Biochemical Approach to Assessing Growth Characteristics in Salmonids

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Growth characteristics were examined for pink, *Oncorhynchus gorbusha*, chum, *O. keta*, and sockeye salmon, *O. nerka*, caught in the Okhotsk Sea, North Pacific, and the Bering Sea from biochemical aspects. Adult pink, chum, and sockeye salmon had higher condition factor, protein content, and RNA:DNA and protein:DNA ratios than the juveniles. In adult fishes, while both RNA:DNA and protein:DNA ratios increased with condition factor commonly in the three species, positive correlations of both RNA:DNA and protein:DNA with fork length were seen only in chum salmon. In contrast with adults, juvenile chum salmon exhibited negative correlations of both RNA:DNA and RNA:protein ratios with fork length, and the RNA:DNA ratio decreases with triacylglycerol content. These correlations suggest that juvenile chum salmon build up lipid reserves at the expense of protein synthesis just prior to the onset of winter. However, in juvenile pink salmon alternative strategy was shown in which continued rapid growth is favored. These results suggest that Pacific salmon adopt species-specific strategies for allocating resources for somatic growth, and hence exhibit varying growth characteristics as measured by biochemical parameters that depend on species and life history stage during their years at sea.

INTRODUCTION

In the 1990's sockeye, *Oncorhynchus nerka*, chum, *O. keta*, and pink salmon, *O. gorbuscha* in the North Pacific have been at high levels of abundance due a combination of environmental changes favorable to salmon (Beamish and Bouillon 1993; Miller et al. 1994; Francis and Hare 1994), and an increase in hatchery production by Pacific Rim countries. However, these increases have been accompanied by increases in age of maturation and decreases in size at age, especially for chum salmon originating from Japan and Russia (Ishida et al. 1993; Ida and Hayashizaki 1994). These trends suggest that density dependent decreases in growth rate and an overall decline in nutrition may be taking place in the North Pacific. Additionally, since the different species of Pacific salmon are known to frequently occupy common feeding areas (Mishima et al 1966; Mishima and Shimazaki 1969; Takagi 1971 and 1996; Brodeur 1989; Azuma 1991; Perry et al. 1996), interspecific relationships associated with their growth condition also deserve further investigation. It is important, therefore, to clarify species specific growth and physiological condition, so that we can have an improved qualitative assessment of the salmon stocks.

Biochemical approaches for estimating growth characteristics have been adopted for many fish species including salmonids since the 1980's (Nakano et al. 1985: Fukuda et al. 1986; Houlihan et al. 1993; Shearer et al. 1994). However, for Pacific salmon in the marine stages of their life history, the biochemical parameters associated with growth are poorly understood. The purpose of this study is to assess the growth characteristics of Pacific salmon through examining triacylglycerol and protein content, and nucleic acid ratios.

MATERIALS AND METHODS

A total of 653 salmon (363 pink, 227 chum, and 63 sockeye) were examined in this study. They were caught using gillnets and surface trawls in the Okhotsk Sea and the eastern North Pacific Ocean in the fall of 1993 by the *Wakashio maru* and the *Kaiyo maru*, gillnets and longlines in the Bering Sea in the summer of 1994 by the *Wakatake maru*, and gillnets in the central North Pacific Ocean in the summers of 1994 and 1995 by the *Hokko maru* and the *Wakatake maru*.

In this study, pink, chum, and sockeye salmon caught from the Okhotsk Sea and western North Pacific in the fall are defined as juveniles, while those from the Bering Sea and central North Pacific in the summer of 1994 and 1995 are called adults.

Processing of salmon at sea included measurement of fork length, body weight, gonad weight, identification of sex, and collection of scale samples. Whole fish were frozen at -40°C immediately following ship board processing and stored at -80°C in the laboratory until analysis.

Using white muscle tissue taken from the lateral part just beneath the dorsal fin of each fish, we measured triacylglycerol, protein, RNA and DNA contents, and then calculated RNA:DNA, RNA:protein, and protein:DNA ratios individually. Triacylglycerol was extracted from the muscle with ethanol-ether solution (3:1, v/v) and estimated by the GPO-p-chlorophenol method using Wako's kit which includes standard solution (Triglyceride G-Test Wako, Wako Ltd). Protein content was determined by the method of Lowry et al. (1951) on a white muscle homogenate buffered in a 0.25 M sucrose -20mM Tris -HCl -1mM EDTA solution at 7.5 pH, using bovine serum albumin as a standard. RNA and DNA were extracted from the same buffered homogenate and determined spectrophotometrically by the Schmit-Thanhauser-Schneider method, as modified by Nakano (1988), using both ibonucleic acid from yeast and deoxyribonucleic acid extracted from salmon sperm as standards. Nucleic acid contents published by Azuma (1995) for juvenile salmon collected from Okhotsk Sea in 1993 were re-estimated according to the renewed assay system. Determinations of triacylglycerol and protein contents were expressed as μg per mg of wet weight of white muscle tissue.

Data were grouped by species and by the life history stage mentioned above. Statistically significant differences for biological and biochemical parameters among seasonal and life history groups within each species were determined with the Student's t-test for unequal sample size; and statistically significant correlations between biochemical parameters, and both fork length, and condition factor (CF, $CF=BW(g)*FL(mm)^{-3}*10^{6}$) were determined with the F-test. When there was ten percent or higher coefficient of determination in significant correlation between any two parameters, the regression line was drawn with a solid line in the relevant figure. When significant correlations with coefficient of determination was less than 10 %, the regression line was expressed with a broken line.

RESULTS

Pink Salmon

Juveniles captured in November were larger ($P<0.05$) but thinner ($P<0.05$) than those in October (Table 1). November - caught fish also exhibited much higher values in protein content ($P<0.0001$)

Table 1. Biological characteristics of pink, chum, and sockeye salmon examined. The values indicate the mean and (in brackets) one standard error.

Species	Stage	Location	Date	Number of Fish	Fork Length (mm)	Body Weight (g)	Condition Factor
Pink Salmon	Juvenile	Okhotsk & Western North Pacific	Oct., 1993	135	239(1.3)	142(2.7)	10.25(0.06)
			Nov., 1993	172	244(1.3)	149(2.7)	10.07(0.06)
	Adult	Bering Sea & North Pacific	June & July in 1994 & 1995	56	468(4.1)	1297(38.8)	12.51(0.15)
Chum Salmon	Juvenile	Okhotsk & Western North Pacific	Aug. & Sep., 1993	25	215(5.0)	119(9.0)	11.42(0.18)
			Oct., 1993	79	245(3.1)	172(6.6)	11.23(0.08)
			Nov., 1993	70	234(2.4)	136(4.2)	10.40(0.09)
	Adult	Bering Sea & North Pacific	June & July in 1994 & 1995	53	524(8.2)	1866(85.7)	12.39(0.16)
Sockeye Salmon	Juvenile	Okhotsk & Western North Pacific	Oct. & Nov., 1993	17	262(7.8)	224(25.1)	11.87(0.21)
	Adult	Bering Sea & North Pacific	June & July in 1994 & 1995	46	501(8.5)	1633(105.0)	12.34(0.19)

and protein:DNA ratio ($P<0.0001$),while exhibited much lower values in RNA:protein ratio ($P<0.0001$) and triacylglycerol content ($P<0.001$) than those in October (Table 2). Adult pink showed even higher values in condition factor ($P<0.0001$), triacylglycerol content ($P<0.005$), protein content ($P<0.0001$), RNA:DNA ratio ($P<0.0001$), and protein:DNA ratio ($P<0.005$) compared with juveniles (Tables 1 and 2). The RNA:protein ratio of adult pink was intermediate between the two juvenile groups (Table 2).

Chum Salmon

Juveniles captured in November were smaller than those in October ($P<0.005$ in fork length and $P<0.0001$ in body weight), although they were larger than those captured in late August to early September ($P<0.005$), although the latter had the highest CF of the three juvenile groups (Table 1). Condition factor of adult chum was higher than for juveniles, similar pink salmon ($P<0.0005$). Triacylglycerol content of juveniles in October was highest but did not differ from that of adult chum significantly (Table 2). Protein content of juveniles decreased with season but again rose in adult stage ($P<0.0001$) (Table 2). RNA:DNA ratio was highest in adult chum, while RNA:protein ratio was highest in juveniles captured in early fall (Table 2). Protein:DNA was higher in adults than in any juvenile groups ($P<0.001$).

Sockeye Salmon

No significant difference was shown in condition factor between juveniles and adults (Table 1). Triacylglycerol and protein contents, RNA:DNA and protein:DNA ratios were much higher in adults than juveniles, while RNA:protein ratio was higher in juveniles than adults ($P<0.005$) (Table 2).

Table 2. Biochemical characteristics of dorsal white muscle of pink, chum, and sockeye salmon examined (means and, in parentheses, standard errors).

Species	Stage	Location	Date	Number of Fish	Triacylglycerol (%)	Protein(%)	RNA:DNA	(RNA:Protein) $\cdot 10^3$	Protein: DNA
Pink Salmon	Juvenile	Okhotsk & Western North Pacific	Oct., 1993	135	++0.75(0.05)	+13.6(0.18)	4.84(0.09)	+29.9(0.65)	+174.8(5.4)
			Nov., 1993	172	0.51(0.03)	15.6(0.07)	4.84(0.08)	21.5(0.19)	226.6(3.4)
	Adult	Bering Sea & North Pacific	June & July in 1994 & 1995	56	0.96(0.10)	16.3(0.16)	6.16(0.20)	24.8(0.43)	249.9(7.9)
Chum Salmon	Juvenile	Okhotsk & Western North Pacific	Aug. & Sep., 1993	25	0.88(0.11)	16.1(0.29)	4.93(0.13)	30.3(0.73)	164.0(4.6)
			Oct., 1993	79	1.67(0.17)	15.5(0.20)	3.75(0.06)	17.6(0.48)	221.2(5.3)
			Nov., 1993	70	+++0.85(0.09)	14.9(0.19)	3.63(0.09)	19.7(0.60)	187.9(3.5)
	Adult	Bering Sea & North Pacific	June & July in 1994 & 1995	53	1.25(0.15)	16.1(0.16)	5.18(0.15)	20.3(0.40)	262.1(10.1)
Sockeye Salmon	Juvenile	Okhotsk & Western North Pacific	Oct. & Nov., 1993	17	0.30(0.04)	14.7(0.19)	3.96(0.17)	20.0(0.47)	197.8(6.7)
	Adult	Bering Sea & North Pacific	June & July in 1994 & 1995	46	1.63(0.17)	16.9(0.23)	5.27(0.18)	17.7(0.41)	305.5(12.8)

+N=133, ++N=130, +++N=69

Relationships Among Biochemical and Biological Characteristics

Positive relationships between triacylglycerol content and fork length was seen only in juvenile chum and in adult sockeye (Fig. 1). Significant regressions between triacylglycerol content and condition factor were observed in juvenile pink and chum salmon and adult pink and sockeye salmon, but the coefficient of determinations were low, particularly in juvenile pink (Fig 1).

Protein content increased with both fork length and condition factor in juvenile pink and chum but not sockeye, although the coefficient of determinations were low in pink and chum salmon (Fig. 2). Positive relationships were observed only between protein content and fork length in adult chum and sockeye salmon (Fig. 2).

RNA:DNA ratio increased with condition factor except for juvenile sockeye (Fig. 3). Although significant relationships between RNA:DNA ratio and fork length were shown in chum salmon, the slope of regression lines was negative in juveniles and positive in adults (Fig. 3).

Negative correlations were observed between RNA:protein ratio and fork length for juvenile chum and pink, although the correlation was very weak in pink (Fig. 4). Between RNA:protein and condition factor, only adult pink exhibited a positive correlation (Fig. 4).

Protein:DNA ratio increased with fork length in juvenile pink and both juvenile and adult chum salmon (Fig. 5). Protein:DNA ratios increased with condition factor in all cases except juvenile sockeye (Fig. 5).

Only juvenile chum exhibited negative correlations of triacylglycerol content with both RNA:DNA ratio and RNA:protein ratios, although the coefficient of determination was below 10% in the former case (Figs. 6 and 7).

DISCUSSION

In the present study, we examined triacylglycerol and protein contents and RNA:DNA, RNA:protein, and protein:DNA ratios in white muscle tissue to assess the growth characteristics of salmonids. Triacylglycerol is the major constituent of neutral fat, which is the primary material for energy storage. Protein is directly related to somatic body construction and can be viewed as a measure of growth condition. Both RNA:DNA and RNA:protein ratios reflect protein synthetic rates (Foster et al. 1993; Houlihan et al. 1993). Since the former is standardized by DNA, the value is affected by the change of cell size in contrast with the RNA:protein ratio. Protein:DNA ratio reflects relative cell size (Bulow, 1987), and muscle development can be evaluated from it. The

Fig. 1 **Relationship of triacylglycerol content of white muscle with fork length and condition factor by species and by life history stage.**

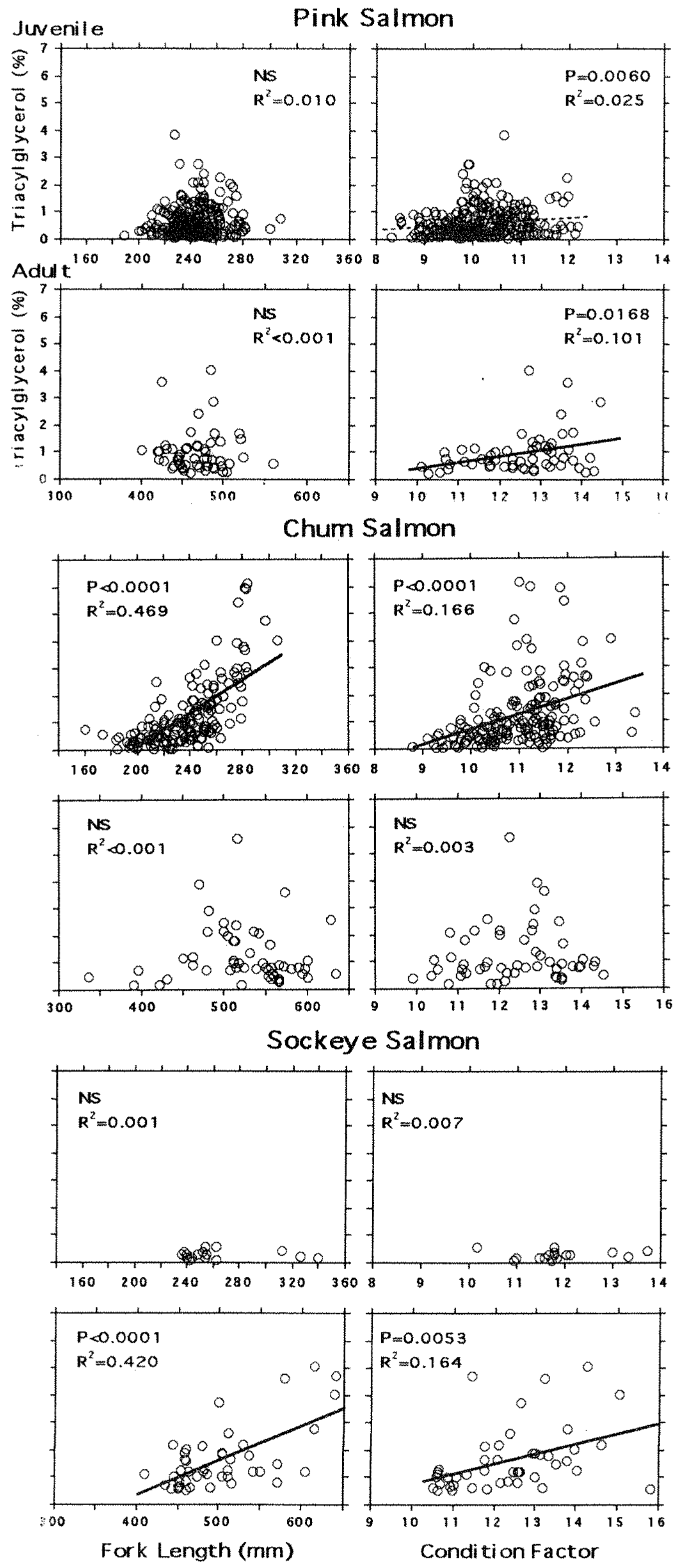


Fig. 2 Relationship of protein content of white muscle with fork length and condition factor by species and by life history stage.

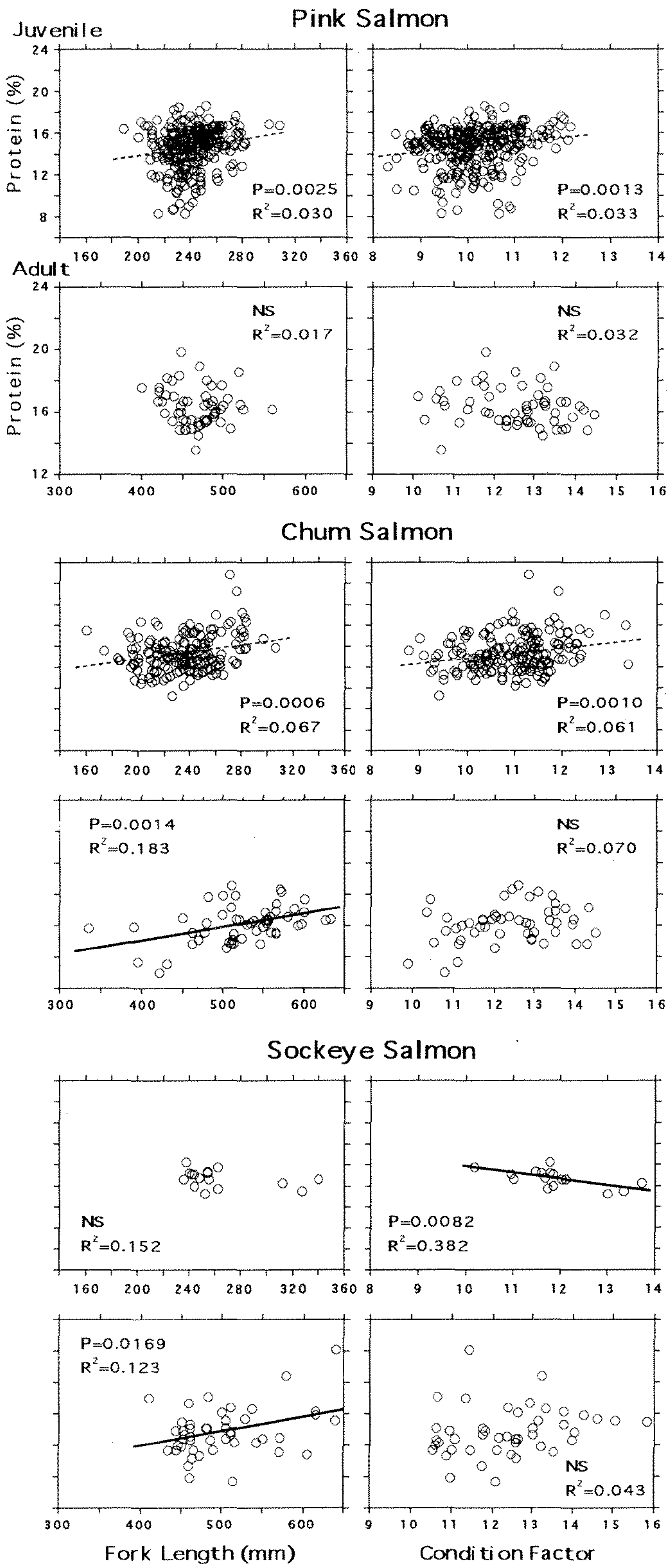


Fig. 3 Relationship of RNA:DNA ratio of white muscle with fork length and condition factor by species and by life history stage.

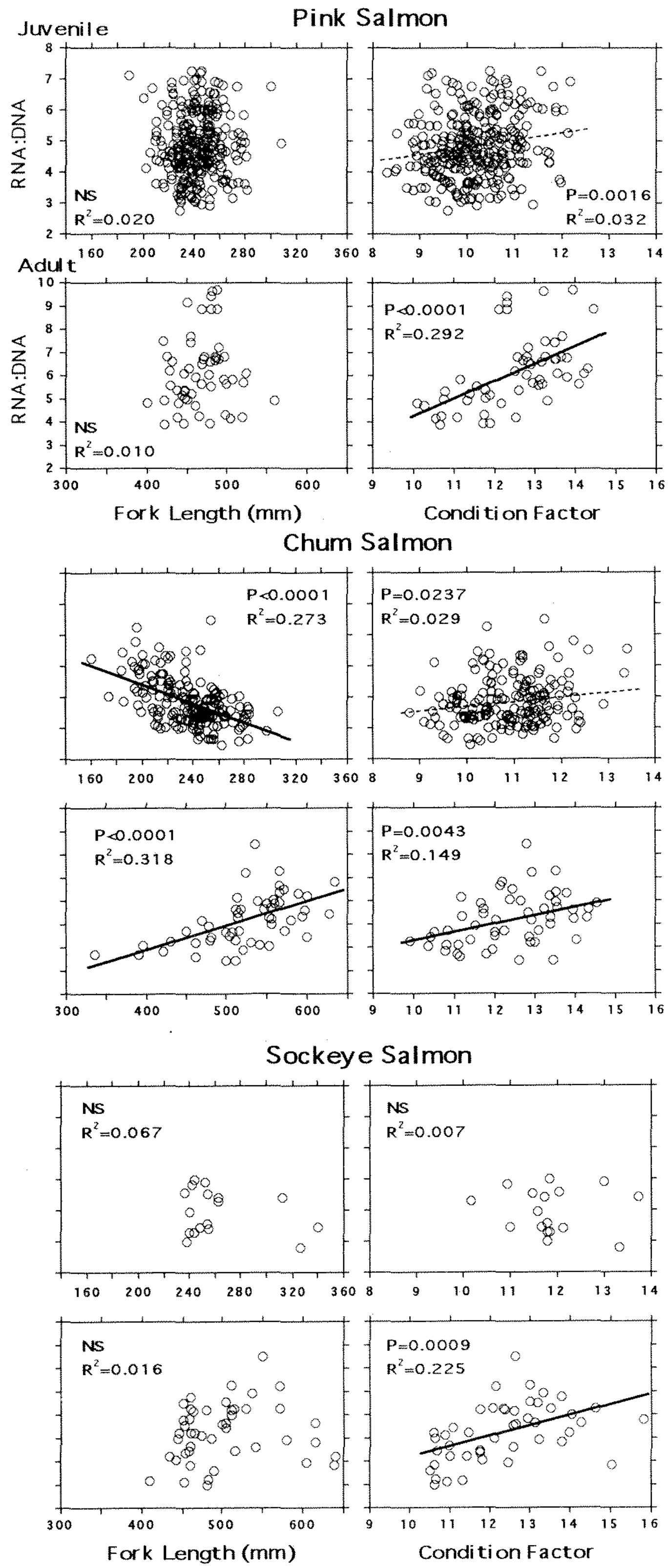


Fig. 4 Relationship of RNA:protein ratio of white muscle with fork length and condition factor by species and by life history stage.

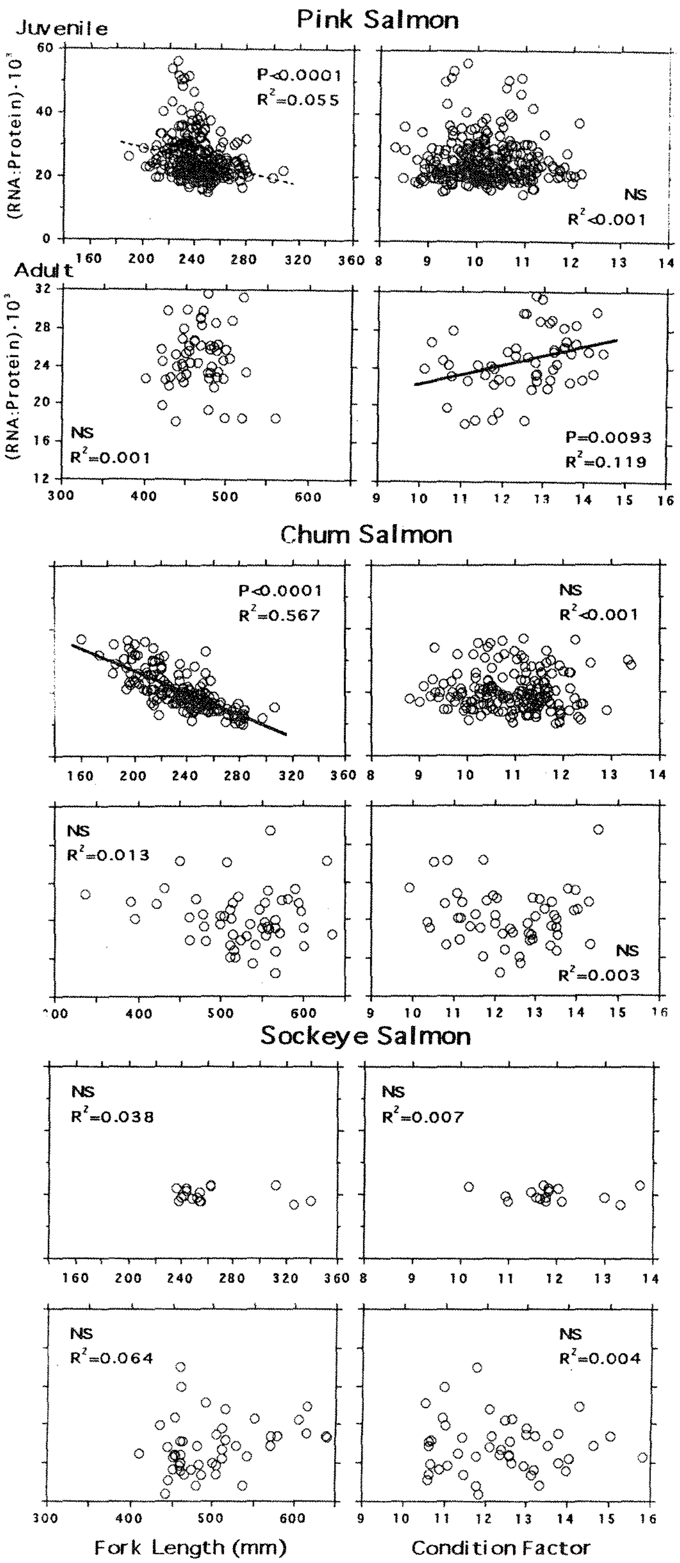


Fig. 5 Relationships of protein:DNA ratio of white muscle with fork length and condition factor by species and by life history stage.

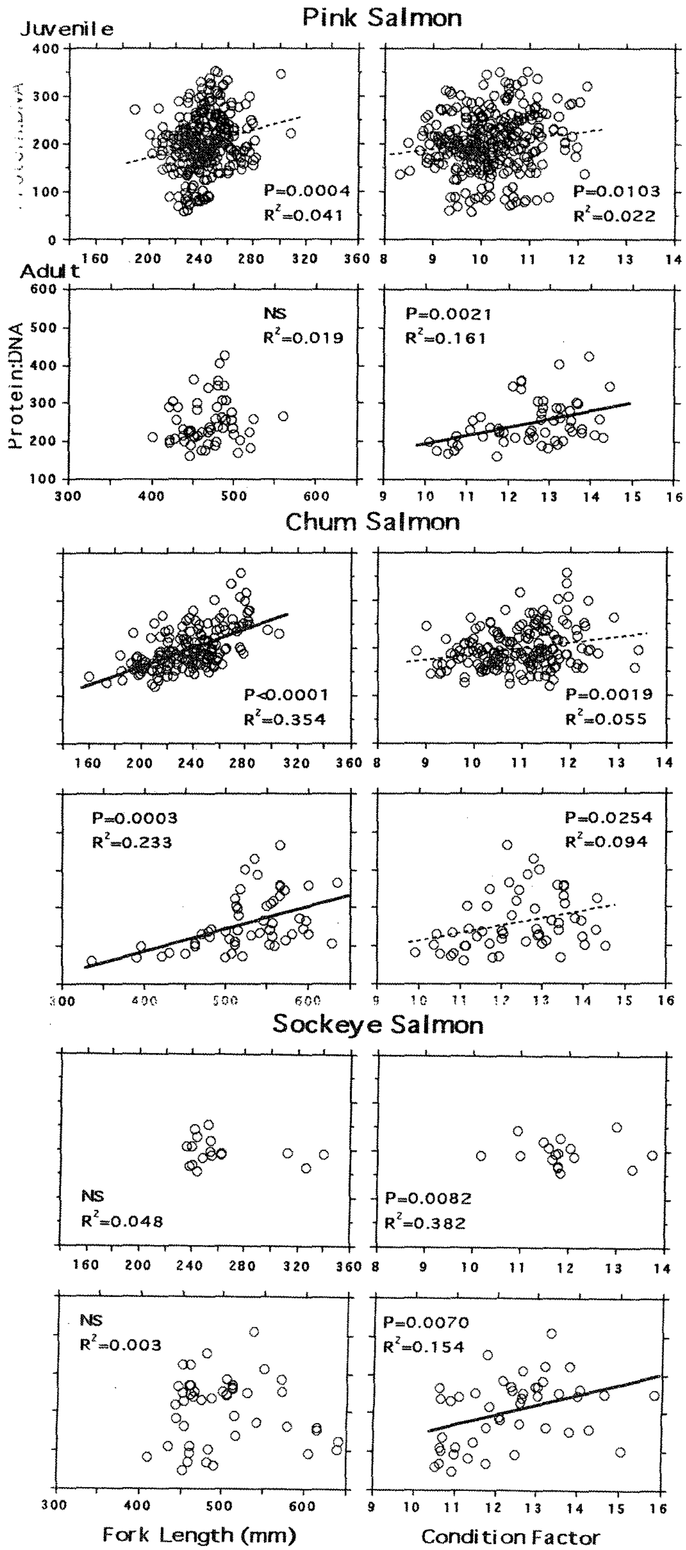


Fig. 6 Relationship of RNA:DNA ratio with triacylglycerol content of white muscle by species and by life history stage.

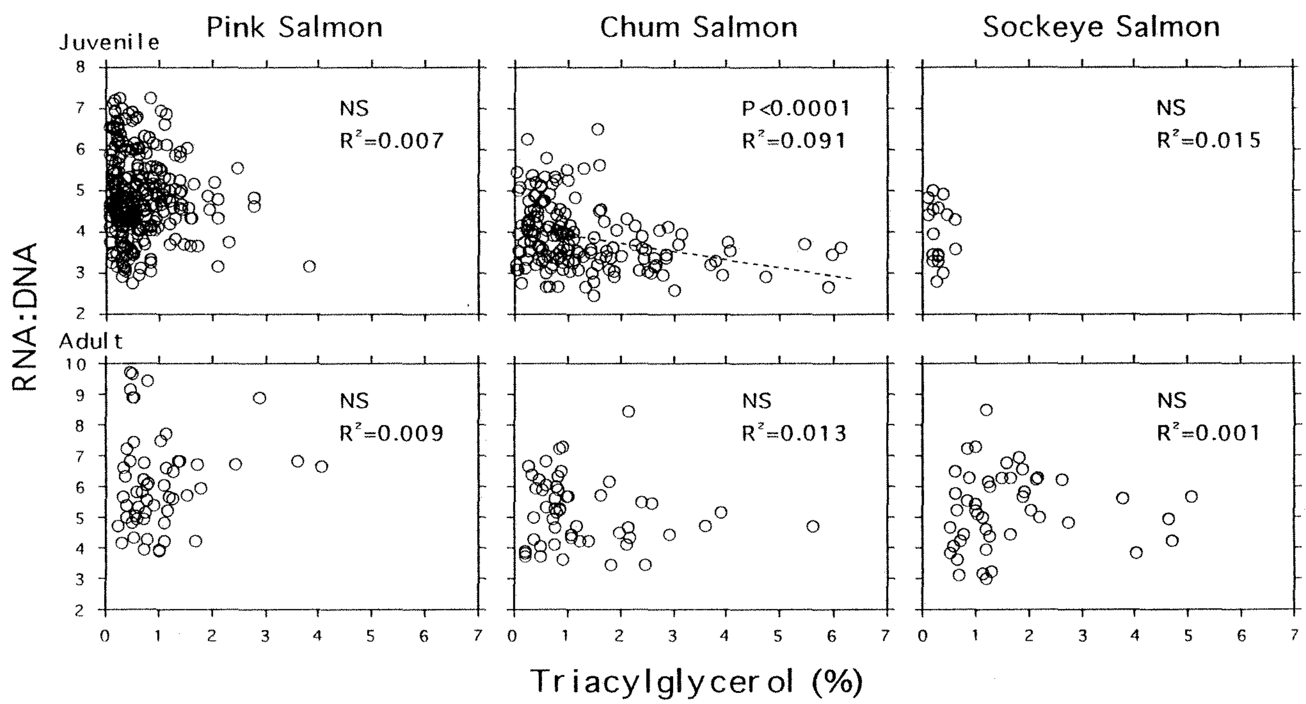
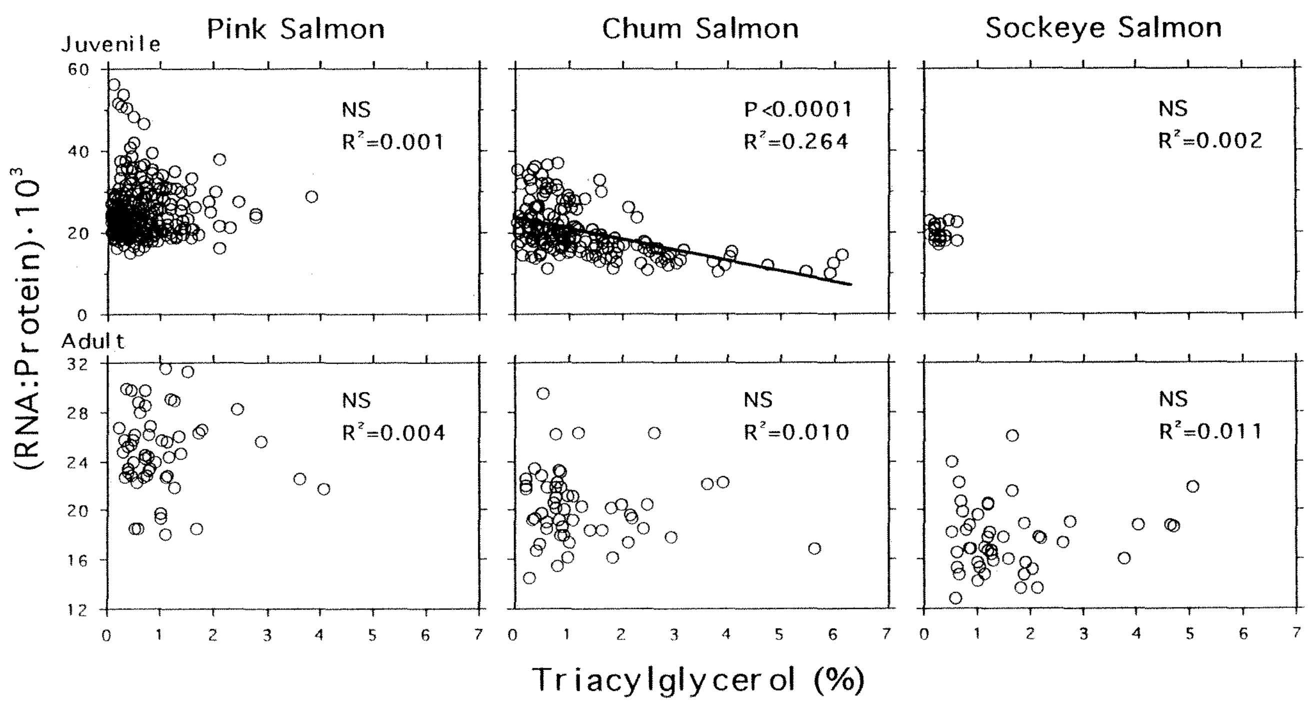


Fig. 7 Relationship of RNA:protein ratio with triacylglycerol content of white muscle by species and by life history stage.



RNA:DNA and RNA:protein ratios in white muscle have been demonstrated to be positively correlated with specific growth rate in salmonids (Thorpe et al. 1982; Wilder and Stanley 1983; Miglavs and Jobling 1989; Ferguson and Dazmann 1990; Varnavskiy et al. 1991; Wang et al. 1993; Grant 1996) These parameters are therefore supposed to indicate the growth potential of fish. In practice, white muscle comprises most of the body mass (Grant 1996), and Houlihan et al.(1993) reported that the protein synthesis in white muscle is more strongly correlated with somatic growth rate than other tissues.

In adult salmonids, we found that condition factor is positively correlated with RNA:DNA and protein:DNA ratios in all three species. This indicates that condition factor generally reflect growth potential expressed as protein synthetic rate per unit DNA content, and cell development in white muscle. However, it was found that relationships of either triacylglycerol content, protein content, or RNA:protein with condition factor differ from species to species. Correlations of biochemical parameters with body size seem to reflect species-specific growth characteristics. A positive correlation between triacylglycerol content and body size was seen in sockeye alone, and correlations of RNA:DNA ratio and protein:DNA ratio with body size were seen in chum.

Biological and biochemical characteristics of juvenile pink salmon indicate that development of skeletal muscle through the fall was supported by hypertrophy of muscle at the expense of energy reserves, because the protein:DNA ratio increased and triacylglycerol content decreased from October to November. However, since the correlation between protein:DNA and fork length was not strong, it cannot be clearly said that somatic growth is associated with cell enlargement of white muscle during the juvenile stage. Comparing juvenile chum and sockeye salmon, juvenile pink exhibited larger variation in protein and nucleic acid contents. This might be due to the broader distribution and wider range of water temperatures at which pink salmon were caught (National Research Institute of Far Seas Fisheries, 1993), and higher variability of such components due to temperature (Ferguson and Danzmann 1990; Houlihan et al. 1993). Judging from their biochemistry, juvenile pink appear to adopt a strategy of not storing the energy but maintaining a high somatic growth rate instead. The higher growth potentials also seen in adult stage is consistent with their faster growth rate during the oceanic period of their life history.

Juvenile chum salmon, in contrast with juvenile pink, clearly deposit much greater energy reserves inside the white muscle as they grow. Although cell enlargement of white muscle was presumed to happen through growth, judging from the relationship between the protein:DNA ratio and fork length, the rate of protein synthesis unit per DNA decreased with body size. These results indicate that the decrease in the ribosomal capacity for protein synthesis was more significant during the juvenile stage in chum salmon entering winter. The negative correlation of triacylglycerol content with RNA:DNA and RNA:protein ratios demonstrates that juvenile chum slow down their growth rate to maintain their energy reserve. This may a be suitable strategy for chum salmon which do not exhibit as fast a growth rate as pink salmon when winter zooplankton abundance will be low. These results are consistent with the finding that juvenile pink migrating northward on the west coast of Vancouver Island exhibit higher potential growth rates than do co-migrating juvenile chum (Perry et al. 1996).

No strategy was evident for juvenile sockeye salmon, probably because of small sample sizes.

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