Transactions of the American Fisheries Society 134:184–191, 2005

[Note]

A Nonlethal, Rapid Method for Assessing the Somatic Energy Content of Migrating Adult Pacific Salmon

GLENN T. CROSSIN*

Forest Sciences Department, Forest Sciences Centre, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

SCOTT G. HINCH

Forest Sciences Department and Institute for Resources, Environment, and Sustainability, Forest Sciences Centre, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

Abstract.—Traditional methods for determining the energy content of fish involve either chemical assays of lipid and protein levels (proximate analyses) or tissue combustion (bomb calorimetry). In studies of migrating Pacific salmon *Oncorhynchus* spp., entire fish need to be homogenized prior to analysis, as energy reserves are stored along a head-to-tail gradient and change along this gradient depending on the stage of upriver migration. The logistics and costs associated with transporting carcasses to the laboratory can be prohibitive. Also, many populations of Pacific salmon are at risk of extinction, so lethal sampling is often not an option. Few reliable and practical methods exist that enable rapid and nonlethal energy determinations of large fish in the field. We evaluated a handheld microwave energy meter as a means of estimating whole-body energy concentrations. In 2002 and 2003, we collected sockeye salmon *O. nerka* from several stocks during their coastal and upriver migration through the Fraser River watershed (British Columbia). For each stock, we sampled fish from various locales ranging from ocean to spawning areas. Fish somatic tissues were interrogated at four body positions; however, the two most anterior positions produced the most accurate energy information. It took less than 30 s per fish to collect these data. We found strong regression relationships between somatic lipid percentage ($R^2 = 0.93$; $P < 0.001$) and gross somatic energy density ($R^2 = 0.94$; $P < 0.001$) measured by whole-carcass proximate analyses and $\log_e$ transformed energy meter readings. The slopes and intercepts of these relationships did not differ among stocks or years.

Anadromous adult Pacific salmon *Oncorhynchus* spp. undertake remarkable migrations, traveling long distances from ocean feeding areas to freshwater spawning beds. Their upriver migrations are particularly fascinating, as they are powered exclusively with endogenous energy reserves accrued at sea. Thus, adult Pacific salmon provide an excellent model for studying energy use and allocation patterns and life history variation. Several recent studies (Hendry and Berg 1999; Kinnison et al. 2001, 2003; Crossin et al. 2003, 2004) have measured the energy content of Pacific salmon sampled at different locales along upriver migration routes as a means to assess how environmental factors (e.g., river distance, elevation gain, river velocity) influence patterns of energy use, metabolism, and fecundity. Similar studies of Atlantic salmon *Salmo salar* have also been conducted (Jonsson et al. 1991, 1997; Jonsson and Jonsson 2003).

The two standard methods for determining energy content of fish (and those used in the studies above) are proximate body constituent analyses and bomb calorimetry (Higgs et al. 1979). In proximate body constituent analyses, energy concentrations in a target tissue (e.g., soma, gonads) are derived from lipid, protein, water, and carbohydrate estimations. The bomb calorimetry method involves the combustion of tissue samples to derive its caloric content. Both methods provide reliable energetic estimates but have several limitations. In studies of migrating salmon, these methods necessitate killing fish to obtain the required tissues for analysis. The entire fish needs to be homogenized prior to analyses, as body constituents tend to be stored along a head-to-tail gradient (Herbinger and Friars 1991) and constituent levels change along this gradient depending on the stage and locale of upriver migration. Thus, using only sections of tissue in the analysis could result in bias. In addition, large numbers of salmon carcasses must be transported, often from remote locales, to the laboratory for processing. The logistics and costs associated with this can be prohibitive, and the homogenization of whole carcasses is a time-consuming and odious task. Moreover, many populations of Pacific salmon are at risk of extinction, so lethal sampling is often not an option. Techniques are needed for rapid, nonlethal in

* Corresponding author: crossin@interchange.ubc.ca

Received May 7, 2004; accepted July 27, 2004

situ assessments of somatic energy concentrations in migrating adult fish.

There are several techniques available for nondestructively assessing energy levels in fish. Total body electrical conductivity estimates somatic water concentrations (which is inversely related to lipid concentrations). Though allowing in situ measures, this approach has not been proven accurate (e.g., Brown et al. 1993; Lantry et al. 1999). Near-infrared spectroscopy measures the reflectance, interactance, and absorption of specific wavelengths that can be related to water, lipid, and protein concentrations. Though a promising technology (e.g., Downey 1996), equipment for this approach is expensive and not appropriate for field use. Computerized tomography (X-rays; Rye 1991) and nuclear magnetic resonance (Burgetz et al. 1998) approaches have also been used to examine lipids and other by-products of energy use in whole fish. However, these approaches are also expensive and the equipment is not designed for field use.

Microwave transmission is used widely in the finfish aquaculture industry for assessing the fat content of market fish. Handheld microwave meters are commercially available and relatively inexpensive, but there has been little evaluation of this tool in studies of wild, freely migrating fish. A recent study demonstrated that a microwave meter could be used to assess lipid levels in adult Chinook salmon *O. tshawytscha*; however, the authors (Colt and Shearer 2001) concluded that the accuracy of the device could resolve fish into only ''high'' or ''low'' lipid content categories. Their study had several design limitations that affected the power of their analyses and interpretations. Most notably, many of their measurements were taken from postspawned fish with energy levels near the lower detection limits of the meter. Additionally, sex ratios were skewed toward females.

We collected sockeye salmon *O. nerka* from several stocks at various locales along their coastal and upriver migration through the Fraser River watershed (British Columbia; Figure 1). We then compared lipid content of somatic tissues (determined by a handheld microwave energy meter) to lipid and somatic energy values from the same fish (determined through whole-carcass proximate analysis). Our objectives were to determine if this tool could provide accurate predictions across the range of somatic energy levels observed in wild, migrating adult salmon and, specifically, to develop a protocol and numerical models for rapid, accurate energy assessments of wild sockeye salmon. We also tested the robustness of these models among stocks, genders, and years.

Methods

Study system and animals.—We collected 171 (in 2002) and 54 (in 2003) adult sockeye salmon from 10 Fraser River stocks at several locations throughout coastal British Columbia and upriver areas of the Fraser River watershed (Figure 1). All stocks collected had approximately equal sex ratios in each year during their spawning migrations. Of these stocks, three were sampled in 2002 across their full migratory range, from marine to freshwater spawning environments. The Adams (n = 74), Horsefly (n = 25), and Early Stuart (n = 22) stocks traverse broad distances and elevation gradients to reach spawning areas, and all possess unique morphological and energetic traits that economize energy use in the face of difficult migrations (Crossin et al. 2004). Adams sockeye salmon migrate 484 km upriver and ascend 366 m in late summer, when river flows are relatively low and temperatures are high. Early Stuart and Horsefly sockeye salmon migrate considerably longer distances and to higher elevations in early summer under converse environmental conditions. Early Stuart sockeye salmon migrate 807 km upriver and ascend 762 m and Horsefly sockeye salmon migrate 1,100 km upriver and ascend 701 m. Fish were captured in (1) continental shelf waters at the entrance to Juan de Fuca Strait; (2) the Strait of Georgia as they approach the Fraser River mouth; (3) upstream of the Fraser River mouth at Whonnock, British Columbia (termed here ''river entry''); (4) the Thompson River at Ashcroft, British Columbia (Adams stock only); and (5) at their respective spawning grounds prior to spawning, Adams River and Stuart–Takla. Marine area fish were collected by Pacific Salmon Commission (PSC) purse seine and gill-net test-fishing vessels. River fish were collected by beach seine or dip net. Stock identification was determined by a combination of run timing, DNA (Beacham et al. 2004), and scale analyses (Gable and Cox-Rogers 1993) conducted by the PSC. At each site, 10 males and 10 females were collected from each stock.

Microwave device and energy measurements.—We used a Distell Model 692 Fish Fatmeter (Distell Inc., West Lothian, Scotland), termed the ''energy meter'' in our analyses. This cordless, handheld unit houses a microwave oscillator that emits a low-powered wave (frequency, 2 GHz ± 2,000 MHz; power, 2 mW) that interacts with water in the somatic tissues at a given location (Figure 2).

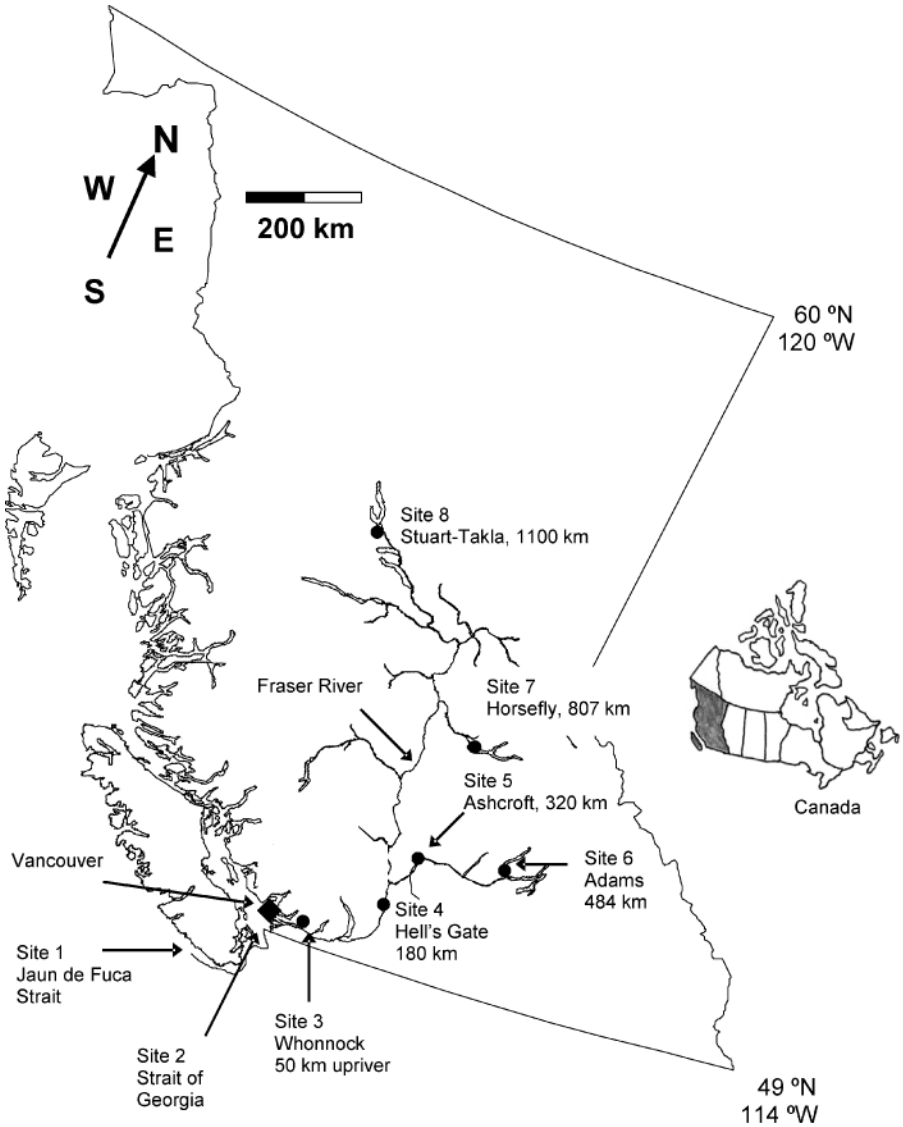

FIGURE 1.—Map of British Columbia indicating the sampling locations of the wild sockeye salmon used to assess somatic energy content.

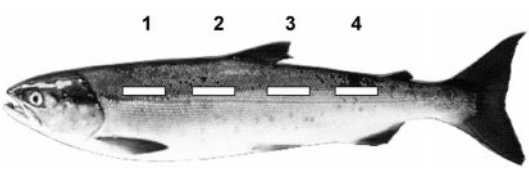

FIGURE 2.—Side-view drawing of a salmon that indicates the four positions at which energy meter readings were taken to assess the somatic energy content of wild sockeye salmon during their upriver migrations through the Fraser River watershed.

Drawing from the strong inverse relationship between the water and lipid content in fish tissues (Craig et al. 1978; Higgs et al. 1979), microwave sensors convert water concentration to estimates of lipid concentration. The energy meter contains four to six channels for estimating somatic lipids in different species (which can be chosen from the manufacturer). The manufacturer's species calibrations are preprogrammed in the meter's microprocessor and were derived through regressions of somatic lipid percentages determined through chemical methods (Foss-let method; e.g., Doman-

ski et al. 1974) against energy meter readings. However, the Foss-let method of lipid determination employed by the manufacturer only determined lipid concentrations in a muscular section of the fish; thus, the calibrations preprogrammed in the energy meter relates meter readings to the lipid content in a particular fillet section rather than for the whole animal. In ecological and energetic studies of salmon, it is common to estimate lipid and gross energy content in the entire fish (e.g., the somatic compartment) because migratory salmon draw on reserve energy stored not only in the muscle ''fillets,'' but also in other places, like the visceral cavity. Thus, whole fish (less gonads) tend to be homogenized, and subsamples of homogenized tissue are used in analyses. To relate energy meter readings to the whole somatic compartment of salmon in this study, we developed our own sockeye salmon calibration by relating energy meter readings to lipid and gross energy estimates derived from proximate constituent analyses in whole salmon (methods outlined below). We also felt the need to generate our own calibration, as we were unsure of the identity of the sockeye salmon (i.e., wild or hatchery) used in the manufacturer's calibration, as well as the range of lipid–water values covered in their analyses.

Upon capture, live sockeye salmon were weighed and measured for body length (postorbital-to-hypural length) and interrogated with the energy meter at four positions (Figure 2). Interrogation did not require anesthesia. Salmon were placed in a live-box cradle containing ambient, circulating water that submerged the fish to its lateral line and allowed water to cover the gills. One person held the fish, exposing its left side, while another swept the exposed dorsal surface with a wet hand to remove excess water. Following suggestions in Colt and Shearer (2001), the energy meter's sensor was placed along the dorsal surface at four positions just above the lateral line (Figure 2). Interrogation generally took less than 30 s. Salmon were then euthanized by concussion, sealed in airtight bags, and transported on ice to the laboratory for comparative proximate analyses. To determine which position (or positions) provided the best reflection of a whole salmon's somatic lipid and energy content, we regressed energy meter readings at each individual position (and at mean values calculated for all possible position combinations) against proximate somatic lipid and gross energy values.

Proximate analyses.—Carcasses (less gonads) were homogenized in an industrial food processor (Robot Coupe Blixer, Model BX6V). An approximately 250-g subsample of each homogenate was packed separately in airtight plastic freezer bags and stored at −20°C until analysis. We then examined 2 g of tissue for proximate constituency (lipids, protein, water, and ash) according to the methods outlined by Higgs et al. (1979). Body constituents were calculated as percentages (and derivatively as g/kg). Water content was determined by drying a 2-g sample of each homogenate at 100°C for 24 h. Carbohydrate (ash) was determined by combusting the dried sample at 600°C for 2 h. Water and ash content were calculated as percentages by wet mass. Lipid content was determined by wet mass through a chloroform: methanol (1:1) extraction of a 2-g subsample of tissue homogenate. Protein percentage was calculated as the difference between 100 and the summed water, ash, and lipid percentages. This indirect protein method has been employed in previous studies and has been found to be accurate and reliable (Berg et al. 1998; Hendry and Berg 1999; Hendry et al. 1999; Crossin et al. 2004). Protein and lipid percentages were converted to their energetic equivalents by multiplying percentages (by wet mass) by 0.02364 MJ/kg for protein and 0.03954 MJ/kg for lipid (Higgs et al. 1979). Converted lipid and protein values were then summed for gross somatic energy (MJ/kg).

Statistical analyses.—We used analysis of covariance (ANCOVA; SAS 1998) to adjust within-year, stock-specific lipid and gross energy estimates for differences in fish length, as in Hendry and Berg (1999). We found homogeneous slopes in each analysis ($P > 0.05$), thus least-squares means were used to generate stock-specific, length-corrected means and standard errors for use in subsequent analyses. Differences in lipid and gross energy estimates between males and females were examined though analysis of variance (ANOVA). This was done within stocks, locales, and years. Data were pooled if no significant differences between the sexes were found. The energy meter technical specifications indicate that when fish soma have extremely low energy levels (e.g., ~2.5% lipid or less), the meter's sensors may ''saturate.'' In practice, this means that energy meter readings do not show the same linear response to fish water content at low lipid levels as they do when lipid levels are modest to high. Colt and Shearer (2001) identified a logarithmic relationship between energy meter estimates and proximate lipid measures in adult Chinook salmon due to heterogeneity of energy meter data. We log-

normally transformed all energy meter data prior to analyses and were able to generate strong linear relationships between energy meter output and proximate energy data across the full range of meter values in our study. All proximate constituent data (lipid percentages and gross somatic energy) were normally distributed and, thus, were left untransformed.

Focusing just on the 2002 data, as the sample size in 2002 was much larger than that in 2003, we used linear regression to examine the relationships between lipids (%) and water (%), gross energy (MJ/kg) and lipid (%), and gross energy (MJ/kg) and water (%) for the intensively sampled stocks (Adams, Horsefly, and Early Stuart). Using ANCOVA, we compared the slopes and intercepts of the regressions among those stocks to determine if stock-specific differences existed in these body constituent relationships.

With the 2002 multistock data set, we used linear regression to examine relationships between mean energy meter readings from individual positions and combinations of multiple positions (averages of readings for two, three, and four positions) with somatic lipid (%) and gross somatic energy values (MJ/kg) derived through proximate constituent analyses. The purpose of these analyses was to evaluate which positions, or combinations of positions, generate the strongest predictive relationships and are best to use for rapid field applications of the energy meter. For each of the intensively sampled stocks, we separately regressed somatic lipid and gross energy on energy meter readings using data based on the ''best'' position, or positions, identified above, to evaluate whether stocks differed in these predictive relationships. Slopes and intercepts of the regressions were compared among stocks using ANCOVA. If no differences among stocks were identified, we compared the pooled multistock regression relationships from 2002 to the pooled multistock regression relationships from 2003 using ANCOVA.

Results

All proximate constituent data (lipid percentages and gross somatic energy) were normally distributed and thus were not transformed. Energy meter readings were $\log_e$ transformed as per Colt and Shearer (2001; see Methods). Within stocks and years, males and females did not differ in their somatic lipid, water, or gross energy levels at any locale during their migration (ANOVA, all $P > 0.05$), thus sexes were pooled for all subsequent analyses. There were no differences among stocks either in the slopes or intercepts of the regressions of lipid and water, gross energy and lipids, and gross energy and water (all $P > 0.05$). Because water content is so strongly related to lipid and gross energy content, water estimates can be used to predict these parameters. Pooled 2002 multistock data ($n = 171$) revealed strong negative correlations between lipids and water ($r = -0.96$; $P < 0.001$) and gross energy and water ($r = -0.99$; $P < 0.001$) and a strong positive correlation between gross energy and lipids ($r = 0.98$; $P < 0.001$; Figure 3).

We used the entire 2002 data set to evaluate which positions produced the strongest predictive relationships for lipids and gross energy. Regressions of somatic lipids and gross energy on lognormally transformed energy meter readings produced very strong coefficients of determination, regardless of whether single or multiple positions were used in obtaining energy meter readings (all $R^2 > 0.82$, $P < 0.001$; Table 1). The best coefficients were generated from regressions using the average of positions 1 and 2 (lipid, $R^2 = 0.93$; gross energy, $R^2 = 0.94$; Figure 4). Therefore, we used energy meter data based on the average of positions 1 and 2 for subsequent analyses. We found no differences in intercepts (ANCOVA; $n = 121$, $F = 408$, $P = 0.09$) for regressions of somatic lipid and gross energy on energy meter readings among the three intensively sampled stocks. We also found no differences in intercepts (ANCOVA; $n = 225$, $F = 429$, $P = 0.11$) for regressions of somatic lipid and gross energy on energy meter readings between pooled stocks collected in 2002 and 2003.

Discussion

Our results demonstrate that handheld microwave energy meters can be used to generate highly accurate measures of gross somatic energy and lipid level percentages in migratory Pacific salmon. This technology is effective because microwave meters can accurately estimate the water content of somatic tissues. In addition, strong proportional relationships between water, lipid, and gross somatic energy exist in fish, relationships that we confirmed in sockeye salmon and that have been reported in several other salmon energetic studies (e.g., Gilhousen 1980; Brett 1995; Hendry and Berg 1999; Kinnison et al. 2001; Crossin et al. 2003, 2004). As salmon migrate upriver, they draw predominantly on somatic lipid reserves to power migration and develop reproductive tissues. At the cellular level, the space generated by lipid catab-

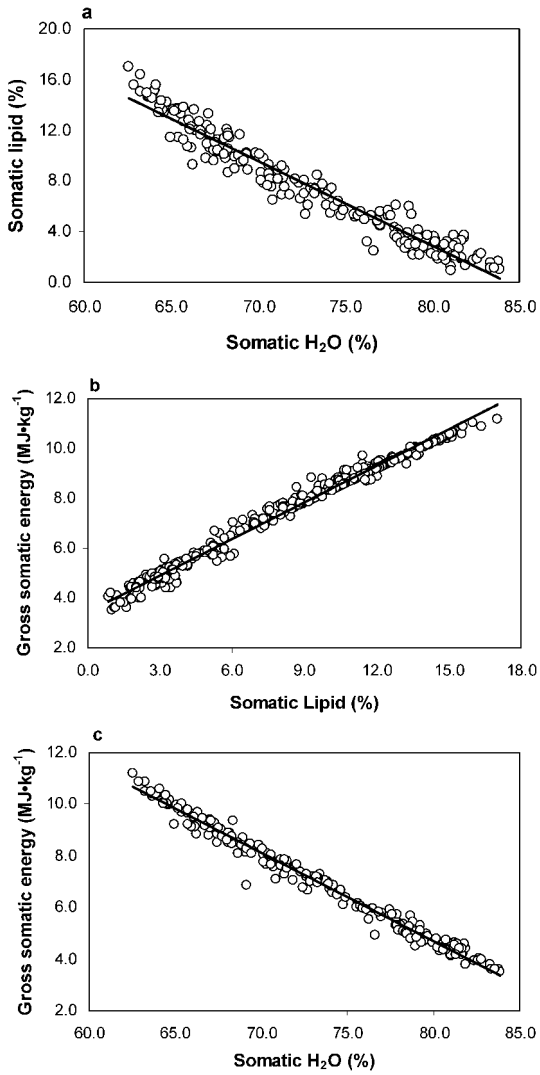

FIGURE 3.—Relationships between **(a)** percentage lipid and percentage water, **(b)** gross energy density and percentage lipid, and **(c)** gross energy density and percentage water for all sockeye salmon collected in 2002 from various locations in British Columbia to assess somatic energy content. Linear regression lines are presented for each panel.

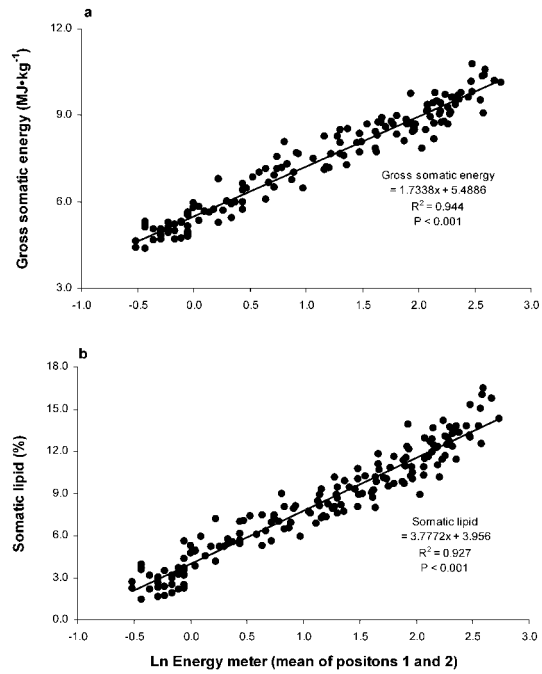

FIGURE 4.—Relationships of **(a)** gross somatic energy density and **(b)** percentage somatic lipid to $\log_e$ transformed energy meter readings (average of positions 1 and 2) based on all sockeye salmon collected in 2002 from various locations in British Columbia to assess somatic energy content. The linear regression lines, equations, coefficients of determination, and probability values are presented for each relationship.

olism is occupied by water, thus water levels rise in salmon as they migrate upriver. Lipid levels are usually near depletion by the time salmon reach their spawning grounds. Protein catabolism thus ensues to provide the necessary energy for final maturation, courtship, and spawning. Water again fills the space generated when proteins oxidize. Thus, energy meter estimates of gross somatic energy reflect the contribution of both lipid- and protein-derived water. Interestingly, the lipid and gross energy relationships we examined had nearly identical coefficients of determination, indicating that protein catabolism was a relatively small component of the total energy budget used by the sockeye salmon in this study.

Unlike calorimetry or proximate analyses, microwave energy meters enable a rapid assessment of energy states. We measured each fish at four positions, requiring approximately 30 s per fish. However, our results indicate that the strongest predictive relationships between gross energy density (or lipid percentage) and energy meter readings occurred at the average of two positions (positions 1 and 2). By taking only two readings, sampling time can be reduced to less than 15 s per fish. Because the handling time is so short, the energy meter can be used to estimate energy density on live salmon with negligible handling effects. Using a flow-through cradle to hold individual fish, we have used the energy meter to measure and release hundreds of sockeye salmon from

TABLE 1.—Coefficients of determination (R^2) from linear regressions of somatic lipid (%) and gross somatic energy (MJ/kg) versus $\log_e$ transformed energy meter readings from individual positions and averages of readings for two, three, and four positions. The entire 2002 multistock dataset ($n = 171$) is used. For all regressions, $P < 0.001$.

Position(s)	Fish position	Lipid	Gross energy
Individual positions	1	0.917	0.941
	2	0.919	0.934
	3	0.883	0.891
	4	0.821	0.840
Two positions	1, 2	0.927	0.944
	1, 3	0.910	0.931
	1, 4	0.919	0.940
	2, 3	0.915	0.927
	2, 4	0.915	0.929
	3, 4	0.880	0.890
Three positions	1, 2, 3	0.923	0.941
	1, 2, 4	0.922	0.942
	1, 3, 4	0.910	0.929
	2, 3, 4	0.912	0.924
Four positions	1, 2, 3, 4	0.924	0.940

sampling platforms aboard vessels and on streamsides (S. J. Cooke, University of British Columbia, unpublished data). Because this approach is noninvasive and relatively benign, it is appropriate for energy determinations on wild stocks that are endangered, and on fish that are to be used in experiments in which estimates of initial energy could help explain performance or success of fish throughout a trial (e.g., swim tunnel respirometry, radiotelemetry). Though their sample sizes were small, Colt and Shearer's (2001) preliminary studies showed that the microwaves emitted from the energy meter did not have negative effects on egg production in maturing salmon, on egg viability, or on the performance of radio transmitters and passive integrated transponders.

We have shown that the relationships between lipid percentage, gross energy density, and $\log_e$ transformed energy meter readings did not differ among stocks of sockeye salmon, despite the fact that these stocks differed considerably in size, shape, rate of migratory energy use, and initial energy densities (Crossin et al. 2004). Moreover, there were no differences in these relationships between years. These results suggest that our calibration relationships are broadly applicable among Fraser River sockeye salmon and may be applicable to the entire species. The manufacturer can preprogram this particular energy meter with lipid calibrations for some species. This enables the meter to estimate lipid percentages without the investigator needing to conduct proximate analyses, as we did, though investigators should bear in mind that the manufacturer's calibrations are derived from fillet cuts rather than from whole carcasses (see Methods). Calibrations for gross somatic energy are not produced by the manufacturer, so if this variable is important, investigators will have to generate the appropriate calibrations. If investigators cannot generate calibration relationships (because, for example, a stock or species is endangered or because the costs of proximate analyses are prohibitive) and the manufacturer cannot provide them, it may be reasonable to apply calibrations from closely related species. For example, our sockeye salmon calibrations accurately predicted the energy density of adult Fraser River coho salmon *O. kisutch* (G. Alexander, Forest Sciences Department, University of British Columbia, Vancouver, unpublished data).

According to the manufacturer's technical specifications, meter sensors begin to saturate when somatic energy densities are very low (~2.5% lipid) due to high levels of catabolic water accumulated in the tissues; thus, accuracy begins to deteriorate. This is one potential limitation of the microwave energy meter. Such low levels of energy are typical of spawning Pacific salmon (Hendry and Berg 1999; Crossin et al. 2004), thus caution needs to be exercised when using this type of meter to estimate lipid levels in spawning salmon. However, recent work shows some promise and utility in studies of spawning fish (Hendry and Beall 2004).

Acknowledgments

We extend thanks to John Colt, Steve Cooke, David Fulton, and Glenn Wagner for input on this manuscript. We are grateful to Vicki Maloney, Trevor Nowak, and Mark Whelly for laboratory assistance. Thanks also to Fisheries and Oceans Canada for support. Funding was provided by Fisheries and Oceans Canada and by Natural Sciences and Engineering Research Council of Canada Discovery and Strategic Grants. A stipend to G.T.C. was provided by the Strategic Grant and a partial international student tuition waiver was provided by the Faculty of Forestry, University of British Columbia.

References

Beacham, T. D., M. Lapointe, J. R. Candy, B. McIntosh, C. MacConnachie, A. Tabata, K. Kaukinen, L. Deng, K. Miller, and R. Withler. 2004. Stock identification of Fraser River sockeye salmon using microsatellites and major histocompatibility complex

variation. Transactions of the American Fisheries Society 133:1117–1137.

Berg, O. K., E. Thornæs, and G. Bremset. 1998. Energetics and survival of virgin and repeat spawning brown trout (*Salmo trutta*). Canadian Journal of Fisheries and Aquatic Sciences 55:47–53.

Brett, J. R. 1995. Energetics. Pages 3–68 *in* C. Groot, L. Margolis, and W. C. Clarke, editors. Physiological ecology of Pacific salmon. University of British Columbia Press, Vancouver.

Brown, M. L., D. L. Gatlin III, and B. R. Murphy. 1993. Nondestructive measurement of sunshine bass, *Morone chrysops* × *Morone saxatilis*, body composition using electrical conductivity. Aquaculture and Fisheries Management 24:2528–2592.

Burgetz, I. J., A. Rojas-Vargas, S. G. Hinch, and D. J. Randall. 1998. Initial recruitment of anaerobic metabolism during submaximal swimming in rainbow trout (*Oncorhynchus mykiss*). Journal of Experimental Biology 201:2711–2721.

Colt, J., and K. D. Shearer. 2001. Evaluation of the use of the Torry Fish Fatmeter to nonlethally estimate lipid in adult salmon. Report of Research to the U.S. Army Corps of Engineers, Portland District, Contract Report W66QKZ00805700, Seattle.

Craig, J. F., M. J. Kenley, and J. F. Talling. 1978. Comparative estimations of the energy content of fish tissue from bomb calorimetry, wet oxidation, and proximate analysis. Freshwater Biology 8:585–590.

Crossin, G. T., S. G. Hinch, A. P. Farrell, D. A. Higgs, A. G. Lotto, J. D. Oakes, and M. C. Healey. 2004. Energetics and morphology of sockeye salmon: effects of migratory distance and elevation. Journal of Fish Biology 65:1–23.

Crossin, G. T., S. G. Hinch, A. P. Farrell, M. P. Whelly, and M. C. Healey. 2003. Pink salmon migratory energetics: response to migratory difficulty and comparisons with sockeye salmon. Canadian Journal of Zoology 81:1986–1995.

Domanski, G., K. Aitsetmuller, and E. von Jan. 1974. Rapid determination of fat in herring: discussion of different methods—results obtained with a Foss-let apparatus. Zeitschrift für Lebensmittel-Untersuchung und -Forschung 154(3):129–134.

Downey, G. 1996. Noninvasive and nondestructive percutaneous analysis of farmed salmon flesh by near infrared spectroscopy. Food Chemistry 55:305–311.

Gable, J., and S. Cox-Rogers. 1993. Stock identification of Fraser River sockeye salmon: methodology and management application. Pacific Salmon Commission Technical Report No 5:1–36.

Gilhousen, P. 1980. Energy sources and expenditures in Fraser River sockeye salmon during their spawning migration. International Pacific Salmon Fisheries Commission Bulletin 22.

Hendry, A. P. and E. Beall. 2004. Energy use in spawning Atlantic salmon. Ecology of Freshwater Fish 13: 185–196.

Hendry, A. P., and O. K. Berg. 1999. Secondary sexual characters, energy use, senescence, and the cost of reproduction in sockeye salmon. Canadian Journal of Zoology 77:1663–1675.

Hendry, A. P., O. K. Berg, and T. P. Quinn. 1999. Condition dependence and adaptation-by-time: breeding date, life history, and energy allocation within a population of salmon. Oikos 85:99–514.

Herbinger, C. M. and G. W. Friars. 1991. Correlation between condition factor and total lipid content in Atlantic salmon, *Salmo salar* L., parr. Aquaculture and Fisheries Management 22:527–529.

Higgs, D. A., J. R. Markert, D. W. MacQuarrie, J. R. McBride, B. S. Dosanjh, C. Nichols, and G. Hoskins. 1979. Development of practical dry diets for coho salmon, *Oncorhynchus kisutch*, using poultry-by-product meal, feather meal, soybean meal, and rapeseed meal as major protein sources. Pages 191–218 *in* J. E. Halver and K. Tiews, editors. Finfish Nutrition and Fishfeed Technology. Heenemann Verlagsgesellschaft, Berlin.

Jonsson, N., L. P. Hansen, and B. Jonsson. 1991. Variation in age, size, and repeat spawning of adult Atlantic salmon in relation to river discharge. Journal of Animal Ecology 60:937–947.

Jonsson, N., and B. Jonsson. 2003. Energy allocation among developmental stages, age groups, and types of Atlantic salmon (*Salmo salar*) spawners. Canadian Journal of Fisheries and Aquatic Sciences 60: 506–516.

Jonsson, N., B. Jonsson, and L. P. Hansen. 1997. Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic salmon *Salmo salar*. Journal of Animal Ecology 66:425–436.

Kinnison, M. T., M. J. Unwin, A. P. Hendry, and T. P. Quinn. 2001. Migratory costs and the evolution of egg size and number in introduced and indigenous salmon stocks. Evolution 55:1656–1667.

Kinnison, M. T., M. J. Unwin, and T. P. Quinn. 2003. Migratory costs and contemporary evolution of reproductive allocation in male Chinook salmon. Journal of Evolutionary Biology 16:1257–1269.

Lantry, B. F., D. J. Stewart, P. S. Rand, and E. L. Mills. 1999. Evaluation of total-body electrical conductivity to estimate whole-body water content of yellow perch, *Perca flavescens*, and alewife, *Alosa pseudoharengus*. Fisheries Bulletin 97:71–79.

Rye, M. 1991. Prediction of carcass composition in Atlantic salmon by computerized tomography. Aquaculture 99:35–48.

SAS. 1988. SAS/STAT user's guide, release 6.03 edition. SAS Institute, Cary, North Carolina.