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FIELD EXPERIMENT REVEALS NO RELATION BETWEEN SUBSTRATE COMPOSITION AND *CHIRONOMUS* ABUNDANCE

ABSTRACT: In the field experiment no relation between organic matter content (0–83%) in substrates provided and *Chironomus plumosus* (L.) abundance was found. Numbers of larvae and their age structure were usually similar on the mineral substrate and various bottom sediments but sometimes differed up to 2–3 times (at the same site and date of sampling); there was however no correlation with the quality of substrate. This indicates that the feeding of *Chironomus plumosus* did not depend on the bottom deposit the larvae lived in, but mostly on sedimenting tripton, which formed a thin layer on the top of substrates provided. Also mutual relations of individuals and the period of exposure of the sediment (from 3 weeks to few months) did not matter. Very high abundance of *Ch. plumosus* (up to 90 thousands ind. m^{-2}) found already after 3 weeks exposition in the sediment initially without that species indicates a very high growth potential of the *Chironomus* population at the study site.

KEY WORDS: *Chironomus*, substrate quality, intra-population relations

1. INTRODUCTION

The variation of *Chironomus* abundance and of the pattern of its dynamics in various environments is enormous and the role of sediment for it is unclear. Many authors stress the high flexibility of this species (review of papers – Brinkhurst 1974, Sokolova 1983, Resh and Rosenberg 1984, Kajak 1987, Armitage *et al.* 1995). So far no studies on these topic have been carried out in the field conditions.

The main goal of this paper was to compare *Chironomus* populations (total numbers as well as numbers of size classes) on substrates differing in organic matter contents (the mineral, artificial substrate and bottom sediments), in the same study site, under natural conditions – in the field experiment. We also wanted to check the importance of several other factors as the exposure period and the term of the experiment, the thickness of the substrate layer and the relations between larvae.

As the study site a lowland, eutrophic shallow reservoir was selected. The *Chironomus plumosus* population has been investigated there for several years (Dusoge 1989, Kajak and Dusoge 1996, Kajak 1997) and the general pattern of its dynamics (with two peaks of abundance – in the spring and late summer) was found to be similar in each year, despite great differences of larval abundance.

Much higher abundance of *Chironomus* larvae have been found in previous studies (Kajak and Dusoge 1996, Kajak 1997) in the sediment located in plastic containers (30 × 30 × 12 cm) installed 0.4–1.2 m above the bottom than in the bottom itself. It was most probably due to better oxygen conditions above the bottom than at the bottom surface. This is why in that work we located containers with different substrates 0.4–0.8 m above the bottom. It made possible the comparison of the abundance and the development of larvae on different substrates in favourable conditions and eliminated the possible influence of oxygen deficits, which can occur at the bottom of the reservoir.

2. STUDY AREA

The study site was located in the lowland eutrophic dam reservoir Zegrzyński (30 km from Warsaw), supplied by rivers Narew and Bug. A point of a slight water flow (up to few centimetres s^{-1} at the medium and low water flow), with a depth of 5.5–6.0 m, in the middle, broad part (2 × 4 km) of the reservoir, some 200 m from the closest shore was choosen. The bottom was flat, muddy, the organic matter content in the deposit was about 9.0%, SiO_2 – 36.0, Ca – 16.0, Mg – 2.1, Fe – 0.6, P – 0.2, N – 0.6, C – 4.4% (Kajak and Dusoge 1989, Kajak 1990a, b). The average P_{tot} concentration in the water during the vegetation season was 150–200 $\mu g\ l^{-1}$, N_{tot} – 2.7 mg l^{-1}, Secchi disc visibility 0.6–0.7 m. In the inflowing rivers the chlorophyll concentration was in 1997 up to 120 $\mu g\ l^{-1}$, BOD_5 – 15.0, oxidability $KMnO_4$ – 18.0 mg $O_2\ l^{-1}$, COD – $K_2Cr_2O_7$ – 57.0 mg $O_2\ l^{-1}$ and C_{org} up to 18 mg l^{-1}. Small diatoms (mainly *Cyclotella meneghiniana* Kütz. and *Stephanodiscus hantschii* Grün. and green algae (in summer) were dominant in the phytoplankton (Bubień 1989, Simm 1990). About 50% of the phytoplankton biomass sedimented, mainly in the part of the reservoir the study was carried out (Bubień 1989). The sedimenting tripton was a bit richer than bottom deposits in the study site: accordingly organic matter 13.6 and about 9–10, P_{tot} – 0.34 and 0.23, N_{tot} – 0.72 and 0.58%.

The benthos in the reservoir was very abundant, the biomass reaching sometimes 1000 g m^{-2}. *Chironomus* abundance in containers with the sediment above the bottom was as a rule, during the whole season, several times higher (occasionally reaching 90 thousands ind. m^{-2}) than in the bottom, where the highest numbers reached were 30 thousands ind. m^{-2}, during a short period only, in the spring (Kajak and Dusoge 1996, Kajak 1997). The proportion of the *Chironomus* (the main dominant) in the total benthos biomass at the study site was on average about 80%, Tubificidae – *Pothamothrix hammoniensis* (Mich.), *Limnodrillus hoffmeisteri* (Clap.), *Tubifex tubifex* (O. F. Mill.) – 20%. Other chironomids and *Pisidium* sp. were sporadic (Dusoge *et al.* 1985, Dusoge 1989, Kuklińska 1989, Kajak 1990a, b).

3. METHODS

Square plastic containers (30 × 30 cm and 12.5 cm deep) were filled with substrates to 0.5–1.0 cm below the upper edge of the container. They were installed by a diver in 3 metal stands, 80 cm high, 4 containers in each stand in 1997. In 1993 (a pilot study) 2 containers in 1 stand, 40 cm high were used. The rationale of locating containers with substrates above the bottom was to obtain favourable and stable conditions and high abundance of *Chironomus*; both were proved in other papers (Kajak and Dusoge 1996, Kajak 1997). The distance of containers from the bottom was relatively small (80 or 40 cm), as compared to the depth of this site (about 6 m), so the supply of tripton, eggs and the youngest larvae was the same to the containers as to the bottom.

Containers were located in the reservoir a short time (12 days) before the mass appearance of the spring *Chironomus* generation and the highest abundance of larvae in the season (Table 1). The following substrates were used (data for lakes deposits – Rybak 1969):

- the mineral substrate – 70% bentonite plus 30% chalk (0% of the organic matter).
- bottom deposit from the study site i.e. the eutrophic, polimictic Zegrzyński reservoir (9% of the organic matter),
- bottom deposit from the eutrophic, dimictic lake Mikołajskie (23% organic matter),
- bottom deposit from the eutrophic, polimictic lake Śniardwy (38% organic matter),
- bottom deposit from dimictic, polyhumic lake Flosek (83% organic matter).

The local bottom deposit with its benthos was located in containers by the diver on the spot. The substrates from other water bodies (3 lakes) were taken with an Ekman dredge from their surface layer 2 months before the experiment and kept in the laboratory. They contained no *Chironomus* at the start of the experiments (Table 1). In the original locations of these deposits numbers of *Chironomus* were very low – no more than a few hundred per m^2.

Table 1. Scheme of field experiments 1997; trays with different substrates located 0.8 m above the bottom, in one study site in the Zegrzyński reservoir

Dates of installations and sampling	Variants of experiments (trays with different substrates) Trays No											
	1	2	3	4	5	6	7	8	9	10	11	12
	% of organic matter											
15 May – first installation of trays – 6 variants	**0**		**9***		**23**		**38**		**83**		**9***	
10 June	+	+	+	+	+	+	+	+	+	+	–	–
											installation of **cuboids**	
10 July installation of 1 variant	+	+	+	+	+	+	+	+	–	–	+	+
									9*			
1 August installation of 2 variants	+	+	+	+	–	–	–	–	+	–	+	+
							resed. (9)		**9***			
1 September installation of 6 variants	–	–	–	–	–	–	+	–	+	–	+	+
	0	**9**	**23**	**38**	**deep cont. (9)***	**pre-heated (9)**						
1 October – end of experiments	+	+	+	+	+	+	–	–	+	–	–	–

+ – sampling; – no sampling
Organic matter content in substrates: 0% – the mineral substrate: 9% – mud from the study site (marked as "control" on Fig. 2.), 23, 38 and 83% muds from other lakes. Other experimental variants (in the substrate from the study site, 9% org. matter): "cuboids" – the mud divided into small cuboids; "resed." – the resedimented mud; "deep. cont." – the container with the mud layer twice thicker (25 cm deep) than in all other variants; "preheated" – the mud deprived of zoobenthos by heating. Variants with *Chironomus* larvae present in the substrate at the beginning of the exposition are marked with asterisk (*), all other substrates contained initially no *Chironomus*.

Apart from variants with substrates of different organic matter contents (Table 1, see also Fig. 1), several other variants with the local bottom deposit were applied (Table 1, see also Fig. 2):
- deposit divided into small cuboids (2 × 2 × 12.5 cm) – to check the importance of mutual contacts of larvae on their density,
- deposit deprived of organisms by heating (60° C, 24h) – the different approach to the same problem as mentioned above,
- deposit in a deep container (25 cm – twice deeper than all other containers used) – to check the significance of the substrate layer for *Chironomus*,
- deposit exposed for various periods – to check the influence of the exposure time of containers on *Chironomus* numbers,
- an empty container, which constituted a kind of trap for the tripton and was filling gradually during the exposition with the resedimenting deposit and sedimenting tripton – to study the colonisation of the newly sedimented substrate.

Two containers in each treatment were used at the beginning of the experiments, later usually one container (Table 1). Most of the time, during relatively calm weather the substrate in containers remained undisturbed, and only thin layer of the sedimenting tripton was settling on the top of it. However occasional heavy winds resulted in washing out the substrate from containers.

Samples, 5 from each container, were taken by the diver, with 20 cm^{-2} plexiglass tube, once in 1993 (see Fig. 1) and 5 times in 1997 (Table 1, see Figs 1 and 2). Samples were never taken more than 3 times from the same container, usually twice before the end of the exposure (third sampling was the last one, and the container was not exposed any longer). Each time from each container a relatively small portion of the substrate was taken (5 samples of 20 cm^2 = 100 cm^2, that is 11%

of the container area). From the nearby bottom samples were taken on average once a week between May and September, using a 20 cm^2 core sampler. The samples were sieved alive (through a 0.2 × 0.2 mm mesh sieve) within a few hours, preserved in alcohol, and sorted in the laboratory. Total numbers of *Chironomus plumosus* larvae and numbers in size classes ≤ 6 mm, 7–12 mm, 13–17 mm and ≥ 18 mm were analysed. The abundance of larvae ≤ 6 mm informs about hatching from eggs and survival of the youngest larvae, and that ≥ 18 mm about numbers of larvae close to emergence.

Kruskall-Wallis ANOVA statistics was applied to compare larval numbers between experimental variants, differences with *P* value ≤ 0.05 were treated as statistically significant.

Two-three times monthly Secchi disc visibility was measured, and a temperature-oxygen vertical profile was made, the lowest point of measurement being about 50 cm above the bottom (see Fig. 3).

4. RESULTS

4.1. EFFECT OF DIFFERENT ORGANIC MATTER CONTENT IN SUBSTRATES

Great differences in *Chironomus* numbers and size structure were found between sediments from the polyhumic lake and the eutrophic reservoir in 1993 (Fig. 1A). Much higher numbers of older larval classes in the bottom deposit from the polyhumic lake indicate the lower reduction of larval numbers and much quicker growth of larvae in this substrate. These results were however not repeated in 1997, when some (occasionally quite high) differences of *Chironomus* numbers on particular sediments were found, but no correlation with organic matter content in the substrate, and no consequent similarity between the same substrate on different sampling dates. For example numbers in the sediment poor (9%) in the organic matter were as a rule similar to that with 23% organic matter (Fig. 1B, C, E), and sometimes similar (Fig. 1B) or higher (Fig. 1C) than in that with 38% organic matter. In the polyhumic sediment, the richest (83%) in the organic matter, *Chironomus* numbers in June were lower than in deposits poorer in organic matter from the other 3 water bodies, and almost identical (no significant difference) with numbers in the mineral substrate (Fig. 1B).

In the fully mineral substrate (bentonite and chalk, 0% organic matter) *Chironomus* numbers were higher (Fig. 1D) or not lower (Fig. 1B, C, E) than in some bottom deposits with various (up to the highest – 83%) organic matter content. Also numbers of the oldest class of larvae on the mineral substrate were similar or higher than on any other substrates (Fig. 1B, C, D, E), indicating a high growth rate of larvae on the mineral substrate.

4.2. EFFECT OF THE EXPOSURE TIME

Differences of *Chironomus* numbers in experimental variants of various exposure time were not significant. Similar numbers and size structure were found in August 1997 in variants "control (1 Jul.)" and "control (15 May)" exposed for 3 weeks (10 July – 1 August) and 11 weeks (15 May – 1 August), respectively (Fig. 2). The same result was obtained in October 1997: *Chironomus* numbers in variant "control (1 Aug.)" exposed for 2 months (1 August – 1 October) were not significantly different from those in three other variants with the same sediment exposed for 1 month (1 September – 1 October): "control (1 Sep.)", "deep cont. (1 Sep.)", and "preheated (1 Sep.)" (Fig. 2) as well as in the deposit of 23% organic matter, exposed for the same period (Fig. 1E).

In the bottom deposit initially without *Chironomus* its numbers were very high after a short time of exposure. *Chironomus* abundance reached 62.5 thousands of individuals in the polyhumic deposit (83% organic matter) after 3 weeks (Fig. 1A) and 50–86 thousands ind. m^{-2} in various substrates (0; 23; 38; 83% organic matter) after 4 weeks (Fig. 1B).

4.3.EFFECTS OF MODIFIED INTERRELATIONS OF LARVAE

Chironomus numbers in October in the sediment initially without the zoobenthos (preheated in 60° C, 24h) did not differ significantly from 3 variants with the benthos: "control (1 Aug.)", "control (1 Sep.)" (exposed for different periods), and "deep container"(with thick sediment layer) (Fig. 2). The same was true in June for the sediment initially with the benthos (9% organic matter)

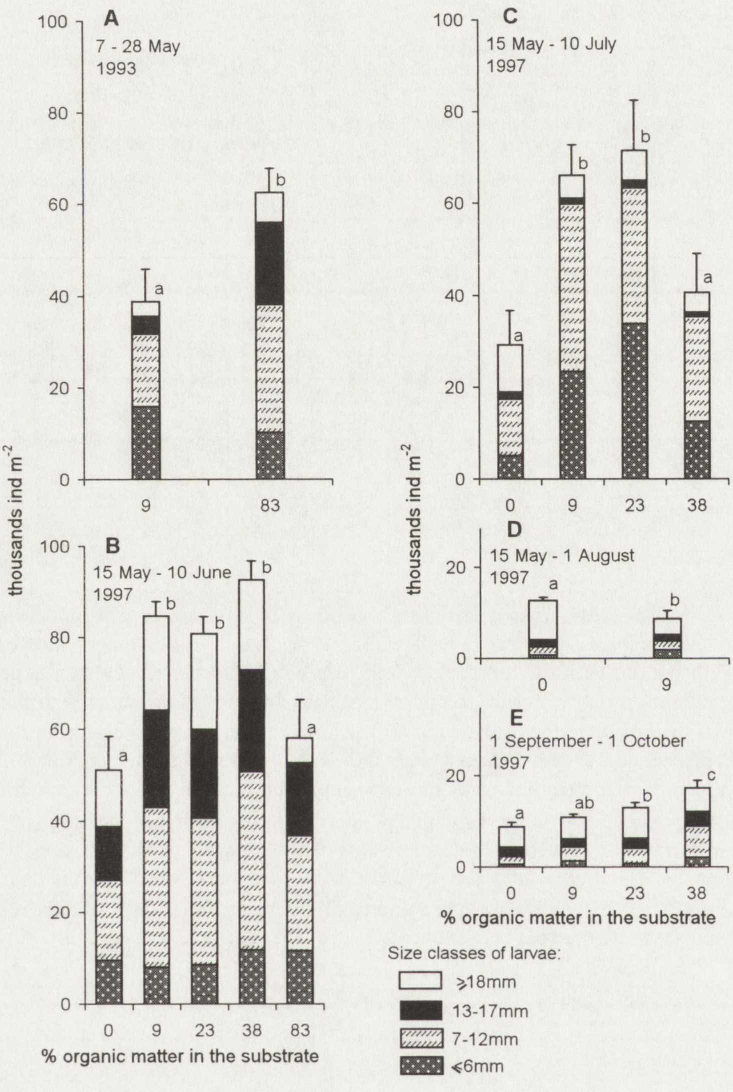

Fig. 1. Numbers of *Chironomus plumosus* – total and in size classes in plastic containers filled with various substrates and located 0.4 m (1993 – A) and 0.8 m (1997 – B, C, D, E) above the bottom in the same study site (Zegrzyński Reservoir, about 6 m depth). Periods of exposure are given at the top of each picture. Particular substrates: 0% organic matter – mineral substrate, 9, 23, 38 and 83% – bottom deposits from different water bodies (see "Methods" for details). The same letter at the top of the columns (a or b or c) means that numbers are not significantly different ($P > 0.05$); different letters indicate significant ($P \leq 0.05$) differences. Vertical bars at the top of each column – standard errors.

and two others (23 and 38% organic water) without it (Fig. 1B).

The partitioning of the sediment in the container into cuboids by locating vertical plastic compartments (2 × 2 × 12 cm – the variant "cuboids"), to constrain contacts of larvae, had no consequent influence on *Chironomus* numbers and the size structure. Numbers of larvae were higher in that variant than in the three others in August (Figs 2 and 1D), while in July they were lower than in the control (Fig. 2) and also than in the variant with 23% organic matter but similar to the variant with the mineral substrate (0% organic matter) and that with 38% organic matter (compare Figs 1C and 2). In September *Chironomus* numbers in the variant with "cuboids" were lower than in two others (Fig. 2).

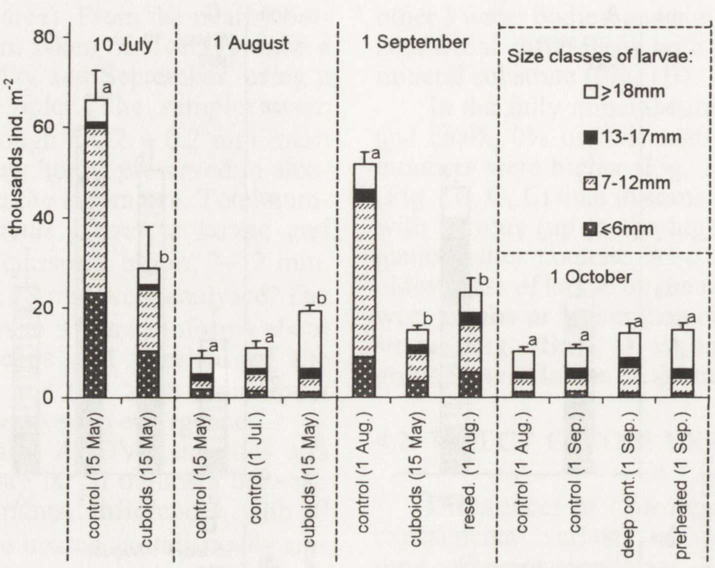

Fig. 2. Numbers of *Chironomus plumosus* – total and in size classes in containers located 0.8 m above the bottom and filled with bottom deposit from Zegrzyński reservoir (9% organic matter), experiments conducted in 1997. Particular variants: "control" – the sediment with the benthos taken directly from the bottom of the reservoir; "cuboids" – the sediment (as in the control) partitioned with plastic sheets into cuboids: 2 × 2 × 12.5 cm; "deep cont." – the container with 25 cm (twice the depth of the standard containers) layer of sediment; "resed." – container empty at the beginning of the exposure, then naturally filled with sedimented tripton and resedimented deposit; "preheated" – sediment deprived of benthic organisms by heating (60° C, 24h). Dates of settings of particular variants are given in brackets. Dates of finishing of particular series shown at the top of the figure. The same small letter at the top of the columns (a or b or c) means that numbers are not significantly different ($P > 0.05$); different letters indicate significant ($P \leq 0.05$) differences. Vertical bars at the top of each column – standard errors.

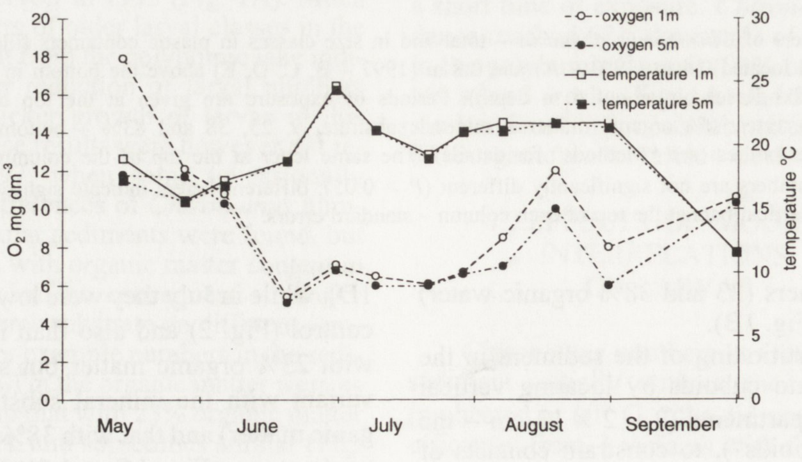

Fig. 3. Temperature and oxygen concentration values under the surface (1 m depth) and near the bottom of the experimental site (5 m depth) during the season 1997.

4.4. INFLUENCE OF THE THICKNESS OF SUBSTRATE LAYER

The relatively thin (about 12 cm) layer of deposit used in containers was obviously fully sufficient for *Chironomus*. Its numbers and also size class structure in the container with the thick (25 cm) layer of deposit (the variant "deep cont.") were not significantly different from those in three variants in containers with the 12 cm layer of sediment (9% organic matter) (Fig. 2, October). They also did not differ from that in the variant with 23% organic matter content in the deposit (Fig. 1E). In September 1997, in the variant with a thinner (about 8 cm) layer of substrate (composed of the resedimented bottom deposit and tripton) the variant "resed. (1 Aug.)" – numbers were about twice lower than in containers full of the sediment from the bottom (Fig. 2). This was however probably not due to the thin sediment layer, but to the fact that the container was empty for some time at the beginning, so larvae had less time to colonise it.

4.5. OXYGEN AND TEMPERATURE

Oxygen concentrations above the bottom were always relatively high on the dates of their measurement (Fig. 3); this however does not exclude the short-term deficits between these dates at the end of night, especially at the bottom surface; it was not possible to make continuous and permanent measurements.

Temperatures were relatively low at the end of May and in June (Fig. 3), which did not impede the mass appearance of the spring *Chironomus* generation. At the end of September 1997 numbers of larvae decreased strongly parallely to the temperature (compare Figs 1, 2 and 3).

5. DISCUSSION AND CONCLUSIONS

No consequent relationship between the organic matter content (0–83%)in the substrate and *Chironomus* numbers have been found in the field experiment in the polytrophic reservoir, at 6 m depth. However the differences in numbers (up to 2–3 times within particular terms) occurred. In some laboratory observations (Kajak, unpublished) mature *Chironomus* larvae fulfilled their development to imago on the fully mineral substrate – the chalk. That substrate was however soon covered by a tiny (< 0.1mm) layer of tripton from the circulating water with some organic particles resuspended from other small containers with the bottom deposit in the same bigger aquarium. Similar phenomenon was observed in the present study, where the mineral substrate exposed in field conditions was also covered with quite thick (up to few mm) layer of sedimenting tripton and resedimented bottom deposit. This implies that larvae use mostly the sedimenting tripton as the main source of the food in the environment under study. Some authors suggest that the dependence of *Chironomus* development on the type of soft bottom deposits does exist (see reviews by Brinkhurst 1974, Resh and Rosenberg 1984, Kajak 1988, Armitage *et al.* 1995). The size (age) structure of the population under conditions of this study (a polytrophic reservoir with slight water flow) was usually similar in the variants with substrates of different organic matter content (Figs 1 and 2), what contradicts the dependence mentioned above.

The substrate layer of 12 cm used in containers was evidently sufficient as proved by much higher numbers of larvae in containers, than in the bottom (Kajak and Dusoge 1996, Kajak 1997) but also by the fact that in the much thicker (25 cm) deposit layer (Fig. 2) numbers were not higher than in containers with 12 cm substrate layer, used in all other variants of experiments.

The similar numbers and age structure in variants initially with or without *Chironomus*, as well as in that with limited contacts (due to plastic cuboids inserted into the sediment) indicate small influence of interactions between larvae on their abundance in the situation under study. In other situations however interrelations between larvae can be essential for their abundance (Kajak 1987, Nakazato and Hirabayashi 1998).

The final abundance was much higher in containers than in the bottom and was independent from the type (organic matter content) of the substrate used. *Chironomus* abundance was in this study obviously determined by the favourable feeding conditions - abundantly sedimenting, valuable tripton carried in by two polytrophic rivers supplying the reservoir (Bubień 1989, Kajak 1997) and by the oxygen situation, which was better and more stable 0.4–0.8 m over the bottom than at it very surface. The main mechanism of colonising the environment is probably the settling of eggs and young larvae, but immigration of older larvae is also possible (Prus and Kajak 1999). The exposure of containers for three weeks was sufficient to achieve the normal (not different from containers exposed longer), high abundance of the population.

The favourable feeding conditions in the reservoir are proved by very high, as compared to other water bodies, *Chironomus* abundance in the reservoir. Much better oxygen conditions over the bottom are confirmed by several times higher *Chironomus* numbers noted in containers with deposits located over the bottom versus the bottom itself (Kajak and Dusoge 1996, Kajak 1997). Perhaps in such circumstances both the type of substrate and the mutual relations of larvae are of secondary importance.

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6. SUMMARY

The main goal of this study was to compare *Chironomus plumosus* populations on substrates with different organic matter content (0–83%) exposed in containers located 0.4–0.8 m above the bottom, in the shallow, lowland, eutrophic, polimictic Zegrzyński reservoir. Substrates used in experiments originated (apart from the mineral one) from different water bodies (Table 1). Some other factors influencing *Chironomus* population, like initial abundance and interactions between larvae were also studied, as well as containers exposure period, term of the experiment and substrate layer thickness (Table 1).

In the pilot series of experiments, conducted in the spring 1993 (Fig. 1A), numbers of *Chironomus* larvae were significantly higher in the polyhumic deposit (83% of organic matter) than in the deposit from the study site (9% org. matter). These results were however non repeated in the main series of experiments, conducted in 1997 (May–September), where no correlation was found between the kind of substrate used and *Chironomus* abundance (Fig. 1B, C, D, E). It indicates that the substrate is not the main source of food for *Chironomus*. Larvae fed mainly on the sedimenting tripton, which was sufficient for their development. This was proved by similar abundance and size structure of *Chironomus* population on the mineral substrate and on deposits with different organic matter content (Fig. 1).

Chironomus numbers and size structure after the 3–4 weeks of the exposition were similar in sediments initially without the benthos and in those with the natural population abundance for the study site (Figs 1 and 2, see also the text for detailed information). This indicates a very high growth potential of *Chironomus* population in the reservoir. The lack of the influence of the lowered initial population abundance and also of reduced contacts between larvae on *Chironomus* abundance and development was probably caused by the comfortable feeding and oxygen (Fig. 3) conditions, which overweighed the negative interactions of larvae.

The substrate layer provided (12 cm) was fully sufficient, what is proved by several times higher *Chironomus* numbers in containers, as compared to population abundance in the bottom (Kajak and Dusoge 1996, Kajak 1997), and by similar numbers of larvae in a deep (25 cm) container and in normal containers (Fig. 2). The diversified exposure time of experimental containers (3–10 weeks) had no serious influence on *Chironomus* abundance (Fig. 2, Table 1).

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