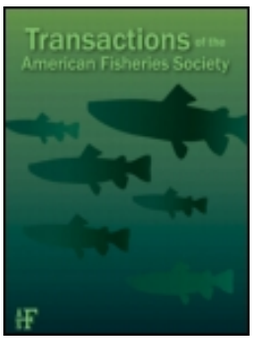

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[Note]

Nonlethal Assessment of Juvenile Pink and Chum Salmon for Parasitic Sea Lice Infections and Fish Health

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Abstract.—Industrial salmon farming has been correlated with infestations of parasitic sea lice *Lepeophtheirus salmonis* in adjacent wild juvenile salmonids and declines of sympatric wild salmonid populations. Prohibitively large financial, human, and logistical resource requirements prevent the implementation of long-term, large-scale monitoring programs to assess the effect of farms on wild salmonids. We report a novel nonlethal sampling procedure for quantifying louse abundances and measures of fish health on wild juvenile pink salmon *Oncorhynchus gorbuscha* and chum salmon *O. keta* during their early marine life history phase. The method significantly reduces the resource requirements of sampling programs and provides a desirable nonlethal alternative for studying depressed or threatened populations. The simplicity of the protocol facilitates public participation, further decreasing costs while increasing the potential spatiotemporal coverage and resolution of future research–monitoring programs.

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

Current methods demand extensive human, logistical, and financial support because they require the transport, accommodation, and organization of personnel, equipment, and samples in remote coastal locations. Analyses are then deferred to postmortem examination of frozen specimens. For example, the 2003 Fisheries and Oceans Canada (FOC) monitoring program in the Broughton Archipelago, British Columbia, lethally sampled a total of 21,524 sticklebacks (family Gasterosteidae) and juvenile pink salmon *Oncorhynchus gorbuscha* and chum salmon *O. keta* throughout the Broughton Archipelago over a 3.5 month period using beach and purse seines (S. Jones, Pacific Biological Station, personal communication), at a cost of Can$32.52 per fish (total cost = $0.7 million). These resource demands make the sustained implementation of such programs unlikely.

Here we report a nonlethal sampling procedure that significantly reduces resource demands; analyze the methodology for precision, accuracy, and mortality impacts; and show how the protocol can be extended to inform on fish health. Samples are analyzed on site, eliminating the need for organization, storage, transport, and postmortem laboratory analysis. Previous application of this method throughout Knight Inlet and Tribune Channel (within the Broughton Archipelago) over a 2-month period in 2003 yielded a total sample size of ~7000 juvenile pink and chum salmons caught by beach seine and assayed for lice at a cost of

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less than $1.81 per fish (Krkošek, unpublished data). The method is applicable to monitoring programs of juvenile pink salmon, chum salmon, or both during their nearshore life history phase and provides a nonlethal alternative to study depressed or threatened populations. The simplicity of the protocol facilitates public participation in sampling programs, and public interest exists in many coastal communities. Greater community involvement would further reduce costs while increasing spatiotemporal resolution and coverage.

Natural History

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

Currently in British Columbia wild juvenile pink and chum salmons are of particular concern. Both species share a unique life history among anadromous salmonids: juveniles emerge from gravel and immediately enter the marine environment at approximately 28–35 mm and 30–40 mm fork length, respectively (Groot and Margolis 1991; A. Morton, personal observation). This makes them the smallest salmonids to contend with marine parasites. They rear in nearshore habitats during their seaward migration typically in mixed schools, which often brings them within the immediate vicinity of salmon farms that may amplify copepodid densities (Morton et al. 2004; Krkošek et al., in press).

Methods

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

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

A period of 30–90 s per fish was required for body measurements and louse counts, dependent on the size and infestation level of the fish (the mean infestation levels in our analysis of 106 sets of 100 salmon ranged from 0 to 10.5 lice per fish). Handling time was minimized by tasking one to three people with analysis and one with data recording.

Morphometrics and condition factor.—Fork length and a proxy measure of weight were used to estimate the Fulton condition factor (k = weight · [fork length]$^{-3}$ · 100). It was difficult to obtain weight measurements directly from live juvenile salmon, but weight was inferred from fork length and body depth measurements. Body depth is the maximum linear distance between ventral and dorsal surfaces, and if this corresponds to the head of the fish, it is measured halfway between the posterior of the head and anterior of the dorsal fin. A simple geometric argument relates these metrics: juvenile salmon morphology is crudely cylindrical or rectangular, and fish weight should

be proportional to volume by density. This suggests a power relationship

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

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

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

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

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

Analysis of methods.—We analyzed 40 juvenile pink and chum salmons using the nonlethal sampling technique and then lethally reanalyzed these fish with a dissecting microscope under 8–20× 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 5 h of the nonlethal 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 in the following ways: (1) is the nonlethal technique biased to underdetect lice (because it is less thorough); (2) are morphometrics equal between the two techniques; and (3) does the infection level of the fish affect the accuracy of the nonlethal 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 nonlethal technique that is a function of the infestation level of the fish.

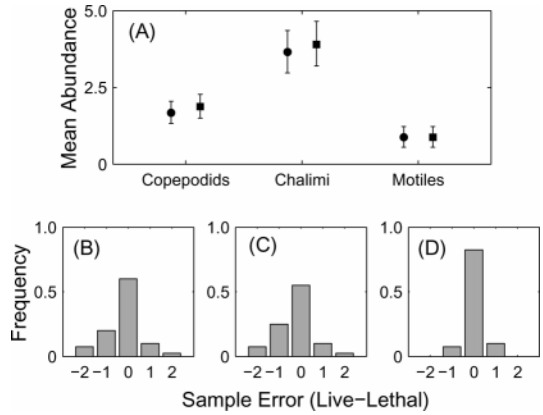

FIGURE 1.—A comparison of louse counts between live and lethal sampling methods. Panel **(A)** shows the mean abundances of louse stages estimated by nonlethal (circles) and lethal (squares) methods. Error bars are bootstrapped 95% confidence intervals on the mean and sample sizes are 40 each. Error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

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

Results

Nonlethal and lethal sampling methods provided similar estimates of louse abundances (Figure 1A). However, the nonlethal method is biased to underdetect copepodids ($p = 0.056$) and chalimi ($P = 0.028$), but not motiles ($P = 0.65$; one-tailed nonparametric bootstrap paired sample *t*-test for each stage). This is reflected in the frequency dis-

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tributions of measurement error (Figure 1B–D); the histograms are negatively skewed for copepodid and chalimus lice, but not for motiles. The mean abundances of lice stages are presented in Figure 1, and those estimates ranged zero to four copepodids per fish, 0–10 chalimi per fish, zero to five motiles per fish, and 2–15 total lice per fish.

Regression analyses between differences in live and lethal counts indicated the *y*-intercept was not different from zero for all lice stages; *y*-intercepts with 95% confidence bounds were 0.34 (–0.09, 0.77), −0.11 (−0.40, 0.61), 0.09 (–0.20, 0.04) for copepodids, chalimi, and motiles, respectively. The slopes in the regressions were not different from zero for chalimi and motiles (slopes with 95% confidence bounds were 0.09 [–0.20,0.02] and −0.08 [–0.08,0.26]), but the slope was less than zero for copepodids (−0.29 [−0.48, −0.10]; $P < 0.005$). This indicates that as infestation levels increase the nonlethal technique will underestimate copepodid abundances, but counts in the other stages will be unaffected.

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

We fit equation (2) to log-transformed fork length–body depth–weight data from both lethal ($n = 1059$) and nonlethal ($n = 768$) techniques. The log-transformed data showed a strong linear relationship and equation (2) explained 95% and 93% of the variance in these data, respectively (Figure 2C–D). The regressions were strongly significant ($P < 0.001$ for both), and parameter estimates with 95% confidence limits are: (1) live: $\log_e(\alpha) = -9.07$ (−9.36, −8.78); $\gamma_1 = 1.97$ (1.84, 2.09); $\gamma_2 = 0.74$ (0.63, 0.85); and (2) lethal: $\log_e(\alpha) = -12.48$ (−13.28, −12.68); $\gamma_1 = 3.09$ (2.98, 3.21); $\gamma_2 = 0.21$ (0.18, 0.25).

The average postassay mortality rate was 0.74% per sample. That is, 99.26% of fish subjected to the nonlethal method recovered and were subsequently released at the location of capture. Long-term survivorship was equally good. From 86 pink and chum salmon retained in ocean enclosures following analysis, only one died in the following 30 d.

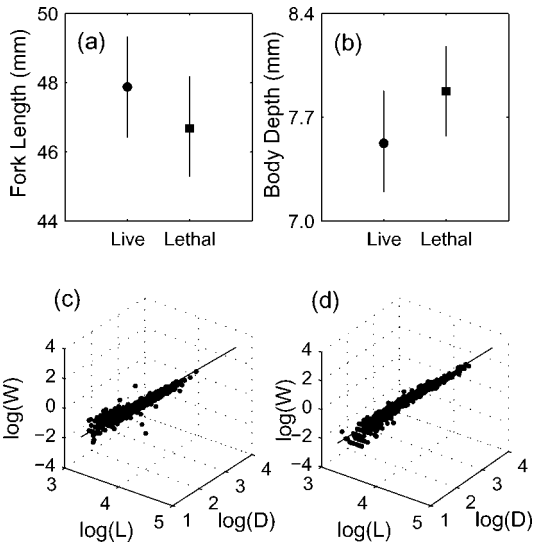

FIGURE 2.—Juvenile salmon morphometrics. Top panels: comparison of **(A)** mean fork length and **(B)** body depth between nonlethal (circles) and lethal (squares) sampling techniques. Error bars are bootstrapped 95% confidence intervals on the mean and sample sizes are 40 each. Bottom panels show linear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

Discussion

Both nonlethal and lethal sampling techniques produced similar estimates of louse stage abundances despite a bias to underdetect copepodid and chalimus lice in nonlethal samples. Measurement error can be attributed to a reduced detectability of small chalimus and copepodid lice, misidentification of louse stages, and reduced integrity of lethal samples. Similar abundance estimates were obtained because errors had a strong central tendency at zero (with only a slight negative skew), and error variability occurs at a lower scale than variability in the data (e.g., chalimus data showed the strongest bias and var = 5.89 for lethal chalimus counts, whereas var = 0.71 in the paired differences between live and lethal chalimus counts). Regression analyses indicated there was a bias to underestimate copepodids as copepodid abundances on sampled fish increased, but no corresponding bias was detected in counts of chalimus and motile lice. It is known that increasing noise in response variables (i.e., louse abundances) does not confound statistical analyses as it does for ex-

planatory variables (e.g., farm proximity, temperature, or salinity; Gustafson 2004). This leads us to conclude that the nonlethal technique provides a biologically viable data collection method for analyzing temporal and spatial patterns of louse population structure, but likely underestimates the true abundance of sea lice.

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

The sampling methods we developed only apply to the early marine life history stages of pink and chum salmons when they occupy nearshore habitats. As these fish grow in size they move into deeper waters inaccessible to a beach seine (Groot and Margolis 1991). Salmon susceptibility to the disease impacts of lice likely decreases with increasing body size and it is therefore the early marine stages that are most important for monitoring objectives. Fortunately the nearshore phase persists for 1–2 months allowing an application of these methods (Groot & Margolis 1991; M. Krkošek, personal observation).

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

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

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