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RESPONSES OF CHIRONOMID LARVAE (INSECTA, DIPTERA) TO ECOLOGICAL QUALITY IN MEDITERRANEAN RIVER MESOHABITATS (SOUTH ITALY)

LAURA MARZIALI[a*], DAVID GIORDANO ARMANINI[a], MARCELLO CAZZOLA[a], STEFANIA ERBA[a], ELISA TOPPI[a], ANDREA BUFFAGNI[a] and BRUNO ROSSARO[b]

[a] *CNR – IRSA, Water Research Institute, Via del Mulino 19, I-20047, Brugherio (MI), Italy*
[b] *Department of Agri- food and Urban Systems Protection (DiPSA), University of Milan, Via Celoria 2, I-20133 Milan, Italy*

[*]Correspondence to: PhD Laura Marziali, CNR – IRSA, Water Research Institute, Via del Mulino 19, I-20047, Brugherio (MI), Italy. E-mail: marziali@irsa.cnr.it

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ABSTRACT

Chironomid larvae and pupae were studied in selected Mediterranean rivers with the aim of identifying pool and riffle taxa assemblages and of analyzing their response to ecological quality gradients. Macroinvertebrate samples were collected in 6 Italian rivers along a pool-riffle sequence in 3 seasons following a multihabitat sampling technique. Chironomids were identified as genus/species, other macroinvertebrates as family/genus. The main physico-chemical, hydromorphological and geographical data were collected. Samples were ascribed to 5 quality classes according to the STAR_ICM index. Based on Chironomid taxa, PCA axis 1 represented an organic pollution gradient, axis 2 represented seasonality. Pool and riffle samples were significantly different according to taxa assemblages. Similar results were obtained with PCA based on the whole macrobenthic community. Indicator Value analysis facilitated the detection of the Chironomid indicators of high/good quality pools (e.g. *C. pallidula*, *R. ornata*, *E. ephemerae*) and riffles (*T. calvescens, E. gracei*). The Berger-Parker dominance index based on Chironomid assemblages in pools was correlated to PCA axis 1 and performed well in discriminating between quality classes. In riffles no correlations to PCA axes were detected and a wide overlap between quality classes was present. Thus, assessment in the analyzed river type may focus on pool mesohabitat as this seems to represent best the ecological gradient of sites.

INTRODUCTION

Mediterranean rivers are characterized by high hydrological instability and marked seasonality (e.g. Sabater *et al.,* 2008). A strong dynamism between lentic and lotic conditions during the year, resulting from climatic factors, elicits a high variability in environmental parameters, such as suspended solids, conductivity, and hardness. These factors can cause important changes in principle energy sources and biological communities (Graça and Coimbra, 1998).

At the reach-scale, portions of the river channel can become partly or totally dry in low flow conditions and can be disrupted by a sudden increase of water velocity and discharge during high flow. These conditions represent dramatic stress for aquatic communities (e.g. Boulton and Lake, 1990; Townsend and Hildrew, 1994).

At the mesohabitat scale, water velocity, flow rate, depth and substrate type may show pronounced differences in pool and riffle areas (Brown and Brussock, 1991; Ward and Wiens, 2001), determining important differences between mesohabitats as far as macroinvertebrate community structure, trophic assemblages and biotic indices are concerned (Scullion *et al.*, 1982; Brabec *et al.*, 2004; Bonada *et al.*, 2008).

The succession of pool and riffle sequences along river longitudinal profiles has been identified as one of the primary physical characteristics in the structuring of macrobenthic communities (e.g. Church, 1992; Parsons and Norris, 1996). In general terms, pools can be described as depositional areas characterized by slower flows and thinner substrates, while riffles are characterized by larger size substrates and occur in relatively shallower and faster flowing areas (e.g. Sear *et al.*, 2003). Important differences can also be observed in terms of allochthonous detritus accumulation, with higher retention in the pool areas, directly influencing benthic invertebrate distribution (Lemly and Hilderbrand, 2000). Differences may be enhanced in Mediterranean rivers characterized by high hydrological instability, especially where longitudinal connectivity between mesohabitats is temporary lost during the drought (Bonada *et al.*, 2006a; Bonada *et al.*, 2008).

These aspects need to be taken into account when considering quality assessment and the use of macroinvertebrates in monitoring Mediterranean rivers (Buffagni *et al.*, 2004). As natural factors are highly relevant in those rivers, methods are needed to discriminate between natural and anthropic sources of variability (Buffagni *et al.*, 2009a). Moreover, taxa preferences for specific conditions can lead to a different colonization of pools and riffles (Bonada *et al.*, 2008), resulting in a different response to anthropic impacts. In the past, in most Mediterranean rivers, quality assessment has been achieved using assessment systems based on data collected at reach scale with a multihabitat approach (Iberian BMWP, Alba-Tercedor and Sánchez-Ortega, 1988), or only in riffle areas (Italian Extended Biotic Index, Ghetti, 1997), which were considered the riverine areas most sensible to alterations. In recent years, new multimetric approaches (Hering *et al.*, 2004) have been proposed, based upon samples collected in pools (Buffagni *et al.*, 2004). This mesohabitat may be the most representative in Mediterranean rivers, especially in low flow periods i.e. when riffles tend to disappear and the hydraulic difference between mesohabitats is more evident (Boulton and Lake, 1990; Sear, 1996). Nevertheless it is also possible that under high discharge regimes flow conditions in pools can be similar to riffle conditions if pools are not very deep. In general terms, the representativeness of single mesohabitats is river-type dependent and the convenience in sampling one or both habitats should be adapted to the river type and to the purpose of the sample collection (Buffagni *et al.*, 2004; Bonada *et al.*, 2006b).

Among macroinvertebrate taxa, Chironomidae is one of the richest groups in species that are found in lotic habitats (Cranston, 1995). Due to their ubiquity and different species preferences, Chironomids are well known as indicators of organic enrichment and heavy metal contamination in running waters (e.g. Rosenberg, 1992). Even though few studies have been addressed to integrating these species in quality metrics, in general it is recognized that taxa assemblages tend to be mostly dominated by a few species in impaired sites (e.g. Astin *et al.*, 2006) and that Chironomid species can greatly contribute to this increased dominance (Camargo *et al.*, 2004). Nevertheless they are often considered in biotic indices at family level because of the difficulty of identifying the species in their larval stage (Armitage *et al.*, 1983; Armitage and Blackburn, 1985; Marziali *et al.*, 2006).

With their high resistance and resilience, Chironomids are widely present in Mediterranean rivers, being among the first colonizers after dry periods or flooding events. This has been demonstrated by several studies that have considered the collection of floating pupal exuviae (Malo *et al.*, 1998; Langton and Casas, 1999; Ubero-Pascal *et al.*, 2000; Laville and Langton, 2002; Calle-Martinez and Casas, 2006). This technique facilitates easier identification but does not provide information on taxa habitat preferences.

However, Chironomid larvae collections in specific habitats have rarely been carried out in Southern Europe (e.g. Calle-Martinez *et al.*, 1995; Gendron and Laville 1997; Puntì *et al.*, 2009) and Italy (Prato and Rossaro, 1991; Rossaro and Di Francesco, 1992; Rossaro and Pannunzio, 1992; Rossaro *et al.*, 1996; Rossaro and Mietto 1998). In particular, few studies have considered small spatial scales in order to characterize taxa habitat preferences. It is known that Chironomid larvae are mainly distributed according to substrate composition, organic matter deposits, water depth and velocity: different taxa assemblages would therefore, be expected to occur in pools and riffles (Pinder, 1995; Lencioni and Rossaro, 2005). Recently Principe *et al.* (2008) found different Chironomid assemblages in riffle and run mesohabitats in Argentinean streams, which were characterized by different indicator taxa.

The main aims of the present paper are: a) to investigate Chironomid taxa assemblages at the mesohabitat scale in selected Mediterranean rivers, b) to compare the Chironomid response to environmental parameters with that of the overall community response and c) to evaluate the possible use of Chironomid taxa at the larval stage as indicators of ecological quality in pool and riffle mesohabitats.

STUDY AREA

A total of 11 sites were chosen in 6 rivers in the Southern Apennines (Campania Region, Salerno Province, Italy) (Figure 1) and were sampled in spring (May), early autumn (October) 2000 and in winter (March) 2001 (Buffagni *et al.*, 2004). Selected rivers were representative of a single river type according to System A of the WFD (EC/2000/60): small-sized rivers in calcareous basins, with catchment areas between 10 and 100 Km^2 and altitudes between 200 and 800 m a.s.l.. Only 1 site, i.e. Tanagro Ascolese, had catchment area larger than 100 km^2. Rivers were generally non-intermittent, except for possible dry periods in summer.

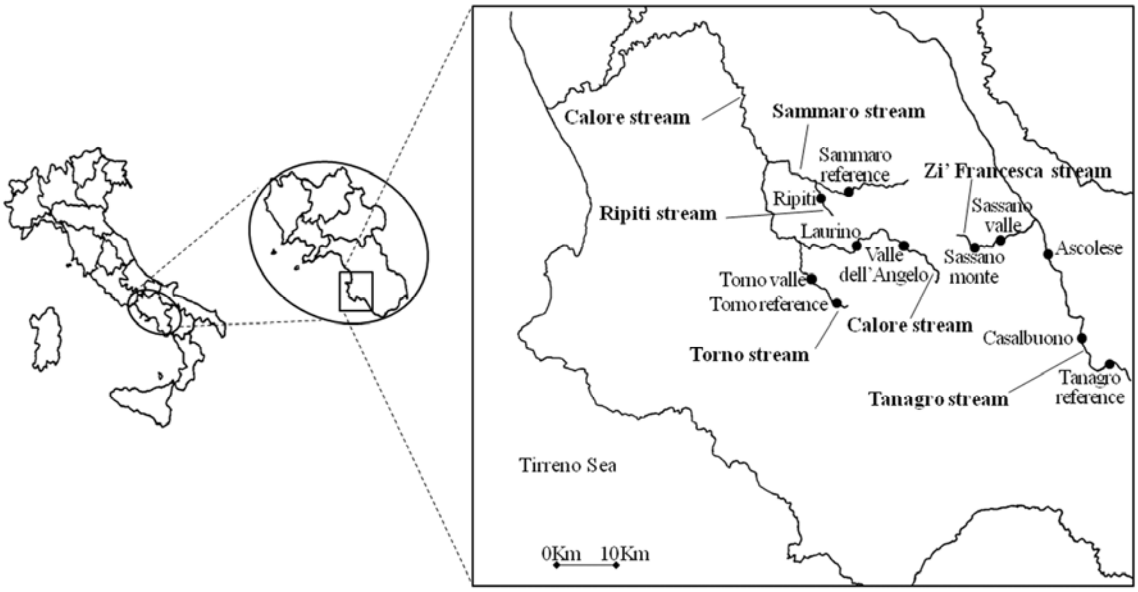

Figure 1. Map of study area and sampling sites (Cilento, Salerno province, Southern Italy; modified from Buffagni *et al.*, 2004).

Sites were chosen according to the AQEM sampling design, to cover the environmental gradient from reference to heavily impacted sites and to avoid strong altitudinal/longitudinal gradients (from 288 to 581 m a.s.l.) (Buffagni *et al.*, 2004; Hering *et al.*, 2004). According to the AQEM Project approach, 11 sites are considered enough to detect an overall degradation gradient, if those sites are selected along the environmental grade, even when stressor-specific metrics are under investigation (Hering *et al.*, 2003; 2004). Even though two sampling seasons are considered enough in the AQEM protocol to detect the seasonal gradient (summer and winter), our sampling design included a third sampling season (spring), in order to better characterize the strong seasonal gradient of Mediterranean rivers.

The main stressor observed in the area was organic pollution, often associated with the degradation of river morphology (Buffagni *et al.*, 2004). A reference site was chosen along the Sammaro River

(Sammaro reference), which was characterized by high 'naturalness', i.e. a low incidence of influence from anthropic activities. The Tanagro River presented reference conditions in its upper section (Tanagro reference). Downstream it was impacted at Casalbuono by the morphological alteration of its banks and channel and by re-sectioning at Ascolese, where chemical pollution was present mainly due to agricultural land use. A reference site was located in the upper part of Torno River, while Torno Valle was influenced by stock farm sewage. Ripiti River was characterized by reduced discharge. Calore Valle dell'Angelo presented a natural morphology of banks and channel but was characterized by heavy organic pollution. The downstream site Calore Laurino was impacted by waste water inflow and pasture. Zì' Francesca River was characterized by artificial banks and was severely affected in its upper section (Sassano monte) by urban waste water inflow and downstream (Sassano Valle) by the presence of a treatment plant.

A further description of the sampling sites can be found in Balestrini *et al.* (2004) and a table showing mean and standard deviation of the main environmental parameters is included in Buffagni *et al.* (2004).

SAMPLING METHODS

Macroinvertebrate samples were collected according to the AQEM sampling strategy (Hering *et al.*, 2004), a multihabitat proportional method with specifications for Southern European rivers (Buffagni *et al.*, 2001). Within each site a 25-50 m long reach characterized by a pool-riffle sequence, i.e. a sequence of a depositional and an erosional areas, was defined. Different microhabitat types were located within each of the two areas and their relative occurrence was visually evaluated (Buffagni *et al.*, 2001). A total of 20 sample units (i.e. microhabitat units) were collected: 10 units were distributed within the pool habitat and 10 within the riffle habitat, depositional and erosional areas being similarly represented. Each sample unit consisted of a 0.05 m^2 area and was sampled with a Surber net or a hand net (500 µm mesh size) in the deepest habitats. Thus, in this paper a total number of 66 samples was considered (= 11 sites x 3 seasons x 2 mesohabitats).

Samples were sorted *in situ*, examining single quotes of material in white trays. A part of the sediment collected during the sampling, for each sample, was brought to the laboratory to further check for the presence of small taxa. Only for 7 cases, where high numbers of specimens (e.g. *Chironomus* larvae) were present, 1/3 of sampled material was examined.

Macroinvertebrates were identified to family or genus, except for Ephemeroptera, Odonata, Mollusca and Coleoptera, which were identified to species (see Buffagni *et al.*, 2004).

Chironomid larvae and pupae were examined under a stereomicroscope and grouped according to empirical characters (e.g. size, colour, body setation, larval head capsule shape, pupal thoracic horn shape). Few individuals (1-5) for each empirical group were dissected and slide mounted with Canada Balsam according to Wiederholm (1983; 1986). Prior to slide preparation, large-size larval specimens and pupae were cleared in 10 % KOH at 85 °C. Specimens were identified to the finest taxonomic level (e.g. species, species group, genus) according to Janececk (1998), Rossaro (1982), Wiederholm (1983; 1986), Langton and Visser (2003), Stur and Ekrem (2006), Lencioni *et al.* (2007). Identification of mature pupae, some of which associated to larval exuviae, allowed species determination of Tanypodinae, *Orthocladius* and *Cricotopus* larvae. Examination of larvae and associated mature pupae collected in Italy (collection B. Rossaro) allowed the identification to species of most larval material. Young larval stages were generally absent because large-mesh nets (500 µm) were used for samplings. Species total abundance per sample unit (= 0.05 m^2 area) was recorded.

The main physico-chemical parameters were measured during the sampling using field multi-probes or following standard methods (APAT-IRSA, 2004): water temperature (°C), conductivity ($\mu S\ cm^{-1}$), hardness (°F), dissolved oxygen ($mg\ l^{-1}$), pH, N-NO_3 ($mg\ l^{-1}$), N-NH_4 ($mg\ l^{-1}$), total phosphorus

(TP, µg l^{-1}), P-orthophosphate ($P\text{-}PO_4$, µg l^{-1}), total chloride (mg l^{-1}), *Escherichia coli* (CFU 100 ml^{-1}), BOD_5 (mg l^{-1}).

The physico-chemical alteration of sites was assessed calculating *Livello di Inquinamento dei Macrodescrittori* (LIM, DL 152/99), an index of organic pollution that increases with increasing water quality.

Geographical and typological information were collected for each site, for example: stream order, source distance, catchment area, stream density (a measure of the total length of streams per unit area of drainage basin), valley and thalweg slope and wet channel width. For each sampling unit, water velocity, depth and flow type (EA, 2003) were determined. Mean water velocity and mean water depth were calculated separately for pool and riffle samples.

The CARAVAGGIO method (Buffagni *et al.*, 2005), formerly known as the *South European River Habitat Survey* (Buffagni and Kemp, 2002) and derived from the *River Habitat Survey* protocol (RHS, Raven *et al.*, 1998) was adopted to collect morphological, hydraulic and hydrological information. The data were used to calculate: the *Habitat Quality Assessment* (HQA, Raven *et al.*, 1998), an index of habitat diversification able to infer on general stream quality (Balestrini *et al.*, 2004); the *Habitat Modification Score* (HMS, Raven *et al.*, 1998), an index of morphological impairment (artificial features such as bridges, weirs, channel resectioning, etc); and the *Lentic-lotic River Descriptor* (LRD, Buffagni *et al.*, 2009b), to define the overall flow habitat condition of the sites, based on the ratio between the lentic and lotic areas (i.e. riffle-pool) found along the stream channel. Both natural and artificial morphological characteristics are considered to calculate LRD. Artificial features obtain positive values, because they are supposed to create flow impedance. Since the presence of such fixed artificial structures is considered as invariant (HMS values are generally stable in sites), the variation that can be observed in LRD values at a river site will depend on seasonal discharge and natural channel features (e.g. substrate, macrophytes) (Buffagni *et al.*, 2009b).

DATA ANALYSES

Ecological status classification

Samples were classified into five classes (from high to bad status) using the STAR_ICM index, i.e. the official Italian method for classification based on macroinvertebrate communities, which was developed for WFD Intercalibration purposes (Buffagni *et al.*, 2007; EC, 2008). The STAR_ICM index is a multi-metric index based on 6 different metrics. The identification level required for calculation is family. After normalization by the median value of reference sites' samples, these metrics are combined into the STAR_ICM index. The STAR_ICM index score is calculated separately for pool and riffle samples: the score of the site is represented by the mean of the two values and was used to characterize both mesohabitats at each site. To classify samples, as far as the Biological Quality Element of macroinvertebrates is concerned, the official Italian boundaries were used (EC, 2008). Metric and index calculations were run with ICMeasy software, version 1.2 (Buffagni and Belfiore, 2006).

Mesohabitat Indicator taxa

To detect Chironomid taxa preferences for pool or riffle mesohabitat, the Indicator Value (IndVal) (Dufrêne and Legendre, 1997) method was run using PC-Ord for Windows 4.1 (McCune and Mefford, 1999). This method combines the relative abundance of a species with its relative frequency of occurrence in the different sample groups. Indicator species are defined as the most characteristic species of each group, i.e. found mostly in a single group and present in most sites of that group. This index can range from a minimum of 0% (not found in the habitat) to a maximum of 100%, obtained when the species is found only in one group of samples and when it occurs in all samples of that group. In general terms, only species obtaining an IndVal value ≥ 25% are considered characteristic of that habitat. Only high/good status pools and riffles (24 pools vs. 24

riffles) were included in the analysis, in order to eliminate the confounding gradient produced by anthropic impact. The significance of the obtained values was tested with a Monte Carlo randomization procedure that re-allocates samples among sample groups (9999 permutations) (Dufrêne and Legendre, 1997).

Multivariate ordination technique and response of Chironomids to environmental gradients

To detect the main ecological gradients influencing Chironomid taxa assemblages in the study area, a multivariate ordination analysis was run. Data were used at the mesohabitat scale, considering riffle and pool samples collected at the same site as distinct samples: a total of 66 cases were thus considered (= 11 sites x 3 seasons x 2 mesohabitats). Species abundances were ranked according to Preston's log-normal classes (Preston, 1948) to reduce the influence of extreme values and to normalize distributions. Environmental data values (29 variables) were Log_{10} transformed except for pH, HMS, LRD, HQA, LIM, stream order and season.

Ordination analysis was run by means of the CANOCO program, version 4.51 (Ter Braak and Šmilauer, 2002). In order to reduce the total number of biological variables only species present in the dataset with a frequency ≥ 10 (i.e. present in more than 10 samples) were included (37 taxa out of a total of 79), as rare species were not considered necessary in defining the main ecological gradients (Cao *et al.*, 2001). A Detrended Correspondence Analysis (DCA) was run first to detect if the data had an unimodal or linear structure according to the gradient length of axes. As gradient length was comprised between 3 and 4 SD for axes 1-3 and shorter than 3 for axis 4 a Principal Component Analysis (PCA) was performed, being a linear method considered appropriate (Ter Braak and Šmilauer, 2002). Four Principal Components were calculated. The resulting ordination axis scores were interpreted according to abiotic and biotic parameters by calculating Pearson's product-moment correlation coefficients.

Pool sample scores were compared with corresponding riffle sample scores using the Wilcoxon Matched Pairs Test to detect any significant differences between the two mesohabitats (software Statistica 7.0, Statsoft, 2007).

The same analyses had previously been run by Buffagni *et al.* (2004) including data on the whole macroinvertebrate community and considering Chironomids at family level. For detailed methods see Buffagni *et al.* (2004). The results were compared.

The effect of seasonality on taxa assemblages was explored comparing subfamily composition (relative abundance of subfamilies) between seasons using the Friedman ANOVA for multiple dependent samples and then the Wilcoxon Matched Pairs Test as post-hoc comparison test (software Statistica 7.0, Statsoft, 2007). The same test was used to compare the total number of individuals per sample between seasons and the t-test for dependent samples was used to compare taxa richness per sample between seasons (after running a Shapiro-Wilk's W test for normality and testing homogeneity of variance with Levene's test).

To test Chironomid response to ecological quality status at mesohabitat scale, the Berger-Parker dominance index (i.e. number of individuals of the most abundant taxon on the total number of individuals) (Berger and Parker, 1970) was calculated for each sample. The median value of the index was calculated for the pool samples belonging to the same STAR_ICM index class and the Kruskal-Wallis ANOVA by ranks was used to find differences between the median values of the classes (software Statistica 7.0, Statsoft, 2007); Pearson's correlation coefficient was calculated between Berger-Parker index values and PCA axis 1 scores for pools. The same analyses were performed for riffle samples.

RESULTS

Overall environmental and habitat conditions

Reference sites showed the lowest values of BOD_5, TP, N-NH_4, N-NO_3 and *E. coli* due to the absence of organic pollution (see Buffagni *et al.*, 2004 for details). HMS values up to 11 were calculated for reference sites, as a slight morphological alteration was present in Torno and Sammaro reference. HQA values were ≥ 59, representing good habitat diversification.

In non-reference sites water pollution was often associated with morphological alterations of the fluvial habitats, except in some sites, where only organic pollution (e.g. Calore Valle dell'Angelo) or morphological alteration (e.g. Tanagro Casalbuono) was present. Organic enrichment was sometimes very intense (e.g. Calore Valle dell'Angelo and Zi' Francesca rivers) as shown by the high values of BOD_5, TP, $N\text{-}NH_4$ and *E. coli* (respectively up to 10.58 mg l^{-1} BOD_5, 2100 µg l^{-1} TP, 2 mg l^{-1} $N\text{-}NH_4$ and 42000 CFU 100 ml^{-1}) and low oxygen levels (down to 1.9 mg l^{-1} O_2). HMS values ranged from 0 to 84, indicating that morphological alteration was very slight in Ripiti Sacco and Calore Valle dell'Angelo, while it was strong along the Tanagro and Zi' Francesca rivers. HQA values were ≥ 43 for most sites, representing a good habitat diversification. Only Zi' Francesca River showed low values of HQA (24-39), being characterized by artificial banks.

LRD values were generally negative for all seasons, highlighting the lotic character of most sites (42 out of 66 samples with LRD < -10) (Buffagni *et al.*, 2009b), with positive values i.e. lentic conditions (LRD > 10) observed in only a few sites during the autumn (8 samples); 16 samples showed intermediate LRD values (from -10 to 10). Nevertheless pool-riffle sequence was present and obvious in all seasons, with pool and riffle mesohabitats represented in a comparable way and always connected (i.e. no portions of the river channel were dry).

Flow types recorded in pool and riffle microhabitat units in the three sampling seasons are represented in Figure 2.

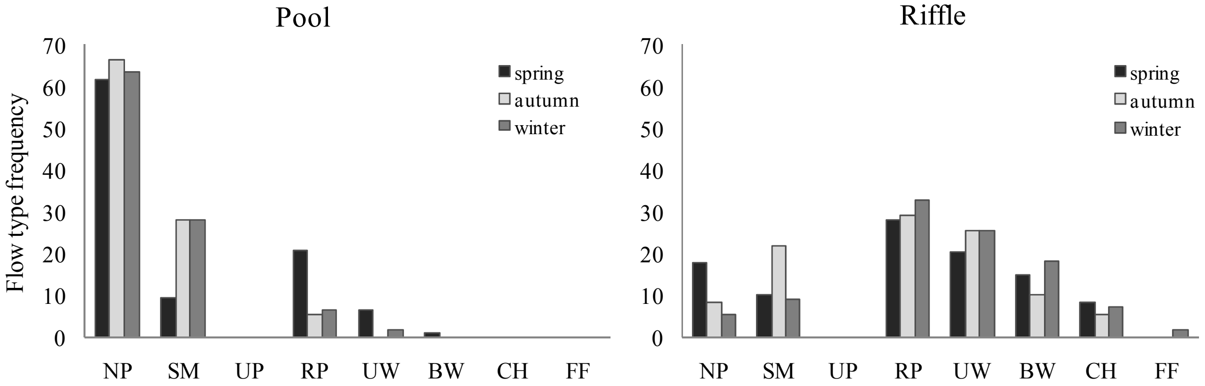

Figure 2. Frequency histograms of flow types recorded in pool (left graph) and riffle (right graph) microhabitats for three sampling seasons, as recorded for the invertebrate sample units (10 records in pool and 10 records in riffle, for each site/season). Flow types: NP = no perceptible; SM = smooth; UP = upwelling; RP = rippled; UW = unbroken standing waves; BW = broken standing waves; CH = chute; FF = free fall.

Flow types were very different between pool and riffle units and similar between the three sampling seasons (Fig. 2). No perceptible, smooth, rippled flow, unbroken and broken standing waves were present in pool, with no perceptible flow representing more than 60%. In riffle units flow types were more diversified, ranging from no perceptible flow to free fall with a higher frequency of rippled flow (about 30%) and unbroken standing waves (20-25%).

Chironomid taxa assemblages at mesohabitat scale

A total of 25630 Chironomid specimens were collected and 79 taxa were identified, belonging to 51 genera and 5 subfamilies (Appendix 1). About 1400 individuals were slide prepared.

In pools 81% of specimens were Chironominae and 16% Orthocladiinae, while in riffles Chironominae accounted for 59% and Orthocladiinae for 34%. Prodiamesinae and Tanypodinae were more abundant in pools, while Diamesinae were more abundant in riffles. A higher number of taxa were found in pool mesohabitats (75 taxa), while 67 taxa were found in riffles.

An IndVal analysis including 75 taxa was run to describe the Chironomid community in the two studied mesohabitats in natural and slightly impacted conditions (Table I). 27% of the observed taxa were present exclusively in pools while 7% were present exclusively in riffles. Moreover, 47% of taxa showed the highest number of collected specimens (> 75%) in pools while 18% of taxa were mostly present in riffles. The residual 35% of taxa were almost equally present in the two mesohabitats.

According to taxa frequency, six taxa were present in more than 50% of pool samples: *Conchapelopia pallidula*, *Paratrissocladius excerptus*, *Orthocladius rhyacobius*, *Rheopelopia ornata*, *Zavrelimyia melanura* and *Polypedilum convictum*. One taxon only was present in more than 50% of riffle samples: *Tvetenia calvescens*.

According to the Indicator Values, 6 species were indicators of the pool mesohabitat (*C. pallidula*, *R. ornata*, *Epoicocladius ephemerae*, *Macropelopia nebulosa*, *Polypedilum pedestre* and *Rheocricotopus effusus*) (Table I). *T. calvescens* and *Eukiefferiella gracei* were significant indicators of the riffle mesohabitat.

Table I. Indicator values for Chironomid taxa from pools vs. riffles considering only high/good quality samples. Only taxa with significant values are shown.

Habitat	Taxa	IndVal (%)	p-value
Pool	*Conchapelopia pallidula*	48	0.029
	Rheopelopia ornata	47	0.033
	Epoicocladius ephemerae	38	0.011
	Macropelopia nebulosa	38	0.013
	Polypedilum pedestre	30	0.008
	Rheocricotopus effusus	30	0.021
Riffle	*Tvetenia calvescens*	59	0.009
	Eukiefferiella gracei	25	0.042

Multivariate analysis and response of Chironomids to environmental gradients

PCA was run to detect the main ecological gradients within the Chironomid taxa dataset (Table II). Axis 1 accounted for 20.9 % of total variance and represented an environmental quality gradient, mainly related to water quality. In fact, it was positively correlated to *E. coli*, TP, BOD_5, $N\text{-}NH_4$ and Cl^- and negatively to stream density, dissolved oxygen and quality indexes LIM and HQA. The species most associated with low water quality were *Chironomus riparius*, *Micropsectra atrofasciata* and *Cricotopus intersectus*, which were dominant in Sassano monte, Sassano Valle and Calore Valle dell'Angelo, while *P. excerptus*, *E. ephemerae* and *Microtendipes rydalensis* were abundant in high quality sites, such as the reference sites of Tanagro Ascolese and Ripiti Sacco.

PCA axis 2 (12.3 % of total variance) was interpreted as a seasonal gradient, being positively correlated to stream width, pH, discharge, dissolved oxygen and negatively to season, water temperature and conductivity (Table II). Seasonal variation of subfamily composition is represented in Table III. Orthocladiinae were dominant in winter and Chironomini in autumn due to peaks of thousands of individuals of *C. riparius* in Calore and Zi' Francesca rivers: at Calore Valle dell'Angelo and Sassano Valle diversity showed a minimum, with total number of taxa 2 and 4 respectively. Tanypodinae and Tanytarsini exhibited the highest numbers in spring and autumn. Percent of Tanypodinae and Orthocladiinae varied significantly between seasons ($\chi_{2\ (22,\ 2)} = 25.07$ and $\chi_{2\ (22,\ 2)} = 17.82$ respectively, $p < 0.01$). In particular, Tanypodinae were different between spring and winter and between autumn and winter, while Orthocladiinae were different between all seasons ($p < 0.01$ for all comparisons). Total number of individuals per sample varied significantly between seasons ($\chi_{2\ (22,\ 2)} = 6.90$, $p < 0.05$), in particular between spring and autumn and between autumn and winter ($p < 0.05$), while total number of taxa per sample didn't vary between seasons ($p > 0.05$). Axis 2 was also slightly correlated to water quality, being positively correlated to LIM and negatively to TP and N-NH_4. A weak negative correlation to altitude was also detected, but all taxa found are retained to cover the considered altitudinal range (between 288 and 581 m a.s.l) (Moog, 1995; Rossaro pers. comm.), except for *Eukiefferiella brevicalcar* (usually > 550 m a.s.l., e.g. Puntì *et al.*, 2009), which was excluded by PCA as rare taxon.

Table II. Axis eigenvalues and correlations between selected variables and sample scores for PCA including Chironomid taxa (left columns) and including macroinvertebrate community (Chironomids at family level) (right columns; mod. from Buffagni *et al.,* 2004). Only significant correlation coefficients are shown ($p < 0.05$).

	Chironomid taxa				Macroinvertebrate community (with Chironomids at family level)			
	Axis 1	Axis 2	Axis 3	Axis 4	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues:	0.21	0.12	0.11	0.08	0.16	0.11	0.10	0.07
Species-environment correlations:	0.98	0.95	0.92	0.89	0.98	0.98	0.98	0.97
Cumulative percentage variance:								
- of species data:	20.9	33.2	43.9	51.8	16.3	26.9	36.7	44.4
- of species-environment relation:	25.1	38.8	50.0	57.7	17.3	29.0	39.7	47.7
Variables	Pearson correlation coefficients ($p < 0.05$)							
Descriptive variables								
Stream density	-0.80				0.68			
Stream width	-0.27	0.49						
Stream order	-0.28		0.25					
Distance to source			0.29	0.36			0.50	
Altitude		-0.28						0.42
Catchment area			0.27	0.39			0.57	
Discharge		0.44			0.44			
Water temperature	0.24	-0.35		-0.33		0.32		
Season		-0.60		-0.28		0.48		
Mean water velocity							0.46	
LRD	0.34				-0.63	0.45		
Quality variables								
O_2 mg/l	-0.54	0.41			0.55			
N-NH_4	0.66	-0.28			-0.48			
BOD_5	0.59				-0.41			
P-PO_4	0.56	-0.43			-0.61			
TP	0.57	-0.34			-0.67			
Total chloride	0.44		-0.26		-0.58			
E. coli	0.69				-0.66			
pH	-0.28	0.45			0.34			
Conductivity		-0.35	-0.26			0.47		
HMS	0.27				-0.44			
HQA	-0.69				0.73			
LIM	-0.73	0.34			0.65			

PCA axes 3 and 4 accounted for a small part of the total variance (respectively 10.7 % and 7.9 %) and were positively correlated to catchment area and distance from source, being associated with the slight upstream-downstream gradient. Axis 3 was also characterized by high concentrations of the total chloride measured in Calore Valle dell'Angelo in May, probably due to urban waste water inflow.

Table III. Mean and standard deviation (SD) of Chironomid subfamily percent composition in each sampling season. Total number of taxa and of specimens for each subfamily considering all seasons together (n) are reported.

Subfamily	Taxa (n)	Specimens (n)	Spring (mean±SD)	Autumn (mean±SD)	Winter (mean±SD)
Tanypodinae	14	1330	23.2 ± 25.2	21.4 ± 25.8	2.3 ± 3.5
Diamesinae	2	87	0.9 ± 3.1	0.1 ± 0.4	2.5 ± 8
Prodiamesinae	1	43	0.9 ± 1.6	0	0.5 ± 1.6
Orthocladiinae	43	5468	36.5 ± 25.4	20.6 ± 24	57.4 ± 27.4
Tanytarsini	5	1639	11.1 ± 8.1	13 ± 10.9	11.4 ± 14.1
Chironomini	14	17064	27.1 ± 27.2	44.7 ± 40.9	25.7 ± 28.3

Factor scores for pool and riffle samples for axis 1 and axis 2 were significantly different according to Wilcoxon Matched Pairs Test ($N = 33$; $p < 0.05$), emphasizing the presence of different taxa assemblages in depositional and erosional mesohabitats along each detected ecological gradient.

PCA analysis including data on the whole macroinvertebrate community was run by Buffagni *et al.* (2004), considering Chironomids at family level. Results are reported in Table II. Approximately 215 macroinvertebrate taxa were found in both mesohabitats, with about 30 exclusive taxa in riffles and 30 in pools.

PCA axis 1 represented a quality gradient, being positively correlated to dissolved oxygen, HQA and LIM and negatively to TP, *E. coli*, $N\text{-}NH_4$ and HMS. Reference sites showed high positive score values.

Axis 2 was interpreted as a seasonality gradient, being correlated to season, water temperature and hydrological instability (Buffagni *et al.*, 2004). The third axis was associated with a longitudinal gradient, being correlated to distance from source and catchment area. The fourth axis accounted for a small amount of total variability and probably related to local characteristics.

The Wilcoxon Matched Pairs Test was used to compare the scores of riffle and pool samples according to PCA axes. A significant difference between groups was detected for all axes ($N = 33$; $p < 0.05$) (Buffagni *et al.*, 2004).

The Berger-Parker dominance index value was calculated for each sample based on Chironomid taxa assemblages. The index values were significantly correlated to PCA axis 1 scores for pools ($r = 0.78$, $p < 0.01$), while they were not for riffles. An increase of the index values according to STAR_ICM index class was shown for the pool mesohabitats (Fig. 3); median values of the index in the classes were significantly different ($KW_{(4,33)} = 17.63$, $p < 0.01$). On the contrary, for riffle samples, no difference was found between classes ($KW_{(4,33)} = 5.11$, $p = 0.27$) due to the similar median values of the dominance index and the broad overlap between high, good and moderate status classes.

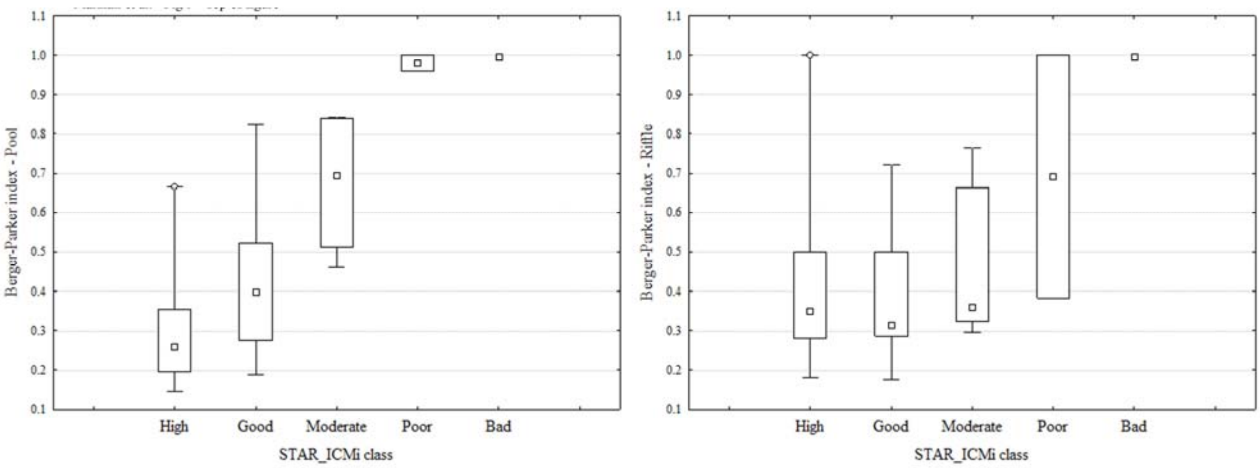

Figure 3. Variability of the Berger-Parker dominance index in the five STAR_ICM index quality classes. Squares = median values; boxes = 25th-75th percentile range; whiskers = non-outlier range (coefficient = 1); circles = outliers.

DISCUSSION

Different taxa assemblages were found between riffles and pools both for Chironomids and macroinvertebrates along anthropic and natural gradients (e.g. water quality and seasonality). Previous studies associated these results with the different hydrological character of depositional and erosional areas (e.g. substrate composition, flow types, etc), which may prevail over the differences between sites (e.g. Brown and Brussok, 1991; Parsons and Norris 1996; Lencioni and Rossaro, 2005; Principe *et al*., 2008). Moreover, deposits of fine organic sediment are generally present in pools, while coarser substrates may characterize riffles, determining the habitat selection according to species ecological preferences (Lemly and Hilderbrand, 2000).

As regards Chironomids, IndVal analysis showed that in natural and slightly impacted conditions, pools were characterized by assemblages with many exclusive or characteristic taxa, while riffles hosted few exclusive taxa and were characterized by the presence of few indicator species. This may be due to the fact that in this river type, pools are less influenced by seasonal hydrological changes than riffles and thus may host more structured communities (Brown and Brussock, 1991). Moreover, most Chironomid taxa and specimens were found in pool areas as they provide the most suitable mesohabitat for detritivorous organisms (Pinder 1995; Arimoro *et al*., 2007).

Multivariate analyses including Chironomid taxa (mostly species) and including the whole macroinvertebrate community (mostly at family/genus level) gave similar results, showing the potential of those Diptera in biomonitoring. Armitage and Blackburn (1985) found similar results in their pioneering study.

Chironomids were shown to be valid indicators of the overall quality status of sites, showing different taxa assemblages according to ecological gradients linked to anthropic impact (Rosenberg, 1992). In more detail, in the area under examination Chironomids as well as macroinvertebrate community were shown to be good indicators of organic enrichment, being in agreement with the LIM index and individual pollution variables, and to a lesser extent with the habitat diversification expressed by HQA score. Chironomids, however, were less influenced by morphological alteration and habitat structure than other macroinvertebrates (see e.g. HMS in Table II).

According to PCA results, some taxa showed a clear response to ecological quality conditions of mesohabitats. *C. riparius* and *Rheocricotopus fuscipes* showed an opportunistic response dominating in impaired riffles and pools: they are known to be euryeciuos detritivorous species dominant respectively in poly- and mesosaprobic waters (Wiederholm, 1983; Moog, 1995). *C. intersectus* and *Eukiefferiella claripennis* were characteristic of impaired pools, being grazers and detritus feeders resistant to low oxygen levels (they can be found also the littoral zone of lentic waters) (Wiederholm, 1983; Moog, 1995); *R. effusus* and *Eukiefferiella ilkleyensis* characterized impaired riffles, being resistant to saprobic waters with a relatively high oxygen content (Moog, 1995). Tanypodinae - *C. pallidula*, *Z. melanura*, *R. ornata*, cold stenothermic lentic taxa (Wiederholm, 1983) - were abundant in high/good status pools, along with detritivorous *P. excertpus* and *P. convictum*. *Tanytarsus* sp. was abundant in high/good riffles, being less tolerant to low oxygen levels than most Chironomini (Thienemann, 1953).

Another important driving force for both Chironomids and macroinvertebrates was seasonality, i.e. temporal heterogeneity characterising Mediterranean rivers (Puntì *et al*., 2009). Nevertheless Chironomids were shown to be less influenced by hydrological parameters than other macroinvertebrates, showing no correlation to water velocity and a weak correlation to discharge and the LRD descriptor i.e. to the overall lentic-lotic character of the river site. It is known indeed that some species are highly resistant and resilient, adapting rapidly to environmental changes (Langton and Casas, 1999). Therefore, different taxa assemblages between riffles and pools may relate more to the trophic preferences of species than to hydrological conditions.

Moreover, in comparison with other Mediterranean rivers (Puntì *et al.*, 2009) a narrow variation of taxa assemblage was found between seasons. According to their ecological preference, some taxa were mostly abundant and frequent in spring (e.g. *Macropelopia* spp., *T. calvescens*), some were mostly found in autumn such as *C. riparius* and *Tanytarsus* sp., others were characteristic of winter (e.g. *Orthocladius* spp., *Euorthocladius rivicola*, *R. effusus*). Spring exclusive taxa (e.g. *Eukiefferiella* spp. and *Eudactylocladius fuscimanus*) were species adapted to flowing waters (Rossaro, 1982); some lentic (e.g. *Ablabesmyia monilis*, *Psectrotanypus varius*, *Acricotopus lucens*) and semiterrestrial species (e.g. *Limnophyes* sp., *Metriocnemus* sp.) (Moog, 1995) were found only in autumn; cold-adapted *Boreheptagyia*, *Heleniella serratosioi*, *Euorthocladius rivulorum* (Rossaro, 1982) were exclusive in winter. According to our results, the correlation to the season may, therefore, result more from species voltinism and ecological requirements than hydrological change during the year.

The Berger-Parker dominance index based on the Chironomid taxa assemblages of pools was correlated to PCA axis 1 scores for pools, representing a potential metric responding to anthropic alteration gradients. Moreover, the index values calculated for pools were in agreement with the biological conditions of the whole site, i.e. with the STAR-ICM index class, which is based on site macroinvertebrate communities. On the contrary the index values calculated for riffles were not correlated to PCA axes and showed a broad overlap between STAR-ICM index classes. Therefore, as already observed for the whole community (Buffagni *et al.*, 2004), the Chironomid assemblages of pools were shown to be better indicators of anthropic alteration in this river type than riffle faunas.

CONCLUSION

Chironomids are potentially good indicators of ecological status in Mediterranean rivers (Puntì *et al*., 2009), also because they seem to be less influenced by hydrological conditions than other macroinvertebrates (Langton and Casas, 1999). This result may prove relevant, as studies have recently demonstrated the need to develop quality metrics that are less influenced by hydrological variability than standard ones (i.e. ASPT, Armitage *et al*., 1983), especially as regards Mediterranean rivers (Buffagni *et al*., 2009a).

Few studies have been carried out to study Chironomid larval stages at species level because of identification difficulties. Our results showed that in some cases species belonging to the same genus showed rather different ecological responses (e.g. *M. atrofasciata*, characterizing low quality sites vs. *Micropsectra aristata* and *Micropsectra recurvata*, which were found in good quality sites) and therefore identification at the finest level is seen as necessary. However, some species or genera that are difficult to separate showed a similar distribution in our dataset, potentially being indicators of the same quality conditions: e.g. the genera *Rheopelopia* and *Conchapelopia*, characteristic of good quality sites; *R. fuscipes* and *R. effusus*, along with and *E. ilkleyensis* and *E. claripennis*, indicators of impaired conditions. In these cases Operational Units composed of taxa with similar morphological and ecological traits could be introduced into biomonitoring programmes, as previously suggested for mayflies (Buffagni, 1997). This could facilitate taxa identification and avoid a loss of information. To support the process, specific studies at small spatial scales should be carried out to clarify taxa preferences and tolerance.

As an alternative, identification difficulties may be overcome by developing specific metrics reflecting species assemblages: for example, the Berger-Parker dominance index may prove to be a valid indicator of organic pollution (Camargo *et al.*, 2004).

As suggested by many authors, data from different mesohabitats should not be merged before the evaluation of water quality status (e.g. Buffagni *et al.*, 2001), because the combination of such data could increase the natural variability effects and confuse the impairment assessment (Parsons and Norris, 1996; Brabec *et al*., 2004). Great caution should be taken in establishing which mesohabitat has to be considered in ecological status and water quality evaluation. In the analyzed Mediterranean river type, Chironomids in the pool area seem to respond better to changes in ecological status. Thus, assessment methods in the area should focus on pool mesohabitat, as has been previously confirmed in analyses of the whole macroinvertebrate community (Buffagni *et al*., 2004).

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Appendix 1. Checklist of Diptera Chironomidae sampled in 6 Southern Italian rivers in Cilento (Salerno province), with sum of individuals collected in riffle and pool mesohabitats (Ab.= number of specimens per 16.5 m^2) and number of samples (tot. = 66) where species was present (Freq. = frequency).

Taxa	Author	Pools		Riffles	
		Ab.	Freq.	Ab.	Freq.
Subfamily Tanypodinae					
Ablabesmyia monilis	(Linnaeus, 1758)	3	2	41	3
Conchapelopia pallidula	(Meigen, 1818)	78	17	32	9
Krenopelopia binotata	(Wiedemann, 1817)	2	2	0	0
Larsia atrocincta	(Goetghebuer, 1942)	6	3	0	0
Macropelopia nebulosa	(Meigen, 1804)	108	13	13	3
Macropelopia notata	(Meigen, 1818)	71	7	127	7
Procladius choreus	(Meigen, 1804)	74	5	1	1
Paramerina cingulata	(Walker, 1856)	25	12	81	7
Psectrotanypus varius	(Fabricius, 1787)	18	1	1	1
Rheopelopia ornata	(Meigen, 1838)	74	13	19	10
Thienemannimyia laeta	(Meigen, 1818)	4	1	0	0
Thienemannimyia lentiginosa	(Fries, 1823)	1	1	0	0
Trissopelopia longimana	(Stæger, 1839)	73	9	14	7
Zavrelimyia melanura	(Meigen, 1804)	333	15	123	8
Subfamily Diamesinae					
Boreoheptagyia sp.		1	1	0	0
Diamesa insignipes	Kieffer, 1908	27	2	59	9
Subfamily Prodiamesinae					
Prodiamesa olivacea	(Meigen, 1818)	34	9	9	3
Subfamily Orthocladiinae					
Acricotopus lucens	(Zetterstedt, 1850)	1	1	3	1
Brillia bifida	(Kieffer, 1909)	21	10	21	10
Brillia longifurca	Kieffer, 1921	4	4	10	3
Chaetocladius piger	(Goetghebuer, 1913)	6	1	0	0
Corynoneura sp.		10	6	4	2
Cricotopus annulator	Goetghebuer, 1927	2	1	1	1
Cricotopus bicinctus	(Meigen, 1818)	237	7	220	4
Cricotopus fuscus	(Kieffer, 1909)	6	3	0	0
Cricotopus intersectus	(Stæger, 1839)	123	7	147	9
Cricotopus similis	Goetghebuer, 1921	7	4	56	5
Epoicocladius ephemerae	(Kieffer, 1924)	82	10	3	3
Eudactylocladius fuscimanus	(Kieffer, 1908)	1	1	2	1
Eukiefferiella ancyla	Svensson, 1986	1	1	0	0
Eukiefferiella brevicalcar	(Kieffer, 1911)	0	0	4	1
Eukiefferiella claripennis	(Lundbeck, 1898)	13	5	111	5
Eukiefferiella cyanea	Thienemann, 1936	2	1	0	0
Eukiefferiella devonica	(Edwards, 1929)	0	0	2	2
Eukiefferiella gracei	(Edwards, 1929)	1	1	152	8
Eukiefferiella ilkleyensis	(Edwards, 1929)	14	1	145	4
Eukiefferiella minor	(Edwards, 1929)	9	3	24	8
Euorthocladius frigidus	(Zetterstedt, 1838)	1	1	3	3

Euorthocladius luteipes	Goetghebuer, 1938	1	1	0	0
Euorthocladius rivicola	Kieffer, 1911	21	6	531	7
Euorthocladius rivulorum	Kieffer, 1909	32	3	19	3
Heleniella serratosioi	Ringe, 1976	1	1		0
Limnophyes sp.		1	1	2	1
Mesopsectrocladius barbatipes	Kieffer, 1923	6	1	0	0
Metriocnemus sp.		1	1	0	0
Orthocladius oblidens	(Walker, 1856)	25	3	9	3
Orthocladius rhyacobius	Kieffer, 1911	303	16	273	17
Orthocladius rubicundus	(Meigen, 1818)	123	13	290	17
Parakiefferiella bathophila	(Kieffer, 1912)	0	0	1	1
Parametriocnemus stylatus	(Spärck, 1923)	22	7	10	5
Paratrichocladius rufiventris	(Meigen, 1830)	142	7	47	8
Paratrissocladius excerptus	(Walker, 1856)	96	14	41	8
Psectrocladius psilopterus	(Kieffer, 1926)	1	1	0	0
Rheocricotopus chalybeatus	(Edwards, 1929)	23	8	71	7
Rheocricotopus effusus	(Walker, 1856)	526	8	85	5
Rheocricotopus fuscipes	(Kieffer, 1909)	61	7	257	5
Synorthocladius semivirens	(Kieffer, 1909)	25	6	24	4
Thienemanniella sp.		1	1	2	2
Tvetenia calvescens	(Edwards, 1929)	128	11	733	22
Tvetenia sp. A	*sensu* Wiederholm, 1983	0	0	46	4
Subfamily Chironominae					
(tribe Chironomini)					
Chironomus riparius	Meigen, 1804	11147	11	4502	11
Dicrotendipes nervosus	(Stæger, 1839)	1	1	1	1
Micropsectra aristata	Pinder, 1976	52	7	291	4
Micropsectra atrofasciata	(Kieffer, 1911)	377	14	377	14
Micropsectra recurvata	Goetghebuer, 1928	92	10	78	8
Microtendipes pedellus	(De Geer, 1776)	40	5	67	4
Microtendipes rydalensis	(Edwards, 1929)	80	10	33	7
Paratendipes albimanus	(Meigen, 1818)	382	8	323	2
Phaenopsectra flavipes	(Meigen, 1818)	7	4	3	1
Polypedilum convictum	(Walker, 1856)	140	13	46	8
Polypedilum nubeculosum	(Meigen, 1804)	81	8	55	7
Polypedilum pedestre	(Meigen, 1830)	72	7	1	1
Polypedilum sordens	(van der Wulp, 1874)	23	7	4	2
Saetheria sp.		44	9	12	5
(tribe Tanytarsini)					
Cladotanytarsus atridorsum	Kieffer, 1924	22	5	3	2
Paratanytarsus sp.		24	5	68	9
Rheotanytarsus sp.		4	4	12	4
Tanytarsus sp.		87	12	149	11
Virgatanytarsus sp.		0	0	2	1