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EFFECTS OF THE DENSITY OF LARVAE AND TYPE OF SUBSTRATE ON *CHIRONOMUS PLUMOSUS* L. (DIPTERA: CHIRONOMIDAE) POPULATION. LABORATORY EXPERIMENTS

ABSTRACT: Strong dependence of larval tube numbers on the population density, the condition of larvae and feeding situation occurred, but no correlation with the type of substrate was found.

Numbers of larval tubes per individual were on the average 2.6 times lower at high (20 and 40 thousands ind. m^{-2}) than at low (2–2.5 thousands ind. m^{-2}) initial densities of larvae. They fluctuated strongly, but only at low densities, indicating the very high activity of larvae; high densities probably restrained this activity.

The grown up larvae succeeded in finishing their full development in the mineral substrate with the tiny (< 0.1 mm) layer of the natural mud at the top of it. No conical nettings (inside larval tubes) for food filtration were observed. The larvae fed mostly on the internal walls of their tubes; sometimes they also collected particles from the surface of the substrate (especially of substrates poor in the organic matter), after the food was furnished.

The emergence of imagines depended on the larval density but quite differently than the numbers of tubes. The threshold for the decreased tube numbers per individual was between 2.5 and 20 thousands individuals m^2, while that for the emergence of imagines – between 20 and 40 thousands individuals m^2.

KEY WORDS: *Chironomus*; benthos laboratory experiments; populations; competition; behaviour; density dependence; type of substrate

1. INTRODUCTION

Chironomus larvae, common in surface freshwaters, are important both for the circulation of matter (exchange of substances between bottom deposits and water) and as a food for fish.

The numbers of *Chironomus* larvae in various natural environments (muddy bottom in water bodies and some slowly flowing rivers) vary enormously – from few individuals to hundred thousands individuals per m^2 (Sokolova 1983, Kajak 1987; 1997, Armitage *et al.* 1994). They happen to be especially high in cultures (Konstantinov 1958, Yashouv 1970, Shaw and Mark 1980). Although the importance of the substrate (bottom deposit) and also of the population density for *Chironomus* larvae is obvious there are practically no papers on this subjects. Our understanding of causes and mechanisms of these problems is far from satisfying.

The tubes built by *Chironomus* larvae are essential for their life activity – the feeding, the respiration, the protection against predators. So the numbers of tubes and its changes can be treated as an important index of the population. However despite of a number of observations on the building of

tubes and their functions (Walshe 1951, McLachlan and Cantrell 1976, Hodkinson and Wiliams 1980, Sokolova 1983, Resh and Rosenberg 1984, Leuchs and Neumann 1990, Armitage *et al.* 1994) there are almost no papers on tube numbers, kind, distribution, etc. in chironomid populations. In the field conditions at very high densities of larvae, the tubes touched each other (there was no free space between them) forming a compact, several cm thick layer, often yellow (due to good oxygenation resulting from the activity of larvae).

That is why we decided to analyse the influence of the type of substrate and the initial density of larvae on *Chironomus* populations in laboratory experiments, where other factors influencing the population were favourable, stable and controlled. The number of larval tubes, rate of mortality and emergence were chosen as indicators of the state of the population.

2. MATERIAL AND METHODS

Experiments were done in "experimental cylinders"– pieces of a plexiglass tube, with internal diameter 50 mm, surface area 20 cm^2, 13 cm high. They were stoppered at the bottom, filled with a substrate to the level 0.5 cm below the upper edge of the cylinder, and located in the aquarium 30 × 40 cm and 30 cm deep. Two days later larvae, 19–22 mm long, originating from the Vistula oxbow lake, were introduced on the surface of the substrate in the cylinders; within few minutes they hided in the mud.

The water level in the aquarium was 5 cm above the top of cylinders. Cylinders were prolonged with tubes of netting (mesh size 1 × 1 mm) protruding 5 cm above the water surface. This enabled counting of pupal exuviae separately in each cylinder. The water was aerated and slowly flowing (due to simple aquarial device – see Kajak and Prus 2000), providing sufficient oxygen concentration at the mud surface in the cylinders.

Numbers of tube holes of *Chironomus* larvae (tube numbers per ind. were always related to the initial number of larvae in each experimental variant), emergence of imagoes and mortality of larvae were registered.

Kruskal-Wallis ANOVA statistics was applied for all series of the data; the differences with *p* value ≤ 0.05 were treated as statistically significant.

2.1. VARIOUS DENSITIES OF LARVAE ON VARIOUS NATURAL MUDS

Three types of natural muds, and four population densities were used. The mud used for experiments was deprived of *Chironomus* larvae and homogenized. It originated from 3 sources: eutrophic reservoir (E), mesoeutrophic lake (M) and humic lake (H); five cm surface layer of the mud taken from lakes and the reservoir was used in experiments. The contents of organic matter in the mud was accordingly about 9, 23 and 83%.

Two basic series of experiments were done, each with mentioned 3 kinds of mud and 2 initial densities of larvae (6 variants altogether). Series I – with densities of larvae 4 and 40 ind. 20 cm^{-2} (2 and 20 thousands ind. m^{-2}) in each kind of mud, exposed between 26.01 and 26.04; and series II – 5 and 80 ind. 20 cm^{-2} (2.5 and 40 thousands ind. m^{-2}) exposed between 3.02 and 26.04. Six experimental cylinders in each variant were used.

2.2. MINERAL AND MINERAL-ORGANIC SUBSTRATES

Numbers of tubes were registered in two mineral substrates: the bentonite (series A) and the chalk (series B) and several combinations of these substrates and some mud or food, as compared to the natural mud from the eutrophic reservoir.

Series of six experimental cylinders, 8 larvae in each of them (4 thousands ind. m^{-2}) were used in each variant.

The following treatments were carried out:

– natural mud from the eutrophic reservoir (as the reference treatment, marked "E" on Fig. 3, series III and IV);

A. "Bentonite treatments":

– fully mineral substrate – bentonite;

– combinations of the bentonite and mud:

– 1 cm of the mud at the top of bentonite (B-1 on Fig. 3),

– 1 cm of the mud 3 cm under the surface of the bentonite,

– 0.5 mm of the mud at the top of the bentonite,

– 0.2 mm of powdered *Daphnia* (as the food for *Chironomus*) at the top of the bentonite.

B. "Chalk treatments":

– fully mineral substrate – the chalk;

– the mixture of the chalk and 10% of the natural mud;

– the chalk and 0.5 mm of the mud at the top of the chalk.

– the chalk and 1.0 cm of the mud at the top of the chalk (C-1 on Fig. 3);

2.3. OBSERVATIONS OF FEEDING BEHAVIOUR OF LARVAE

The observations were made through the glass wall of aquarium to which an other thin (1.0–1.5 mm) glass plate was attached from the internal side, making a microaquarium; the distance of its walls was about 1.5 mm – only slightly more than the diameter of the *Chironomus* larvae. The larvae made their tubes in the mud between the glass walls, so that their activity in tubes could be relatively easily observed.

3. RESULTS

3.1. FOUR DENSITIES OF LARVAE IN THREE KINDS OF NATURAL MUDS

3.1.1. AVERAGE NUMBERS OF LARVAL TUBES

Numbers of tubes were sometimes relatively similar (although some irregular fluctuations occurred) for a long time in particular muds (e.g. in the series II at 5 ind. per experimental cylinder 20 cm^2). However usually they were significantly different on various substrates (although without any stable correlation with the type of the substrate):

– in the series I at 4 ind. 20 cm^{-2} the highest in the humic mud (H) while at 40 ind. 20 cm^{-2} – in the mud E (Fig. 1).

– in the series II at 80 ind. 20 cm^{-2} the highest in the muds H and M, and the lowest in the mud E. (Fig. 1).

The pattern of changes of tube numbers differed greatly; e.g. in the mud E at 40 ind. numbers were high for two months, while at 80 ind. 20 cm^{-2} – they dropped to a very low level already at the end of the first month. So there was also no any consequent dependence of the number of tubes and its changes in time on the type of the mud (Fig. 1).

There was the strong dependence of tube numbers on the initial density of larvae. The number of tubes per individual during the first week of the experiment (when numbers of larvae were practically stable) was on the average 2.6 times lower (the range for particular muds 2.2 – 2.8) at the higher (40 and 80 ind. 20 cm^{-2}) than at the lower (4 and 5 ind. 20 cm^{-2}) density of larvae (Table 1.). This
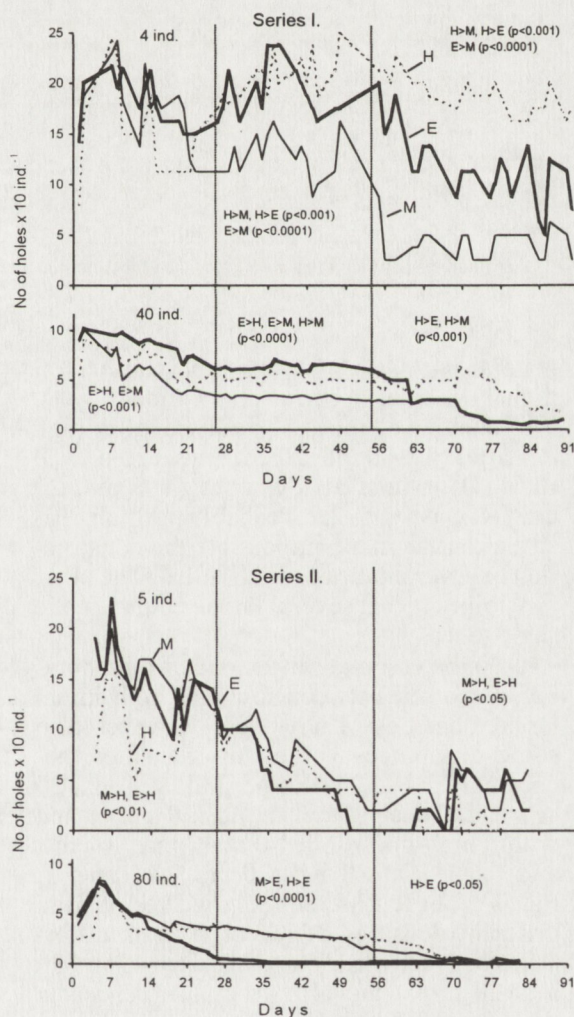

Fig. 1. Number of tubes holes of *Chironomus* (identical larvae from the same environment) in 3 muds from: eutrophic reservoir – E, mesotrophic – M and humic – H lake, at various densities of larvae in 2 parallel series of experiments (series II set 7 days later than series I). Series I. – 4 and 40 ind. 20 cm^{-2}, II. – 5 and 80 ind. 20 cm^{-2}. The exposure time was divided into 3 periods (with similar pattern of tubes numbers dynamics), marked with vertical dotted lines. The significant differences between treatments (muds) for particular periods are marked on graphs.

Table 1. Numbers of Chironomus tubes holes (per individual*) in various muds and at different (low – 4 and 5 ind. 20 cm^{-2} and high 40 and 80 ind. 20 cm^{-2}) initial density of larvae during the first week of the experiment. The deposit from humic – H and mesotrophic – M lakes, and from eutrophic reservoir – E

Mud from	Initial density ind. 20 cm^{-2}	Average no. of holes/ind.	Ratio of average holes no. at high/low density
H	4	2.1	2.6
	40	0.8	
M	4	2.2	2.8
	40	0.8	
E	4	2.2	2.2
	40	1.0	
H	5	1.8	2.6
	80	0.7	
M	5	1.6	2.7
	80	0.6	
E	5	1.5	2.5
	80	0.6	

* tubes numbers per individual were calculated for the initial number of larvae in each experimental variant.

general regularity – lower tube numbers at higher densities occurred all the time (Fig. 1). The number of tubes per individual at high densities (40–80 ind. 20 cm^{-2}) was below 1.0 (0.6–1.0), whilst at low ones (4–5 ind. 20 cm^{-2}) it was – 1.5–2.2. Probably the high density of larvae made impossible the construction of several tubes per individual. The other possibility is that several larvae could use the same tubes.

So the essential difference of tube numbers occurred between low and high initial larval densities. There were however also some differences within low densities (accordingly 2.1–2.2 holes per ind. at 4 ind. 20 cm^{-2} and 1.5–1.8. at 5 ind. 20 cm^{-2}) and within high-ones (0.8–1.0 at 40 ind. 20 cm^{-2} and 0.6–0.7 at 80 ind. 20 cm^{-2}). (Table 1, Fig. 1). These differences could result from different densities of larvae (that is differences between 4 and 5 and between 40 and 80 ind. 20 cm^{-2}) but also from differences in their physical condition (see the chapter 3.1.3.).

3.1.2. TYPES OF THE DECLINE AND FLUCTUATIONS OF TUBE NUMBERS

In all variants (both series, all muds and all larval densities) there was some decrease of tube numbers during the time; it started from few days (at the most – a week) since the beginning of exposition. It was different (from very small to great) in particular vari-

ants. Although there was relatively similar mortality and emergence in most types of muds used in experiments, the character of the decline of tube numbers differed strongly. There was practically no decline during 3 months in the mud H, 4 ind. 20 cm^{-2}, and during 2 months – in the mud E, 4 ind. 20 cm^{-2}. There was also no decline during the second month in all types of muds, 40 ind. 20 cm^{-2}, and in muds H and M, 80 ind. 20 cm^{-2}; in variants with 5 ind. 20 cm^{-2} there was the gradual decline all the time, since the beginning of the second week of the exposition (Fig. 1).

The reduction of tube numbers was as a rule much smaller than the reduction of numbers of larvae. It was very irregular (with great fluctuations) at small densities of larvae. The tubes were very long lasting, with some dependence on the density of larvae, the kind of mud, and the condition of larvae. The course of the decline of tube numbers could differ in the same type of mud at similar densities, and at the same density in different muds. It probably depended on the type of mud, the density of larvae and their condition (which was different in the series I and II) but without the clear regularity (Figs 1, 2). The importance of the physical condition of larvae is supported by much quicker decline of tube numbers in the second than in the first series (see the chapter 3.1.3.).

Tubes of single larvae kept in the 20 cm^{-2} cylinders disappeared in 1–3 days after the

larvae died (an observation on 10 single individuals was carried out). This suggests that the presence of some larvae is essential for keeping tubes in a good state.

The number of tubes in variants with many larvae at the beginning often remained high after the number of larvae decreased seriously. After 9 days only 55–70% and after 14 days 30–40% of larvae remained. The number of tube holes was however in two muds in the series I (4 ind. 20 cm^{-2}) as high as at the beginning till the end of the second month of the exposure. It was also high at 40 ind. 20 cm^{-2} during the two months, and in some muds at both densities during three months (Fig. 1), when only single larvae remained.

Maximal number of tube holes was reached in few days since the setting of the experiments.

There was great difference in the fluctuation of tube numbers between low (4–5 ind. 20 cm^{-2}) and high (40–80 ind. 20 cm^{-2}) densities of larvae. Fluctuations were much stronger (up to more than twice) and much more frequent at low than at high densities (Fig. 1). Sometimes the tube numbers dropped to zero for short or quite long (2 weeks) period, and then significantly increased again (Fig. 1 – series II, 5 ind. 20 cm^{-2}). Obviously the high density limited the activity of larvae in tubes construction and consequently – the fluctuation of tube numbers. The number of tubes and their durability depended both on the density and the physical condition of larvae, but only slightly on the kind of mud.

3.1.3. DIFFERENCES BETWEEN SERIES I AND II RESULTING FROM THE PHYSICAL CONDITION OF LARVAE

The series II was set a week later than the series I. Both the pool of larvae and the muds were the same in both series. However the differences in tube numbers between these two series were great. The average (for all types of muds) number of tubes during the first week of the exposition was significantly higher in the series I than II – accordingly at 4–5 ind. 20 cm^{-2} – 2.2 versus 1.6 and at 40–80 ind. 20 cm^{-2} 0.9 versus 0.6 tube holes per individual (Table 1).

In the series I numbers of tubes stabilised (after the period of great fluctuations) in particular types of muds at very different levels at each of the two densities of larvae (Fig. 1).

In the series II the reduction of tube numbers was much greater both at low and high densities of larvae. It dropped to almost zero already in a month, and the decline started from the almost very beginning.

The experiments were carried out at the beginning of the emergence of imagoes. Probably individuals of the best condition emerged before the series II began; so the condition of larvae in this series was weaker than in the series I. This could be the main reason of all differences between the two series, including the durability and the course of changes of tube numbers.

Although the larvae used were at the final stage of their development (close to emergence) the importance of the density was great.

The larvae in the two series compared reacted differently on the same type of mud obviously due to the different larval condition, although no regularity in the dependence on the mud type was found.

3.1.4. EMERGENCE OF IMAGINES

In both series I and II the emergence began already a few days after locating the larvae in the mud in experimental cylinders, just after larvae set themselves up in the new environment.

In the series I (4 and 40 ind. 20 cm^{-2}) the course of emergence was similar at both densities of larvae and from all muds. The only difference was that the emergence from the variant with the high density (40 ind. 20 cm^{-2}) lasted a few days longer than from the low one (4 ind. 20 cm^{-2}) (Fig. 2, Table 2). It was however the emergence of just single specimen.

The percentage of emergence was rather high at both densities and in all muds; however it was the same at both densities only in the humic mud, and lower at the higher density in the two other muds (Fig. 2).

There was also some difference of the emergence from different muds at the beginning of the exposure; during the first 5 days the emergence from the humic mud (H) was almost twice more intense than from 2 others coupled together (92% ± S.E.=3% vs. 52 ± 9%). It occurred despite the fact that the construction of tubes in the mud H lasted a bit longer than in other muds – the peak number of tubes in this mud was delayed about 2 days (Fig. 1). Also the emergence from this mud at 4 ind. 20 cm^{-2} was 1.5 times more intense
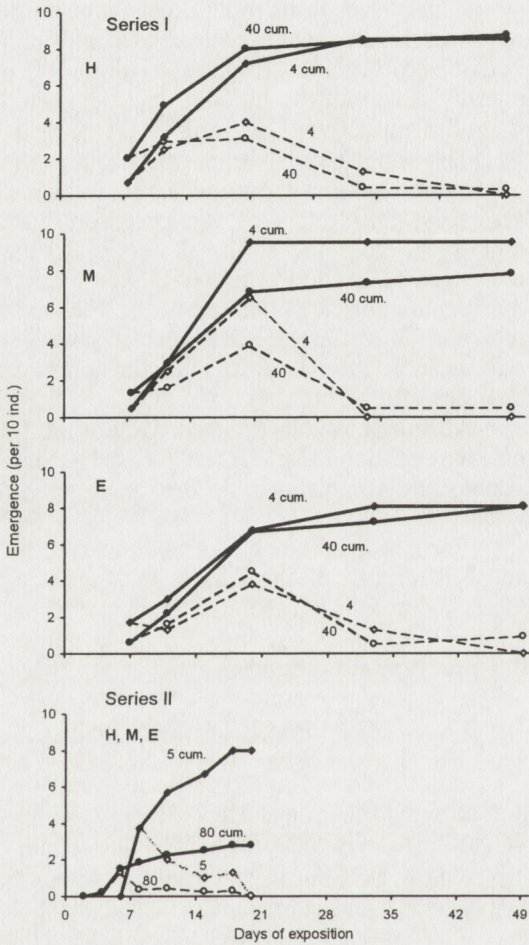
Fig. 2. Emergence of *Chironomus* (grown up larvae, about 20 mm long, from the same environment) in laboratory conditions from muds of eutrophic reservoir (E), mesotrophic (M) and humic (H) lake at various densities of larvae: Series I.: 4 and 40 ind.20 cm^{-2} (2 and 20 thousand ind.m^{-2}). Series II.: 5 and 80 ind.20 cm^{-2} (2.5 and 40 thousand ind.m^{-2}). Data from daily observations. Pattern of emergence – broken lines; cumulative ("cum.") emergence since the beginning of the exposition – solid lines. The non-cumulative data are averaged for periods (few days) with relatively uniform emergence (particular "dots" on the figure located in the middle of each period).

than at 40 ind. 20 cm^{-2} (92 ± 3% vs. 65 ± 16%; p<0.05).

In the series II (which was set a week later than the series I, and the larvae in it had a "weaker condition" – see above) the total number of emerged imagines (per 10 larvae) was on the average almost 3 times lower at the high (80 ind. 20 cm^{-2}) than at the low (5 ind. 20 cm^{-2}) density, and the course of the emergence was slightly different (Fig. 2, Table 2): it began a bit earlier, and lasted few days longer (similarly as in the series I, at the high density 40 ind.). Obviously very high density 80 ind. 20 cm^{-2} was too high, causing much lower emergence and very high mortality of larvae. The mortality in most variants was very low – up to about 10–20% (see Fig. 2 – the final cumulative emergence per 10 individuals making about 80–90% of initial numbers of larvae).The only exclusion was the variant with the highest density (80 ind. 20cm^{-2}) of larvae where only 30% of larvae emerged and the mortality was very high – about 70%. Such density is obviously too high (independently of the type of mud) and causes the high mortality of larvae. It is possible that such high mortality resulted from two cumulative reasons: very high density and the weak condition of larvae. We must stress however that such (and higher) densities do happen both in nature (Kuklińska 1989, Kajak 1997) and in cultures (Konstantinov 1958, Kajak 1987, 1988, Shaw and Mark 1980, Yashouv 1970).

The emergence lasted longer in the series I (about 1 month altogether) than in the series II (about 20 days). Probably the larvae in the series II were a bit more advanced in their development, as far as this series started a week later than the series I.

More prolonged (a few days) emergence at 40 than at 4 and at 80 than at 5 ind. 20 cm^{-2} may indicate disadvantageous influence of the high population density.

Table 2. Emergence of imagines from various types of muds (see Figs 1 and 2) and at various densities of larvae

Series	Initial density (ind. 20 cm^{-2})	Total emergence in % of initial numbers of larvae Mean (and range for 3 types of muds)	Period of emergence (days)
I	4	**81.0** (77.0–86.0)	29
	40	**86.0** (82.5–92.5)	32
II	5	**80.0** (60.0–100.0)	13
	80	**27.7** (23.8–31.9)	19

Lower emergence at 80 ind. 20 cm^{-2} in all 3 muds is significantly different (p<0.001– 0.01) from all other densities in all 3 muds. Differences between all other variants (types of mud and densities of larvae) are small and insignificant (p>0.1–0.2).

Very few larvae remained after the period of emergence – on the average 2.5% of the initial density of larvae. So in most variants most individuals emerged as imagines and in the variant 80 ind. 20 cm^{-2} – most died and only about 30% emerged (Fig. 2).

3.2. MINERAL AND VERY POOR IN ORGANIC MATTER SUBSTRATES AS COMPARED TO ORGANIC ONES

3.2.1. TUBE NUMBERS

Series III (the bentonite)

Numbers of tubes in two substrates – the eutrophic mud (E on Fig. 3) and the bentonite with 1 cm of this mud at the top of it (B1 on Fig. 3) were the highest – accordingly more than 2.0 and 1.5 times higher than in all other substrates (coupled under V on Fig. 3 – the difference significant at p<0.00001– 0.0001); these coupled variants comprised: one cm layer of mud below 3 cm layer of the bentonite, very thin layer – about 0.5 mm of mud at the top of the bentonite, the addition of the food (powdered *Daphnia*) on the bentonite surface and the pure bentonite.

The 1 cm layer of the mud at the top of the mineral substrate (the bentonite), although important, was obviously not sufficient for the full success in the tubes building – the number of tubes was lower in the bentonite +1 cm of the mud than in the pure mud. The number of *Chironomus* tubes in 4 other variants was not significantly different from the pure bentonite (see Fig. 3 – all variants coupled under V). Obviously the larvae need some minimal amount of the "normal" organic substrate, at least the relatively thick (>1 cm) layer of it at the top of mineral one for their high activity expressed by the number of tubes.

Series IV (the chalk)

Numbers of tubes have been compared in the mud (E), pure chalk (C) and the homogenized mixture of the chalk and 10% of the mud, 0.5 and 1.0 mm of the mud at the top (the last 3 variants coupled under V). Tube numbers were much higher in the variants coupled under V (1.2 tube per ind.) than in the pure chalk (C) – 0.5 tube per ind. (p<0.05). The increasing thickness of the thin layer of the mud on the surface in the variants V resulted in increasing tube number – accordingly 0.8 tube per ind. at 0.0 mm, 1.1 at

0.5 mm and 1.3 at 1.0 mm of the mud layer at the surface of the substrate (p<0.05).

Tube numbers in the mud (E) were 3.3 times higher than in the chalk (C) and 1.4 times higher than in all other variants coupled together under V (Fig. 3).

The emergence of imagines occurred from all substrates, being most intense from the eutrophic mud.

3.2.2. GETTING OUT OF TUBES FOR FEEDING

Chironomus larvae are supposed to feed essentially in their tubes, by the filtration of the seston (Sokolova 1983, Armitage *et al.* 1994). Our observations suggest that they mainly collect particles deposited on the internal walls of tubes. We never saw sophisticated conical nets (as suggested by Walshe 1951), but primitive nettings were possible.

Apart from the activity in tubes, larvae were also periodically getting out of tubes, sometimes for almost whole their length, collecting particles around the aperture of the tube and hiding again.

In the special series of observation in experimental cylinders, on the mud from the old river bed, the addition of food (powdered *Daphnia*) increased getting out from tubes 3
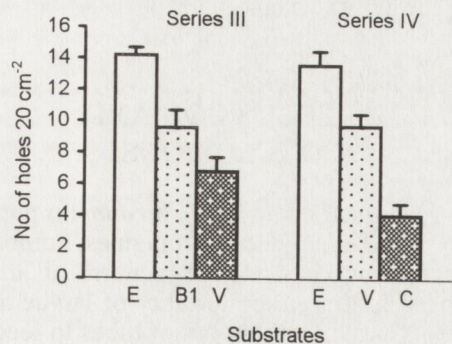

Fig. 3. Number of tubes holes per experimental cylinder (20 cm^{-2}, 8 larvae in each cylinder) in 2 parallel series III and IV in the natural substrate – mud from the eutrophic reservoir, the mineral one (the bentonite or the chalk) and combinations of natural and mineral substrates. In the series III the highest number of tubes holes was in the mud from the eutrophic reservoir – E, a bit lower in the bentonite covered with 1 cm of this same mud – B1, much lower in all other variants (coupled under "V" as not significantly differing each from others p>0.05. In the series IV the number of tubes holes was also the highest in the mud (E), lower in mixtures of the chalck and the mud (coupled under V), the lowest in the pure chalck (C). See the text for detailed descriptions of particular variants.

times (average 6.0 vs. 2.1 in the control, both during 15 min, at the density of 8 ind. 20 cm^{-2}; p$\leq$0.05). After 3 hours the difference still occurred, although getting out was less intense (1.4 vs. 0.7; p$\leq$0.05). Getting out of larvae (after the addition of the food) was less intense from the reservoir mud than from the old river bed mud (1.6 vs. 6.0; p$\leq$0.05). Probably the amount of the valuable food in the old river bed mud was small and of law quality – only that sedimenting from the water collumn, while in the reservoir mud it was plentiful, as continuously and permanently brought in by rivers extremely rich in the phytoplankton, including especially valuable small diatoms. The larvae getting enough food in the tubes by filtration, did not need to get out of them.

In the other series of observations larvae also did not get out from their tubes in the reservoir mud after the addition of the food; they did in the mud covered with 4 cm of chalk, and also, twice more intensively, on the pure chalk (accordingly 2.0 and 4.0 ind. per 15 min at 8 ind. 20 cm^{-2}; p$\leq$0.05). It suggests there was enough food in tubes in the pure mud, but not enough if it was covered with the chalk; in the pure chalk getting out of tubes made the only chance to get the food from the tiny deposit layer, which sedimented at the top of the chalk.

4. DISCUSSION AND CONCLUSIONS

Examined features of *Chironomus* populations: the emergence of imagines, numbers of larval tubes and the pattern of their changes in time, the mortality of larvae and their activity in getting out of tubes to feed – strongly depended on the density of larvae, their condition and the type of substrate. None of these features, except the last one, depended clearly on the kind of substrate, although the variation of them on various substrates was great.

There were relatively small differences of emergence in the range of densities 4–40 ind. 20 cm^{-2}, the essential threshold being between 40 and 80 ind. 20 cm^{-2}; the emergence between densities of larvae 40 and 80 ind. 20 cm^{-2} dropped from about 90 to about 30%, and the mortality increased from about 10% to about 70% of the total numbers of larvae. Obviously essential disadvantageous inter-actions of larvae intensified strongly between these two high densities. The disadvantageous interactions expressed themselves especially strongly in the situation with larvae of weaker physical condition in the series II (the strongest larvae emerged before the series II was set).

The emergence of imagines at high (40–80 ind. 20 cm^{-2}) densities of larvae lasted few days longer than at low ones (4–5 ind. 20 cm^{-2}) (Fig. 2). This resulted probably from disadvantageous interactions between larvae at their higher densities; such interactions are indicated at various densities of larvae (Rasmussen 1985, Watten and Armitage 1994, Kajak 1988).

The variation of tube numbers found in this study was great. The most clear dependence revealed was that between the average tube number and the density of larvae; in high densities – 40 and 80 ind. 20 cm^{-2} the number of tubes per individual was on the average 2.6 times lower than at low (4 and 5 ind. 20 cm^{-2}) ones (Table 1, Fig. 1).

The inconsistency between emergence of imagines and tube numbers is worth to be stressed. The emergence of imagines was similar at 4 and 40 larvae 20 cm^{-2}, while the number of larval tubes was more than twice lower already at 40 ind. 20 cm^{-2} (and of course also at 80 ind. 20 cm^{-2}) than at the low (4–5 ind. 20 cm^{-2}) density. Obviously much lower number of tubes per individual at 40 ind. 20 cm^{-2} did not hinder the normal emergence of imagines. It must be stressed however that all this took place with grown up larvae, almost ready for the emergence; lower tube numbers could be more important at earlier stages of the larval development. At high densities (40–80 ind. 20 cm^{-2}) the tube number per an individual was 0.6–1.0, so in this situation some tubes were obviously used by several larvae, what could intensify the competition between them.

Numbers of tubes, apart from the strong dependence on the density of larvae, clearly depended also on the series of the experiment, probably due to the different physical condition of larvae. In the second series, which started a week later, larvae were probably (as explained earlier) of the poorer condition; the strongest ones had already emerged as imagines during the week between the beginning of the first and the second series. The main difference between two series (at all densities) was, that the decline of tube numbers in the second series began much earlier

and it was much stronger than at the first one. Few larvae which remained in the middle and final phase of the experiment probably used several tubes (abandoned by other larvae due to emergence or mortality) keeping them in good state and prolonging their existence; in the absence of larvae tubes disappeared in few days. The condition of larvae seems to have the essential importance for the firmness of tubes. In the series II (where probably weaker larvae remained) the tubes were much less durable; their numbers at all densities were declining from the beginning of the series, while in the series I often their numbers remained high for a long time. This indicates the high importance of the physical condition of larvae for the quality of tubes.

The inconsistency of different tubes numbers in various types of muds is actually impossible to explain; it is probably the resultat of a number of strongly interconnected features – the kind of muds, the condition of larvae, their density etc.

One of the most unexpected events, difficult to understand, was the frequent, and often great fluctuation of tube numbers. It was great especially at small densities of larvae; obviously the high density of larvae is limiting their activity in the tubes construction. The tube number at low densities (4 and 5 ind. 20 cm^{-2}) fluctuated up to several times, also in the period when there were only single larvae in a cylinder; there were also periods with no tubes and then again relatively high number of tubes appeared (Fig. 1). Few larvae seem to be able to maintain numerous tubes and keep them in good state; in a special series of experiments the tubes without larvae were disappearing in one to few, at the most five days. Keeping many tubes in a good state undoubtedly costs the larvae a lot of energy. It is probable however that they have some profit of this – e.g. collecting the food from the walls of tubes left by other larvae. At high densities of larvae the situation can be opposite: occupation of the same tube by more than one larva (it took place when the tube number per individual was lower then 1.0).

The mineral substrates used in experiments (bentonite and chalck) were found to be not sufficient for the *Chironomus* larvae developement and for the succesfull tubes building. However the addition of some mud (0.5–10 mm) at the top of these substrates increased larval activity expressed by tubes building. It corresponds well with the results obtained in field experiments (Kajak and

Prus 2001), where *Chironomus* numbers were similar on the mineral substrate (bentonite) covered with 1–3 cm layer of sedimented tripton and on the organic mud (83% org. matter). Obviously the most important for *Chironomus* feeding is the few mm top layer of the substrate, composed (in field conditions) mainly of sedimented tripton. The increased getting out from tubes observed after the addition of the valuable food (powdered *Daphnia*) to experimenal cylinders seems to confirm the above statement about the importance of the top layer of the substrate for *Chironomus* feeding.

5. SUMMARY

Experiments on the influence of various initial population densities, various substrate type and various organic matter content on *Chironomus plumosus* population were carried out in laboratory conditions. Cylinders with muds originating from different water bodies and with mineral (bentonite or chalk) substrates and that with extremely low organic matter content were exposed in aquaria with water slowly circulating and constantly aerated. Experimental series I and II were conducted during 3 months, other additional series – during few weeks. Series I and II dealt with *Chironomus* densities in range 4–80 ind. per 20cm^2 cylinder (2–40 thousands ind. m^{-2}). All series (I–IV) dealt also with the organic matter content in substrate (the mineral one and three natural muds) in the range 0–83%. Some observations of larval feeding behaviour were also done, using the technique of microaquaria (their width only slightly exceeded the diameter of grown up larvae).

Strong influence of population density on larval tube numbers was found (Fig. 1). The average tube holes numbers per individual were, as a rule, higher than 1.0 at low (4–5 ind. 20 cm^{-2}) densities of larvae (during the first two months of exposition in series I of the experiments and during one month in series II). These numbers were usually much lower than 1.0 at high (40–80 ind. 20cm^{-2}) densities (during whole exposition period), what indicates strong competition between larvae at their higher densities (Fig. 1, Table 1).

Tube holes numbers did not usually depend on mud type (Fig. 1), but in semi-mineral and fully mineral substrates they were significantly lower than in the natural mud (Fig. 3). The construction of tubes in the humic mud (H) was a bit delayed as compared to other substrates, but the maximal number of tubes was the highest in this mud.

The emergence of imagines occurred from all substrates, including the fully mineral one, with only thin (<1 mm) layer of sedimented mud at the top of it, but it was the highest in natural deposits. The emergence and survival depended strongly on population density, the threshold value was between 40 and 80 ind. 20 cm^{-2} (20–40 thousands ind. m^{-2}). In these variants about 90 and 30% of larvae emerged (survived) respectively (Fig.2, Table 2).

The emergence from the humic mud (H) during initial 5 days of the exposure was about twice more intensive than from other two muds. This happened despite the fact that tubes construction in the mud H was a bit delayed as compared to other two muds. So the humic mud was the only one which had some influence both on tube numbers and the emergence of imagines. The other two muds although quite different each from the other did not influence the registered features of *Chironomus* populations

The feeding of *Chironomus* larvae by collecting particles from internal walls of their tubes was observed in microaquaria. Larvae were periodically getting out from tubes to collect the food particles around the apertures of their tubes. The addition of food (powdered *Daphnia*) increased getting out of tubes 3-times. Larvae were only sporadically collecting food while getting out of tubes in the reservoir deposit (of high nutritive value), but they did it more often in the old-riverbed deposit and (much more intensively) in the pure chalk (substrates of low and very low nutritive value).

The condition of larvae seems to have great importance at their high densities. In the series II, where the condition of larvae was worse than in the series I, the indices of the population, like the mortality, emergence, numbers of tubes were much worse at higher than at lower density of larvae.

All the described differences (dependence on the density of larvae, their condition and the substrate type) were found in populations of grown up ready to emergence larvae. It is very probable, that these differences would be much stronger in populations of younger larvae and these with more diversified age structure.

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