Reproductive Cycle and Associated Changes in Energy Content of Body Tissues in Rainbow Trout from the South Fork Holston River, Virginia

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Abstract: The spawning period and caloric density of body tissues during gonadal recrudescence were studied in a naturalized population of rainbow trout (*Salmo gairdneri*) from September 1979 to August 1980. As judged by gonosomatic indices and the percentage of spent females in semimonthly samples, the fish spawned from mid-February to early April, when water temperature and stream flow were both increasing. Decreases in calorific equivalents (cal mg^{-1} dry weight) of selected tissues during gonadal maturation in fall and winter were greatest in fat reserves along the alimentary tract.

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Studies on the reproductive biology of rainbow trout have demonstrated distinct differences among populations; spawning periods differ according to water temperature, photoperiod, and strain of fish (Needham 1938). Rainbow trout were initially spring spawners, but selective breeding and manipulation of photoperiod in hatcheries have greatly altered spawning times. Hatchery stocks can now spawn between November and June (Carlander

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1969). Wild trout from these hatchery stocks have exhibited the same or different spawning times as parental stocks, presumably in response to local climatic conditions (Nikolsky 1963). The spawning periods of naturalized populations therefore requires documentation at the regional level.

The seasonal partitioning of energy into somatic and gonadal growth is a common phenomenon in fish (Delahunty and deVlaming 1980). Storage and translocation of food materials is probably most pronounced in fish whose reproductive development occurs at a time of reduced food availability or consumption. Gonadal maturation may therefore deplete stored energy reserves and draw nutrients and energy from somatic tissue. Pacific salmon (*Oncorhynchus* spp.) exhibit the most extreme case of spawning-induced energy depletion, in which 99% of body lipids, 72% of protein, and 63% of ash reserves are lost (Love 1970). The loss of these body constituents is compensated by an increase in the water content of affected tissues (Greene 1926), although most species experience a reduction in total body weight.

Lipid deposits and muscle protein are the important components of the annual cycle of energy storage in salmonids (Greene 1919, Idler and Bitners 1958, Shul'man 1974), since carbohydrates do not contribute significantly to the gross chemical constituents of somatic or gonadal tissue (Craig 1977). Fat reserves of the alimentary tract are the most mobile source of energy in brown trout (*Salmo trutta*) (Swift 1955). In Pacific salmon, fat and protein in muscle tissue is mobilized to the gonads or used directly for energy. Other energy depots in fishes include the liver, other viscera, and components of the head, skin, and tail (Swift 1955, Idler and Bitners 1959, Diana and Mackay 1979, Delahunty and deVlaming 1980). Mature ovaries are rich in protein and lipids and contain much more energy than testicular tissue. Differences in the magnitude of timing of gonad development between sexes are well documented for many species (Love 1970, Shul'man 1974), although the degree of energy depletion from male and female body tissues has not been examined. This study was conducted to define the spawning period of a naturalized population of rainbow trout in Virginia and to record changes in the energy content of selected tissues associated with gonad maturation.

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Methods

The South Fork Holston River is a fourth order stream that originates from several springs in Smyth County, western Virginia and flows 181 km to

the Holston River, upper Tennessee River drainage. A naturalized population of rainbow trout has resided in the headwaters of the South Fork for at least 20 years and is considered to be one of the most productive trout populations in Virginia. Fish for our study were collected from an 8.3-km upstream section beginning 0.8 km below the origin of the South Fork. Water temperatures were recorded weekly with a Ryan 30-day thermograph or a Taylor maximum-minimum thermometer from January to May 1980. Stream discharge was obtained weekly from mean water velocity and depth measurements along a stream channel profile. Quarterly measurements of water chemistry in this section provided the following range of values: pH, 6.8-7.7; total hardness 60–90 mg/l; conductivity, 80–131 μmhos.

Monthly collections of 12 to 26 rainbow trout were made from July 1979 through January 1980 and May through August 1980. Trout were collected twice monthly between February and April 1980 to more adequately define the period of maximum reproductive activity. All specimens were captured with a portable backpack electrofisher and placed on ice for transport to the laboratory. In the laboratory, fish were blotted dry, measured (nearest 1.0 mm) and weighed (nearest 0.01 g). Gonads were removed, sexed, weighed (0.1 mg), and classified according to developmental stage. Temporal changes in gonadal maturation were assessed using a gonosomatic index (weight of gonads $\times$ 100/weight of fish – weight of gonads).

Fish from monthly samples were also used to assess temporal fluctuations in energy content of selected body tissues. After gonads were processed, each fish was dissected further to remove the alimentary tract (including caecae and mesenteries), liver, and a sample of muscle from the left epaxial muscle mass. The alimentary tract was opened and flushed clean of ingested material. All fractions were then blotted dry, placed in pre-weighed aluminum pans, and weighed (0.1 mg). Livers, gonads, and muscle samples were frozen at −18 C, freeze-dried in a Labconco freeze dryer for a minimum of 24 hours, dessicated for 24 hours, and weighed. These tissue fractions were ground and homogenized with a mortar and pestle and returned to the dessicator prior to calorific analysis. Homogenizing of alimentary tracts was handled differently due to the high content of oils and connective tissue. Each alimentary tract was homogenized in deionized water with a Waring microblender, frozen at −18 C, freeze dried for at least 48 hours, and stored in a dessicator prior to energy determination.

Calorific determinations were made on individual tissue samples using microbomb calorimetry. Representative subsamples of tissue (<25 mg) were pressed into pellets and bombed with a Phillipson Microbomb Calorimeter (Gentry Instruments, Incorporated, Aiken, S.C.). Procedures and calculations followed those recommended by Gentry Instruments. A preliminary

trial of 6 replicate energy determinations per tissue type yielded sufficient precision to justify single determinations of each sample (Brayton 1981).

Most statistical analyses were conducted on data sets of relatively small size, and normality of distributions was tested when adequate sample size permitted (Kolmogorov-Smirnov and Kuiper tests). Caloric density of rainbow trout tissues was tested for dispersion differences among time periods with the Kruskal-Wallis 1-Way Layout (Hollander and Wolfe 1973). Multiple comparisons of caloric density were conducted using distribution-free multiple comparisons based on Kruskal-Wallis rank sums as modified by Dunn (1964) for large number of treatments and unequal treatment sample size. An error rate of 0.20 was used for multiple comparisons (Sokal and Rohlf 1969).

Results

The reproductive cycle of rainbow trout in the South Fork Holston River was discerned from the gonosomatic index (GSI) and percentage of spent females in each monthly collection. Median GSI values for males ranged between 1.8% and 5.3% from September 1979 to March 1980, and declined to 0.2% by summer (Table 1). Development of testes occurred primarily during fall, and testes remained in a ripe condition until spawning began in February. Small sample sizes probably accounted for the GSI variability dur-

Table 1. Median Gonosomatic Indices (GSI) of Male and Female Rainbow Trout in the South Fork Holston River, September 1979 through August 1980

	Male		Female	
Date	No.	Median GSI	No.	Median GSI
1979				
Sep 26	10	5.3	8	0.3
Oct 19	6	5.0	6	6.9
Nov 14	5	2.5	1	5.4
Dec 20	7	2.2	5	10.5
1980				
Jan 25	6	2.3	4	9.4
Feb 19	4	2.0	4	8.7
Mar 5	8	1.8	3	12.7
Mar 31	5	2.7	3	11.1
Apr 19	4	0.6	4	0.5
Apr 24	9	1.2	4	0.1
May 19	4	0.7	8	0.5
Jun 23	5	0.2	7	0.5
Jul 14	3	0.2	7	1.0
Aug 6	4	0.4	8	0.6

Table 2. Weekly Range of Water Temperatures (C) and Discharge (m^3s^{-1}) in the South Fork Holston River, January to May 1980

Week Ending	Temperature Range (C)	Discharge (m^3s^{-1})
Jan 5	3.9– 7.8	
12	2.8– 8.3	
21	2.2– 8.9	2.6
28	1.1– 8.8	1.4
Feb 4	1.1– 7.1	0.8
11	1.1– 7.8	0.8
19	0.6– 8.3	1.9
25	2.2–11.1	4.3
Mar 3	0.6– 6.7	1.0
10	6.7–11.1	2.5
16	2.2–11.1	1.5
24	3.3–10.0	4.3
31	5.0–11.1	4.0
Apr 7	5.6–14.4	1.8
16	5.0–13.3	4.0
21	5.6–14.4	1.6
27	8.3–14.4	1.5
May 2	8.9–16.7	1.2
11	11.1–17.2	1.2
19	11.4–16.7	0.8

ing fall and winter months. Median GSI values for females gradually increased from 0.3% in September to a peak of 12.7% in March (Table 1). Eggs ripened during the fall months, and ovary weights stabilized from December until the initiation of spawning in February. Females exhibited an extreme range of GSI values on February 19 and March 5, because 27% and 40%, respectively, of these females were spent. Between April and August, GSI values remained at less than 1.0%. There was no evidence of female spawning prior to February, but all females were spent in the April 19 collection.

The spawning period of rainbow trout in the South Fork extended from mid-February to early April, and occurred when water temperature and flow were increasing. Water temperatures reached minimum values during February, then gradually increased through May (Table 2). Stream discharge was also minimal in early February (0.8 $m^3\ s^{-1}$) and then steadily increased to a maximum of 4.3 $m^3\ s^{-1}$ in late March.

Calorific Equivalents

A total of 65 male and 64 female rainbow trout were pooled into 6 2-month sample periods to compare changes in energy content of tissues during the year (Table 3). Sample sizes were insufficient to quantify tissue

Table 3. Median Energy Values (cal mg^{-1} Dry Weight) of Body Tissues in Rainbow Trout, South Fork Holston River, September 1979 to August 1980

Tissue Type	Sample Period					
	1 Sep–Oct	2 Nov–Dec	3 Jan–Feb	4 Mar–Apr	5 May–Jun	6 Jul–Aug
Liver						
Male	5.183(16)[a]	5.268(12)	5.285(10)	5.309(11)	5.395(9)	5.513(7)
Female	5.281(14)	5.190(6)	5.263(8)	5.075(6)	5.360(15)	5.699(15)
Muscle						
Male	5.033(16)	4.881(12)	5.041(10)	4.910(11)	5.072(9)	5.306(7)
Female	5.100(14)	4.846(6)	4.878(8)	4.844(6)	4.887(15)	5.191(15)
Alimentary tract						
Male	6.327(10)	5.154(12)	5.358(10)	5.364(11)	5.975(9)	5.457(7)
Female	7.334(8)	6.385(6)	4.934(8)	6.418(6)	6.472(15)	6.564(15)

[a] Sample size is in parentheses.

weight differences either by average weight in the collection or by the standard fish technique (Idler and Bitners 1958, MacKinnon 1972, Craig 1977, Medford and Mackay 1978). Changes in tissue energy content were therefore expressed as caloric densities or calorific equivalents (cal mg^{-1} dry weight).

Liver and muscle tissue of male and female rainbow trout exhibited small but significant differences in calorific equivalents during the year (Table 3). Median energy content of liver tissue in males increased significantly ($P < 0.05$) from September-October (5.183 cal mg^{-1}) to July-August (5.513 cal^{-1} mg). Calorific equivalents of female liver tissue decreased between September-October (5.281 cal mg^{-1}) and March-April (5.075 cal mg^{-1}) and then increased significantly ($P < 0.05$) through July-August (5.699 cal mg^{-1}). Energy values for both male and female liver tissue peaked during the July-August period. Energy content of male epaxial muscle was similar between September-October and March-April. Median calorific values then increased significantly ($P < 0.05$) between March-April (4.910 cal mg^{-1}) and July-August (5.306 cal mg^{-1}). Calorific equivalents for epaxial muscle in females declined from September-October (5.100 cal mg^{-1}) to a relatively constant value (4.887 cal mg^{-1}) through May-June, and peaked in July-August (5.191 cal mg^{-1}). There was no evidence that caloric densities in either liver or muscle tissue decreased significantly during gonadal maturation in fall and winter.

The greatest change in median energy content occurred in alimentary tissue (Table 3). Calorific equivalents of male alimentary tracts were significantly different ($P < 0.05$) among periods. Energy values peaked during September-October (6.327 cal mg^{-1}) and were lowest during November-

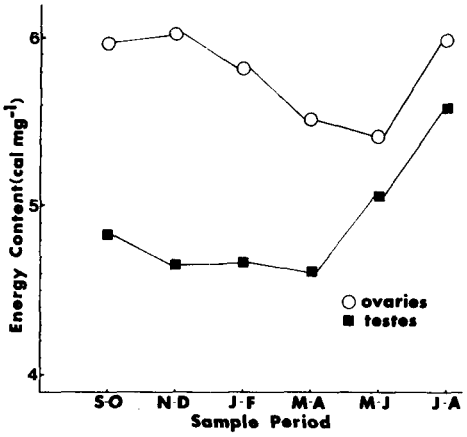

Figure 1. Median calorific equivalents of gonads from rainbow trout, South Fork Holston River, September 1979 to August 1980.

December (5.154 cal mg^{-1}). Median values for females were also significantly different ($P < 0.005$) among periods; equivalents peaked in September-October (7.334 cal mg^{-1}) and declined to their lowest value in January-February (4.934 cal mg^{-1}). Mesentary fat in sacrificed specimens of both sexes peaked in late summer, then gradually decreased as gonads developed. During the period of active gametogenesis (fall and winter), the caloric content of male and female alimentary tissue decreased significantly ($P < 0.05$). There were no fat deposits evident along the alimentary tract of females examined during the spawning period.

Caloric densities of gonads appeared to follow an annual cycle, and the difference in caloric equivalent for protein (5.65 cal mg^{-1}) and fat (9.45 cal mg^{-1}) contributed to the disparity between energy levels in male and female gonads (Fig. 1). Testes and ovaries exhibited significant differences ($P < 0.05$) in energy content among sample periods. Energy values of testes declined slightly between September-October (4.815 cal mg^{-1}) and March-April (4.609 cal mg^{-1}), increased through May-June (5.069 cal mg^{-1}), and peaked in July-August (5.594 cal mg^{-1}). Recrudescence of testes in examined males was dramatic in September, and exhibited little additional size increase thereafter. Based on male GSI values for fall and winter, the relatively constant energy equivalents from November to April were probably due to the high protein content of mature spermatozoa. Median calorific equivalents of ovaries decreased from November-December (6.017 cal mg^{-1}) to May-June (5.422 cal mg^{-1}). Ovaries exhibited a substantial size increase in October and continued to mature into February. As the ova matured and

deepened in color, ovarian membranes began to separate just prior to the spawning period.

Discussion

Previous studies have identified photoperiod and water temperature as major environmental stimuli for gonadal recrudescence in fishes (Nikolsky 1963, Hoyt 1971, deVlaming 1972, Davis 1977). Length of photoperiod apparently activates gonadotropin production (Whitehead et al. 1978a) and changes in water temperature affect total metabolic rate (Nikolsky 1963) or alter photoperiodic response (deVlaming 1972). In brook trout (*Salvelinus fontinalis*), short photoperiods and acceleration of the natural photoperiod cycle induced early gonadal maturity (Henderson 1963). Decreasing photoperiod and warmer temperatures accelerated spermatogenesis more than decreasing photoperiod and colder temperatures in rainbow trout (Breton and Billard 1977). When normal yearly cycles of photoperiod were compressed into 6- and 9-month periods, rainbow trout spawning was advanced by 12 weeks and 6 weeks, respectively (Whitehead et al. 1978a). Most laboratory results with salmonids have indicated that decreasing day length mediates gonadal recrudescence, and photoperiod manipulation is commonly practiced in hatcheries to induce rainbow trout spawning (Kunesch et al. 1974). However, the potential role of low water temperatures in initiating gametogenesis during fall and winter has been largely unexplored (Peter and Crim 1979). A detailed description of reproductive endocrinology in rainbow trout is provided by Whitehead et al. (1978b).

Increasing water temperature and flow have previously been identified as stimuli for spawning readiness in trout (Mottley 1933, Dodge and MacCrimmon 1971, Erman and Hawthorn 1976). Our observations on rainbow trout in the South Fork appear to corroborate the importance of these environmental cues for the commencement of spawning. The upper South Fork is fed by numerous springs which maintain slightly elevated winter water temperatures compared to other streams in southwestern Virginia. Concurrent monitoring of rainbow trout reproduction in 2 smaller streams in adjacent counties of Virginia indicated that gonadal recrudescence and spawning occurred slightly later in these populations (Brayton 1981). Flow rates in these streams were inconsistent and exhibited no general trends. The role of water temperature in initiating spawning therefore appears to be of major importance in rainbow trout.

Accumulation and storage of fats prior to gonadal recrudescence is commonly reported in fishes, and this sexual maturation is often, perhaps always, accomplished at the expense of body tissues (Love 1970). Fish species that

spawn during late winter or early spring usually have low GSI values during summer but undergo active gametogenesis through fall and winter (Wootton 1979). During gonadal development there is a protein increase in testes and a protein and fat (oil) increase in ovaries. Our GSI and calorific data corroborate these physiological trends in the reproductive cycle of rainbow trout. Energy mobilization associated with these changes in sexual maturity comes from stored reserves, but the source of these reserves can differ among species groups. Mature fish have been shown to store energy in the liver (Love 1970, Larson 1973), muscle (Idler and Bitners 1958) and along the alimentary tract Swift 1955) in preparation for the spawning season. Diana and Mackay (1979) reported that the depletion of body energy associated with gonadal recrudescence in northern pike (*Esox lucius*) occurred without any change in body calorific equivalents. Depletion of total body energy during spawning may therefore be due to catabolism of whole tissue rather than metabolism of specific constituents such as protein or fat within tissues.

Within the Salmonidae, fat deposits along the alimentary tract serve as a major energy reserve. Swift (1955) reported that fat along the mesentaries and pyloric caecae were the main food reserve in brown trout. Similarly, the alimentary tract is the major source of fat and protein from the internal organs during the initial phase of salmon upstream migration (Idler and Bitners 1959). Our results demonstrated that the alimentary tract of rainbow trout exhibited the greatest change in caloric density of any analyzed tissue, and that the loss of energy occurred during the period of gonadal recrudescence in fall and winter. However, the direct correlation between these 2 events was not demonstrated, and the source of energy for gonadal maturation can only be inferred. During the processing of alimentary tissue, it was evident that fish were feeding at a reduced rate during winter and supplementing endogenous energy reserves. Previous studies have concluded that no single method can adequately assess energy mobilization during the reproductive cycle of fishes. During this period gonadal recrudescence, migration, spawning activities (courtship, nesting), reduced feeding, physiological stress, and many other factors all constitute a drain on stored energy. The source of energy for each of these activities is therefore a complex issue that can only be resolved by specific research.

Literature Cited

Brayton, S. 1981. Reproductive biology, energy content of tissues, and annual production of rainbow trout (*Salmo gairdneri*) in the South Fork of the Holston River, Virginia. M.S. Thesis, Va. Polytech. Inst. State Univ., Blacksburg. 110pp.

Breton, B., and R. Billard. 1977. Effects of photoperiod and temperature on plasma

gonadotropin and spermatogenesis in the rainbow trout *Salmo gairdneri* Richardson. Ann. Biol. Anim. Biochem. Biophys. 17:331–340.

Carlander, K. D. 1969. Handbook of freshwater fishery biology. Vol. I. Iowa State Univ. Press, Ames. 752pp.

Craig, J. F. 1977. The body composition of adult perch, *Perca fluviatilis*, in Windermere, with special reference to seasonal changes and reproduction. J. Anim. Ecol. 46:617–632.

Davis, T. L. 1977. Reproductive biology of the freshwater catfish, *Tandanus tandanus* Mitchell, in the Gwydir River, Australia. II. Gonadal cycle and fecundity. Aust. J. Mar. Freshwat. Res. 28:159–169.

Delahunty, G., and V. L. deVlaming. 1980. Seasonal relationships of ovary weight and fat stores with body weight in the goldfish, *Carassius auratus* (L.). J. Fish Biol. 16:5–13.

deVlaming, V. L. 1972. Environmental control of teleost reproductive cycles: a brief review. J. Fish Biol. 4:131–140.

Diana, J. S., and W. C. Mackay. 1979. Timing and magnitude of energy deposition and loss in the body, liver, and gonads of northern pike (*Esox lucius*). J. Fish. Res. Board Can. 36:481–487.

Dodge, D. P., and H. R. MacCrimmon. 1971. Environmental influences of extended spawning of rainbow trout (*Salmo gairdneri*). Trans. Am. Fish. Soc. 100:312–318.

Dunn, O. J. 1964. Multiple comparisons using rank sums. Technometrics 6:241–252.

Erman, D. C., and V. M. Hawthorn. 1976. The quantitative importance of an intermittent stream in the spawning of rainbow trout. Trans. Am. Fish. Soc. 105:675–681.

Greene, C. W. 1919. Biochemical changes in the muscle tissue of king salmon during the fast of spawning migration. J. Biol. Chem. 39:435–456.

———. 1926. The physiology of the spawning salmon. Physiol. Rev. 6:201–241.

Henderson, N. E. 1963. Influence of light and temperature on the reproductive cycle of the eastern brook trout, *Salvelinus fontinalis* (Mitchill). J. Fish. Res. Board Can. 20:859–897.

Hollander, M., and D. A. Wolfe. 1973. Nonparametric statistical methods. John Wiley and Sons, New York. 503pp.

Hoyt, R. D. 1971. The reproductive biology of the silverjaw minnow, *Ericymba buccata* cope, in Kentucky. Trans. Am. Fish. Soc. 100:510–519.

Idler, D. R., and I. Bitners. 1958. Biochemical studies on sockeye salmon during spawning migration. II. Cholesterol, fat, protein, and water in the flesh of a standard fish. Can. J. Biochem. Physiol. 36:793–798.

———, and ———. 1959. Biochemical studies on sockeye salmon during spawning migration. V. Cholesterol, fat, protein, and water in the body of the standard fish. J. Fish. Res. Board Can. 16:235–241.

Kunesch, W. H., W. J. Freshman, M. Hoehm, and N. G. Nordin. 1974. Altering the spawning cycle of a rainbow trout by control of artificial light. Prog. Fish-Cult. 36:225–226.

Larson, G. L. 1973. Liver weight of brook trout in a high-mountain lake in Washington state. Prog. Fish-Cult. 35:234–236.

Love, R. M. 1970. The chemical biology of fishes. Academic Press, London. 547pp.

MacKinnon, J. C. 1972. Summer storage of energy and its use for winter metabolism and gonad maturation in American plaice (*Hippoglossoides platessoides*). J. Fish. Res. Board Can. 29:1749–1759.

Medford, B. A., and W. C. MacKay. 1978. Protein and lipid content of gonads, liver, and muscle of northern pike (*Esox lucius*) in relation to gonad growth. J. Fish. Res. Board Can. 35:213–219.

Mottley, C. M. 1933. The spawning migration of rainbow trout. Trans. Am. Fish. Soc. 62:80–84.

Needham, P. R. 1938. Trout streams: conditions that determine their productivity and suggestions for stream and lake management. Holden-Day, San Francisco. 241pp.

Nikolsky, G. V. 1963. The ecology of fishes. Academic Press, New York. 352pp.

Peter, R. E., and L. W. Crim. 1979. Reproductive endocrinology of fishes: gonadal cycles and gonadotropin in teleosts. Ann. Rev. Physiol. 41:323–335.

Shul'man, G. E. 1974. Life cycles of fishes: physiology and biochemistry. John Wiley and Sons, New York. 258pp.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco. 776pp.

Swift, D. R. 1955. Seasonal variations in the growth rate, thyroid gland activity, and food reserves of brown trout (*Salmo trutta* Linn.). J. Exp. Biol. 32:751–764.

Whitehead, C., N. R. Bromage, B. Breton, and R. Billard. 1978a. Effects of altered photoperiod on serum gonadotrophin levels and spawning in female rainbow trout. J. Endocrinol. 79:29–30.

———, ———, and J. R. M. Forster. 1978b. Seasonal changes in reproductive function of the rainbow trout (*Salmo gairdneri*). J. Fish Biol. 12:601–608.

Wootton, R. J. 1979. Energy costs of egg production and environmental determinants of fecundity in teleost fishes. Symp. Zool. Soc. Lond. 44:133–159.