The Effects of Growth on Development of Precocious Male and Smolts in Sockeye Salmon

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Abstract: The effects of alterations in growth on the incidence of precocious male maturation (PM) and seawater tolerance of sockeye salmon (Oncorhynchus nerka) were investigated. Juvenile sockeye salmon were separated into four groups (Groups A-D) and reared under different feeding regimes from November of age-0 to May of age-1. The growth regimes of groups A, B, C and D were the following: low growth throughout the duration, low from November to March and high from March to May, high from November to January and low from January to May, and high throughout the duration, respectively. The PM was determined by gonadosomatic index in May. Seawater tolerance was examined by monthly measurement of gill Na+, K+-ATPase (ATPase) activity. Proportions of PM among male fish of the groups A, B, C and D were approximately 0%, 50%, 80% and 80%, respectively. The ATPase activities of group-A remained consistently low, while that of the groups B, C and D was elevated significantly in April unrelated to differences of mean fork length and past growth. These results indicate that high growth in early winter of age-0 induces PM and consistent low growth during winter and spring hinders development of both seawater tolerance and smolt development.

Key words: Oncorhynchus nerka; Growth; Precocious male; Smolt

In various species of salmon, males can become sexually mature at an earlier age than females of the same brood. These precociously mature male fish (PM) are observed in masu (Oncorhynchus masou) (Kato 1991), Chinook (O. tshawytscha) (Gebhards 1960; Taylor 1989), coho (O. kisutch) (Foerster and Ricker 1953), and sockeye salmon (O. nerka) (Ricker 1938; 1959). The percentage and age of PM is dependent on population (Baily et al. 1980; Myers 1984), growing conditions (Saunders et al. 1982) and/or genetic factors (Glebe et al. 1978; Iwanoto et al. 1984). PM is a viable and biologically important alternate tactic in the life history of many salmonids (Saunders and Schom 1985).

Studies have shown that higher growth and fat deposition prior to sexual maturation may contribute to the proportion of PM (Rowe and Thorpe 1990; Rowe et al. 1991), being higher among faster growing individuals in a population. The effects of size and adiposity on the incidence of sexual maturity are significant (Silverstein et al. 1998; Shearer and Swanson 2000). Furthermore, many reports point out the importance of growth at specific times of year and the existence of critical periods for initiation and development of PM (Campbell et al. 2003; Larsen et al. 2004; Shearer et al. 2006). Manipulation of photoperiod and associated changes in melatonin mediate photoperiodic information to the reproductive axis (Bromage et al. 2001). As a result, development of PM may be induced by synergistic effects of high growth and stimulation of the brain-pituitary-gonadal axis with changes in day length.

The sexual maturation process frequently impairs seawater tolerance and smoltification (Saunders et al. 1982), due to hormonal and metabolic effects in masu (Aida et al. 1984) and Chinook salmon (Foote et al. 1990).
Administration of sex steroids inhibit the natural smoltification of masu salmon (Ikuta et al. 1987). Saunders et al. (1994) studied survival and growth of mature male and smolted immature male Atlantic salmon in seawater and concluded that sexual maturation and smoltification were in developmental conflict. However, in wild sockeye and coho salmon there is a precocious life-history variant referred to as “jacks” (Foester and Ricker 1953; Burgner 1991). Some hatchery-reared populations of sockeye salmon showing precociousness also experience a pelagic residence in their life cycle as well (Urawa and Kaeriyama 1999). They migrate seaward simultaneously with age-1 smolts while undergoing gonadal development and return for spawning the following autumn at age-1 the same time as full sized adults. Thus, it seems that the interrelationship between sexual maturation and seawater tolerance of the PM varies depending on species.

The National Salmon Resources Center in Japan (NSRC) has released juvenile sockeye salmon for more than 40 years in order to enhance the resources of this species. These fish are usually reared for about 15 months and released as smolts in late April. However a significant number of males (20 – 80% depending on brood year) mature precociously at age-1. These PM are likely to experience low growth and survival in the marine phase affecting adult return rates and gender ratios (Bailey et al. 1980). Therefore, it is important to understand and regulate the proportion of PM. This study focused on the effect of growth on the incidence of PM and on seawater tolerance in the NSRC sockeye salmon population.

**Materials and methods**

**Fish and rearing conditions**

In November 1991, sockeye salmon of age-0 were taken from a concrete pond at NSRC and divided into four groups of approximately 150 individuals (groups A – D). Each group was placed in one of four small tanks (100l capacity supplied with 10°C well water) and maintained until the following May. These four groups were fed commercial pellets at variable feeding rates

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as a proportion of body weight per day (bw/d) (Table 1). Throughout the experiment, the groups A and D were consistently fed at 0.5% and 3.0%, respectively. The feeding rate of groups B and C were changed from 1.0% to 3.0% in March and from 3.0% to 1.0% in January, respectively.

**Procedure of sampling and identification of PM and smolt**

Fork length (FL, mm) of all fish from each group was measured monthly from November to May following anesthesia with tricaine methane sulfonate (MS222). At each sampling times, gill filaments were excised from 10 fish from each group and frozen with homogenizing solution (250 mM sucrose, 6 mM EDTA 2Na, 20 mM imidazole, pH 6.8) at −40°C until assay of gill Na+, K+-ATPase activity (ATPase activity). The fish used for ATPase activity were chosen randomly from each tank during November – March. In April and May, only smolted fish were chosen for this assay. Throughout the experiment, PM was not used for the assay of ATPase activity. The estimation of smolt and PM were conducted as follows.

Smolt development was estimated visually based on the appearance of distinct blackening on the dorsal and caudal fin margin (Gorbman et al. 1982). Depending on the degree of pigmentation, the process of changes in fin margin blackening was classified into three stages: (i) not visible; (ii) vague; (iii) distinct. In this study, fish with distinct fin margin blackening were classified as smolts.

PM was determined by designating all males with a gonad somatic index (GSI) greater than 0.15% based on preliminary studies (Fig. 1). The GSI of male fish in April varied from 0.05% to 3.40% and there was no distinct boundary to separate immature male (IM) and PM. However,
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Fig. 1. Scatter plot of GSI versus fork length of male sockeye salmon in April (open circles) and June (closed circles). The horizontal line indicates the GSI of 0.15% used as a threshold for designating PM.

The GSI of male fish in June was clearly separable with IM having a GSI lower than 0.15%. At the end of this experiment in May, body weight (BW, g) and gonad weight (GW, g) of all fish (approximately 90 fish) remaining in each tank was measured. The GSI, gender ratio and proportion of PM were calculated by the following formula. GSI = (GW × 100)/BW, gender ratio = (number of male fish × 100)/number of monitored fish, proportion of PM = (number of PM × 100)/number of male fish.

Assay of gill ATPase activity

Gill ATPase activity was determined according to the method of Folmar and Dickhoff (1979), modified by Ban and Yamauchi (1991). Defrosted gill filaments were homogenized in the homogenizing solution and centrifuged at 2000 g for 5 min at 4°C. The supernatant was incubated for 20 min at 37°C. Inorganic phosphate (Pi) produced from the incubation was measured according to the method of Goldenberg and Fernandez (1996). Protein concentrations were determined by the method of Lowry et al. (1951). Enzyme activity was expressed as micromoles Pi per mg protein per hour (μmol Pi/mg pro./h).

Statistical analysis

Data were subjected to a t-test to determine significant differences in FL and ATPase activity among four groups at each sampling time. A probability level of less than 0.05 was considered significant.

Results and discussion

The gender ratios of PM’s, IM’s and females were approximately 50:50 (Table 2). There were no significant differences in mean FL between IM and female. However, mean FL of PM observed in groups B, C, D were significantly larger than that of IM. Similar to previous studies (Eriksson et al. 1979; Dalley et al. 1983; Myers et al. 1986), these results show PM in sockeye salmon generally occurs in larger fish in the population.

In May, the male fish were classified into one of three categories: PM, smolt, or smolt like PM that show blackening of fin margins and a GSI in excess of 0.15%. The proportion of PM in the four treatments could be generally divided into three broad classes, group-A was 0% PM, group-B was approximately 50% PM, and groups C and D were approximately 80% PM (Fig. 2). Despite the fact that FL of PM in the group-B was significantly larger than that of the group-C (see table 2), the proportion of PM in group-B was lower than that of group-C, suggesting that body size is probably not the only differentiating factor influencing PM.

Changes in the mean FL of PM, IM and female of groups A-D are shown in Fig. 3. Differences in growth reflected differences in feed ratio. Group-A and group-D showed

| Table 2. Gender ratio (%M) and fork length (mean ± SEM) of precociously mature male, immature male and female sockeye salmon of groups A-D at the end of the experiment in May |
|-----------------|-----------------|-----------------|-----------------|
| Group | Gender ratio | Male Precocious | Male Immature | Female Precocious | Female Immature |
| A     | 51.7          | 124.3 ± 2.0     | 122.1 ± 3.1    |
| B     | 52.2          | 183.8 ± 4.0     | 160.8 ± 4.6    | 161.7 ± 2.6 |
| C     | 48.7          | 166.6 ± 2.0     | 155.3 ± 3.6    | 157.8 ± 2.0 |
| D     | 51.8          | 196.4 ± 3.5     | 174.4 ± 5.3    | 171.5 ± 2.7 |
extreme low and high growth throughout the experiment, respectively. The growth of group-B was low from November to January and high from March to May, and the growth of group-C was high from November to January and low from November to January. Treatments with similar proportions of PM exhibited similar growth from November to January, while growth from February to May did not correlate with the proportion of PM. It has been hypothesized that development of PM is influenced by two periods, autumn at age-0 and spring at age-1 and rapid growth during these periods facilitates sexual maturation of male Spring Chinook salmon (Clarke and Blackburn 1994; Shearer and Swanson 2000; Campbell et al. 2003; Larsen et al. 2004). The present findings imply that precocious male maturation is significantly influenced by high growth during November of age-0 – January of age-1 in male sockeye salmon. Taken together, by suppression of the growth of sockeye salmon reared at the NSRC from November to January, we may help reduce the proportion of PM.

This investigation demonstrated that growth in winter has a significant effect on gonadal development of male sockeye salmon. Sexual maturation is under the control of GnRH and GTH (Amano et al. 1994; 1997). Amano et al. (1994) reported that shortened photoperiod in early summer may be required for activation of GnRH synthesis in PM masu salmon, and Berrill et al. (2003) showed that a period of artificial short days from May to July increased the percentage of PM Atlantic salmon. Furthermore, Björnsson et al. (1994) indicated a functional relationship between plasma GH levels controlled by photoperiod and the process of sexual maturation. Those previous findings and the current study suggest that short photoperiod and higher growth during November to January promote gonadal maturation of PM.

In this study, 11% of smolt like PM were observed in the group-D alone. Previous studies have suggested that sexual maturity interferes with smoltification in Chinook salmon (Foote et al. 1990) and reduces the probability of future seaward migration in Atlantic salmon (Hansen et al. 1989). In the case of masu salmon, PM do not show blackening of fin margins. These results may be caused by the antagonistic effect of sexual maturation on smoltification. However, some male fish referred to as “jacks” have been observed in the wild and NSRC populations of sockeye salmon (Burgner 1991, Urawa and Kaeriyama 1999), and coho salmon (Sandercock 1991). They migrate seaward while undergoing gonadal development in spring and they return to the natal river within a few months. The smolt like PM observed in the group-D may, in fact, represent this “jack” phenotype and be caused by high growth from winter to spring.

Pigmentation of fin margins, indicative of smoltification, was not observed in any groups from November to February. In late March, some of the fish in every group showed vague blackening of their fin margins. This color

Fig. 2. The proportions of PM (black column), male smolt (white column) and smolt like PM (gray column) of groups A-D in May.

Fig. 3. Changes in fork length (mm) of groups A-D. Vertical bars indicate mean ± S.E.M. Different letters adjacent to symbols indicate significant differences (p < 0.05) among groups at a sampling date.
Gradually became distinct with the progression of smoltification in April and May. The ATPase activities of all groups were below $10\mu$ mols Pi/mg pro./h from December to March (Fig. 4). During this period, higher growth induced significantly higher enzyme activity. However, the ATPase activity of the groups B, C, D showed a dramatic increase and reached $18-21\mu$ mols Pi/mg pro./h in April unrelated to differences in their fork length and past growth. These results indicate that the ATPase activity is influenced by both growth related and non-growth related factors. Ban (2006) reported that an increase in photoperiod improves seawater tolerance unrelated to growth in sockeye salmon. Rapid increases of the ATPase activity observed in groups B, C, D in April seem to be influenced by increasing photoperiod in spring.

The ATPase activity of group-A remained at $4.5-6.2\mu$ mols Pi/mg pro./h during April and May, despite the fact that these fish displayed distinct blackening of fin margins. This result suggests that consistent and extreme low growth from winter to spring inhibits normal development of seawater tolerance in smolting fish. Dickhoff et al. (1997) and Beckman et al. (1999) reported the importance of high growth to successful smoltification in spring Chinook salmon. High growth in spring may be used to improve smolt development in sockeye salmon as well.

It should be pointed out that the peak of ATPase activity is transient and this activity declines rapidly following smolting. The release of sockeye salmon with higher seawater tolerance appears to improve ocean survival, and frequent monitoring of ATPase activity should be conducted in the spring to determine optimum release times in the hatchery systems.

In conclusion, the current study examined the effects of growth on development of PM and smolting in sockeye salmon. The present results indicate that high growth from November of age-0 to January of age-1 has significant effects while high growth from March to May of age-1 has limited effects on the incidence of age-1 PM. However, low growth during the spring may hinder smolt development. In general, in order to suppress the incidence of PM and increase seawater tolerance of smolts prior to release, it is recommended that the growth in winter of age-0 should be reduced and growth in spring of age-1 should be accelerated.

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**References**


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成長差がベニザケの早熟雄とスモルトの発達に与える影響

伴 真俊

ベニザケ幼魚（Oncorhynchus nerka）の成長差が早熟雄（PM）とスモルトの発現過程に与える影響を調べるため、4 群（A-D 群）に分けた魚を異なる給餌率で 0 年魚の11月から1年魚の5月まで飼育した。各群の成長は、実験期間を通じて低成長（A 群）、11月-3月が低成長、3月-5月が高成長（B群）、11月-1月が高成長、1月-5月が低成長（C群）、実験期間を通じて高成長（D群）だった。PMの確認は生殖腺体指数に基づいて5月に行った。スモルトの発現過程は海水適応能の変化を基に把握した。海水適応能は鰓の Na⁺, K⁺-ATPase (ATPase) 活性を指標に調べた。その結果、雄に占める PM の割合は A 群、B 群、C 群、D 群が、各々 0%, 50%, 80%, 80%だった。ATPase 活性は A 群が実験期間を通じて低かったのに対し、B-D 群は 4 月に平均尾叉長および成長遅延と無関係に有意な上昇を示した。これらの結果から、ベニザケは0年魚の冬季における高成長が早熟雄を惹起すること、また冬季から春季にかけた低成長は海水適応能とスモルトの発達を阻害することが示唆される。