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Respiration rate and respiratory electron transport system (ETS) activity of chironomid larvae from mountain lakes (NW Slovenia)

Stopnja dihanja in aktivnost dihalnega elektronskega transportnega sistema (ETS) pri ličinkah trzač iz gorskih jezer (SZ Slovenija)

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Abstract. Respiration rate (R) and electron transport system (ETS) activity were measured in larvae of four chironomid species, *Chironomus thummi* (Kieffer), *Paratanytarsus austriacus* (Kieffer), *Procladius (Holotanypus)* spp., and *Zavrelimyia barbatipes* (Kieffer) from three mountain lakes. ETS activity and respiration rate, measured at a standard temperature of 20 °C, differed significantly between species (ANOVA: $p<0.001$). ETS activity ranged from 10.3 μL O_2 mg^{-1} DW h^{-1} for *Zavrelimyia* to 27.6 μL O_2 mg^{-1}DW h^{-1} for *Paratanytarsus*. The lowest respiration rate was observed for *Procladius* (4.0 μL O_2 mg^{-1}DW h^{-1}) and the highest for *Paratanytarsus* (12.4 μLO2 mg^{-1} DW h^{-1}). ETS activity and respiration rate of chironomids decreased with increasing size of the animals ($p<0.001$); b values for ETS activity and respiration rate were 0.82 and 0.66, respectively. Respiration rate correlated significantly with ETS activity for all four species investigated. This study revealed that the ETS/R ratio differed between species (ANOVA: $p<0.001$). A low ratio (0.98) was determined for *Zavrelimyia*, while *Procladius* showed the highest value (3.88). The differences can be explained by body size, and the ecological preference and tolerance of each chironomid species.

Key words: Chironomidae, Diptera, benthos, ETS/R ratio, ETS activity, respiration, metabolism

Izvleček. Stopnjo dihanja in aktivnost elektronskega transportnega sistema (ETS) smo določali pri ličinkah štirih različnih vrst trzač, *Chironomus thummi* (Kieffer), *Paratanytarsus austriacus* (Kieffer), *Procladius (Holotanypus)* spp., in Zavrelimyia barbatipes (Kieffer), ki smo jih nalovili v treh gorskih jezerih. Aktivnost ETS in stopnja dihanja, ki smo ju merili pri standardni temperaturi 20 °C, sta se značilno razlikovali med vrstami (ANOVA: $p<0.001$). Aktivnost ETS se je gibala med 10.3 µL O_2 mg^{-1}DW h^{-1} pri ličinkah Zavrelimyia in 27.6 µL O_2 mg^{-1} DW h^{-1} pri ličinkah Paratanytarsus. Najnižjo stopnjo dihanja smo določili pri ličinkah *Procladius* (4.0 µL O_2 mg^{-1}DW h^{-1}), najvišjo pa pri ličinkah *Paratanytarsus* (12.4 µL O_2 mg^{-1}DW h^{-1}). Aktivnost ETS in stopnja dihanja ličink sta se zmanjševali z naraščajočo velikostjo živali ($p<0.001$); vrednosti b sta znašali 0.82 za aktivnost ETS in 0.66 za dihanje. Opazili smo pozitivno korelacijo med stopnjo dihanja in aktivnostjo ETS pri vseh štirih vrstah. Raziskava je pokazala značilne razlike v razmerju ETS/R med vrstami (ANOVA: $p<0.001$). Nizko razmerje (0.98) je bilo določeno pri ličinkah *Zavrelimyia*, medtem ko je bilo pri ličinkah *Procladius* določeno najvišje razmerje (3.88). Različna razmerja so posledica razlik v vrstno-specifični telesni velikosti ter različne ekološke preference in tolerance pri posamezni vrsti trzač.

Ključne besede: Chironomidae, Diptera, bentos, razmerje ETS/R, aktivnost ETS, dihanje, metabolizem

Introduction

The family Chironomidae is widely distributed in all types of aquatic environments, where they form the major part of nearly all freshwater communities, and they occupy a variety of niches. The relatively short life cycle and large total biomass of the numerous larvae confer ecological energetic significance on this taxon, as both consumers and prey, and the partitioning of ecological resources by a large number of species presumably enhances the biologic stability of aquatic ecosystems (Merritt & Cummins 1984).

As chironomid larvae play an important role in aquatic food webs, an estimation of their respiratory energy loss is essential in order to estimate the energy flow through the ecosystems. Direct determination of respiration usually involves incubating animals in a controlled environment, and determining the time-dependent change in oxygen concentration. These methods are time consuming and impractical. The development of enzymatic techniques, however, has allowed metabolic activity to be estimated from the electron transport system activity (Owens & King 1975, Packard 1985). The electron transport system (ETS)-assay, based on the reduction of tetrazolium salt (INT) in the presence of a cell-free homogenate of the organisms and excess substrates of the ETS, has proved to be useful for estimating the metabolic activity of different organisms (Packard 1985, del Giorgio 1992, G.-Tóth & al. 1995). It is simple, rapid and extremely sensitive and has been used extensively with marine plankton (Kenner & Ahmed 1975, Bamstedt 1980, Vosjan & Olanczuk-Neyman 1991) and freshwater plankton (Borgmann 1978, James 1987, del Giorgio 1992), benthic organisms (Cammen & al. 1990, Muskó & al. 1995, 1998), marine sediments (Vosjan & Olanczuk-Neyman 1977, Relexans 1996), and freshwater sediments (Trevors 1984, G.-Tóth & al. 1994). The slow response of ETS activity to short-term variations in environmental factors makes the method superior to direct respiratory measurements on incubated animals (Bamstedt 1980). It is, however, necessary to determine empirically the relation between the highest potential metabolic activity and realised oxygen consumption in order to interpret the ETS data properly (del Giorgio 1992). Much has been published on the respiration of chironomids (Erman & Helm 1970, Konstantinov 1971, Bairlein 1989, Hamburger & Dall 1990, Hamburger & al. 1994), but there is no study concerning their ETS activity.

Previous investigations have shown that the chironomid larvae are the dominant group of the macrozoobenthos in mountain lakes in NW Slovenia (Simčič unpubl. data). These lakes are shallow and, except the eutrophic lakes Krnsko jezero and Jezero na Planini pri Jezeru, transparent to the bottom. Zoobenthos are reported to have a greater effect on energy flux through secondary production in shallow lakes than in deeper ones (JÓNASSON & al. 1990, LINDEGAARD 1994). Therefore, chironomids should be taken into account when energy flow through the food webs in these lakes is investigated.

The purpose of this paper was to determine ETS activity and respiration rate of four different chironomid species from mountain lakes, and to calculate the ETS/R ratios of these species in order to contribute to the estimation of energy flux through secondary production of benthic organisms.

Materials and methods

Sampling of animals: ETS activity and respiration rate were measured in the laboratory for *Paratanytarsus austriacus* (Kieffer) and *Procladius (Holotanypus)* spp. from the oligotrophic lake Zgornje Kriško jezero, *Zavrelimyia barbatipes* (Kieffer) from the oligotrophic lake Srednje Kriško jezero, and *Chironomus thummi* (Kieffer) from the eutrophic lake Krnsko jezero. Zoobenthos samples were taken using van Veen grab (Eijkelkamp). It was important to handle the larvae as little as possible during identification. Single animals were used for each analysis. Different sets of animals were taken to be investigated separately in the respirometric measurements and separately in ETS activity measurements in the first part of experiments. To determine relationship between ETS activity and respiration rate, the individual animal was examined firstly by microrespirometer and then by ETS-assay. Before experiments animals were rinsed with filtered water to remove bacteria adhering to the body surface, and weighed on an electrobalance. The dry mass of animals was calculated using factors determined by drying five samples of animals for 24 h at 60°C.

Respiration measurements: Respiration rate was estimated using a twin-flow microrespirometer (CYCLOBIOS, Innsbruck, Austria) (GNAIGER 1983). In the open-flow system the concentration of oxygen was measured before and after the chamber containing the animal. Oxygen consumption was calculated from the reduction of oxygen concentration and the flow rate of the water with a computer program Dat.Graf 2.1 Analysis.

ETS activity measurements: ETS activity was measured using the method proposed by PACKARD (1971), modified by OWENS & KING (1975) and improved by G.-TÓTH (1999). Each freshly weighed animal was homogenized in 4 ml of cold homogenization buffer (0.1 M sodium phosphate buffer pH = 8.4, 75 μM $MgSO_4$, 0.15% (w/v) polyvinyl pyrrolidone, 0.2% (v/v) Triton-X-100) for 2 min. The homogenate was then sonicated using an ultrasound homogenizer (Cole-Parmer) for 20 sec and centrifuged in refrigerated centrifuge (Sigma) for 4 min at 0 °C at 8500 x g. Three 0.5 ml samples from each homogenate were incubated in 1.5 ml substrate solution (0.1 M sodium phosphate buffer pH = 8.4, 1.7 mM NADH, 0.25 mM NADPH, 0.2% (v/v) Triton-X-100) with 0.5 ml of 2.5 mM 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) solution for 40 min at a standard temperature of 20 °C. The reaction was stopped by adding 0.5 ml of stopping solution (formalin(conc.):H_3PO_4(conc.)=1:1). Formazan production was determined spectrophotometrically from the absorbance of the sample at 490 nm against the blank (1.5 ml substrate solution and 0.5 ml INT solution were incubated as for the samples, and 0.5 ml of homogenate was added after the stopping). ETS activity was measured as the rate of tetrazolium dye reduction and converted to equivalent oxygen utilised per dry mass (DW) in a given time interval as described by KENNER & AHMED (1975).

Analysis of data: ETS/R ratios were calculated as the ratio of the calculated maximum oxygen consumption (ETS), as measured by *in vitro* enzymatic rates, to the rate of respiration (R) *in vivo* (OWENS & KING 1975). An analysis of variance (ANOVA) was carried out to test for differences between species. Linear regression between dry mass (DW) and the parameters investigated (ETS activity, respiration rate, ETS/R ratio), and between ETS activity and respiration rate were calculated after logarithmic transformation of data, according the power function $Y = a X^b$ ($\log Y = \log a + b \log X$) (DEL GIORGIO 1992). The statistical tests were performed using Microsoft Excel.

Results

ETS activity

ETS activity was found to differ significantly between species (Tab. 1). The highest value (27.6 µL O_2 mg^{-1} h^{-1}) was measured in *Paratanytarsus* (Fig. 1a). Significantly lower values were observed in *Procladius* (18.5 µL O2 mg^{-1} h^{-1}), followed by *Chironomus* (14.0 µL O_2mg^{-1} h^{-1}) and *Zavrelimyia* (10.3 µL O_2 mg^{-1} h^{-1}).

The relationship between ETS activity and body mass is shown in Fig. 2a. The mass-specific ETS activity of chironomids decreased with increasing size of the animals ($\log ETS = 1.06 - 0.18 \log DW$; $r=-0.53$; $n=68$; $p<0.001$). The b value was 0.82. The body mass of the animals used in the experiments differed significantly between species (Tab. 1). The mean values (± SD) were 0.07 ± 0.04 mg DW for *Paratanytarsus*, 0.08 ± 0.03 mg DW for *Zavrelimyia*, 0.35 ± 0.27 mg DW for *Procladius* and 1.06 ± 0.94 mg DW for *Chironomus*.

Table 1: Results of analysis of variance applied to ETS activity, respiration rate (R), ETS/R ratio and body mass (DW) in chironomids with species as factor; d.f. - degree of freedom.
Tabela 1: Rezultati analize variance za aktivnost ETS, stopnjo dihanja (R), razmerje ETS/R in telesno maso (DW) pri ličinkah trzač z vrsto kot faktorjem; d.f.-stopinje prostosti

	d.f. between group	d.f. within group	F-value	P-value
ETS	3	64	14.29	<0.001
R	3	39	7.94	<0.001
ETS/R	3	38	15.34	<0.001
DW	3	75	17.94	<0.001

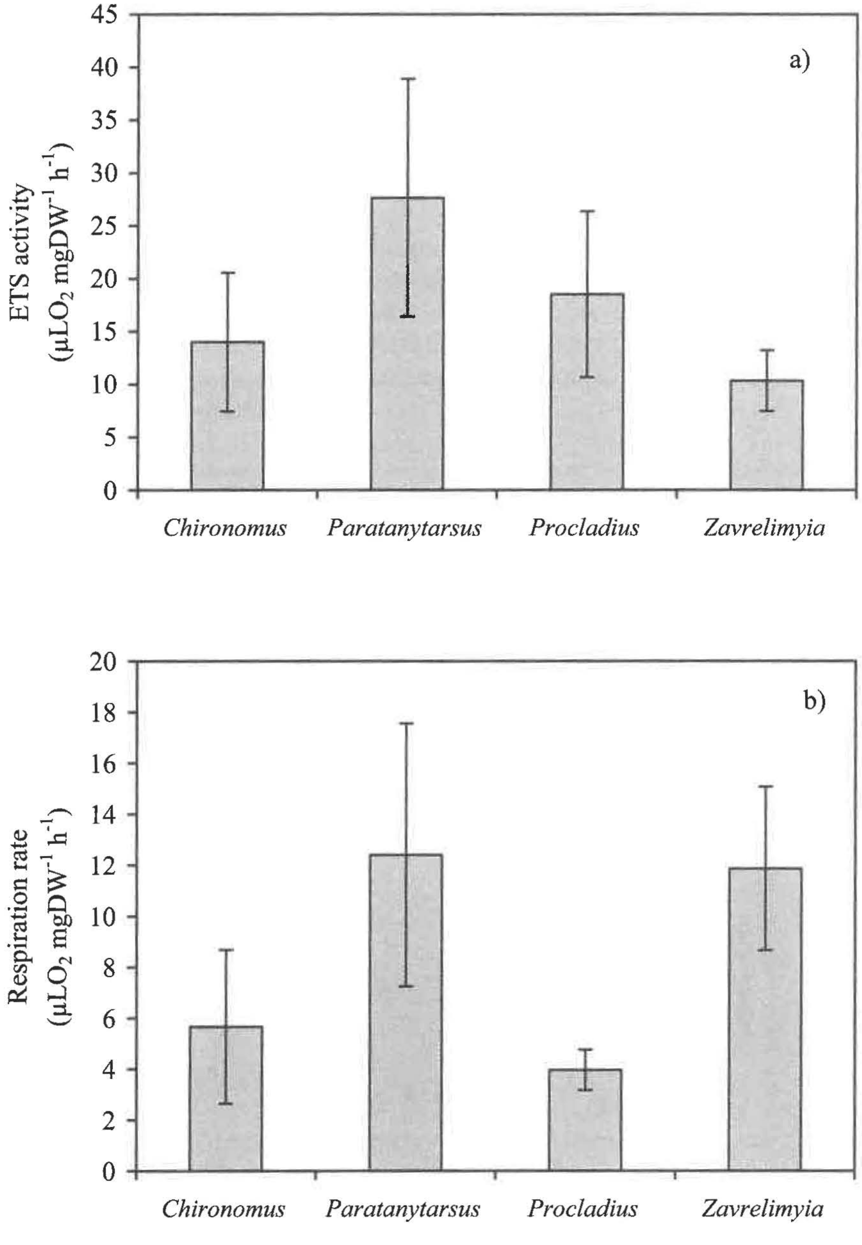

Figure 1: (a) ETS activity and (b) respiration rate of different chironomid larvae (mean ± standard deviation) at 20 °C.

Slika 1: (a) Aktivnost ETS in (b) stopnja dihanja pri ličinkah različnih vrst trzač (srednja vrednost ± standardna deviacija) pri 20 °C.

Table 2: The ratios ETS/R for different species of chironomids, measured at 20 °C.
Tabela 2: Razmerja ETS/R za različne vrste trzač, izmerjena pri 20 °C.

	n	ETS/R ratio	Standard deviation
Chironomus thummi	18	2.73	0.93
Paratanytarsus austriacus	9	2.44	0.86
Procladius (Holotanypus) spp.	8	3.88	0.75
Zavrelimyia barbatipes	7	0.98	0.21

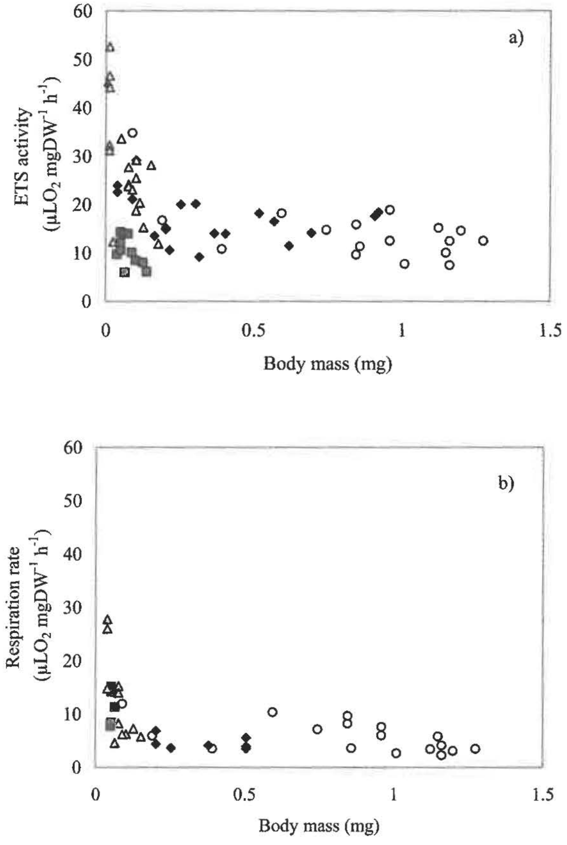

Figure 2: The relationship between (a) ETS activity and body mass (DW), and (b) respiration rate (R) and body mass of different chironomid larvae at 20 °C.
Slika 2: Razmerje med (a) aktivnostjo ETS in telesno maso (DW) ter (b) stopnjo dihanja in telesno maso pri ličinkah različnih vrst trzač pri 20 °C.

Respiration rate

Respiration rate differed significantly between species (Tab. 1). The highest values were obtained for *Paratanytarsus* (12.4 µL O_2 mg^{-1} h^{-1}) and *Zavrelimyia* (11.9 µL O_2 mg^{-1} h^{-1}), while respiration rates of *Chironomus* (5.7 µL O_2 mg^{-1} h^{-1}) and *Procladius* (4.0 µL O_2 mg^{-1} h^{-1}) were approximately two and three times lower (Fig. 1b). The regression between respiration rate and body mass of chironomids was statistically significant ($\log R = 0.60 - 0.34 \log DW$; $r=-0.71$; $n=43$; $p<0.001$; Fig. 2b); b value was 0.66.

The ETS/R ratio

The ratio ETS/R differed significantly between species (Tab. 1). A relatively low value, close to 1, was determined for *Zavrelimyia*, while the highest ratio of 3.88 was obtained for *Procladius* (Tab. 2). Positive correlation between ETS/R ratios and body mass was observed ($r=0.61$; $n=27$; $p<0.001$).

Respiration rate correlated strongly with ETS activity for all four species (Fig. 3). Correlation coefficients and regression constants for relationships between ETS activity and respiration rate for each species are shown in Tab. 3.

Table 3: Relationship between ETS activity and respiration rate of four chironomid species (data were log transformed).
Tabela 3: Razmerje med aktivnostjo ETS in stopnjo dihanja pri štirih vrstah trzač (podatki so bili logaritmirani).

	n	Correlation coefficient	b (slope)	log a (intercept)	P-value
Chironomus thummi	18	0.73	0.56	0.72	<0.001
Paratanytarsus austriacus	9	0.82	1.47	-1.02	<0.01
Procladius (Holotanypus) spp.	8	0.71	0.41	0.09	<0.05
Zavrelimyia barbatipes	7	0.85	0.74	0.30	<0.05

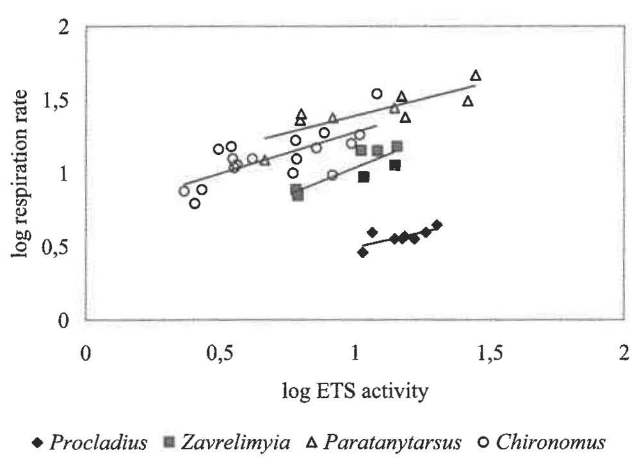

Figure 3: The relationship between ETS activity and respiration rate of different chironomid larvae. Correlation coefficients and regression constants are given in Tab. 3.
Slika 3: Razmerje med aktivnostjo ETS in stopnjo dihanja pri ličinkah različnih vrst trzač. Korelacijski in regresijski koeficienti so podani v Tab. 3.

Discussion

Results of the present study revealed that ETS activity and respiration rate differed significantly between chironomid species (Fig. 1; Tab. 1). Chironomid species vary significantly in body size (Tab. 1) and thus characters related to body size would be expected to vary between species. KONSTANTINOV (1971) reported that the mass-specific respiration rate of chironomid larvae was inversely related to body size. An effect of size on ETS activity was also found in previous investigations, with larger species having lower ETS activities (SIMČIČ & BRANCELJ 1997, 2001). The larger average body sizes of *Chironomus* and *Procladius* were probably one of the reasons for the lower metabolic activity than in the smaller larvae of *Zavrelimyia* and *Paratanytarsus*. The reasons for higher metabolic activity in smaller animals are well discussed in PETERS (1983).

The significant correlations between body mass and ETS activity and between body mass and respiration rate confirmed the effect of body size on metabolic activity in chironomids (Fig. 2). A b value of 0.82 was found in the case of ETS activity and 0.66 for respiration rate. HAMBURGER & DALL (1990) found a value of 0.73 for respiration rate for four different species of chironomids. Similar values of ETS activity were reported for the benthic macrofaunal species *Corophium volutator*, 0.86 (CAMMEN & al. 1990), for *Nereis virens*, 0.84 (CAMMEN & al. 1990), for *Chelicorophium curvispinum*, 0.66 (MUSKÓ & al. 1995), and for Anostraca *Chirocephalus croaticus*, 0.787 (SIMČIČ & BRANCELJ 2000). In general, b values ranged from 0.58 to 0.96 (see LAMPERT 1987). However, the distribution of ETS activity of *Zavrelimyia* compared to similar sized individuals of *Paratanytarsus, Chironomus* and *Procladius* (Fig. 2a) indicated that other factors, beside body size, had influenced on ETS activity. Larvae of *Zavrelimyia* are considered as cold-stenotherms (FERRARESE 1983, FITTKAU & ROBACK 1983). Therefore, one of the reasons for low ETS activities of *Zavrelimyia* was probably respiratory enzyme systems with narrow temperature optima which caused their inactivation at temperature of 20 °C. Contrary to ETS activities, respiration rates of *Zavrelimyia* were close to those of similar-sized individuals of *Paratanytarsus*.

ETS activity correlated significantly with respiration rate in all species investigated (Fig. 3). These results are in accord with those of KING & PACKARD (1975) and SIMČIČ & BRANCELJ (2001) who also found a correlation between ETS activity and respiration rate in invertebrate species. However, ETS activity is a direct enzymatic process, depending on the concentration (BAMSTEDT 1980) and characteristics (PACKARD 1971) of the enzymes, whereas respiration is a complex physiological process. It means that respiration is also influenced by the intact intracellular environment, substrate concentrations, and structure and properties of intact lipid membranes (WITHERS 1992). As investigated species differed in body size (Tab. 1) and their ecological tolerance (FITTKAU & ROBACK 1983, PINDER & REISS 1983), the differences in the ETS activity-respiration rate relationship between them were expected.

One of the purposes of the present study was to obtain an ETS/R ratio that could be used for estimating actual metabolism in chironomids from ETS activity. As shown in Tab. 2 the values of this ratio differed significantly between chironomid species (Tab. 1). In the present experiments, the basal metabolism and expenditure on locomotion were measured. The expenditure on feeding and specific dynamic action (SDA) was minimal, because the animals were not fed just prior to or during the experiments. The low ETS/R ratio in *Zavrelimyia* means that this species exploited 100% of its metabolic potential for basal metabolism and locomotion. *Procladius* had a much higher ETS/R ratio, which means less intensive exploitation of metabolic potential for basal metabolism and activity (~25%). These ratios are similar to the values found in other studies concerning invertebrate species.

Ratios approaching 2 are characteristic of zooplankton (Bamstedt 1980, James 1987, Simčič & Brancelj 1997) but, in the case of benthic species, higher values were determined. Muskó & al. (1995) found that the amphipod *Chelicorophium curvispinum* exhibited an ETS/R ratio of 4.07 at 20 °C. Cammen & al. (1990) reported an ETS/R ratio for *Corophium volutator* of 2.38, and for the polychaete *Nereis volutator* 11.11 (measured at 10 °C). The latter extremely high value was explained by *N. volutator´s* low activity. ETS/R ratio was also influenced by the size of organisms. Present results are in accord with those of Cammen & al. (1990) who found that larger animals have higher ETS/R ratios. The reason for higher ETS/R ratios is the greater decrease in respiration relative to ETS activity with increasing body size. Previous studies have also shown that the ETS/R ratio differs between related species having different ecological tolerance and preference, and consequently they exploit their metabolic potential differently (Muskó & al. 1995, Simčič & Brancelj 1997). Fanslow & al. (2001) reported that, in organisms with high ratios, the capacity for elevated metabolism is maintained so that maintaining the enzyme machinery for increased metabolic activity is an advantage. Investigated chironomid species have different temperature and food preference and tolerance (Fittkau & Roback 1983, Pinder & Reiss 1983), and it is reasonable to assume that ETS/R ratio is influenced by these differences as well. Low ETS/R ratio of *Zavrelimyia* suggest that its energy metabolism is less adaptable to environmental changes compared with the more widely distributed *Chironomus, Paratanytarsus* and *Procladius*. However, further studies will provide additional data in order to obtain detailed conclusions related to relationship between ETS/R ratio and different ecological demands of chironomid larvae. Nevertheless, as ETS/R ratio differed significantly between chironomid species, the energy flux through macrozoobentos has to be estimated by using different conversion factors. Preliminary studies on the estimation of energy flow through respiration in mountain lakes revealed that contribution of chironomids to total respiration differed between shallow oligotrophic and eutrophic lakes.

Conclusions

1. ETS activity and respiration rate, measured at a standard temperature of 20 °C, differed between chironomid species; the body size of species is one of the factors that affected metabolic activity; b value was 0.82 for ETS activity and 0.66 for respiration rate. This indicates the greater decrease in respiration rate relative to ETS activity with increasing body size.

2. The ETS-assay is shown to be a convenient method for estimating respiration rate in chironomid larvae.

3. ETS/R ratios differ between species, so the use of different conversion factors to calculate respiration rate from ETS activity in different chironomid species is recommended. The differences can be explained by different species-specific body size, and different ecological tolerance and preference of chironomid species.

Povzetek

Ličinke trzač (Chironomidae) so široko razširjena skupina, ki ima pomembno vlogo pri pretoku energije skozi prehranjevalne splete, še zlasti v plitvih jezerih. Predhodne raziskave so pokazale, da ličinke trzač številčno prevladujejo v makrozoobentoški združbi gorskih jezerih SZ Slovenije, zato je pri oceni pretoka energije v jezeru potrebno upoštevati tudi njihov delež. Ker je neposredna določitev stopnje dihanja običajno zamudna in nepraktična, so razvili biokemijsko metodo, s katero merimo aktivnost dihalnega elektronskega transportnega sistema (ETS), ki predstavlja metabolni potencial organizmov. Številne raziskave so pokazale, da obstaja med aktivnostjo ETS in dihanjem pozitivna korelacija, tako da lahko na osnovi izmerjene aktivnosti ETS s pomočjo razmerja ETS/R hitro ocenimo intenzivnost dihanja organizmov. Stopnjo dihanja in aktivnost ETS smo določali pri ličinkah štirih različnih vrst trzač, ki smo jih nabrali v treh gorskih jezer: ličinke *Chironomus thummi* v Krnskem jezeru, ličinke *Paratanytarsus austriacus* in *Procladius (Holotanypus)* spp. v Zgornjem Kriškem jezeru in ličinke *Zavrelimyia barbatipes* v Srednjem Kriškem jezeru. Aktivnost ETS in stopnja dihanja, ki smo jo določali pri standardni temperaturi 20 °C, sta se značilno razlikovali med vrstami. Aktivnost ETS in stopnja dihanja trzač sta se zmanjševali z naraščajočo velikostjo živali, kar kaže na vpliv telesne velikosti na intenziteto metabolizma. Opazili smo pozitivno korelacijo med stopnjo dihanja in aktivnostjo ETS pri vseh štirih vrstah, kar pomeni, da je metoda ETS primerna za oceno dihanja tudi pri ličinkah trzač. Raziskava je pokazala značilne razlike v razmerju ETS/R med preiskovanimi vrstami trzač. Različna razmerja so posledica razlik v vrstnospecifični telesni velikosti trzač ter njihove različne ekološke preference in tolerance.

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