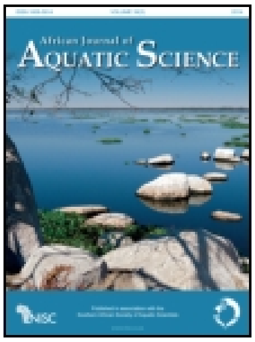

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Patterns of chironomid body-size distribution in an effluent-impacted river in the Eastern Cape, South Africa

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Body size is an important determinant of assemblage structure in rivers and streams impacted by elevated concentrations of pollutants such as salts and metals. In the present study, because of the larger surface-area-to-volume ratio of small-bodied chironomid species compared with large-bodied species, it was hypothesised that the relative abundance of the small-bodied species would decrease at the impacted sites by elevated concentrations of total dissolved solids compared with that at the less-impacted control site. The aim of this study was to analyse and compare patterns of chironomid final instar body-size classes at impacted sites with those at the control site. Chironomid larvae were sampled seasonally from August 2009 to September 2012 from one control site and three impacted sites (Sites 2, 3 and 4) in the Swartkops River, Eastern Cape. Site 2 was impacted by diffuse pollution sources, whereas Sites 3 and 4 were impacted by wastewater effluent discharges in addition to diffuse pollution sources. Small-bodied species dominated the assemblage at the control site and declined significantly at the impacted sites, suggesting that chironomid body size responds predictably to deteriorating water quality in the Swartkops River.

Keywords: aquatic pollution, biomonitoring, biological traits, Chironominae, habitat template, Orthocladiinae, Swartkops River, Tanypodinae

Introduction

The habitat template concept, the fundamental theory underpinning the use of species traits in freshwater biomonitoring, provides the basis for predicting species traits in relation to human disturbances in the aquatic environment (Southwood 1977, 1988; Townsend and Hildrew 1994; Menezes et al. 2010). The use of species traits in biomonitoring is based on the premise that habitat constraints select for specific traits and eliminate others so that there is a correspondence between the habitat and the traits (Townsend and Hildrew 1994; Poff 1997). Thus, species survive and thrive only in environments that meet their biological and ecological requirements. Therefore, the use of species traits in biomonitoring provides an indication of species ecological function and, if the traits are carefully selected, they can be used to link biotic responses to environmental conditions (Statzner and Beche 2010; van den Brink et al. 2011).

A biological trait such as body size is important because it constrains several other traits related to reproduction, adult lifespan, locomotion and metabolic rate (Robson et al. 2005). It is also regarded as an 'integrative taxon-free' attribute that can provide clues to understanding species interaction with their environments (Blanckenhorn 2000; Robson et al. 2005; Siqueira et al. 2008). Body size is often included amongst traits used to assess pollution impacts in freshwater ecosystems (Dolédec and Statzner 2008; Tomanova et al. 2008). Five hypotheses, energetic, phylogenetic, bio-geographical, community interaction and textural discontinuity, have been postulated to explain factors responsible for shaping animal body-size distribution (Allen et al. 2006). Of these, textural discontinuity, which views local habitat characteristics, including physico-chemical conditions, as the main drivers of animal body-size distributions (Stead et al. 2005; Allen et al. 2006), is the most relevant for relating patterns of chironomid body-size distribution to pollution at the scale of the present study.

Animal body size is a critical adaptive trait because it can influence exposure to and absorption of dissolved pollutants such as salts and metals in the external environment due to its relationship with body surface-area-to-volume ratio (Statzner and Béche 2010). Therefore, freshwater pollution resulting in elevated concentrations of dissolved pollutants such as salts could influence patterns of animal body-size distribution. Aquatic macroinvertebrates are exposed to dissolved pollutants through external contact and food ingestion. Thus, in rivers with elevated dissolved salts and other toxicants such as metals, a body size that reduces the body surface-area-to-volume ratio would be favoured (Dolédec and Statzner 2008; Statzner and Béche 2010).

The Swartkops River, where the present study was undertaken, is located in an urbanised and industrialised catchment in the Eastern Cape, South Africa, and receives both wastewater effluent and other non-point discharges leading to elevated concentrations of total dissolved solids (TDS) in the downstream sections of the river (Odume et al. 2012). Consequently, it was hypothesised that, because of the larger surface-area-to-volume ratio of small-bodied chironomid species, their relative abundances would be higher at the upstream control site than at the downstream effluent-impacted sites.

Therefore, the aim of this study was to test this hypothesis by analysing and comparing patterns of chironomid final instar larvae body-size classes at the impacted sites with those at the less-impacted upstream control site. Chironomids were selected for this study because of their high species richness, representing several body-size spectrums, their extraordinary ecological range, and their physiological and behavioural adaptations, such as the possession of haemoglobin and of tube-building in some species, which enable them to inhabit diverse environments with different levels of perturbation (Harrison 2003; Ferrington 2008).

Materials and methods

Study area and sampling sites

The Swartkops River, in the Eastern Cape province of South Africa, has a catchment size of about 1 555 km^2 and the industrial town of Uitenhage and residential towns of Despatch and Perseverance are all within the catchment. The river originates in the foothills of the Groot Winterhoek Mountains, meandering across shale-filled synclines in the upper catchment, and largely gravel-and-stone flood plains of poorly consolidated rocks of the Uitenhage Group in the lower catchment, before discharging into the Indian Ocean at Algoa Bay (Figure 1) (Fromme 1988). The river is an important ecological and socio-cultural asset, which supports a permanently open estuary that has the third largest intertidal salt mash of South African estuaries (Enviro-Fish Africa 2011). The estuary serves as breeding habitats for many bird species (Enviro-Fish Africa 2011). Resources within the Swartkops River catchment are utilised for several socio-cultural purposes, including baptism, spiritual rituals and cleansing, as well as medicinal plant harvesting. However, several sources of pollution, including wastewater effluent discharges, runoffs from informal settlements, farms, surrounding road and rail networks, and industrial sites, severely influence the water quality of the river (Odume and Muller 2011; Odume et al. 2012).

Four sites with similar physical habitat characteristics were selected for sampling over a three-year period between August 2009 and September 2012. Site 1 (33°45′08.4″ S, 25°20′32.6″ E), in the upstream section of the river, was selected as the control site. It was less influenced by residential, industrial and intense agricultural activities. Site 2 (33°47′29.0″ S, 25°24′26.4″ E) and Site 3 (33°47′11.8″ S, 25°25′53.97″ E) were both in the industrial town of Uitenhage, while Site 4 (33°47′34.0″ S, 25°27′58.7″ E) was in the residential town of Despatch. Site 2 was mainly impacted by diffuse sources of pollution, including runoff from surrounding road networks. Site 3 received point source pollution from a wastewater effluent treatment work in Uitenhage. In addition, Site 3 was also impacted by runoff from surrounding informal settlements, road and rail networks. Site 4 was about 2.5 km further downstream of Site 3 and the impact of the effluent still indicated by physico-chemical variables and the benthic community structure (Odume et al. 2012). A further downstream site could not be selected because the tidal limit between the estuary and the freshwater section of the river is only a short distance downstream of Site 4.

Chironomid sampling, body-size and physico-chemical measurements

Chironomid larvae were collected at each of the four sampling sites in spring and summer 2009, autumn and winter 2010, spring and summer 2011, and in autumn and spring 2012, using the South African Scoring System version 5 (SASS5) protocol (Dickens and Graham 2002). Collected chironomid larvae were preserved in 70% ethanol, transported to the laboratory for sorting, mounting, body length measurements and species identification. Although a SASS5 net, mesh size 1 mm, may not be suitable for collecting very small-bodied chironomid species, it was still used in this study, because the SASS5 protocol is the standardised method for macroinvertebrate sampling in South African streams and rivers (Dickens and Graham 2002). The procedure described in Odume and Muller (2011) was followed during the mounting of chironomid larvae. Species were identified using the keys described by Wiederholm (1983) and Harrison (2003). The total body lengths (TL) of 20–30 individuals of the most abundant chironomid species at each sampling site per sampling season were measured under a dissecting Wild M5 microscope with calibrated eyepiece graticule. Measurements were undertaken for only the third and fourth instar larvae. Total body length (TL) measurements were undertaken for all third and fourth instar larvae of the less abundant species. Length measurements based on the ventral view of the headcapsule and mentum were used to determine larval instar stages in this study. Similar measurements have been used to determine chironomid larval instar stages in other studies (e.g. Ford 1959; Frouz et al. 2002; Richardi et al. 2013). Voucher specimens of chrionomids on slides will be lodged in the Albany Museum, Grahamstown, for which catalogue numbers ZWK 121–217 have been assigned. In some instances, a slide contains more than one species.

Concurrent with chironomid sampling, physico-chemical variables were measured at the four sampling sites. On site, subsurface, mid-channel dissolved oxygen (DO), electrical conductivity (EC), turbidity, temperature and pH were measured using Cyberscan DO 300, Cyberscan Con 300, Orbeco-Hellige 966, mercury-in-glass thermometer and Cyberscan pH 300, respectively. Concentrations of total dissolved solids (TDS; mg l^{-1}) were obtained by multiplying EC values (mS m^{-1}) by a factor of 6.6 (Dallas and Day 2004).

Water samples for metal analysis were collected in spring and summer 2011, and autumn and spring 2012, and transported to InnoVenton Analytical laboratory in Port Elizabeth where they were analysed for zinc (Zn), manganese (Mn), copper (Cu), lead (Pb) and chromium (VI) (Cr^{6+}). Apart from Cr^{6+}, metals were analysed using inductively coupled plasma spectrometry according to Martin et al. (1994) EPA method number 200.7. Chromium (VI) was analysed using Merck chromate test kit according to the manufacturer's instructions.

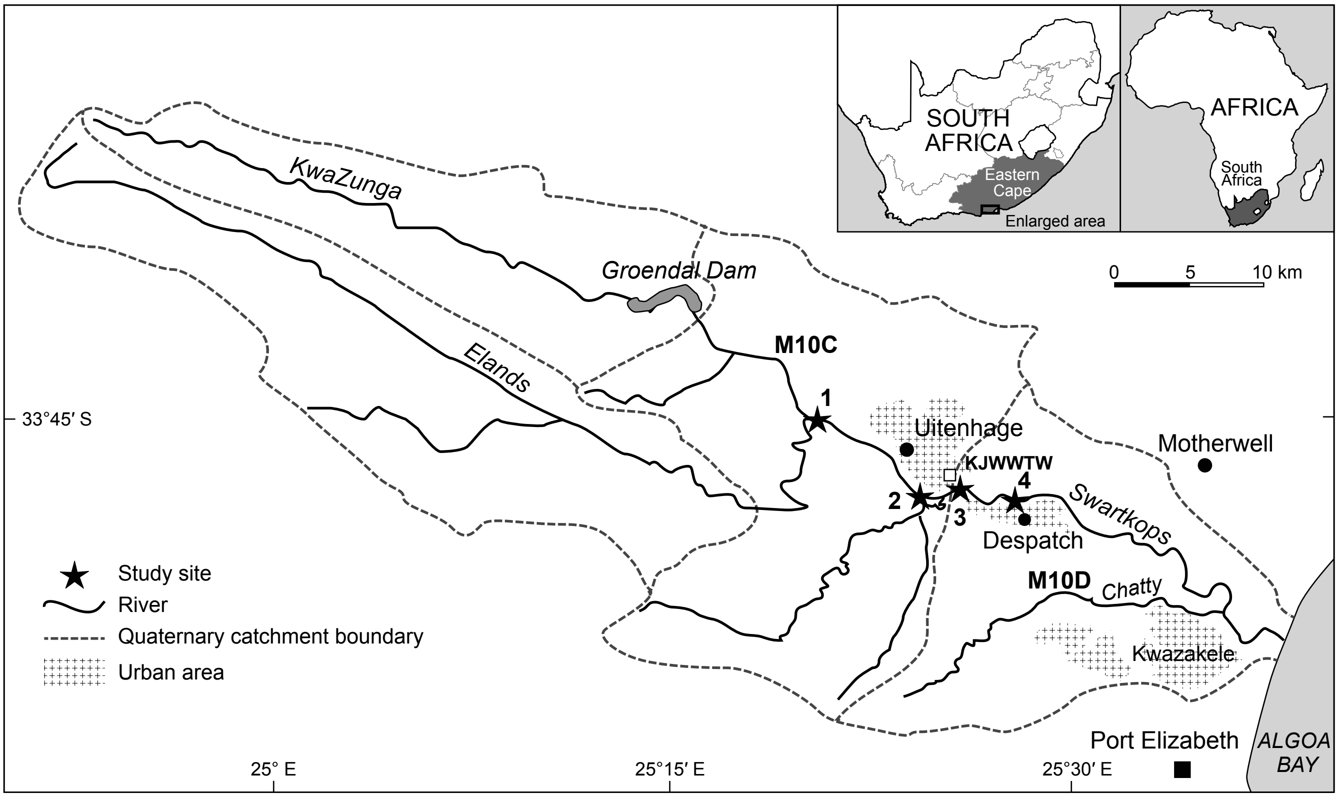

Figure 1: Map of the Swartkops River showing locations of the four sampling sites, Kelvin Jones Wastewater Treatment Works (KJWWTW) and major towns

Statistical and data analysis

All body-size measurements during the study period were used to produce a kernel curve (Havlicek and Carpenter 2001) fitted onto a histogram of size classes in mm. Body-size classes were then defined based on the modes and troughs of the kernel curve. The kernel curve has previously been used to analyse animal body-size distribution in freshwater ecosystems (Havlicek and Carpenter 2001). The kernel curve was computed using Paleontological Statistics (PAST) software version 2.17 (Hammer 2012). The chironomid assemblage was analysed in terms of body size by calculating the relative abundance of individuals belonging to each of the defined body-size classes obtained from the kernel curve. The body-size classes were compared between the sampling sites using the Kruskal–Wallis multiple comparison test (Statistica software package version 9).

Prior to using analysis of variance (ANOVA), the basic assumptions of normality and homogeneity of variance were tested using the Shapiro–Wilk and the Levene's tests, respectively. When it appeared that the assumptions were violated, data were logarithmically transformed. One-way ANOVA was used to compare the concentrations of the physico-chemical variables between the sampling sites ($p < 0.05$). If a significant difference was indicated by ANOVA, then the *post hoc* Tukey's honest significant different (HSD) test was used to indicate the sites that differed. ANOVA and related statistics were computed using Statistica software package version 9.

Results

Physico-chemical variables

The mean, standard deviation (except for pH) and range of each of the measured physico-chemical variables at the four sampling sites during the study period are shown in Table 1. With the exception of pH and temperature, one-way ANOVA indicated that the mean concentrations of the measured variables differed significantly between the sampling sites ($p < 0.05$). The highest mean dissolved oxygen (DO) concentration was recorded at Site 2 and the lowest at Site 3. Dissolved oxygen (DO) concentrations were significantly lower at Site 3 compared with those at Sites 1 and 2 ($p < 0.05$). The overall highest DO concentration of 9.48 mg l^{-1} was recorded at Site 2 and the lowest concentration of 0.9 mg l^{-1} was recorded at Site 4. Generally, DO concentrations were mostly higher at Site 2 during the study period and in most of the sampling events lowest at Site 3. The pH values at the four sampling sites indicated minimal departure from the neutral value of 7. Sites 2, 3 and 4 were slightly alkaline and Site 1 was slightly acidic.

Mean EC values were higher at Sites 3 and 4 than at Sites 1 and 2. The mean EC value at Site 1 was significantly lower compared with those at Sites 2, 3 and 4. Turbidity values were consistently higher at Site 3 throughout the study period. The Tukey's HSD *post hoc* test indicated that turbidity values at Sites 1, 2 and 4 were significantly lower than the values recorded at Site 3 ($p < 0.05$).

Concentrations of analysed metals

The concentrations of the analysed metals were generally low (Table 2). The concentrations of Cr^{6+} and Cu were not significantly different between the four sampling sites ($p > 0.05$). The values of Pb, Zn and Mn were significantly different between the sampling sites ($p < 0.05$). The mean concentrations of Zn and Mn at Sites 1 and 3 were significantly higher than those at Site 2. The mean concentrations of Pb were also significantly lower at Site 2 than at Sites 1 and 4.

Patterns of chironomid body-size distribution

Thirty-five chironomid species in three subfamilies, Chironominae, Orthocladiinae and Tanypodinae, were recorded during the study period (Appendix). The TL of all the measured individuals (3rd–4th instars) were used to produce a kernel curve distribution (Figure 2). The overall body-size distribution of the Swartkops River chironomids was multimodal, with three modes (Figure 2). Based on the kernel curve, the chironomids were divided into five body-size classes A–E, corresponding to the modes and troughs of the curve: A (<3–7 mm), B (>7–9 mm), C (> 9–12 mm), D (>12–16 mm), and E (>16 mm).

The species of Orthocladiinae were mostly small- and medium-bodied chironomids belonging to Classes A (<3–7 mm) and B (>7–9 mm). However, species such as *Cricotopus* sp.1, *Cricotopus trifasciata* gr. (Edwards 1929) and *Cardiocladius* sp. had relatively large individuals belonging to Class C (>9–12 mm) (Figure 3).

Of the eight species belonging to the subfamily Chironominae whose body length were measured, individuals belonging to species in the tribe Chironomini were mostly medium- and very large-bodied chironomids in Classes C (>9–12 mm), D (>12–16 mm) and E (>16 mm) (Figure 4). Individuals of the species in the tribe Tanytarsini, subfamily Chironominae, were of small body sizes belonging mostly to Class A (<3–7 mm). These species include *Tanytarsus* sp., *Rheotanytarsus* sp., *Virgatanytarsus* sp. and *Paratanytarsus* sp. (Figure 4). Species belonging to the predatory Tanypodinae were mostly medium- and large-bodied chironomids in Classes B (>7–9 mm) and C. However, the larvae of *Coelotanypus* sp. and *Thienemannimyia* sp. were small-bodied, belonging to Class A (Figure 5).

The relative abundance of species at Site 1 was dominated by the small-bodied chironomids belonging to Class A (<3–7 mm) (Figure 6). The relative abundance of species in Class A was higher at Site 1 and declined significantly at Sites 2, 3 and 4 ($p < 0.05$). Between the three downstream sites, the relative abundance of the

Table 1: Means ± SD (except for pH) and ranges (in parentheses) of physico-chemical variables ($n = 8$) at sampling sites in the Swartkops River in August 2009–September 2012. ANOVA *p*- and *F*-values are indicated. Different superscripts per variable across sites indicate significant differences ($p < 0.05$) (Tukey HSD *post hoc* test); identical superscripts between sites per variable indicate no significant differences ($p > 0.05$)

Variable	Site 1	Site 2	Site 3	Site 4	*p*-value	*F*-value
Dissolved oxygen (mg l^{-1})	6.99 ± 1.15^a (4.73–9.5)	7.4 ± 1.52^a (5.53–9.48)	3.19 ± 1.47^b (1.81–6.36)	4.81 ± 3.01^{ab} (0.9–8.31)	0.001	7.18
pH	(4.69–7.75)	(5.69–8.99)	(6.56–7.9)	(6.31–8.01)		
Temperature (°C)	17.48 ± 5.46 (7.31–24.0)	17.27 ± 7.17 (6.11–27.3)	20.88 ± 3.29 (14.3–25.2)	18.9 ± 4.14 (12.2–24.0)	0.415	0.98
Electrical conductivity (mS m^{-1})	32.45 ± 17.74^a (8.23–62.0)	160.75 ± 146^b (30–460)	262.51 ± 76.14^b (154.8–333)	259.63 ± 56.28^b (171–354)	0.000	22.57
Total dissolved solids (mg l^{-1})	214 ± 17.74^a (54.3–409.2)	1 061 ± 967^b (198–3 036)	1 732.6 ± 102.4^b (1 022–2 198)	1 713.5 ± 371^b (1 129–2 236)	0.000	12.24
Turbidity (NTU)	5.3 ± 2.22^a (3.0–10.1)	6.33 ± 2.44^a (3.0–11.2)	72.7 ± 102.36^b (10.5–320)	7.08 ± 8.06^a (2.2–26)	0.000	15.67

Table 2: Means ± SD and ranges (in parentheses) of metal concentrations (mg l^{-1}; $n = 4$) in the Swartkops River in August 2009–September 2012. ANOVA *p*- and *F*-values are indicated. Different superscripts per variable across sites indicate significant differences (Tukey HSD *post hoc* test); identical superscripts between sites per variable indicate no significant differences ($p > 0.05$)

Variable	Site 1	Site 2	Site 3	Site 4	*p*-value	*F*-value
Chromium (VI) (Cr^{6+})	0.035 ± 0.024 (0.02–0.07)	0.041 ± 0.021 (0.025–0.07)	0.032 ± 0.01 (0.025–0.048)	0.032 ± 0.001 (0.025–0.046)	0.243	1.475
Copper (Cu)	0.004 ± 0.002 (0.002–0.006)	0.006 ± 0.002 (0.003–0.008)	0.007 ± 0.001 (0.006–0.009)	0.006 ± 0.001 (0.005–0.007)	0.065	2.947
Lead (Pb)	0.005 ± 0.003^b (0.001–0.008)	0.002 ± 0.001^a (0.001–0.003)	0.003 ± 0.001^{ab} (0.002–0.004)	0.005 ± 0.005^b (0.001–0.012)	0.006	5.050
Zinc (Zn)	0.016 ± 0.001^a (0.015–0.016)	0.003 ± 0.002^b (0.002–0.006)	0.011 ± 0.004^c (0.004–0.016)	0.004 ± 0.00^b (0.004–0.004)	0.000	77.96
Manganese (Mn)	0.102 ± 0.08^{ab} (0.039–0.216)	0.06 ± 0.09^a (0.001–0.194)	0.144 ± 0.016^b (0.128–0.166)	0.068 ± 0.046^a (0.015–0.126)	0.007	4.906

small-bodied chironomids in Class A was lowest at Site 3 and highest at Site 2 (Figure 6).

The relative abundance of species in Class B (>7–9 mm) were also higher at Site 1 and declined significantly at Site 3 ($p < 0.05$) (Figure 6). The large- and very large-bodied chironomid species in Classes D (>12–16 mm) and E (>16 mm) dominated the relative abundances of species at Sites 3 and 4. Chironomid species in Class C (>9–12 mm) dominated the species relative abundance at Site 2. The relative abundance of species in Class C was significantly lower Sites 1 and 3 compared with Site 2. The relative abundances of species in Classes D and E were significantly higher at Sites 3 and 4 compared with Site 1 ($p < 0.05$). At Site 1, species in Classes D and E contributed the least to total species. Generally, the abundance of small-bodied species dominated the assemblage at Site 1 and declined significantly at the downstream sites, suggesting that chironomid body size responded predictably to deteriorating water quality in the Swartkops River (Figure 6).

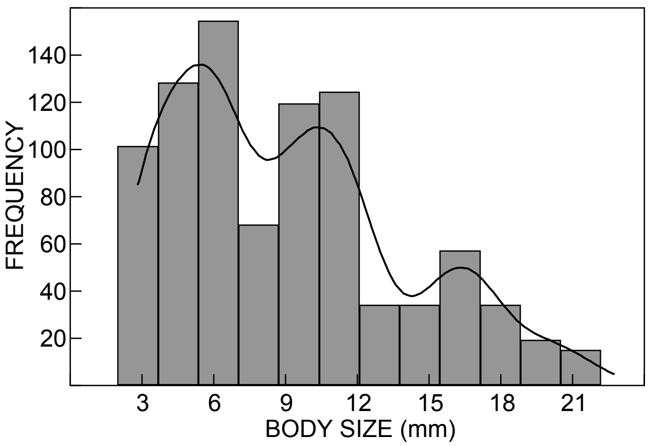

Figure 2: Multimodal histogram with fitted kernel curve of chironomid larval body lengths, in 2 mm intervals, from the Swartkops River in August 2009–September 2012

Discussion

Physico-chemical variables

Physico-chemical variables are important factors capable of influencing the biological assemblages of freshwater ecosystems (Sundermann et al. 2013). The concentrations of the measured physico-chemical variables indicated evidence of deteriorating water quality in the Swartkops River. The elevated turbidity, EC and TDS values, and the relatively low DO concentrations recorded at the downstream sites, particularly at Sites 3 and 4, which were downstream of the discharge point of the WWTW, were indicative of deteriorating water quality in the Swartkops River. A single once-off overall lowest DO concentration was recorded at Site 4 but, in general, the lowest DO values during most of the sampling events were recorded at Site 3. This explained why DO was not significantly different between Sites 2 and 4. Electrical conductivity and TDS are important variables that can shape pattern of body-size distribution of aquatic macroinvertebrates because of their effects on osmotic balance. Organisms may therefore evolve mechanisms including large body size to minimise external exposure to dissolved salts through reduced body surface-area-to-volume ratio. Thus, the elevated EC and TDS concentrations at the downstream sites may have contributed to the observed low relative abundance of the small-bodied chironomid species at Sites 2, 3 and 4 with compared with the control site. The observed relatively high EC and TDS concentrations at the three downstream sites

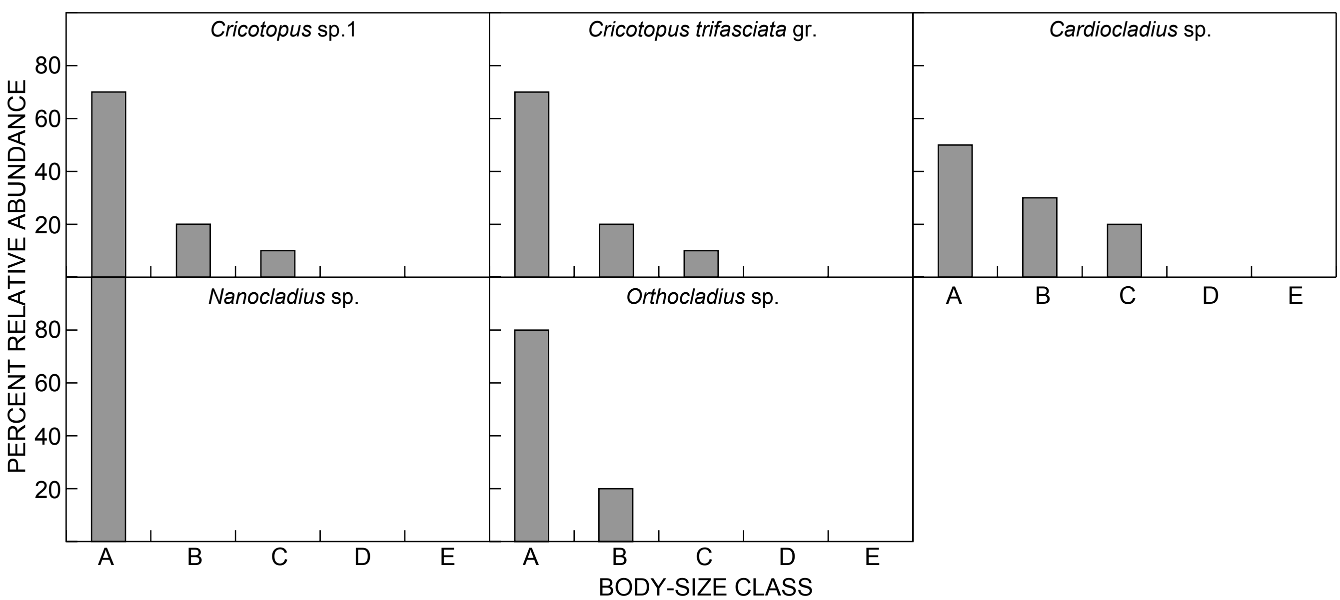

Figure 3: Body-size distributions of final instar larvae of the five most abundant orthocladiine chironomid species collected in the Swartkops River between August 2009 and September 2012. Body-size classes: A = <3–7 mm, B = >7–9 mm, C = >9–12 mm, D = >12–16 mm, E = >16 mm

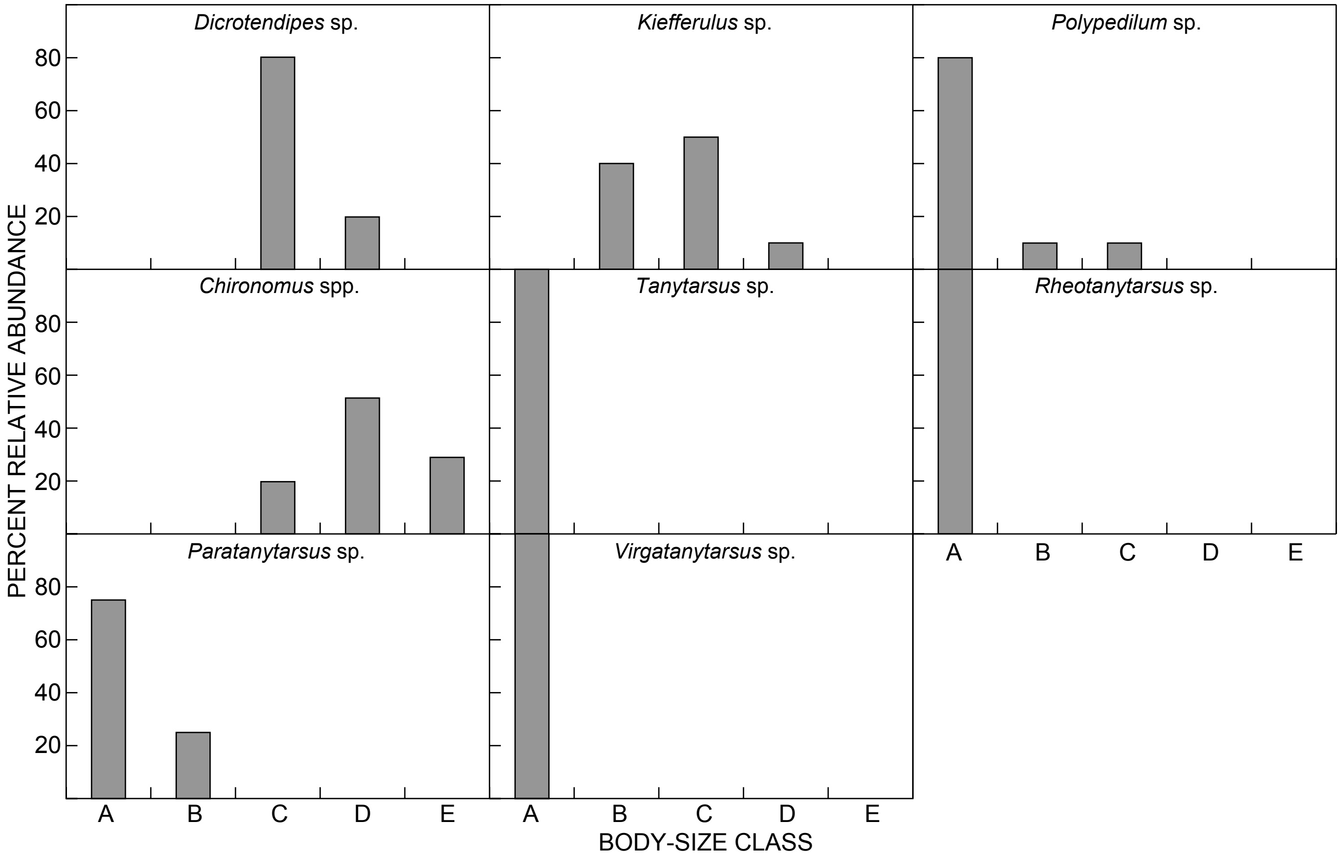

Figure 4: Body-size distributions of final instar larvae of the eight most abundant chironomine chironomid species collected in the Swartkops River between August 2009 and September 2012. Body-size classes A = <3–7 mm, B = >7–9 mm, C = > 9–12 mm, D = >12–16 mm, E = >16 mm

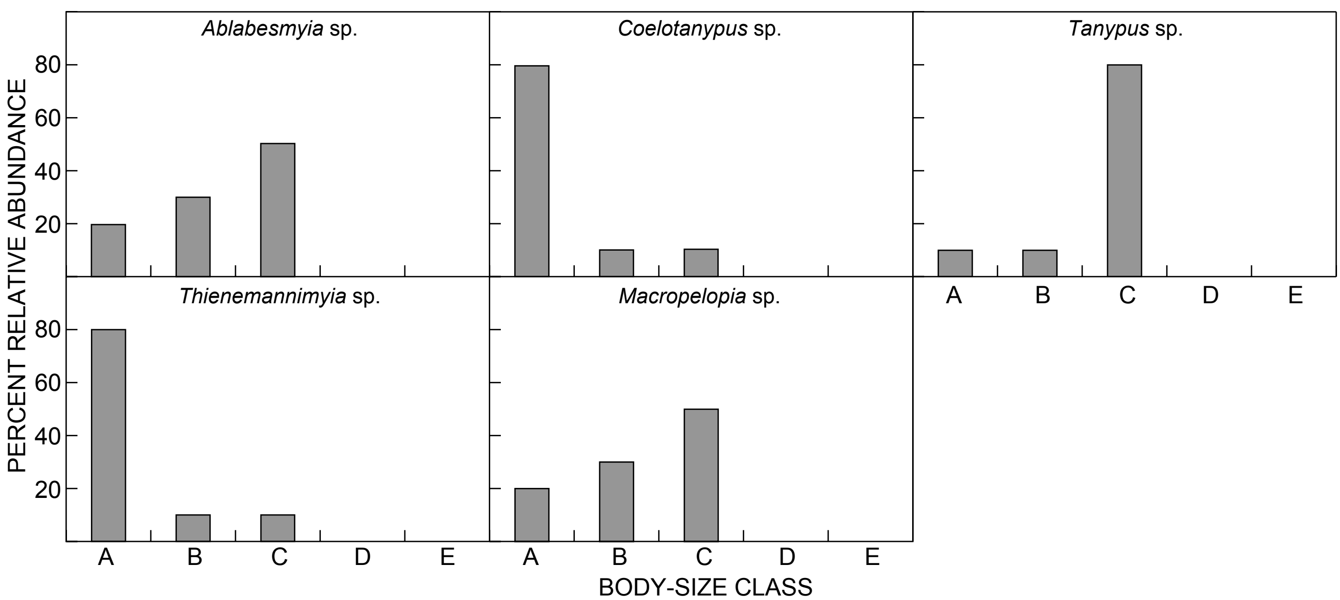

Figure 5: Body-size distributions of final instar larvae of the five most abundant tanypodine chironomid species collected in the Swartkops River between August 2009 and September 2012. Body-size classes A = <3–7 mm, B = >7–9 mm, C = > 9–12 mm, D = >12–16 mm, E = >16 mm

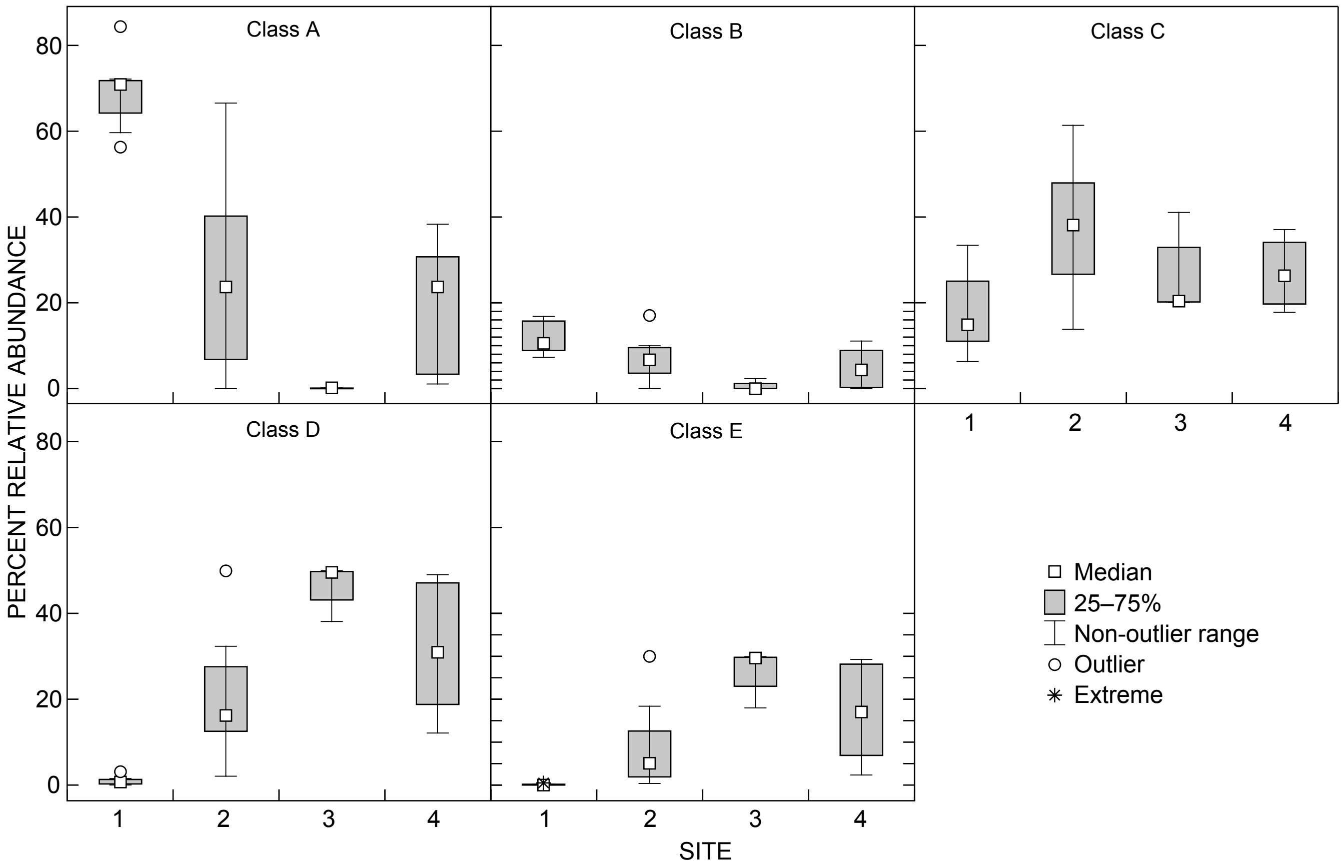

Figure 6: Percentage relative abundances of chironomid species per body-size class of chironomid assemblages per sampling site in the Swartkops River in August 2009–September 2012. Body-size classes A = <3–7 mm, B = >7–9 mm, C = > 9–12 mm, D = >12–16 mm, E = >16 mm

could be attributed to discharges from WWTW, runoff from surrounding rail and road networks and the natural geological formations of the Swartkops River, which are of marine origin (DWAF 1996a).

Although elevated concentrations of metals have previously been reported in the sediment of the Swartkops River (Binning and Baird 2001), the results in the present study indicated low concentrations of metals in the water column. The concentrations of the analysed metals were in ranges that could cause chronic rather than acute effects in aquatic biota (DWAF 1996b). The South African water quality guidelines for aquatic ecosystems set limits above which certain metals may become either chronically or acutely toxic (DWAF 1996b). Of the measured metals in this study, only the concentrations of Mn were consistently within the target water quality range (TWQR) (i.e. range in which no measurable effects were expected). Apart from Site 2, where the mean value of Zn was within the TWQR, the mean values of Cr^{6+}, Pb, Zn and Mn were in ranges that could potentially be chronically toxic to biota. Since most chironomid species live in close association with the bottom sediments, which act as a sink for most metals and accumulates them to levels several-fold higher than the concentrations in the water column (Beasley and Kneale 2002), concentrations of metals in sediments could influence chironomid body-size distribution. In addition, sediment-bound metals may become mobilised due to changes in pH.

Pattern of chironomid body-size distribution

In this study, it was hypothesised that the relative abundance of the small-bodied chironomid species would be lower at the downstream sites with elevated EC and TDS values compared with the upstream control site. Chironomid body size responded as hypothesised; the relative abundance of species in Classes A and B were higher at Site 1 and declined significantly at Sites 2, 3 and 4 (Figure 6). Conversely, the large- and very large-bodied-species in Classes D and E dominated the relative abundance of species at Sites 3 and 4 compared with Site 1. Theoretically, the habitat template concept predicts small body size to be favoured by human disturbances because it is a trait perceived to confer resilience on biological assemblages (Townsend and Hildrew 1994). However, in the present study, the relative abundances of the large-bodied chironomid species were higher at the polluted sites compared with the less-polluted upstream site. The nature of disturbance characterising the Swartkops River leading to increased concentration of TDS is critical to small-bodied individuals because of their large surface-area-to-volume ratio. Statzner and Béche (2010) postulated that elevated dissolved salts were likely to act against small-bodied invertebrates because of their large surface-area-to-volume ratio. The results in this study are in agreement with the Statzner and Béche (2010) postulation.

A multimodal body-size distribution was observed for the Swartkops River chironomid species (Figure 2). The observed distribution in this study is consistent with the findings of Havlicek and Carpenter (2001) who found multiple gaps (troughs) and lumps (modes) within phytoplankton, zooplankton and fish community functional groups. Holling (1992) had attributed the existence of multiple modes and troughs in body-size distribution to adaptation of species to the habitat, though individuals within a species would show different tolerances. The observed distribution in the present study can be attributed to environmental factors including physico-chemical conditions favouring specific body-size classes corresponding to the modes and negatively influencing other class sizes, corresponding to the troughs. The textural discontinuity hypothesis (Allen et al. 2006) viewed the habitat conditions, including water physico-chemical conditions, as the main drivers of animal body-size distributions and many studies have given empirical evidence to support this claim (e.g. de Bruyn et al. 2002; Stead et al. 2005). For example, de Bruyn et al. (2002) related fish body-size distribution to organic enrichment and found that the abundance of large-bodied fish species increased with increased levels of organic pollution. In the present study, the observed differences in chironomid body-size classes between the sampling sites suggest that changes in water physico-chemical conditions between the sites influence the body-size distributions of the Swartkops River chironomid assemblage. Although the hypothesis stated in this study was accepted for the Swartkops River chironomid assemblage, it needs further investigation in other river systems receiving similar water quality impacts to explore whether similar patterns would emerge. Furthermore, the observed responses in this study do not necessarily apply across all macroinvertebrate taxa and therefore the phylogenetically diverse assemblage of the family Chironomidae was informative, enabling the use of body size as a potential biomonitoring tool.

Most of the large-bodied species that were dominant at the polluted sites belong to the subfamily Chironominae, tribe Chironomini (Figure 4). Apart from their large body sizes, they possess physiological and behavioural traits such as haemoglobin formation and tube-building behaviour that enable them to tolerate high levels of aquatic pollution (Van Kleef 2010). For example, chironomid tubes built either with sand or silt have been found to protect the inhabitants against dissolved toxic substances (Halpern et al. 2002). Therefore, although the results presented in this study suggest that differences in water physico-chemical conditions between the sites influenced the chironomid body-size distributions in the Swartkops River, it is important to note that body size is not the only trait that could potentially shape the distribution of chironomids in relation to pollution (Franquet 1999; Van Kleef 2010). Other traits, including gill size, food and feeding habits, tube building and haemoglobin could play an important role in shaping chironomid responses to pollution. For example, under experimental conditions McLachlan (1976) found that individuals of *Chironomus transvaalensis* responded to increased dissolved salts by reducing anal gill size and increasing the size of the ventral tubuli.

Conclusion

The observed patterns revealed that body size is an important morphological trait that can be linked to freshwater pollution. However, more field and laboratory studies are needed to link body size of chironomid species to specific pollutants. In addition, combining physiological and behavioural traits with body size in future studies could provide better insights into chironomid traits-based response to pollution.

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Appendix: List of chironomid species identified from the Swartkops River in August 2009–September 2012, and their body length size classes: class A = <3–7 mm, B = >7–9 mm, C = >9–12 mm, D = >12–16 mm, E = >16 mm

Taxon	Size class	Taxon	Size class
Subfamily Orthocladiinae		Subfamily Chironominae (cont.)	
Cricotopus sp.1	ABC	Tribe Chironomini (cont.)	
Cricotopus trifasciata gr.	ABC	*Glyptotendipes* sp.	CD
Paratrichocladius sp.	ABC	*Dicrotendipes* sp.2	CD
Nanocladius sp.	A	Tribe Tanytarsini	
Eukiefferiella sp.	A	*Tanytarsus* sp.	A
Cardiocladius sp.	ABC	*Rheotanytarsus* sp.	A
Parakiefferiella sp.	A	*Cladotanytarsus* sp.	A
Orthocladius sp.	AB	*Virgatanytarsus* sp.	A
Orthocladius sp.2	AB	*Paratanytarsus* sp.	AB
Subfamily Chironominae		Subfamily Tanypodinae	
Tribe Chironomini		*Ablabesmyia* sp.	ABC
Dicrotendipes sp.	CD	*Coelotanypus* sp.	ABC
Kiefferulus sp.	BCD	*Procladius* sp.	ABC
Polypedilum sp.	ABC	*Trissopelopia* sp.	AB
Cryptochironomus sp.	C	*Clinotanypus* sp.	BC
Chironomus sp. 1	CDE	*Tanypus* sp.	ABC
Chironomus sp. 2	CDE	*Nilotanypus* sp.	A
Chironomus sp. 3	CDE	*Thienemannimyia* sp.	ABC
Microchironomus sp.	A	*Macropelopia* sp.	ABC
Polypedilum sp.2	ABC	*Conchapelopia* sp.	A