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**Non-Lethal Assessment of Juvenile Pink and Chum Salmon for Parasitic Sea
Lice Infections and Fish Health**

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1 **Abstract**

2 Industrial salmon farming has been correlated with parasitic sea lice infestations of adjacent wild
3 juvenile salmonids and declines of sympatric wild salmonid populations. Prohibitively large
4 financial, human, and logistical resource requirements prevent the implementation of long-term
5 large-scale monitoring programs to assess the effect of farms on wild salmon. We report a novel
6 non-lethal sampling procedure for quantifying louse abundances and measures of fish health on
7 wild juvenile pink and chum salmon during their early marine life-history phase. The method
8 significantly reduces the resource requirements of sampling programs, and provides a desirable
9 non-lethal alternative for studying depressed or threatened populations. The simplicity of the
10 protocol facilitates public participation, further decreasing costs while increasing the potential
11 spatio-temporal coverage and resolution of future research/monitoring programs.

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13

14 **Keywords:** aquaculture; salmon; salmon conservation; sea lice; macroparasite; fish health;
15 emergent disease.

16

1 **Introduction**

2 Industrial salmon farming now occurs sympatrically across the native and introduced
3 ranges of most anadromous salmonids. Many studies have correlated the presence of salmon
4 farms with increased infection levels of parasitic sea lice on adjacent wild salmonids (e.g.
5 Scotland: Mackenzie et al. 1998; Ireland: Tully et al. 1999; Norway: Bjorn and Finstad 2002;
6 Canada: Morton et al. 2004). These correlations are concurrent with declines in wild populations
7 but their causal nature is highly contentious (McVicar 1997; McVicar 2004). Given these
8 findings and the current intensity and anticipated growth of marine industrial salmon culture,
9 large-scale monitoring programs are needed to assess the effect of farms via sea lice on wild
10 salmon.

11 Current methods demand extensive human, logistical, and financial support because they
12 require the transport, accommodation, and organization of personnel, equipment, and samples in
13 remote coastal locations. Analyses are then deferred to post-mortem examination of frozen
14 specimens. For example, the 2003 Fisheries and Oceans Canada (FOC) monitoring program in
15 the Broughton Archipelago, BC, lethally sampled a total of 21,524 sticklebacks and juvenile pink
16 and chum salmon throughout the Broughton Archipelago over a 3.5 month period using beach
17 and purse seines (S. Jones, Pacific Biological Station, Nanaimo, BC, pers. com.), at a cost of
18 \$CAN 55.75 per fish (total cost = \$CAN 1.2 million). These resource demands make the
19 sustained implementation of such programs unlikely.

20 Here, we report a non-lethal sampling procedure that significantly reduces resource
21 demands and analyze the methodology for precision, accuracy, mortality impacts, and show how
22 the protocol can be extended to inform on fish health. Samples are analyzed on site, eliminating
23 the need for organization, storage, transport, and post-mortem laboratory analysis. Previous
24 application of this method throughout Knight Inlet and Tribune Channel (within the Broughton

1 Archipelago) over a 2 month period in 2003 yielded a total sample size of ~7000 juvenile pink
2 and chum salmon caught by beach seine and assayed for lice at a cost of < CAN\$ 1.81 per fish
3 (Krkošek unpubl. data). The method is applicable to monitoring programs of juvenile pink and/or
4 chum salmon during their near-shore life-history phase and provides a non-lethal alternative to
5 study depressed or threatened populations. The simplicity of the protocol facilitates public
6 participation in sampling programs, and public interest exists in many coastal communities.
7 Greater community involvement would further reduce costs while increasing spatio-temporal
8 resolution and coverage.

9

10 *Natural History*

11 Two common sea louse species coexist on salmonids in Pacific waters off North America:
12 *L. salmonis* and *C. clemensi* (Parker and Margolis 1964). Both species have planktonic larval
13 stages, and parasitic juvenile and adult stages. Planktonic nauplii hatch from gravid parasitic
14 females and develop into infective copepodids. After settling on a host fish, copepodids develop
15 through distinct chalimus and motile pre-adult and adult stages. Attached stages feed on the
16 mucus, scales, and blood of the host fish leading to osmotic stress and emaciation of sufficiently
17 infected hosts. The ecology of these species differ: *L. salmonis* are salmonid specialists, whereas
18 *C. clemensi* are generalists occurring on members of several piscine Families (Parker and
19 Margolis 1964; Pike and Wadsworth 2000).

20 Currently in British Columbia wild juvenile pink salmon (*Oncorhynchus gorbuscha*) and
21 chum salmon (*Oncorhynchus keta*) are of particular concern. Both species share a unique life
22 history among anadromous salmonids: juveniles emerge from gravel and immediately enter the
23 marine environment at approximately 28-35 mm and 30-40 mm fork length, respectively (Groot
24 and Margolis 1991; pers. obs.). This makes them the smallest salmonids to contend with marine

1 parasites. They rear in nearshore habitats during their seaward migration typically in mixed
2 schools, which often brings them within the immediate vicinity of salmon farms that may amplify
3 copepodid densities (Morton et al. 2004).

4

5 **Description of Methods**

6 *Parasite Loads and Fish Health Observations*

7 Juvenile pink and chum salmon were captured with a beach seine off rocky intertidal
8 shorelines. The minimum recommended dimensions of the seine net are 20 m x 1.5 m with 4 mm
9 mesh size for salmon < 5.5 cm fork length and 35 m x 3 m with 4 mm mesh bunt for salmon 5.5-
10 10 cm. Upon capture, live samples were stored in buckets where appropriate water temperature
11 and dissolved oxygen levels were maintained. Stress to fish was minimized during transfer to
12 buckets, both to protect fish health and to minimize louse loss.

13 Juvenile salmon were analyzed on site. Individual fish were placed inside a clear plastic
14 envelope without water for analysis. A sufficient envelope is a standard large ziplock storage bag
15 (3.7 L, 27 x 28 cm) with the top portion of the bag cut and removed to create a 27 x 12 cm
16 envelope. The position of the fish was controlled by the surface tension of the envelope and all
17 surfaces and fins of the fish were viewed. A hand lens (e.g. Ruper 16X, 25 mm diameter) was
18 sufficient to differentiate copepodid, chalimus, and motile stages (see Kabata (1972) and Johnson
19 and Albright (1991)). It was difficult to distinguish the two louse species with this technique for
20 most parasitic stages except gravid females – which is when obvious morphological differences
21 emerge (Kabata 1972; Johnson and Albright 1991). However, since both species exist on salmon,
22 they were grouped and assayed together, as has been practiced in other studies (Morton et al.
23 2004). Fish health observations were recorded (haemorrhaging, scarring, predation marks,
24 lesions, fin erosion), and were not confounded by sacrificing and freezing specimens as occurs in

1 traditional methods. After analysis, fish were allowed to recover and then released at the location
2 of capture.

3 A period of 30-90 seconds per fish was required for body measurements and louse counts,
4 dependant on the size and infestation level of the fish (the mean infestation levels in our analysis
5 of 106 sets of 100 salmon ranged from 0 to 10.5 lice per fish). Handling time was minimized by
6 tasking 1-3 people with analysis and another with data recording.

7

8 *Morphometrics and Condition Factor*

9 Fork length and a proxy measure of weight were used to estimate the Fulton condition
10 factor: $k = \text{weight} \cdot (\text{fork length})^{-3} \cdot 100$. It was difficult to obtain weight measurements directly
11 from live juvenile salmon, but weight was inferred from fork length and body depth
12 measurements. Body depth is the maximum linear distance between ventral and dorsal surfaces,
13 and if this corresponds to the head of the fish, it is measured halfway between the posterior of the
14 head and anterior of the dorsal fin. A simple geometric argument relates these metrics: juvenile
15 salmon morphology is crudely cylindrical or rectangular, and fish weight should be proportional
16 to volume by density. This suggests a power relationship,

$$17 \quad w = \alpha L^{\gamma_1} D^{\gamma_2}, \quad (1)$$

18 where w is weight, L is fork length, and D is body depth. The remaining parameters α , γ_1 , and γ_2 ,
19 are left to be determined. Taking the natural logarithm of both sides we have

$$20 \quad \ln(w) = \ln(\alpha) + \gamma_1 \ln(L) + \gamma_2 \ln(D), \quad (2)$$

21 which we fit to log transformed fork length-body depth-weight data (see Results) . The Fulton
22 condition factor then becomes

$$23 \quad k = (\alpha L^{\gamma_1 - 3} D^{\gamma_2}) \cdot 100. \quad (3)$$

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Analysis of Methods

We analyzed 40 juvenile pink and chum salmon using the non-lethal sampling technique and then lethally re-analyzed these fish with a dissecting microscope under 8-20X magnification. Live samples were sacrificed by retaining them within individually marked storage bags without water and placing them immediately on ice. Lethal samples were analyzed within five hours of the non-lethal assay. Copepodid, chalimus, and motile stages were distinguished and counted, and fork length and body depth measurements were recorded. The identity of each fish was tracked and the resulting data were paired: live and lethal louse counts and morphometric measurements for each fish. Differences between live and lethal data pairs allowed an analysis of measurement error: Is the non-lethal technique biased to under-detect lice (because it is less thorough)? Are morphometrics equal between the two techniques? Does the infection level of the fish affect the accuracy of the non-lethal technique? Louse count data were discrete, which violates normality assumptions, so we applied one-tailed nonparametric bootstrap paired-sample *t*-tests to test the null hypotheses that louse counts from live samples were not less than those from lethal samples. Morphometric data did conform to normality assumptions so we applied two-tailed paired-sample *t*-tests to test for measurement error in morphometrics. To test the effect of infestation level on the accuracy of the methods we regressed differences between live and lethal counts per fish against the lethal counts on those fish for each louse stage: copepodids, chalimi, and motiles. Statistically significant differences from zero in the y-intercept indicate a fundamental bias between the two techniques while a statistically significant difference from zero in the slope indicates a bias in the non-lethal technique that is a function of the infestation level of the fish.

1 We analyzed the short-term and long-term survivorship of sampled fish. Short-term
2 survival was determined by recording the number of mortalities incurred during the analysis of
3 10,600 (106 sets of 100) juvenile pink and chum salmon in May, 2004, in Tribune Channel and
4 Knight Inlet, British Columbia. The long-term survival was determined by subjecting 86 juvenile
5 pink and chum salmon to the non-lethal assay and retaining them for 30 days in a 189 L plastic
6 ocean enclosure at the Raincoast Research Station, Simoom Sound, BC. Those fish had an
7 average infection burden of 0.24 copepodids, 0.067 chalimi, and 0.077 motiles, and were on
8 average 67 mm fork length. Fish were fed commercial salmon feed in excess of satiation every 2-
9 4 hours daily and mortalities were recorded and removed every 2-4 hr daily. Daily sea surface
10 temperatures were on average $12.0 \pm C$, and ranged from 9.6 to $14.2 \pm C$.

11

12 **Results**

13 Non-lethal and lethal sampling methods provided similar estimates of louse abundances
14 (Figure 1A). However, the non-lethal method is biased to under-detect copepodids ($p=0.056$) and
15 chalimi ($p=0.028$), but not motiles ($p=0.65$; one-tailed nonparametric bootstrap paired sample t -
16 test for each stage). This is reflected in the frequency distributions of measurement error (Figure
17 1 B-D); the histograms are negatively skewed for copepodid and chalimus lice, but not for
18 motiles. The mean abundances of lice stages are presented in figure 1, and those estimates ranged
19 0-4 copepodids per fish, 0-10 chalimi per fish, 0-5 motiles per fish, and 2-15 total lice per fish.

20 Regression analyses between differences in live and lethal counts and lethal counts
21 indicated the y-intercept was not different from zero for all lice stages: y-intercepts with 95%
22 confidence bounds were 0.34 (-0.09, 0.77), -0.11 (-0.40, 0.61), 0.09 (-0.20, 0.04) for copepodids,
23 chalimi, and motiles, respectively. The slopes in the regressions were not different from zero for
24 chalimi and motiles (slopes with 95% confidence bounds were -0.09 (-0.20,0.02) and -0.08 (-

1 0.08,0.26)), but the slope was less than zero for copepodids (-0.29 (-0.48, -0.10); $p < 0.005$). This
 2 indicates that as infestation levels increase the non-lethal technique will underestimate copepodid
 3 abundances but counts in the other stages will be unaffected.

4 There were statistically significant differences in morphometrics between live and lethal
 5 sampling techniques (length: $p = 3.78 \times 10^{-7}$; body depth: $p = 0.065$; two-tailed paired sample t -test
 6 with $df = 39$ for each). Fork length estimates were greater in non-lethal analyses than lethal
 7 analyses and estimates in body depth from non-lethal analyses were less than those from lethal
 8 analyses (Fig 2 A-B).

9 We fit equation (2) to log transformed fork length-body depth-weight data from both
 10 lethal ($n = 1059$) and non-lethal ($n = 768$) techniques. The log-transformed data showed a strong
 11 linear relationship and equation (2) explained 95% and 93% of the variance in these data,
 12 respectively (Figure 2 C-D). The regressions were strongly significant ($p < 0.001$ for both) and
 13 parameter estimates with 95% confidence limits are: live: $\ln(\alpha) = -9.07$ (-9.36,-8.78), $\gamma_1 = 1.97$
 14 (1.84, 2.09), $\gamma_2 = 0.74$ (0.63, 0.85); and lethal: $\ln(\alpha) = -12.48$ (-13.28, -12.68), $\gamma_1 = 3.09$ (2.98, 3.21),
 15 $\gamma_2 = 0.21$ (0.18,0.25).

16 The average post-assay mortality rate was 0.74% per sample. That is, 99.26% of fish
 17 subjected to the non-lethal method recovered and were subsequently released at the location of
 18 capture. Long-term survivorship was equally good. From 86 pink and chum salmon retained in
 19 ocean enclosures following analysis, only 1 died in the following 30 days.

20

21 **Discussion**

22 Both non-lethal and lethal sampling techniques produced similar estimates of louse stage
 23 abundances despite a bias to under-detect copepodid and chalimus lice in non-lethal samples.

1 Measurement error can be attributed to a reduced detectability of small chalimus and copepodid
2 lice, misidentification of louse stages, and reduced integrity of lethal samples. Similar abundance
3 estimates were obtained because errors had a strong central tendency at zero with only a slight
4 negative skew and error variability occurs at a lower scale than variability in the data (e.g.
5 chalimus data showed the strongest bias and $\text{var} = 5.89$ for lethal chalimus counts whereas
6 $\text{var}=0.71$ in the paired differences between live and lethal chalimus counts). Regression analyses
7 indicated there was a bias to underestimate copepodids as copepodid abundances on sampled fish
8 increased but no corresponding bias was detected in counts of chalimus and motile lice. It is
9 known that increasing noise in response variables (i.e. louse abundances) does not confound
10 statistical analyses as it does for explanatory variables (e.g. farm proximity, temperature, salinity;
11 Gustafson 2004). This leads us to conclude that the non-lethal technique provides a biologically
12 viable data collection method for analyzing temporal and spatial patterns of louse population
13 structure, but likely underestimates the true abundance of sea lice.

14 Differences in morphometrics between lethal and non-lethal sampling techniques were
15 evident from direct measurements and also from differences in parameter estimates of equation
16 (2). Differences in body condition between live and dead fish may produce this; live fish retain a
17 firm cylindrical profile whereas dead fish become flaccid. However, the tight linear relationship
18 among log-transformed fork length, body depth, and weight data make it possible to infer weight
19 from fork length and body depth measurements using equation (2). The same measurements of
20 fish condition (Fulton condition factor) can be obtained from both non-lethal and lethal sampling
21 techniques.

22 The sampling methods we developed only apply to the early marine life-history stages of
23 pink and chum salmon when they occupy near-shore habitats. As these fish grow in size they
24 move into deeper waters inaccessible to a beach seine (Groot and Margolis 1991). Salmon

1 susceptibility to disease impacts of lice likely decreases with increasing body size and it is
2 therefore the early marine stages that are most important for monitoring objectives. Fortunately
3 the near-shore phase persists for 1-2 months allowing an application of these methods (Groot &
4 Margolis 1991; *pers. obs.*).

5 For simplicity we grouped both species of salmon and lice together in the present analysis
6 but this is not a necessary feature of the non-lethal methods – juvenile pink and chum salmon can
7 be easily identified (Pollard *et al.* 1997) and monitoring programs can assess their infection levels
8 independently. Differences in gravid female counts between the two louse species can provide an
9 index of the presence/absence of these species, if not an index of their relative abundance. If
10 greater resolution of the relative abundance between louse species at earlier life-history stages is
11 desired a lethal subsampling procedure is required since laboratory analysis is needed for the
12 proper identification of louse species in their early life-history stages.

13 The potential impacts of sea lice transmission from salmon farms on wild salmon have
14 raised public concerns, particularly in coastal communities that have the necessary resources to
15 implement these methods. The requirements are minimal: a crew of 3, a beach seine, a hand lens,
16 and access to appropriate intertidal habitat. Engaging communities by providing scientific
17 training and involving them in properly designed monitoring programs would increase the
18 potential spatio-temporal coverage of future monitoring and/or research programs. The method
19 eliminates all logistical constraints associated with lethal sampling, which alone expands research
20 potential. While the method likely underestimates the true abundance of sea lice it holds promise
21 as a simple, precautionary, and adaptive approach to studying the effects of industrial salmon
22 farming via sea lice on wild Pacific salmon.

23

1 **Acknowledgments**

2 We are grateful to Eric Nelson for his generous hospitality and logistical support of the
3 fieldwork. Special thanks to Brendan Connors, Scott Rogers, Peter Molnar, Vendulka Krkošek,
4 Laird Herbert, Louise Dykslag, Katrina Assonitis, Jamie Pepper, and Jennifer Gee for their hard
5 work in the field. Discussions with Mark Lewis improved this manuscript. This work was
6 supported by the David Suzuki Foundation; Raincoast Conservation Society; Raincoast Research
7 Society; an NSERC Discovery Grant to JPV; and a Walter H. Johns Graduate Fellowship and
8 NSERC Industrial Postgraduate Scholarship to MK.

9

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- 15

1 **Figure Headings**

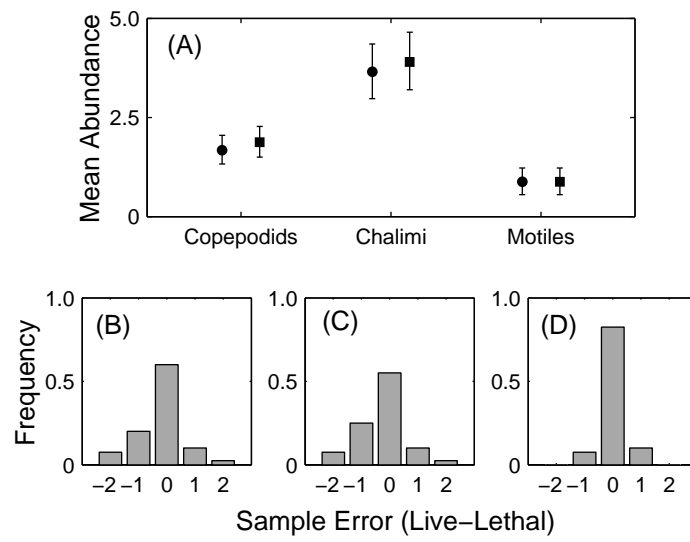
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3 Figure 1. A comparison of louse counts between live and lethal sampling methods. Panel (A)
4 shows the mean abundances of louse stages estimated by non-lethal (circles) and lethal (squares)
5 methods. Error bars are bootstrapped 95% confidence intervals on the mean and sample sizes are
6 40 each. Error frequencies in louse counts are shown for (B) copepodids, (C) chalimi, and (D)
7 motiles, calculated as the difference in counts between paired live-lethal samples.

8

9 Figure 2. Juvenile salmon morphometrics. Top panels: comparison of mean fork length (A) and
10 body depth (B) between non-lethal (circles) and lethal (squares) sampling techniques. Error bars
11 are bootstrapped 95% confidence intervals on the mean and sample sizes are 40 each. Bottom
12 panels show linear relationships among log transformed morphometric data for juvenile pink and
13 chum salmon: fork length (L, mm), body depth (D, mm), and weight (W, g). Solid lines are
14 equation (2) fit independently to (C) live sampled fish ($n=736$; $R^2 = 0.93$) and (D) lethally
15 sampled fish ($n=1059$; $R^2 = 0.95$). Parameter estimates are given in the main text.

Krkosek et al. Figure 1.



Krkosek et al Figure 2

