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Reproductive Characteristics of Precocious Male Parr in Salmonids: Morphology, Physiology, and Behavior

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Abstract

Morphological, physiological, and behavioral characteristics of precociously mature male parr were examined in the two representatives of salmonid species in Japan, masu salmon, *Oncorhynchus masou* and Miyabe charr, *Salvelinus malma miyabei.*

Selection on morphological characters during breeding activity was quantified on mature masu salmon parr. Larger body size was favored for sneaking success, contrary to the prediction that sneaking behavior favors small body size. This selection pressure was caused by the dominance hierarchy among parr and may have been facilitated by indifference of dominant migratory males to parr. No morphological characters other than body size contributed to the reproductive success of parr. This non-contribution may have been responsible for the lack of development of sexual characters in parr.

Energy allocation to soma and testes of mature parr was compared with that of migratory males in masu salmon. Energy contents per unit mass of soma and testes did not show the consistent difference between mature parr and migrants; rather, they differed between two populations, likely reflecting the difference in food availability. By contrast, both relative testes investment in mass and energy were higher in mature parr than in migrants, consistently in the two populations. This result supports sperm competition theory that predicts the higher gametic investment of competitively inferior male (e.g., sneaker) for improving fertilization efficiency.

Breeding behavior of mature parr of the Miyabe charr was compared under the different availability of refuges in the experimental and natural conditions. If mature parr are suitable to sneak, refuges may contribute to avoid aggression from competitors and improve the sneaking success. Contrary to this expectation, high refuge availability did not reduce aggression from other participants or facilitate intrusion into the nest either in experiment or in the wild. Moreover, the effect of refuges on the proximity of precocious parr to the nest was limited to the experiment where the refuge availability was probably much higher than in the natural stream. Consequently, the availability of refuges did not affect the probability of successful sneaking. Even though these results do not reject directly the behavioral specialization in sneaking, it is doubtful whether mature parr are realized the ideal specialization in sneaking. The other possible benefits by using refuges are discussed.

Lack of selection on morphological characters other than body size and energy allocation pattern in soma and gonad were compatible with the predictions from specialization-in-sneaking. Selection for larger body size and insensitivity of components of reproductive success to the refuge availability could not be explained by specialization-in-sneaking. These discrepancy may be modified by incorporating the formally discounted selective agent (among-parr competition) and physiological and life history constraints into specialization-in-sneaking.

Key Words: alternative reproductive tactics, breeding competition, reproductive success, sneaking, sexual selection

Chapter 1
Introduction

Organisms are expected to be selected to maximize fitness, or lifetime reproductive success (i.e., number of surviving offspring), within the constraints imposed by phylogeny, genetics, development, physiology, and stochastic environment. Reproductive success depends largely on the pattern of reproduction, which includes where and when an individual organism reproduces and how it allocates finite resources (e.g., time and energy) within and among reproductive bouts (Clutton-Brock, 1988; Wootton, 1990). As a consequence of selection, reproductive pattern or phenotype adopted by an individual will confer higher fitness of the individual.

In many animal breeding systems, more than one phenotypes (i.e., alternative reproductive phenotypes) are found within sexes (Taborsky, 1994; Gross, 1996). Most of them are conditional (alternative tactics) while it is purely genetic (alternative strategies) in a few cases (e.g., Shuster and Wade, 1991; Ryan *et al.*, 1992; Lank *et al.*, 1995). For example, 'sneaking' and acting as a 'satellite' are common non-competitive reproductive tactics which are alternatives to fighting or courting for access to mates (e.g., insect: Crespi, 1988; Emlen, 1997; anuran: Sullivan, 1982; Fukuyama, 1991; fish: Kodric-Brown, 1986; Reynolds *et al.*, 1993). Such

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alternative tactics suggest that a tactic yielding higher fitness of an individual is not successful for different individuals as well or even for the same individual if exposed to different environments. In other words, occurrence of alternative tactics suggests the heterogeneity of sexual selection and the plasticity of organisms to response to it. Hence, alternative reproductive tactics may provide an excellent opportunity for understanding the set of evolutionary mechanisms, selection and adaptation. Furthermore, study of evolutionary mechanisms using alternative tactics may afford unambiguous interpretations of the results because, unlike interspecific comparison, they are not affected by phylogenetic constraint (Harvey and Pagel, 1991).

Many salmonid species have alternative reproductive tactics, 'precocious males' that mature at small body size either after a short migration (i.e., jacks) or without migration (i.e., mature male parr) and 'migratory males' that mature at large body size after a long seaward or lakeward migration. During breeding, small precocious males may sneak into the spawning of a large migrant while the large migrant fights for access to a mate (Jones, 1959; Hanson and Smith, 1967; Maekawa, 1983; Gross, 1985, 1991; Myers and Hutchings, 1987). Alternative tactics in salmonids have been considered to be specialized in alternative behavior during breeding (Gross, 1984, 1985). That is, precocious and migratory males may be evolutionarily favored by sneaking and fighting, respectively. However, this inference has not been evaluated adequately. Only a few empirical studies on migratory males generally support the theoretical predictions drawn from specialization in fighting (Fleming and Gross, 1994; Quinn and Foote, 1994). Moreover, little is known about the degree to which precocious males are specialized in sneaking. Previous studies on precocious males have been concentrated on the morphological comparison with migrants (e.g., Maekawa, 1978; Foote *et al.*, 1997) and molecular examination of the fertilization success relative to dominant migrant (e.g., Maekawa and Onozato, 1986; Hutchings and Myers, 1988; Morán *et al.*, 1996; Foote *et al.*, 1997; Thomaz *et al.*, 1997). For understanding the evolutionary pathway of precocious males, it is primarily necessary to evaluate the degree of specialization in sneaking.

In this paper, I examine the morphological, physiological, and behavioral characteristics of precociously mature male parr in the two representatives of salmonid species in Japan, masu salmon, *Oncorhynchus masou* and Miyabe charr, *Salvelinus malma miyabei*. The studies in respective chapters are not mutually supportive, or rather independent, but the consistent objective is to investigate the reproductive characteristics of mature male parr from the viewpoint of specialization in breeding behavior. Based on my results and previous studies, I discuss the applicability, limitation, and modification of the stereotyped explanation by specialization-in- sneaking for the evolution of mature male parr.

Chapter 2
Morphological Characteristics: Morphological Specialization to Alternative Reproductive Tactic *

2.1 Introduction

It is the most case that the adoption of alternative reproductive tactics depends on body size and/or morphological characters which are representative of competitive ability or social status (Gross, 1984; Hayashi, 1985; Hughes, 1985). Individuals which adopt non-competitive tactic have a smaller body size and/or less-developed morphological characters than those that compete directly. Moreover, in some species, intrasexual variation in morphology consists of two or more discrete frequency distribution modes, the upper and lower modes being associated with competitive and non-competitive behaviors, respectively (Eberhard, 1982; Danforth, 1991; Shuster and Wade, 1991; Emlen, 1997). Such di- and poly-morphism may represent morphological specialization corresponding to the alternative behaviors.

Precocious and migratory males in salmonid species is the typical case of male dimorphism. Morphological differences between migratory and precocious males exist in body size and other characters, such as snout length and hump size, which are secondarily developed in migrants at maturity (Maekawa, 1978, 1984). As for other dimorphic species, salmonid dimorphism is interpreted as morphological specialization for alternative behaviors. Large body size with well-developed sexual characters and small body size without these characters appear to be suited for fighting and sneaking, respectively (Gross, 1985, 1991). Indeed, the large body, hump, and snout sizes of migratory males in Pacific salmon (*Oncorhynchus* spp.) are favored by sexual selection through intrasexual competition (Fleming and Gross, 1994; Quinn and Foote, 1994). However, despite a large number of studies concerning fertilization success (e.g., Maekawa and Onozato, 1986; Hutchings and Myers, 1988; Jordan and Youngson, 1992; Morán *et al.*, 1996; Foote *et al.*, 1997), the contribution of morphology to reproductive success remains unclear for precocious males (but see Thomaz *et al.*, 1997). Gross (1985) showed that smaller jacks can get closer to females in coho salmon (*O. kisutch*). In contrast, Maekawa (1983) and Myers and Hutchings (1987) reported that a dominance hierarchy was established among mature male parr, with the largest gaining closest access to females in Miyabe charr (*Salvelinus malma miyabei*) and Atlantic salmon (*Salmo salar*). Furthermore, Thomaz *et al.* (1997) found that fertilization success was positively correlated with body size in Atlantic salmon parr. More studies are needed to conclusively explain the morphological specialization of precocious males in salmonids.

In this study, I examined, in mature male parr of

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masu salmon *Oncorhynchus masou*, (1) what morphology is favored by sexual selection, or which morphological characters contribute to reproductive success, and (2) how the characters contribute to the reproductive success, or what mechanisms operate on the contribution of morphology to the reproductive success. My hypothesis was that, since a less-conspicuous morphology should reduce aggression from dominant migrant (Gross, 1985), small body size will contributes to the sneaking success but the other morphological characters will not. Under this hypothesis, I predicted that selection favoring small body size intensifies with the dominant's aggression. To test this prediction, I conducted a semi-natural mating experiment in which the density of migrants was manipulated to vary the dominant's aggression against parr. I observed the reproductive behavior and success of parr during the experiment to quantify selection acting on morphology.

2.2 Materials and Methods

(1) Fish studied

Masu salmon (*O. masou*) are distributed throughout the Far Eastern Asian region of the Pacific Ocean (Tsiger *et al.*, 1994). Almost all females migrate to the sea from the natal stream but males have three life history forms: large, late-maturing migratory males which descend to the sea like females, small mature male parr residing in the natal stream throughout their lives, and intermediate-sized migrants whose life history is similar to that of coho salmon jacks (the frequency of latter form is extremely low, Tsiger *et al.*, 1994). In Hokkaido, northern Japan, migrants and parr usually mature at age 3 or 4 and at age 1 or 2, respectively (Sano, 1951; Uto, 1976). All migrants die after breeding while some parr breed in 2 or more years (Tsiger *et al.*, 1994).

I used landlocked masu salmon in Toya Lake, Hokkaido, Japan. The masu salmon in this lake were introduced in the early 1930's from some river systems in Hokkaido (T. Yamamoto, personal communication). Several inlets of this lake provide spawning grounds for the fish, so that they have similar life-histories to anadromous masu salmon (Yamamoto *et al.*, 2000). Mature male parr commonly occur in these inlets (personal observation). In the breeding season, the pairs of migratory fish are present patchily at the spawning ground and each forms a spawning aggregation with several mature male parr.

(2) Fish collection

Migratory fish were collected from the Sobetsu Stream (42°38'N, 140°51'E) flowing into Toya Lake, in September 1997. From 5 to 14 September, fish were caught with a weir set across the stream, while on 23 September an Electrofisher (Smith-Root Inc.) was used. The collected fish were stocked in water tanks at the Toya Lake Station (TLST) of Hokkaido University until transportation. Mature male parr artificially bred from wild fish were collected from the TLST tanks. I confirmed that parr were mature by softly pushing their bellies and releasing a very small amount of semen. The fish were transported in a cooled and aerated tank truck on September 16 and 24 to the Tomakomai Experimental Forest (TOEF) of Hokkaido University. The transported fish were kept in holding facilities at the Horonai Stream (42°40'N, 141°36'E) running within the TOEF prior to the experiment.

(3) Experiment

The mating experiment was conducted from September 18 to 25 in a reach of the Horonai Stream. The reach is about 4 m in width, 10–26 cm in depth, with a flow rate of 0.3–0.7 m/s, and a substrate consisting mainly of gravel. Several salmonids including masu salmon have reproduced in this stream (e.g., Kitano *et al.*, 1993; Taniguchi *et al.*, 1996). I constructed six enclosures (4 × 4 m each) using plastic fences and drew black vinyl curtains along the reach banks to observe fish behavior without disturbance. Small colored ribbon-tags (3 cm long, 2 mm wide) were sewn on the back of all parr for individual identification after being anesthetized with 2-phenoxyethanol. In the enclosures, fish were allowed to spawn and mating behavior was observed at least once every 2 h from dawn to dusk (0500–1800 hours). Restricting observations to daylight hours did not affect the results – there was no evidence that spawning occurred at night. After a spawning aggregation had formed, I videotaped the attending males to record all aggressive encounters and determine the sneaking success of parr (defined as rushing into the nest at oviposition). I also measured the distance from each parr to the nest (egg pit) when the female crouched for oviposition. The distance from parr to the nest (cm) was estimated relative to fish size or colored markers on the gravel (1 × 1 m grids).

To test whether the aggression from dominant migrants intensifies selection acting on parr, the mating experiment comprised three migrant-density treatments: one, two, or four migratory males with five parr and one female. I expected that, while the aggression among migrants increases with an increase in the density of migrants (Quinn *et al.*, 1996), this decreases the aggression of dominant migrant against parr. Five new parr were used in each trial, although several migratory fish were re-used; one of nine females was used twice and two were used three times, and 7 of 24 males were used twice, and 1 was used three times. This was done because not enough fish were available to use fresh individuals in each trial. The behavior of the re-used fish did not appear to differ among the successive trials. I minimized the differences of parr body size (postorbital–hypural length) among trials; the variance did not differ among either the trials or the treatments (Bartlett's test for homogeneity of variances: among trials, $\chi^2 = 7.26$, $df = 13$, $P = 0.89$; among treatments, $\chi^2 = 1.36$, $df = 2$, $P = 0.51$), nor did the mean (ANOVA: among trials, $F_{13,55} = 0.76$, $P = 0.70$; among treatments, $F_{2,66} = 1.20$, $P = 0.31$) (see Table 2-1). To minimize the effects of time-dependent factors, the three treatments (× two replicates) were performed simultaneously in six enclosures. The enclosures were randomized for the treatments. Spawning took place in

Table 2-1. Experimental design and body length and body weight (mean ± SD) of the fish used in each trial.

Treatment[a]	Enclosure	Trial day in September 1997	Mature male parr		Migratory male		Female	
			Length (mm)	Weight (g)	Length (mm)	Weight (g)	Length (mm)	Weight (g)
1 (1)	2	19	88.1 ± 15.3	17.4 ± 7.6	396	1,454	397	1,332
	3	25	102.4 ± 11.2	26.9 ± 8.0	369	1,250	423	1,338
	4	21	100.9 ± 7.0	24.0 ± 4.7	399	1,506	374	903
	5	19	97.7 ± 11.3	23.6 ± 5.9	389	1,447	420	1,448
	6	21	93.6 ± 11.6	23.0 ± 9.1	346	1,018	373	1,264
2 (2)	1	21	90.1 ± 7.1	18.8 ± 4.9	384 ± 13	1,353 ± 101	433	1,493
	2	19	88.9 ± 8.5	19.5 ± 5.1	401 ± 2	1,715 ± 64	397	1,332
	4	21	95.6 ± 11.8	23.6 ± 8.8	344 ± 4	1,165 ± 219	374	903
	5	18	97.4 ± 10.4	24.1 ± 6.7	369 ± 23	1,175 ± 227	408	1,524
3 (4)	1	21	102.1 ± 13.1	25.1 ± 5.7	362 ± 19	1,209 ± 174	433	1,493
	2	20	99.8 ± 14.2	25.2 ± 11.0	371 ± 18	1,330 ± 108	397	1,332
	3	18	92.0 ± 18.0	20.3 ± 10.9	366 ± 10	1,250 ± 277	453	1,980
	4	20	96.9 ± 8.3	24.0 ± 6.7	342 ± 25	1,167 ± 208	374	903
	6	25	102.4 ± 9.2	25.6 ± 5.3	366 ± 10	1,250 ± 277	418	1,586

[a] In parentheses, the number of migratory males

five, four, and five trials for the treatments including one, two and four migratory males, respectively. Table 2-1 presents details of the experiment and the size of fish used in each trial.

In contrast to my expectation, the density of migrants had no effects on the aggression of dominant migrant against parr or on the mating behavior of parr (see 2.3 Results). Therefore, I combined the three treatments (14 trials in total) to analyze the data of 69 individuals (one parr was lost) simultaneously.

(4) Morphological characters and their transformation

Parr recaptured after the experiment were weighed (nearest gram) and the following ten morphological characters were measured (nearest 0.1 mm): body (postorbital–hypural) length, body height (the longest distance from the back to the belly, perpendicular to body length), caudal–peduncle depth, snout length, dorsal fin height, anal fin height, pectoral fin length, pelvic fin length, adipose fin length and caudal fin length (Fig. 2-1). Dorsal fin height, adipose fin length, and caudal fin length were not measured for some individuals due to damage, and were excluded from the analyses. Multicollinearity – strong correlations among the characters – due to allometric growth was detected (Table 2-2). Many authors have indicated that multicolinearity confuses the estimation of selection gradients (Lande and Arnold, 1983; Mitchell-Olds and Shaw, 1987; Crespi and Bookstein, 1989). It was therefore reduced by a size-adjustment procedure (see Fleming and Gross, 1994). In this procedure, all characters are log (ln)-transformed and each character except body size is then regressed on body size. The value of each individual character relative to standard size is expressed as residual from this ln-ln regression. Like Fleming and Gross (1994), I chose body weight as an useful measure of body size because its explanatory power during regression analyses was the best. Prior to the regression analyses, body weight (g) was cube root-transformed to reduce its dimensions. After this size-adjustment procedure, only the correlation between pectoral and pelvic fins was still significant at the multiple comparison level (Table 2-2). All characters were then standardized (mean = 0, variance = 1) to compare the strength of selection (i.e., selection gradient) among characters and across components of reproductive success.

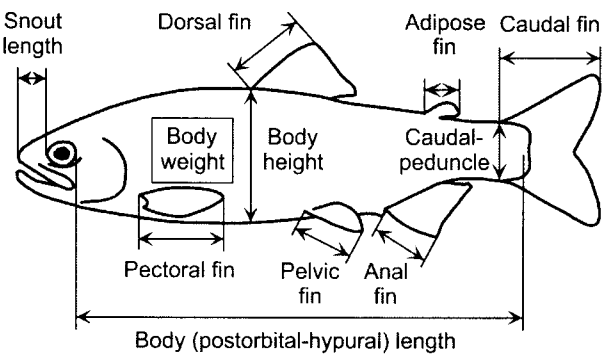

Fig. 2-1. Characters measured on masu salmon parr.

Table 2-2. Correlations among the characters of masu salmon parr before and after a size-adjustment procedure (see text). Correlation Coefficients (sample size in parentheses) before and after size adjustment are represented in the upper and lower triangle, respectively.

	Body weight	Body length	Body height	Caudal-peduncle depth	Snout length	Pectral fin	Pelvic fin	Dorsal fin	Anal fin	Adipose fin	Caudal fin
Body weight		0.951***	0.912***	0.915***	0.892***	0.777***	0.737***	0.759***	0.636***	0.526***	0.751***
		(69)	(69)	(69)	(69)	(69)	(69)	(68)	(69)	(53)	(65)
Body length	–0.094		0.869***	0.892***	0.900***	0.821***	0.750***	0.768***	0.674***	0.562***	0.744***
	(69)		(69)	(69)	(69)	(69)	(69)	(68)	(69)	(53)	(65)
Body height	–0.056	–0.138		0.884***	0.836***	0.790***	0.758***	0.732***	0.662***	0.587***	0.734***
	(69)	(69)		(69)	(69)	(69)	(69)	(68)	(69)	(53)	(65)
Caudal-peduncle depth	0.032	0.049	0.163		0.869***	0.814***	0.767***	0.775***	0.643***	0.564***	0.752***
	(69)	(69)	(69)		(69)	(69)	(69)	(68)	(69)	(53)	(65)
Snout length	–0.045	0.240	0.002	0.168		0.737***	0.679***	0.710***	0.586***	0.471**	0.719***
	(69)	(69)	(69)	(69)		(69)	(69)	(68)	(69)	(53)	(65)
Pectral fin	–0.017	0.305	0.255	0.303	0.035		0.791***	0.727***	0.706***	0.478**	0.745***
	(69)	(69)	(69)	(69)	(69)		(69)	(68)	(69)	(53)	(65)
Pelvic fin	–0.029	0.132	0.219	0.260	–0.055	0.479*		0.669***	0.670***	0.592***	0.718***
	(69)	(69)	(69)	(69)	(69)	(69)		(68)	(69)	(53)	(65)
Dorsal fin	–0.023	0.154	0.009	0.199	0.008	0.279	0.224		0.660***	0.442**	0.711***
	(68)	(68)	(68)	(68)	(68)	(68)	(68)		(68)	(52)	(64)
Anal fin	0.002	0.166	0.166	0.106	–0.033	0.388	0.322	0.304		0.524***	0.593***
	(69)	(69)	(69)	(69)	(69)	(69)	(69)	(68)		(53)	(65)
Adipose fin	0.117	0.116	0.206	0.149	–0.101	0.050	0.295	0.004	0.220		0.408**
	(53)	(53)	(53)	(53)	(53)	(53)	(53)	(52)	(53)		(50)
Caudal fin	–0.001	0.073	0.111	0.148	0.162	0.322	0.319	0.264	0.173	–0.008	
	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(64)	(65)	(50)	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ in sequential Bonferroni tests (Rice 1989).

(5) Measuring selection

Selection was estimated for each of two components of reproductive success: proximity to nest (ranked in each trial in order of actual distance) and sneaking success (binary: one or zero). The value of each individual in the components was divided by the mean value based on five individuals in the corresponding trial for transformation to relative success. To detect directional selection, I estimated the standardized linear selection gradients for the set of characters measured for all individuals (Lande and Arnold, 1983; Arnold and Wade, 1984). Standardized linear selection gradients were calculated as the partial regression coefficients from the multiple regression of relative success on the set of standardized characters. To determine whether selection was non-linear (stabilizing or disruptive), I estimated standardized non-linear selection gradients for the characters (Lande and Arnold, 1983). Standardized non-linear selection gradients were calculated from the multiple regression including the linear and quadratic terms of the standardized characters. Each partial regression coefficient of the quadratic term is the non-linear selection gradient for the character.

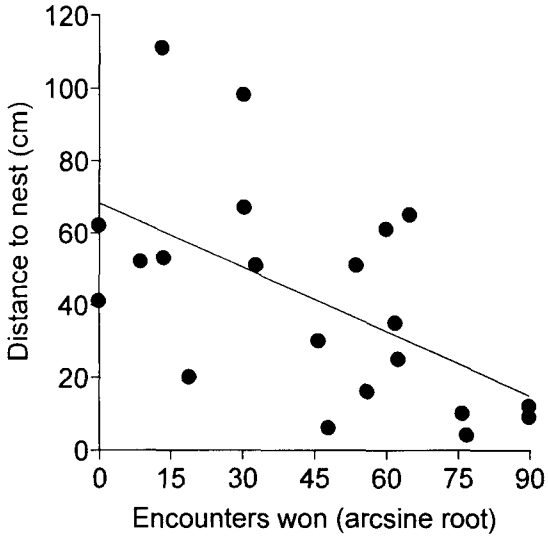

Fig. 2-2. Relationship between the percentage of aggressive encounters won and the distance to the nest in satellite parr. Regression line: $y = 68.2 - 0.6x$, $R^2 = 0.32$, $F_{1,19} = 8.96$, $P < 0.01$.

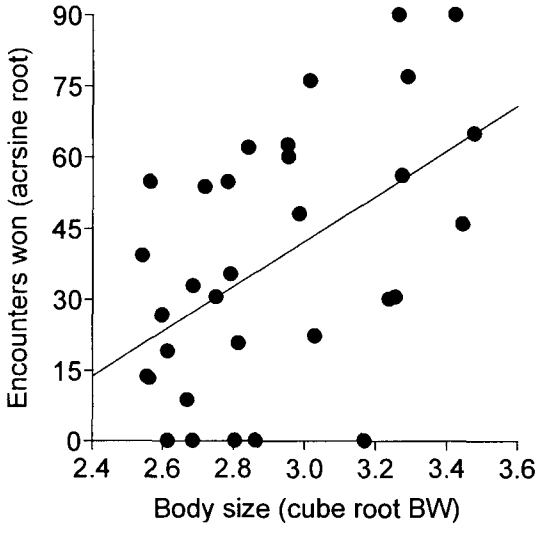

Fig. 2-3. Relationship between body size and the percentage of aggressive encounters won by parr which participated in aggressive interactions. Regression line: $y = -100.5 + 47.5x$, $R^2 = 0.27$, $F_{1,30} = 10.84$, $P < 0.01$.

(6) Statistical analyses

The frequency of aggressive encounters (number of encounters per 10 minutes) and the percentage of encounters won (number of victories/number of encounters) were ln- and arcsine root-transformed for parametric testing, respectively. Analyses of variance (ANOVA) were performed for comparison among treatments. Pearson's correlation coefficient was used for examining some correlations. A large number of the correlation coefficients among characters were tested by sequential Bonferroni tests for multiple comparisons (Rice, 1989). The significance tests of selection gradients were performed with a randomization test, due to the non-normality of the relative success (Manly, 1997). In this test, I constructed 999 pseudo-data matrices by randomizing the relative success of individuals for their character set in each matrix. The expected distribution of each multiple regression coefficient was generated from the 999 coefficients of the matrices, and then used for quantifying the unlikelihood of the coefficient of the original data matrix.

2.3 Results

(1) Reproductive behavior of masu salmon with special reference to parr

In the treatments including two or four migratory males, the dominants completely defeated the other migrants immediately after the initiation of trials, and the subordinates never participated in the spawning aggregation, except in the case of one migrant in one trial. No aggressive interactions were observed between subordinate migrants and parr, or between females and males, including parr. Although the dominants exhibited 'threatening' behavior, quickly shaking their head toward parr swimming close to their heads, this behavior rarely developed into more intense aggression, such as chases and bites. Therefore, the frequency of aggressive behaviors from dominant migrants to parr was low (mean ± SD = 1.9 ± 1.7 times per 10 min, N=14), and did not differ among the treatments ($F_{2,11} = 0.37$, $P = 0.70$). Neither the number of satellite parr nor the number of parr successful in sneaking was affected by the treatments (number of satellites, $F_{2,11} = 1.56$, $P = 0.25$; number of successful parr, $F_{2,11} = 0.82$, $P = 0.47$).

Aggressive encounters among parr were frequently

Table 2-3. The standardized selection gradients on the morphological characters of parr for the two components of reproductive success.

Component of reproductive success	Body size	Body height	Caudal-peduncle depth	Snout length	Pectoral fin	Pelvic fin	Anal fin
Proximity	0.32***	0.04	−0.03	0.08	0.11	−0.03	−0.10
Sneaking success	0.43*	0.04	−0.05	0.25	0.19	−0.22	−0.02

* $P < 0.05$; *** $P < 0.001$ in randomization tests (Manly 1997).

observed (mean ± SD = 7.2 ± 6.3 times per 10 min, N = 14) and resulted in the formation of dominance hierarchies. Twenty five (36%) parr were present as satellites around females at spawning in 12 (86%) of 14 trials, and 12 (17%) parr succeeded in sneaking in 8 (57%) trials. The distance from each satellite parr to the nest at spawning ranged from 4–111 cm (mean ± SD = 42.3 ± 28.9, N = 25). The distance to the nest was negatively related with the percentage of aggressive encounters won (linear regression, $R^2 = 0.32$, $F_{1,19} = 8.96$, $P < 0.01$; Fig. 2-2). The percentage of encounters won was positively related with body size (linear regression, $R^2 = 0.27$, $F_{1,30} = 10.84$, $P < 0.01$; Fig. 2-3).

(2) Selection on morphology of parr

A significant standardized linear selection gradient was found for body size, both for proximity to the nest and for sneaking success (proximity: $\beta = 0.32 \pm 0.08$, $P < 0.001$; sneaking success: $\beta = 0.43 \pm 0.24$, $P < 0.05$; Table 2-3). The standardized non-linear selection gradients (i.e., multiple regression coefficients for quadratic terms) were not statistically significant ($P > 0.05$).

2.4 Discussion

I expected selection to favor smaller body size in mature male parr during breeding as small size may be suitable for sneaking (Gross, 1985). In contrast to my expectations, I found that selection favors larger body size which allows a position closer to the nest to be gained and consequently increases sneaking success. Behavioral observations showed that selection on body size acted through competition within parr rather than aggression of the dominant migratory male. Like other salmonid species (Maekawa, 1983; Myers and Hutchings, 1987; Hutchings and Myers, 1988), aggressive encounters within parr were observed in masu salmon and this resulted in the formation of a dominance hierarchy according to body size. However, unlike other salmonids (Jones, 1959; Maekawa, 1983; Maekawa and Onozato, 1986; Myers and Hutchings, 1987), the migratory males of masu salmon rarely attacked parr although the dominant migrants quickly chased away subordinates from the spawning aggregations. Therefore, I suggest that the size-structured dominance hierarchy within parr is the main cause of selection favoring large body size of parr. The indifference of migrants to parr may also have facilitated the advantage for parr in being larger to gain closer access to the nest.

In this experiment, I studied the reproductive success of parr during only a short portion of the breeding season (i.e., one spawning bout). In natural populations, male salmon (both parr and migrants) become exhausted as the breeding season progresses (Jonsson *et al.*, 1991), and environmental conditions may vary. Moreover, no components of reproductive success other than proximity to nest and sneaking success (e.g., number of females sneaked, and fertilization success based on DNA paternity analysis) were examined. Nevertheless, selection favoring larger parr is probably invariable, because the contribution of body size toward fertilization success in the successive spawnings in Atlantic salmon parr (Thomaz *et al.*, 1997) and toward the reproductive activity and mating success through the entire breeding season in male fluvial Dolly Varden (Kitano, 1996) has been documented.

Given this, why do mature male parr not attain even larger body size? First, selection pressure may change as parr evolve toward larger body size. This study showed that selection pressure by migrants was absent because migrants were almost indifferent to satellite parr. However, migrant indifference may decrease as parr size increases to a point where they become potential competitors of migrants. In a natural stream running into Toya Lake, dominant migratory masu salmon aggressively chase jack-like males of intermediate size between migrants and parr, along with subordinate migrants (T. Yamamoto and K. Edo, unpublished data). The sneaking success of jack-like males appears to be much lower than that of parr. Therefore, the benefit of being larger, or enjoying dominance within parr, may be canceled or exceeded by the cost of increased aggression by migrants (disruptive selection, Gross, 1985; Fleming and Gross, 1994). The potentially conflicting selection pressures on body size may have driven evolution to an optimal body size for parr. Second, ecological constraints may

prevent parr from an evolutionary response to selection. A major constraint for parr may be restricted resource abundance in their habitat. In the fluvial stage of the early life-history of salmonids, populations are regulated by density-dependent mechanisms (Elliott, 1985, 1990), and resource use is unequal among individuals (Nakano, 1995). These phenomena suggest that resources for individual growth are limited in streams..

This study indicates that selection pressures on the morphology of precocious male parr differs from that on migratory males, partially supporting my original expectations. In migratory males, the elongated, hooked snout and humped back, which represent dominance in fighting as well as body size, apparently contribute to reproductive success (coho salmon: Fleming and Gross, 1994; sockeye salmon: Quinn and Foote, 1994). In contrast, snout length and body height did not contribute to reproductive success in masu salmon parr. Therefore, conspicuous sexual characters may not have evolved in mature male parr. The exaggerated sexual characters associated with male-male competition should result in a high energy cost for individuals. In dimorphic brown trout (*Salmo trutta*), the somatic energy content is lower in small resident fish than in large migrants, probably due to the poor resources in streams (Jonsson and Jonsson, 1997). Therefore, mature male parr should invest energy toward improving behavioral and/or physiological characters concerned with reproductive success rather than toward developing morphological characters which make no contribution to sneaking success. The quantitative and qualitative characteristics of gametes of Atlantic salmon parr may be such a strategic energy allocation. Atlantic salmon parr have a larger gonadosomatic index than migratory males (Gage *et al.*, 1995; Fleming, 1996; but see Jonsson and Jonsson, 1997) and their ejaculated sperm are more motile and survive longer than those of migrants (Gage *et al.*, 1995). Such gamete characteristics have been shown for sneakers in other fish species as well (de Fraipont *et al.*, 1993; Taborsky, 1998). The results of my study and the above information suggest that mature male parr increase their reproductive success through sperm competition rather than behavioral competition (Gage *et al.*, 1995; see also Simmons *et al.*, 1999).

Chapter 3
Physiological Characteristics: Energy Allocation Pattern*

3.1 Introduction

Reproduction is most severely exhausting event of life for many organisms, thereby reproducing organisms are often faced with energetic constraints. As a result, organisms may have to invest in current reproduction at the cost of future growth, survival, and reproduction (Roff, 1983, 1992; Wootton, 1984; Stearns, 1992). Moreover, they may also have to allocate the finite energy into different reproductive requirements (e.g., intrasexual competition, courtship behavior, and parental care) at a time of reproduction (Wootton, 1990). In theory, selection favors the pattern of energy allocation that maximizes individual fitness (Sibly and Calow, 1986). Therefore, if selection acts on individuals heterogeneously, differential energy allocations among individuals may evolve. For example, males often invest higher into sexual ornamentation and display at the higher risk of mortality compared with females (Andersson, 1994). Even within sexes, energy allocation of individuals may differ as a response to the heterogeneity of selective pressure. Alternative life-history forms may be the case that exhibits differential energy allocations within sexes (Taborsky, 1994, 1998).

In many salmonid species, males have alternative life-history forms associated with alternative reproductive tactics: migrants and mature parr (Balon, 1980; Groot and Margolis, 1991; Elliott, 1994). Migratory males mature at larger body size and older age after seaward/lakeward migration, and contest intensely for the dominant position to the female (see 2.3 Results). Postbreeding mortality of migrants is great (Fleming, 1996), and the extreme case is fatal death in semelparous Pacific salmon (*Oncorhynchus* spp.). In contrast, mature male parr attain sexual maturity precociously with smaller size in the natal stream, and sneak into migrant's mating mostly in a group (see 2.3 Results). The subsequent mortality of mature parr may be lower than that of migrants (Fleming, 1996) and, more clearly in semelparous Pacific salmon, not all mature parr die after breeding (Tsiger *et al.*, 1994 in masu salmon, *O. masou*; Unwin *et al.*, 1999 in chinook salmon, *O. tshawytcha*).

Given the salmonid breeding system, competitively superior migrants and unfavorable mature parr may be expected to invest more energy in competitive advantage (body condition and sexual characters) and fertilization efficiency (gamete production), respectively (Taborsky, 1994). That is, gonadal investment relative to somatic investment may be higher in mature parr than in migrants. This intuitive expectation is theoretically confirmed and refined by sperm competition game models (Parker, 1990a, b; Gage *et al.*, 1995). The opposite prediction may be drawn from the trade-off between current reproduction and the subsequent life-history demands of growth, survival, and reproduction (Roff, 1992; Stearns, 1992). This life-history trade-off predicts that, in general, iteroparous organisms invest less reproductive effort in a bout of reproduction than semelparous species. If the postbreeding survival is higher for mature parr than migrants (Fleming, 1996), mature parr may possess more iteroparous life-history features than migrants. That is, lower reproductive effort (i.e., relative gonadal investment) of mature parr in a reproductive bout may be expected. Such a contrast of relative gonadal investment, or energy allocation to gonadal development, between life-history forms may be striking in Pacific salmon because the migrants

* The Chapter 3 was reproduced from *Koseki, Y., and K. Maekawa. 2002. Differential energy allocation of alternative male tactics in masu salmon Oncorhynchus masou.* Canadian Journal of Fisheries and Aquatic Sciences *59: 1717-1723*, with kind permission of National Research Council Canada.

inevitably die as the cost of breeding as described above. So far, empirical data of the energy allocation of alternative life-history forms were obtained from two semelparous salmonids, Atlantic salmon, *Salmo salar* and brown trout, *S. trutta* (Gage *et al.*, 1995; Jonsson and Jonsson, 1997). Those results are however contradictory, the former showing the higher relative gonadal investment of mature parr but the latter giving the opposite. As far as I know, no comparison of relative gonadal investment of alternative life-history forms has been conducted in Pacific salmon.

The objectives of this study are (1) to examine the relative gonadal investment of mature male parr and migratory males in a Pacific salmon, masu salmon, (2) to evaluate which of sperm competition and life-history trade-off is the predominant factor of energy allocation of the two life-history forms, and (3) to give an understanding of the contradictory results of the previous studies comparing with the results of the present study. For these objectives, the somatic and gonadal investment were estimated in mass and energy for the two forms in the lake-run populations of masu salmon.

Lake-run populations were selected because they are considered advantageous for the comparison of energy allocation between the life-history forms in the following points. First, returning migrants are generally required to allocate their energy not only to competition and fertilization but also to upstream migration (Fleming and Gross, 1989). Lake-run migrants may however spend less energy for upstream migration because, unlike anadromous (sea-run) migrants for which migration takes several months (Mayama, 1992; Kiso, 1995; Tago, 2000), they undertake a relatively short migration from feeding habitat (lake) to spawning sites (inlet streams) and even enter the streams within a few weeks of the breeding (Yamamoto *et al.*, 2000). Therefore, the somatic and gonadal investment of lake-run migrants may more clearly reflect the energy allocation to competitive advantage and fertilization efficiency than that of anadromous migrants. Second, since the lakes are not as favorable environments as the ocean for the growth of migrants (Tamate and Maekawa, 2000a), surplus energy is not likely to mask the trade-off between the somatic and gonadal investment in lake-run migrants. Except such differences in migrants, the general life histories of the two forms of lake-run masu salmon are the same as those of anadromous populations (Tamate and Maekawa, 2000b).

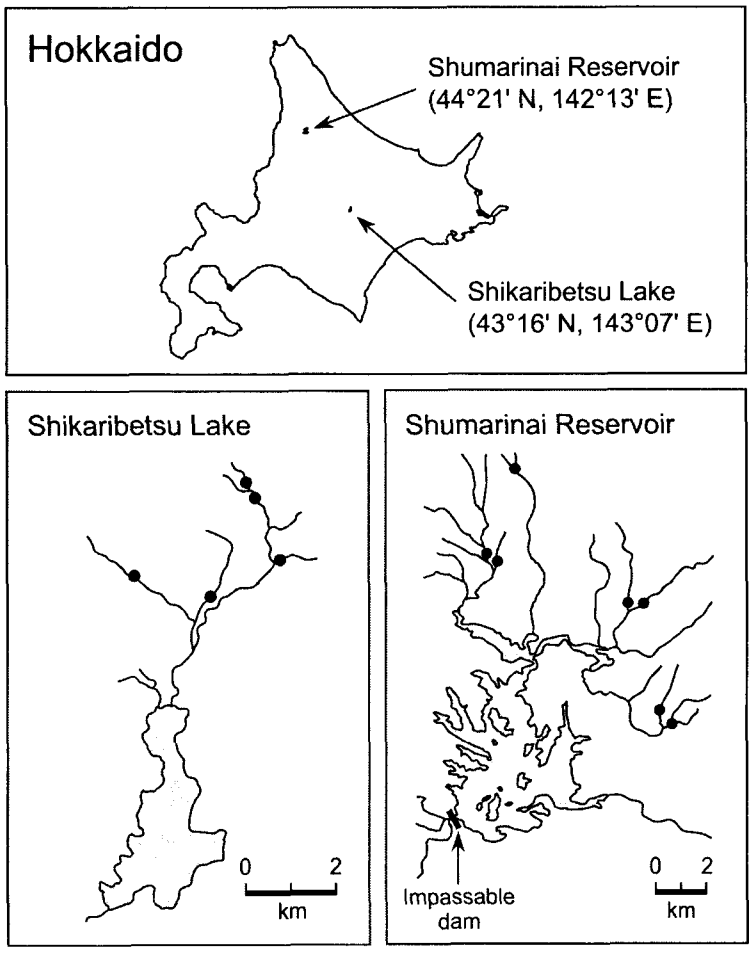

Fig. 3-1. Location of the two masu salmon populations and the sampling sites (•) in each population.

3.2 Materials and Methods

(1) Sample collection

Sample fish were collected from two lake-run populations of masu salmon in Hokkaido, Japan: Shikaribetsu Lake (43°16' N, 143°07' E) and Shumarinai Reservoir (44°21' N, 142°13' E) (Fig. 3-1). The Shikaribetsu population was established by introduction in 1980s and the Shumarinai population originates from native masu salmon landlocked by damming in 1940s (Tamate and Maekawa, 2000a). From 26 August to 4 September 1999, mature male parr and migratory males were collected on the spawning grounds of the two populations using an Electrofisher (Model 12-B POW, Smith-Root Inc., Vancouver, Washington, USA) (Fig. 3-1). The timing of sampling was just before the commencement of breeding because no nests was observed nor females damaged on the caudal fin (due to nest construction) was captured during sampling. All the fish sampled were sealed in polyethylene bags and stored at –20°C until analysis.

(2) Energy content

Energy stores were measured for 154 mature parr and 28 migrants in Shikaribetsu Lake, and 88 and 11 in Shumarinai Reservoir. After partly defrosted, the fish were divided into gonad (testes) and soma (all body part excluding testes). At the time, otoliths were extracted for age determination (Jones, 1992), and consequently age compositions of the two populations were confirmed as similar (G = 2.25, df = 2, P = 0.33 for mature parr; G = 0.02, df = 2, P = 0.88 for migrants). The separated soma and testes were weighed (nearest 0.01 g except for soma of migrants, for which nearest g) and then homogenized using a meat grinder and blender. Within the two forms, soma and testes of similar-sized individuals were sometimes pooled to obtain sufficiently large samples for a series of analyses (ca. 20 g).

Somatic and gonadal energy contents were determined by measuring water, ash, fat, and protein in the homogenates (proximate analysis, Brett, 1995). I did not measure carbohydrates because its amount in tissues of salmonid fishes is small enough to be negligible (< 0.5%, Jonsson and Jonsson, 1997; Jonsson *et al.*, 1997). Water was measured by drying about 20-g homogenate for 36 h in a freeze dryer (FD-5N, TOKYORIKAKIKAI Co., Tokyo, Japan). Ash was determined by combustion of 2-g dried sample at 550°C for 12 h. Fat was determined after extracting 2-g dried sample for 8 h with diethyl ether in a Soxhlet fat extractor (for appropriateness of the solvent and extraction time, see Dobush *et al.*, 1985). Ash and fat proportions by dry mass were multiplied by the proportion of dry material in the original homogenate to translate into the proportions by wet mass. Protein proportion was estimated by subtracting the proportion of water, ash, and fat from one (Hendry and Berg, 1999; Hendry *et al.*, 1999). Somatic and gonadal energy contents per unit mass ($kJ \cdot g^{-1}$) were then estimated by multiplying the proportions of fat and protein by the energy equivalent (36.4 $kJ \cdot g^{-1}$ for fat and 20.1 $kJ \cdot g^{-1}$ for protein, Brett, 1995) and adding the products. Total energy content (kJ) of the soma and testes of each fish was calculated as the product of the energy content per unit mass and total mass. Estimation of energy content using proximate analysis with energy equivalents is the standard approach in studies on reproductive energetics of salmonids (see Appendix in Hendry and Berg, 1999), and gives similar results as that using bomb calorimetry (Craig *et al.*, 1978).

(3) Analyses

Prior to analysis, all variables concerning soma and testes were $\log_{10}$-transformed. The effects of life-history form and population on the fat, protein, and energy content per unit mass of soma and testes were examined using two-way ANOVAs at first. However, owing to the significant effect of interaction term between life-history form and population (see 3.3 Results), I tested the effect of life-history form on the mass-specific fat, protein, and energy content in soma and testes by population using simple factorial ANOVA.

Gonadal investment (testes mass and testes energy) relative to somatic investment was compared among life-history forms and populations. Despite the gonadosomatic index (GSI, gonad weight as a percentage of somatic weight) is typically used as the measure of reproductive investment, particularly in fish (Taborsky, 1994), the use of GSI is not appropriate because it does not take into account the allometric relationship between the soma and gonad sizes (Wootton, 1990; Roff, 1992). When the exponent of allometric function (slope of log gonad size on log soma size) is more/less than one, GSI increases/decreases with an increase in soma size independent of some factor of concern. I avoided this problem by employing ANCOVA, in which the effects of life-history form and population on log gonadal investment were tested with the covariate effect of log somatic investment. Prior to this ANCOVA, homogeneity of the slope of log gonadal investment on log somatic investment (S) among life-history forms (L) and populations (P) was checked as nonsignificant effects of the interactions (S × L, S × P, and S × L × P) in the preliminary model including these terms with the others (S, L, P, L × P) composing the final model. Moreover, for graphical illustration, the gonadal investment of each male was adjusted to the grand sample mean somatic investment by the following equation (Ihssen *et al.*, 1981):

$$G_a = G_o(S_m/S_o)^b,$$

where G_a is the adjusted gonadal investment, G_o is the original gonadal investment, S_m is the grand sample mean somatic investment, S_o is the original somatic investment, and b is the common within-group slope obtained from the above ANCOVA.

3.3 Results

(1) Energy content

The effect of life-history form on energy content per unit mass of soma differed depending on population

(interaction term, $F_{1,147} = 27.70$, $P < 0.001$). In the Shikaribetsu population, migratory males had a lower energy content per unit mass of soma than mature male parr ($F_{1,94} = 64.55$, $P < 0.001$; Table 3-1). In the Shumarinai population, however, the two tactics had similar energy content per unit mass of soma ($F_{1,53} = 1.33$, $P = 0.254$). The lower somatic energy content of migrants in the Shikaribetsu population was caused by both of the lower fat and protein contents ($F_{1,94} = 79.62$, $P < 0.001$ for fat; $F_{1,94} = 20.47$, $P < 0.001$ for protein).

Similarly, the effect of life-history form on energy content per unit mass of testes differed between populations (interaction term, $F_{1,50} = 12.36$, $P = 0.001$). The mass-specific testes energy content of migrants was lower than that of mature parr in the Shikaribetsu population ($F_{1,33} = 27.67$, $P < 0.001$; Table 3-2) but not in the Shumarinai population ($F_{1,17} = 0.38$, $P = 0.545$). Both the lower fat and protein contents contributed the lower mass-specific testes energy content of the Shikaribetsu migrants ($F_{1,33} = 18.07$, $P < 0.001$ for fat; $F_{1,33} = 23.08$, $P < 0.001$ for protein).

(2) Gonadal investment

Neither slopes of testes mass nor testes energy on the corresponding somatic values differ among life-history forms and populations (all interaction terms, $P > 0.69$ for mass; $P > 0.49$ for energy; Fig. 3-2). The subsequent ANCOVAs revealed that both testes mass and energy for the corresponding somatic values were higher in mature parr than in migrants without the confounding effect of interaction term between life-history form and population (Table 3-3; see also Fig. 3-3). Population did not affect either testes mass or testes energy. Testes mass (mean ± SD) adjusted to the grand sample mean somatic mass of 62.4 g ($N = 281$) was 8.2 ± 2.0 g ($N = 242$) for mature male parr and 5.0 ± 1.2 g ($N = 39$) for migratory males (Fig. 3-3). The corresponding value of testes energy (adjusted to 267.7 kJ) was 29.0 ± 7.6 kJ for mature parr and 19.0 ± 4.4 kJ for migrants.

3.4 Discussion

Migratory male masu salmon in Shikaribetsu Lake had a lower energy content per unit mass of testes than mature male parr, while the mass-specific testes energy content of migrants in Shumarinai Reservoir was similar to that of mature male parr. However, the lower mass-specific testes energy content of the Shikaribetsu migrants does not appear to be a strategic gonadal investment because it was associated with the lower

Table 3-1. Proximate composition (by wet mass) of the somatic tissues of alternative male life-history forms (mature parr and migrants) in two lake-run masu salmon populations.

Population Life history (N)	Water (%)	Ash (%)	Fat (%)	Protein (%)	Energy ($kJ \cdot g^{-1}$)
Shikaribetsu					
Mature parr (68)	76.9 ± 1.0	2.6 ± 0.2	2.6 ± 0.7	17.9 ± 0.5	4.5 ± 0.3
Migrants (28)	79.2 ± 1.4	2.3 ± 0.4	1.2 ± 0.9	17.3 ± 0.7	3.9 ± 0.4
Shumarinai					
Mature parr (44)	78.1 ± 1.3	2.6 ± 0.2	2.5 ± 1.0	16.8 ± 0.6	4.3 ± 0.4
Migrants (11)	78.1 ± 0.9	2.0 ± 0.3	2.9 ± 1.0	17.0 ± 0.4	4.5 ± 0.4

Values are mean ± SD.

Table 3-2. Proximate composition (by wet mass) of the testes of mature male parr and migratory males in two lake-run masu salmon populations.

Population Life history (N)	Water (%)	Ash (%)	Fat (%)	Protein (%)	Energy ($kJ \cdot g^{-1}$)
Shikaribetsu					
Mature parr (18)	80.0 ± 0.9	2.9 ± 0.3	1.5 ± 0.1	15.6 ± 0.9	3.7 ± 0.2
Migrants (17)	81.6 ± 1.2	3.1 ± 0.3	1.3 ± 0.1	13.9 ± 1.1	3.3 ± 0.2
Shumarinai					
Mature parr (9)	78.5 ± 1.5	3.3 ± 0.4	1.5 ± 0.1	16.8 ± 1.3	3.9 ± 0.3
Migrants (10)	78.0 ± 1.3	3.2 ± 0.3	1.4 ± 0.1	17.3 ± 1.3	4.0 ± 0.3

Values are mean ± SD.

mass-specific somatic energy content. Moreover, the lower mass-specific energy content in the soma and testes resulted from both of the lower fat and protein contents. Therefore, the lower mass-specific somatic and gonadal energy contents of the Shikaribetsu migrants may have simply reflected lower food availability in the lake, although the difference of growth rate between the two populations is small and nonsignificant in females (Tamate and Maekawa, 2000a). By comparing alternative life-history forms in different two populations, I revealed that the difference in mass-specific energy content between life-history forms could change among populations. Moreover, in anadromous populations, migratory males may have a higher mass-specific energy content than mature male parr because of food abundance in the marine environments. The higher mass-specific somatic energy content of migrants in anadromous populations of brown trout is consistent with this expectation (Jonsson and Jonsson, 1997). Unfortunately, in masu salmon, there is no data of the energy contents of the two life-history forms in anadromous populations.

Sperm competition theory predicts that mature male parr unfavorable for fighting for mates invest relatively more into sperm production for improving fertilization efficiency in sneaking (Parker, 1990a, b; Gage *et al.*, 1995). On the contrary, life-history trade-off between current reproduction and the future survival and reproduction predicts lower investment in sperm production for mature parr that may survive and breed in the following years. In this study, mature male masu salmon parr and the migratory males presented the distinctive energy allocation patterns consistently in the two populations, as the mean gonadal, relative to somatic, investment of mature parr was 1.6-times higher than that of migrants in mass and 1.5-times in

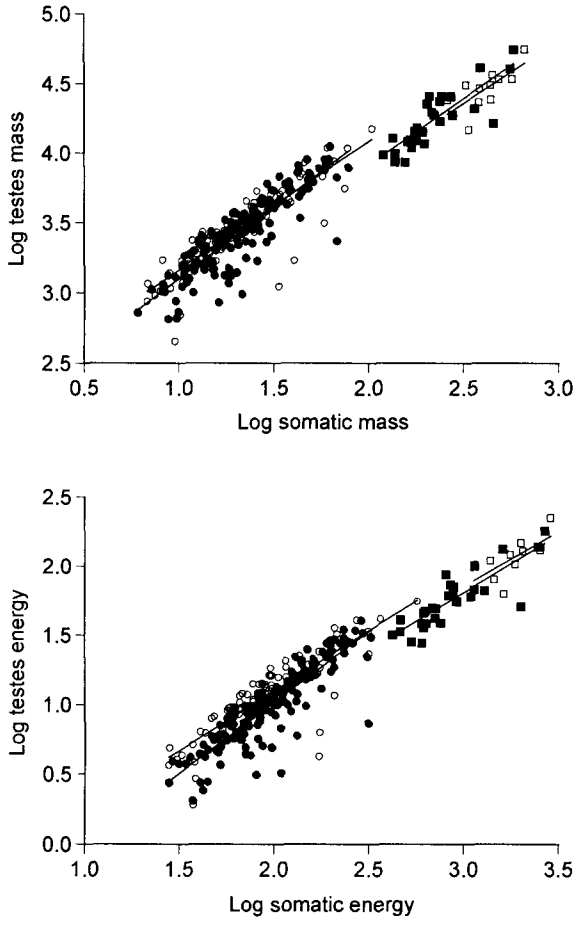

Fig. 3-2. Allometric relationships between (a) the somatic and testes mass, and (b) the somatic and testes energy in mature parr (•) and migrants (■) of Shikaribetsu Lake, and mature parr (○) and migrants (□) of Shumarinai Reservoir. Slopes of regression did not differ among groups (see text).

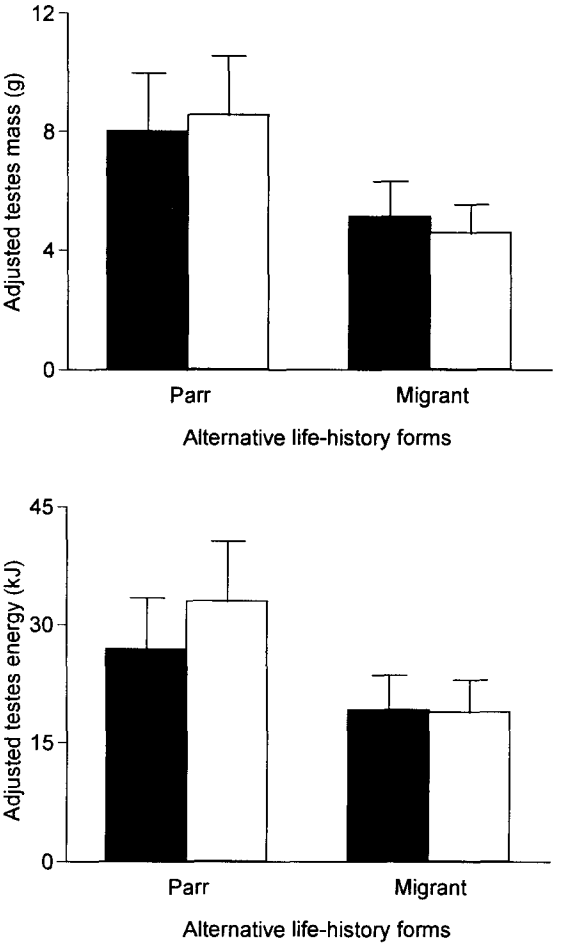

Fig. 3-3. Comparison of the mean (± SD) adjusted (a) testes mass and (b) testes energy of mature parr and migrants in Shikaribetsu Lake (■) and Shumarinai Reservoir (□). The original testes mass and energy of each fish was adjusted to the grand sample mean somatic mass (62.4 g) and energy (267.7 kJ), following the equation of Ihssen *et al.* (1981). Numbers above bars refer to sample sizes.

Table 3-3. ANCOVAs for the effects of alternative life-history form (mature parr or migrants) and population (Shikaribetsu Lake or Shumarinai Reservoir) on log-transformed testes investment (mass and energy) with the covariate effect of log-transformed somatic investment. See also Fig. 3-3.

Testes investment Source of variation	*df*	*MS*	*F*	*P*
Testes mass				
Somatic mass (S)	1	14.537	871.424	< 0.001
Life-history forms (L)	1	0.420	25.169	< 0.001
Population (P)	1	0.002	0.123	0.726
L × P	1	0.038	2.279	0.132
Error	276	0.017		
Testes energy				
Somatic energy (S)	1	14.266	819.779	< 0.001
Life-history forms (L)	1	0.309	17.773	< 0.001
Population (P)	1	0.049	2.809	0.095
L × P	1	0.056	3.239	0.073
Error	276	0.017		

energy. This result fits well with the prediction from sperm competition, not from the life-history trade-off. Therefore, the present study suggests that sperm competition is of predominance as the evolutionary cause forming the differential energy allocations of alternative life-history forms in masu salmon.

Similar result has been shown in iteroparous Atlantic salmon as the mean GSI (wet mass %) of mature parr was 2-fold higher than that of migrants (Gage *et al.*, 1995). Moreover, mature parr have a greater concentration of spermatozoa, sperm motility, and sperm life span, which may also show the higher gametic investment of parr for improving fertilization efficiency (Daye and Glebe, 1984; Gage *et al.*, 1995). Empirical supports for the significance of sperm competition on gametic investment of males have also been provided from several other species (de Fraipont *et al.*, 1993; Schärer and Robertson, 1999; Simmons *et al.*, 1999). Contradictory results have been provided from the polymorphic brown trout, of which resident males (i.e., mature male parr, Elliott, 1994) have a similar GSI to that of migrants in energy (kJ %) and even a lower GSI than migrants in mass (Jonsson and Jonsson, 1997). Jonsson and Jonsson (1997) gave an interpretation that somatic investment may be important to residents as well as to migrants because even small residents compete each other for proximity to the female. However, this interpretation is probably inappropriate because competition among mature parr equally occurs in both masu and Atlantic salmon (Chapter 2; Hutchings and Myers, 1988).

Instead, the lower gonadal investment of resident brown trout and the consequent discrepancy in gonadal investment between the salmon and trout may be explained by the degree to which residents or mature parr are specialized in sneaking and thus affected by sperm competition. The adoption of sneaking behavior depends greatly on the relative body size of individual to potential competitors in the breeding aggregation. That is, the smaller body size, the more probability the individual acts as sneaker. In the brown trout, because the total somatic energy contents, or body sizes, of residents and migrants overlap largely (Fig. 3 in Jonsson and Jonsson, 1997), residents have almost fair chance to mate as dominant male. However, in both masu and Atlantic salmon of which alternative life-history forms are completely dimorphic and never overlap the body sizes (Fig. 3-2 in this study; Fig. 2 in Gage *et al.*, 1995; see also Table 1 in Hutchings and Myers, 1988), sneaking is the only way for mature parr to mate with the female. Therefore, mature male parr of masu and Atlantic salmon may be affected by sperm competition more than resident brown trout and thus selected to invest more into gonadal development or gamete production.

Chapter 4
Behavioral Characteristics: Breeding Behavior under Different Stream Environments*

4.1 Introduction

IIt has been known that mature male parr or jacks often hold their positions in or beside 'refuges' such as rocks, debris, and shallow areas (Maekawa, 1983; Gross, 1985, 1991). This behavior may be advantageous to escape aggression from the mating pair

* The Chapter 4 was reproduced from *Koseki, Y., I. Koizumi, H. Kobayashi, and K. Maekawa. 2002. Does the refuge availability influence the spawning behavior of mature male parr in salmonids? A test in the Miyabe charr.* Environmental Biology of Fishes *64: 87-93*, with kind permission of Springer Science and Business Media.

and from other participants while remaining close to the nest. In this context, Gross (1991) suggested that the abundance of these refuges in the breeding ground limits the opportunities for sneaking, and predicted that increased refuges would be associated with the increase of the frequency of jacks in coho salmon (*Oncorhynchus kisutch*) populations. However, no study has yet investigated whether sneaking behavior and success depends on the availability of refuges. In this study, I hypothesized a behavioral mechanism for the benefit of high refuge availability to the sneaking success, based on several predictions of previous studies (Gross, 1985, 1991). This hypothetical mechanism was tested in mature male parr of the Miyabe charr, *Salvelinus malma miyabei*, by conducting a breeding experiment and also by analyzing natural spawning.

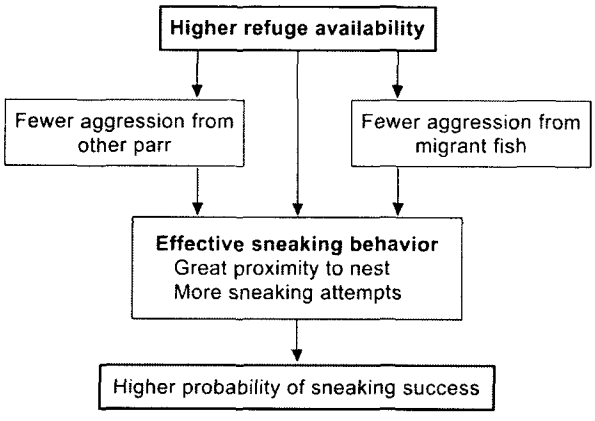

Fig. 4-1. Hypothesized behavioral mechanism for the effect of the refuge availability on the sneaking success of mature male parr.

4.2 Materials and Methods

(1) Fish studied

Miyabe charr (*Salvelinus malma miyabei*) inhabit Shikaribetsu Lake, central Hokkaido, Japan (43°16' N, 143°07' E) and its inlet rivers. They (both males and females) have alternative life histories, either 'migrants' which migrate to the lake to mature at larger body size and older age (3+ or over) or 'residents' which mature in the natal streams at smaller size and younger age (1+ or over) (Maekawa, 1984). As in other salmonid species, alternative life histories are associated with different reproductive tactics in males, where large migrant males fight for mates while small resident males (i.e., mature male parr) attempt to sneak into the nests of migrant pairs (Maekawa, 1983). An average of 3.0 mature parr (range: 0–5) may aggregate around a migrant pair, staying still on the bottom or hiding in refuges such as plants or woody debris but sometimes being involved in aggressive interactions with the paired male and female or the other parr (Maekawa, 1983). Females usually make 1–3 nests during the breeding season and oviposit 1–3 batches in each nest (Maekawa and Hino, 1990). Mature male parr sneak into the nest not only to fertilize eggs but also to eat them (Maekawa, 1983; Maekawa and Hino, 1990). Breeding activity lasts from September to November (Maekawa, 1984).

(2) Predictions

My hypothetical behavioral mechanism providing a benefit of high refuge availability on sneaking success is composed of the following components (Fig. 4-1). First, mature parr suffer from aggression of the migrant pair and also from the other parr during spawning activities (Jones, 1959; Maekawa, 1983; Hutchings and Myers, 1985; see also Chapter 2). If refuges are

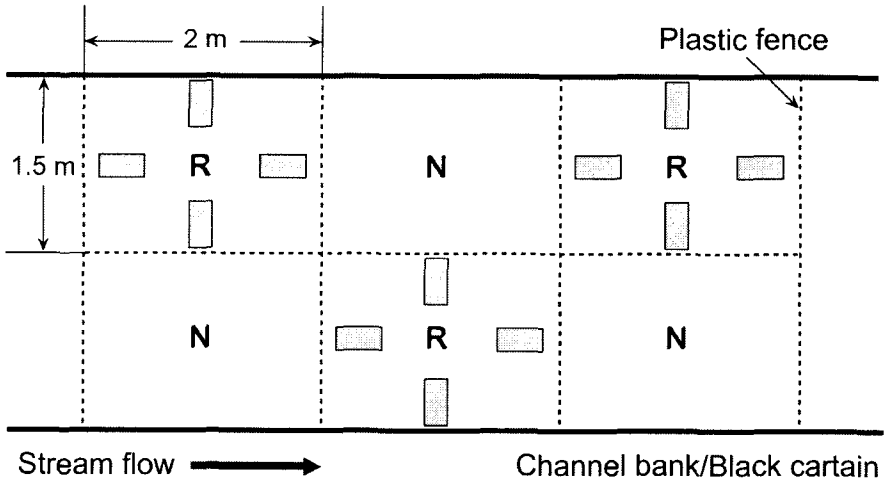

Fig. 4-2. Schematic diagram of the enclosures in the experimental channel. In each enclosure, the refuge (R) and no-refuge (N) treatments were conducted by turns. Four building blocks (39 × 18 × 10 cm) were placed into the enclosures for the refuge treatment. Black vinyl curtains were hung along the channel banks for observing fish without disturbance.

available, mature male parr may be less subjected to the aggressions from other fish (Gross, 1985). In juvenile brown trout, for instance, the presence of refuges reduces aggressions among individuals through visual isolation (Sundbaum and Näslund, 1998). Second, refuges may favor sneaking behavior directly or indirectly through the decrease in aggression from the other fish. Specifically, refuges may allow parr to hold positions close to the nest (Gross, 1984, 1985) and/or intrude into the nest more easily so that parr exercise more sneaking attempts. Finally, such an effective sneaking behavior may in turn result in a high probability of sneaking success (i.e., successful intrusion at spawning). In fact, sneaking probability increases with proximity to the nest in both Dolly Varden, *Salvelinus malma*, and Japanese charr, *Salvelinus leucomaenis* (Hino *et al.*, 1990; Maekawa *et al.*, 1994).

(3) Experiment

I conducted an experiment in an artificial rearing channel of the Shikaoi Fish Hatchery (SFH) from September 21 to October 15, 1999. The channel was approximately 20 m long by 4 m wide and covered with stream gravels. A regulated water flow was provided from Yamada Creek, a tributary of Yambetsu River, a natural spawning stream of the Miyabe charr, at a flow rate of 5.2 ± 0.5 cm/s (mean ± SD) with a depth of 16.2 ± 1.3 cm. I constructed six enclosures (2 m long, 1.5 m wide) in the channel using plastic fences and hung black vinyl curtains along the channel banks to prevent disturbance by observers (Fig. 4-2).

Two treatments were conducted: *refuge* and *no-refuge* (as a control). In the refuge treatment, four building blocks (38 cm wide, 19 cm deep, 10 cm high) with three tunnels on the larger vertical sides were placed in the enclosures as potential refuges for parr (Fig. 4-2). No blocks were provided for parr in the no-refuge treatment. In both treatments, I introduced one pair of migrants and five mature male parr in each enclosure. Prior to the introduction, I confirmed maturation of anaesthetized (2-phenoxyethanol) parr by gently pushing the abdomen and releasing a very small amount of semen. Parr were also individually identified by color combinations of small ribbon-tags (3 cm long, 2 mm wide) sewn on the back. The experimental fish were collected by an electrofisher (Model 12-B POW, Smith-Root Inc., Vancouver, Washington, USA) and a dip net throughout the experimental period from the Yambetsu River.

The fish were observed every hour between 0500 and 1700 (daytime) and between 1800 and 2200 (nighttime). In daytime, I observed the breeding behavior of migrant pairs. When the female began to excavate a nest while being courted by the male, I recorded aggressive behaviors (chases and bites) among fish for 10 min, and mapped the positions of every parr and nest. Every daytime hour, I recorded the pair and the attending parr by video camara to count the sneaking attempts (defined as intrusions into the nest before spawning) during 1 hour before spawning and to determine sneaking success (defined as an intrusion into the nest just at spawning). Intrusions for pecking eggs after spawning (Maekawa and Hino, 1990) were also observed but not counted as sneaking attempts because my primary concern in this study was whether the refuge availability affected the breeding behavior of parr. In the nighttime, I used a camcorder equipped with noctovision (CCD-TR290PK, Sony Corp., Tokyo, Japan) to observe whether the spawning occurred or not, but quantitative data was not to be recorded. After spawning all fish were removed and measured the body size (fork length nearest 0.1 mm). The enclosures were used for the two treatments by turns.

Six trials of the refuge treatment and eight trials of the control treatment were recorded yielding data on aggression and the parr's positions. Observations of sneaking attempts and sneaking success were available from 5 trials in both treatments, trials in which spawnings took place during daytime and were videotaped. There were no differences among treatments in fish (female, migrant male and mature parr) sizes in the trials where the breeding behavior was observed or spawning took place (one-way ANOVAs for females and migrant males, nested ANOVAs for parr where trials were nested within treatment, all $P > 0.05$).

(4) Analysis of video recordings of spawning groups in the wild

Observations of spawning behaviors in the wild were made on nine clear videotapes, each of which recorded an entire spawning group (a migrant pair and all of the attending parr) in Yamada Creek in 1984 or 1985. Some of the observations in 1985 were analyzed elsewhere (Maekawa and Hino, 1987, 1990). All aggressive and sneaking behaviors in the spawning groups were recorded for 13–50 (average: 28.6) min just before spawning, and the availability of refuges, proximity to the nest and sneaking success were determined for each parr. Refuges were classified into woody debris, rocks, undercut of stream bank, plant and shallow area (3, 2 ,2, 1, and 1 cases, respectively). Proximity to the nest was estimated relative to fish size, except for 15 parr in 4 spawning groups in which fish had not been captured and measured.

(5) Data analyses

All frequency data were log-transformed before parametric testing. In the experiment, I performed nested ANOVAs where trial was treated as random factor within the fixed factor of treatment, in order to compare the following behavioral data of mature male parr between the experimental treatments:

(1) frequency of aggressions from the migrant pair,
(2) frequency of aggressions from the other parr,
(3) frequency of sneaking attempts, and
(4) proximity to the nest. The number of parr successful in sneaking in each trial was compared between treatments by Mann-Whitney *U*-test. In the analysis of video recordings, the same four behavioral data as in the experiment were compared among mature parr that held a refuge or did not, using *t*-tests. Prior to the comparisons, allowing for the interdependence among

mature parr within the same spawning groups, I divided the original values by the mean value based on the individuals in the corresponding spawning group to transform into the relative values. The dependence of the sneaking success on the refuge availability was tested by Fisher's exact test.

4.3 Results

(1) Experiment

In the refuge treatment, total of 26 (86.7%) out of 30 mature male parr in 6 trials used the blocks as refuges at least once during the observation. The parr approached the pair (nest) from behind the block, waited to intrude into the nest beside the block, or fled from aggression by other fish into the block. In the no-refuge treatment, mature parr often froze motionless when attacked by the pair. Migrant pairs never showed further aggression toward parr adopting this posture.

The frequency of aggressive behaviors from migrant pair to parr was not different between the refuge treatment (mean ± SE = 7.18 ± 1.74 times per 10 min, $N = 30$) and the no-refuge treatment (8.06 ± 2.04 times per10 min, $N = 40$) (Table 4-1). Similar results (i.e., insignificant differences between treatments) were attained when the aggressions from migrant male and female were analyzed separately. The frequency of aggressions by the other parr was low in both treatments (refuge: 1.13 ± 0.37 times per10 min, $N = 30$; no refuge: 0.43 ± 0.12 times per10 min, $N = 40$), and did not differ significantly between treatments (Table 4-1). Proximity to the nest was greater in the refuge treatment (0.77 ± 0.09 m, $N = 28$) than in the no-refuge treatment (1.10 ± 0.08 m, $N = 40$) (Table 4-1). On the other hand, there was no significant difference in the frequency of sneaking attempts between treatments (refuge: 1.36 ± 1.10 times per10 min, $N = 25$; no refuge: 0.49 ± 0.22 times per10 min, $N = 25$; Table 4-1). As a result, the number of parr successful in sneaking in each trial did not differ between treatments (refuge: average = 0.8, range = 0–3; no refuge: average = 0.6, range = 0–1; $U = 11.5$, $N = 10$, $P = 0.82$).

Table 4-1. Results of mixed model nested ANOVAs for the fixed effects of experimental treatments (refuge and no refuge) on the behavior of mature male parr: frequency of aggression from migrant pair, frequency of aggression from other parr, frequency of sneaking attempts, and proximity to the nest.

Variable Source of variation	*df*	*SS*	*MS*	*F*	*P*
Freq. aggression from migrant pair					
Experimental treatment	1	0.002	0.002	0.012	0.914
Trial (nested within treatment)	12	2.010	0.167	0.541	0.879
Error	56	17.348	0.310		
Freq. aggression from other parr					
Experimental treatment	1	0.185	0.185	3.646	0.080
Trial (nested within treatment)	12	0.610	0.051	1.063	0.408
Error	56	2.678	0.048		
Freq. sneaking attempts					
Experimental treatment	1	0.210	0.210	1.245	0.297
Trial (nested within treatment)	8	1.347	0.168	2.884	0.012
Error	40	2.336	0.058		
Proximity to the nest					
Experimental treatment	1	1.990	1.990	7.573	0.017
Trial (nested within treatment)	12	3.163	0.264	1.214	0.298
Error	54	11.730	0.217		

Table 4-2. Results of *t*-tests for the effects of refuge availability on behavior of mature male parr in natural spawnings: frequency of aggression from migrant pair, frequency of aggression from other parr, frequency of sneaking attempts, and proximity to the nest.

Variable	*df*	*t*	*P*
Freq. aggression from migrant pair	30	0.144	0.886
Freq. aggression from other parr	30	0.173	0.864
Freq. sneaking attempts	30	0.331	0.743
Proximity to the nest	15	0.460	0.652

(2) Analysis of video recordings of spawning groups in the wild

A total of 32 mature male parr (average = 3.6, range = 1–6) participated in the 9 spawning groups analyzed. Nine (28.1%) of them used refuges in a manner similar to parr in the experiment. Neither the relative values of aggressions from the pair nor aggressions from other parr was different among the mature parr that either held a refuge or did not (Table 4-2). No significant differences were found among the refuge holders and the non-holders in the relative proximity to the nest and their relative frequency of sneaking attempts (Table 4-2). The percentage of successful sneakings was 77.8% (7 of 9) in the refuge holders and 91.3% (21 of 23) in the non-holders. Therefore, sneaking success did not depend on the availability of refuges ($P = 0.31$).

4.4 Discussion

I predicted that the availability of refuges would affect the sneaking behavior of mature male parr and therefore improve their sneaking success. The predictions were, however, not apparently fulfilled in mature parr of the Miyabe charr. First, I did not observe an effect of refuge availability on reducing aggressions from the migrant pair in the experiment. Miyabe charr parr fled from aggressions into the refuges in the presence of them. However, even in the absence of refuges, they could escape the escalation of the pair's aggression by freezing motionless, as has been observed in Atlantic salmon parr (Jones, 1959; Myers and Hutchings, 1987). Therefore, fleeing into refuges may not be more advantageous than the freezing behavior. In addition, the presence of refuges did not reduce aggression among the other parr. This may be explained by the parr's limited aggression among themselves under the pressure of a pair's aggression (Myers and Hutchings, 1987). Natural spawning data suggests that, also in nature, refuges provide mature male parr with little advantage in aggression avoidance; mature parr that held a refuge incurred the aggression from the pair and other parr as much as the parr that did not hold a refuge did. Second, I did not find any consistent effects of refuge availability on sneaking behavior either in the experiment or in the natural spawning. Although the experiment showed that mature parr potentially get closer to the nest in the presence of refuges, this effect did not operate in natural spawning probably due to the sparseness of the refuges convenient to nests in the stream. Moreover, the frequency of sneaking attempts did not change depending on refuge availability either under experimental conditions or under natural ones. Hence, a benefit of refuge availability for sneaking behavior is unlikely. Finally, refuge availability did not affect the sneaking success of parr either in the experiment or in nature. Taken together, opposite to the initial predictions, refuge availability is not beneficial to the breeding behavior and success of mature male parr at least in the Miyabe charr.

Nevertheless, the use of refuges may be advantageous to breeding male parr; benefits of high refuge availability may exist in some aspects of the breeding behavior different from those in this study. For instance, I have hypothesized in this study that the high refuge availability improves the probability of sneaking success, and therefore did not examine a more accurate measure of breeding success (i.e., fertilization success). Fertilization success depends on the following proximate factors: timing of nest entry and subsequent spawning, and male positions at spawning (Mjølnerød *et al.*, 1998). It is possible that the refuge availability does not affect sneaking success but enhances fertilization success by improving proximate factors.

Energy expenditure is a factor not taken into consideration in this study. Staying still in or beside refuges will cost less energetically than holding positions in open areas against direct stream flow (Sundbaum and Näslund, 1998). Such difference in energetic cost may be little in each breeding opportunity but will accumulate over successive breeding events (Jonsson *et al.*, 1991, 1997). The degree of exhaustion may influence parr breeding behavior particularly in the late breeding season.

The risk of incurring fatal injuries from aggressive behavior was also not considered in this study, although the intensity of aggression against parr was measured. Indeed, aggressive behavior of the dominant migrant males toward parr can result in mortal wounds (Hutchings and Myers, 1987). In this sense, sneaking behavior using refuges may be safer or more risk-averse measure. It may also serve to avoid predation risk considering the high vulnerability of breeding animals to predation (Magnhagen, 1991; Sih, 1994). Predator-sensitive male mating behaviors are well documented in other fishes (e.g., Magurran and Nowak, 1991; Berglund, 1993; Godin, 1995; Magnhagen, 1995; Candolin, 1997; Candolin and Voigt, 1998).

In conclusion, I could not find a positive effect of high refuge availability on the breeding behavior in mature male parr of the Miyabe charr. However, this study merely shows that the refuge availability was of insignificance in terms of my hypothesis. Further studies may be needed to ascertain whether the high refuge availability is advantageous to mature male parr in other aspects of the breeding behavior, such as fertilization success, energy expenditure, and risk taking.

Chapter 5
General Discussion

5.1 Applicability of specialization-in-sneaking

In this paper, I examined consistently how well or to what extent specialization in sneaking explain the reproductive characteristics of mature male parr in salmonids. Specialization-in-sneaking hypothesis explained sufficiently some reproductive characteristics of mature male parr. First, specialization-in-sneaking predicts that, unlike secondary sexual characters for migratory males (i.e., hooked snout and humped back; Fleming and Gross, 1994; Quinn and Foote, 1994), the corresponding morphological characters (i.e., snout length and body height) are not particularly favored for mature parr during breeding (Gross, 1985). In this study,

no selection was detected appropriately on such characters of mature masu salmon parr (Chapter 2). Therefore, parr morphology can be considered as a product of specialization in sneaking, in part (see below).

Second, if mature parr are specialized in sneaking, they should invest relatively more into gonad because the increased risk of sperm competition caused by sneaking favors the heavier production of sperm (Parker, 1990a, b; Gage *et al.*, 1995). Indeed, gonadal investment relative to somatic investment was higher in mature parr of masu salmon than in the migrants (Chapter 3). Moreover, such an energy allocation of masu salmon parr is consistent with that of distinctly small mature parr in Atlantic salmon but did not with that of relatively small resident type in brown trout (Gage *et al.*, 1995; Jonsson and Jonsson, 1997). Since the alternative adoptions of breeding behaviors (i.e., fighting for dominant position to the female or sneaking the mating) in male salmonids depends mostly on the body size relative to the potential competitors (Hino *et al.*, 1990; Kitano, 1996), the difference in the level of gonadal investment between mature parr in the salmons and resident males in the trout may be associated with their difference in the degree of dependency on sneaking. This association will illustrate the evolutionary transition to specialization in sneaking. I therefore conclude that the relatively high gonadal investment of mature male masu salmon parr is a physiological character specialized in sneaking to improve fertilization success by the higher fertilization efficiency. However, the higher gonadal investment and less-developed sexual characters may not have been evolved entirely independently but resulted from energetic trade-off under the finite reproductive investment to some extent. The potential influence of the trade-off may be indirectly emphasized if the negative relationship between investment into gonad and development of sexual characters is seen in migratory males.

5.2 Insufficiency and modification of specialization-in-sneaking

Some characteristics could not be explained fully by the specialization-in-sneaking which has been considered so far. First, I could not confirm the prediction that selection favors smaller body size in mature parr during the sneaking activity (Chapter 2). By contraries, selection favored larger body size for gaining more proximate position to the nest and intruding into the nest. Furthermore, this advantage of larger body size was caused by the higher status in dominance hierarchy. These results suggest that, within small mature parr, competitive ability is an important determinant of reproductive success as well as in migrants. Such unexpected results do not deny totally the specialization of mature parr in sneaking, but it is sufficient to doubt the completeness of the previous interpretation by disruptive selection. Disruptive selection on the two tactics in salmonid simply assumes the alternative requirements of suitability for sneaking or competitive ability for precocious and migratory males. Under disruptive selection, competitive ability should not be favored for mature parr. Therefore, I suggest that the previous studies have overlooked another selective agent on mature parr, and propose that the selective agent is the competition among mature parr. Taken it into account, body size can be interpreted as the outcome of antagonistic selective agents, disruptive selection and among-parr competition.

Second, this study did not support the expectations on behavioral specialization that parr may avoid aggression from migrants and improve the sneaking success by using refuges on the breeding grounds (Chapter 4). The interpretation of the unexpected parr behavior is beyond this study and remains to be elucidated. After the entire examination of the possibilities such as I mentioned in Chapter 4, we should direct our consideration to some constraints (e.g., energy limitation and life history trade-offs) preventing mature parr from perfect reaction to selection (Halliday, 1987; Partridge and Endler, 1987). For example, it is possible that because of their iteroparity mature parr in the Miyabe charr may be favored to allocate more energy to future survival and therefore future reproduction to attain a higher lifetime success.

Finally, this study documented the adaptive significance of some parr reproductive characteristics for the sneaking activities. However, the benefits of many other reproductive characteristics are still unclear. Understanding on the apparently non-beneficial characteristics may be advanced in the context of the cost-benefit relationship. Thus, future studies considering the costs and benefits of having a certain reproductive characteristics or a certain set of characteristics may be fruitful.

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