Rapid estimation of lipid content of immature chum salmon in the ocean with a handheld microwave meter

Toshiki Kaga[1], Shunpei Sato[1], Toru Nagasawa[1], Masa-aki Fukuwaka[2], Tetsuichi Nomura[3], and Shigehiko Urawa[1, 4]

[1]*National Salmon Resources Center, Fisheries Research Agency,*
2-2 Nakanoshima, Toyohira-ku, Sapporo 062-0922, Japan
[2]*Hokkaido National Fisheries Research Institute, Fisheries Research Agency,*
116 Katsurakoi, Kushiro, Hokkaido 085-0802, Japan
[3]*National Research Institute of Aquaculture, Fisheries Research Agency,*
2-2 Nakanoshima, Toyohira-ku, Sapporo 062-0922, Japan
[4]*North Pacific Anadromous Fish Commission, 502-889 West Pender Street, Vancouver, BC, V6C 3B2, Canada*

Abstract: Chemical analysis for determining the lipid or energy content of fish is often complex and time consuming, because this analysis requires transporting entire fish to a laboratory and homogenizing prior to analysis. Here, we evaluated a handheld microwave meter as an effective tool for the rapid estimation of the total lipid content in the muscle of immature chum salmon (*Oncorhynchus keta*). A total of 175 immature chum salmon caught in the Bering Sea were examined to compare the estimated lipid contents and response values of microwave meter. A regression relationship was obtained between the total lipid content of fish muscles measured by chemical analyses and microwave meter values. It took less than 30 second per one fish to examine by microwave meter during high-seas surveys. Rapid estimation of total lipid content using a microwave meter can enable us to collect huge number of data, and will permit increased precision in physiological study of high-seas chum salmon.

Keywords: chum salmon, microwave meter, total lipid content, rapid estimation

INTRODUCTION

Lipid plays an important role in providing and accumulating energy in the carnivorous fish like salmonids (Watanabe 1982; Weatherly and Gill 1987; Novotony and Beeman 1990). Lipid content in the body of many species of fish shows a wide variability in the annual cycle, affected by living conditions. Therefore lipid content is probably a reliable index of trophic status of fish (Shulman and Love 1999).

However, the lipid analysis requires a lengthy procedure like homogenizing the muscle prior to analysis, extracting lipid using chlorofolm and methanol, and determining the lipid content by weight. In the ecological and energetic studies of salmon, it is general to estimate lipid content or gross energy in the whole fish, because the body constituents tend to be stored unevenly throughout their body. It is necessary to transport the whole body to a laboratory in order to avoid bias in analysis. However, the transporting and homogenizing of the whole body is a time-consuming and unpleasant task.

Recently, microwave transmit method is used popularly in the finfish aquaculture manufacturing for estimating the total lipid content of commercial fish. Microwave meter can estimate the water content of tissue accurately. Strong negative relationship between the water content and total lipid content (Craig et al. 1978; Nomura et al. 2005) enables the microwave meter to generate highly accurate measures for total lipid content. There has been several examples of using microwave meter in studies of wild fish such as Chinook salmon (*Oncorhynchus tshawytscha*, Colt and Shearer 2001), brook trout (*Salvelinus fontinalis*, Cox and Hartman 2005), and sockeye salmon (*O. nerka*, Crossin and Hinch 2005). However, there were few examples of using this tool for high-seas chum salmon (*O. keta*). In addition, a conversion formula between total lipid contents and microwave meter values has not been evaluated yet for chum salmon.

We attempted to evaluate the conversion formula between microwave meter values and total lipid contents for chum salmon by comparing the microwave meter values and total lipid contents determined through chemical analysis. We aimed to determine if this tool could provide accurate predictions in chum salmon and also to propose the standard protocol for rapid total lipid estimation of high-seas chum salmon.

MATERIALS AND METHODS

Microwave Meter

We used a Distell Model FM-992 Fish Fat Meter (Distell Inc., West Lothian, Scotland) for rapid lipid measurement, termed as microwave meter in our study. The microwave meter measures the attenuation of microwave (2 GHz) energy in decibels (dB) responding to the amount of water in a sample and the meter converted the attenuation of microwave to the response values ranged from 0.1 (distilled water) to 99.9 (lard).

Conversion Formula for Microwave Meter

In order to evaluate the conversion formulae we used 175 immature chum salmon which were captured during the research survey of R/V *Hokko maru* in the Bering Sea during the summer of 2008. We measured the response values of these fish with microwave meter at three positions (Fig. 1). We also measured body length, body weight, age, gonad weight of the fish samples. Age of fish was determined by examination of scale patterns. "European" system was used for age designation. The samples consisted of 98 age 0.1, 72 age 0.2, and 5 age 0.3 fish. Tissues for chemical analysis were kept frozen at -30℃ until analysis. After thawing, a fillet of half body was removed from each fish and was homogenized in a food processor. Lipid was extracted from an approximately 5 g of each homogenate with chloroform/methanol (2:1 v/v) following the method outlined by Folch et al. (1957). Lipid extract was collected and solvent was evaporated with a rotary evaporator. Then the total lipid content was measured gravimetrically.

To determine whether three positions which were measured by the microwave meter were appropriate for application of rapid lipid estimation of microwave meter or not, the difference in lipid content between the mean for three positions and half body fillet. From six immature chum salmon (40-60 cm in fork length), three pieces of muscle from the different positions of fish body (Fig. 1) and a fillet of another side of half body were collected. Lipid contents in these samples were measured according to the method by Folch et al. (1957). The difference between total lipid contents of the half body fillet and average total lipid contents among three positions was tested with a t-test and the correlation between the total lipid content of the half body fillet and the average of total lipid contents at three positions was compared with a Pearson correlation coefficient test.

A non-linear relationship between microwave meter values and total lipid contents in fish were identified (Kent et al. 1993). The conversion formulae was obtained by the regression of the microwave meter values against total lipid contents of half body fillet determined through chemical analysis using non linear least squares method.

RESULT

Average of total lipid contents at three positions were highly correlated with the average lipid content of half body fillet (Table 1, $r = 0.98$, $p < 0.001$). No significant difference was found between the average of total lipid contents at three positions and total lipid contents of half body fillet (Table 1, $p = 0.68$). We determined these positions were appropriate for estimating the total lipid content of half body fillet by a microwave meter.

Significant positive correlation was obtained between total lipid contents of half body fillet and microwave meter values ($p < 0.01$). Regression formula was $Y = 1.993e^{0.032x}$ for immature chum salmon ($r^2 = 0.71$) (where Y: total lipid content determined through chemical analysis [%], x: microwave meter response values).

DISCUSSION

Our results suggest that microwave meter can be used as an effective tool for the rapid estimation of total lipid contents in chum salmon. Microwave meter can estimate the water content of tissue accurately. Strong negative relationship between the water content and total lipid content (Craig et al. 1978) enables the microwave meter to generate highly accurate measures for total lipid content. With decreasing water accompanied by high lipid content, the penetration depth of microwave energy into the flesh increased and reflections of power could occur at boundaries both between flesh and bone or flesh and whatever surface the fish is lying on (Kent 1990). It is possible that the dynamics of microwave is attributed to the variability of readings in the measurement of the response value of the sample which has high lipid content. According to the manufacture’s technical manual, a meter sensor begins to saturate when energy densities or lipid content (~2.5%) are very low due to high levels of catabolic water in the tissue (Crossin and Hinch 2005). Salmon just before spawning or during overwintering have low energy content, therefore, it is necessary to pay attention to examine such kind of fish.

Our results demonstrate that the strong relationship between the total lipid content of half body fillet and microwave meter values. Unlike chemical analysis, microwave meter enables us to assess the total lipid content rapidly. It requires approximately 30 second per one fish for measuring at three positions. Microwave meter can enable us to collect more number of data and increased data will permit increased precision in physiological studies. The trophic status of high-seas Pacific salmon is variable depending on their ocean habitats which related to climate changes. Thus it is important to continue the long-term trophic monitoring of Pacific salmon in order to understand their ocean survival, growth, and fecundity.

ACKNOWLEDGEMENT

We extend our thanks to the captain, officers, and crews of R/V *Hokko maru* for fish collections. We express our sincere appreciation to Y. Aoyama for support in proceeding of analysis. This study was supported by the Promotion Program for International Resources Surveys of the Fisheries Agency of Japan.

REFERENCES

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 Enginners, Portland District, Contrace Report W66QKZ00805700, Seattle.

Cox, M.K., and K.J. Hartman. 2005. Nonlethal estimation of proximate composition in fish. Can.

J. Fish. Aquat. Sci. 62: 269-275.

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., and S.G. Hinch. 2005. A non lethal, rapid method for assessing the somatic energy content of migrating adult Pacific Salmon. Trans. Amer. Fish. Soc. 134:184-191.

Folch, A.J., M. Lees, and G.H. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.

Kent, M. 1990. Hand-held instrument for fat/water determination in whole fish. Food Control 1: 47-53.

Kent, M., A. Lees, and A. Roger. 1993. Estimation of the fat content of minced meat using a portable microwave fat meter. Food Control 4: 222-225.

Nomura, T., S. Urawa, M. Kawana, S. Sato, T. Azumaya, M. Fukuwaka, K. Hida, A. Nakajima, T. Tojima, and N.D. Davis. 2005. Seasonal changes in the total lipid content of immature chum salmon in the Bering Sea during the summer and fall of 2002-2004. N. Pac. Anadr. Fish Comm. Doc.899: 27pp. (Available at http://www.npafc.org).

Novotony, J.F., and J.W. Beeman. 1990. Use of a fish health condition profile in assessing the health and condition of juvenile Chinook salmon. Prog. Fish-Cult. 52: 162-170.

Shulman, G.E., R.M. Love. 1999. Indicators of Fish Condition. In Advances in Marine Biology. The Biochemical Ecology of Marne Fishes. Edited by A.J. Southward, P.A. Tyler, and C.M. Young. Academic Press, London. pp. 205-220.

Watanabe, T. 1982. Lipid nutrition in fish. Comp. Biochem. Physiol. B. 73: 3-75.

Weatherley, A.H., and H.G. Gill. 1987. Protein, lipid and caloric contents. *In* The biology of fish growth. Academic Press. London. pp. 101-146.

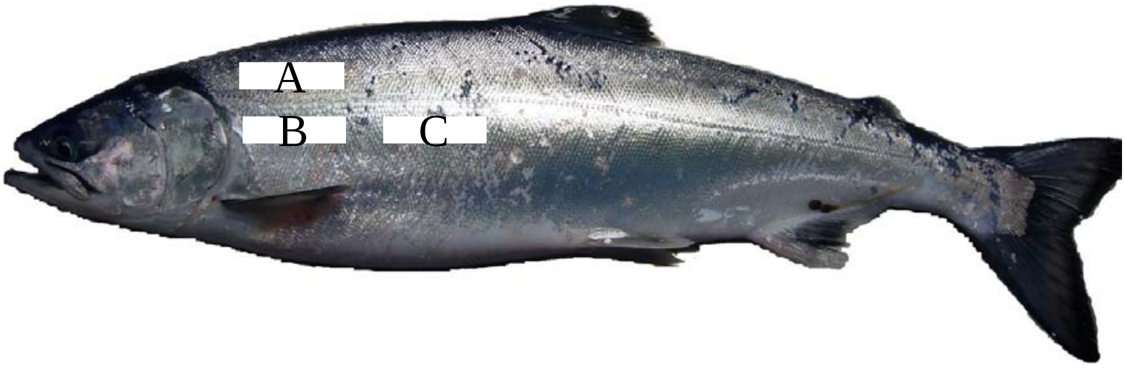

Fig. 1. Three positions which were measured by the microwave meter were taken to assess the total lipid content of immature chum salmon which was caught in the Bering Sea during the summer of 2008.

Table 1. Biological characteristics of the immature chum salmon and average lipid content of half body fillet and lipid contents (%) at different positions. Immature chum salmon were captured at the Bering Sea during the summer of 2008.

Ocean age	2	2	2	x	3	3
Fork length	448	461	464	506	530	501
Body weight	1020	1280	1050	1280	1760	1400
Position						
A	6.9	12.5	1.8	2.5	12.1	6.7
B	11.8	17.3	4.2	5.7	15.8	11.9
C	9.3	15.4	2.3	3.4	16.2	8.1
Average of half body fillet	11.0	19.8	2.6	4.9	15.3	9.7

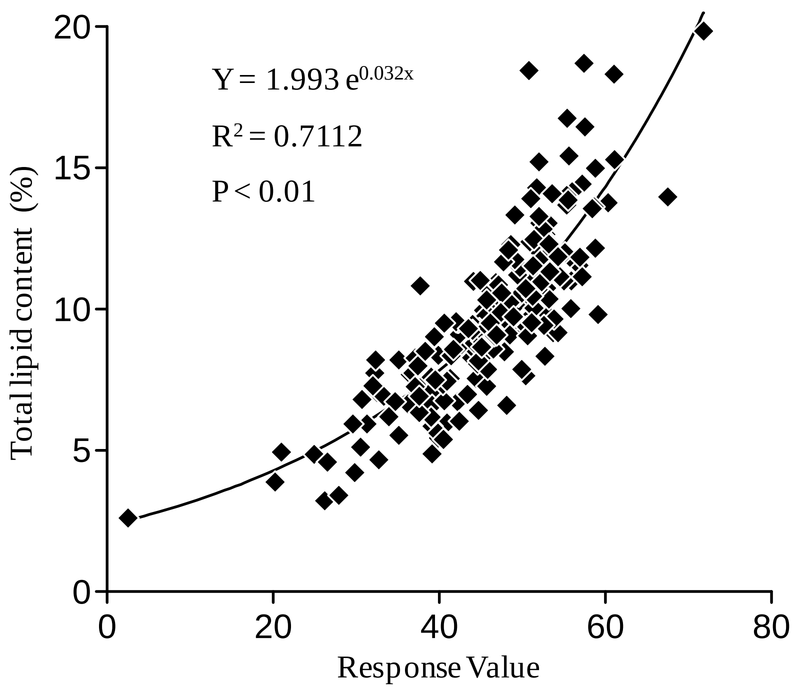

Fig. 2. Total lipid contents (% wet weight) versus response value of immature chum salmon which were caught in the Bering Sea during the summer of 2008. Lipid content was measured by Folch's method and response value were obtained by microwave meter.