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Regular research paper

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INFLUENCE OF THE POPULATION DENSITY AND THE AMOUNT OF FOOD ON *CHIRONOMUS PLUMOSUS* (L.) AND TUBIFICIDAE. LABORATORY EXPERIMENTS

ABSTRACT: The aim of this paper has been to examine experimentally the importance of the density of larvae and of the addition of the food for *Chironomus* and Tubificidae using selected parameters and indices of their populations.

Increase of the density of *Chironomus plumosus* larvae (0.5–50.0 thousands ind. m⁻²) in laboratory experiments resulted in the decrease of emergence of imagos, number of tube apertures (3.5–0.4 apertures ind.⁻¹), and in the lower rate of tubes building. The addition of the food (powdered dry daphnids or food tablets for aquarial fish) had only slight effect on tube numbers but it decreased clearly the getting out of larvae from tubes (probably due to improved feeding conditions inside tubes). It had also a slight negative effect on the survival of larvae. Numbers and individual growth of Tubificidae were positively dependent on the addition of the food (also in the form of naturally dead *Chironomus* larvae) and negatively – on the density of *Chironomus*.

KEY WORDS: benthic communities, density dependence, interspecific interactions, feeding behaviour, *Chironomus*, Tubificidae

1. INTRODUCTION

Chironomus plumosus (Diptera: Chironomidae) and few species of Tubificidae belong to the most common and important species in eutrophic freshwaters outside the littoral. They are important as the food for fish (Konstantinov 1958, Hruška 1966, Kajak 1998), and also as components seriously influencing the exchange of substances between bottom deposits and the overlying water (Wiśniewski and Planter 1985). *Chironomus* numbers in natural waters vary enormously – from dozens ind. m⁻² to about hundred thousands ind. m⁻² (Sokolova 1983, Kajak 1987). The causes of this as well as of fluctuations of numbers during the year are often not clear. Undoubtedly oxygen and food conditions are important (Sokolova 1983, Kajak 1990, Armitage *et al.* 1995, Kajak 1997, Kajak and Prus 2001b, 2003). Density *per se*, especially when it is very high, is certainly essential for the survival of individuals and the population

dynamics. A number of authors showed the importance of mutual relations of *Chironomus* for the development of its populations at various (including quite low) densities, as well as the importance of relations *Chironomus* – Tubificidae for numbers and development of the latter (Konstantinov 1958, Kajak 1963, 1987, Yashuow 1970, Jonasson 1977, Marian and Pandian 1985, Kangur 1989, Lindegaard *et al.* 1993, Nakazato and Hirabayashi 1998).

The aim of this paper has been to examine experimentally the importance of the density of larvae and of the addition of the food for *Chironomus* and Tubificidae using selected parameters and indices of their populations.

2. METHODS

The experiments were mostly carried out with grown up *Chironomus* larvae (18–23 mm) from the Vistula r. oxbow lakes. Only in one series, with intact cores of mud from the Zegrzyński dam reservoir (on Bug and Narew Rivers, 40 km from Warsaw), larvae were smaller (on average 17 mm, range 6–20 mm).

The experiments were conducted in "miniaquaria" – plastic cylinders of 20 cm² of the surface area and 15 cm high. They were filled with the mud from the dam reservoir (9% of organic matter), up to 5 mm below the upper edge of the cylinders; benthic organisms had previously been removed from the mud by sewing. The cylinders were placed in the glass aquaria (40 × 70 cm, and 25 cm high) with the water (its level was 5 cm above the upper edges of cylinders) circulating due to aquarial turbines. Horizontal plastic sheets located in the aquaria just below upper edges of cylinders ensured the circulation of the surface water layer in aquaria, so that the mud surface and the water over it in miniaquaria were perfectly aerated. See Kajak and Prus (2000) for more detail description of the methods.

Densities of larvae applied in experiments were between 0.5 and 50 thousands ind. m⁻²; that is within the range of densities occurring in nature (Sokolova 1983, Kajak 1987).

Three types of experiments were carried out:

- without additional feeding (18 series),
- the addition of food (powdered dry daphnids, or powdered food tablets for aquarial fish) at various densities of *Chironomus* larvae (11 series),
- influence of various densities of *Chironomus*, together with the food addition on Tubificidae numbers and growths (5 series).

Altogether 34 series were conducted, 10 cylinders were used in each variant of particular series. Experiments lasted 2–4 weeks. The following indices of the *Chironomus* population were observed:

- numbers of tube apertures per individual,
- getting out of larvae from their tubes to feed,
- survival of larvae,
- emergence of imagos.

These very important features of the population are very difficult or impossible to be examined in nature. Numbers of tube apertures were observed every few days, getting out of larvae – daily or every second-third day, 0.5–1.0 hour after the addition of the food, during 15 minutes each time. Survival was estimated at the end, sometimes also in the middle of the exposure period. Emergence of imagos was observed every day, by counting exuviae of pupae floating at the surface of the water in tubes made of plastic netting with the mesh size 1×1 mm, encircling tightly each of the cylinders, and stretching few cm over the water surface (Kajak and Prus 2000). In some series the influence of the food addition and of various *Chironomus* densities on the growth and numbers of Tubificidae was also observed. Kruskal-Wallis ANOVA statistics was applied for all series of the data;

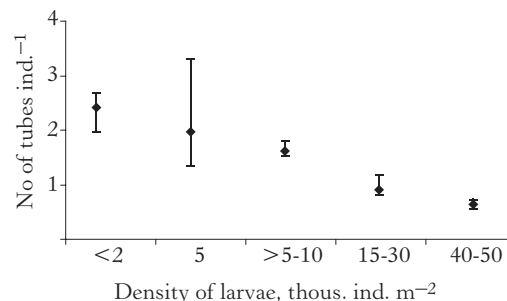


Fig. 1. Numbers of tube apertures (per individual) at different densities of *Chironomus plumosus* larvae. The average values for several series at each density are given; the range marked with the vertical bars.

the differences with P value ≤ 0.05 were treated as statistically significant.

3. RESULTS AND DISCUSSION

3.1. Influence of the density of larvae and the addition of the food on *Chironomus* population

Number of tubes per individual was decreasing regularly with the increasing density of larvae (Fig. 1). The highest value equal to 3.5 tubes ind.⁻¹ was found at low (5 thousands ind. m⁻²) but not the lowest density. Next values like 2.8 tubes ind.⁻¹ were found at lowest density (≤ 2 thousands ind. m⁻²). The average number of tubes was the highest (2.6 tubes ind.⁻¹) at the lowest density. The lowest number of tubes (0.4 per ind.) was found at 40–50 thousands ind. m⁻² (Fig. 1). Similar reduction of tube number per individual at high population densities (20–40 thousands ind. m⁻²) was observed in previous experiments (Kajak and Prus 2001a). The addition of the food resulted in the increased tube number only in some series and to the small degree (Fig. 2A).

Number of tubes built on the first day was the highest (about 90% of the maximal numbers, which occurred on the 4. day) only in one variant, that one with the lower density without additional feeding. In all other variants the number of tubes on the first day reached only 40–60 % of the maximal ones (Fig. 2B.) So both the very high density and additional feeding diminished the rate of tubes building, although probably mechanisms were completely different. The additional feeding both at low and high densities resulted in about 40% tubes built on the first day. Probably the larvae did not need to build the tubes, because they could get enough food at lower tube numbers. Lower tube numbers (about 60% of maximal) at very high density of larvae (40 thousands ind. m⁻²) with no additional feeding resulted probably from the limiting strong mutual influences of larvae at this high densities.

The influence of the food addition on getting out of larvae from tubes was examined at densities of larvae 10 and 40 thousands ind. m⁻² (Fig. 2C). Usually at the beginning of the experiment only the short front part of the body was slipping out the

tube; later larvae slipped out of tubes for almost their whole length. The hind "legs" and the very posterior part of the larva always remained in the tube. Only in two of 11 series larvae did not slip out of their tubes; so in about 80% of series they did, to collect the food particles from the surface of the substrate. Getting out of larvae from tubes in variants with the addition of the food was much less intense than in control ones at both densities (Fig. 2C; the differences in all series were significant at

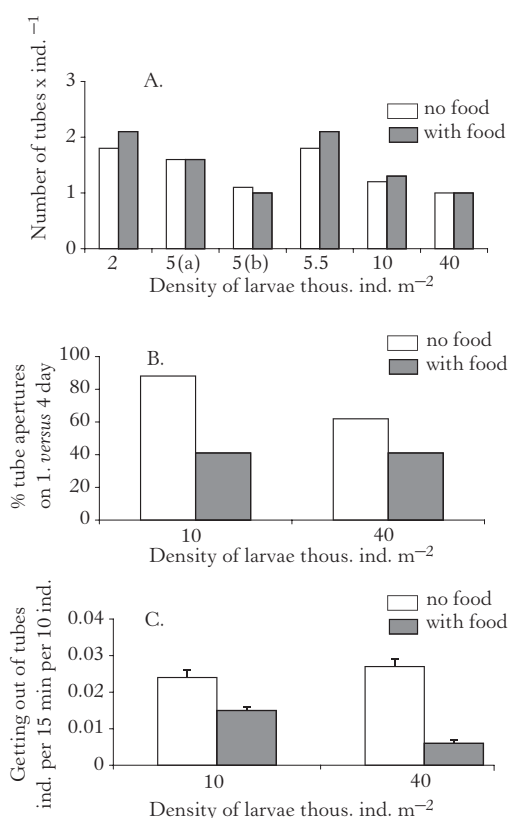


Fig. 2. Influence of the addition of the food and population density on numbers of tube apertures of *Chironomus plumosus* and getting out of larvae from tubes:

A – Average tube numbers (per individual) for the exposition period (3 weeks). Two series with the density of 5.0 thousands ind m⁻² which differed significantly – marked as (a) and (b); differences "no food" – "plus food" are significant for 2.0 and 5.5 thousands ind. m⁻² ($P < 0.05$ and $P < 0.0000$, respectively), all other differences between feeding variants are insignificant ($P > 0.5$);
 B – % of tubes built at 1 day versus 4 day (when their numbers were maximal);
 C – Getting out of larvae from tubes, calculated for 10 larvae and 15 min. of observation.

$P \leq 0.050$). Obviously the food was getting inside the tubes and probably it intensified the development of the bacteria also inside the tubes. Larvae, having enough food in tubes, could slip out less frequently, because the filtration causes much less risk of predation, than collecting of food particles from the surface of the substrate. However in some experiments (Kajak and Prus 2001a) the opposite effect of additional feeding, i.e. more intense getting out from tubes, was observed. Therefore the mechanism described above is probably not a rule. Higher frequency of getting out of tubes was observed in previous experiments on substrates poor in organic matter (e.g., bentonite and chalk) in comparison with substrates providing good feeding conditions (like reservoir mud, which has 9% of organic matter) (Kajak and Prus 2001a). This is an evidence for reduction of getting out of tubes in improved feeding conditions.

The emergence of imagos was lower (about 45%) at the higher density of 5 thousands ind. m^{-2} than at 2 thousands ind. m^{-2} (62%) during the whole monthly exposure. However during the first 10 days the effect was opposite: the higher density of larvae did speed up the emergence of imagos (Fig. 3). Obviously the higher density brought about some mechanisms speeding up the emergence at the beginning of the exposure.

Evidently at these relatively low densities interconnections and mutual relations of larvae strongly influence the important parameters of the population like number of tubes (essential for feeding, respiration, protection from predators), growth rate, emergence, survival. Effects of mutual relations in nature were observed at even much lower densities of *Chironomus* larvae i.e.

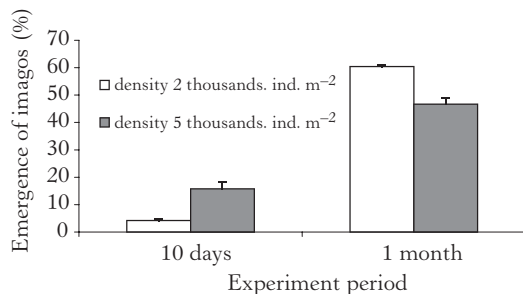


Fig. 3. Emergence of imagos at two densities of larvae and during first 10 days of exposure and during 1 month. Differences significant at $P = 0.0000$ for 10 days exposure and at $P = 0.03$ for monthly exposure.

below 1 thousand ind. m^{-2} (Kajak 1963). Nevertheless sometimes densities of larvae both in nature (Kajak 1987) and in cultures (Konstantinov 1958, Yashouy 1970) are enormous - up to 100 thousands ind. and about 1 kg fresh weight of the biomass per square meter. Decreased rate of tubes building (from 2 to 0.5 tube per ind.) and survival (from 90 to about 30%) were observed in previous experiments at very high (40–80 thousands ind. m^{-2}) densities, as compared to moderate ones (4–5 thousands ind. m^{-2}) (Kajak and Prus 2001a).

The larvae used in experiments were big (about 20 mm or more) so their growth rate was slow and its differences between variants were negligible. Only in the series with intact cores of the mud and natural benthic community the larvae were smaller (average length about 17 mm; range 6–20 mm). The growth of larvae in this series was remarkable: the final average size of larvae was 21.5 mm in the variant with additional feeding, while 18.5 mm in the control. So the younger larvae grew much quicker when additionally fed.

The survival of larvae was lower in variants with the addition of the food than in the control at densities up to 20 thousands ind. m^{-2} (Fig. 4). This was probably caused by less favourable oxygen conditions in variants with food addition, resulting from the increased oxygen consumption due to decomposition of the remains of food, not eaten by larvae. Positive influence of the additional feeding on larval growth,

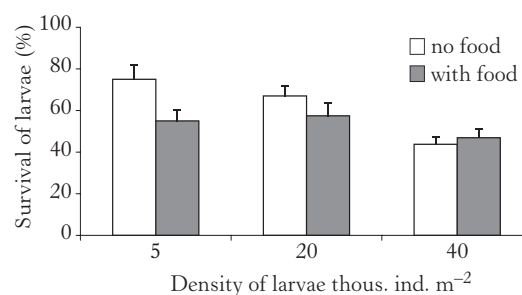


Fig. 4. Survival of larvae at their various densities in feeding treatments: controls (blank bars) and with the addition of the food (hatched bars). Differences between treatments (control and with the additional feeding) at 5 and 20 thousands ind. m^{-2} are significant at $P < 0.03$. Differences between densities 5 and 20 thousands ind. m^{-2} together versus 40 thousands ind. m^{-2} are significant at $P = 0.05$ for both feeding treatments. Standard error marked with vertical bars.

emergence and survival was stated in other experiments, both in the laboratory (Kajak and Prus 2000) and in the field (Prus unpublished), at very different population densities (<1–40 thousands ind. m⁻²). In the present study only at the highest density (40 thousands ind. m⁻²) the survival was equal in both feeding variants, and the lowest of all density variants (Fig. 4). Obviously in this situation it was the high density itself which decided about the survival, rather than feeding and oxygen conditions.

3.2. Influence of the addition of food and of various *Chironomus* densities on Tubificidae

Increasing density of *Chironomus* caused clearly decreasing density of Tubificidae (Fig. 5A, B). The strong negative influence of *Chironomus* on Tubificidae was sometimes observed (Yashouv 1970, Marian and Pandian 1985). In the experiment with the data monitored every 5 days it was observed almost immediately i.e. during first few days of the exposure (Fig. 5B). The decreased level of the density of Tubificidae lasted for next 10 days (till the end of the experiment) at lower (2.5 thousands ind. m⁻²) densities of *Chironomus*. At next two highest *Chironomus* densities (10–20 thousands ind. m⁻²) the numbers of Tubificidae became to rise after few days since their decrease. The most probable cause of this rise after 10 days of exposure is the mortality of *Chironomus*. This mortality increases with the increasing density of *Chironomus* (from 0% at 0.5 thousands ind. m⁻² to about 50% at 40 thousands ind. m⁻²), supplying the high value organic matter from the dead *Chironomus* larvae - the source of the food for Tubificidae. At the same time the negative influence of *Chironomus* on Tubificidae decrease, due to the decreasing numbers of *Chironomus* larvae. At 2.5 thousands *Chironomus* larvae per m² numbers of Tubificidae declined and stayed at the low level. Obviously the "manuring" of the environment by dead *Chironomus* larvae was here too small to cause the increase of Tubificidae abundance. Only the lowest density of *Chironomus* (0.5 thousand ind. m⁻²) had no influence on the density of Tubificidae, which was stable during all the exposure time (Fig 5B).

The addition of the food affected positively and very distinctly numbers and growth of Tubificidae. The share of big individuals was greater in the variant where the food was added (Fig. 5C). It was so although the food was added on the surface of substrate, while Tubificidae feed a few cm under the surface. Obviously the food added influenced trophic conditions not only at the surface but also at the depth of

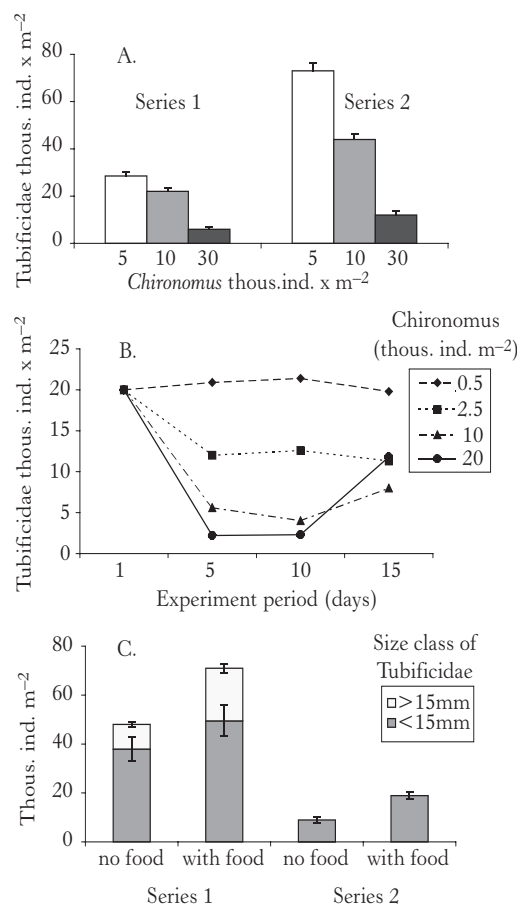


Fig. 5. The effect of the increasing density of *Chironomus plumosus* (A, B) and addition of the food (C) on the density of Tubificidae. A. Two series of experiments; three *Chironomus* densities in each, two weeks of the exposure; differences between treatments are significant at $P = 0.001-0.05$. B. Four variants of *Chironomus* density, two weeks exposure time; all differences between treatments are significant at $P < 0.0000-0.03$. C. Tubificidae cultivated without *Chironomus* larvae; two series of experiments with the addition of the food on the surface of the mud. In series no 2 the size of individuals was not registered. All differences are significant at $P = 0.02-0.03$; standard errors (marked with vertical bars) never exceeded 15% of the average.

the feeding of Tubificidae. No difference between the effects of powdered dry *Daphnia* and powdered tablets of the food for aquarial fish was observed.

4. CONCLUSIONS

1. Numbers of tube apertures were decreasing regularly with the increasing density of larvae – from about 2.6 apertures per individual (maximal value 3.5) at the low (few thousands ind. m⁻²) density of larvae, to 0.4 aperture ind.⁻¹ at their high density (40–50 thousands ind. m⁻²) (Fig. 1). Also the emergence of imagos was lower at higher densities of larvae. However, during first decade of the experiment the emergence at the high density was much higher than at the low one (Fig. 3).

2. At lower density without the additional feeding the maximal number of tubes was achieved during the first day of the experiment, while at higher density and in the variant with the additional feeding – only after a few days (Fig. 2B).

3. The addition of the food sometimes had the slight positive, but insignificant effect on number of tubes at various densities (Fig. 2A).

4. The addition of the food decreased distinctly the intensity of getting out of larvae from their tubes (Fig. 2C), evidently by the improved feeding conditions inside the tubes. It also influenced negatively the survival of larvae (Fig. 4), probably due to less favourable oxygen conditions caused by the decay of food remains.

5. Numbers and growth of Tubificidae reacted very positively on the addition of organic matter, both in the form of additional feeding and of dead *Chironomus* larvae (as a result of their natural mortality) and negatively – on the density of *Chironomus* (Fig. 5).

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5. SUMMARY

Three types of experiments were carried out: 1) various densities of *Chironomus* larvae without additional feeding (18 series), 2) the addition of food (powdered dry daphnids, or powdered food tablets for aquarial fish) at various densities of *Chironomus* larvae (11 series), 3) influence of various densities of *Chironomus*, and of the addition of the food on Tubificidae (5 series).

Increase of the density of *Chironomus plumosus* larvae (0.5–50.0 thousands ind. m⁻²) in laboratory experiments resulted in the decrease of: number of tube apertures (Figs 1 and 2A), rate of tubes building (Fig. 2B), emergence of imagos (Fig. 3) and survival of larvae (Fig. 4). The addition of the food had only slight effect on tube numbers but it decreased clearly the getting out of larvae from tubes (Fig. 2C), probably due to improved feeding conditions inside tubes. It had also a slight negative effect on the survival of larvae (Fig. 4). Numbers and growth of Tubificidae were positively dependent on the addition of the food (also in the form of naturally died *Chironomus* larvae) (Fig. 5B, C) and negatively – on the density of *Chironomus* (Fig. 5A).

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