time (time entangled in the beached seine before removal) would have a negative effect on fish vitality, we evaluated whether RAMP score would indicate lower vitality (higher RAMP score) using a Spearman rank correlation test comparing fishery handling time with RAMP score. We used the same test to evaluate the effect of researcher handling/holding time on RAMP score. Because we made four different comparisons here using RAMP scores, we used a Bonferroni-corrected significance threshold to account for type I errors. As such, we assessed significance at α = 0·0125.

Entanglement time and handling time are relatively conventional, intuitive predictors of bycatch mortality, and therefore, we evaluated whether these variables affected mortality outcomes. ANOVA was used compare both fishery and researcher handling time among immediate mortalities, successfully migrants and unsuccessful migrants. These tests were assessed as significant at $P \leq 0.05$.

We used migration rate as a metric to determine whether tagged coho salmon incurred any behavioural impairments as a result of the fishery and whether reflex impairment would be predictive of postrelease behaviour. For simplicity, the only two migration rates we used for statistical testing were the initial migration rate (the first 20 km) and overall migration rate (release site to the Thompson River, the final survival checkpoint 130 km upstream). First, we tested whether size (FL) had an effect on migration rate using simple linear regression. Secondly, we compared researcher handling time and log-transformed fishery handling time with both initial and overall migration rates, also using linear regression. Thirdly, we grouped fish by RAMP score and used one-way ANOVA with initial and overall migration rates as dependent variables to evaluate the predictive effect of reflex impairment on migration rate. Finally, we wanted to determine whether migration rate was important in terms of success in reaching natal subwatersheds, and so we used ANOVA to compare initial migration rate between successful and unsuccessful migrants (migration rate to Thompson not available for unsuccessful migrants). Because we made multiple comparisons with initial and overall migration rates, we corrected our significance criterion to α = 0·025.

We used multiple linear regression with type III (orthogonal) sums of squares, including fishery handling time and researcher handling time as independent variables, and plasma measurements as the dependent variable. There was a large range in researcher handling times (between removals from the seine to blood sampling), so we included researcher handling time as a variable. We grouped fish by RAMP scores and used one-way ANOVAs to compare mean concentrations of each plasma variable to evaluate whether there was a relationship between blood parameters and reflex impairment. We used a Bonferroni-corrected α of 0·007 because of multiple comparisons with blood physiology data. Researcher handling time, fishery handling time and cortisol concentration each failed to pass a Shapiro–Wilk test and were therefore log-transformed for parametric tests. All data are presented as means ± SEM unless otherwise noted.

Results

SUMMARY

The study encompassed 26 beach seine net sets in which 13 060 pink salmon were caught. The bycatch comprised 105 coho salmon, six sockeye salmon *Oncorhynchus nerka*, one chinook

salmon *Oncorhynchus tchawytcha* and one white sturgeon *Acipenser transmontanus*. The total time required to deploy and pull in a beach seine averaged 7 min 28 ± 29 s (range: 3 min 55 s–12 min 40 s). The net was always pulled into shallow water where fish reacted by thrashing vigorously for 1–2 min. Coho salmon used for telemetry and biopsy were in the seine (i.e. fishery handling time) for an average of 8 min 18 s ± 42 s (0 min 5 s–41 min 0 s) before being removed. Telemetry-tagged fish spent an additional 6 min 22 ± 21 s (1 min 37 s–11 min 38 s) in the possession of the research team (mostly holding time in hypalon fish bags while waiting to be processed). Biopsied coho salmon required an additional 9 min 41 ± 75 s for holding and processing (1 min 0 s–28 min 40 s; hereafter referred to as researcher handling time). There was a significant positive correlation between fishery handling time and reflex impairment at release (Fig. 3; Spearman rank correlation, $r_s = 0.40$, $P < 0.01$). In contrast, researcher holding/handling time was negatively associated with reflex impairment ($r_s = -0.37$, $P < 0.01$; Table 1). Of the 105 incidentally captured coho salmon, five died before removal from the net (i.e. immediate mortality; Table 2). Of
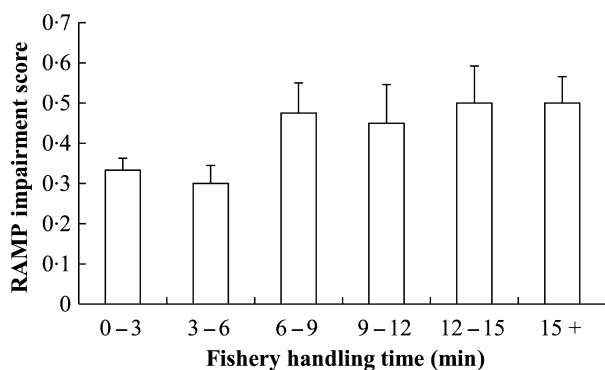
the 50 radio-tagged fish we released, 37 (74%) migrated successfully and 13 (26%) died en route. Short-term mortality (i.e. died within 96 h of release) was evident in 12% of radio-tagged coho.

RAMP SCORES, HANDLING TIME AND MIGRATION SUCCESS

Reflex action mortality predictors score exhibited a significant relationship with postrelease survival, whereas handling times were not significant predictors of survival. Coho salmon that failed to reach natal subwatersheds (mortalities) exhibited significantly higher reflex impairment upon release than did fish who migrated successfully (Mann–Whitney *U*-test: $P = 0.005$). However, neither log-transformed fishery handling time (one-way ANOVA; $F_{2,52} = 2.57$, $P = 0.09$) nor researcher handling time ($F_{2,52} = 0.04$, $P = 0.96$) differed significantly among successful migrants, unsuccessful migrants and immediate mortalities (Table 2).

With successively increasing RAMP score from 0 to 0.6, the proportion of telemetry-tagged fish successfully migrating to natal subwatersheds decreased (though these differences were not statistically significant; Kruskal–Wallis ANOVA, $H_{4,45} = 9.0$, $P = 0.06$). For example, coho salmon with one reflex impaired (RAMP = 0.2) had 93% survival, whereas this figure was 68% for fish with two reflexes impaired, and 44% for fish with three reflexes impaired (Table 1). The pattern of individual reflex impairment showed that body flex was most commonly impaired, closely followed by tail grab: these were the reflexes impaired in nearly every fish with an RAMP score of 0.4 (Table 3). While body flex and tail grab were often impaired, orientation was virtually always the next reflex to become impaired with decreasing vitality. Impairment of the orientation reflex alone was a nonsignificant predictor of premature mortality: a lower proportion (0.54) of fish with impaired orientation at release successfully migrated to the Thompson than did fish with positive orientation (0.81;

Fig. 3. Fishery handling times (time in landed seine net) and mean proportion of reflexes impaired at release (reflex action mortality predictors impairment index; $P < 0.001$). Error bars represent standard error.

Table 1. Relationships between reflex impairment (RAMP score) and fishery handling time, researcher holding time, postrelease survival (migration success), short-term migration rate (speed from release to Hope) and long-term migration rate (speed from release to Thompson)

RAMP impairment index (proportion)	Fishery handling time (min : ss)	Researcher holding time (min : ss)	Postrelease survival (%)	Migration rate short term (km day^{-1})	Migration rate long term (km day^{-1})
0	$n = 12$ 3 : 12 ± 1 : 02	$n = 12$ 10 : 30 ± 1 : 32	$n = 4$ 1.00 ± 0	$n = 4$ 21.15 ± 0.65	$n = 4$ 18.65 ± 1.76
0.2	$n = 22$ 4 : 01 ± 0 : 57	$n = 22$ 8 : 14 ± 1 : 16	$n = 15$ 0.93 ± 0.07	$n = 11$ 11.20 ± 2.06	$n = 10$ 14.45 ± 0.99
0.4	$n = 39$ 3 : 27 ± 1 : 01	$n = 39$ 9 : 40 ± 1 : 09	$n = 19$ 0.68 ± 0.11	$n = 14$ 16.31 ± 1.50	$n = 12$ 17.39 ± 1.39
0.6	$n = 18$ 8 : 31 ± 1 : 42	$n = 18$ 6 : 14 ± 1 : 18	$n = 9$ 0.44 ± 0.18	$n = 5$ 7.46 ± 2.36	$n = 3$ 13.03 ± 1.48
0.8	$n = 5$ 8 : 45 ± 2 : 50	$n = 5$ 8 : 35 ± 3 : 17	$n = 2$ 0.5 ± 0.5	$n = 2$ 13.62 ± 2.92	$n = 1$ 12.57

Values are given in means ± standard error.
RAMP, reflex action mortality predictors.

Table 2. Immediate and postrelease survival of coho salmon *Oncorhynchus kisutch* captured in an aboriginal beach seine fishery. Handling time data are means given in min : s $\pm$ 1 SE RAMP values represent the average proportion of reflexes impaired in individuals of that group

| | All fish pooled | Telemetry-tagged fish | | | |
| | | Died before Hope | Died before Hell's Gate | Died before Thompson | Reached Thompson |
	Survived <1 h	Survived <24 h	Survived <96 h	Migration failure	Migration success
Percentage of fish	5% (5 of 105)	6% (3 of 50)	12% (6 of 50)	26% (13 of 50)	74% (37 of 50)
Fishery handling time	17 : 10 $\pm$ 5 : 24	9 : 01 $\pm$ 4:14	6 : 33 $\pm$ 2 : 17	8 : 36 $\pm$ 1 : 46	6 : 45 $\pm$ 1 : 15
Researcher handling time	5 : 22 $\pm$ 4 : 36	5 : 18 $\pm$ 1 : 53	4 : 52 $\pm$ 1 : 01	5 : 08 $\pm$ 0 : 35	5 : 21 $\pm$ 0 : 25
Total handling time	22 : 32 $\pm$ 4 : 08	14 : 19 $\pm$ 5 : 16	11 : 25 $\pm$ 2 : 42	13 : 44 $\pm$ 1 : 43	12 : 06 $\pm$ 1 : 09
RAMP impairment index	1·00	0·46 $\pm$ 0·07	0·47 $\pm$ 0·04	0·49 $\pm$ 0·04	0·31 $\pm$ 0·03

RAMP, reflex action mortality predictors.

Table 3. Impairment of individual reflexes with increasing overall reflex impairment (RAMP Index). Values represent the proportion of individuals with a particular reflex impaired within each group

RAMP impairment index (proportion)	Tail grab	Body flex	Orientation	Head complex	VOR
N = 12 0	0	0	0	0	0
N = 22 0·2	0·36	0·59	0·05	0	0
N = 39 0·4	1·00	1·00	0·03	0·03	0
N = 18 0·6	1·00	1·00	1·00	0	0
N = 5 0·8	1·00	1·00	1·00	1·00	0

RAMP, reflex action mortality predictors; VOR, vestibular-ocular response.

Mann–Whitney U-test, P = 0·06). Head complex was rarely impaired, while impairment of VOR was not observed among live fish in this study (Table 3).

MIGRATION RATE

Upstream migration rate for radio-tagged coho salmon was highly variable and did not show clear patterns with respect to the predictor variables measured (see Fig. S1, Supporting information). Migration rate did not vary significantly with size (FL; both P > 0·15), and therefore, we calculated migration rate independent of FL (in km day^{-1}). We did not detect any patterns in initial or overall migration rates with respect to log-transformed fishery handling time (both P > 0·15), researcher handling time (both P > 0·30) or final migration success (only initial migration tested: P > 0·40), possibly due to the high variation in migration rates. RAMP score was a significant predictor of migration rate for the initial 20 km, from the release site to Hope ($F_{4,31}$ = 4·44, P = 0·006; all other stretches P > 0·10), but not in post hoc comparisons (Tukey HSD, all P > 0·10).

PLASMA PHYSIOLOGY

There was high inter-individual variation in blood plasma measures among the 43 coho salmon we sampled. Using multiple regression, both fishery handling time ($F_{1,40}$ = 8·77, P = 0·005, β = 0·97) and researcher handling time ($F_{1,40}$ = 34·66, P < 0·001, β = 0·75) were significantly (α = 0·007) predictive of log-transformed cortisol titres (model R^2 = 0·46). Likewise, plasma lactate concentration in captured coho salmon was significantly affected by fishery ($F_{1,40}$ = 20·80, P < 0·001, β = 13·14) and researcher handling times ($F_{1,40}$ = 22·39, P < 0·001, β = 5·25; model R^2 = 0·42). Only researcher handling was a significant predictor of plasma glucose ($F_{1,40}$ = 14·42, P = 0·005, β = 1·44, R^2 = 0·21) and osmolality concentrations ($F_{1,40}$ = 18·16, P < 0·001, β = 15·85, R^2 = 0·33). Plasma chloride was not significantly affected by either fishery (P = 0·23) or researcher handling time (P = 0·62). Similarly, sodium was not significantly affected by fishery (P = 0·57) or researcher (P = 0·03) handling time and likewise for potassium (P = 0·46, P = 0·03, respectively). Grouping coho by RAMP scores did

not reveal any differences in plasma constituents (all $P > 0.05$). Females exhibited notably higher cortisol concentrations (not significant; $F_{1,40} = 7.00$, $P = 0.01$), while glucose was significantly higher in males ($F_{1,40} = 16.13$, $P < 0.001$; all others plasma variables $P > 0.10$; Table 4).

Discussion

Reflex action mortality predictors was developed as a tool for rapid, simple and inexpensive evaluation of fish vitality and fisheries-induced mortality without the use of conventional research methods (i.e. holding pen experiments, telemetry and blood physiology). This study demonstrates that RAMP scores can be used to predict postrelease mortality in salmon captured as bycatch while migrating to spawning grounds. A higher proportion of reflexes were impaired at release in radio-tagged coho salmon that failed to reach their natal subwatersheds than for individuals that migrated successfully. This result is particularly interesting given the lack of concordance between the intensity of the stressor (here, fisheries handling time) and delayed mortality. In fact, RAMP score was the only measurement we made for radio-tagged fish that predicted postrelease mortality. Further, RAMP integrated the organismal response to varying degrees of capture stress, showing a significant positive correlation with stressor intensity (i.e. fishery handling time; Fig. 3, Table 1). Research elsewhere has shown similar correlations between capture stress intensity, reflex impairment and mortality in Atlantic cod *Gadus morhua*, walleye pollock *Theragra chalcogramma*, northern rock sole *Lepidopsetta polyxystra*, Pacific halibut *Hippoglossus stenolepis*, sablefish *Anoplopoma fimbria*, and coho salmon smolts (Davis 2005, 2007; Davis & Ottmar 2006; Humborstad, Davis & Lokkeborg 2009). However, in addition to being the first study to use RAMP in a freshwater environment and on adult salmonids, our data are the first to show a correlation between RAMP score and mortality for a fish released into the wild, thus validating this approach.

Table 4. Physiological measures of stress compared between resting coho salmon *Oncorhynchus kisutch* (borrowed from Donaldson *et al.* 2010b) and coho salmon sampled following a aboriginal beach seining experience (current study). Both sexes are pooled where sex is not indicated. Values are means ± SE

Plasma variable	Baseline reference	Beach seined (current study)
Cortisol (ng mL^{-1})		
Male	78·5 ± 19·8	159·7 ± 40·1
Female	94·1 ± 21·9	328·5 ± 43·0
Glucose (mmol L^{-1})	5·6 ± 0·3	5·2 ± 0·2
Lactate (mmol L^{-1})	1·9 ± 0·1	12·3 ± 0·6
Chloride (mmol L^{-1})	134·5 ± 0·8	138 ± 0·6
Sodium (mmol L^{-1})	155·2 ± 0·9	163·5 ± 1·0
Potassium (mmol L^{-1})	1·9 ± 0·2	4·1 ± 0·14
Osmolality (mOsm kg^{-1})	321·4 ± 1·3	341·2 ± 1·8

Physiological variables were indicative of both fishery and researcher handling durations but did not show concordance with RAMP scores, which is not surprising as most of the variables measured increased following initiation of a stressor for up to an hour (Barton 2002). Regardless, the biopsy data showed that the captured coho had experienced substantial stress, as plasma values were substantially different than those considered routine for nonstressed fish (Table 4). Indeed, capture and crowding in the beach seine would constitute a combination of exhaustive exercise and hypoxia, two stressors known to result in severe physiological disturbance (e.g. Ferguson & Tufts 1992; Kieffer 2000). Longer time entangled in fishing gear was predictive of higher lactate accumulation in plasma (controlling for researcher handling time). Lactate values (i.e. ~12 mmol L^{-1}) revealed that fish were clearly using anaerobic metabolism which is characteristic of both exhaustive exercise and hypoxia. Physiological exhaustion was probably a driver of reflex impairment, although plasma lactate (our best measure of exhaustion here) was not significantly different among RAMP scores. Like lactate, cortisol titres were positively correlated with fishery handling times but did not demonstrate a pattern with respect to RAMP score. This result was surprising, given our assumption that reflex impairment has a basis in physiological stress (Davis 2010). It is possible that alternative measures could better relate to RAMP scores (e.g. muscle physiology, plasma pH and arterial PO$_2$). More importantly, had every fish been blood sampled instantly upon removal from the seine, we would probably have found a stronger relationship between plasma physiology, RAMP score and fishery handling time. Although basic physiological analysis is not as widely accessible as RAMP, it can offer researchers an added understanding of capture stress. Future efforts to predict mortality in Pacific salmon could attempt to combine blood physiology and reflex measures (e.g. Campbell *et al.* 2010). Regardless, our study demonstrates that in an applied context, RAMP appears to be more useful in field studies of bycatch than traditional physiological tools.

At this time, we can only speculate about the mechanistic links between reflex impairment, fish vitality and the physiology of morbidity and death. Reflexes are neurological responses to external stimuli although in our assessment we included two responses (head complex – the pattern of regular ventilation – and VOR) that are functions of the autonomic nervous system. Neurological control of respiration in fish is complex and originates in the brain where neurons discharge rhythmically in time with respiration (De Graaf & Roberts 1991). The proximate motor nerve controlling the opening and closing of the lower jaw in fish (the pattern we observed to assess this reflex) is the trigeminal fifth (Taylor *et al.* 2006). VOR was not impaired in any fish in this study, although its impairment has been observed in sockeye and pink salmon air exposed for more than 3 min in other studies in the lower Fraser River (G. Raby, unpublished data). Analogous to 'ocular counter-rolling' in humans (MacDougall *et al.* 1999), the VOR reflex in teleosts occurs when static signals in the otolith reach the extraocular motoneurons (Suwa, Gilland & Baker 1999). When fish lose equilibrium, it may be caused by a

combination of the breakdown of neural and muscle function broadly, but perhaps in particular at the fins involved in controlling balance (e.g. the pectoral fins). Body flex and tail grab both involve use of white myotomal musculature innervated by local motoneurons (Lauder 2005): these reflexes are likely to be impaired as a result of white muscle exhaustion (e.g. high lactate loading) rather than neurological dysfunction. Collectively, this suite of reflexes became progressively more impaired with increasingly severe capture stress. Our supposition is that, as a whole-animal index of vitality, a higher RAMP score predicts delayed mortality by indicating a state further from homeostasis from which a complete recovery is less probable (and mortality is more likely). However, the proximate causes of fish death following capture and release have yet to be identified (Wood, Turner & Graham 1983; Davis 2002). Future research that uncovers the links between physiological disturbance, reflex impairment and delayed mortality would be valuable from both an applied and fundamental perspective.

Arguably, the most important contribution of the present study is compelling evidence that RAMP deserves further consideration as a tool for predicting delayed mortality of fish released into the wild. Particularly for comparing vitality among fish, assessing handling techniques and determining postrelease mortality, RAMP should be considered as a widespread tool in Pacific salmon management and research. In the case of fisheries in the lower Fraser River, RAMP assessments could be conducted on coho prior to release to determine whether capture conditions are impairing fish condition to a point that could lead to mortality. Fishers could change their behaviour in real-time by conducting shorter net sets and leaving fish in deeper water if fish condition is poor. RAMP could also be used to determine the likelihood that a fish will survive if released, facilitating decisions on whether or not the fish should be released, retained or recovered using recovery tools (e.g. Farrell *et al.* 2001). Education programs could be used to develop capacity for RAMP assessment among fishers and management agencies. Most importantly, RAMP could be used to rapidly generate inexpensive mortality estimates for different combinations of fishing conditions, handling techniques, species and gear types that otherwise would be impossible given the prohibitive costs of conducting numerous telemetry studies. Such mortality estimates are valuable in accounting for incidental mortality in management models (Baker & Schindler 2009).

Although the data we have presented here represent a single case study, RAMP may be widely applicable in fisheries and potentially in other areas of wildlife management, where animal welfare and mortality outcomes are monitored. A weight of evidence has already accumulated that RAMP works across taxa, systems and in a variety of laboratory and field conditions. The most obvious value of RAMP lies in bycatch management, and although only tested on fish and shellfish thus far, all animals have reflexes that can become impaired. Thus, it is conceivable that conservation practitioners could use RAMP for managing the welfare and mortality of birds, turtles and mammals caught as bycatch. There may even be potential for the application of reflex measures of some form outside of fisheries, such as in monitoring the welfare and predicting the survival of translocated wildlife (e.g. Pinter-Wollman, Isbell & Hart 2009). The use of reflex measures has the potential to play an important role in assessing and managing fisheries-induced mortality and, in turn, contribute to the conservation of Pacific salmon populations and possibly other species elsewhere.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Upstream migration rate for radio tagged coho salmon released from an aboriginal beach seine fishery in the lower Fraser River.

Appendix S1. Additional methods details.

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