

## Physiological Responses of Juvenile Rainbow Trout to Fasting and Swimming Activity: Effects on Body Composition and Condition Indices

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**Abstract.**—The physiological traits that allow fish to survive periods of limited food resources are poorly understood. We assessed changes in proximate body composition, relative organ mass, blood metabolites, and relative weight ( $W_r$ ) of sedentary and actively swimming (15 cm/s) juvenile rainbow trout (154–182 mm total length) over 147 d of fasting. Fasting caused measurable responses that were augmented when fish were swimming. Lipids and plasma triacylglycerides declined over time. Proteins were catabolized simultaneously with lipid reserves, but ammonia concentrations in plasma did not increase. The liver somatic index (LSI) did not change substantially over 105 d, suggesting that gluconeogenesis maintained blood glucose concentrations and hepatic glycogen reserves for a substantial period of fasting. The gut somatic index (GSI) and  $W_r$  declined linearly during fasting, but the LSI did not decline until after 105 d of fasting. Consequently, the use of different body condition indices could lead to different conclusions about the condition of juvenile rainbow trout. Swimming activity caused fish to have lower lipid and protein reserves than those of sedentary fish. No mortalities were observed among sedentary fish, but mortalities occurred among actively swimming fish after 97 d of fasting when 3.2% or less lipid remained in their bodies. Body condition indices did not account for differences in proximate body composition between sedentary and actively swimming fish and were relatively poor predictors of lipid content and risk of mortality. The probability of mortality was most accurately predicted by percent lipid content. Therefore, we suggest that fisheries scientists consider using percent lipid content when evaluating the physiological status and risk of mortality due to starvation among juvenile rainbow trout.

North-temperate salmonids typically experience periods of limited food availability and consumption. Food availability has been linked to variation in body condition and survival of salmonids (see Cunjak and Power 1987; Filbert and Hawkins

1995; Simpkins and Hubert 2000), but little consideration has been given to physiological mechanisms that enable salmonids to survive periods with little or no food. In general, salmonids are adapted to mobilize energy reserves and survive extensive periods without food (Toneys and Coble 1980; Navarro and Gutiérrez 1995).

Fasting involves the ability of an animal to adapt physiologically to limited consumption and maintain a level of metabolic homeostasis that can preserve vital organ function. Starvation occurs when homeostatic control is lost and vital organ function is compromised (Young and Scrimshaw 1971; Van Itallie and Yang 1984). Measures of proximate

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body composition (i.e., lipid, protein, and water) respond quickly to variation in food consumption, and changes in proximate body composition can be reflected in organ weights (e.g., liver and gut) and blood metabolites (e.g., glucose, triacylglyceride, and ammonia concentrations; Love 1970; Henderson and Tocher 1987; Navarro and Gutiérrez 1995).

Physiologists typically describe three phases of fasting for vertebrate animals (Castellini and Rea 1992; Hervant et al. 2001). Responses to fasting have not been characterized according to phases for salmonids, but phases probably occur there also (Navarro and Gutiérrez 1995). During the first few days of food deprivation, that is, phase 1, hepatic glycogen reserves are used as the body defends the concentrations of circulating glucose (Hilton 1982; Moon et al. 1989; Vijayan and Moon 1992) needed as critical fuel for the central nervous system (Young and Scrimshaw 1971; Vander et al. 1994; Soengas et al. 1998). To conserve glucose, the body switches to fat oxidation while preserving proteins (Navarro and Gutiérrez 1995). If possible, bodily movements are reduced and overall metabolic rates begin to decline (Cook et al. 2000). Phase 2 involves further use of lipids, partial use of proteins, and additional metabolic depression (Castellini and Rea 1992). The use of lipids involves the catabolism of triacylglycerides in adipose tissue and liberation of free fatty acids and glycerol into blood circulation. Fatty acids are then processed in the liver into ketone bodies and circulated as energy sources for use by the brain (Soengas et al. 1998). Protein catabolism liberates amino acids, which are catabolized into keto acids and ammonia in the liver (Robinson and Mead 1973; Mommsen et al. 1980; Van Waarde 1983). Glycerol and protein-derived keto acids are precursors for glucose production by way of gluconeogenesis (Morata et al. 1982; Moon et al. 1989). Phase 3 entails an increased use of protein, which compromises vital tissue function and leads to death (Castellini and Rea 1992; Navarro and Gutiérrez 1995). The extent to which salmonids can use proteins before starving is unknown. The effects of fasting on salmonid survival are accentuated by swimming activity, but the effects on body composition, blood metabolites, and organ mass have not been examined (Moyes and West 1995).

We were not aware of any research that describes the time course of fasting and the effect of swimming activity on physiological responses among salmonids. Our purpose was to examine the

effects of fasting and swimming activity on proximate body composition (whole body lipid, protein, and water), organ mass (liver and gut), and blood metabolites (triacylglycerides and ammonia) of juvenile rainbow trout *Oncorhynchus mykiss*. Our objectives were to (1) describe changes in proximate body composition, relative organ sizes, and blood characteristics over time among fasted rainbow trout; (2) determine the extent that body reserves, relative organ sizes, and blood characteristics change before death from starvation; and (3) assess differences in physiological responses between actively swimming and sedentary fish being fasted. Additionally, our data permitted us to examine whether three body condition indices (relative weight [ $W_r$ ], liver somatic index [LSI], and gut somatic index [GSI]) were accurate in assessing physiological condition, as estimated by proximate body composition, and in predicting mortality. Relative weight (Wege and Anderson 1978) has been applied widely in fisheries management. It is popular because it does not vary with fish length and is assumed to serve as a surrogate measure of lipid reserves (Brown and Murphy 1991; Blackwell et al. 2000).

### Methods

Juvenile rainbow trout (154–182 mm total length [TL]) were obtained from the Wyoming Game and Fish Department's Como Bluffs Fish Hatchery and transported to the University of Wyoming's Red Buttes Environmental Research Laboratory near Laramie. Two-hundred seventy-five fish were marked with soft visual-implant tags (Northwest Marine Technologies, Inc.); 75 fish were placed randomly in each of three 240-L circular tanks (height = 0.85 m; radius = 0.30 m) that received a continuous flow (10 L/min) of aerated well water maintained at 7.5°C. An automated timer and fluorescent lights were used to produce a light:dark photoperiod of 12:12 h. Fish were allowed to acclimate for 21 d during which they were fed an excess ration of Silver Cup pelleted trout chow (Nelson and Son's, Inc.). After the acclimation period, fish were sedated with MS-222 (3-aminobenzoic acid ethyl ester methanesulfonate), individually identified, weighed to the nearest gram, and measured for TL to the nearest millimeter. We continued to feed the fish in one tank, but fish in the other two tanks were subjected to two experimental treatments to affect proximate body composition and condition. The latter fish were deprived of food and were held either in standing water or in a current (approximately 15

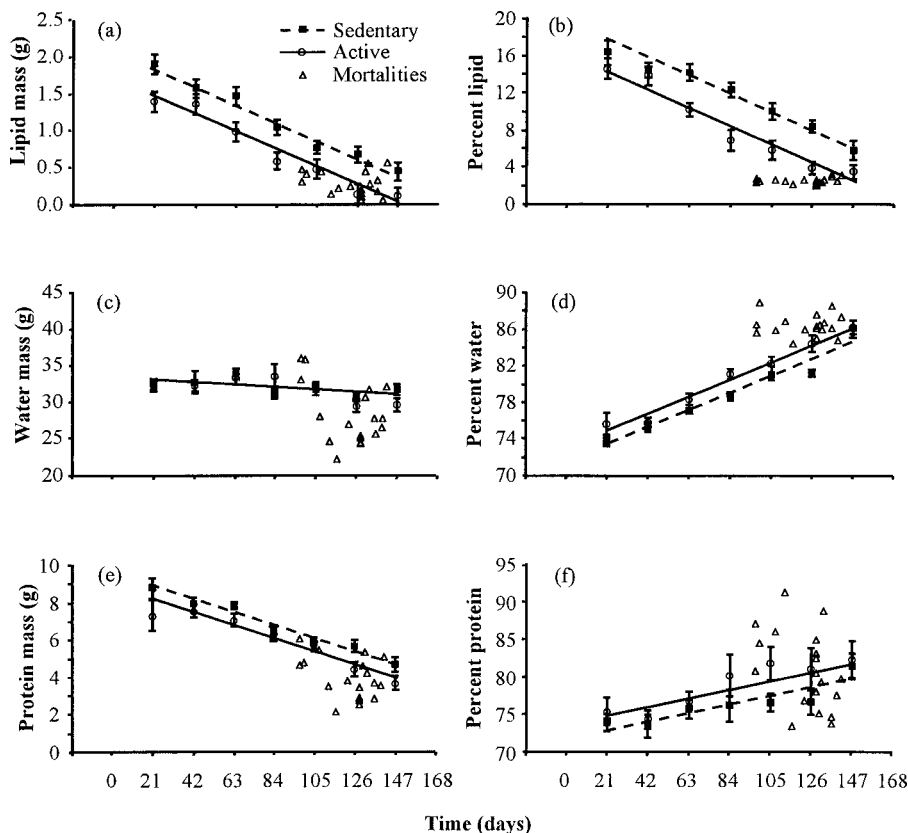


FIGURE 1.—Trends in mean masses and percentages of (a–b) lipid, (c–d) water, and (e–f) protein among sedentary and actively swimming (15 cm/s) juvenile rainbow trout (154–182 mm TL) over 147 d of fasting.

cm/s) produced by circulating water pumps (March Manufacturing, Inc., Model 2U).

All of the fish from each tank were sedated, individually identified, weighed, and measured every 21 d. Six fish were randomly selected from each tank and killed to measure blood metabolites, liver and gut weights, and proximate body com-

position on each sampling date. Blood was collected from the dorsal aorta, transferred into a 1-mL Eppendorf tube, weighed, and centrifuged for 5 min to separate plasma. Plasma was cryogenically preserved in plastic tubes before analysis. Triacylglyceride and ammonia concentrations in plasma were assayed colorimetrically with medical diagnostic kits (Sigma-Aldrich Inc.; triacylglyceride procedure 337; ammonia procedure 171).

Sedated fish were killed with a blow to the head and ventrally lacerated to remove the liver and gut, which consisted of the esophagus, stomach, pyloric caeca, intestine, rectum, and anus. After weighing, the organs were returned to the body cavities before the fish were quartered and dried at 60°C to a constant mass (approximately 48 h). Whole body water content was determined as the difference between wet and dry tissue weight. Dry tissues of individual fish were ground to a fine powder and stored in airtight plastic containers at -20°C away from light. The total lipid content of dry tissue was assessed by measuring the total fatty

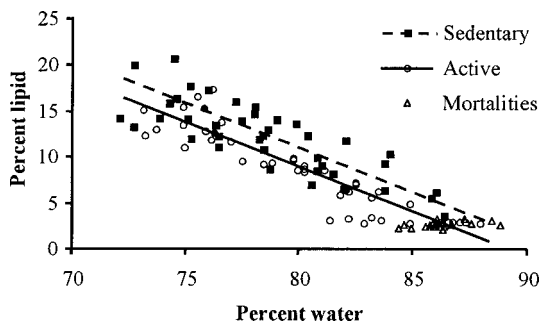


FIGURE 2.—Percent lipid relative to percent water for both sedentary and actively swimming juvenile rainbow trout.

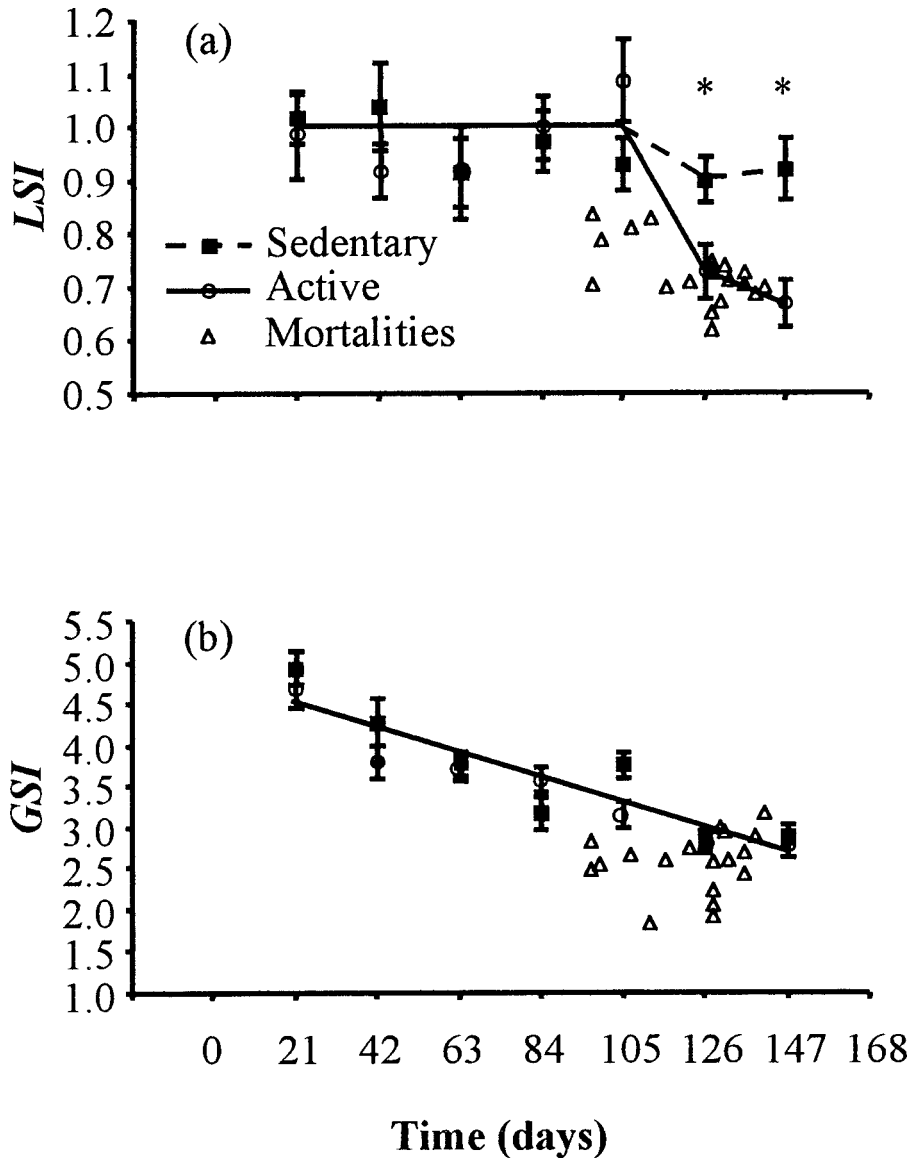


FIGURE 3.—Trends in mean (a) liver somatic index (LSI) and (b) gut somatic index (GSI) among sedentary and actively swimming juvenile rainbow trout over 147 d of fasting. Asterisks denote significant differences between treatments in a two-sample *t*-test.

acid content. Duplicate 200-mg samples of dried tissue were subjected to direct transesterification with borontrifluoride in methanol to prepare fatty acid methyl esters (Rule 1997). Fatty acid methyl esters were quantified by gas-liquid chromatography (Hewlett-Packard 5890) as described by Rule (1997). The nitrogen content of dry tissue, determined in 5–10-mg samples with a CHNS analyzer (Fisons Instrument EA 1108), was converted to total crude protein (percent crude protein

= percent nitrogen  $\times$  6.25). Total lipid and crude protein were expressed as dry weight percentages.

The liver somatic index and GSI were calculated as the proportion of liver or gut mass relative to the wet mass of the sampled fish ( $100 \times$  organ weight/wet mass). Relative weight was calculated as

$$W_r = 100 \times W/W_s$$

where  $W$  was the wet weight (g) of the individual

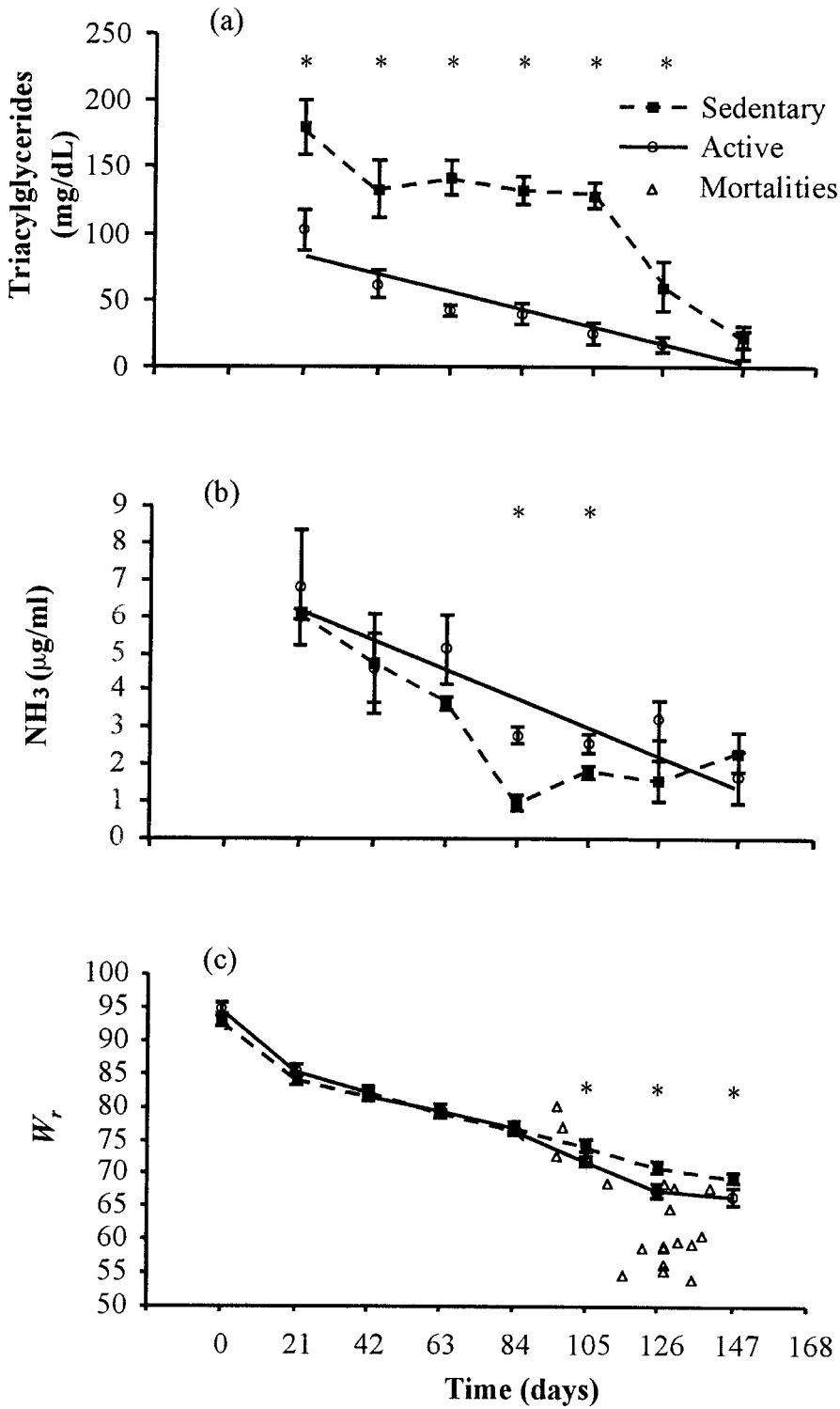


FIGURE 4.—Trends in (a) triacylglyceride concentrations in blood plasma, (b) ammonia (NH<sub>3</sub>) concentrations in blood plasma, and relative weight (*W<sub>r</sub>*) among sedentary and actively swimming juvenile rainbow trout over 147 d of fasting. Asterisks denote significant differences between treatments in a two-sample *t*-test.

fish and  $W_s$  was the length-specific standard weight. We used the lotic  $W_s$  equation for rainbow trout ( $\log_{10} W_s = -5.023 + 3.024 \log_{10} \text{TL}$ ) from Simpkins and Hubert (1996) to calculate  $W_r$ .

Two-sample  $t$ -tests were used to test for differences in the means of initial lengths, weights, and  $W_r$  between fasted treatments. Data collected from fed fish were used to account for allometric variation in measures collected from fasted treatments. Least-squares regression and correlation analyses were used to evaluate relationships between  $\log_{10}$  transformed values of TL and measures of proximate body composition, blood characteristics, LSI, and GSI for fed fish. Slopes and back-transformed intercepts were used in a Gauss–Newton nonlinear regression algorithm to empirically derive constants ( $a$  and  $b$ ) for the power function relating dependent variables with total length ( $Y = a\text{TL}^b$ ; Motulsky and Ransnas 1987; Haddon 2001). To minimize variation in measures associated with length for fasted fish, residuals were calculated as the difference between the observed values for fasted fish and  $a\text{TL}^b$ .

We assessed changes over time in residuals of proximate body composition, blood characteristics, LSI, GSI, and  $W_r$  for fasted fish. Coefficients of determination, residual plots, and  $F$ -tests for lack of fit were used to evaluate the suitability of applying linear models to assess changes over time in proximate body composition, blood metabolites, LSI, GSI, and  $W_r$  (Neter et al. 1996). Mortalities were excluded from analyses, but we included them in the accompanying illustrations to identify the extent to which proximate body composition, blood metabolites, LSI, GSI, and  $W_r$  changed before mortality occurred. For linear relationships, we used the general linear model (GLM; Neter et al. 1996) to determine whether the intercepts and rates of change in response variables differed between treatments. The GLM took the following form:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \varepsilon_p$$

where  $Y$  was the response variable of interest,  $X_1$  was a nominal variable equal to 0 if fish were inactive and 1 if fish were active, and  $X_2$  was a continuous variable equal to the time of treatment (d). The value of  $\beta_0$  was a derived constant, equal to the common intercept of the relationship between the dependent variable and time. For nonlinear relationships between response variables and time, we used Spearman rank correlation coefficients ( $r_s$ ). Two-sample  $t$ -tests were used to

compare means in response variables for each sampling date between treatments. Although we conducted statistical analyses on residuals, we report changes in response variables over time for fish of average length (residual +  $a\overline{\text{TL}}^b$ ) to facilitate interpretation.

The GLM was also used to assess differences in intercepts and slopes of relationships between percent water ( $X_2$ ) and percent lipid ( $Y$ ) as well as between body condition indices ( $X_2 = W_r$ , LSI, or GSI) and percent lipid for active and sedentary fish. Mortalities were excluded from the analyses. Coefficients of determination, residual plots, and  $F$  lack-of-fit tests were used to evaluate the suitability of applying linear models to assess relationships for active and sedentary fish (Neter et al. 1996). For the  $F$  lack-of-fit test, we grouped values of percent water and  $W_r$  by rounding to the nearest whole number; for LSI and GSI, we rounded to the nearest tenth.

We used logistic regression (Neter et al. 1996) to evaluate the ability of proximate body composition, LSI, GSI, and  $W_r$  to predict mortality. The logistic regression model took the following form:

$$\text{Probability of survival} = e^{\text{logit}} / (1 + e^{\text{logit}}),$$

where the logit was the linear model  $\text{logit} = \beta_0 + \beta_1 X_1$ ,  $X_1$  was the variable of interest (i.e., percent water, percent lipid, percent protein, LSI, GSI, or  $W_r$ ), and  $\beta_0$  and  $\beta_1$  were empirically derived regression coefficients. A  $z$ -test was used to judge the significance of each regression coefficient in logistic models. A likelihood ratio chi-square test ( $G$ -test) was used to determine the significance of a model. Log-likelihood values, Hosmer and Lemeshow's goodness-of-fit test, and percent concordance were used to determine the best logistic model. Log-likelihood values were used to compare models on the basis of the weight of evidence in favor of a model given the data, where better models had higher log-likelihood values than poorer models did (Neter et al. 1996; Haddon 2001). For the Hosmer and Lemeshow test,  $P$ -value greater than 0.10 suggested a significant difference between the observed and model-predicted values of the dependent variable. Consequently, the strength of fit of the data to the logistic model was evaluated by the magnitude of the Hosmer and Lemeshow  $P$ -value, with larger  $P$ -values indicating highly significant model fit (Hosmer and Lemeshow 1989). Percent concordance was a mea-



sure of association between observed values and predicted probabilities.

Logistic regression analyses were performed by using MINITAB 10.2 (Minitab 1994), but other statistical computations were performed with JMP 4.0.4 (SAS Institute 2001). Significance was determined at  $P < 0.05$  for all tests, and means ( $\pm$ SE) are presented unless otherwise noted.

## Results

### *Effects of Length*

At the onset of the experiment, fish did not differ significantly in mean total length ( $168 \pm 0.5$  mm), weight ( $49 \pm 0.5$  g), or  $W_r$  ( $94 \pm 0.5$ ) among treatments. Lipid mass, water mass, protein mass, percent protein, LSI, and GSI increased allometrically with body length for fed fish. Consequently, we calculated the residuals of these measures for our experimental fish, and changes over time in these measures are presented for fish of average initial length. Percent lipid, percent water, triacylglyceride concentrations, and ammonia concentrations were not significantly related to total length, so statistical computations were performed with observed values.

### *Changes in Body Composition*

Trends in mean lipid mass over time did not differ significantly from a linear model for sedentary or actively swimming fish (Figure 1a). From 21 to 147 d, mean lipid mass in a fish of average length significantly decreased, but at a rate that did not differ significantly between sedentary and active fish (common slope =  $-0.012 \pm 0.001$  g/d). However, the intercepts of this relationship were significantly different between treatments. After 21 d, mean lipid mass was  $0.4 \pm 0.1$  g lower in active fish than in sedentary fish. Mean percent lipid significantly decreased in a manner that did not significantly depart from linearity among both active and sedentary fish (Figure 1b). Although the slope of the relationship between percent lipid and time did not differ significantly between treatments (common slope =  $-0.088 \pm 0.006\%/d$ ), the intercepts were significantly different. After 21 d, percent lipid was  $3.4 \pm 0.5\%$  lower in active fish than in sedentary fish.

Changes in mean water mass over time for actively swimming and sedentary fish of average length did not differ significantly from a linear pattern (Figure 1c). The slope and intercept of the relationship between water mass and time did not differ significantly between treatments (common slope =  $0.020 \pm 0.006$  g/d; common intercept =

$33.5 \pm 0.6$  g), and water mass declined significantly over the course of the study. Conversely, percent water significantly increased with time in a fashion that did not depart significantly from linearity for both active and sedentary fish (Figure 1d). The rate at which percent water increased did not differ between treatments (common slope =  $0.092 \pm 0.004\%/d$ ), but water was  $1.4 \pm 0.3\%$  greater in active fish after 21 d.

Negative relationships between percent lipid and percent water did not differ significantly from a linear model for actively swimming or sedentary fish (Figure 2). Although swimming activity had no significant effect on the slope (common slope =  $-0.912 \pm 0.053$ ), the effect on the intercepts was significant. The intercept was  $83.7 \pm 0.3\%$  lipid for sedentary fish ( $r^2 = 0.70$ ) and  $81.5 \pm 0.3\%$  lipid for active fish ( $r^2 = 0.84$ ).

Actively swimming and sedentary fish of average length lost protein mass in a fashion that did not differ significantly from a linear model and at a rate that did not differ between treatments (common slope =  $-0.031 \pm 0.003$  g/d; Figure 1e). Active fish averaged  $0.7 \pm 0.2$  g less protein than sedentary fish after 21 d. Changes in percent protein over time did not differ significantly from linearity for both treatments (Figure 1f). Even though the slope of the relationship did not differ significantly between treatments (common slope =  $0.062 \pm 0.012\%/d$ ), the intercept was significantly higher ( $2.1 \pm 1.0\%$ ) for active fish.

### *Changes in Organ Size*

Trends in LSI for sedentary fish of average length did not differ significantly from a linear model over the course of the experiment but did significantly differ from linearity among actively swimming fish (Figure 3a). The relationship with time did not differ significantly from a linear pattern for as long as 105 d for LSI in either active or sedentary fish. Active and sedentary fish of average length maintained a mean LSI at  $1.0 \pm 0.02$  over 105 d. After 105 d, the LSI declined and active fish had significantly smaller livers and significantly lower LSI values than sedentary fish. Relationships between GSI and time for active and sedentary fish of average length did not differ significantly from linear models (Figure 3b). No significant differences in intercepts or slopes were observed between treatments (common intercept =  $4.8 \pm 0.1$  g; common slope =  $-0.015 \pm 0.001$  g/d).

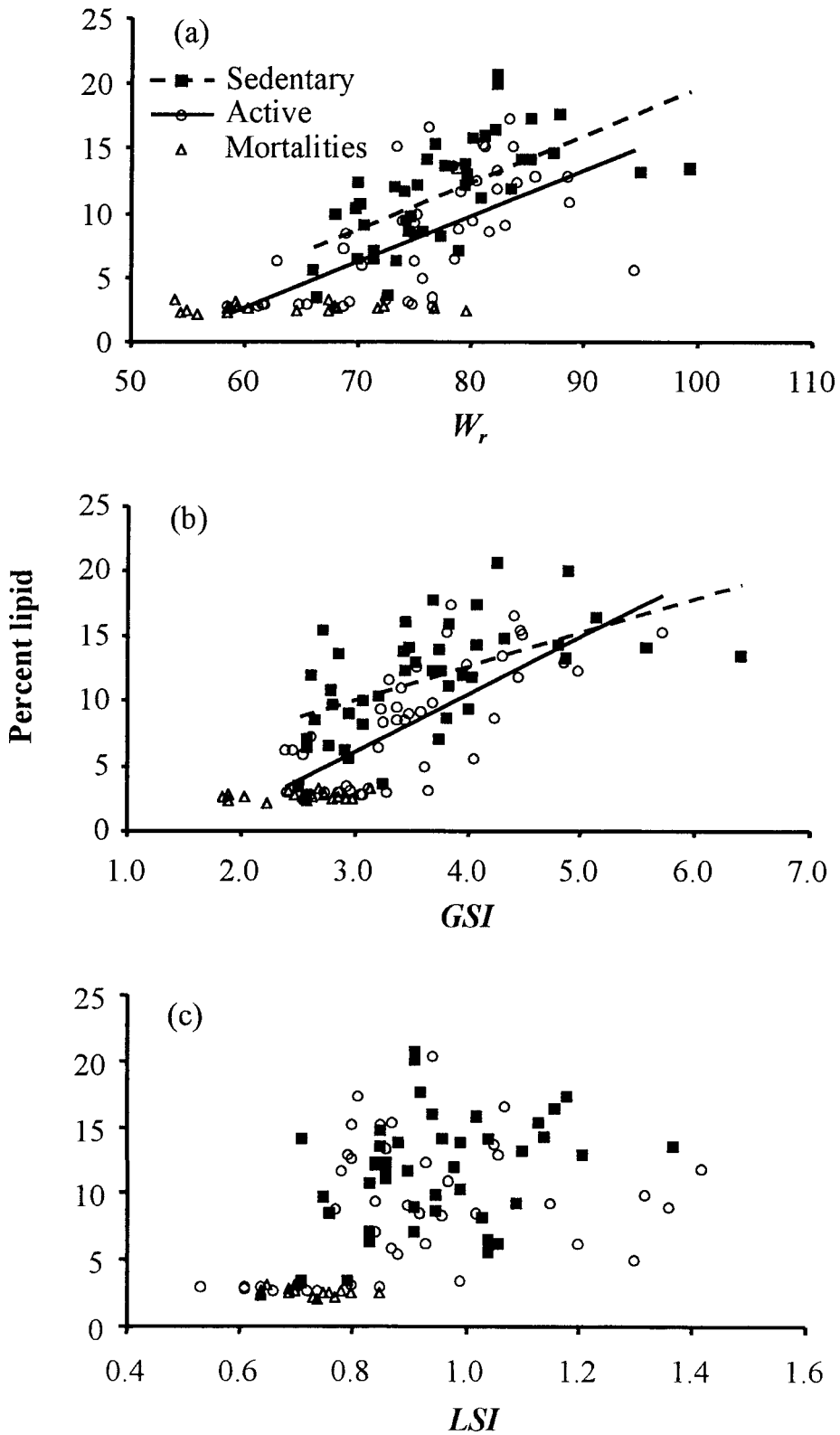


FIGURE 5.—Relationships between percent lipid and (a) relative weight ( $W_r$ ), (b) gut somatic index (GSI), and (c) liver somatic index (LSI) for both sedentary and actively swimming juvenile rainbow trout over 147 d of fasting.



TABLE 1.—Logistic regression equations and associated statistics describing the relationship between survival and percent lipid, percent water, percent protein, gut somatic index (GSI), liver somatic index (LSI), and relative weight ( $W_r$ ) for both actively swimming, (15 cm/s) and sedentary juvenile rainbow trout (154–182 mm total length) fasted 147 d.

Predictor variable	Intercept				Slope			
	$\beta_0$	SD	$z$	$P$	$\beta_i$	SD	$z$	$P$
Percent lipid	−12.38	4.49	−2.76	<0.01	4.51	1.66	2.72	<0.01
Percent water	56.23	14.19	3.96	<0.001	−0.65	0.17	−3.93	<0.001
Percent protein	9.47	3.65	2.60	<0.01	−0.10	0.05	−2.21	<0.05
LSI	−7.63	2.24	−3.41	0.001	11.29	2.90	3.89	<0.001
GSI	−10.02	2.80	−3.58	<0.001	3.97	1.02	3.88	<0.001
$W_r$	−14.87	2.67	−5.56	<0.001	0.26	0.04	6.45	<0.001

### Changes in Blood Metabolites

Triacylglyceride concentrations declined linearly over time for actively swimming fish (slope =  $-0.644 \pm 0.091 \text{ mg} \cdot \text{dL}^{-1} \cdot \text{d}^{-1}$ ), but nonlinearly for sedentary fish (Figure 4a). We compared mean triacylglyceride concentrations between treatments for each sampling date by using two-sample *t*-tests. The triacylglyceride concentrations were significantly higher in sedentary fish than in active fish, except at 147 d. Ammonia concentrations declined for sedentary fish in a manner that differed significantly from a linear model but declined in a linear fashion for active fish (slope =  $-0.035 \pm 0.008 \text{ } \mu\text{g} \cdot \text{mL}^{-1} \cdot \text{d}^{-1}$ ; Figure 4b). Two-sample *t*-tests indicated that active fish had significantly greater ammonia concentrations than sedentary fish did at 84 and 105 d of fasting.

### Relationships between Body Condition and Lipid Content

Relative weight declined over time in both actively swimming and sedentary fish in a fashion that differed significantly from a linear relationship (Figure 4c). Mean  $W_r$  did not differ significantly between active and sedentary fish until after 105 d. Relationships between  $W_r$  and percent lipid did not differ significantly from linearity for active or sedentary fish (Figure 5a), and there was no significant difference in the slope (common slope =  $0.375 \pm 0.049\% \text{ lipid}/W_r$ ), but the intercepts differed significantly. The intercept for sedentary fish was  $-17.6 \pm 0.5\% \text{ lipid}$  ( $r^2 = 0.41$ ) and for active fish was  $-20.1 \pm 0.6\% \text{ lipid}$  ( $r^2 = 0.38$ ).

The GSI was related to percent lipid in a fashion that did not differ significantly from a linear pattern for active and sedentary fish, but the slopes and intercepts of the relationships differed significantly (Figure 5b). Percent lipid increased more slowly for sedentary (slope =  $3.17 \pm 0.14$ ;  $r^2 = 0.31$ ) than for active fish (slope =  $7.33 \pm 2.35$ ;

$r^2 = 0.52$ ). The intercept was not significant for sedentary fish but was for active fish ( $-7.3 \pm 2.4$ ).

Relationships between LSI and percent lipid did not differ significantly from linearity for sedentary fish but did differ significantly for active fish (Figure 5c). However, the relationships between LSI and percent lipid were not significant.

### Mortality

No mortalities occurred among sedentary fish, but 19 active fish died during the 147-d experiment. Fish that died had 3.2% or less lipid (range, 2.0–3.2%) and 84% or more water (range, 84–89%). Their LSI values ranged from 0.6 to 0.8, GSI values ranged from 1.8 to 3.2, and  $W_r$  values ranged from 54 to 80.

Probabilities computed from logistic regression models indicated that the probability of survival decreased significantly as percent lipid, LSI, GSI, and  $W_r$  decreased as well as when percent water and percent protein increased (Table 1; Figure 6). The percent lipid model was considered to be the best because it had the greatest log-likelihood values, Hosmer and Lemeshow *P*-value, and percent concordance.

## Discussion

### Phases of Fasting

The phases of fasting described by physiologists for birds and mammals suggest that energy is initially derived from hepatic glycogen, followed by lipid reserves, and finally body proteins (Le Maho et al. 1981; Cherel et al. 1988; Castellini and Rea 1992). We expected similar patterns in fasted rainbow trout over time with preferential use of energy reserves.

Reductions in hepatic glycogen levels have been associated with declines in LSI during fasting by salmonids (Barton et al. 1988; Vijayan et al. 1993; Larsen et al. 2001), so we based our assessments

TABLE 1. Extended.

Predictor variable	Model			Hosmer–Lemeshow		Concordance (%)
	$G_1$	$P$	Log-likelihood	$\chi^2$	$P$	
Percent lipid	65.20	<0.001	−17.25	2.05	0.98	97.4
Percent water	43.35	<0.001	−28.17	6.36	0.61	91.1
Percent protein	4.97	<0.05	−47.36	7.94	0.44	67.4
LSI	26.80	<0.001	−36.45	13.80	0.09	87.1
GSI	39.77	<0.001	−29.96	2.91	0.94	89.2
$W_r$	68.07	<0.001	−59.90	6.87	0.55	89.0

of glucose and glycogen use by juvenile rainbow trout during fasting on their LSI values. We observed that the mean LSI remained relatively constant over 105 d of fasting, suggesting that gluconeogenesis maintained hepatic glycogen reserves and defended circulating blood glucose concentrations. Trends in LSI did not indicate any change from predominately glycogen to lipid ca-

tabolism. Conversely, reductions in hepatic glycogen were associated with declines in LSI for small (100–125 mm TL) coho salmon *O. kisutch* during short (60-d) periods of fasting that had little effect on lipid reserves (Larsen et al. 2001). Rainbow trout appear to maintain circulating glucose and liver glycogen concentrations over long periods of fasting because the liver and kidney can

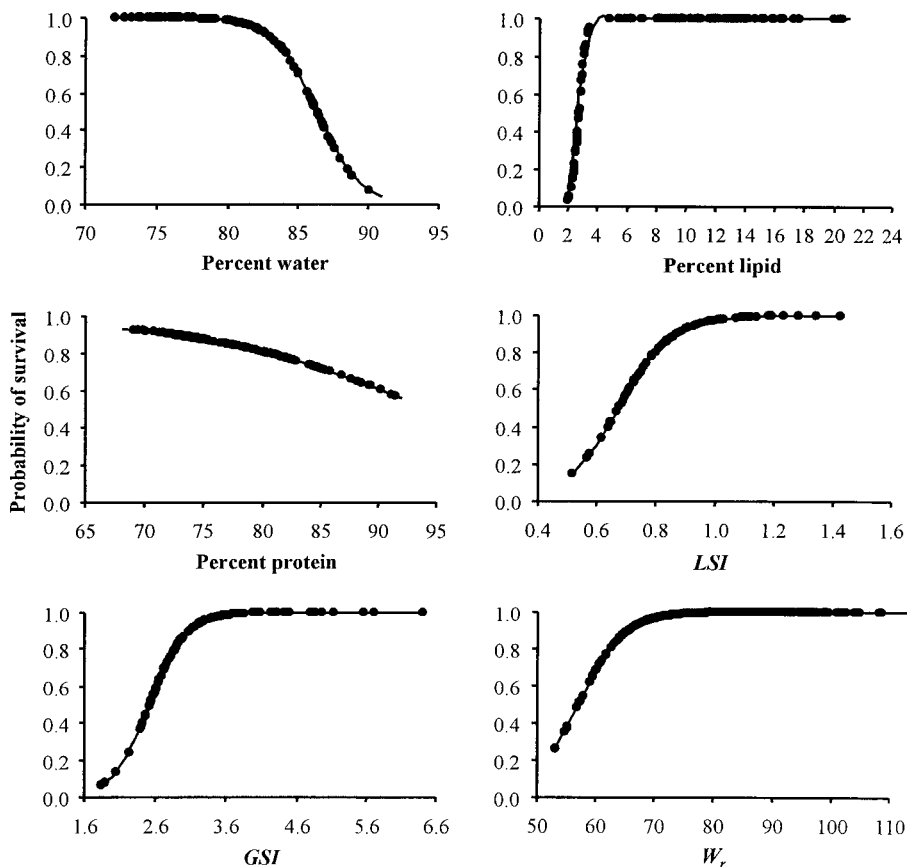


FIGURE 6.—The probability of survival relative to percent lipid, water, and protein, and liver somatic index (LSI), gut somatic index (GSI), and relative weight ( $W_r$ ) among juvenile rainbow trout before death from starvation. Dots denote measurements from sampled fish.

increase the rate of gluconeogenesis from noncarbohydrate precursors (Morata et al. 1982; Moon et al. 1989).

The most evident change in body composition that occurred during fasting in juvenile rainbow trout was in lipid content. Fish lost 80–90% of their initial lipid mass over 147 d. Lipids were mobilized sufficiently to maintain relatively constant triacylglyceride concentrations for as long as 105 d among sedentary fish, but these concentrations declined linearly among active fish over the entire 147-d experiment. The decline in triacylglyceride concentrations among sedentary fish after 105 d indicates that they probably shifted from lipid to protein catabolism. However, lipid and protein mass decreased continuously during the experiment. Although declines in protein mass were less severe than declines in lipid mass, sedentary fish lost an average of 50% of their initial protein mass over 147 d, whereas active fish lost 60%. Protein catabolism was not sufficient to substantially increase ammonia concentrations in plasma of either active or sedentary fish. Because excess ammonia can be efficiently excreted, ammonia concentrations appear not to increase when protein catabolism occurs during fasting in rainbow trout (Morata et al. 1982; Walton and Cowey 1982; Mommsen and Walsh 1989; Moon et al. 1989). Nonetheless, there was little evidence suggesting that fasted rainbow trout changed from solely lipid to solely protein catabolism during our 147-d experiment.

Lipid and protein reserves declined linearly during 147 d of fasting, but the LSI remained relatively unchanged for 105 d, supporting the concept that liver function is defended (Moon et al. 1989). Apparently, other sources of lipid and protein were being depleted before liver tissues (Milne et al. 1979; Navarro and Gutiérrez 1995). Lipids in muscle of rainbow trout have been observed to decrease after visceral adipose tissue has declined, lipids being mobilized more easily from white muscle tissue than dark muscle tissue (Robinson and Mead 1973). Trout muscles are rich in proteolytic enzymes that can mobilize body proteins for fuel when lipid reserves decline (Walton and Cowey 1982; Mommsen and Walsh 1989).

The GSI declined linearly over the course of the 147-d experiment, suggesting that gut tissue was being used preferentially to liver tissue. Similarly, gut atrophy during periods with no food has been observed among some snake species that experience long and unpredictable intervals between meals (Secor et al. 1994; Secor and Diamond

1997). Mobilization of energy reserves from the gut probably acts to preserve the liver and contributes to the ability of juvenile rainbow trout and some snakes to survive without food for several months.

#### *Effects of Activity*

Because of the increased metabolic demands associated with swimming activity (Adams and Breck 1990), we expected and observed more pronounced changes among actively swimming fish than in sedentary fish. Swimming caused lipid mass to be 20% lower and triacylglyceride concentrations to be 40% lower than in sedentary fish after 21 d, the result of high rates of lipid catabolism in swimming fish before 21 d. Protein mass was 8% lower in swimming fish than sedentary fish after 21 d, suggesting that juvenile rainbow trout did not conserve body protein for muscular activity. Conversely, Idler and Bitners (1959) and Mommsen et al. (1980) observed that body proteins were conserved in sockeye salmon *O. nerka* until the spawning migration was almost completed. Protein catabolism may have occurred in swimming rainbow trout throughout our study because fish were forced to swim continuously, which may not accurately represent the migration behavior of sockeye salmon.

#### *Physiological Limits*

It has been suggested that there is a natural limit as to the amount of structural lipids that must be retained before essential membrane functions are compromised and survival is questionable (Navarro and Gutiérrez 1995). We observed no mortalities among sedentary rainbow trout that were fasted for 147 d, despite the observation that sedentary fish lost 80–90% of their initial lipid mass. However, swimming activity caused some juvenile rainbow trout to die after 97 d of fasting. The fish that died had 3.2% or less lipid remaining in their bodies. At lipid levels below this, LSI sharply declined and mortality was highly probable. Consequently, there appears to be a natural limit to the amount of lipid that can be used during fasting by rainbow trout before liver function is compromised. Similarly, Newsome and Leduc (1975) found that mortality occurred among yellow perch *Perca flavescens* when the amount of lipid remaining in their bodies reached 2.2%.

#### *Body Condition Indices*

It has been suggested that body condition indices in fish may not be sensitive to fasting if body

weight is maintained by replacing lipids with water (Love 1980; Navarro and Gutiérrez 1995; Mommensen 1998). Inverse relationships have been found between percent lipid and percent water content in sockeye salmon (Idler and Bitners 1959; Brett et al. 1969), brown trout (Elliot 1976), striped bass *Morone saxatilis*, palmetto bass (striped bass  $\times$  white bass *M. chrysops*; Brown and Murphy 1991), and several other species (Love 1970). Similarly, we found highly correlated inverse relationships between percent water and percent lipid in both sedentary and swimming juvenile rainbow trout. However, we observed that water mass declined as lipid mass, percent lipid, and  $W_r$  declined. Consequently, our results do not confirm that body weight and body condition are maintained through the replacement of lipids with water.

Studies have described relationships between body condition indices and food availability among fish stocks (see Gas and Noailiac-Depeyre 1976; Love 1980; Goede and Barton 1990; Blackwell et al. 2000), but little attention has been given to validating relationships between body condition indices and body composition or the risk of mortality (Hayes and Shonkwiler 2001). The LSI and GSI have been related to measures of body composition among fasted golden perch *Macquaria ambigua* (Collins and Anderson 1995). Brown and Murphy (1991) observed relatively strong relationships between  $W_r$  and measures of proximate body composition among striped bass and palmetto bass and concluded that  $W_r$  was a viable alternative to proximate body analysis for estimating body composition. Conversely, Jonas et al. (1996) found poor relationships between  $W_r$  and whole-body energy in juvenile muskellunge *Esox masquinongy*. Our findings suggest that LSI, GSI, and  $W_r$  have relatively weak relationships with measures of body composition and with the probability of survival among fasted juvenile rainbow trout. The use of different body condition indices may lead to different conclusions about the condition of fish. Although the probability of survival was related to body condition indices in our study, distinct thresholds were not observed. Percent lipid was a much better predictor of the probability of survival. Consequently, we suggest that LSI, GSI, and  $W_r$  are not highly accurate predictors of energy reserves or mortality risk in juvenile rainbow trout, and we encourage fisheries scientists to investigate the potential application of proximate lipid content as an assessment tool for salmonids.

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