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Proximate Composition, Reproductive Development, and a Test for Trade-Offs in Captive Sockeye Salmon

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Abstract.—Energy limitations during reproduction should lead to the evolution of adaptive patterns of energy use and should cause trade-offs in the expression of different traits. We addressed these issues by measuring secondary sexual development, gonad investment, and proximate composition for sockeye salmon *Oncorhynchus nerka* maturing in captivity. Each of the last 3 months before maturity was characterized by a different pattern of reproductive development and energy use. From June to July, gonad mass increased (1.1% to 5.2% of male body mass; from 1.3% to 2.7% of female body mass), muscle fat decreased (15.1% to 8.6% sex-specific values averaged), and viscera fat decreased (23.9% to 16.7%). From July to August, male gonad mass did not change appreciably, but female gonad mass nearly doubled (to 5.5% of body mass). Muscle fat and viscera fat continued to decrease (to 6.0% and 8.8%, respectively), but muscle protein remained relatively constant. From August to maturity (September–October), female gonad mass more than tripled (to 18.6% of body mass) and secondary sexual characters increased in linear dimension by as much as 20.0% (male snout length). Viscera fat continued to decline (to 3.3%), but muscle fat did not decrease appreciably. The conservation of muscle protein until after fat was depleted may postpone reductions in performance that would accompany muscle degeneration. Mass-specific energy decreased between June and maturity in muscle (9.5–5.6 kJ · g^{-1}) and viscera (11.2–4.9 kJ · g^{-1}). We found no evidence for trade-offs in allocation between stored somatic energy, the size of secondary sexual characters, and gonad investment. An important area requiring further research is the effect of variation in energy stores prior to maturity on reproductive development at maturity. This prebreeding energy variation may obscure phenotypic trade-offs.

Animals can obtain only a limited amount of energy from their environment, and this energy must then be allocated among competing physiological processes, including metabolism, somatic growth, and reproductive development (Calow 1985; Sibly and Calow 1986). To compensate for these inevitable resource-based constraints, many animals store energy when food is plentiful and mobilize that energy during less productive periods (Reznick and Braun 1987; Sandberg and Moore 1996; Doughty and Shine 1997; Jönsson 1997). Energy reserves are often taxed most severely during breeding, when time and energy are diverted from procuring food and into tasks associated with successful reproduction (Wootton 1985). Because reproductive investment has important consequences for fitness, natural selection should favor optimization of energy storage, allocation, and use (Calow 1985; Sibly and Calow 1986).

Sockeye salmon *Oncorhynchus nerka* experience severe energy constraints during reproduction. They often undertake prodigious migrations from feeding areas in the open ocean to spawning sites in fresh water (Burgner 1991). Females allocate large amounts of energy to egg production (mature ovaries compose about 20% of body mass, Hendry et al. 1999), compete for nest sites (Foote 1990; Hendry et al. 1995), dig a nest for their eggs (Steen and Quinn 1999), and defend their nest from encroachment by other females (Quinn and McPhee 1998). Males develop large secondary

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sexual characters (Quinn and Foote 1994; Hendry and Berg 1999) and compete vigorously for access to breeding females (Quinn et al. 1996). All sockeye salmon die following a single reproductive season, which varies in length from a few days to several weeks (McPhee and Quinn 1998; Hendry et al. 1999). Despite considerable demands on energy stores, salmon cease feeding when they leave the ocean (sometimes months before spawning) and rely thereafter on endogenous energy stores (Brett 1995).

The influence of energy constraints on life history and behavior has been studied in several natural populations of Pacific salmon (reviewed by Brett 1995; Hendry and Berg 1999; Hendry et al. 1999). Unfortunately, studies in the wild are invariably limited in their ability to obtain a long and regular temporal sequence of samples from a single population. At best, such studies examine several different stages during freshwater migration and spawning, as well as a sample just prior to the cessation of feeding in the ocean. The only way to obtain a longer and more precise temporal sequence from a single population is to hold them captive and sample them regularly. We adopted this approach using sockeye salmon held in captivity as part of a program examining methods for artificial propagation of endangered populations. Our results provide the first published information on proximate composition for sockeye salmon maturing in captivity, and the first temporal sequence to include morphology, gonad development, and energy stores in a captive population of Pacific salmon.

Objectives

Our first objective was to determine how energy stores are partitioned among different tissues (muscle, viscera, gonad) and chemical constituents (fat, protein) and to determine when these stores are mobilized to fuel reproductive development. At a coarse level, salmon can store energy as either fat or protein (Hardy et al. 1984; Jonsson et al. 1997) in their viscera or muscle tissue (or as subcutaneous fat deposits; Aursand et al. 1994; Zhou et al. 1996). Fat deposition is the most efficient way to store energy for future metabolic use (Jobling 1994) because it holds the most energy per unit mass, it can be readily converted to energy, and it can be depleted without directly compromising physical performance. Conversely, protein deposition is the most effective way to increase body size because each gram of protein also binds 3–4 g of water (Jobling 1994), but mobilization of muscle protein will lead to muscle deterioration and reduced physical performance (e.g., Davison and Goldspink 1977). Mobilization of viscera fat stores does not hamper physical performance, but viscera storage capacities are limited. Different properties of fat and protein and different storage potentials of muscle and viscera suggest that selection for efficient energy transfer would lead to coupling between aspects of reproductive development and depletion of specific energy stores.

Our second objective was to test for trade-offs among different aspects of reproductive investment. The theoretical importance of allocation trade-offs has long been recognized (‘‘. . . if nourishment flows to one part or organ in excess, it rarely flows, at least in excess, to another part . . . ,’’ [Darwin 1859:147]), and the effects of such trade-offs now form a central tenant of life history theory (Sibly and Calow 1986; Stearns 1992). In mature salmon, energy has been allocated among three major categories of reproductive investment aside from migration (gonads, secondary sexual characters, stored somatic energy). Increased investment into any of these categories without decreasing investment into the others should have positive effects on fitness. Allocation trade-offs are expected, however, because energy reserves are limited. We investigated allocation trade-offs in captive sockeye salmon by testing for negative correlations between aspects of secondary sexual development, gonad investment, and somatic energy at maturity.

Methods

Study design.—Our experimental fish were the progeny of wild adults captured from the Wenatchee River, Washington, in fall 1991. The fish were reared for their entire lives in freshwater holding tanks with isothermal water (10°C) and were exposed to a natural photoperiod. They were fed Biodiet pellets (Bioproducts, Warrenton, Oregon), including Biodiet Grower (14.5% fat, 43.0% protein) for the first 3 years and Biodiet Brood (13.0% fat, 47.5% protein) for the last year. By the middle of August 1995, maturing fish had stopped feeding, and food was no longer provided. Most of the fish matured in late September or early October 1995 at 4 years of age.

In February 1995, all fish were tagged with passive integrated transponder (PIT) tags (Prentice et al. 1990). Body size, reproductive development (gonad size, secondary sexual trait sizes), and energy stores (fat and protein in the muscle and viscera) were then measured for a subset of these tagged fish over the 7 months leading up to ma-

turity. At approximately monthly intervals (March 8, April 6, May 10, June 7, July 17, August 16, September 27–October 11), each tagged fish was measured for length (fork length, mm) and weighed (body mass, g). Each month, 16–41 fish were killed (using an overdose of tricaine, MS-222), sexed, and sampled for morphology and proximate composition (as below). The final sampling period (September/October) was for mature fish and was spread over 2 weeks because of temporal variation in maturation.

The freshwater-reared, maturing fish described above form the basis of our study. We also opportunistically sampled (1) a few fish that were reared identically except that they were held in saltwater tanks for several years and then transferred to freshwater tanks on August 17, 1995, and (2) a few fish that were not maturing (they would have matured in the next year). These saltwater-reared fish and nonmaturing fish were sampled and processed in the same manner as the freshwater fish but were so few that they have been left out of our analysis. We have, however, provided a single summary table of some of their reproductive characteristics (Table 6) for the purposes of qualitative comparison to the freshwater fish. Table 6 is referred to in the discussion, but all other methods, results, tables, and figures refer to the freshwater-reared fish only.

Morphology.—For all fish that were killed, we used calipers to measure aspects of body shape, including body length (middle of eye to end of hypural plate), snout length (tip of snout to middle of eye), caudal peduncle depth (at its shallowest point), body depth (anterior insertion of dorsal fin to bottom of abdomen, perpendicular to lateral line), hump height (portion of body depth above lateral line), and adipose fin length (anterior insertion to posterior margin). Of these traits, snout length (males and females), adipose fin length (males), body depth (males), and hump height (males) are considered secondary sexual characters (Hendry and Berg 1999). Analysis of covariance (ANCOVA) was then used to assess morphological change over time, standardizing for variation in body length. All measurements were $\log_{10}$ transformed, and the relationship between each trait and body length was tested for heterogeneity of slopes across the last four months before maturity (the period of most interest). When slopes did not differ, the interaction term was removed from the ANCOVA model, and adjusted means were calculated using the common within-group slope. We used antilogarithms of adjusted means for presentation. Our approach to the measurement, analysis, and presentation of morphological variation was identical to that taken by Hendry and Berg (1999) for wild sockeye salmon.

Tissue sampling.—After measuring morphology, we (1) removed and weighed the testes or ovaries, (2) removed and weighed remaining viscera (intestines, liver, stomach, etc.) after discarding any stomach contents, and (3) dissected a piece of skeletal muscle from one side of the hump just anterior to the dorsal fin (see Hendry and Berg 1999, figure 2). Viscera, gonad, and muscle samples were stored at −20°C until processing. Our samples did not include several sites of energy storage, such as the dorsal fat deposit, ''belly flap,'' and subcutaneous fat deposits (Aursand et al. 1994; Zhou et al. 1996). These omissions limited our analysis for muscle tissue to changes in proximate composition and mass-specific energy, instead of total energy stores. We used standardized muscle samples rather than homogenizing the entire carcass because doing so saved considerable time and space during the field collections and sample processing. Similar muscle samples have also been used for wild sockeye salmon (Hendry and Berg 1999) and for chinook salmon *Oncorhynchus tshawytscha* (Unwin et al. 1999). For wild sockeye salmon, patterns of mass-specific energy depletion determined using the muscle samples mirrored patterns observed for the entire somatic tissue (Hendry and Berg 1999).

Proximate composition.—Viscera, gonad, and muscle samples were dried for 24 h at 105°C to determine percent solids (ratio of dry mass to wet mass) and percent water (100 − % solids). Fat content was then measured for each dry sample using the Soxhlet method with methylene chloride as the solvent. Methylene chloride extracts both neutral lipids (energy reserves) and structural lipids (phospholipids). Phospholipids, however, make up only about 0.5% by wet mass of sockeye salmon muscle tissue (Hatano et al. 1995). Ash content was determined for each sample by combustion in a muffle furnace at 550°C for 16 h. To determine percent fat and percent ash by wet mass, the proportion of dry tissue composed of fat and ash was multiplied by percent solids from the original sample. These water, fat, and ash estimation procedures are standard for the National Marine Fisheries Service laboratory in which they were performed (e.g., Shearer et al. 1997).

We estimated protein content by subtraction (% protein = 100 − % water − % fat − % ash) because (1) protein estimated by subtraction is usu-

ally within a percentage point of protein estimated using the standard Kjedahl procedure (e.g., Jonsson et al. 1991, table 1), (2) the Kjedahl procedure is subject to considerable error and subtraction may actually be a more reliable way to estimate protein, and (3) by forgoing the expensive and time-consuming Kjedahl procedure, our sample sizes could be increased. When calculating protein content by subtraction, we ignored carbohydrates because they make up less than 0.5% of the somatic tissue of salmonid fishes (e.g., Jonsson et al. 1991, 1997). Other studies estimating protein content by subtraction include Berg et al. (1998), Hendry et al. (1999), and Hendry and Berg (1999).

Energy content.—Mass-specific fat and protein energy ($kJ \cdot g^{-1}$) was estimated for each tissue (viscera, gonad, muscle) by multiplying percent fat and percent protein (wet mass) by the appropriate energy equivalents (fat = 36.4 $kJ \cdot g^{-1}$, protein = 20.1 $kJ \cdot g^{-1}$; Brett 1995). Fat and protein energy were then summed within each tissue to determine the combined mass-specific energy. Energy content estimated from proximate analysis (with correct energy equivalents) is essentially identical to that estimated using bomb calorimetry (Craig et al. 1978) and is the standard approach used in studies of salmonid reproductive energetics (reviewed by Hendry and Berg 1999).

Monthly averages for total viscera energy and total gonad energy were estimated using mass-specific energy and the total mass of each tissue. For this calculation, we did not use tissue mass measurements for the fish killed each month because these samples were made up of different individuals (total values would therefore have been influenced by variation in body size owing to sampling variation). Instead, we used the PIT tag database to extract body mass measurements for each of the fish that were ultimately killed and sampled at maturity. Monthly body mass averages for these fish (Figure 1) were then multiplied by the proportion of body mass comprised of viscera and gonad tissue (from fish killed each month) to estimate the mass of these tissues. These average tissue mass values were then multiplied by average mass-specific energy to estimate total gonad and total viscera energy each month. We did not estimate total muscle energy because only a piece of skeletal muscle was sampled.

Allocation trade-offs.—We tested for allocation trade-offs at maturity by examining correlations between different categories of reproductive investment, including two measures of relative secondary sexual development (residual hump, residual snout), two measures of relative gonad investment (residual gonad mass, residual gonad energy), and two measures of relative somatic energy (mass-specific muscle energy, mass-specific viscera energy). Residual hump, residual snout, and residual gonad mass were residuals from regressions of $\log_{10}$ body length at maturity versus $\log_{10}$ hump height (females, $r^2 = 0.73$, $P < 0.001$; males, $r^2 = 0.66$, $P < 0.001$), $\log_{10}$ snout length (females, $r^2 = 0.72$, $P < 0.001$; males, $r^2 = 0.69$, $P < 0.001$), and $\log_{10}$ gonad mass (females, $r^2 = 0.71$, $P < 0.001$; males, $r^2 = 0.73$, $P < 0.001$). Residual gonad energies were residuals from regressions of total gonad energy on body mass (females, $r^2 = 0.52$, $P = 0.002$; males, $r^2 = 0.74$, $P < 0.001$).

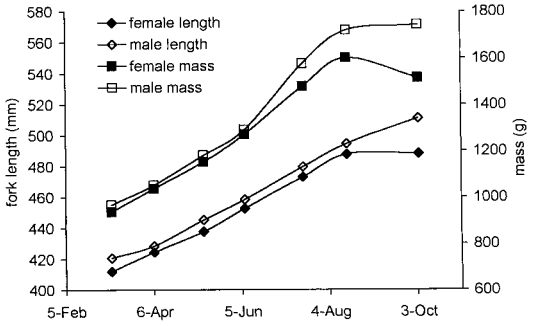

FIGURE 1.—Trends in body size (fork length and body mass) for fish ultimately sacrificed at maturity (i.e., from monthly measures of the same individual fish). Points represent monthly means, connected by smoothed lines.

Pearson's correlation coefficients were calculated for each pairwise comparison among the six variables and were tested for one-tailed significance at $\alpha = 0.05$ (one-tailed because positive correlations were predicted within each category of reproductive investment, and negative correlations were predicted among them). Four tests were performed when examining each pair of investment categories for evidence of trade-offs. For example, the four correlations tested for a trade-off between secondary sexual development and gonad investment were residual hump versus residual gonad mass, residual hump versus residual gonad energy, residual snout versus residual gonad mass, and residual snout versus residual gonad energy. The sequential Bonferroni procedure was used to control the overall alpha level at 0.05 for these multiple tests (i.e., $\alpha/4 = 0.0125$).

Results

Body Size, Morphology, and Gonad Mass

Body length, based on repeated measures of individually tagged fish that were ultimately killed

TABLE 1.—Physical characteristics of fish killed and sampled for morphology and proximate composition each month (mean ± SD). Length measurements (mm) are fork length (FL) and middle of eye to end of hypural plate (MEH).

Sample period	*N*	Length (mm)		Body mass (g)	Gonad mass (g)
		FL	MEH		
			Females		
Mar	7	403 ± 25	342 ± 22	880 ± 171	6.4 ± 1.3
Apr	10	415 ± 27	364 ± 25	1,000 ± 191	7.3 ± 1.7
May	12	435 ± 26	378 ± 22	1,098 ± 167	9.5 ± 3.5
Jun	7	443 ± 15	380 ± 14	1,145 ± 148	15.0 ± 2.0
Jul	7	475 ± 14	400 ± 15	1,492 ± 156	40.4 ± 9.1
Aug	11	493 ± 22	423 ± 20	1,621 ± 228	105.6 ± 21.9
Sep–Oct	20	481 ± 39	411 ± 30	1,467 ± 406	272.4 ± 80.7
			Males		
Mar	11	416 ± 25	356 ± 23	968 ± 169	0.7 ± 0.5
Apr	8	416 ± 22	358 ± 20	930 ± 162	1.0 ± 0.8
May	6	437 ± 29	374 ± 30	1,140 ± 303	4.7 ± 2.4
Jun	10	452 ± 26	387 ± 23	1,247 ± 204	13.4 ± 11.1
Jul	11	458 ± 33	383 ± 26	1,379 ± 271	72.1 ± 19.0
Aug	5	485 ± 29	404 ± 30	1,563 ± 380	86.6 ± 28.5
Sep–Oct	21	506 ± 34	420 ± 24	1,651 ± 354	69.0 ± 19.5

and sampled at maturity (September/October) increased for females between March and August and for males between March and maturity (Figure 1). Body mass also increased between March and August and then remained relatively constant or decreased from August to maturity (Figure 1). Similar, albeit less consistent, temporal trends were evident for fish killed and sampled each month (Table 1). Ovaries and testes increased in mass only marginally between March and May (Table 1; Figure 2). Afterward, ovary mass increased exponentially through maturity, and testes mass increased to July before leveling off in August and decreasing to maturity (Figure 2B). Decreasing testes size during the final month before maturity has also been observed in wild sockeye salmon (Hendry and Berg 1999) and may reflect the release of some sperm after males mature but before they are sampled.

All morphological traits (and gonad mass) were positively correlated with body length in the final 4 months leading to maturity: June to September/October (Table 2). (Female adipose fin length was not correlated with body length until after the interaction term was removed.) ANCOVA models explained most of the variation in trait sizes (R^2 = 0.33–0.98; Table 2) and revealed that some increased in relative size during maturation (Table 3). Most dramatically, snout length increased from July to August by 11.6% for females and 29.0% for males and from August to maturity by another 10.0% for females and 20.0% for males (Table 3). For males, the final month before maturity was also characterized by increases in adipose fin length (19.1%) and hump height (9.0%). Male caudal peduncle depth did not show an obvious temporal trend. For females, relative hump height decreased steadily from June to maturity, whereas adipose fin length, body depth, and caudal peduncle depth did not show consistent trends in relative size (Table 3). A decrease in relative hump height with maturity has also been documented for wild female sockeye salmon (Hendry and Berg 1999), and its cause is unknown.

Proximate Composition

Ash (% by wet mass) varied little within each sex and month and did not vary among months (all samples combined; muscle = 1.43%, SD = 0.4%, viscera = 1.2%, SD = 0.2%). Muscle fat (% by wet mass) increased from March to May, decreased almost linearly from May to August, and then remained fairly constant at a low level until maturity (Figure 3). Viscera fat (% by wet mass) in females remained constant from March to May and then decreased linearly until maturity (Figure 3). Viscera fat in males decreased almost linearly from March to maturity, with the exception of a slight increase in June (Figure 3). Muscle protein (% by wet mass) remained relatively constant until August, after which it decreased markedly to maturity (Table 4). Viscera protein (% by wet mass) increased from June to maturity, owing to the rapid depletion of the initially large fat stores (Table 4). On an absolute basis, viscera fat and viscera protein both decreased dramatically over the last 3 months before maturity (data not shown).

Fat content (% by dry mass) was positively cor-

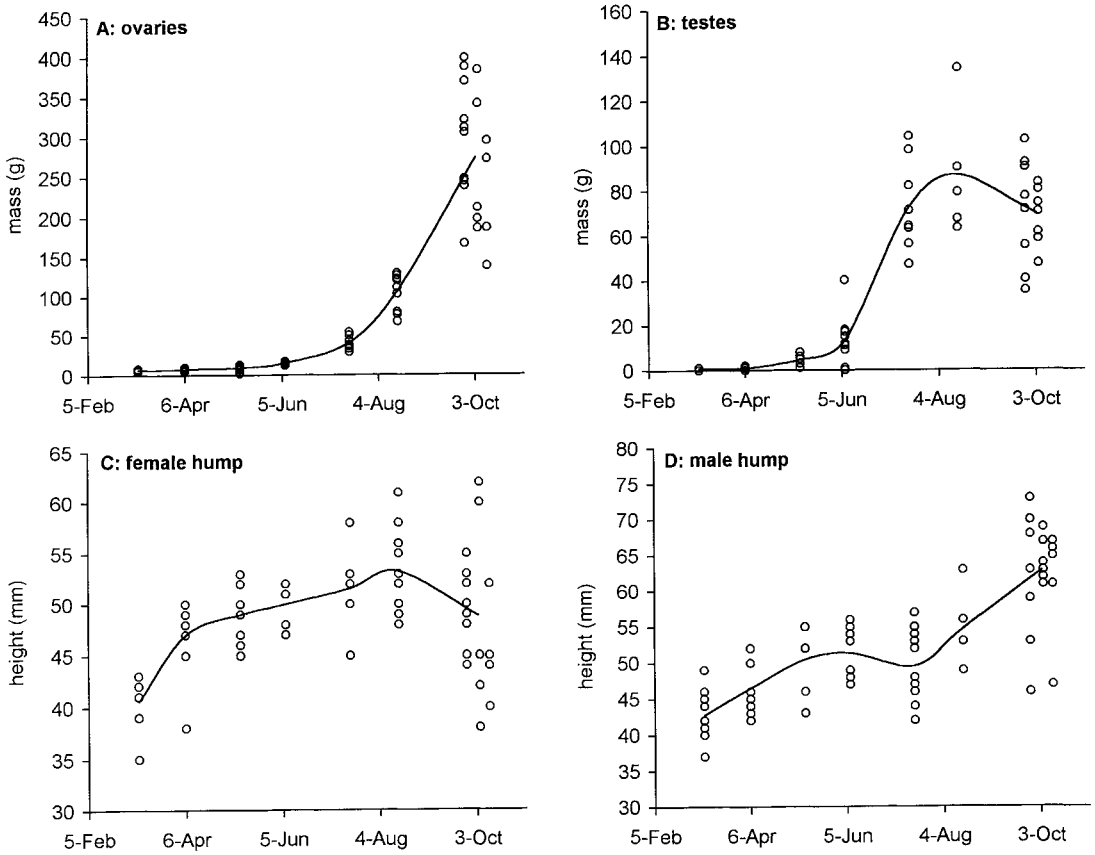

FIGURE 2.—Gonad mass (g) and hump height (mm) in maturing sockeye salmon. The smoothed lines connect mean values for the different months.

TABLE 2.—Results of analysis of covariance for $\log_{10}$-transformed trait values on $\log_{10}$-transformed body length. The first three columns show statistics for the full model (including an interaction to test for slope heterogeneity). For those traits for which slopes did not differ among collections ($P > 0.05$), the interaction term was removed to calculate adjusted means. The last three columns show slope coefficients and statistics for models without the interaction term; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Trait	*F*-value			Slope coefficient	*F*-value, adjusted means	Model R^2
	Collection	Length	Interaction			
Females						
Gonad mass	7.23*	13.13**	0.41	3.191	445.82***	0.98
Body depth	0.04	12.35**	0.04	0.770	1.78	0.53
Hump height	0.55	29.21***	0.53	1.392	11.60***	0.71
Snout length	0.54	23.50***	0.59	1.327	27.35***	0.85
Adipose length	1.57	1.08	1.58	0.931	0.75	0.33
Caudal depth	0.57	18.14***	0.58	0.977	4.89**	0.71
Males						
Gonad mass	1.56	1.53	1.67	2.150	23.94***	0.72
Body depth	4.50**	26.48***	4.58**			
Hump height	1.57	44.70***	1.63	1.278	7.24**	0.82
Snout length	0.85	33.33***	0.90	1.173	82.98***	0.94
Adipose length	0.65	7.21*	0.70	0.915	7.29***	0.64
Caudal depth	1.24	27.87***	1.25	0.843	4.67**	0.62

TABLE 3.—Gonad mass (g) and morphological trait size (mm) after standardizing to a common body length (408.4 mm for females; 401.5 mm for males). Values are antilogarithms of adjusted means from the log–log relationships used in analysis of covariance (Table 2). Adjusted means for body depth were not calculated for males because slopes varied among the collections (Table 2).

Trait	Jun	Jul	Aug	Sep–Oct
Females				
Gonad mass	18.7	42.5	92.5	250.6
Body depth	112.3	116.3	110.8	115.2
Hump height	55.2	53.1	50.6	48.1
Snout length	42.3	37.8	42.2	46.4
Adipose length	22.6	23.0	22.6	23.7
Caudal depth	33.6	36.8	34.4	35.0
Males				
Gonad mass	11.8	76.8	82.6	60.3
Hump height	53.9	52.6	54.3	59.2
Snout length	43.3	40.0	51.6	61.9
Adipose length	24.0	23.4	23.5	28.0
Caudal depth	33.3	35.4	36.7	34.0

related with percent solids (100 − % water) in the muscle and viscera tissue of females and males (Figure 4). This correlation was evident prior to the onset of maturation (March–June pooled) and within each of the subsequent months (July to maturity). This pattern indicated that fish with a higher proportion of water in their tissue had a lower proportion of fat per unit of dry tissue. In the viscera, both percent solids and percent fat by dry mass declined each month until maturity (June to September/October), reflecting a loss of both fat and protein in each of the months leading up to maturity (Table 4). In muscle, percent solids also decreased each month, but percent fat by dry mass actually increased slightly from August to maturity (Figure 4), reflecting a greater loss of protein than of fat from muscle tissue during the final stages of maturation (Table 4). Thus, major differences between muscle and viscera tissue were (1) fat composed a greater proportion of viscera than of muscle prior to maturity and (2) viscera fat con-

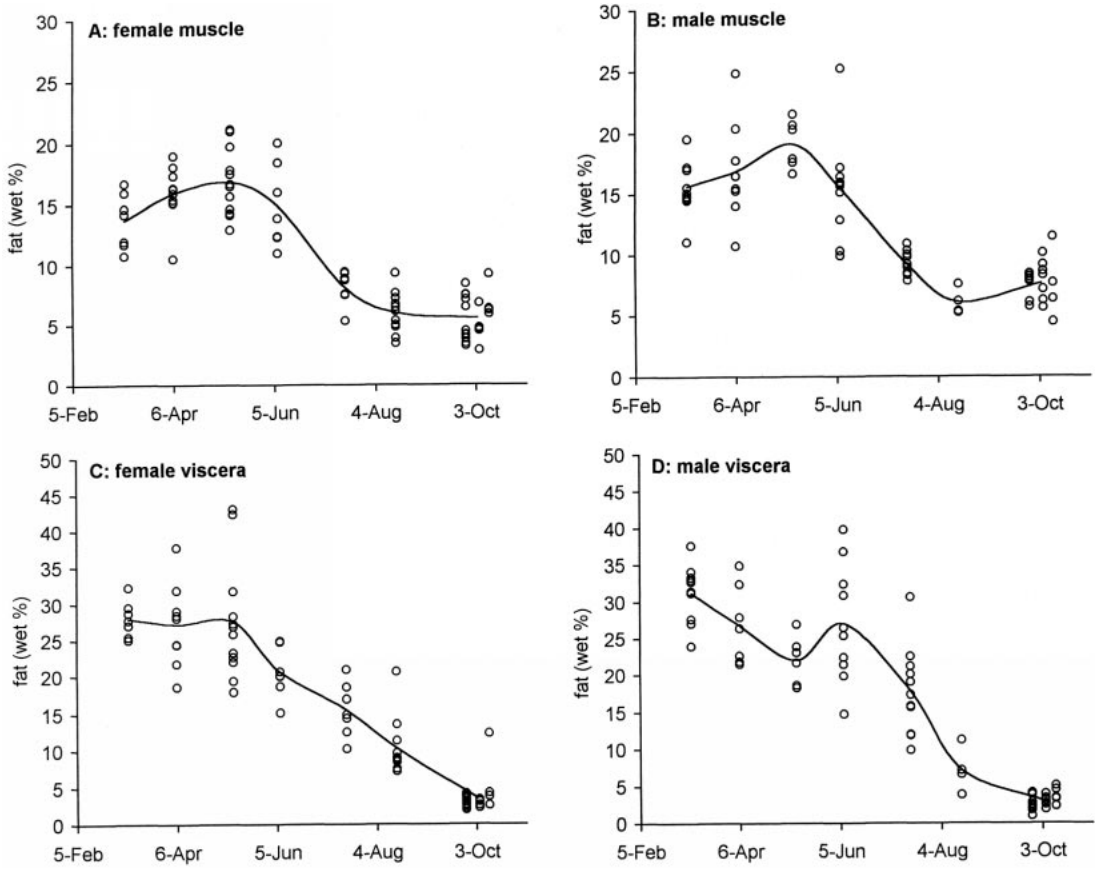

FIGURE 3.—Fat content (% by wet mass) of muscle and viscera in maturing sockeye salmon. The smoothed lines connect mean values for the different months.

TABLE 4.—Proximate composition (% water, % fat, % protein; by wet mass) and mass-specific energy ($kJ \cdot g^{-1}$) in tissue of sockeye salmon over the final months before maturity. Values are given as the mean (± SD) for a sample of white skeletal muscle or viscera. Protein content was determined as 100 − % water − % fat − % ash.

Component	Jun	Jul	Aug	Sep–Oct
		Females		
Muscle				
Water	63.9 ± 2.4	70.0 ± 1.2	72.5 ± 1.1	76.2 ± 1.5
Fat	14.8 ± 3.4	8.1 ± 1.5	6.0 ± 1.7	5.5 ± 1.8
Protein	19.9 ± 1.0	20.5 ± 0.4	20.3 ± 0.8	17.1 ± 0.8
Energy	9.4 ± 1.0	7.1 ± 0.5	6.3 ± 0.5	5.4 ± 0.6
Viscera				
Water	65.5 ± 4.9	69.3 ± 2.6	72.7 ± 3.3	77.3 ± 1.7
Fat	20.7 ± 3.4	15.5 ± 3.6	10.4 ± 3.9	3.5 ± 2.2
Protein	12.7 ± 2.4	14.1 ± 1.3	15.5 ± 0.8	17.7 ± 1.1
Energy	10.1 ± 1.5	8.5 ± 1.1	6.9 ± 1.3	4.8 ± 0.7
		Males		
Muscle				
Water	63.4 ± 3.0	69.1 ± 1.6	72.4 ± 0.7	76.1 ± 2.2
Fat	15.4 ± 4.3	9.1 ± 1.0	6.0 ± 1.1	7.5 ± 1.7
Protein	19.8 ± 1.2	20.3 ± 1.4	20.3 ± 0.5	15.3 ± 1.6
Energy	9.6 ± 1.3	7.4 ± 0.5	6.3 ± 0.3	5.8 ± 0.7
Viscera				
Water	59.6 ± 7.7	68.1 ± 5.3	74.1 ± 3.0	76.2 ± 1.0
Fat	27.0 ± 7.9	17.9 ± 5.9	7.2 ± 3.1	3.0 ± 1.0
Protein	12.3 ± 5.5	12.9 ± 0.9	16.4 ± 0.3	19.5 ± 1.1
Energy	12.3 ± 2.6	9.1 ± 2.0	5.9 ± 1.1	5.0 ± 0.3

tinued to decline until maturity, whereas muscle fat showed little change over the last month.

Gonad water remained fairly stable in females from March to April (66.4–67.4%), decreased from April to July (67.4, 65.2, 62.6, 56.8%; values separated by commas here and afterward represent a chronological sequence of monthly means), then increased from July to maturity (56.8, 58.0, 61.9%). In males, gonad water increased from March to June (61.1, 77.5, 78.4, 81.1%) and varied between 74.8 and 78.3% thereafter. Gonad fat (by wet mass) increased in females from March to June (16.8, 17.6, 19.1, 20.8%) then decreased from June to maturity (20.8, 17.3, 12.3, 6.5%). In males, gonad fat varied between 1.0 and 1.9% over the period that testes were large enough for analysis (May to maturity).

Energy Content

From March to June, mass-specific energy remained high in muscle (females, 8.9, 9.6, 9.8, 9.4 $kJ \cdot g^{-1}$; males, 9.5, 9.8, 10.5, 9.6 $kJ \cdot g^{-1}$) and viscera (females, 12.6, 12.4, 12.5, 10.1 $kJ \cdot g^{-1}$; males, 13.8, 12.2, 10.8, 12.3 $kJ \cdot g^{-1}$). Mass-specific energy then decreased in both sexes and both tissues until maturity (Table 4). Mass-specific gonad energy in females remained stable from March to April (9.4, 9.2 $kJ \cdot g^{-1}$), increased from April to July (9.2, 9.9, 10.7, 11.3 $kJ \cdot g^{-1}$), then decreased from July to maturity (11.3, 10.2, 8.5 $kJ \cdot g^{-1}$). In males, gonad energy decreased from May to July (3.7, 3.6, 3.4 $kJ \cdot g^{-1}$) then increased from July to maturity (3.4, 4.0, 4.5 $kJ \cdot g^{-1}$).

Total viscera energy in females increased from March to May (760, 856, 979 kJ) then decreased from May to maturity (979, 758, 674, 516, 142 kJ). In males, total viscera energy was 913 kJ in March then increased from April to June (777, 865, 984 kJ) and decreased from June to maturity (984, 690, 321, 236 kJ). Total gonad energy in females increased slowly from March to May (60, 68, 97 kJ) then rapidly from May to maturity (97, 159, 453, 1048, 2416 kJ). In males, gonads were too small to measure energy content until May, after which total gonad energy increased to August (23, 53, 248, 344 kJ) then decreased to maturity (309 kJ).

Allocation Trade-Offs

Within each of the three major categories of reproductive investment (stored somatic energy, secondary sexual development, gonad investment), the two different measures of investment were often correlated with each other, although not always significantly so. Mass-specific muscle energy was positively correlated with mass-specific viscera energy (females, $r = 0.42$, $P = 0.03$; males, $r = 0.18$, $P = 0.31$); residual hump size was pos-

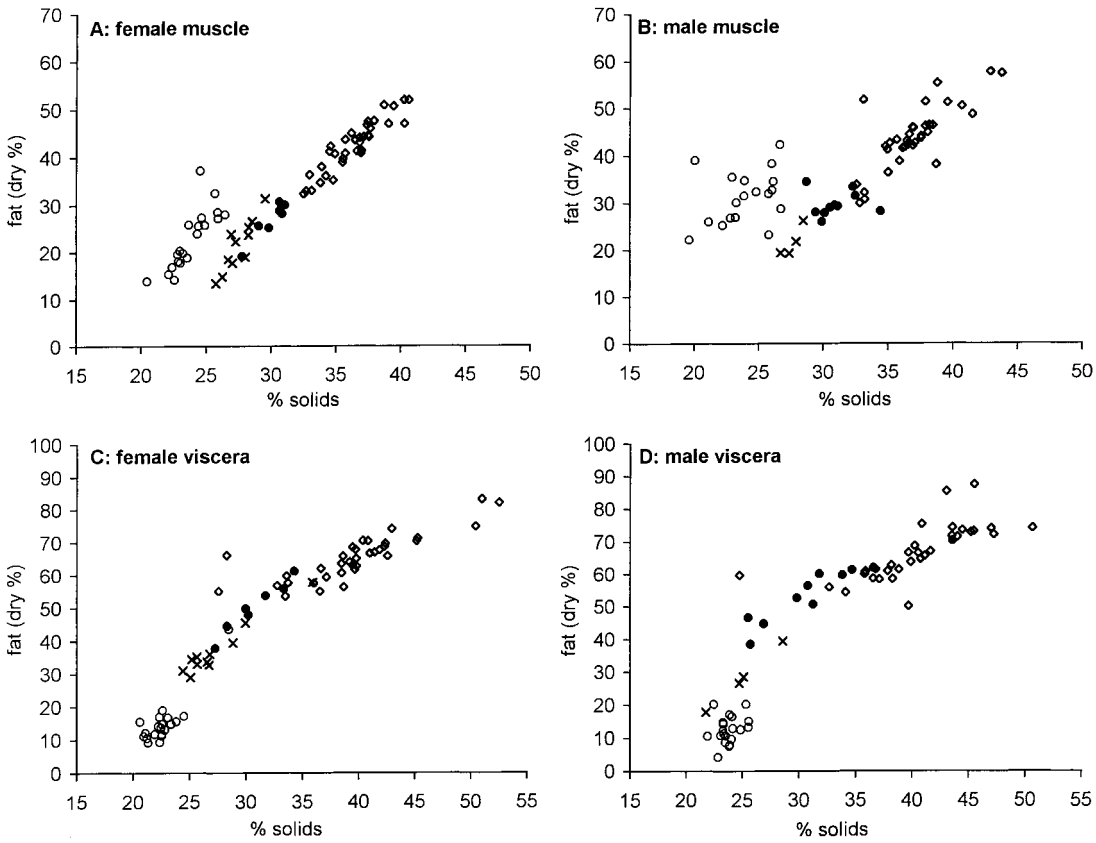

FIGURE 4.—Relationship between % solids (100 − % water) and % fat (by dry mass) in muscle and viscera tissue of maturing sockeye salmon. Fish sampled during different periods are denoted with open diamonds (March–June), closed circles (July), crosses (August), and open circles (September–October).

itively correlated with residual snout size (females, $r = 0.33$, $P = 0.06$; males, $r = 0.57$, $P = 0.004$), and residual gonad mass was correlated with residual gonad energy (females, $r = 0.89$, $P < 0.001$; males, $r = 0.87$, $P = 0.001$). Thus, our two different measures of investment for each major category were consistent. Negative correlations were not evident between any of the major investment categories (Table 5), indicating an apparent lack of allocation trade-offs among the different categories of reproductive investment.

Discussion

Pacific salmon accumulate energy while feeding in the ocean and use that energy while breeding in fresh water. Most of the stored energy is muscle protein, which also provides important structural and performance-related functions (e.g., swimming). Hence, salmon store most of their *mobilizable* energy as fat. Prebreeding fat storage is a common strategy in species for which food resources are isolated in space or time from breeding sites (e.g., mosquitofish, Reznick and Braun 1987; migratory birds, Sandberg and Moore 1996; lizards, Doughty and Shine 1997). Once maturation begins, differential mobilization of fat or protein from muscle or viscera tissue becomes increasingly important to survival and reproductive success. We first consider several apparently adaptive patterns of energy mobilization then discuss results of our test for allocation trade-offs.

Energy Storage and Mobilization

Each of the last 3 months before maturity was characterized by a different pattern of reproductive development and energy use. From June to July, gonad mass began to increase noticeably in both sexes (Figure 2), and muscle fat was depleted at a high rate (Figure 3). From July to August, male gonad mass remained relatively constant, but fe-

TABLE 5.—Pearson's correlation coefficients between secondary sexual characters, gonad traits, and energy stores at maturity. Muscle and viscera refer to mass-specific energy in a standard sample of each tissue. Residual hump, residual snout, and residual gonad refer to residuals from regressions of $\log_{10}$ hump height, $\log_{10}$ snout length, and $\log_{10}$ gonad mass on $\log_{10}$ body length. Residual gonad energy refers to residuals from a regression of total gonad energy on body mass. Values for females are above the diagonal and values for males are below. The correlation between residual gonad and residual hump was no longer significant after correction for multiple comparisons (sequential Bonferroni); * $P < 0.05$, ** $P < 0.01$, *** $P = 0.001$.

	Energy		Residual			
Trait	Muscle	Viscera	Hump	Snout	Gonad	Gonad energy
Energy						
Muscle		0.42*	0.21	0.30	−0.24	−0.17
Viscera	0.18		−0.01	0.10	−0.14	−0.08
Residual						
Hump	−0.06	0.13		0.33	0.10	0.03
Snout	−0.49	−0.04	0.57**		−0.22	−0.11
Gonad	0.27	−0.19	0.44*	0.28		0.89***
Gonad energy	0.10	−0.27	0.20	0.27	0.87***	

male gonad mass more than doubled. Muscle and viscera fat continued to decrease, but muscle protein remained relatively constant (Table 4). From August to maturity (September/October), female gonads more than doubled in mass again and, although viscera fat continued to decline, muscle fat did not decrease appreciably. Thus, a major difference during this final month, relative to those previous, was that muscle protein was depleted at a higher rate (Table 4). These results indicate that salmon tend to use energy differently early in maturation than they do late in maturation. These patterns of energy use are qualitatively similar to those observed for sockeye salmon in the wild (Hendry and Berg 1999), but are not seen in nonmaturing fish (Table 6).

Early in maturation, fat seems to be used in preference to protein (Table 4; Brett 1995; Hendry and Berg 1999). We believe that this pattern arises because (1) fat is a better energy source for metabolism and is required for the early stages of egg production and (2) protein depletion would be detrimental to physical performance. In the wild, salmon often migrate long distances from the ocean to their natal spawning sites (Burgner 1991). During these migrations, stored fat would provide the most efficient energy source for metabolism (Ballantyne et al. 1996), whereas depletion of mus-

TABLE 6.—Summary information for maturing fish reared in saltwater tanks, and for nonmaturing fish. Proximate composition was determined as described in the text. Morphological trait sizes were standardized to the average body size of freshwater fish using the slope coefficients in Table 2. Water and fat averages can be compared to those reported in Table 4 for fish reared in freshwater. Gonad mass and snout length averages can be compared to those reported in Table 3 for fish reared in freshwater.

	Fish reared in saltwater			Nonmaturing fish		
Variable	Jul	Aug	Sep–Oct	Jul	Aug	Sep–Oct
Females						
Sample size	2	5	6	4	3	7
Muscle water (%)	69.3	73.8	80.0	66.3	70.3	66.5
Muscle fat (%)	8.3	4.1	5.0	11.3	6.4	12.0
Viscera water (%)	71.0	77.1	78.5	57.2	58.8	53.4
Viscera fat (%)	13.5	4.7	2.8	27.1	27.2	35.5
Relative gonad mass (g)	45.3	109.4	213.3	6.0	5.9	6.5
Relative snout length (mm)	41.0	44.0	45.2	36.4	40.6	39.5
Males						
Sample size	2	1	7	1	3	7
Muscle water (%)	67.9	72.8	76.6	64.8	67.7	64.3
Muscle fat (%)	8.3	5.1	6.8	13.1	9.7	14.4
Viscera water (%)	66.1	73.5	78.3	47.3	59.1	45.6
Viscera fat (%)	18.2	9.8	2.7	41.7	28.1	44.7
Relative gonad mass (g)	86.9	64.9	50.6	< 1.0	< 1.0	< 1.0
Relative snout length (mm)	37.4	46.3	55.2	36.3	37.2	39.2

cle protein would reduce swimming performance (Davison and Goldspink 1977). Fish held captive in our study could not undertake their normal upstream migration (842 km, 569 m elevation), but they still depleted most of their fat energy before drawing on muscle protein. In females, mobilized fat was presumably channeled into eggs (see also Ballantyne et al. 1996), but in males, gonad fat was negligible and secondary sexual characters did not change much in size during this period. Thus, early fat loss in both sexes appears to be a genetically based response to metabolic demands.

Late in maturation, muscle protein appears to provide the major energy source (Table 4; Brett 1995; Hendry and Berg 1999). This shift from fat to protein may arise simply because very little fat remains or perhaps because protein is required for reproductive development during this period. Depletion of muscle protein was largely matched in females by an increase in ovary protein (see also Hardy et al. 1984) and, in males, by rapid secondary sexual development (Figure 2D; Table 3). We infer that the final month before maturity was characterized by the transfer of protein from muscle to ovaries and by the use of protein in constructing secondary sexual characters. Protein is particularly important for accomplishing morphological changes because secondary sexual characters are composed mainly of cartilage, which cannot be synthesized from fat. The energy requirements of secondary sexual development have been largely ignored despite evidence that they constitute a substantial fraction of the reproductive energy budget. For salmon that migrate exceptionally long distances, protein may also be used to fuel the final stages of migration (Mommsen et al. 1980).

Allocation Trade-Offs

We failed to find evidence for reproductive trade-offs at maturity: negative correlations were not detected between somatic energy stores, secondary sexual trait sizes, or gonad investment (Table 5). Several hypotheses may be advanced to account for this negative result. First, the measures of investment we used may not accurately reflect energy allocation. For example, we did not directly measure the cost of secondary sexual development and instead used relative trait size as a surrogate measure (indeed, no study has yet directly measured these costs). Another potential limitation was that our measures of somatic energy were mass-specific rather than absolute (total muscle mass as well as energy density may vary among individuals). However, mass-specific energy estimated by drying samples of muscle tissue has proven sufficient for identifying trade-offs in other studies; GSI in captive male chinook salmon is negatively correlated with energy density at maturity (Unwin et al. 1999). Also, statistical power was probably not an insurmountable problem because correlations between traits *within* reproductive categories were highly significant and because half the correlations among categories (12 of 24) were actually positive in direction. We conclude that our methods were likely sufficient to detect any actual phenotypic trade-offs between the three categories of reproductive investment (they were obviously not sufficient to detect trade-offs with unmeasured aspects of reproductive investment, such as migration).

Second, our study was carried out under artificial conditions: the fish were held their entire lives in freshwater, fed an unnatural diet, and not allowed to migrate or spawn. Trade-offs evident in natural populations may not have been manifested under these circumstances. For example, Hendry et al. (1999) found a trade-off between somatic and gonadal energy stores in a wild population of sockeye salmon, a pattern not evident in the present study. Unnatural conditions certainly influenced our results, but (1) fish transferred between freshwater and saltwater tanks at appropriate times had similar patterns of energy depletion and reproductive development (Table 6), (2) diets of sockeye salmon in the wild can be quite high in caloric content (Nishiyama 1977; Davis et al. 1998), (3) the proximate composition of muscle tissue in the present study was similar to that of wild sockeye salmon (Hendry and Berg 1999), and (4) prevention of migration and spawning can be considered a control for variation in these behaviors and should have increased our ability to detect trade-offs among other traits. Regardless, further tests in wild populations will be critical for understanding reproductive trade-offs.

A third possible reason for our failure to detect trade-offs is that individuals may vary in the amount of energy they acquire before maturation, and this variation may translate into correlated variation in energy allocation. Specifically, energy-rich individuals may be able to sustain greater investment into multiple characters than can energy-poor individuals, thereby obscuring underlying genetic trade-offs (van Noordwijk and de Jong 1986; de Jong and van Noordwijk 1992). Evidence for effects of energy stores on reproductive development *within* groups of salmon has been scant. Ad-

ams and Huntingford (1997) inferred an association between fat stores (estimated using morphology) before maturity and egg number at maturity in Arctic char *Salvelinus alpinus*, but (1) the reported relationship was negative and very weak ($r^2 = 0.09$), (2) relative gonad mass was not similarly correlated with body shape ($r^2 = 0.08$), and (3) the body shape variables are often a poor surrogate for energy stores (see r^2 values in Adams et al. 1995). We feel that a link between variation in energy stores among individuals and their ultimate reproductive development has yet to be convincingly demonstrated for salmon. Nonetheless, the substantial evidence that different diets can influence energy stores and reproductive development (e.g., Bromage et al. 1992; Shearer et al. 1997), suggests that such links are indeed present and that investigators have simply not yet devised a good enough method to detect them.

An important area for future research is the consideration of effects that variation in energy stores have on the expression of reproductive trade-offs. To examine this effect, reproductive development at maturity can be compared with active energy stores in individuals before maturity. Doughty and Shine (1997) provide an excellent example of this approach in lizards. For fish, equipment has been developed to estimate fat content using microwaves that measure water content (Kent 1990). Kadri et al. (1995) used this device for dead fish, and it should be equally effective for live fish. Another potentially informative technique is ''phenotypic manipulation'' (Sinervo and Basolo 1996). For example, it may be possible to surgically remove visceral fat deposits from live fish and then monitor subsequent reproductive investment. We encourage further study into how energetics influence reproductive development because such effects may play an important role in life history evolution.

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References

Adams, C. E., and F. A. Huntingford. 1997. Growth, maturation and reproductive investment in Arctic charr. Journal of Fish Biology 51:750–759.

Adams, C. E., F. A. Huntingford, and M. Jobling. 1995. A non-destructive morphometric technique for estimation of body and mesenteric lipid in Arctic charr: a case study of its application. Journal of Fish Biology 47:82–90.

Aursand, M., B. Bleivik, J. R. Rainuzzo, L. Jørgensen, and V. Mohr. 1994. Lipid distribution and composition of commercially farmed Atlantic salmon (*Salmo salar*). Journal of Scientific Food Agriculture 64:239–248.

Ballantyne, J. S., and seven coauthors. 1996. Plasma nonesterified fatty acid profiles in male and female sockeye salmon, *Oncorhynchus nerka*, during the spawning migration. Canadian Journal of Fisheries and Aquatic Sciences 53:1418–1426.

Berg, O. K., E. Thronæs, and G. Bremset. 1998. Energetics and survival of virgin and repeat spawning brown trout (*Salmo trutta*). Canadian Journal of Fisheries and Aquatic Sciences 55:47–53.

Brett, J. R. 1995. Energetics. Pages 1–66 *in* C. Groot, L. Margolis, and W. C. Clarke, editors. Physiological ecology of Pacific salmon. University of British Columbia Press, Vancouver.

Bromage, N., and seven coauthors. 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). Aquaculture 100:141–166.

Burgner, R. L. 1991. Life history of sockeye salmon (*Oncorhynchus nerka*). Pages 3–117 *in* C. Groot and L. Margolis, editors. Pacific salmon life histories. University of British Columbia Press, Vancouver.

Calow, P. 1985. Adaptive aspects of energy allocation. Pages 13–31 *in* P. Tytler and P. Calow, editors. Fish energetics: new perspectives. Johns Hopkins University Press, Baltimore, Maryland.

Craig, J. F., M. J. Kenley, and J. F. Talling. 1978. Comparative estimations of the energy content of fish tissue from bomb calorimetry, wet oxidation and proximate analysis. Freshwater Biology 8:585–590.

Darwin, C. 1859. On the origin of species by means of natural selection. John Murray, London.

Davis, N. D., K. W. Myers, and Y. Ishida. 1998. Caloric value of high-seas salmon prey organisms and simulated salmon ocean growth and prey consumption. North Pacific Anadromous Fish Commission Bulletin 1:146–162.

Davison, W., and G. Goldspink. 1977. The effect of prolonged exercise on the lateral musculature of the brown trout (*Salmo trutta*). Journal of Experimental Biology 70:1–12.

de Jong, G., and A. J. van Noordwijk. 1992. Acquisition and allocation of resources: genetic (co)variances, selection, and life histories. American Naturalist 139:749–770.

Doughty, P., and R. Shine. 1997. Detecting life history trade-offs: measuring energy stores in ''capital'' breeders reveals costs of reproduction. Oecologia 110:508–513.

Foote, C. J. 1990. An experimental comparison of male and female spawning territoriality in a Pacific salmon. Behaviour 115:283–314.

Hardy, R. W., K. D. Shearer, and I. B. King. 1984. Proximate and elemental composition of developing eggs and maternal soma of pen-reared coho salmon (*Oncorhynchus kisutch*) fed production and trace element fortified diets. Aquaculture 43:147–165.

Hatano, M., K. Takahashi, O. A. Mathisen, and G. Amma. 1995. Biochemical differences in sockeye salmon that are ocean feeding and on the spawning grounds. Journal of Food Biochemistry 19:269–284.

Hendry, A. P., and O. K. Berg. 1999. Secondary sexual characters, energy use, senescence, and the cost of reproduction in sockeye salmon. Canadian Journal of Zoology 77:1663–1675.

Hendry, A. P., O. K. Berg, and T. P. Quinn. 1999. Condition dependence and adaptation-by-time: breeding date, life history, and energy allocation within a population of salmon. Oikos 85:499–514.

Hendry, A. P., F. E. Leonetti, and T. P. Quinn. 1995. Spatial and temporal isolating mechanisms: the formation of discrete breeding aggregations of sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Zoology 73:339–352.

Jobling, M. 1994. Fish bioenergetics. Chapman and Hall, New York.

Jönsson, K. I. 1997. Capital and income breeding as alternative tactics of resource use in reproduction. Oikos 78:57–66.

Jonsson, N., B. Jonsson, and L. P. Hansen. 1991. Energetic cost of spawning in male and female Atlantic salmon (*Salmo salar* L.). Journal of Fish Biology 39:739–744.

Jonsson, N., B. Jonsson, and L. P. Hansen. 1997. Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic salmon *Salmo salar*. Journal of Animal Ecology 66:425–436.

Kadri, S., N. B. Metcalfe, F. A. Huntingford, and J. E. Thorpe. 1995. What controls the onset of anorexia in maturing adult female Atlantic salmon? Functional Ecology 9:790–797.

Kent, M. 1990. Hand-held instrument for fat/water determination in whole fish. Food Control 2:47–53.

McPhee, M. V., and T. P. Quinn. 1998. Factors affecting the duration of nest defense and reproductive lifespan of female sockeye salmon, *Oncorhynchus nerka*. Environmental Biology of Fishes 51:369–375.

Mommsen, T. P., C. J. French, and P. W. Hochachka. 1980. Sites and patterns of protein and amino acid utilization during the spawning migration of salmon. Canadian Journal of Zoology 58:1785–1799.

Nishiyama, T. 1977. Food-energy requirements of Bristol Bay sockeye salmon *Oncorhynchus nerka* (Walbaum) during the last marine life stage. Research Institute of North Pacific Fisheries, Faculty of Fisheries, Hokkaido University, Hokodate, Japan. Special Volume 1977:289–320. (In Japanese; English summary.)

Prentice, E. F., T. A. Flagg, and C. S. McCutcheon. 1990. Feasibility of using implantable passive integrated transponder (PIT) tags in salmonids. Pages 317–322 *in* N. C. Parker, A. E. Giorgi, R. C. Heidinger, D. B. Jester, Jr., E. D. Prince, and G. A. Winans, editors. Fish-marking techniques. American Fisheries Society, Symposium 7, Bethesda, Maryland.

Quinn, T. P., M. D. Adkison, and M. B. Ward. 1996. Behavioral tactics of male sockeye salmon (*Oncorhynchus nerka*) under varying operational sex ratios. Ethology 102:304–322.

Quinn, T. P., and C. J. Foote. 1994. The effects of body size and sexual dimorphism on the reproductive behaviour of sockeye salmon, *Oncorhynchus nerka*. Animal Behaviour 48:751–761.

Quinn, T. P., and M. V. McPhee. 1998. Effects of senescence and density on the aggression of adult female sockeye salmon. Journal of Fish Biology 52: 1295–1300.

Reznick, D. N., and B. Braun. 1987. Fat cycling in the mosquitofish (*Gambusia affinis*): fat storage as a reproductive adaptation. Oecologia 73:401–413.

Sandberg, R., and F. R. Moore. 1996. Fat stores and arrival on the breeding grounds: reproductive consequences for passerine migrants. Oikos 77:577–581.

Shearer, K. D., J. T. Silverstein, and W. W. Dickhoff. 1997. Control of growth and adiposity of juvenile chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 157:311–323.

Sibly, R. M., and P. Calow. 1986. Physiological ecology of animals: an evolutionary approach. Blackwell Scientific Publications, Oxford, UK.

Sinervo, B., and A. L. Basolo. 1996. Testing adaptation using phenotypic manipulations. Pages 149–185 *in* M. R. Rose and G. V. Lauder, editors. Adaptation. Academic Press, New York.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, New York.

Steen, R. P., and T. P. Quinn. 1999. Egg burial depth by sockeye salmon (*Oncorhynchus nerka*): implications for survival of embryos and natural selection on female body size. Canadian Journal of Zoology 77: 836–841.

Unwin, M. J., M. T. Kinnison, and T. P. Quinn. 1999. Exceptions to semelparity: postmaturation survival, morphology, and energetics of male chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 56: 1172–1181.

van Noordwijk, A. J., and G. de Jong. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. American Naturalist 128:137–142.

Wootton, R. J. 1985. Energetics of reproduction. Pages 231–254 *in* P. Tytler and P. Calow, editors. Fish energetics: new perspectives. Johns Hopkins University Press, Baltimore, Maryland.

Zhou, S., R. G. Ackman, and C. Morrison. 1996. Adipocytes and lipid distribution in the muscle tissue of Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 53:326–332.