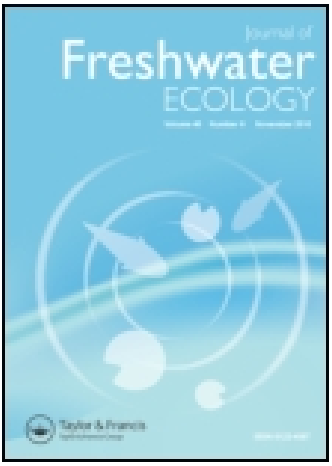

Journal of Freshwater Ecology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tjfe20

Energy Densities of Brown Trout (Salmo trutta) and Its Main Prey Items in an Alpine Stream of the Slizza Basin (Northwest Italy)

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Published online: 06 Jan 2011.

To cite this article: E. Pizzul , M. Bertoli , A. Basset , F. Vignes , M. Calligaris & E. Tibaldi (2009) Energy Densities of Brown Trout (Salmo trutta) and Its Main Prey Items in an Alpine Stream of the Slizza Basin (Northwest Italy), Journal of Freshwater Ecology, 24:3, 403-410, DOI: 10.1080/02705060.2009.9664312

To link to this article: http://dx.doi.org/10.1080/02705060.2009.9664312

Energy Densities of Brown Trout (*Salmo trutta*) and Its Main Prey Items in an Alpine Stream of the Slizza Basin (Northwest Italy)

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ABSTRACT

In the present study, energy densities of 80 adult brown trout (*Salmo trutta*), seasonally sampled in an alpine stream in the eastern Alps and energy densities of their main prey items, were determined. The energy density (J/g wet mass) and dry weight content (%) of fish were highly correlated ($p<0.001$) and averaged 5,611.6 ± 857.9 J/g wet mass and 25.3 ± 2.1% dry weight, respectively. Energy density values were significantly higher in fish sampled in spring than in other seasons. No major changes in the energy content were observed due to age or sex. Macroinvertebrates, particularly Ephemeroptera and Diptera, were the major food source of brown trout in the sampled area. Their gross energy content varied within a wide range of values (1,654-5,110 J/g wet weight), depending on the taxa and family or genus within a given taxon.

INTRODUCTION

The brown trout (*Salmo trutta*), being a major target species for recreational fishing, is subject to a high fishing pressure in Italy. For this reason, it has been used in restocking programs even into streams not included in its original distribution area. Stocking programs often use specimens which come from other European countries, and so brown trout populations of the Atlantic strains are widespread and largely predominate over the Mediterranean populations, which are isolated and vanishing (Lucentini et al. 2006, Pensierini et al. 2006, Splendiani et al. 2006, Razpet et al. 2007). Moreover, restocking often involves massive introductions of juvenile or adult specimens, regardless of the actual trout population structure and often exceeding the carrying capacity of streams.

Bioenergetics models based on the energetics of feeding, metabolism, and growth, have been proposed for brown trout by Elliott (1976a, 1976b, and 1976c), Elliott and Hurley (1998, 1999, 2000, and 2001) and Dieterman et al. (2004). Jonsson and Jonsson (1997, 1998, 2003, and 2005) and Jonsson et al. (1997) investigated body composition, energy allocation, and the role of body lipid depots as energy sources throughout life-history stages of *Salmo trutta* and *Salmo salar*. Energy densities have been reported for many freshwater and marine fish species, including salmonids, and 72 taxa of macroinvertebrates collected in some lakes of China by Yan and Liang (2002). Recent studies on energy flows based on energy densities of fish and their macroinvertebrate prey have been conducted in freshwater streams in Patagonia by Ciancio and Pascual (2006) and Ciancio et al. (2007).

The present investigation was conducted on an alpine stream, where *Salmo trutta* is the only fish species present, either as a native population or as the result of stocking actions. Energy densities of adult trout and its main prey items were measured to provide information for possible use in defining an energy flow model as a basic tool for a correct management of brown trout in the streams of the Italian alpine region.

METHODS AND MATERIALS

This study was carried out at the Rio del Lago, Eastern Alps (UTM: Easting 398978.07; Northing 5149901.02, 754 m.a.s.l.), a tributary of the Slizza Stream within the Donau River watershead system. The investigation was preceded by a quantitative fish sampling (electrofishing) conducted in May 2004, along a net-closed 100 m tract of the stream. *Salmo trutta* was the only fish species present in the stream, with a density of 0.3 ind/m^2.

Fish samplings were than conducted in different seasons as December 2004, May 2005, July 2005, and October 2005 by electrofishing described above. A total of 75 specimens was collected each sampling time. Sampled fish were measured for total length (TL ± 0.1 cm) and live weight (TW ± 0.1 g). Sex was determined by gross visual inspection, except for some specimens, which were classified as sexually-undetermined due to the impossibility of sex identification without histologycal analysis. The age was determined by scale reading. In the winter sample there were 24 males, 33 females and 18 sex-undetermined fish; their age ranged from age 2 to age 5, TL was 23.3 ± 4.2 cm, and TW was 128.6 ± 72.4 g. In spring, the sample resulted in 12 females, eight males, and 55 sex-undetermined specimens; their age ranged from age 2 to age 8; TL ± standard deviation (SD) and TW ± SD were respectively 24.9 ± 5.7 cm and 181.6 ± 131.4 g. Fish sampled in summer were 32 females, 26 males, and 17 sex-undetermined, aging from age 2 to age 6, with TL ± SD and TW ± SD respectively of 22.5 ± 4.5 cm and 133.4 ± 69.7 g. In fall the sample included 55 females and 20 males between age 2 and age 6; TL ± SD was 21.0 ± 5.0 cm and TW ± SD was 105.3 ± 75.5 g. Fish were then killed by an overdose of anaestethic (ethanol solution of clove oil 20% v:v) and brought to the laboratory in refrigerated bags. Eighty fish out of the 300 sampled specimens were subjected to individual dry weight, water, ash contents analysis, and energy density measurements. Each sampling time was represented by 20 specimens, equally distributed among four age classes (i.e., age 2, 3, 4, and 5) and, when possible, between males and females (Table 1). Individual specimens were minced in a cutter and the resulting homogenates and weighed prior to being freeze-dried, weighed after equilibration to ambient moisture, and then ground in a microcutter. Subsamples of pulverized homogenates were dried at 105°C to a constant weight and then combusted at 600° to determine dry weight (DW), water (W), and the ash contents (AW). Preweighed pellets (pt, 0.5 - 0.7g) obtained from further subsamples of pulverised specimens were used to measure the energy content using an adiabatic bomb calorimeter (Ika C7000- Ika Werke GMBH & Co. KG, Stanfen, Germany). All analytical determinations were carried out in duplicate, and percentage dry weight (DW%), water (W%), and ash (AW%), and energy content (J/g) of individual specimens were calculated based on the wet weight.

A prey taxonomical analysis was carried out on the remaining 220 sampled fish, whose stomachs were removed and put into 4% formaldehyde solution. Prey items were identified to the family level, and when possible to the genus level. Macroinvertebrates

Table 1. Mean values ± S.D. of total length (TL), total weight (TW), sex, and age (years) of the 80 specimens used for the analyses of energy density.

Period	n	TL(cm)	TW(g)	Males	Females	Undetermined	Age
Dec.	20	23.2 ± 4.5	126.8 ± 74.8	7	9	4	2-5
May	20	23.2 ± 4.9	149.5 ± 96.4	6	7	7	2-5
July	20	22.4 ± 4.3	121.7 ± 60.8	6	9	5	2-5
Oct.	20	23.2 ± 4.5	138.6 ± 73.3	8	12	0	2-5

Table 2. Prey found in the stomach contents of the 220 specimens and their frequencies.

Classes	Orders	No. of taxa	No. of specimens	Frequency (%)
Insecta	Ephemeroptera	7	1,283	31.1
	Plecoptera	5	118	2.9
	Trichoptera	9	428	10.4
	Diptera	9	1,089	26.4
	Coleoptera	7	63	1.5
	Hymenoptera	1	831	20.2
Oligochaeta		2	48	1.2
Other taxa[a]		19	262	6.4

[a]Included Insecta, Crustacea, Chilopoda, Diplopoda, Arachnida, Adenophorea, Gordioidea, and Turbellaria represented by only one or a few specimens with frequencies ≤1%.

were the only prey items observed (Table 2). The taxa with greatest frequency of ocurrence were chosen for the energy density (hereafter ED) investigation.

Contemporary to fish captures, samples of macrobenthic fauna were collected, using a Surber sampler (0.5 x 0.5 m, 500 μm mesh). The samples were frozen until analysis. Taxa were identified to the same levels as those found in stomach contents and measured with a millimetric grid to the nearest 1 mm, to obtain a dimensional range for every taxon. In total, 16 taxa of Insecta and one of Oligochaeta were identified. The wet weight (WW ± 0.1 mg) of the whole sample was measured for every taxon. Then the samples were dried at 70°C for 72 h or until constant weight. The dried samples were weighed, and DW% was calculated. Dried samples were pulverised with a mortar and pestle, and a pellet obtained thereafter was used for the measure of ED with an adiabatic semimicro bomb calorimeter (Parr 1425 semimicro, Parr Instrument Co., U.S.A.). ED values were obtained in Cal/g dry weight and then converted to J/g dry weight and to J/g wet weight. Due to the small amount of dry material available, calorimetric measurements on macroinvertebrate samples were carried out as single determinations. The energy density, dry weight, and ash contents of trout whole body were first subjected to a three-way analysis of variance (ANOVA; season, age-class and sex) according to the linear models of the SPSS-P statistical package resulting in a non-normal data distribution (Levene's test). Hence, the data set was subjected to the nonparametric test of Kruskal-Wallis to evaluate the effects of the season and age-class, while the nonparametric test U Mann-

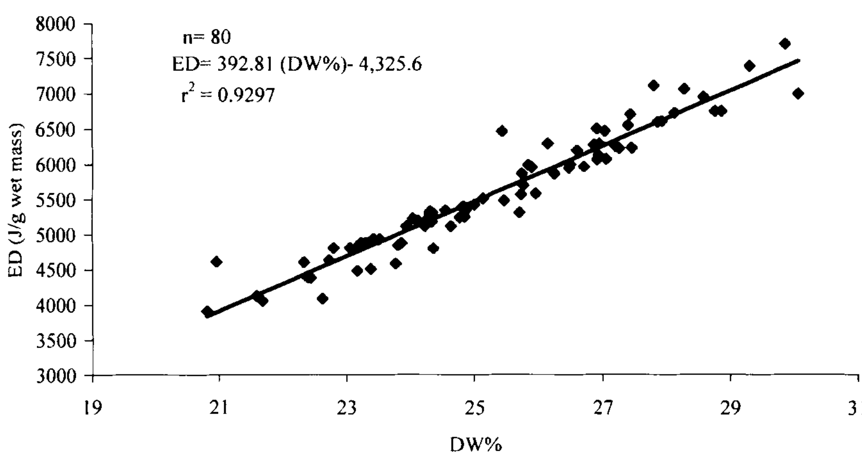

Figure 1. Relationsihp between percentage of dry mass (DW%) and energy density ED (J/g wet mass) in *Salmo trutta*.

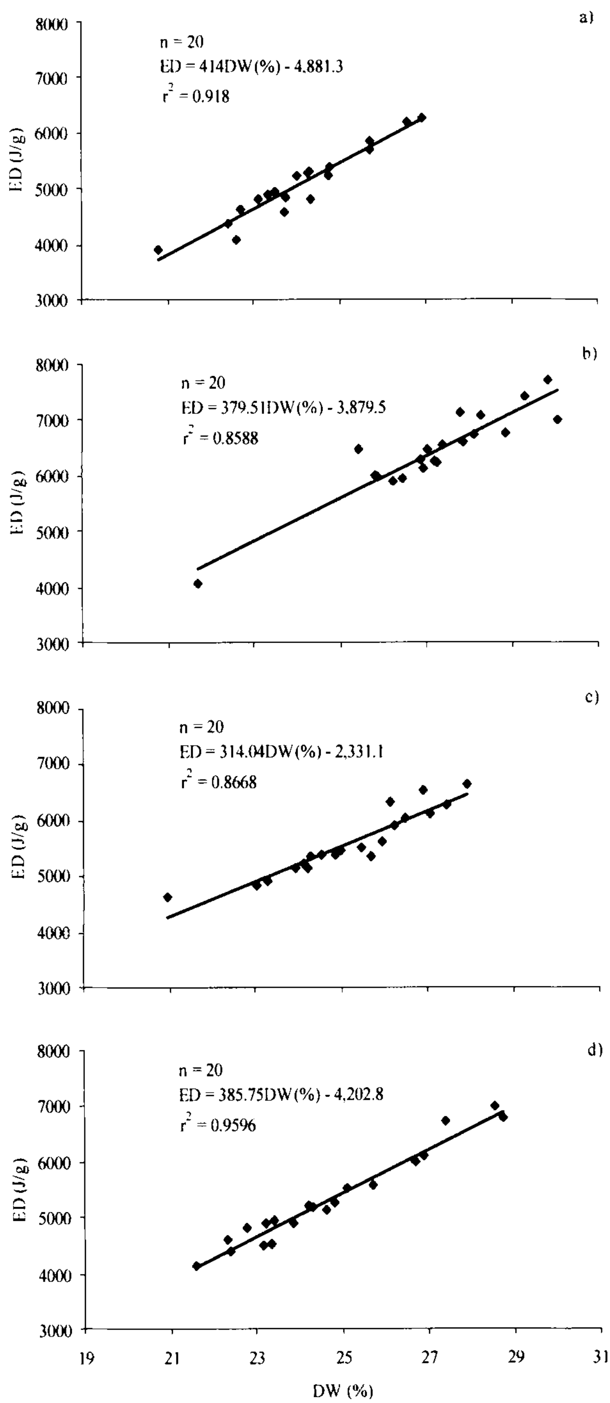

Figure 2. Relationsihp between percentage of dry mass (DW%) and energy density ED (J/g wet mass) of *Salmo trutta* in (a) winter (December 2004), (b) spring (May 2005), (c) summer (July 2005), and (d) fall (October 2005).

Whitney was applied for testing differences due to sex (software Statistica 8.0). A least square regression analysis was adopted to fit the relationship between fish DW% and ED values. The ED data of macrobenthic prey as classified by order were compared by the nonparametric Kolmogorov-Smirnov's test using the Statgraphics 1.6 software.

RESULTS

Energy density of Salmo trutta

The average values ± SD of ED, DW%, and AW% of the 80 analyzed brown trout were respectively 5,611.6 ± 857.9 J/g wet weight, 25.3 ± 2.1%, and 3.0 ± 0.5% (Table 3). The relationship between ED and DW% of the whole fish sample resulted in a significant ($p<0.001$) linear regression (Fig. 1). This same relationship was investigated in every sampling season (Fig. 2), and four least square regressions obtained were all highly significant, resulting in r^2 values ranging from 0.86 to 0.96. The Kruskal-Wallis test showed significant differences between ED data of samples collected in spring and those obtained in the other seasons (spring vs. winter or fall $p<0.001$; spring vs. summer $p<0.05$). Nonparametric tests were used also for the other comparisons [i.e., males *vs.* females (U Mann-Whitney) and, regardless of the sex and season, among different age classes (Kruskal-Wallis)] and showed no significant differences ($p>0.05$) in ED.

Table 3. Mean values ± SD of total length (TL), total weight (TW), percentage of dry weight (DW %), water content (W%), and ash weight (AW%), and energy density ED of the 80 brown trout specimens analyzed.

Period	n	TL (cm)	TW (g)	DW%	W%	AW %	ED (J/g wet mass)
Dec.	20	23.2 ± 4.5	126.8 ± 74.8	24.1 ± 1.5	75.9 ± 1,5	3.1 ± 0.3	5,107.2 ± 635.4
May	20	23.2 ± 5.0	149.5 ± 96.4	27.2 ± 1.8	72.8 ± 1.8	2.6 ± 0.5	6,448.4 ± 752.7 [a]
July	20	22.4 ± 4.3	121.7 ± 60.8	25.9 ± 1.7	74.8 ± 1.7	3.1 ± 0.4	5,574.8 ± 573.1
Oct.	20	23.2 ± 4.5	138.6 ± 73.3	24.7 ± 2.1	75.3 ± 2.1	3.1 ± 0.5	5,316.2 ± 814.4

[a] Significantly different ($p<0.05$ or $p<0.001$) from the other column mean values.

Table 4. Mean value ± S.D. of wet weight (WW), dry weight (DW), percentage of dry weight (DW %), and energy density ED of the macrozoobenhic taxa analyzed.

	WW (g)	DW (g)	DW%	ED (J/g wet weight)
Ephemeroptera	2.04 ± 1.43	0.29 ± 0.18	15.12 ± 2.27	3,664.3 ± 472.4
Plecoptera	0.72 ± 0.43	0.13 ± 0.08	17.90 ± 4.10	4,125.4 ± 1025.9
Trichoptera	1.56 ± 1.59	0.15 ± 0.23	16.20 ± 2.69	4,356.0 ± 462.6
Diptera	1.96 ± 0.30	0.25 ± 0.12	12.52 ± 5.12	3,084.8 ± 1272.0
Oligochaeta [a]	24.30	3.52	6.96	2,395.25

[a] Oligochaeta represented only by the taxon Lumbricidae.

Energy density of macrobenthic invertebrates

The analysis of the stomach content of the 220 brown trout specimens captured in different seasons revealed that macroinvertebrates were the primary prey items composing the diet. In particular, in winter and fall the most eaten taxon was Trichoptera, in spring Ephemeroptera and Diptera showed high frequencies; in summer Hymenoptera was the dominant taxon in the stomach contents. Except for this last taxon, exclusively represented by the family Formicidae, the ED values of the major insect orders and the oligochaetes were determined (Table 4). The nonparametric test of Kolmogorov-Smirnov did not show significant differences among this taxa (p>0,05). Within the insect orders, families and genera exhibited ED values that were very different (Table 5). For example, in the order Plecoptera, ED values ranged between 2,700.5 J/g (genus *Perlodes*) and 5,109.9 J/g (family Nemouridae). However, a statistical analisys to this levels was not possible due to the small amount of material available.

Table 5. Range in size- length, wet weight (WW), dry weight (DW), percentage of dry weight (DW %), and energy density ED (J/g wet mass) of single families or genera within macrozoobenthic taxa.

		n	Lenght range (mm)	WW (g)	DW (g)	DW %	ED (J/g wet mass)
Ephemeroptera	*Baetis*	736	4 - 13	3.50	0.43	12.35	3,296.5
	Rhitrogena	893	3 - 13	3.46	0.46	13.31	3,437.6
	Ecdyonurus	42	5 - 13	0.39	0.07	17.08	3,997.1
	Epeorus	112	5 - 18	1.92	0.34	17.51	4,324.9
	Ephemerella	335	4 - 12	0.91	0.14	15.34	3,265.6
Plecoptera	*Perlodes*	15	8 - 24	1.13	0.15	12.85	2,700.5
	Isoperla	65	6 - 11	0.33	0.06	18.21	4,520.0
	Nemuridae	926	3 - 11	1.05	0.24	22.88	5,110.0
	Leuctra	138	5 - 12	0.36	0.06	17.66	4,171.3
Trichoptera	Rhyacophilidae	205	5 - 28	3.79	0.58	15.26	3,971.9
	Hydropsychidae	87	4 - 25	1.60	0.28	17.34	4,353.6
	Sericostomatidae	8	13 - 19	0.51	0.10	20.52	5,007.4
	Limnephilidae	9	8 - 22	0.32	0.07	20.89	4,091.0
Diptera	Chironomidae	668	5 - 15	2.27	0.37	16.09	4,089.6
	Limoniidae	135	4 - 30	1.93	0.13	6.66	1,654.2
	Athericidae	71	6 - 20	1.67	0.25	14.82	3,508.9
Oligochaeta	Lumbricidae	307	50 - 200	24.30	3.52	6.96	2,395.3

DISCUSSION

As shown by Hartman and Brandt (1995) for salmonids, the relationship between energy density (ED) and the percentage of dry matter (DW%) was highly significant. The ED values provided by the present study for *Salmo trutta* were been compared with those reported Elliott (1976) for *Salmo trutta* (test proposed by Sokal and Rohlf 1981), and no significant differences were found. The nonparametric tests used in the present study showed no significative differences between males and females or among the age classes (2-5 years), which agreed with Jonsson and Jonsson (1998), who analyzed resident and anadromous trout (*Salmo trutta*). The ED values showed significant differences among the seasons. In particular, ED was higher in spring, and this possibly correlates with yearly peaks in the biomass of benthic fauna of similar alpine streams in this season (De Fiorido et al., 2005). ED values measured here for the orders Ephemeroptera, Plecoptera and for the class Oligochaeta are similar to those reported by Ciancio and Pascual (2006), in their study on Patagonian streams. On the other hand they are higher than those measured by Yan and Liang (2002), for the order Ephemeroptera and the families Chironomidae and Hydropsychidae. Differences could be probably due to the fact that the Chinese samples came from lakes, not from streams. In fact, ED values measured here were quite different among macroinvertebrates families or genera within a given taxon (Table 5). This was mostly the case for Plecoptera (ED range: 2,700-5,100 J/g) and Diptera (1,654-4,089 J/g), the latter being a major component of brown trout diet in the present study (Table 2). This makes it difficult to compare data from various studies and could lead to biased estimates of the actual contribution to the gross energy density of the diet of a target fish species, when macroinvertebrates found in stomach contents are grouped (classified) just general and broader taxa.

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Received: 19 January 2009 Accepted: 23 March 2009