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and the Bering Sea during the Summer of 2009**

by

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# Total Lipid Contents and RNA/DNA ratios of Sockeye, Chum and Pink Salmon in the North Pacific Ocean and the Bering Sea during the Summer of 2009

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## Abstract

This document reports the total lipid contents and RNA/DNA ratios of sockeye, chum and pink salmon which were caught in the North Pacific Ocean and the Bering Sea during the summer (June-July) of 2009. A total of 526 salmon which includes 106 sockeye, 180 chum and 240 pink salmon, was examined for total lipid contents. A total of 180 salmon which includes 51 sockeye, 74 chum and 55 pink salmon, was examined for RNA/DNA ratio. Older sockeye and chum salmon had higher total lipid content than younger fish. Total lipid content correlated positively with fork length in sockeye and chum salmon, but did not in pink salmon. RNA/DNA ratio negatively correlated with fork length in chum salmon. Comparing the RNA/DNA ratios in the present results with a past study, RNA/DNA ratios in 2009 was lower than that in 1994 and 1995. Total lipid content of chum salmon in 2009 was significantly higher than the mean total lipid content from 1998 to 2007, lipid storage state of chum salmon in 2009 was regarded as high. These biochemical parameters of pink salmon were higher than sockeye and chum salmon at the same ocean age. This difference in biochemical parameters among salmon species might reflect the difference in life history that the pink salmon had a shorter ocean life than sockeye and chum salmon.

## Introduction

Dietary lipids play an important role for fish in providing energy, including salmon, because of their limited ability to utilize carbohydrates as an energy source (Weatherly and Gill 1995). Although there have been a large number of lipid studies on cultured fish and artificial feed (Wilson 1991 etc.), few studies have determined lipid contents of high-seas salmon. Lipid content is useful to evaluate the trophic status and energy storage condition of high-seas salmonids. Protein constructs somatic body and RNA is an essential cellular component in the biosynthesis of proteins. On the other hand, DNA content is insensitive to environmental conditions, therefore RNA/DNA ratio is a useful indicator of protein synthesis rate (e.g. Bulow 1970; Buckley 1984; Houlihan et al. 1993).

Nomura et al. (2005) found that the neutral lipid content in the muscle of chum salmon (*Oncorhynchus keta*) during the summer and fall was higher than that in the spring or winter. It was also found that zooplankton biomass in the subarctic water is poor in winter (Nagasawa 2000; Parsons, Lalli 1988; Brodeur et al. 1996; Sugimoto, Tadokoro 1998). In addition, the lipid content of chum salmon was quite low in winter (Nomura et al. 2000, Kaga et al. 2006). Storing lipid in summer is necessary for salmonids to survive through the following severe winter.

This study reports the total lipid contents and RNA/DNA ratios of sockeye (*Oncorhynchus nerka*), chum and pink salmon (*Oncorhynchus gorbuscha*) which were caught in high-seas of the North Pacific Ocean and Bering Sea during the summer (June-July) of 2009.

## Materials and Methods

Salmon were caught in the North Pacific Ocean and the Bering Sea by a drift gillnet during the summer cruise of the R/V *Wakatake-maru*, June 3 to July 19, 2009 (Fig. 1). A total number of 526 salmon which includes 106 sockeye, 180 chum (53: North Pacific Ocean, 127: Bering Sea), and 240 pink salmon, were examined for total lipid content in the white muscle (Table 1). Biological data which included fork length, body weight, gonad weight, identification of sex and collection of scale samples were collected from each fish. Maturation state of sockeye and chum salmon were identified as immature and mature according to gonad weight following Takagi (1961). Only immature fish were used for later analysis because maturation state might have an influence on the condition of fish. Catch number of sockeye and pink salmon was small in the North Pacific Ocean, therefore we excluded sockeye and pink salmon which was caught in the North Pacific Ocean from later analysis. Total lipid contents were measured using a fish fat meter (Model 992 manufactured by Distell Inc., West Lothian, Scotland) on board ships according to the method described in Kaga et al. (2009). For RNA and DNA contents, liver tissues were collected from 23 sockeye, 52 chum (29: North Pacific Ocean, 23: Bering Sea), and 48 pink salmon (Table 2). Liver tissues were frozen at  $-40^{\circ}\text{C}$  immediately after the collection and stored at  $-80^{\circ}\text{C}$  in the laboratory until analysis. Liver tissue was homogenized in a 0.25 M sucrose–20 mM Tris–HCl–1 mM EDTA buffered solution at 7.5 pH. RNA and DNA contents were extracted from the homogenates and determined spectrophotometrically by the Schmit-Thanhauser-Schneider method, as modified by Nakano (1988).

Statistical significance in biological and biochemical parameters was tested among age groups within each species with one-way ANOVA. The differences in these parameters between North Pacific Ocean and the Bering Sea were tested with Student's t-test for unequal sample size. Pearson's product moment correlation was used for the relationship between biochemical parameters and fork length.

## Results

### Total Lipid Content

#### Sockeye Salmon

Average total lipid contents of sockeye salmon which was caught in the Bering Sea were  $4.07 \pm 0.30\%$  ( $n = 37$ , Min. 2.22, Max. 6.38) for age -.1,  $6.78 \pm 0.24\%$  ( $n = 66$ , Min. 2.77, Max. 14.07) for age -.2 and  $11.38 \pm 1.85\%$  ( $n = 3$ , Min. 6.51, Max. 15.12) for age -.3 (Table 1). Total lipid content positively correlated with fork length (Fig. 2,  $p < 0.001$ ,  $R^2 = 0.356$ ). Older fish had higher lipid content than younger fish (Fig. 3, ANOVA,  $F = 35.14$ ,  $p < 0.001$ ).

#### Chum Salmon

Average total lipid contents of chum salmon in the Bering Sea were  $4.30 \pm 2.30\%$  ( $n = 50$ , Min. 2.42, Max. 6.61) for age -.1,  $9.06 \pm 0.57\%$  ( $n = 20$ , Min. 6.13, Max. 14.67) for age -.2,  $12.42 \pm 0.47\%$  ( $n = 55$ , Min. 4.70, Max. 19.87) for age -.3, and  $10.49 \pm 2.81\%$  ( $n = 2$ , Min. 7.67, Max. 13.30) for age -.4 (Table 1). Total lipid content positively correlated with fork length (Fig. 2,  $p < 0.001$ ,  $R^2 = 0.599$ ). Older fish had higher total lipid content than younger fish except for age -.4 (Fig. 3, ANOVA,  $F = 77.82$ ,  $p < 0.001$ ). Average total lipid contents in the North Pacific Ocean were  $2.85 \pm 0.16\%$  ( $n = 28$ , Min. 1.75, Max. 5.47) for age -.1,  $5.93 \pm 0.64\%$  ( $n = 23$ , Min. 1.83, Max. 11.34) for age -.2, and  $6.09 \pm 0.92\%$  ( $n = 2$ , Min. 5.18, Max. 7.01) for age -.3 fish (Table 1). Total lipid content positively correlated with fork length (Fig. 2,  $p < 0.001$ ,  $R^2 = 0.331$ ). Age -.2 fish had significantly higher total lipid content than age -.1 fish (Fig. 3,  $p < 0.001$ ).

#### Pink Salmon

Pink salmon in the Bering Sea had higher total lipid content than sockeye and chum salmon at the same ocean age, i.e. -.1 (Table 1, ANOVA,  $p < 0.001$ ,  $F = 147.83$ ). Average total lipid content of pink salmon was  $8.73 \pm 0.15\%$  ( $n = 240$ , Min. 3.09, Max. 15.49). The variance of pink salmon's total lipid content seemed large, although they were same age. There was no significant correlation between fork length and total lipid content (Fig. 2,  $p > 0.05$ ).

### **RNA/DNA ratio**

#### **Sockeye Salmon**

Average RNA/DNA ratio of was  $3.48 \pm 0.15$  ( $n = 13$ , Min. 2.77, Max. 4.58) for age -.1 sockeye salmon and  $3.17 \pm 0.18$  ( $n = 10$ , Min. 2.40, Max. 4.21) for age -.2 sockeye salmon (Table 2). There was no significant correlation between fork length and RNA/DNA ratio (Fig. 2,  $p > 0.05$ ). Average RNA/DNA ratio of age -.2 fish was slightly lower than that of age -.1 fish, although no statistically significant difference (Fig. 3,  $p > 0.05$ ).

#### **Chum Salmon**

Average RNA/DNA ratios of chum salmon were  $4.28 \pm 0.14$  ( $n = 12$ , Min. 3.52, Max. 5.20) for age -.1,  $3.18 \pm 0.56$  ( $n = 3$ , Min. 2.18, Max. 4.13) for age -.2, and  $3.67 \pm 0.27$  ( $n = 8$ , Min. 2.71, Max. 4.55) for age -.3 fish (Table 2). Larger fish had lower RNA/DNA ratio than smaller fish (Fig. 2,  $p < 0.01$ ,  $R^2 = 0.293$ ). Age -.1 fish had higher RNA/DNA ratio than age -.2 fish (Fig. 3,  $p < 0.05$ ). Average RNA/DNA ratios of chum salmon in the North Pacific Ocean were  $3.02 \pm 0.10$  ( $n = 15$ , Min. 2.26, Max. 3.54) for age -.1,  $2.97 \pm 0.12$  ( $n = 13$ , Min. 2.33, Max. 3.79) for age -.2, and 2.53 ( $n=1$ ) for age -.3 fish (Table 2). There was no significant correlation between fork length and RNA/DNA ratio of chum salmon in North Pacific Ocean (Fig. 2). There was no significant difference in RNA/DNA ratio among age groups (Fig. 3).

#### **Pink Salmon**

Pink salmon had significantly higher RNA/DNA ratio compared with sockeye and chum salmon at the same ocean age, i.e. -.1 (Table 2, ANOVA,  $p < 0.001$ ,  $F = 22.20$ ). Average RNA/DNA ratio of pink salmon in the Bering Sea was  $5.53 \pm 0.17$  (Min. 3.86, Max. 8.17). There was no significant correlation between fork length and RNA/DNA ratio (Fig. 2,  $p > 0.05$ ).

## **Discussion**

In the present study, we examined total lipid contents and RNA/DNA ratios of Pacific salmon in high seas. Lipid is a primary material for energy storage and owns considerable potential as condition marker (Shulman, Love 1999). Protein constructs somatic body and RNA is the organizer of protein synthesis. DNA content remains relatively constant to changes in environmental conditions. Therefore, RNA/DNA ratio is a useful indicator of protein synthesis rate (e.g. Bulow 1970; Buckley 1984; Houlihan et al. 1993).

Azuma et al. (1998) examined the RNA/DNA ratios of salmonids in the North Pacific Ocean and the Bering Sea in June and July, 1994 and 1995. Results in the past study and present study were shown in Table 3. Average RNA/DNA ratios of sockeye, chum and pink salmon were higher in 1994 and 1995 than 2009. Pacific salmon have been at high levels of abundance since 1990s (Irvine et al. 2009). On the other hand, simultaneous decreases in growth rate, increases in age of maturation, and decreases in size at returning have been reported and it seems to be a density dependent effect (Ishida et al. 1993; Ida and Hayashizaki 1994; Kaeriyama 1989, 1998; Kaeriyama and Urawa 1992). Lower values of RNA/DNA ratios in 2009 than those in 1994 and 1995 might indicate that the nutritional limit could be taken place for salmonids in high-seas waters. However, further discussion would be necessary because no statistical comparison was done and single-year sample could have a large bias.

Our past results of total lipid content of chum salmon in the Bering Sea and present results were shown in Fig. 4 and Table 4 (Nomura et al. 2001, 2002, 2004, 2005; Kaga et al.

2007). Total lipid content of chum salmon fluctuated annually in the past. Fish in 2009 had higher total lipid content than the mean lipid content during 1998 and 2007 at the same age except for age -4 ( $p < 0.001$ ). Especially, total lipid content of age -2 fish was recovered in 2009 after 2002 to 2007 when their total lipid content had been low. Total lipid contents of older fish had larger variance than younger fish. Older fish includes various individuals which had different life histories, this characteristics would make it difficult to analyze the nutritional status of pacific salmonids.

Biochemical parameters of pink salmon were obviously higher than sockeye and chum salmon at the same age, i.e. -1. Pink salmon return to their natal river after one-year ocean life, therefore they must achieve higher growth rate and accumulate their lipid content for spawning during the short period. Our biochemical results may reflect such biological characteristics of pink salmon. Asian pink salmon stocks have a tendency for odd/even year fluctuation in the abundance (Takagi et al. 1981). Chum salmon changes their prey items according to the abundance of pink salmon (Tadokoro et al. 1996). Prey availability for chum salmon could be different between odd and even year. It might be necessary to examine the biochemical parameters of these salmon species in even year.

### Acknowledgements

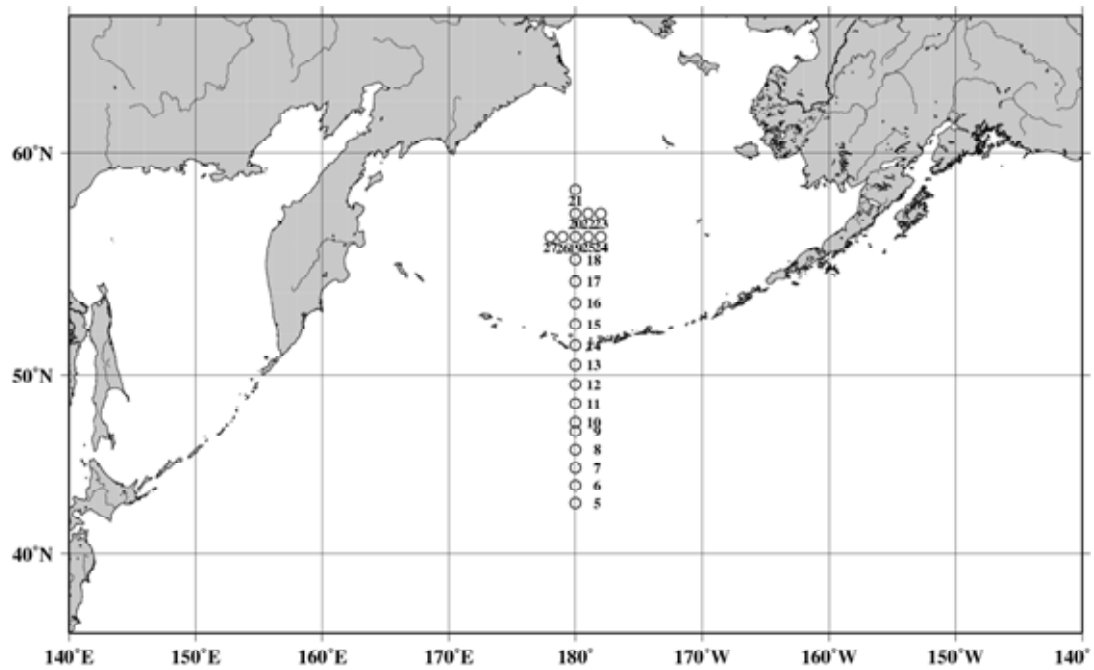
We express our appreciation to the captains, officers, and crew of the R/Vs *Wakatake-maru* and *Kaiyo-maru* for collecting samples during the research cruises.

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**Fig. 1.** Locations of fishing stations for sockeye, chum and pink salmon during the summer research cruises of R/V *Wakatake-maru* in June and July 2009. Surface drift gillnet was used at stations from 5 to 10 in the North Pacific Ocean and at stations 18 to 29 at the Bering.



**Table 1.** Biological and biochemical parameters (mean and standard error in parentheses) of sockeye, chum, and pink salmon examined for total lipid content.

Species	Location	Ocean Age	Number of Fish	Fork Length (mm)	Body Weight (g)	Condition Factor	Lipid(%)
Sockeye Salmon	Bering Sea	1	37	301(4.3)	302(14)	10.77(0.16)	4.07(0.30)
		2	66	469(3.3)	1205(27)	11.59(0.09)	6.78(0.24)
		3	3	546(18.1)	2050(207)	12.50(0.17)	11.38(1.85)
Chum Salmon	North Pacific Ocean	1	28	326(2.9)	381(9)	10.97(0.13)	2.85(0.16)
		2	23	441(3.7)	993(29)	11.55(0.11)	5.93(0.64)
		3	2	494(23.5)	1430(180)	11.85(0.19)	6.09(0.92)
	Bering Sea	1	50	357(4.0)	516(17)	11.19(0.11)	4.30(0.23)
2		20	484(6.6)	1392(62)	12.20(0.20)	9.06(0.57)	
3		55	518(3.9)	1751(39)	12.57(0.18)	12.42(0.47)	
Pink Salmon	Bering Sea	4	2	561(10.5)	2255(155)	12.78(0.16)	10.49(2.81)
Pink Salmon	Bering Sea	1	240	453(2.3)	1204(21)	12.67(0.06)	8.73(0.15)

**Table 2.** Biological and biochemical parameters (mean and standard error in parentheses) of sockeye, chum and pink salmon examined for RNA/DNA ratio.

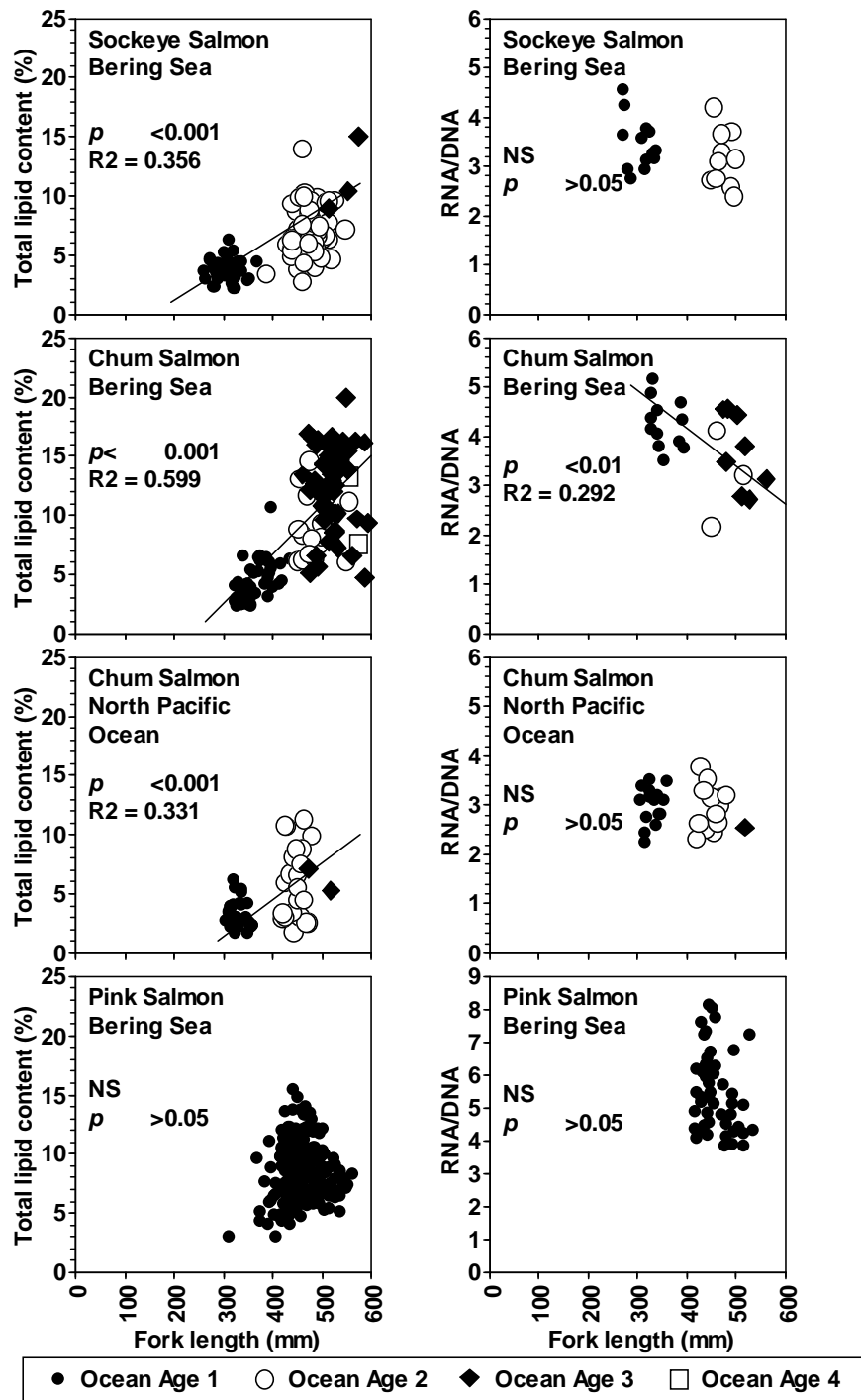
Species	Location	Ocean Age	Number of Fish	Fork Length (mm)	Body Weight (g)	Condition Factor	RNA/DNA
Sockeye Salmon	Bering Sea	1	13	301(7.0)	290(23)	10.31(0.27)	3.48(0.15)
		2	10	470(5.8)	1220(43)	11.71(0.16)	3.17(0.18)
Chum Salmon	North Pacific Ocean	1	15	326(4.3)	375(15)	10.81(0.19)	3.02(0.10)
		2	13	444(5.3)	1019(42)	11.55(0.15)	2.97(0.12)
		3	1	517	1610	11.65	2.53
	Bering Sea	1	12	350(8.1)	499(33)	11.54(0.19)	4.28(0.14)
2		3	471(20.0)	1277(163)	12.12(0.23)	3.18(0.56)	
3		8	506(10.1)	1641(92)	12.61(0.40)	3.67(0.27)	
Pink Salmon	Bering Sea	1	48	455(4.6)	1233(42)	12.92(0.14)	5.53(0.17)

**Table 3.** Biological parameters and RNA/DNA ratios (mean and standard error in parentheses) of sockeye, chum and pink salmon which were caught in the North Pacific Ocean and the Bering Sea in June and July in 1994, 1995 and 2009. Data in 1994 and 1995 were cited from Azuma et al. (1998).

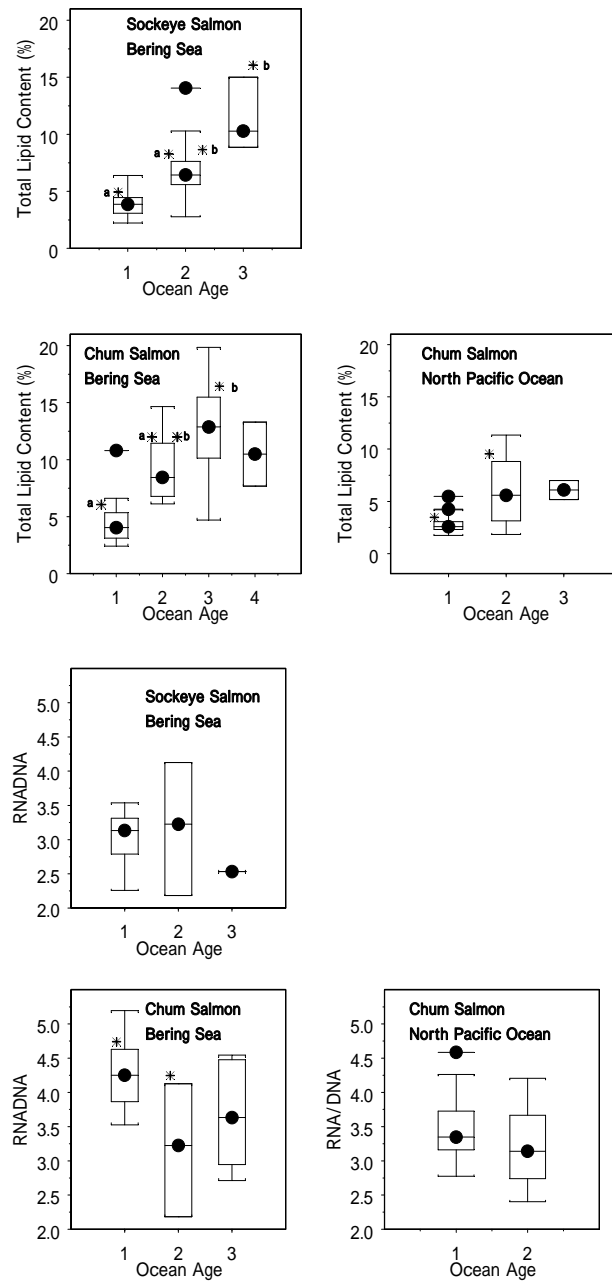
Species	Date	Number of Fish	Fork Length (mm)	Body Weight (g)	Condition Factor	RNA:DNA
Chum Salmon	June & July in 1994 & 1995	53	524(8.2)	1866(86)	12.39(0.16)	5.18(0.15)
	June & July in 2009	74	456(13.2)	1430(131)	12.07(0.15)	3.75(0.14)
Sockeye Salmon	June & July in 1994 & 1995	46	501(8.5)	1633(105)	12.34(0.19)	5.27(0.18)
	June & July in 2009	51	491(19.0)	1875(186)	12.23(0.22)	3.51(0.13)
Pink Salmon	June & July in 1994 & 1995	56	468(4.1)	1297(39)	12.51(0.15)	6.16(0.20)
	June & July in 2009	55	452(4.2)	1192(40)	12.69(0.15)	5.46(0.16)

**Table 4.** Biological parameters and total lipid contents (mean and standard error in parentheses) of chum salmon in the Bering Sea in June and July from 1998 to 2009.

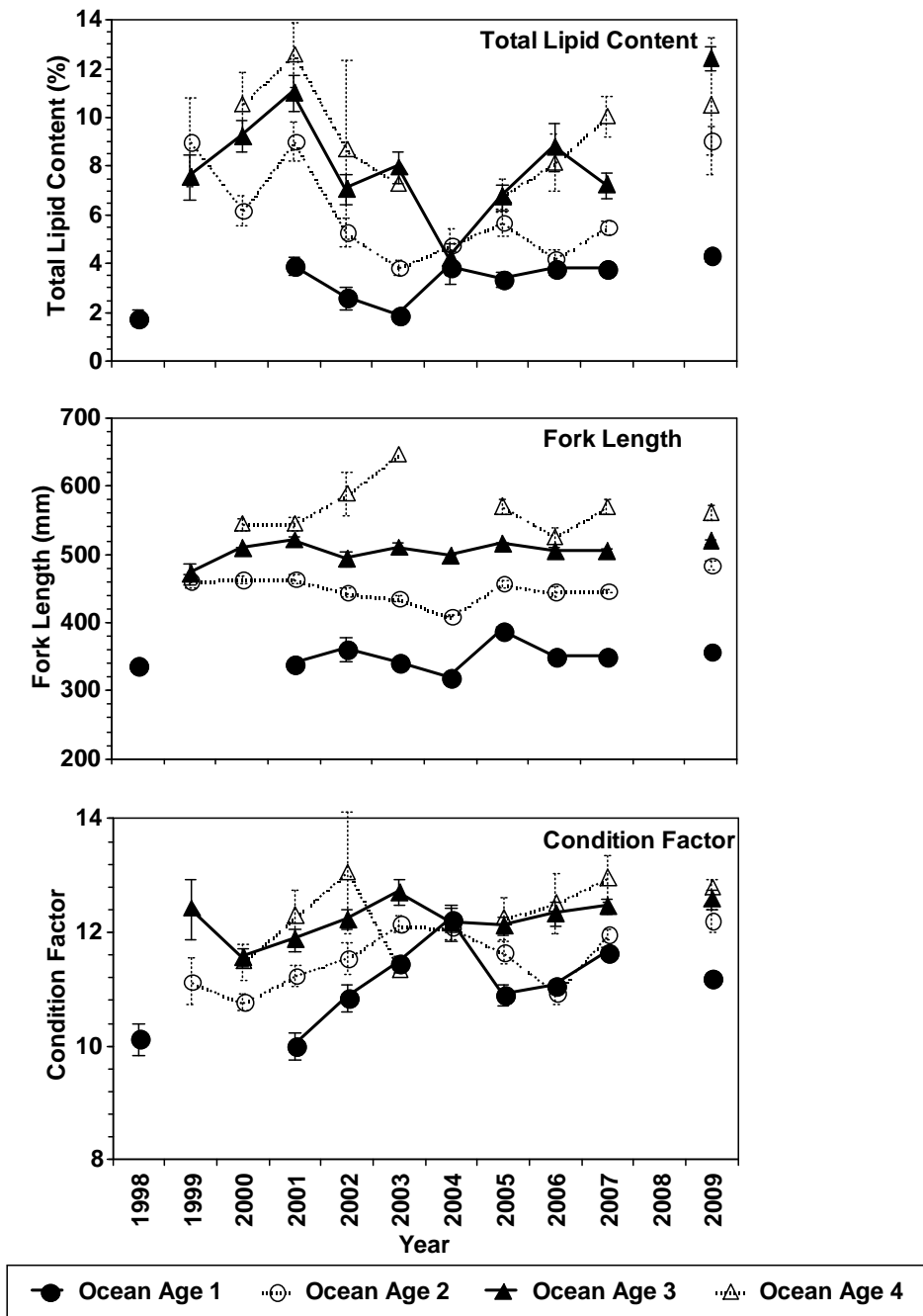
Research Vessel	Date	Ocean Age	Number of Fish	Fork Length (mm)	Body Weight (g)	Condition Factor	Lipid(%)
R/V Wakatake	June & July in 1998	1	10	335(2.3)	380(5.5)	10.12(0.28)	1.75(0.34)
R/V Wakatake	June & July in 1999	2	5	460(10.1)	1088(81.9)	11.13(0.41)	8.99(1.83)
		3	5	470(15.8)	1316(156.9)	12.41(0.53)	7.54(0.95)
R/V Wakatake	June & July in 2000	2	28	463(5.6)	1079(38.2)	10.77(0.15)	6.19(0.62)
		3	39	507(4.1)	1526(52.6)	11.55(0.16)	9.21(0.65)
		4	18	544(8.6)	1862(97.2)	11.48(0.32)	10.54(1.31)
		1	25	339(3.5)	390(13.1)	9.99(0.23)	3.88(0.37)
R/V Wakatake	June & July in 2001	2	33	464(6.0)	1141(48.7)	11.23(0.19)	9.02(0.83)
		3	30	520(6.6)	1702(71.6)	11.86(0.20)	10.99(0.74)
		4	8	545(9.9)	2009(141.5)	12.29(0.46)	12.57(1.31)
		1	18	360(18.5)	600(134.6)	10.83(0.24)	2.58(0.46)
R/V Kaiyo	June & July in 2002	2	25	444(7.6)	1032(57.5)	11.54(0.27)	5.28(0.57)
		3	20	493(11.2)	1509(101.5)	12.22(0.17)	7.04(0.62)
		4	2	588(32.0)	2713(653.0)	13.05(1.07)	8.67(3.67)
		1	43	341(2.7)	456(11.9)	11.44(0.14)	1.88(0.15)
R/V Kaiyo	June & July in 2003	2	68	435(3.8)	1013(29.5)	12.14(0.15)	3.83(0.32)
		3	29	509(8.2)	1712(71.3)	12.70(0.23)	7.94(0.66)
		4	1	646	3056	11.34	7.27
		1	10	319(2.1)	396(10.5)	12.22(0.21)	3.83(0.69)
R/V Kaiyo	June & July in 2004	2	10	409(1.2)	829(14.6)	12.08(0.20)	4.75(0.69)
		3	10	497(2.8)	1493(50.2)	12.16(0.32)	4.19(0.65)
		1	21	387(5.3)	634(24.2)	10.89(0.19)	3.33(0.32)
R/V Wakatake	June & July in 2005	2	23	457(5.5)	1126(50.6)	11.64(0.19)	5.68(0.58)
		3	47	514(3.7)	1648(38.2)	12.11(0.16)	6.71(0.52)
		4	9	570(10.3)	2279(109.9)	12.25(0.37)	6.78(0.67)
		1	38	349(4.1)	474(15.7)	11.05(0.10)	3.75(0.23)
R/V Wakatake	June & July in 2006	2	29	445(6.7)	978(43.9)	10.93(0.21)	4.21(0.38)
		3	17	504(6.3)	1588(65.8)	12.32(0.20)	8.75(0.98)
		4	7	525(13.8)	1843(169.7)	12.50(0.52)	8.13(1.17)
		1	91	349(2.2)	499(9.5)	11.64(0.10)	3.75(0.18)
R/V Wakatake & R/V Hokko	June & July in 2007	2	100	446(2.9)	1073(22.3)	11.96(0.10)	5.49(0.27)
		3	60	503(3.9)	1605(39.3)	12.46(0.13)	7.22(0.53)
		4	9	569(11.4)	2390(130.2)	12.95(0.41)	10.05(0.84)
		1	50	357(4.0)	516(17)	11.19(0.11)	4.30(0.23)
R/V Wakatake	June & July in 2009	2	20	484(6.6)	1392(62)	12.20(0.20)	9.06(0.57)
		3	55	518(3.9)	1751(39)	12.57(0.18)	12.42(0.47)
		4	2	561(10.5)	2255(155)	12.78(0.16)	10.49(2.81)



**Fig. 2.** Relationships between total lipid content, RNA/DNA ratio, and fork length for sockeye, chum and pink salmon by locations and by ocean ages.



**Fig. 3.** Box plots of total lipid contents (upper) and RNA/DNA ratios (lower) of sockeye and chum salmon by ocean ages and by locations. \*:  $p < 0.05$ .



**Fig. 4.** Mean total lipid content, fork length, and condition factor of each ocean age of chum salmon in the Bering Sea during the summer of 1998–2009 (cited from Nomura et al. 2001, 2005, and the present study). Error bars mean standard error.