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Effect of silt, water and periphyton quality on survival and growth of the mayfly *Heptagenia sulphurea*

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Abstract

The herbivorous mayfly *Heptagenia sulphurea* is characteristic of rivers with stony bottoms. Records from the 20th century showed that this species had disappeared from the Common Meuse in the Netherlands, probably due to river regulation or changes in water quality. A field survey in 2003 showed that *H. sulphurea* was present in the Geul tributary, approximately 300 m upstream of its confluence with the Common Meuse. *H. sulphurea* has not recolonized the Common Meuse despite improvements in water quality over the last decades. Concentration of suspended sediments in the River Meuse, however, is still high, much higher than in the beginning of the 20th century. The presence of a silt layer may limit the return of *H. sulphurea* in this river by reducing availability and quality of its food. The prime objective of this study was to investigate the impact of silt on survival and growth of *H. sulphurea* in a laboratory experiment. Furthermore, the impact of water and periphyton quality from the Common Meuse on survival and growth of this mayfly was also investigated. Results showed that neither water quality nor cultured periphyton from the Common Meuse reduced survival and growth of *H. sulphurea*. The presence of a silt layer resulted in a significantly lower growth rate of the mayfly larvae. It is concluded that the silt layer reduces the accessibility of the food. Covering of food is possibly one of the main factors limiting the recolonization of *H. sulphurea* and probably other benthic grazers in the Common Meuse.

Introduction

The mayfly *Heptagenia sulphurea* (Müller 1776) is a typical inhabitant of rivers with a stony substratum (Macan 1957; Hynes 1970). At the end of the 19th century Albarda (1889) first reports this species in the Common Meuse, a small part of the Meuse river in the southern part of the Netherlands forming the frontier between Belgium and the Netherlands. It is a gravel bed river with an armoured bed, with flow velocities up to 4 m s^{-1} and coarse sediment (Duizendstra 2001). According to Redeke (1948) *H. sulphurea* is characteristic of this part of the River Meuse. More recent observations (reviewed by Bij de Vaate 1995) showed that by the 1980s this species had disappeared from the Common Meuse together with a decline or disappearance of species of Plecoptera, Odonata, Hemiptera, Coleoptera, Trichoptera, and other Ephemeroptera. The low aquatic macroinvertebrate biodiversity in the Common Meuse in comparison to other rivers is assumed to be due to pollution, high load of suspended sediments, eutrophication, canalisation, habitat destruction, and artificial discharge fluctuations caused by the hydro-electric power station in the Lixhe weir in Belgium (Bij de Vaate 2003).

Over the last two decades, water quality of the River Meuse has significantly improved (Admiraal et al. 1993; Bij de Vaate 2003). Concentrations of trace metals, polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and mineral oil have decreased whereas dissolved oxygen content has increased (Bij de Vaate 2003). Stuijfzand et al. (1999) reported that despite this improvement, toxicant levels in the water in the mid 1990s were still too high for hydropsychid caddisflies to recolonize and maintain stable populations in Common Meuse. The concentration of suspended sediments in the River Meuse increased threefold between 1880 and 1980 (Micha and Borlee 1989). On average, the concentration of suspended sediments has dropped from 40 mg/l in the 1970s to 25–30 mg/l in the 1990s (unpublished data Dutch State Institute for Inland Water Management and Waste Water Treatment, RIZA) but concentrations are still much higher than observed by Redeke (1948) in 1938 (12 mg/l) and may still be an impediment to benthic macroinvertebrates (Bij de Vaate 2003).

In general, it is well-known that suspended sediments in rivers affect aquatic life (Doeg and Milledge 1991; Walling and Webb 1992) with light silting ($\sim$1 mm thick) on rock surfaces having a variable effect on the insect fauna and heavy silting generally resulting in lower insect species diversity (Minshall 1984). Although, according to Zweig and Rabeni (2001) it is difficult to formulate a comprehensive theory of the effects of suspended sediments on benthic fauna from published data due to differences in objectives and methodology, some general responses to deposited sediments are known. Allan (2004) summarizes that sedimentation among others impairs substrate suitability for periphyton and biofilm production, decreases primary production and food quality, harms crevice-occupying invertebrates and gravel-spawning fishes by filling of interstitial habitat, and coats gills and respiration surfaces. Furthermore, Zweig and Rabeni (2001) report shifts in benthic community composition as a result of increased sedimentation and Osmundson et al. (2002) suggest cascading effects of accumulation of fine sediment in the riverbed of the Colorado River by reducing periphyton and macroinvertebrates standing crops resulting in a limited availability of prey fish for the endangered Colorado Pikeminnow.

Most mayfly species are known as sensitive to pollution (Bauernfeind and Moog 2000). With the water quality improvement in the Common Meuse, it may be expected that mayflies would return to this part of the river. However, despite the improved water quality no observations of *H. sulphurea* in the Common Meuse have been reported since (unpublished data RIZA). Availability and quality of food is important for all secondary consumers and *H. sulphurea* gathers its food by grazing periphyton layers on hard surfaces (Merritt and Cummins 1984). The productivity and composition of periphyton layers is affected among others by water quality (Courtney and Clements 2002; Guasch et al. 2003) and the deposition of fine sediments (Yamada and Nakamura 2002). Decreases in primary production due to sedimentation produce negative cascading effects through depleted food availability (Henley et al. 2000). Although it is known that a relatively thick layer of silt is present on hard surfaces in the Common Meuse (Van den Burg et al. 2000), no investigations have been performed to find out whether this may be an important factor hampering the return of *H. sulphurea* in the Common Meuse.

The prime objective of the present study was to investigate the impact of coverage of periphyton by deposited sediments on survival and growth of *H. sulphurea* in a laboratory experiment. Furthermore, the impact of the quality of water and periphyton from the Common Meuse on survival and growth of this mayfly was also investigated. It was hypothesized that water and periphyton from the Common Meuse would not affect mayfly performance whereas the presence of a silt layer would reduce its survival and growth because food may be less available and quality may be lower.

Material and methods

Preliminary field study

H. sulphurea was reported in a number of Dutch tributaries of the Common Meuse (unpublished data waterboard authority Roer and Overmaas).

A visit in October 2003 to this area indicated that a population (10 ind m^{-2}) of *H. sulphurea* was present in the Geul tributary at a distance of approximately 300 m from the confluence with the Common Meuse (Figure 1).

To determine the thickness of the silt layer in the Common Meuse, stones of different sizes were carefully picked out of the river and washed separately to remove the silt present. It was observed that the stones were not only covered by silt but also with macroalgae. The surface area of each stone was determined as well as the amount (wet weight) of silt on it. There was a significant positive correlation between stone surface area and the amount of silt present ($R^2 = 0.8247$), with an average of 0.225 g wet weight per cm^2. Analyses showed that the proportion of organic matter in the silt was $4.6 \pm 1.6\%$ ($n = 10$). Stones from the Geul tributary were also collected, however, they were not covered either by silt or macroalgae at all.

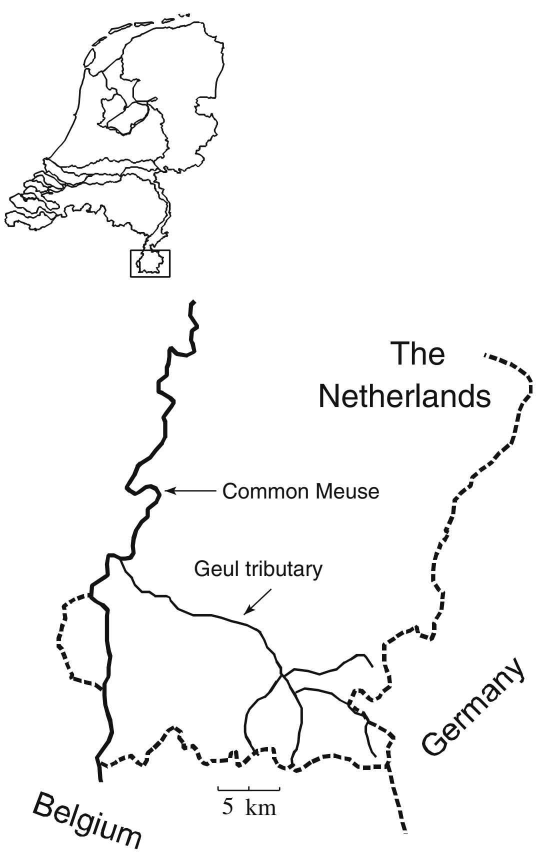

Figure 1. Study area with the Common Meuse and Geul tributary.

Test organisms

In December 2003 (5 weeks prior to the start of the experiments) nymphs of *H. sulphurea* were collected from the Geul. Animals were collected by carefully washing them off stones into a bucket. Animals were brought to the laboratory and kept in aerated water from the Geul. Water temperature in the Geul was 9 °C at the time of sampling, and in the laboratory, water temperature was increased daily by 0.5 °C to a temperature of 15 °C. Animals were fed with leaves and associated biofilms from the Geul. Light–dark regime was 10:14 h with a light intensity of 35 μmol m^{-2} s^{-1}.

Preparation of periphyton layer

Periphyton was obtained by cleaning 5 stones from the Common Meuse and from the Geul tributary. Periphyton was scraped from the stones and kept in a 1 l bottle filled with 0.8 l water from the Common Meuse and Geul respectively. In the experiments, 30 ml of the periphyton suspension was added to aquaria ($17 \times 10.5 \times 11.5$ cm) that were filled with 0.5 l filtered water from either the Common Meuse or the Geul. All aquaria were held at a constant temperature (15 ± 1 °C) for 2 weeks with 24 h of light with an intensity of 35 μmol m^{-2} s^{-1}.

Preparation of silt layer

To exclude effects of contaminants, fine sediment (< 1 mm) was collected from a nearby, unpolluted pond and kept frozen (−20 °C) for 3 weeks to kill any invertebrates present. This sediment had an organic matter content of 30%. To obtain a similar percentage as observed in the Common Meuse (4.6%) clay was added, as clay is an important part of the suspended sediments in the Common Meuse (Van den Burg et al. 2000). Sediment was added to each aquarium 1 day before the start of the experiment by means of a small sieve with a mesh-size of 1 mm. Based on the surface of the aquaria (178.5 cm^2) and the thickness of the silt layer in the Common Meuse (0.225 g cm^{-2}) a total of 40 g wet weight was added to each aquarium.

Preliminary experiments

A small preliminary experiment was performed to test whether *H. sulphurea* could survive in aquaria filled with water either from the Common Meuse or the Geul and a periphyton layer on the bottom. It appeared that after one month all specimens were alive and still growing.

In a second experiment, changes in oxygen were recorded after adding a layer of silt to the aquaria that were not aerated. Oxygen concentrations in the water remained the same (> 9.0 mg l^{-1}) over a period of 15 days, even near the sediment.

Experiment 1. Effect of water- and periphyton quality on H. sulphurea

A 2 × 2 factorial laboratory experiment was performed to investigate the effect of water- and periphyton quality on larvae of *H. sulphurea*. Periphyton originating from the Common Meuse or the Geul was combined with water from the Common Meuse or the Geul. Water was first filtered over 0.45 μm to remove suspended material. With 5 replicates for each treatment, 20 aquaria (17×10.5×11.5 cm) were used and placed in a water bath to maintain a temperature of 15 ± 1 °C. In each aquarium, 5 individuals of *H. sulphurea* were placed. The experiment lasted for 37 days.

Experiment 2. Effect of silt on H. sulphurea

A second experiment was performed to investigate the effect of the presence of a layer of silt on *H. sulphurea*. Periphyton and filtered water from the Geul tributary were used. Twenty aquaria (17×10.5×11.5 cm) were used and placed in a water bath to maintain a temperature of 15 ± 1 °C, and in 10 aquaria silt was added in a concentration of 0.225 g wet weight per cm^2. In each aquarium, 5 individuals of *H. sulphurea* were placed. The experiment lasted for 37 days.

Measurements

For both experiments, all aquaria were inspected daily for dead animals and exuvia. Dead animals and exuvia were counted, and removed from the aquaria and recorded. At the end of the experiment the number of survivors was recorded. The average number of exuvia per specimen was calculated per aquarium.

Total length and head width of living *H. sulphurea* were measured by microscopy with digital imaging (Leica, QM3500, Leice Microsystems, AG, Wetzlan, Germany) before the start and at the end of the experiments. Length was measured as the length from the top of the head to the end of the abdomen and width as the widest part from eye to eye (Tseng 2003). Specimens were not marked individually and, therefore, increase in length or width was averaged per aquarium. Increase in length was calculated as mean length at the end of the experiment minus the mean length at the start and increase in width was calculated similar. During the experiment it was observed that in certain treatments individuals were located at the sides of the aquaria. Therefore, at the end of the experiment the positions of the individuals on the bottom and on the sidewalls were counted and recorded in each aquarium.

Statistical analyses

Shapiro–Wilk tests demonstrated that increase in length and width and the number of exuvia in both experiments were normally distributed and, therefore, Analysis of Variance (ANOVA) was performed to test for significant differences between treatments. The Levene statistic was calculated to test for homoscedasticity. Shapiro–Wilk tests demonstrated also that survival and position of individuals were not normally distributed in both tests even after arc sinus square root transformations and, therefore, Kruskal–Wallis tests were applied to test for significant differences between treatments. All analyses were done with the available options within the software programme SPSS version 10.1.

Results

Experiment 1. Effect of water quality and periphyton on H. sulphurea

Survival was high in all treatments ranging from 84 to 96% and was not significantly different between treatments (Kruskal–Wallis: χ^2:1.583;

df = 3; $p = 0.663$). At the end of the experiment there were clear differences in growth between the treatments (Figure 2). ANOVA showed that increase in head width was not significantly different between treatments ($F_{3,16} = 0.801$; $p = 0.511$), but differences in increase in length were significant ($F_{3,16} = 10.827$; $p = 0.000$). Tukey's *post-hoc* test showed that only in one treatment, Common Meuse periphyton with Common Meuse water, increase in length was significantly higher. The mean number of exuvia per individual ranged from 2.6 to 3.0 and was not significantly different between treatments ($F_{3,16} = 1.092$; $p = 0.381$). Most individuals (between 80 and 100%) were found on the bottom of the aquaria and very few on the sidewalls. Differences in position of the individuals between the treatments were not significant (Kruskal–Wallis: $\chi^2 = 2.369$; df = 3; $p = 0.499$).

Experiment 2. Effect of silt on H. sulphurea

Survival was high (92–94%) and not significantly different between treatments (Kruskal–Wallis: $\chi^2 = 0.209$; df = 1; $p = 0.648$). At the end of the experiment there were clear differences in growth between the treatments (Figure 3). ANOVA showed that the increase in length was significantly lower for the silt treatment ($F_{1,18} = 13.910$; $p = 0.02$) as was the increase in head width ($F_{1,18} = 8.191$; $p = 0.010$). The presence of a silt layer had no significant effect on the number of exuvia ($F_{1,18} = 1.150$; $p = 0.298$) but resulted in a higher percentage of individuals observed on the sidewalls (approximately 55%). These differences in position between the treatments were significant (Kruskal–Wallis: $\chi^2 = 16.493$; df = 1; $p = 0.000$).

Discussion

The fieldwork performed during the present study showed that *H. sulphurea* was present in the Geul tributary approximately 300 m upstream of the confluence with the Common Meuse. Drift, the downstream dispersal of animals, is a common phenomenon in rivers and is an important mechanism of recolonizing downstream areas by aquatic macroinvertebrates (Hynes 1970; Brittain and Eikeland 1988). No information could be found in literature regarding drift distances of *H. sulphurea*, but it seems likely to assume that *H. sulphurea* is able to reach the Common Meuse. Therefore, their apparent absence from the Common Meuse is likely to be due to the conditions in the river itself, rather than in its tributary.

The results of the present study clearly showed that filtered water from the Common Meuse had no negative effect on the survival and growth of *H. sulphurea*. Surprisingly, increase in total length

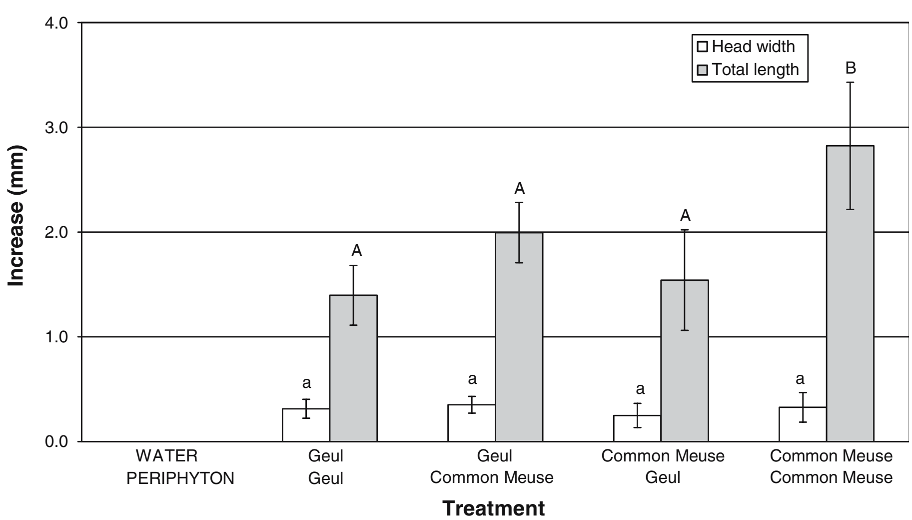

Figure 2. Mean (± SD) total length and head width for the experiment in which *H. sulphurea* was exposed to combinations of filtered water and periphyton from both the Common Meuse and the Geul. Treatments with a similar letter belong to the same Tukey's homogeneous subset (ANOVA, Tukey's *post-hoc* test, $p < 0.005$).

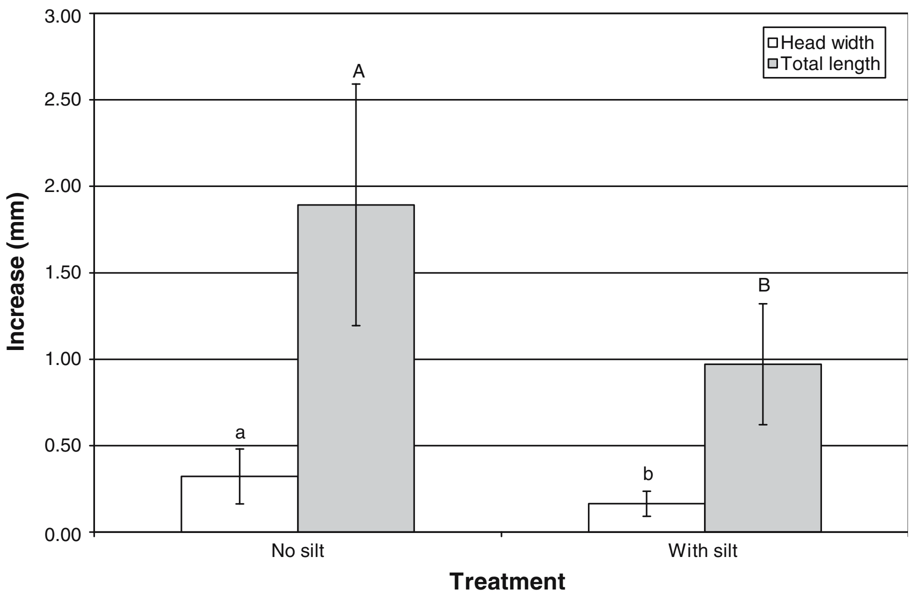

Figure 3. Mean (±SD) total length and head width for the experiment in which *H. sulphurea* was exposed to periphyton with and without a layer of silt. Treatments with a similar letter belong to the same Tukey's homogeneous subset (ANOVA, Tukey's *post-hoc* test, $p < 0.005$).

was highest in the treatment where both water and periphyton came from the Common Meuse. Comparison of water quality data from the Geul tributary and the Common Meuse over the period 1980–2002 showed that for most trace metals concentrations were in the same order of magnitude, except for zinc. Zinc concentrations in the Geul tributary were much higher than in the Common Meuse, approximately 200µg/l and 50µg/l respectively, and mayflies are known to be sensitive to metal pollution (e.g. Hickey and Golding 2002; Carlisle and Clements 2003). This may explain the observed differences in growth of *H. sulphurea* in the present experiments.

The present experiment showed that *H. sulphurea* survived and grew well on cultured periphyton that originates from the Common Meuse and, therefore, the quality of that periphyton seems good enough to sustain growth of *H. sulphurea*. The presence of a layer of silt on periphyton did not affect the survival of *H. sulphurea* in the laboratory experiment, but had a significant effect on growth. Silt may affect the stability of a substratum for macroinvertebrates (Minshall 1984) through various direct or indirect mechanisms (Martin and Neely 2001). Direct mechanisms include loss of suitable habitats and burial of major food sources whereas indirect mechanisms include changes in the productivity of periphyton, reductions in oxygen, and the presence of pollutants absorbed to sediment particles (Lenat et al. 1981; Lemly 1982; Quinn et al. 1992; Henley et al. 2000; Martin and Neely 2001; Allan 2004). Observations on the position of the individuals in the aquaria showed that more individuals were on the sidewalls in the treatment with a silt layer. Furthermore, oxygen concentrations remained at a sufficiently high level during the experiment and accumulation of particles on the animals probably did not take place because the animals were placed in the aquaria 1 day after adding the suspended sediments. Therefore, it is more likely that the reduced growth of the mayflies in the present experiment was due to the covering and thus the accessibility of the food. This is also supported by the observation that some individuals on the sidewalls in the aquaria with silt were grazing, indicating that they might be merely looking for food rather than avoiding bad environmental conditions.

Conclusions

In conclusion, the present laboratory study analyzed the effect of three factors that could have an adverse effect on growth and survival of

H. sulphurea in the Common Meuse. Although the experiments were performed in aquaria, it appeared that *H. sulphurea* survived well probably as a result of the good oxygen conditions. Furthermore, the main mechanism studied in this experiment, covering of food, also occur in lotic environments. The results showed that both filtered water and periphyton from the Common Meuse do not limit survival and growth of this mayfly. However, the presence of a layer of silt on the periphyton significantly reduced growth of *H. sulphurea* by reducing the accessibility and thus the availability of food. This is probably one of the main factors that limit the recolonization of the mayfly *H. sulphurea* and other grazers in the Common Meuse. For the restoration of the ecosystem in the Common Meuse and thus for the return of *H. sulphurea* in this river, the amount of suspended sediment in the river water should be reduced.

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