Life cycle of *Agapetus fuscipes* (Trichoptera, Glossosomatidae) in a first-order upland stream in central Germany

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Abstract

The number of immature stages and the seasonal patterns of development are basic life history features of a stream dwelling species and knowledge about these important components are essential for understanding its adaptations to its dynamic environment. The life cycle of *Agapetus fuscipes* (Trichoptera, Glossosomatidae), one of the dominant scrapers in the upper and middle reaches of the Breitenbach, a first-order upland stream in central Germany, was analysed. The pronotum length and the relationship between pronotum length, larval biomass and case length showed seven distinct larval instars, contrary to earlier findings from the Breitenbach. In addition to a few trichopteran species from other functional feeding groups, *A. fuscipes* is the only scraping caddis fly reported to have more than five larval instars. The moult increments of pronotum length and larval biomass were distinctly lower than in glossosomatid species with five larval instars. *A. fuscipes* is clearly univoltine in the Breitenbach. First-instar larvae were found from July to the beginning of December, and second-instar larvae from July to January. At the beginning of December the population consisted of the instars I to V, and development did not cease during winter. The sixth-instar larvae occurred mostly in January, and the seventh-instar larvae were never present before January. The prepupae and pupae occurred in April. The last pupae were found at the beginning of September, although most of the emergence took place in June and July. At least five different immature stages with different ecological demands were present at any time throughout the year. The ecological advantage having two additional larval instars compared to other glossosomatid species may be to compensate for the high rate of mouthpart wear that occurs while the larvae feed on the rough Bunter Sandstone substratum. A further advantage may be to spread the risk of high mortality under unfavourable environmental conditions.

Keywords: *Agapetus fuscipes*; Life cycle; Seven larval instars; Larval biomass; Case length; Moult increments; Breitenbach; Upland stream

Introduction

Basic information on a species' life history is essential for understanding its adaptation to its environment as well as the functions and interactions of biological communities (e.g. Butler, 1984; Roff, 1992; Stearns, 1992). Knowledge of the number of immature stages and the patterns of growth and development throughout the year is an important component of this basic information.

Glossosomatid larvae are typical scrapers in rivers and streams of all faunal regions (Wiggins, 1996).
Glossosomatid species generally have five larval instars (e.g. Anderson & Bourne, 1974; Irons, 1988; Sameshima & Sato, 1994; Houghton & Stewart, 1998), which is also typical for most other Trichoptera. However, *Agapetus fuscipes* Curtis is an exception. Several authors have reported more than five larval instars (Nielsen, 1942; Benedetto, 1975; Iversen, 1976; Recasens & Murillo, 1986; Sangpradub, Giller, & O'Connor, 1999). *A. fuscipes* is common in European upland and lowland streams (Botosaneanu & Malicky, 1978; Pitsch, 1993; Robert, 2001; Fischer, 2003; Nijboer, 2004) and is one of the dominant trichopteran species in the upper and middle reaches of the Breitenbach, a first-order upland stream in central Germany (Illies, 1978, 1983; Sandrock, 1978; Wagner & Schmidt, 2004). The larvae scrape on the stony substrata of the Breitenbach (Becker, 1990, 1994) and have a significant influence on the structure and species composition of epilithic biofilms (G. Becker, unpublished data).

However, the existing data on this species are insufficient and partly contradictory (Table 1). Nielsen (1942), Iversen (1976), Recasens & Murillo (1986), and Sangpradub et al. (1999) reported seven larval instars in different European streams, while Benedetto (1975) reported eight larval instars in laboratory studies, but could not find all of these instars in the Breitenbach. Frequency histograms of the pronotum length, which enable the individual instars of *A. fuscipes* to be distinguished, and also data on the mean biomass of individual larval stages have not been published. Benedetto (1975) analysed only the biomass of freshly hatched larvae. However, the specific information about instar number and size are essential for understanding the seasonal timing of various life cycle processes, population synchrony, distributional pattern, and the persistence in the face of disturbance. Size (biomass) is especially important with respect to risk factors such as predation and exposure to hydrodynamic forces.

The objective of the current study was to answer the following questions: (i) How many distinct larval stages exist in the Breitenbach? (ii) Can these larval stages be distinguished on the basis of pronotum length, larval biomass, and case length? (iii) Are the moul increments of pronotum length, larval biomass, and case length between the larval instars smaller than in trichopteran species with five larval instars? (iv) What are the durations of the individual larval stages present in the Breitenbach throughout the year?

### Methods

The permanent flowing stretch of the Breitenbach is some 2 km in length and rarely exceeds 1 m in width. The stream is fed by several springs and flows through a grassland valley, mostly unshaded by trees, before entering the River Fulda. The dominant geology in the area is Bunter Sandstone.

The larvae of *A. fuscipes*, used to analyse pronotum length, body mass and case length, were sampled between January 1999 and December 2002 in the upper, perennial reach of the Breitenbach, which has an annual mean temperature of about 8 °C and an annual temperature amplitude of between 4 and 16 °C (H. H. Schmidt, unpublished data). All analyses were undertaken with fresh larvae and cases. After anesthetization with CO₂, pronotum length was measured along the mid-dorsal ecdysial line using a dissection microscope (magnification 100×). Additionally, the length of the corresponding larval case was measured. Pronotum length was the preferred measurement because preliminary investigations had shown that the differences between the larval instars were more distinct than when tarsus length or the more commonly head capsule width were used. Young larvae and their cases (instars I–III)

### Table 1. Comparison of literature data on the life history of *A. fuscipes*

<table>
<thead>
<tr>
<th>Authors</th>
<th>Life history</th>
<th>Larval instars</th>
<th>Larval head capsule width</th>
<th>Larval pronotum length</th>
<th>Prepupal pronotum length</th>
<th>Larval biomass</th>
<th>Larval length</th>
<th>Case length</th>
<th>Country</th>
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<tr>
<td>Nielsen (1942)</td>
<td></td>
<td>7</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td>Denmark</td>
</tr>
<tr>
<td>Benedetto (1975)</td>
<td>Univoltine</td>
<td>8</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>Germany</td>
</tr>
<tr>
<td>Recasens &amp; Murillo (1986)</td>
<td>Bivoltine</td>
<td>7</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td>Spain</td>
</tr>
<tr>
<td>Sangpradub et al. (1999)</td>
<td>Univoltine</td>
<td>7</td>
<td>x</td>
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<tr>
<td>Becker (present study)</td>
<td>Univoltine</td>
<td>7</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td>Germany</td>
</tr>
</tbody>
</table>
were weighed in groups of up to 15 individuals because of their low body mass and the averaged values were used for further calculations. The older larvae (instars IV–VII) and their corresponding cases were analysed individually. Larvae and cases were frozen at \(-20^\circ\text{C}\) and dried at 105\(^\circ\text{C}\) for 24h before they were weighed and reweighed after ashing at 510\(^\circ\text{C}\) for 8h.

Glossosomatid larvae build a new case after each moult and the larval case cannot be altered within an instar (Hansell, 1968; Anderson & Bourne, 1974; Bohle & Fischer, 1983). Thus larvae with light-coloured sclerites, which had moulted shortly before, were excluded from the calculations of the relationships between pronotum length, larval biomass, and case length. This approach was taken because the larvae moult in their old case before building a new case, and it is the new case that characterizes the new larval instar. To ensure that the youngest larval stage was not missed in the field, egg masses were sampled from the upper reach of the Breitenbach in August (see also Becker, 1991) and cultured in laboratory streams at about 14\(^\circ\text{C}\). After hatching, young larvae were fed for 6 weeks with epilithic biofilms from the Breitenbach, with the biofilms being replenished once a week. Larvae from this culture were sampled and measured regularly throughout this period. The pronotum lengths of the young larvae (instars I–III) from the laboratory streams were then compared with larvae from the field samples. Because distinction between sexes in the last larval instar of Trichoptera is not reliable, ranges of prepupal pronotum lengths from the upper reach of the Breitenbach were used to avoid counting the smaller males and the larger females as two different instars.

Qualitative samples were taken on 40 occasions between February 1997 and November 2003 in the upper reach of the stream within the context of various investigations. These data provided information on the occurrence of the different developmental stages of *A. fuscipes* in the Breitenbach.

Statistical analyses were conducted using the software package SPSS 11.5 (SPSS Inc.) Differences between the measurements of the various larval instars were compared using sequential Mann-Whitney *U*-tests. *P*-values were considered significant after adjustment with the Bonferroni method (Sokal & Rohlf, 1995). Thus, in the pairwise comparison of seven instars, six tests were conducted and *p*-values < 0.0083 were considered significant. The regression analyses were conducted using the software package SigmaPlot 7.0 (SPSS Inc.).

**Results**

The frequency histogram of the pronotum lengths shows seven larval stages of *A. fuscipes* that were distinctly separated (Fig. 1a). The pronotum lengths of instars I to V were distinct, whereas the peaks of instars VI and VII overlapped slightly. The frequency diagram of the pronotum lengths of larvae (instars I–III) that had hatched and grown in the laboratory culture (Fig. 1b) corresponded exactly with the field specimens, demonstrating that all the existing larval instars of *A. fuscipes* were present in the field samples. The range of the pronotum lengths of instar-VII larvae corresponded well with the prepupal pronotum lengths (Fig. 1c). The prepupae had reached the end of the larval development, and were enclosed in their pupal cases shortly before pupation. Thus the last two peaks indicated two different larval instars rather than sexual dimorphism in the last larval instar.

The case lengths, and especially the larval biomasses, of the instars partly overlapped (Table 2 and Fig. 2). However, the pairwise comparison of case length and biomass showed significant differences between the seven larval instars (Mann-Whitney *U*-test, all pairs with both parameters *p* < 0.001). When the pronotum length was used to distinguish the larval instars, the correlation between pronotum length, larval biomass, and case length (Fig. 3) showed clear distinction between the four older larval instars (IV–VII). The values of instar-VII larvae are widely distributed compared to the other instars because approximately 55% of the mean larval biomass of the instar-VII larvae was accumulated during the development of this larval stage (see also Table 2 and Fig. 4).

Throughout the ontogeny, the ranges of all three parameters increased with each successive larval stage (Table 2). The mean moult increment of pronotum lengths throughout the ontogeny was 1.35, and the value decreased from 1.67 at the second-instar/first-instar moult to 1.20 at the seventh-instar/sixth-instar moult. The regression between pronotum length and larval instar showed a linear function (Fig. 4). The mean moult increment of the larval AFDM was 2.25, and the value decreased from 2.64 at the second-instar/first-instar moult to 2.28 at the seventh-instar/sixth-instar moult. The regression between larval AFDM and larval instar showed a typical exponential growth curve (Fig. 4). The mean moult increment of case lengths was 1.26 and was relatively constant throughout the ontogeny. The regression between case length and larval instar showed a slightly exponential function (Fig. 4), but a linear regression also had a high correlation coefficient (0.95).

An analysis of the population structure of *A. fuscipes* throughout the year showed a clearly univoltine life cycle in the upper reach of the Breitenbach (Fig. 5). The composite of the data material of 6.5 years of collection showed little variance between the years (Fig. 5, thin lines). Instar-I larvae occurred in mid-July and were found until the beginning of December. Instar-II larvae were found from mid-July until January, instar-III and
instar-IV larvae from mid-August until mid-March and end of April, respectively. Instar-V larvae were found from the beginning of September, rarely from the end of August, until mid-June. At the beginning of December the population consisted of the instars I to V. Detailed observations showed that these larvae were feeding intensively throughout the winter, and that their development was not interrupted. Sixth-instar larvae were generally not found before January. Only in one year were some instar-VI larvae found much earlier in the season, in September. Sixth-instar larvae were found until the end of June. Instar-VII larvae never occurred before January, and the last individuals were recorded at the end of August. The first prepupae developed in mid-April and were present until mid-August. Pupae occurred at the end of April and were found until the beginning of September. However, most of the population pupated between May and July. At least five different developmental stages of A. fuscipes coexisted in the Breitenbach at any given time of the year (Fig. 5).

Fig. 1. Frequency distributions of the pronotum length of the seven larval instars of A. fuscipes (A) in the Breitenbach (n = 1056) and (B) in laboratory culture (n = 50). (C) Frequency distributions of the pronotum length of prepupae from the Breitenbach (n = 86). Explanations: I–VII = larval instars; PP = prepupae.
Discussion

In the Breitenbach, *A. fuscipes* clearly has a univoltine life history and seven distinct larval instars. These observations concur with the findings of several authors, who have described seven larval instars in various parts of Europe (Nielsen, 1942; Iversen, 1976; Recasens & Murillo, 1986; Sangpradub et al., 1999). Because of the continuous increase of the larval biomass throughout the ontogeny, distinguishing the larval instars based on biomass is not possible. The clear distinction of the larval instars in *A. fuscipes* using the correlation of pronotum length, larval biomass, and case length (Fig. 3) may be possible because of the step-like increase not only of the pronotum length, but also of the case length, after each moult. In contrast to many other trichopteran larvae, glossosomatid larvae do not lengthen and widen their case continuously as they grow. Glossosomatid larvae build a new case after each moult and do not alter the size of the case within an intermoult period (Hansell, 1968; Anderson & Bourne, 1974; Bohle & Fischer, 1983).

Although glossosomatid larvae are common and often abundant (Wiggins, 1996), the life cycle and number of larval instars are only documented in a few species. Of the 14 glossomatid species in Central Europe (Pitsch, 1993), the number of instars is known in only three species besides *A. fuscipes*: *Synagapetus iridipennis* (Otter, 1989), *Glossosoma conformis* (Sangpradub et al., 1999), *G. intermedia* (Fjellheim & Raddum, 1998). These three species each have five larval instars, as do *Agapetus yasensis* and *G. inops* in Japan (Sameshima & Sato, 1999).
Eighty glossosomatid species are known in the Nearctic region (Wiggins, 1996). However, the life cycle of only nine of these species has been analysed (overview in Houghton & Stewart, 1998). Two of these nine species are Agapetus species: *A. bifidus* (Anderson & Bourne, 1974) and *A. illini* (Bowles & Allen, 1992). All nine species were found to have five larval instars. Thus *A. fuscipes* is the only scraping caddisfly species in the Northern Hemisphere currently known to have more than five larval instars. That means that one important life cycle feature of this species, the instar number, differs markedly from the typical number of larval instars in Trichoptera and all the other glossosomatid species that have been analysed in detail to date. This finding is of particular importance because major life cycle features such as development and growth patterns underlie a strong lineage-specific control and may be fixed within lineages (e.g. Butler, 1984; Stearns, 1992).

The number of seven larval instars within *A. fuscipes* seems to be fixed, because only Benedetto (1975) found eight larval instars in laboratory cultures of larvae from the Breitenbach, but not in the Breitenbach itself. However, the data he reported for pronotum lengths are difficult to interpret. He noted a mean pronotum length of 200 μm for first-instar larvae, and up to 1550 μm for last instar (Table 3). The current study has shown that larvae with a mean pronotum length of 200 μm belong to the third instar, and that larvae with a pronotum length of more than 630 μm were not found in the Breitenbach. On the other hand, Benedetto (1975) measured head capsule widths in last instar larvae that were distinctly less than the widths reported by Sangpradub et al. (1999) for larvae of *A. fuscipes* in an Irish stream. Thus the findings of Benedetto (1975) do not concur with measurements reported by other authors for larvae in the Breitenbach and other streams.

Few Trichoptera species in the northern hemisphere are known to have more than five larval instars. Iversen (1976) reported six larval instars in the headwater species *Berea maurus*, with a non-synchronous life history, in a Danish spring. In some Sericostoma species the number of larval stages is not clear, and it is not known whether the number of stages is fixed or variable. In *Sericostoma personatum*, different numbers of larval instars have been described: five by Nielsen (1942), six by Elliott (1969), and seven by Iversen (1973) and Wagner (1990). Resh et al. (1981) observed that the larvae of *Gumaga nigricula* (Sericostomatidae) moulted...
Recasens & Murillo (1986) reported values ranging between 200 and 300 mg for last instar larvae in the Breitenbach. These characteristics correspond well with the slow seasonal life cycle in the classification scheme of Hynes (1970). The emergence period in the Breitenbach ranges from May to September (Illies, 1971; Sandrock, 1978; Fischer, Fischer, Schnabel, Wagner, & Bohle, 1998; Wagner & Schmidt, 2004). However, most adults (88%) emerged in June and July (Sandrock, 1978). First-instar

<table>
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<tr>
<th>Authors</th>
<th>Larval instar</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>Prepupa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benedetto (1975)</td>
<td>Mean (N)</td>
<td>200</td>
<td>250</td>
<td>400</td>
<td>500</td>
<td>650</td>
<td>750</td>
<td>1050</td>
<td>1250/1550</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>252</td>
<td>380</td>
<td>1652</td>
<td>3507</td>
<td>3581</td>
<td>1070</td>
<td>1245</td>
<td>1007</td>
<td></td>
</tr>
<tr>
<td>Becker (present study)</td>
<td>Mean (±SD)</td>
<td>99±8.2</td>
<td>165±9.5</td>
<td>221±13.2</td>
<td>297±18.9</td>
<td>386±19.2</td>
<td>475±20.4</td>
<td>569±29.0</td>
<td>573±27.6</td>
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<tr>
<td></td>
<td>N</td>
<td>86</td>
<td>57</td>
<td>94</td>
<td>210</td>
<td>185</td>
<td>175</td>
<td>251</td>
<td>86</td>
<td></td>
</tr>
</tbody>
</table>

14 times, and Denis (1981) assumed 10 larval stages in Sericostoma galeatum. These are detritivore species, most of which have a semivoltine, or longer life cycle. Further, in some trichopteran species with a non-synchronous life history from the southern hemisphere, higher numbers of larval instars than the typical five have been assumed (Winterbourn, 1978; Towns, 1981; Dean & Cartwright, 1987).

The pronotum lengths of the different larval instars in this study concur with the results of Recasens & Murillo (1986). These authors reported pronotum lengths for A. fuscipes in a Spanish stream (Catalonia, SE Spain) that were somewhat smaller than in the Breitenbach population (Table 3). However, they noted that A. fuscipes had a bivoltine life history, and the shorter generation time may explain the smaller size of their larvae. Nielsen (1942) found that case lengths of the first larval instars of A. fuscipes in a Danish stream ranged between 1.33 and 1.85 mm, and that case lengths of the last larval instars ranged between 4.6 and 8.0 mm, both of which concurred with the case lengths reported in the current study for A. fuscipes in the Breitenbach (1.22–2.18 mm and 4.75–7.6 mm, respectively).

The larval biomass of A. fuscipes is relatively low compared to the case mass (Becker, 2001). The AFDM values of the last instar larvae in the current study were relatively low (mean ± SD, 391 ± 84 µg). No comparable data for the AFDM of glossosomatid larvae could be found in the literature. Benedetto (1975) analysed only the dry mass of freshly moulted A. fuscipes, and not the mean dry mass of individual larval instars. Fjellheim & Raddum (1998) and Marchant & Hehir (1999) calculated the mean dry mass of preserved larvae, and reported values ranging between 200 and 300 µg for last instar larvae of three different glossosomatid species, which were lower than the findings of the current study for larvae of A. fuscipes in the Breitenbach.

The moult increment for hard cuticle parts between two larval stages (Dyar’s rule) of trichopteran species with five larval instars amounts to about 1.5 (Nielsen, 1942). In four Japanese glossosomatid species with five larval instars, the mean moult increments for head capsule lengths ranged between 1.41 and 1.49 (calculated from Sameshima & Sato, 1994). The average of 1.35, and the minimum of 1.2, for the moult increment for the pronotum length of the seven larval stages in A. fuscipes were distinctly lower (Table 2). The moult increments of the larval biomass in A. fuscipes showed a typical exponential function and the mean moult increment of 2.25 was distinctly lower than the values of 2.79 and 2.92 in two Australian Agapetus species with five larval instars (calculated from Marchant & Hehir 1999). The relationship between moult increments of case length and larval instars was slightly exponential.

The duration of a life cycle can be highly variable in the Glossosomatidae, ranging from 1.5 months for the summer generation of the trivoltine population of G. inops at water temperatures between 17.5 and 20°C (Sameshima & Sato, 1994) to nearly one year for most species in the northern hemisphere at lower temperatures (e.g. Fjellheim & Raddum, 1998; Sangpradub et al., 1999). A. fuscipes in the upper reach of the Breitenbach showed a clearly seasonal life cycle with distinct annual classes and slowly growing larvae. The composite of 40 qualitative samples over 6.5 years showed little differences of the population structure of A. fuscipes between the years (Fig. 5) due to the relative constant water temperature (average 8°C) at the sampling site nearby the main spring (H. H. Schmidt, pers. comm.). However, the population was relatively asynchronous. Thus individuals in several size classes occurred at all times of the year. Most of the larval biomass was accumulated in late winter and spring. These characteristics correspond well with the slow seasonal life cycle in the classification scheme of Hynes (1970). The emergence period in the Breitenbach ranges from May to September (Illies, 1971; Sandrock, 1978; Fischer, Fischer, Schnabel, Wagner, & Bohle, 1998; Wagner & Schmidt, 2004). However, most adults (88%) emerged in June and July (Sandrock, 1978). First-instar
larvae were present for nearly 4.5 months, from mid-July to the beginning of December, and instar-II larvae were present for 6 months, from mid-July to mid-January, due to the extended emergence period. Individuals of at least five immature stages were present throughout the year. These characteristics of the immature stages do not agree well within Hynes (1970) slow seasonal life cycle classification.

Because the number of glossosomatid species with well known life histories and larval stages is relatively small, it is not known whether a life cycle with more than five larval instars is within the normal phylogenetic variability of Glossosomatidae, or whether the large number of larval instars is a species-specific adaptive strategy of A. fuscipes to headwater streams like the Breitenbach, which would indicate an adaptive response to long-term environmental effects (e.g. Butler, 1984; Sweeney, 1984). The life history traits of A. fuscipes are more complex than the typical lineage-specific constraints in Trichoptera. Thus it is relevant to question the ecological advantage of this atypical number of immature stages to A. fuscipes. Two findings seem to be important in this context. On the one hand, the larvae specifically feed on very thin biofilms in the Breitenbach, even on biofilms with low chlorophyll-α content of as little as 0.2 μg cm⁻² (G. Becker, unpublished data). The high abrasion of the mouthparts due to feeding off the rough surface of Bunter Sandstone substratum (Arens, 1990) may be compensated for by two extra moults. On the other hand, the wide range of larval size classes present at the same time, which is extended by having two additional larval instars, may enhance the ability of A. fuscipes to cope with unfavourable conditions in their abiotic and biotic environment, spreading the risk of high mortality (Den Boer, 1968; Danks, 1987). At least five different immature stages, each with different ecological demands (Majecki, Schot, Verdonschot, & Higler, 1997; Nijboer, 2004), for instance with respect to risk factors such as predation and exposure to hydrodynamic forces and nutritional requirements, were present throughout the year. Between five and eight developmental stages are present at the same time, inside and outside the stream, if the egg and adult stages are included. Further studies are needed to understand how A. fuscipes uses its two additional moults to optimize the allocation of resources to growth, development, survival and reproduction.

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References


