Influence of the surface roughness of hard substrates on the attachment of selected running water macrozoobenthos

Dissertation

zur
Erlangung des Doktorgrades (Dr. rer. nat.)
der Mathematisch-Naturwissenschaftlichen Fakultät
der
Rheinischen Friedrich-Wilhelms-Universität

vorgelegt von

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Mönchengladbach

Bonn, 2009

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn

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Tag der Promotion

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List of abbreviations

ANOVA	analyse of variances
d.f.	degree of freedom
Chi-Sq	Chi-Square
λc	wavelength
μ	friction coefficient
$\Delta \mu$	difference of friction coefficients in larvae with and without gill lamellae
F	friction force
ΔF	difference of friction force in larvae with and without gill lamellae
HMDS	1,1,1,3,3,3-Hexamethyldisilasan
p-profile	unfiltered profile
P	Probability value
r-profile	filtered profile
Ra	arithmetic roughness average
Ra_{H0}	Ra measured in coarse roughness setting
Ra_{H2}	Ra measured in fine roughness setting
Re	Reynolds number
Rk	core roughness depth
RK	core roughness ratio
Rpk	reduced peak height
RPk	peak-valley proportion
Rvk	reduced valley depth
Rz	average maximum height of the profile
S	substrate
S1-S4	substrate types
SEM	scanning electron microscopy
S.D.	standard deviation
ST 1-4	seta types
V	flow velocity
W1-W3	three ranges of normal forces applied in experiments

Preface and acknowledgements

Everybody who ever tried to swim against the flow or simply puts his hand in flowing water gets an impression of the forces acting on animals in running waters. To cope with these flow forces animals developed a fascinating diversity of adaptations like attachment devices supporting bottom dewlling animals to maintain their position in the current. So far little is known about the interplaybetween the microtexture of solid substrates and these attachment devices. Are there surface textures animals cannot hold on? Are there other textures advantageous to cling to? Dealing with these questions I was able to find first interesting answers. Also I realized that we just start to understand this interplay and much more work can (and should) be devoted to this topic in future.

This thesis was carried out at the Nees Institute for Biodiversity of Plants (University of Bonn) in close collaboration with the german Federal Institute of Hydrology (Koblenz). This work was accomplished under the project “Investigation of the relation between surface texture and macrozoobenthos attachment devices against the background of the development of optimized surfaces for hydraulic engineering” which was financially supported by the Federal Ministry of Transport, Building and Urban Affairs.

Many people contributed for this study. First of all, I wish to thank PD Dr. Jochen Koop (Federal Institute of Hydrology, Koblenz), who had the initial idea to investigate the interplay between surface roughness and attachment in macrozoobenthos in running waters. He directed my interest to this fascinating topic, supervised this study and made it possible for me to use their comprehensive technical equipment of the Federal Institute of Hydrology.

Moreover, I am indebted to Prof. Dr. Wilhelm Barthlott (Nees Institute for Biodiversity of Plants, Bonn) for supervision of this thesis, his generous support and helpful discussions. He enabled me to take a deeper look on aquatic attachment devices by the fascinating technique of scanning electron microscopy.

I would also like to express my special thank to Prof. Dr. Stanislav Gorb (Max Plank Institute for Metal Research, Stuttgart; University of Kiel) for inspiring discussions and helpful comments regarding Chapters 4 and 5 and the invitation to Stuttgart for research purposes. This stay allowed me to explore new techniques and gain more understanding about the functioning of attachment devices in general. I thank Conny Miksch (Max Plank Institute for Metal Research, Stuttgart) for her steady technical support during this time. The equal opportunity commissioner of the Max Plank Institute for Metal Research is greatly thanked for arranging a child-care place in Stuttgart for my little daughter that made it possible for me to work their. Moreover, I am indebted to the University of Bonn for financial support of this research opportunity out of the Maria von Linden-programme.

Special thank for assistance goes to the whole department of animal ecology of the Federal Institute of Hydrology. Bernd Mockenhaupt provided excellent technical assistance and was a great help during the field work. Bettina Salinus helped with profilometric measurements. Many thanks to Barbara Anderer for supporting the determination of macrozoobenthos animals. Moreover, Dr. Christian Ortmann, Bettina Salinus, Kathrin Busch, and Stefan Wieland supported some of the field work.

Moreover, I wish to thank the staff of the Nees Institut for the support given in many ways. Dr. Holger Bohn gave helpful comments on Chapter 4. Georg Fisher and Zdravko Colic assisted some of the field trips and helped preparing animals for the SEM.

I am very grateful to Ronald Bellstedt (Museum of Natural History of the city of Gotha) for making available specimens of *E. assimilis* larvae. The Senckenberg Research Institute and Natural History Museum kindly provided *I. alpicola* larvae which are under protection in Germany. More specimens of *I. alpicola* larvae have been collected by Laurent Vuataz (Musee cantonal de zoologie, Lausanne) in the Swiss Alps to whom I express my gratitude. Many thanks to Dr. Janice Peters (University of Florida) for kindly providing examples of *Drunella doddsi*.

I thank Mr. Bernd Uebelmann (Federal Institute of Hydrology) for proof-reading parts of this thesis. My sincere thanks to Dr. Andreas Frutiger (former EAWAG Dübendorf) and Dr. Carola Winkelmann (TU Dresden) for helpful comments to chapter 4. Other helpful comments have been provided by Prof. Dr. Adam Summers (San Diego State University) to the topic of chapter 5, by Dr. Georg Becker (University of Cologne) to chapter 6 and by Dr. Tanja Bergfeld (Federal Institute of Hydrology) to Chapter 3.

Last but not least, I wish to express my warmest gratitude to my family who supported me in so many ways. My mother Ursula and Barbaros took care of my little daughter Celine so often and they even accompanied us to several conferences and research stays. I thank my daughter Celine for her patience with me when I had to work so often. I also thank her for sometimes forcing me to make a break and see the world with her children’s eyes reminding me of the diversity of live in all its ways.

Chapter 1

General introduction

Current is the dominating factor of selection for organisms in running waters (Einsele 1960). Beside benefiting from positive effects that water flow has for respiration, nutrient and food supplies aquatic animals have to cope with the flowing water which can develop an immense force of pushing on them (Allan 1995). During evolution, animals living in running water have developed a great variety of behavioural and morphological adaptations to current (Statzner 1987 according to different authors). To the latter belong specialized body shape and small body size, which have minimising influence on the flow forces acting on the animals (Nachtigall 1982, Vogel 1996). Further, the bottom-dwelling macrozoobenthos developed more or less specialized devices in order to attach themselves to the substrate to withstand the forces of flow. For the torrential fauna which inhabits the current-exposed surfaces of stones in running waters, the following attachment devices are described: suckers, claws, hooks, secretions, glues, friction pads, and increased marginal contact (e.g.: Steinmann 1907, Dodds and Hisaw 1924, Hora 1930, Ruttner 1962, Hynes 1970, Nachtigall 1974, Smith and Dartnall 1980, Kiel et al. 1989, Wichard et al. 1995, Frutiger 2002). Some of these attachment devices are assumed to function only on substrates with a suitable surface roughness. For example, sucker devices require smooth substrates while hooks or claws require irregular surfaces (Hora 1936, Hynes 1970, Nachtigall 1974). Furthermore, from terrestrial animals like flies, beetles, and geckos it is known that the roughness of the substrate plays an important role for the maximal attachment force developed by setose attachment pads (e.g. Gorb 2001, Huber et al. 2007) and claws (Dai et al. 2002). Some values of surface roughness are associated with strongly decreased attachment ability in these terrestrial attachment systems (MPI 2001). Such measurements of the attachment forces in relation to the surface roughness of the substrates are not available for torrential aquatic insects.

Moreover, not much is known about the range of conditions of the surface texture and characteristic roughness of stones in natural streams (Allan 1995). Many investigations have been made about the influence of the substrate on the distribution of aquatic macroinvertebrates (e.g. Cummins 1962, Egglishaw 1964, Macky and Kalff 1969, Reice 1980, Minshall 1984) but most of these studies deal with other characteristics like substrate type, roughness of the riverbed, amount of organic matter or grain size. In some studies the surface roughness of the substrates has been qualitatively described (e.g. Erman and Erman 1984, Downes et al. 2000a, Boyero 2003). A first attempt to quantify the surface roughness of stones was made by Casey and Clifford (1989) using a specially constructed roughness meter. However, just two different kinds of rocks have been investigated. Although modern methods allow the quantitative description of surface roughness by a variety of physical roughness parameters (Pferstorf 1997, Schmoeckel et al. 1998, Volk 2005), until today a quantitative description of the range of surface roughness of hard substrates commonly occurring in running waters is still missing.

Against this background, the objective of the present study was to gain deeper understanding of the influences of surface topography and roughness of solid substrates on the attachment ability of the torrential stone fauna. Due to this context, also the investigation of principle functions of selected attachment devices was necessary to understand the interactions between surfaces and attachment devices. For a larger number of torrential species, their distribution on natural substrates in reference to the surface roughness was investigated. By means of scanning electron microscopy (SEM), videotaping, white-light profilometry, friction measurements, attachment experiments, and replication techniques the following questions are addressed:

- What is the range of surface roughness of natural stones in running waters? (Chapter 3)
- How does the surface roughness influence the distribution of torrential fauna in natural streams? (Chapter 3)
- Are there important inconsistencies in the state of knowledge about attachment devices? Are there other attachment devices in addition to the described ones? (Chapter 4)
- How does the surface roughness of the substrates influence the attachment of selected attachment devices (e.g. setose pads or claws)? (Chapters 5 and 6)
- Which roughness orders and parameters describe the relevant microtopography best? (Chapter 6)

Chapter 2

Background and state of knowledge

Contents

2.1 Current in running waters

Water velocity and the associated physical forces collectively represent the most important environmental factor that affects the organisms living in running waters (Allan, 1995). Beside its direct influence, flow velocity has an impact on the size of particles of the substrates, the oxygen concentration, and the rate of renewal of different substances (e.g. oxygen, nutrients, food).

2.1.1 Laminar and turbulent flow

Usually, a distinction is made between laminar flow and turbulent flow. If flow is laminar, different layers are gliding independently over each other as a consequence of viscosity (Lampert and Sommer 1993). Laminar flow usually requires current velocities below 10 cm/s, especially if the water depth exceeds 0.1 m, as it is just the case in shallow and slowly moving water (Allan 1995).

In free-flowing waters, current becomes turbulent due to the inertness of the water particles, which then are not flowing in parallel, i.e. the whole water body flows in one direction, but the single particles have irregular paths. Some authors (e.g. Schmith 1975, Davis and Barmuta 1989) additionally mention transitional flow, which characterizes the status in-between laminar and turbulent.

The Reynolds number (Re) can be used to distinguish the types of flow and which forces are experienced by an organism. At low Re (< 500), viscous forces predominate and flow is laminar, whereas at high Re (> 2000) inertial forces prevail and turbulence occurs. Physical conditions between the extremes of low and high Re differ profoundly (Vogel, 1996). The Re is defined as

$$Re = \frac{U \cdot l}{\upsilon} \qquad [1]$$

where U is the velocity of the fluid [m/s], l is a characteristic length scale [m], and υ is the cinematic viscosity (1.004×10^{-6} m²/s for freshwater at 20°C). Re can be calculated with regard to the stream channel, the near-bed region, or an individual organism (Allan 1995).

2.1.2 Effects of flow forces on organisms in running water

Organisms living in currents have to cope with drag and lift forces. Drag can be caused by friction of the water flowing over the surface of the animal body (friction drag) or by the flowing water that presses frontally against the animal’s body (pressure drag). While friction drag primary depends on the whole surface area, pressure drag is determined by separation of the boundary layer around the animal (Nachtigall 1982). At low Re, friction drag predominates over pressure drag, and at high Re the reverse is true. So, at low Re a bluff body shape minimizes drag forces and at high Re a streamlined one (Vogel, 1996). The resistance an animal gives to the water flow is primarily determined by body shape, size and the flow velocity of its environment. Benthos organisms have to deal additionally with lift forces.

Bodies without rotational symmetry in the flow are generally subject to lift forces (Nachtigall, 1982). Also vertical velocity or pressure gradients lead to lift forces acting on the animal body according to Bernoulli's theorem.

2.1.3 Flow conditions within boundary layers

Macrozoobenthos organisms have special situation, because they live within a flow-velocity gradient. This boundary layer develops due to friction between the flowing water and the surface of hard substrates. At the interface between a stationary solid and a moving fluid, the velocity of the fluid is zero. Within the fluid, the velocity begins to change from that of the solid towards the free-stream velocity at some distance vertically. Per definition, the height of this boundary layer reaches from the substrate up to the region, where flow velocity has risen to a value of 99 % of the free-stream flow velocity. Bernoulli's equation cannot be applied to the differences in velocity within a boundary layer, because the equation assumes constant total heat (Vogel, 1996).

Within the boundary layer, flow can be laminar or turbulent. But even in a turbulent boundary layer there is a viscous sublayer flowing close to the substrate (Nachtigall 1982). Inside the turbulent part of boundary layer the flow velocity decreases in direction to the substrate, but not as fast as in the laminar part. The height of the boundary layer depends on flow velocity, dynamic viscosity, surface roughness, and location on the substrate. Except under low-flow conditions over flat surfaces, (boundary) layers of greatly reduced flow appear to be less than 1 mm in height (Allan 1995 according to Silvester and Sleigh 1985 and Statzner and Müller 1989). Present study macrozoobenthos organisms are mostly larger and should therefore have to deal with flow forces. Further, according to Vogel (1996) the existence of such a gradient zone means both good news and bad news: It can be a hiding place from drag, but it is also a barrier for the exchange of materials and energy.

2.1.4 Flow conditions on a higher hierarchical order

The near-bed flow conditions in rivers and streams are also influenced by the bed roughness. Behind stones and boulders, regions of slow and undirected flow occur in so called "dead-water regions" (Ambühl 1959). Davis and Barmutta (1989) and Jumars and Novell (1984) distinguish between isolated roughness flow (=independent flow), wake-interference flow (=interactive flow), and quasi-smooth flow (=skimming flow) in relation to the differences in streambed roughness. The bed roughness is determined by the diameter of the streambed material and its distribution. As the direct measurement of near-bed conditions is extremely difficult and time-consuming, different attempts have been made to estimate near-bed conditions (e.g. Smith 1975, Gore 1978, Statzner 1981, Statzner, Gore and Resh 1988, Statzner and Müller 1989, Carling 1992). However, all these calculations of near-bed flow, viscous sublayers depth, and hydraulic stress are based on untested assumptions of the applicability of engineering equations to near-bed conditions in real streams (Allan 1995). Moreover, they give average values for a whole streambed sections and therefore might be applicable to aquatic communities in general. But due to the heterogeneity of many streambeds these values might not say very much about the maximum impacts experienced by some animals on current-exposed places. Mutz (1989) found two flow patterns for flow in a height of 0-8 mm above the substrate: The first pattern was in the order of topographic elements (about 100 m²) and the second on a small-scale level in the order of cm² depending on the roughness of the bed. In the latter case, the relative height of a point in a rough riverbed influences the flow velocity at this point.

Davis and Barmutta (1989) used the Froude number (Fr) additionally to Re for the characterization of the mean flow conditions. Fr was originally developed for hull design of surface ships and is a reasonable ratio of inertial and gravitational forces, if gravity waves are what matters most (Vogel 1996).

On an even higher hierarchical order, regarding the whole running water system, flow velocity in general is high at the spring and decreases downstream (Illies 1961).

2.2 The influence of current on aquatic macroinvertebrates

2.2.1 Effects of current

Current predominates selection in running waters (Einsele 1960). On the one side, flowing water can develop an immense force pushing on aquatic animals. On the other side, water current has a beneficial influence by constantly supplying nutrients and oxygen making the site physiologically more fertile (Ruttner 1962). Food constantly is transported downstream by current. In this context, lotic habitats may have a qualitatively and quantitatively more abundant fauna compared with lenitic habitats (Schönborn 1992). Moreover, water current influences animals in many import aspects: respiration and metabolism (e.g. Ambühl 1959, Feldmeth 1970, Franke 1977), food intake (e.g. Harrod 1964, Schröder 1980, Silvester, 1983, La Barbera 1984), and behaviour, like the construction behaviour of caddies larvae (e.g. Webster and Webster 1943, Edington 1968, Philipson and Moorhouse 1974, Bohle and

Fischer 1983), reproduction behaviour (e.g. Adams and Greenwood 1983) or locomotion and rheotaxis (e.g. Steinmann 1913, Bishop and Hynes 1969, Hultin, Svensson and Ulfstrand 1969, Butz 1975, Williams 1986).

2.2.2 Adaptations to current by behavioural strategies

Currents induce the danger of being drifted away for the animals. In order to cope with this danger animals developed different morphological and behavioural strategies. Some running water animals simply avoid strong currents by searching habitats with lower flow velocity within the running waters, e.g. under or behind stones and boulders. Rhithral species as *Gammarus sp.* and *Ephemerella ignita* do so and do not show any further morphological adaptations (Schönborn, 1992). Species which avoid strong currents, should not be confused with species which hide under stones for some time period (e.g. at daylight), but moving to the surface of the stones from time to time, e.g. at night (West 1929, Elliott 1968 and 1970, Butz 1970, Lehmann 1972, Schweder 1985, Wiley and Kohler 1980, Gonser 1997). Even an occasional stay on current-exposed surfaces needs morphological adaptations! However, hiding under stones at daylight can be a protection from predation (Ward 1992) and might have nothing to do with avoidance of flow forces.

Nevertheless, animals inhabiting current-exposed places might search for a place that is most beneficial for them in respect to mircohydraulic factors. Lacoursiere (1992) found that black fly larvae did not selected the greatest speed or even the steepest gradient at the surface, but the greatest velocity gradient along the lengths of their bodies. This position maximizes particle flux through their cephalic fans (filters) and minimizes drag incurred by the bulbous posterior part of the larval body. Also, Chance and Craig (1985) described a positioning of the larval fan of *Simulium vittatum* relating to the microflow conditions around the larvae.

Despite such adaptations to current, animals are swept away from their substrate passively or let themselves drift away actively (e.g. in order to avoid dangerous situations). These drifting animals are transported downstreams where they can attach themselves again. Running water macrozoobenthos organisms often show rheotaxis and migrate upstreams to compensate drifting away from their habitat (e.g. Steinmann 1913, Neave 1930, Minckley 1964, Bishop and Hynes 1969). Also the typical flight upstreams of adult female merolimnic insects in front of egg positioning are assumed to be a compensatory behaviour to balance the downstream transport by drift (Müller 1954).

2.2.3 Adaptations to current by morphological strategies

Morphological adaptations to current have been intensively investigated already at the beginning of running water research. Steinmann (1907) already interpreted the following characteristics as (morphological) adaptations to current: dorsoventral flattening, small body size, different attachment devices, increased area of contact with the substrate, increased load, reduction of swim hairs or special cases. In his well known “Schubtheorie” Steinmann further suggested that a dorsoventrally flattened animal can be compared to a tilted plane pressed to the ground by flow forces. His assumption caused an intensive controversial discussion in the following years (e.g. Thienemann 1925, Wesenberg-Lund 1943, Nielsen 1950 and 1951, Einsele 1960, Ruttner 1962) until Ambühl (1959) introduced Prandtl’s findings about boundary layers in running water biology. Ambühl stated that the flat body is useful to “hide” in the boundary layer as a region of reduced flow velocity. Therefore, Ambühl concluded that forces assumed in the “Schubtheorie” are not reached inside of the boundary layer and small animals do not need further adaptations to current. Ambühl’s work and opinion strongly influenced limnology for many years (e.g. Hynes 1970, Illies 1961, Uhlmann 1988, Schönborn 1992). Typical dorsoventrally flattened torrential insects are the mayfly larvae of the families Heptageniidae (Fig 2.1), Prosopistomatidae and Baetiscidae or the larvae of the beetle families Psephenidae and Elmithidae (e.g. Ward 1992, Wichard et al. 1995).

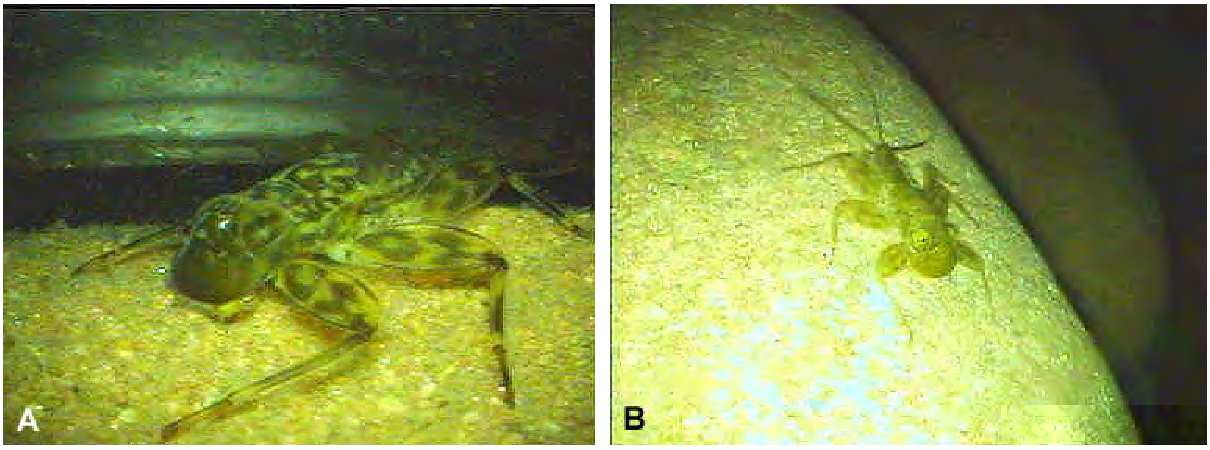

Fig. 2.1. Larvae of the dorsoventrally flattened mayfly *Ecdyonurus sp.* clinging to a stone surface

About 20 years later Statzner and Holm (1982) revealed by means of laser doppler anometrie (LDA) that the hydrodynamic conditions around benthos organisms are much more complicated. They showed that strong currents do not simply pass over the animal and that the boundary layers are not as high as suggested by Ambühl. According to Statzner (1987), even small animals cause resistance to flow, which should be influenced by their shapes and sizes. Moreover, Statzner assumed that the increased flow velocity above the highest point of the animal induces lift forces, and that therefore animals have to develop additional morphological adaptations to flow. While the investigations by Statzner (1987, 1988) and Statzner and Holms (1982) had been made with dead animals, Weißenberger et al. (1991) measured drag and lift forces on living macrozoobenthos in order to include behavioural adaptations to flow. As a result these authors determined drag coefficients for selected organisms between 0.9 and 1.9. The measured drag forces were up to 4.5 mN and lift forces between –1.0 and 8 mN for the maximum possible flow velocity of their flume (1.2 m/s). The authors concluded that the wide biological variations in lift forces are indications of special adaptations of the animals to counteract the danger of being swept away from their support.

Body shape

The literature about morphological adaptations is sometimes confusing and contra dictory. For example, the dorsoventral-flattening of the body is often assumed to be a classical morphological adaptation to flow forces. However, with the knowledge that most running water animals cannot simply hide inside the boundary layer the old discussion raises up again. Nielsen (1950) mentioned that a flattened body is probably connected with the habit of most animals to seek shelter in narrow cavities from predation and is also found in lenitic forms. This of course is true, but it does not necessarily mean that dorsoventral-flattening does not bring advantage in currents. A flattened body shape can help to hide in small cavities for predation (in running and still waters) and can also bring advantage in currents. Biological solutions brought out by evolution are often multifunctional, what means that they may be optimized to two or three different factors (Speck and Harder 2006).

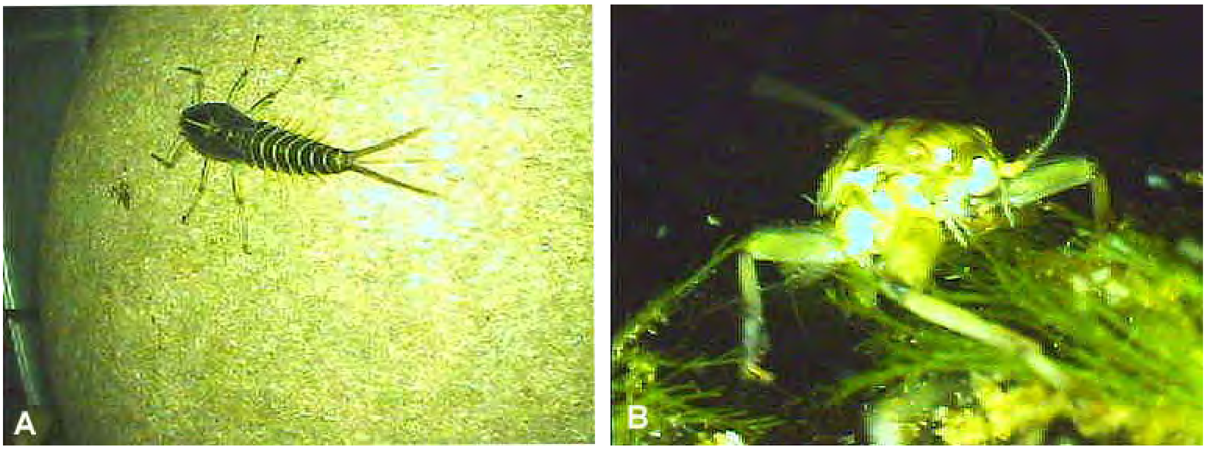

Fig. 2.2. Larvae of *Baetis cf. rhodani* clinging to (A) a stones surface (A) and to (B) moss

Another of Nilson's argument is that one of the most successful mayfly genus inhabiting torrential streams is *Baetis*, which do not show the slightest trace of dorsoventral flattening (Fig 2). This is true, but it also does not say anything about the effects of dorsoventral-flattening on flow forces, because evolution can bring out different solutions. *Baetis* obviously has a streamlined body shape which is known to reduce pressure drag (Vogel 1996). Even femura and tibia have a streamlined cross-sectional shape which should further reduce drag forces (Dodds and Hisaw 1924). Another advantage of such a rounded streamlined body shape is that the larvae do not have to cope much with lift forces. Weißenberger et al. (1991) measured under all conditions lift forces smaller than 0.2 mN for *Baetis*. This might be very important for these larvae which do not only live on the surfaces of stones, but occur also on water plants reaching vertically higher in the water body (Fig. 2.2B). The streamlined body shape also enables fast swimming. So the round streamlined body shape seems to be just another adaptation to flow with its own advantages. Coming back to the question of dorsoventral-flattening in mayfly larvae, it has to be mentioned that e.g. species of the gena *Rhithrogena*, *Epeorus* and *Iron* inhabit torrential streams, too. These gena are usually categorized as dorsoventrally–flattened (e.g. Merritt and Cummins 1996, Haybach and Malzacher 2002). Nevertheless, important criticism remains. Water currents are usually not laminar like assumed by Steinmann (1907) but turbulent. Nielsen (1950) suggested that simultaneously with the progressive movement, the water particles also perform a rolling movement. He concluded that as often as the current exerts a pressure directed downstream, it exerts an upwardly directed suction force. This suction will have its strongest effects on flat bodies. Also Vogel (1995) concluded "flatness is a two-edged sword - on one hand it affords a location deeper within the boundary layer of the substratum and thus lower drag plus a greater surface of attachment, but on the other hand it raises the bothersome bugbear of lift".

However, Weißenberger et al. (1991) found drag coefficients around 1.0 in both streamlined *Baetis* and flattened forms like *Ecdyonurus* and *Epeorus*. Consequently, both types of shape reduce pressure drag effectively. While lift forces were not relevant for *Baetis,* they showed wide variations with even negative values in some cases for flattened mayfly larvae. These wide variations in lift forces indicate that the animals have to deal with lift forces, but additionally should have further adaptations to influence lift forces. An example for the latter could be the assumed deflection of the water in such a way that part of the stream force is used to press the animals to the bottom, for example by the tilted femora (Dodds and Hisaw 1924, Gonser 1990) or the lowered head shields of *Ecdyonurus* (Weißenberger 1991). Nevertheless, very little is known about such shape adaptations beyond basic body shape.

Body size and other adaptations

In dependence on the prevailing Re, the body size determines the kind of drag that affect the animals. Statzner (1988) determined Re between 1 and 10 for very small invertebrates (0.5-1 mm in length), including newly hatched larvae, while in larger individuals (about 10 mm) Re was 10^2-10^3. Thus, according to Statzner (1988) a growing larvae initially is mainly subject to friction drag, while it experiences in later instars mainly pressure drag. Therefore, to minimize drag, differently sized animals must vary in shape. Small animals should be hemispheric and larger ones streamlined. But within one species young and old instars are usually of the same shape, and Statzner therefore concluded that evolution compromises between life at low and high Re. Considering Statzners results, it becomes clear that beside the basic body shape further adaptations as attachment devices should be relevant. Moreover, there might be possibilities to reduce drag through specialized surface structures as known for example from dolphins (Nachtigall 1982) or sharks (Cerman, Barthlott and Nieder 2005).

A very special and interesting adaptation is the boundary layer control of the Psephenidae described by Smith and Dartnall (1980). These very small beetle larvae are a classic example of dorsoventral flattening. Some species are able to suck water through the slots between their abdominal segments and pump it out though their anal gills. By doing so, the larvae delay the separation of the boundary layer on their bodies and thus reduce drag.

As another adaptation to flow Dodds and Hisaw (1925) suggested that caddies larvae of rapid waters use stones as ballast. However, this assumption has been doubted, because stones are merely what is available in fast currents (Resh and Solem 1984) and further on, increase pressure drag (Waringer 1993, Lampert and Sommer 1993). Otto and Johansson (1995) found that the ballast effect made up only 2-5 % of the current resistance while larvae with ballast stones benefitted by increased survival because of reduced frequency of attacks by bullhead and trout.

With the exception of the latter, all the morphological adaptations described above are possibilities to influence and reduce flow forces acting on the animal body. Additionally, the animals developed different attachment devices to cling to the substrate in order to protect themselves of being swept away. These attachment devices are described in a separate chapter due to their importance.

Tab. 2.1. *Overview of attachment force, drag force and the maximal flow velocity of the habitat of several taxa according to different authors*

Taxon	**Attachment device**	**Attachment force [mN]**	**Drag [mN]**	**max. flow velocity [cm/s]**	**Author**
Hapalothrix lugubris (Blephariceridae)	Sucker	84*		450	Frutiger (2002)
Liponeura cinerascens	Sucker	40[1],*		>300[2]	[1]Frutiger (2002) [2]Dittmar (1955)
Ancylus fluviatilis (Mollusca)	Gastropod feed	-		118	Dittmar (1955)
Epeorus sp.	Claws, friction pads	-	0.0-4.5 (Cw=1.0)[1]		Weißenberger et al. (1991)
Ecdyonurus venosus (Heptageniidae)	Claws		0.0-2.0 (Cw=1.0)[1]	170-200[2]	[1]Weißenberger et al. (1991), *Ecdyonurus sp.* [2]Butz (1975)
Sclerocyphon sp. (Psephenidae)	Claws	8.8*	0.2-1.5	-	Smith and Dartnall (1980)
Baetis sp. (Baetidae)	Claws		0.0-0.7 (Cw=1.0)[1]	300[2]	[1]Weißenberger et al. (1991) [2]Dodds and Hisaw (1924)
Rhyacophila sp.	Claws, secretory thread	-		200	Edington and Hildrew (1981)
Simulium sp. (Simuliidae)	Circlet of hooks, secretion	12[1], **	0.03-0.4[1]	280[2]	[1]Eyman (1988), *S. vittatum* [2]Dittmar (1955)
Deuterophlebiidae	Circlets of hooks,	-		250	Hynes (1970) according to Kennedy (1958)
Perla sp.	Claws		0.2-2.5 (Cw=1.9)[1]		Weißenberger et al. (1991)
Brachycentrus montanus	Secretion			108	Dittmar (1955)

* measured in vertical direction; ** measured in horizontal direction; Cw, drag coefficient

2.3 Attachment devices of the torrential macrozoobenthos

2.3.1 Suckers and sucker-like structures

A sucker attaches to the substrate by developing negative pressure under the sucker cup. For the development of negative pressure, a tight contact at the borders of the sucker is necessary. Otherwise no negative pressure can be built up. According to Nachtigall (1974), sucker devices require smooth substrate surfaces. True hydraulic suckers are known from Blephariceridae (Komarek 1914, Hora 1930) which as clingers inhabit the surfaces of stones in stream riffles (Merrit and Cummins 1996). Blephariceridae “walk” on six ventral suckers, with each sucker working similar to a piston pump (Frutiger 1998, 2002). Due to these suckers Belephariceridae larvae can develop high attachment forces (Tab.2.1). According to Hynes (1970) the broad feet of gastropods are rather similar to true suckers. The limpet-like

Ancylidae are very characteristic inhabitants of stony substrates in running waters almost everywhere. Stream-dwelling snails can maintain themselves in very fast waters if they find a suitable solid substrate.

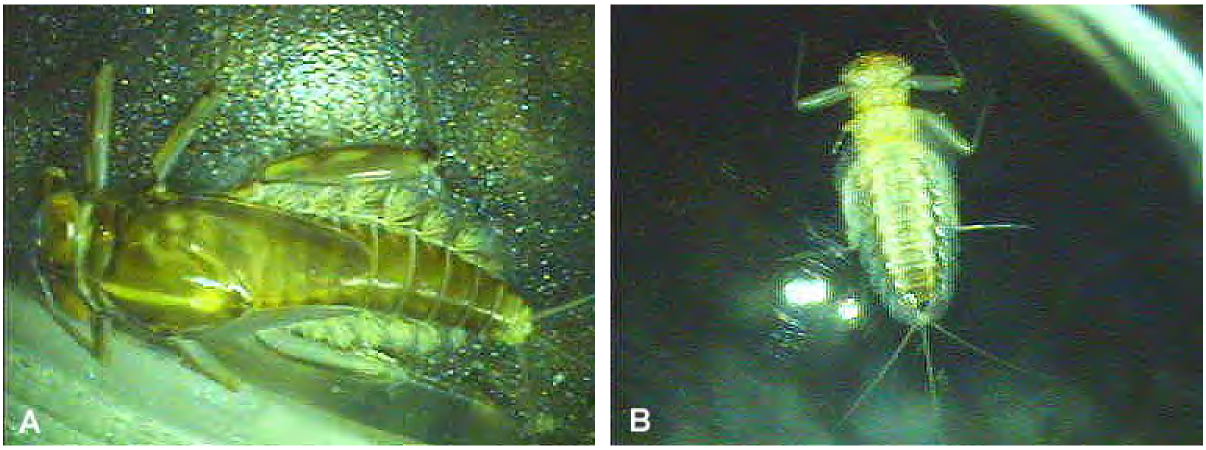

Fig. 2.3. Larvae of *Rhithrogena sp*. (A) clinging to an artificial substrate and (B) a plexiglass pane, ventral view.

Ephemeroptera larvae of the gena *Iron*, *Epeorus* and *Rhithrogena* (Fig. 2.3) are described to have gill lamellae modified to a kind of sucker (e.g. Dodds and Hisaw 1924; Wesenberg-Lund 1943; Ruttner 1962; Uhlmann and Horn 2000; Bauernfeind and Humpesch 2001; Haybach and Malzacher 2002; Staniczek 2003). Ruttner (1962) wrote that *I. alpicola* is able to attach to the substrate by using its gill lamellae to form a sucker apparatus, and he assumes that for this purpose the gill lamellae are arranged like roofing tiles covering the entire ventral side. However, it has not been explained yet in detail how this “gill-sucker apparatus” may work. Hynes (1970) disagrees with the assumption that the gills function like suckers (see above) referring to the point that in most species of *Rhithrogena* the anterior gills do not even meet, and in many *Epeorus* species are not even directed inwards. A similar case seems to be that of *Oligoneuriella rhenana*, which is described to have a suction disc on the ventral side of its labium (Thiemann 1925).

2.3.2 Claws and hooks

Tarsal claws are the most common example of the hook-like attachment devices used for short-time attachment during locomotion by mechanical interlocking with surface texture (Gorb 2008). In running waters, most arthropods have well-developed tarsal claws by means of which they hold on to the rough surfaces of the stones (Hynes, 1970).

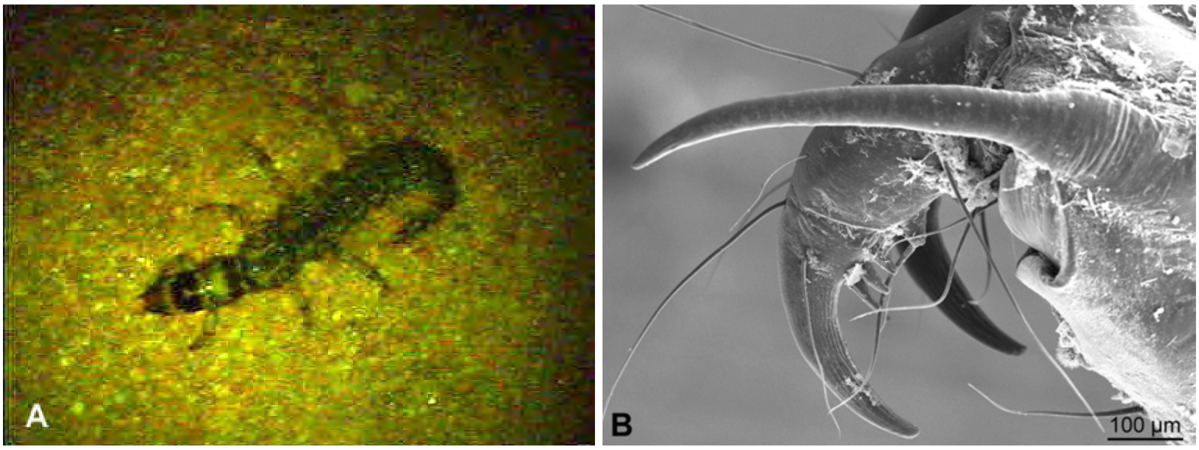

Fig. 2.4. (A) Larva of *Rhyacophila sp*. clinging to a stones surface, (B) Grapple like claws on the posterior prolegs of *Rhyacophila sp.*

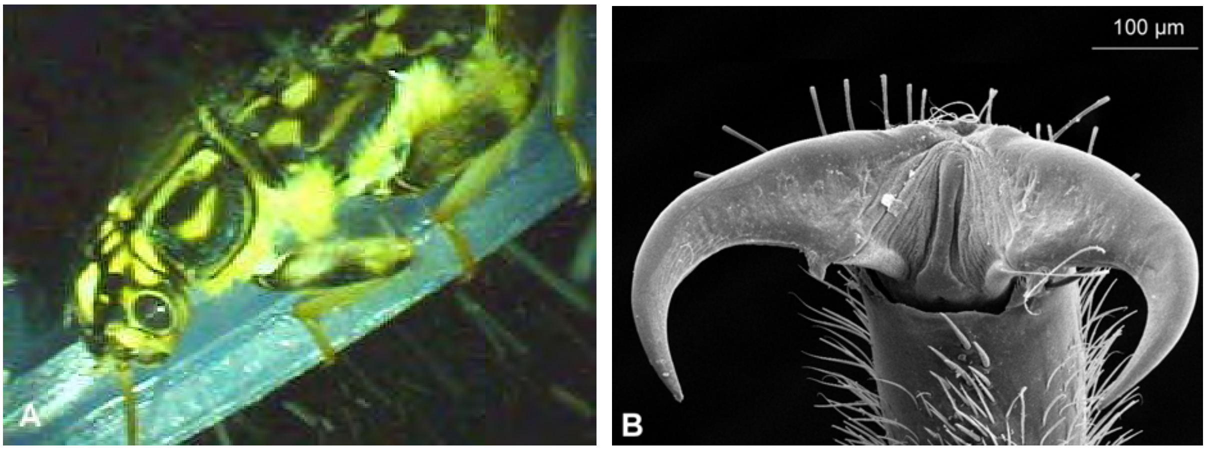

Fig. 2.5. (A) Larva of *Perla marginata* clinging to a glass edge, (B) two tarsal claws of *Perla marginata*

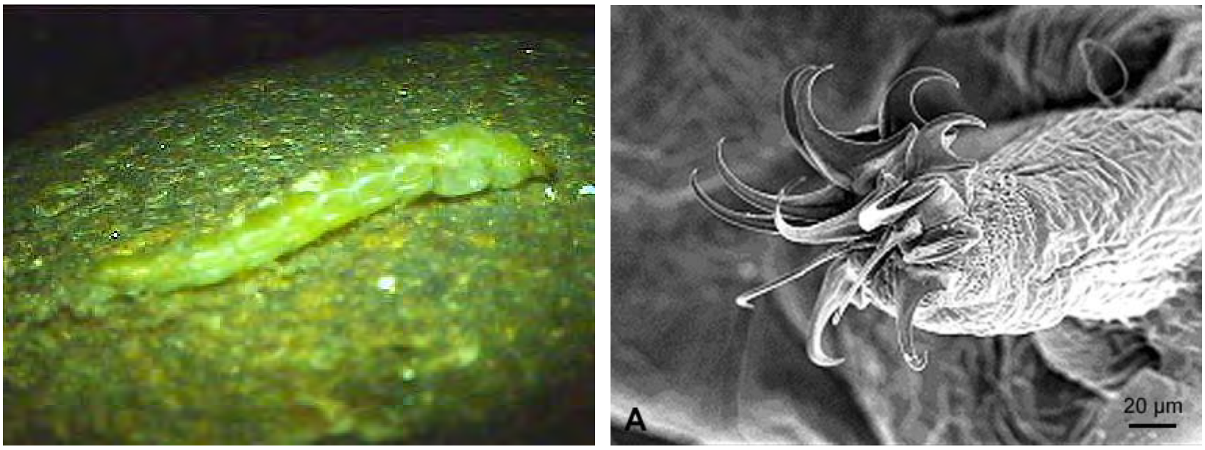

Fig. 2.6. (A) Chironomidae larva clinging to a stone, (B) Circlet of hooks on the proleg of a Chironomidae larva

Most rheophilic insect larvae bear extra strong tarsal claws (Schönborn 1992), e.g. Perlidae, Heptageniidae and Elmidae. Moreover, claws are common attachment devices in some adult aquatic insects like Coleoptea (e.g. Elmitidae, Psephenidae) as well as in Crustacea and Arachnida. While most Ephemeroptera (Nilssen 1996, Kluge 2004) and Trichoptera

(Waringer and Graf 1997) bear just one claw (Fig. 2.4B) on the tarsus, Plecoptera and most Coleoptera have two tarsal claws (Fig. 2.5B). Free-living caddis larvae (Trichoptera) like *Rhyacophila* (Fig. 2.4) have additional claws like grapples on their posterior prolegs. Under normal conditions, the larvae are sprawled on the rocks with both the thoracic and the abdominal claws engaged (Hynes 1970). From *Rhyacophila nubila* it is further known that it secures against the current with a secretory thread (Sauer 1988). Moreover, circlets of outwardly directed hooks can be found in several Diptera (Fig. 2.6). Attachment by circlets of hooks on a forked front proleg and two posterior prolegs is found in larvae of Diamesinae which occur on stones in fast waters (Thienemann 1954). Blackfly larvae (Simuliidae) have circlets of outwardly directed hooks on both anterior and posterior prolegs supporting the larvae in attachment and movement (Allan 1995). Circlets of hooks on the ends of prolegs are used by larvae of Deuterophlebiidae too. While the hook circlets of the simuliidae are engaged only in silk mats, those of the Deuterophlebiidae are used directly on the stones. By protrusion and retraction of the prolegs, the larvae can attain a fairly firm foothold, and they can crawl quite fast in high currents (Tab. 2.1).

2.3.3 Secretions and glue

Many stream-dwelling arthropods employ secretions of silk or similar material to attach themselves to hard substrates exposed to the current (Hynes 1970) and for a variety of other functions (Ward 1992). Under water, where capillary effects should not be relevant secretions attach due to molecular interactions.

Some Psychomyidae (Trichoptera) and *Corophium* (Crustacea) spin silken tubes on the stone surfaces, in which they spend their lives (Hynes 1970). Chironomidae use also silk to built their larval tubes and to attach themselves in order to resist the water current (Tönjes 1989). Some Tanytarsini reinforce their tubes with particles of solid material (Walshe 1950). Simuliid larvae have very large salivary glands that produce the silk by means of which they make a tangled mat on the substratum to which they can attach themselves by hooks on highly modified prolegs (Hora 1927 and 1930, Hynes 1970). Moreover, simuliid larvae secret a sticky thread when swept away that helps them to attach themselves again (Wotton 1986, Kiel et al 1989).

Some cased caddis larvae anchor their cases more or less permanently by silk, as e.g. some Hydroptilidae or Brachycentridae do (Fig. 2.7), which use their legs for another purpose (Hynes 1970) like filtering. Also all stream-dwelling cased caddis-worms and even those which do not have cases as larvae (e.g. Rhyacophilidae, Polycentropidae and Philopotamidae) attach their cases to solid objects when they pupate (Hynes 1970).

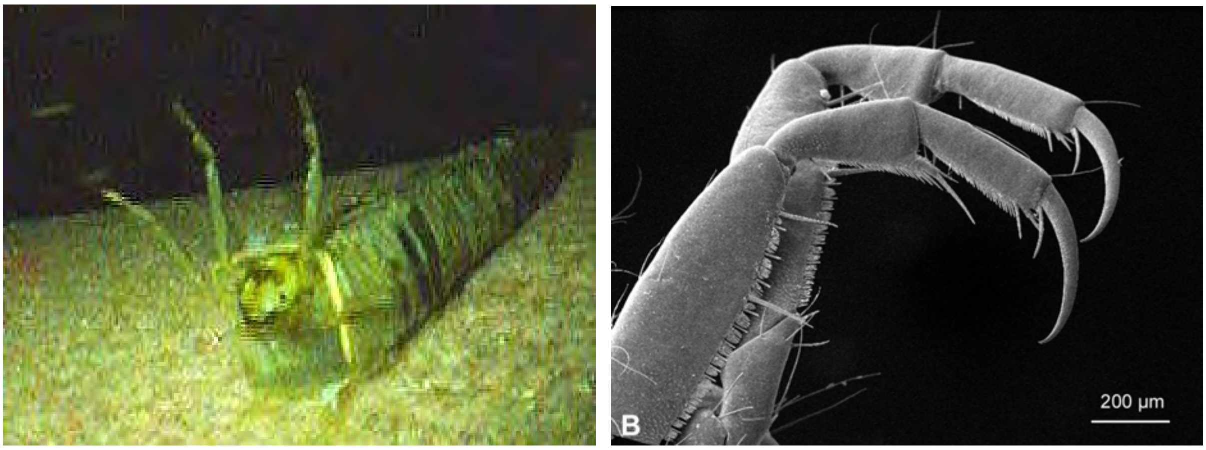

Fig. 2.7. (A) Larva of *Brachycentropus subnubilus* clinging to a stones surface by silk while using the legs for filtration, (B) legs of *Brachycentropus subnubilus*

Moreover, one mollusc should be mentioned in this context. Even if the zebra-mussel *Dreissena polymorpha* is by no means confined to running waters, it is remarkably successful there, and has become abundant on rocks and man-made structures in rivers. This might be possible because of its attachment by byssal threads, what is unique among freshwater clams (Hynes 1970, Crisp et al. 1985, Kilgour and Mackie 1993, Anderson and Waite 1998).

2.3.4 Friction pads and marginal contact

In many insects of the torrential fauna, the flattened ventral surface or some structures round the edge of the animal is modified in such a way in order to make close contact with the substrate. According to Hynes (1970) this increases frictional resistance, and the danger that the animal may be lifted off by the current is reduced. Examples can be found in the soft flexible periostracum round the edges of limpet shells (e.g. *Ancylus fluviatilis*) which fits closely to the irregularities in the surface. Some Coleoptera (Psephenidae, Elmithidae) have a complete peripheral ring of rather complex movable spines that fit the surface and seal off the ventral side of the larvae (Thienemann 1925, Hora 1930) (Fig. 2.8). *Dicercomycon* (from Africa) and *Drunella doddsi* (from North America) have a fringe of outwardly directed hairs which make close contact with the substrate and serve as friction pads (Ventner 1961, Hynes 1970, Ward 1992).

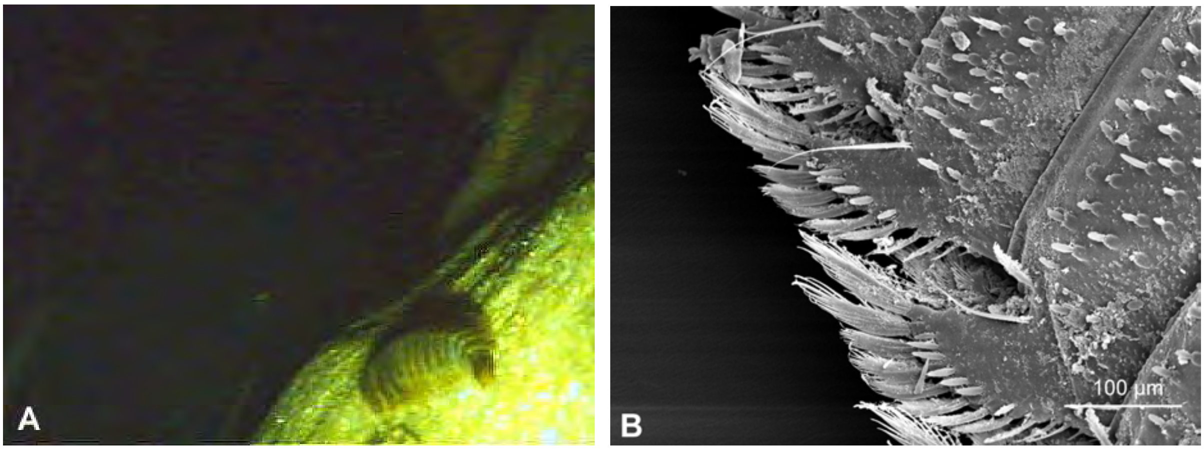

Fig. 2.8. (A) Larva of *Elmis sp.* clinging to a stone surface, (B) Structure on the distal edge of the ventral side of an *Elmis sp.* larva

Moreover, according to Hynes (1970) the Heptageniidae *Rhithrogena* and *Epeorus* belong to this group too. Their gills extend sideways from the abdomen and make direct contact with the substrate. In the genus *Rhithrogena* and some species of *Epeorus,* the front gills are enlarged and turned forward under the body, thus increasing the area of marginal contact and reducing the possibility of water flowing under the larva. Additionally, Hora (1930) described thickened spiny pads on the anterior margin of the gills 2-6 of *Iron* and *Epeorus*.

2.4 Surface texture of hard substrates and its influence on the attachment of macrozoobenthos

For some of these attachment devices (claws, sucker) it has been mentioned that they work only on substrates of appropriate surface roughness. Furthermore, from different attachment devices of some terrestrial animals it is known that the roughness of the substrate has a significant effect on the attachment force of the animals (e.g. Gorb 2001, Dai et al. 2002, Huber et al. 2007). Such experiments have not been performed with aquatic torrential insects so far.

2.4.1 Surface roughness and distribution of macrozoobenthos

Different studies investigated the influence of the surface texture of natural substrates on the distribution of macrozoobenthos organisms in running waters, but the results were often contradictory. Clifford, Gotceita and Casey (1989), Downes et al. (2000A), Downes et al. (2000B) described increased species richness on rough substrates, while Erman and Erman (1984) found no effect in dependence on roughness. Also abundances of total individuals or several taxa (e.g. mayfly, stoneflies, chironomidae) showed different correlations in literature. Higher abundances on rough substrates than on smooth ones were described by Erman and Erman (1984), Clifford, Gotceita and Casey (1989), Boyero (2003), Downes et al. (2000B), while Casey and Clifford 1989, Downes et al. 2000A reported that no effect was found. In several studies the relations of taxa distribution and surface roughness depended on the kind of taxa. For example, Clifford, Gotceita and Casey (1989) described significantly higher abundances on rough substrates in most taxa (amongst others *Baetis,* Chironomidae, *Rhyacophila*) but in a few other taxa like e.g. *Epeorus* they observed the opposite effect. Also Boyero (2003) found a different influence of surface texture in different taxa. He concluded that the surface texture of the substrate has significant effect on colonization by macroinvertebrates, although the effect is complex and needs more careful examination.

The results of the studies discussed above may be influenced by the different methods used. Artificial substrates (tiles and bricks) were examined by Clifford, Gotceita and Casey (1989), Downes et al. (2000A) and Downes et al. (2000B), while Erman and Erman (1984), Downes et al. (2000A), Downes et al. (2000B) and Boyero (2003) investigated stones of several natural rocks. Casey and Clifford (1989) put two kinds of rocks in substrate baskets.

2.4.2 Range of surface roughness of natural substrates

The surface topography of stones can be very different as can be seen in the 3D profiles of two different kind of rocks (Fig. 2.9). Nevertheless, just little can be said about the range of conditions of surface texture occurring in natural streams (Allan 1995).

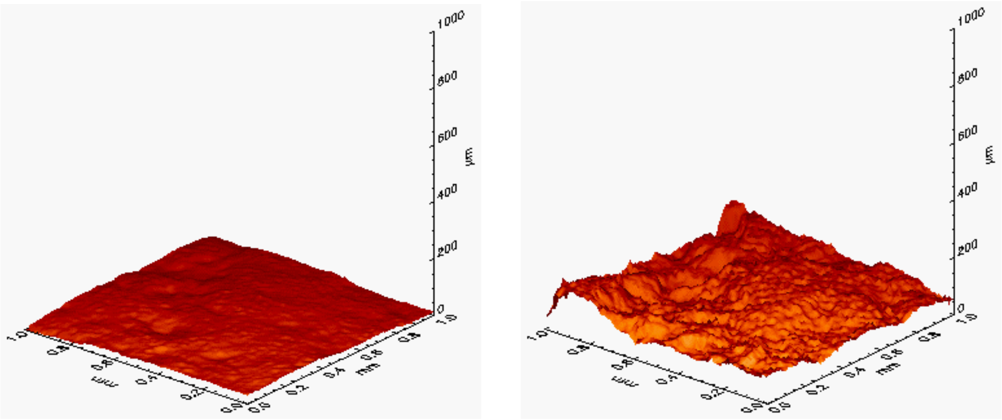

Fig. 2.9. (A) 3D-Profile of the surface of an andestite stone, (B) 3D-Profile of the surface of a bunter stone. Area: 1000 µm x 1000 µm, z-range: 1000 µm

Some studies investigated the influence of different rocks on the distribution of macrozoobenthos. Erman and Erman (1984) used quartzite, granite and sandstone for experiments. Downes et al. (2000A) investigated the colonization of siltstone, sandstones, crystal-poor felsic volcanics, plain paving bricks, granodiorites, and crystal-rich felsic volcanics, and Casey and Clifford (1989) exposed limestone and sandstone.

Confusingly, stones of the same rock have been differently classified. Downes et al. 2000A describe sandstone as smooth, while Casey and Clifford 1989 classify sandstone as rough. It is not clear whether this different classification is caused by wide variations of the surface roughness or whether it is due to the relative (qualitative) comparison between different rocks. Most studies have only qualitatively distinguished between smooth and rough substrates (Erman and Erman 1984, Downes et al. 2000a, Downes et al. 2000b, Boyero 2003). A quantitative method was used only by Casey and Clifford (1989) and Clifford, Gotceita and Casey (1989) who measured the surface roughness of the stones using a stylus-type roughness meter. This specially constructed roughness meter measured roughness that might be considered a degree rougher than microscopic roughness. However, Casey and Clifford (1989) investigated only two kinds of rocks, so that a general characterization of the range of surface roughness of stones commonly occurring in running waters is still missing. In order to understand the interactions of attachment devices and surface topography, a quantification of surface roughness seems relevant.

2.4.3 Determination of surface roughness

Nowadays the quantitative description of surface roughness by a variety of different physical roughness parameters is possible by means of modern techniques (Pferstorf 1997, Schmoeckel et al. 1998). In general, each surface consists of overlapping form variations of different hierarchical orders (Volk 2005). Form variations of first order are variations of the basic shape of the surface. Second order form variations are labelled as waviness, while roughness is defined as form variations of third to fifth order for technical surfaces (DIN 4760). Which roughness orders are relevant for macrozoobenthos is not clear.

Waviness is characterized by undulations that are several times longer than deep. In contrast, the roughness is determined by undulations that are just a few times longer than deep. The limit between roughness and waviness is defined by the wavelength λc (Volk 2005). The length of λc is not predetermined but influences the value of roughness parameters. In praxis the length of the measured profile (p-profile) is usually six or seven times the length of λc. Due to mathematical reasons, a distance comprehending a half or the full length of λc is cut off at the front and at the end of the measured profile (Volk, 2005). Consequently, only a length of five times λc is included in the calculation of roughness parameters. The original or unfiltered profile (p-profile) is “filtered” by the wavelength λc. After this so called “filtering”, the profile comprises only the structures that are smaller than the applied wavelength λc. This filtered profile is called r-profile. Roughness parameters are determined out of the r-profile. The form variations of the profile can be distinguished in peaks and valleys and great many different profile shapes are possible. The technology of roughness measurement and determination provides a number of roughness parameters which describe different roughness properties (e.g. DIN EN ISO 4287, SEP 1940, DIN EN ISO 13565). A set of selected roughness parameters and their properties are shown in Appendix 1. The most commonly used roughness parameters are the “Roughness Average” (Ra), the “Average Maximum Height” of the profile (Rz) and the “Root Mean Square Roughness” (Rq). Ra and Rq describe the average variations of the profile and are relatively insensitive to different shapes of the surface. In contrast, Rz is a value for the common height of the profile and is influenced by the different profile shapes like e.g. single peaks and grooves. Some roughness parameters (Rk-group) give information about the material part of the profile in a certain height range and therefore about the shape of the profile. Roughness parameters like the “Core Roughness Depth” (Rk), “Reduced Peak Heigth” (Rpk) and “Reduced Valley Depth” (Rvk) are determined out of the Abbot-Firestone curve, which is the cumulative probability density function of the surface profile height and a way to describe the shape of the profile.

2.4.4 Influence of biofilm

In aquatic systems, the situation is complicated compared with terrestrial conditions due to the fact that substrates are covered with a layer of biofilm. After just two hours of exposure in aquatic environment organic material, bacteria, and fungi form a primary biofilm on the substrates surface (Korte and Blinn 1983). This primary biofilm makes it easier for autotroph microorganisms and algae to attach as well. Extracellular polymer substances, which are secreted by microorganisms, embed algae, bacteria, fungi, and detrital particles in an organic sublayer. These biofilms can show a wide variety in composition and thickness, and in particular the thickness is influenced by the grazing benthos itself (Eitner 2004).

The influence of the biofilm on the attachment of the animals has been investigated only for a few species. The attachment of *Dreissena* is not influenced by the presence of biofilm. According to Baier et al. (1992) byssal threads replace the biofilm and make direct contact with the substrate. Also Crisp et al. (1985) did not found an influence of biofilm on attachment. In contrast, the colonisation of the substrate seems to be influenced by the presence of biofilm. Biofilm removal reduced mussel colonisation by 10-20 % in *Dreissena* *polymorpha* (Wainman et al. 1996). In the first colonization of substrates by Simuliid larvae no differences were found in substrates with biofilm compared with those without biofilm layer (Kiel 1996). Nevertheless, long term effects could not be excluded for Simuliid larvae.

Chapter 3

The surface roughness of natural hard substrates in running waters and its influence on the distribution of selected macrozoobenthos organisms

Abstract. - While the influence of most substrate properties on macrozoobenthos is well investigated, not much is known about the range of surface roughness of stones occurring in natural streams. Instead of a qualitative approach only distinguishing between smooth and rough substrates, in this study the surface texture of stones was measured by means of white light profilometry in two different settings. The setting in lower magnification (1000 µm x 1000 µm) turned out to be suitable for larger amounts of samples, while the proceeding with the higher magnification (100 µm x 100 µm) was time consuming and failure afflicted.

By calculation of selected roughness parameters, the surface roughness for different rocks collected in several running waters was quantified. The average roughness (Ra) of the roughest rock was about 2.5 times higher than that of the smoothest one in both settings (Ra measured at lower magnification: 3.5-8.6 µm, Ra measured at higher magnification: 0.6-1.4 µm). However, the surface roughness for all rocks strongly varied and the surface roughness of single stones of the same rock differed up to a factor of 4. These results show that a differentiation between the different rocks only can cause considerable inaccuracy for assessing roughness.

The colonization by selected macrozoobenthos organisms showed varying trends in relation to the measured surface roughness for the different seasons. In spring and summer, significantly higher values on smooth substrates compared with rough ones were observed for total taxa number and the taxa *Baetis sp.*, Chironomidae and *Rhyacophila sp.*. In autumn, the opposite trend was present for total taxa number, individual density and *Elmis sp.* larvae. Both trends of macrozoobenthos taxa in regard to surface roughness had been described in previous studies for other species. However, for *Baetis sp.* and Chironomidae exactly the opposite trend in regard to surface roughness was reported and it seems that other factors than roughness as e.g. food resources strongly influence their distribution.

Depending on their attachment devices, animals were assumed to be found on stones with the respective suitable surface roughness. However, only *Elmis sp.* larvae and *Ancylus fluviatilis* indicated such an association. Almost all species were found on very smooth stones. Even animals with claws were obviously able to cling to smooth stones on which at least a few individuals (*Elmis sp*. larvae) occurred. One species attaching only by claws (*Baetis sp*.) even preferred smooth stones. A possible reason can be crevices on the surface of stones independent of the average surface roughness. For a deeper understanding of the interplay of attachment devices and substrate surface, additional experiments with more defined conditions are required.

Keywords: surface texture, roughness, stones, attachment devices, substrate properties, clinger

Contents

3.1 Introduction

The substrate of running waters is one important factor influencing the distribution and abundance of stream invertebrates (Hynes 1970). While the influence of most substrate properties like substrate type, roughness of the river bed, amount of organic matter or grain size on the distribution of aquatic macroinvertebrates have intensively been investigated (e.g. Cummins 1962, Egglishaw 1964, Macky and Kalff 1969, Reice 1980, Minshall 1984), comparatively little can be said about the conditions of surface (micro-) texture occurring in natural streams (Allan 1995). Some attempts have been made to investigate the influence of the surface texture of natural substrates on the distribution of macrozoobenthos organisms in running waters but the results were sometimes contra dictionary. In some studies species richness increased on the rough substrates (Clifford, Gotceita and Casey 1989, Downes et al. 2000A, Downes et al. 2000B) but in others no effect of the surface texture on the number of taxa was found (Erman and Erman 1984). Moreover, some authors found significantly higher abundances of total individuals or several taxa (e.g. mayfly, stoneflies, chironomidae or scrapper functional feeding group in general) on rough substrates than on smooth substrates (Erman and Erman 1984, Clifford, Gotceita and Casey 1989, Boyero 2003, Downes et al. 2000B) while other authors did not (Casey and Clifford 1989, Downes et al. 2000A). Clifford, Gotceita and Casey (1989) also described significantly higher abundance for some taxa on smooth substrates (*Cinygmula sp*. Ephemeroptera; *Epeorus sp.*, Ephemeroptera; *Nephelopsis absucura*, Hirudinea and an unidentified capniid, Plecoptera). Also Boyero (2003) found a different influence of surface texture for different taxa and concluded that the surface texture of the substrate has a significant effect on colonization by macroinvertebrates although the effect is complex and needs more careful examination.

One factor which should influence the ability to attach to different substrates is the kind of attachment device. Macrozoobenthos organisms of the typical torrential fauna developed a large variety of attachment devices in order to cling to the substrates. The latter are appropriately identified as adaptations which support their owners in maintaining position against the current (Allan 1995). In order to attach to the substrates in running waters the animals use suckers and so called sucker like structures (no real hydraulic suckers), claws and hooks, secretions and glue, friction pads and marginal contact (e.g. Hynes 1970, Ward 1992). From some of these attachment devices it is known that they need a certain surface texture in order to function. Sucker devices require smooth substrates while hooks or claws need irregular surfaces in order to attach to the substrate (Nachtigall, 1974). For example Hora (1936) explains the absence of Blephariceridae larvae in Indian streams with the presence of moss and roughened stones. These larvae are well known clingers attaching by true hydraulic suckers (Merrit and Cummins 1996, Frutiger 2002). It can be assumed that animals prefer a certain kind of surface roughness with regard to their attachment strategy. Benthos organisms only attaching by claws might show a preference for rough substrates. On the other hand animals which attach by means of secretions might show no preference for a certain kind of surface roughness.

So far, the influence of surface texture on the colonization by macrozoobenthos in running waters was examined by different methods. Artificial substrates (tiles) were used by Clifford, Gotceita and Casey (1989), while stones of different rocks were investigated from Erman and Erman 1984 (quartzite, granite and sandstone), Downes et al. 2000A and Downes et al. 2000B (siltstone, sandstones, crystal-poor felsic volcanics, plain paving bricks, granodiorites, crystal-rich felsic volcanics, and sand-blasted paving bricks) and Boyero 2003 (cobbles and gravel of unkown rock). Casey and Clifford 1989 put stones (limestone and sandstone) in substrate baskets. Confusingly, the same rock was classified in some studies as smooth (sandstone in Downes et al. 2000a) and in others as rough (sandstone in Casey and Clifford 1989). It is not clear whether this different classification of the same rock is caused by a large variation of the surface texture from smooth to rough within the same rock or whether it is caused by the relative (qualitative) comparison between different rocks. In most investigations it was only qualitatively differentiated between smooth and rough substrates (Erman and Erman 1984, Downes et al. 2000a, Downes et al. 2000b, Boyero 2003). Only Casey and Clifford (1989) and Clifford, Gotceita and Casey (1989) quantified the surface roughness of the stones using a stylus-type roughness meter. This specially constructed roughness meter measures roughness that might be considered a degree rougher than microscopic roughness. However, in these studies only two rocks were investigated, so that a characterization of the range of surface roughness of rocks commonly occurring in running waters is still missing.

The goal of this study was (A) to detect the range of surface roughness of natural hard substrates commonly occurring in running waters and (B) to investigate its effect on the distribution of macrozoobenthos organisms under natural conditions in reference to their attachment strategy. Therefore, stones of a variety of rocks were collected out of three third order streams (classification system according to Strahler 1957) of comparable size and water quality. Additionally, some stones of the shore stabilization of two large rivers were collected. The roughness of these stones was measured in two different magnifications by a white light profilometer. Selected roughness parameters were calculated for each stone. Further, some characters of the biofilm, the surface area of the stones and the total number of each taxon were determined for each stone. Macrozoobenthos species of the torrential fauna with different kinds of attachment devices were selected (claws, secretion plus hooks, mollusc feet) and scanning electron microscopy (SEM) pictures were made from their attachment devices. In this study the following questions are addressed:

- Is the used method suitable for characterisation of the surface roughness of stones? Which settings are appropriate?
- How does the surface roughness of the different stones and kinds of rock (e.g. slate or sandstone) vary in running waters?
- How does the surface roughness of the stones influence the distribution of selected macrozoobenthos species regarding their attachment strategy?

3.2 Study area

The intention was to select running waters with a large variety of different kinds of rocks. At the same time, these streams should have the highest possible comparability with regard to other factors like water quality and discharge because the macrozoobenthos had to be compared for different waters. This was unavoidable since it was the aim to investigate macrozoobenthos colonization under natural conditions but different geological types of rocks naturally do not all occur in the same stream or river. A detailed description of the water catchments of the selected running waters and their geology is given in Busch (2007). Additionally, two large Federal waterways were included in the study in order to get an impression of the surface roughness of stones of the used shore stabilisation. However, the original macrozoobenthos fauna of the federal waterways is mostly displaced by neozoen species (Eggers 2003, Eggers and Martens 2007). Therefore, emphasis was put on the smaller streams which let expect a much higher species richness.

3.2.1 Stream Kyll

The third order stream Kyll flows through the Eifel highland which rocks are predominated by bunter (sandstone) and slate. The sampling point was about 15 km before the stream Kyll flows into the river Mosel (at river-km 183,6) and shortly above the village Kordel where the next water gauge is located. At this point the long term average monthly discharge varies between 1 and 5 m³/s. In the experimental year peaks between 38 and 48 m³/s were measured in February, March, April and May/June while lowest discharge was registered in June (end of the month), July and August (< 4 m³). At the sampling point the water is about 10 m broad and boarded by riparian shrubberies and trees (mostly alder). The water bed is relatively plain at the right side while there is a deep channel at the left. Sediments consist predominately of gravel, stones, boulders and native rocks.

3.2.2 Stream Nahe

The stream Nahe is a tributary of the river Rhine in to which it flows at Rhine river-km 529,1. and flows through the Saar-Nahe-Bergland. The stones of this highland mainly consists of Rotliegendes (Upper Carboniferous to Middle Permian age), alkali and intermediate stones like e.g. Rhyolith. The next water gauge is located in Idar Oberstein and shows an average monthly discharge between 1 and 5 m³/s. During the experimental year the highest discharge values (48-75 m³) were measured in February, March and April. Low discharge (< 2 m³) occurred in July and August. At the sampling point the stream Nahe can be classified as third order stream . It measures about 10 m in width at the sampling point and the shore is covered with grass, several herbs and single bushes. The bed is covered by stones and gravel.

3.2.3 Stream Wied

Another tributary of the river Rhine is the third order stream Wied which flows through the hill lands of the Westerwald whose rocks are predominated by slate and greywacke. The sampling point is located about 15 km above the mouth into the river Rhine at river-km 610,2 close to Koblenz. At the sampling point this low mountain river is about 12 m broad and the shore is boarded with bushes, trees and herbage. The sediment is predominated by stones and boulders. The next water gauge (Friedrichsthal) is located about 1 km upstream. The long term average discharge per month varies between 1 and 5 m³/s. In the experimental year discharge peaks between 50 and 74 m³ were registered in February, March, April and June. The lowest water level was measured in July and August (< 3 m³).

3.2.4 River Rhine

The river Rhine was investigated in its middle part. Samples were taken at two different sampling points: Andernach (river-km 614) at the left riverside and Hammersteiner Werth (river-km 620) at the right riverside. The stones of the shore stabilization were made by rip-rap. The sampling points had a distance of about 15 m from the shore. The long term average discharge of the river Rhine counts between 1520 and 2510 m³/s monthly at the gauge Andernach. In the experimental year 2006 discharge peaks were measured in March (5760 m³) and April (5210 m³) while the lowest discharge was measured in November (826 m³).

3.2.5 River Elbe

The river Elbe was investigated at Gauernitz which is close to the city of Coswig (river-km 236). When the river Elbe passes the border between the Czech Republic and Germany after 105 km the river-km count is reset to zero (Koop et al. 2008). Therefore, at our sampling point the river Elbe actually has passed already 351 km. At the sampling point there is an island in the middle of the river. The sampling point was on the left river side where stones were collected in a distance of about 10-15 m from the river shore. The long term average discharge per month of the river Elbe is between 179 and 375 cm³/s at gauge Wittenberg. In the experimental year highest discharge occurred in February (429 m³) and March (424 m³), while low discharge was measured from May to the beginning of September (118-185 m³).

3.3 Materials and methods

Samples were taken from the rivers Wied, Nahe and Kyll in three seasons of the year 2006 (spring: May/June; summer: July/August; autumn: October). While these waters were examined by foot, the larger rivers Rhine and Elbe were investigated by boat. Here samples were taken only once in late spring (river Rhine spring 2006 and river Elbe in spring 2007). At each sampling point ten cobbles were collected from riffles out of the middle of the water (river Wied, Nahe and Kyll) or the deeper shore (river Rhine and Elbe). For removal a net (mesh size of 1.0 mm x 1.5 mm) was put behind the stones. Then the stone was carefully kicked into the net and taken out off the water. For sampling by boat in the large rivers samples have to be taken by dredge. All animals were collected from the stones (and the net) by means of a featherweight forceps and were fixed in 70 %- Ethanol. In the laboratory, macrozoobenthos organisms were sorted, determined and counted. Altogether, 110 stones were collected and used for further investigations.

3.3.1 Sediment and biofilm coverage of the stones

The stones were transported to the laboratory in separate clean plastic bags. The stones with the previously air dried biofilm were dried at 105°C for 18 h in a drying cupboard and weighed by an special libra up to a scale of 0.01 g. Afterwards, the dried biofilm was brushed from the stones. Dry mass of biofilm was calculated out of the difference of the stones mass with dried biofilm and without biofilm. Additionally, the ignition loss of the biofilm was determined in accordance with DIN 19684 by heating up to 550°C.

3.3.2 Determination of the surface roughness of the stones

Before roughness measurements, stones were cleaned by an ultrasonic desintegrator (Branson Sonifer 250, Heinemann Ultraschall-und Labortechnik, Schwäbisch Gemünd) and brushed up again. Measurements of the surface topography were made by means of the white light interferometer FRT MicroProf (CHR 150N high resolution optical sensor, Fries Research & Technology, Bergisch Gladbach, Germany). In order to detect different orders of surface roughness the surface topography of each stone was determined at two different magnifications. At the lower magnification an area of 1000 x 1000 µm (Pixel size: 10 µm²) was measured while at higher magnification an area of 100 x 100 µm (Pixel size: 1 µm²) was scanned per field. Each substrate and magnification were measured at ten different areas of the stones, while the scan size and pixel size were kept constant. The surface topography was analysed by the software FRT-Mark III and several roughness parameters were calculated. According to Volk (2005), the wavelength (λc) was defined as the sixth part of the length of the measured profiles. Consequently, the wavelength λc was 166.67 µm in the setting with lower magnification and 16.7 µm in the setting with higher magnification.

3.3.3 Determination of the surface area of the stones

The surface area of the stones was needed in order to have a frame of reference for species number and biofilm mass. It was determined by means of aluminium foil which was carefully placed around the stone. The overlapping foil was cut so that one layer aluminium foil covered the whole stone. Afterwards, the foil of each stone was weighted with a detection limit of 0.01 g. Additionally, a straight calibration line was made based on foils of known area between 45.0 cm² and 1489.8 cm². Due to the determined relation of foil weight and foil area, it was possible to calculate the surface area of the stones on the basis of the weight of the foil that covered each stone. A similar method was described by Doeg and Lake (1981) which used plastic foil instead of aluminium foil. The authors determined a mistake of the method of about 10 %.

3.3.4 Scanning electron microscopy

Scanning electron microscope (SEM) pictures were made of the attachment devices of selected species. Therefore, selected specimens were dehydrated in an increasing series of ethanol (80 %, 90 % and 99 %-ethanol, each 10 min). Subsequently, they were dried in a mixture (50:50) of HMDS (1,1,1,3,3,3-Hexamethyldisilasan, Merk-Schuhardt, Hohenbrunn) and Isopropanol (Carl Roth GmbH & CoKG, Karlsruhe) and afterwards in pure HMDS (each 10 min). The dried larvae were glued to a needle, sputter-coated with gold (Blazer Union SCD 034; Blazer Wiesbaden, Germany) and clamped to a special sample holder according to Wichard et al. (1995). This sample holder was rebuilt in reduced height and with expanded diameter. Moreover, the screw was positioned laterally in order to allow an improved flexibility in the SEM. The examinations were made in the scanning electron microscope LEO 1450 (Leica-Zeiss, Oberkochem, Germany) at 15 KV.

3.3.5 Data analyses

The results of surface roughness, sediment/biofilm characters, macrozoobenthos densities and taxa number were summarized for the different kinds of rocks. Data were tested for normal distribution (Anderson-Darling Normality test) and equal variances (Levene’s test). If the data set was normally distributed and variances were homogeneous, the analysis of variance (ANOVA) was carried out. If premises for ANOVA were not fulfilled, the non parametric Kruskall Wallis test was applied. Fisher post hoc test was used to identify the pair wise differences.

The species selected for intensive consideration had to be present in most investigated waters and had to show sufficient abundances. As the variation of roughness within the same rock extremely varied, all macrozoobenthos characteristics were investigated not only for tendencies between the different rocks but also for correlation to the surface roughness parameters. The considerable variation of Ra clearly showed that it was not possible to measure Ra without error. Therefore, it was not suitable to use regression statistics because the premises are not fulfilled (Dytham 1999) even if a cause (roughness) - effect (macrozoobenthos characteristics) association was tested. The correlation was tested by Spearman’s rank-order correlation since data were not appropriate for Pearson’s product-moment correlation (no normal distribution of both variables).

3.4 Results

3.4.1 Surface roughness of the stones

14 different kinds of rocks were collected in the five investigated running waters (Appendix 2). But only seven of these rocks (Fig. 3.1) occurred regularly (9 stones or more in total per rock) while the other seven kinds of rocks were represented with only one or two stones and therefore cannot be analysed in questions of the properties of these rocks. The varying number of stones of different rocks was caused by the fact that the rock could often only be determined after collecting.

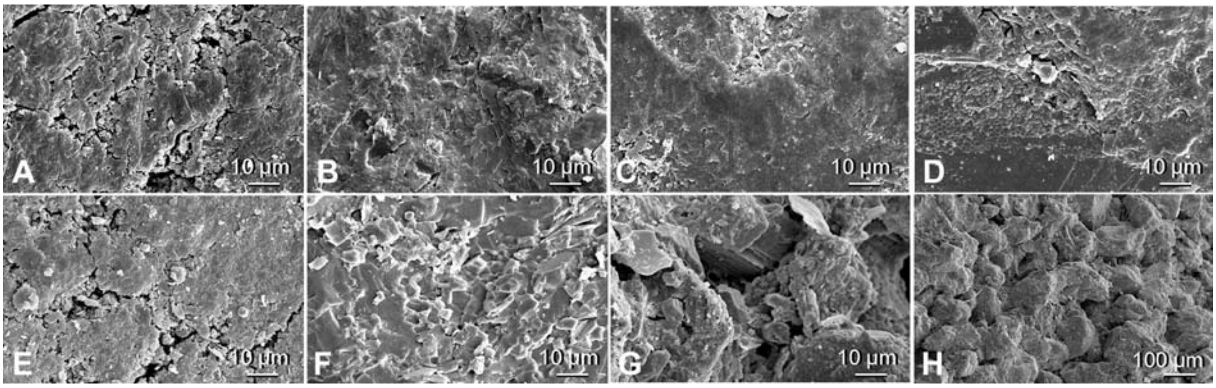

Fig. 3.1. SEM-pictures of the surface of selected rocks: (A) andesite, (B) slate, (C) basalt, (D) quartz gravel, (E) greywacke, (F) quarzite and (G, H) bunter.

Concerning the roughness evaluated at lower magnification, the regularly collected seven rocks showed an increase of the mean arithmetic roughness average “Ra” in the order andesite, slate, basalt, quartz gravel, greywacke, quartzite, bunter (Appendix 3, Fig. 3.2). The mean of the roughness parameter Ra measured at lower magnification (Ra_{LM}) ranged between 3.5 and 8.6 µm. The same ascending order was usually observed for the average maximum height of the profile (Rz) and for the root mean square (Rq). However, significant differences in coarse roughness were only found between bunter and all other mentioned rocks, while between all other rocks no significant differences were present (Kruskall Wallis test: H=56.55, d.f.=6, P<0.001, Fisher post hoc test).

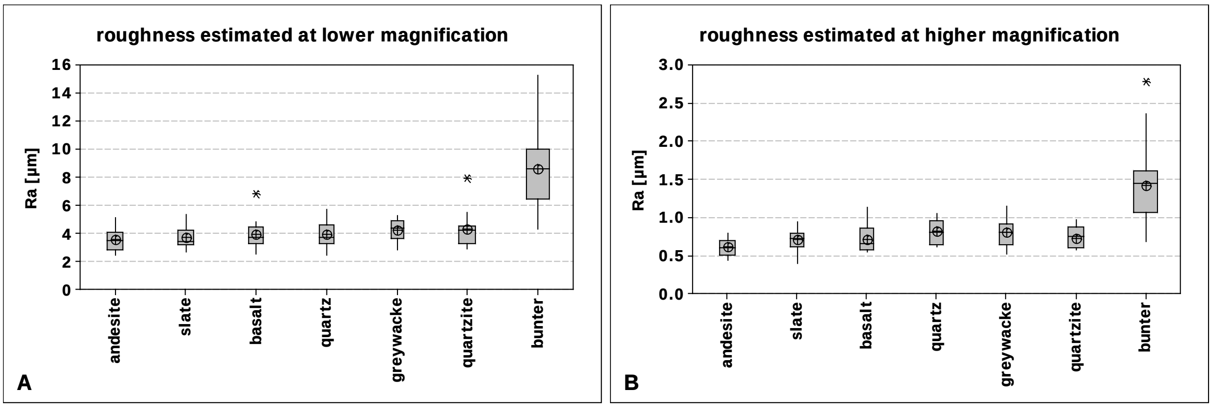

Fig. 3.2. Arithmetic roughness average “Ra” of selected rocks determined (A) at lower magnification and (B) at higher magnification measured by means of white light interferrometer FRT-MircoProf. Figures show box plots with round mean symbol, median line, interquartile range box, wisker bars and outlier symbols. Box width is proportional to sample size

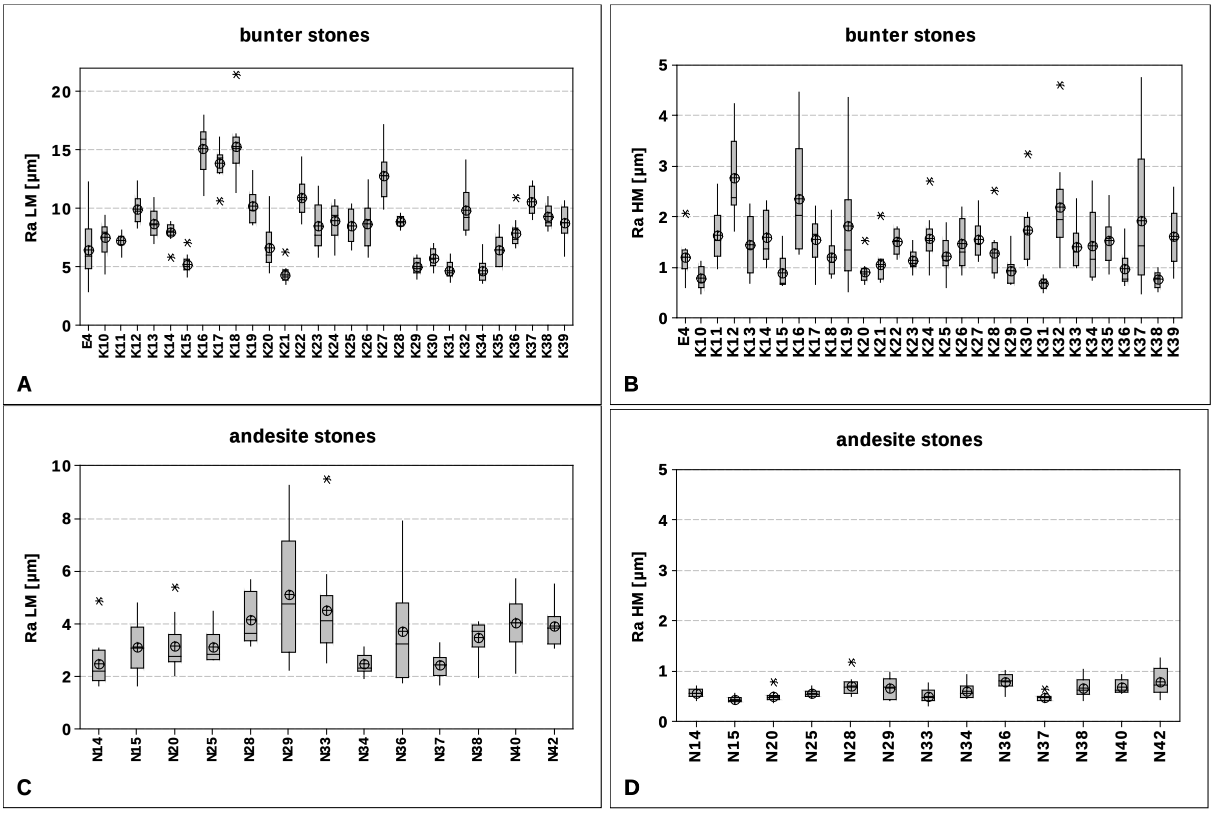

Fig. 3.3. The range of the arithmetic roughness average “Ra” of stones of the same rocks: (A) roughness evaluated at lower magnification for andesite stones, (B) roughness evaluated at lower magnification for bunter stones, (C) roughness evaluated for higher magnification for andesite stones and (D) roughness evaluated at higher magnification for bunter stone. Figures show box plots with round mean symbol, median line, interquartile range box, wisker bars and outlier symbols. Abbreviations: Ra LM, roughness parameter Ra determined at lower magnification; Ra HM, Ra determined at higher magnification.

The stones of the same rock showed a large variation in their surface roughness. These differences in surface roughness were significant between stones of all rocks (Tab. 3.1). An extreme heterogeneity was found on the roughest rock, bunter (Fig. 3.3A). The range of Ra of the collected 31 stones was very large (mean of Ra_{LM}: 4.3–15.3 µm), but also the smoothest rock showed clear variations of Ra within the 10 measured stones (mean of Ra_{LM}: 2.4-5.1 µm) (Fig. 3.3C). Taking all stones into account, the parallel measurements even showed a large variation of Ra up to 7 µm difference for the same stone. The average surface roughness of all collected stones ranged for Ra_{LM} from 2.5 to 15.3 µm.

Tab. 3.1. ***Results of the Kruskal-Wallis test for differences of the roughness parameter Ra on the stones of different rocks***

Comparison	d.f	H	P
Ra_{LM}			
andesite	12	47.89	0.000
slate	11	61.30	0.000
basalt	10	51.69	0.000
quartz gravel	9	27.33	0.001
greywacke	8	34.76	0.000
quarzite	14	71.61	0.000
bunter	29	233.26	0.000
Ra_{HM}			
andesite	12	46.72	0.000
slate	11	41.22	0.000
basalt	10	33.78	0.000
quartz gravel	9	28.56	0.001
greywacke	8	34.91	0.000
quarzite	14	42.10	0.000
bunter	30	126.28	0.000

d.f., degrees of freedom; H, test statistik; P, probability value.

For the roughness evaluated at lower magnification, very similar trends as described for the coarse roughness were observed (Fig. 3.2B). Ra calculated for the setting of higher magnification (Ra_{HM}) was significantly higher on rocks of bunter compared with all other rocks (Kruskall Wallis test: H=56.99, d.f.=6, P<0.001, fisher post hoc test). The mean of Ra_{HM} counted between 0.6 and 1.4 µm for the different rocks (Appendix 4). Further, the roughness was very heterogeneous within the same rock (Fig. 3.3B and D). The differences between the stones were significant for all rocks (Tab. 3.1). Also the roughness parameter Ra often strongly varied on the same stone. Considering all stones collected the average surface roughness of single stones varied between 0.4 and 2.8 µm for Ra_{HM}.

3.4.2 Sediment and biofilm coverage of the stones

The dry mass of the total coverage of the stones (DM_{total}) as well as the organic content measured as ignition loss ($DM_{ignition\ loss}$) were different on the investigated rocks (Fig. 3.4A and B). Both characters were significantly higher on andesite (mean: DM_{total} 72 g/m², $DM_{ignition\ loss}$ 4.8 g/m²) compared with all other rocks. Further, dry mass and ignition loss were significantly higher on basalt (mean: DM_{total} 27.7 g/m², $DM_{ignition\ loss}$ 5.5 g/m²) than on quartz gravel (mean: DM_{total} 7.4 g/m², $DM_{ignition\ loss}$ 1.2 g/m²) (Kruskall Wallis test: total coverage: H=36.90, d.f.=6, P<0.001, fisher post hoc test; ignition loss: H=27.00, d.f.=6, P<0.001, fisher post hoc test).

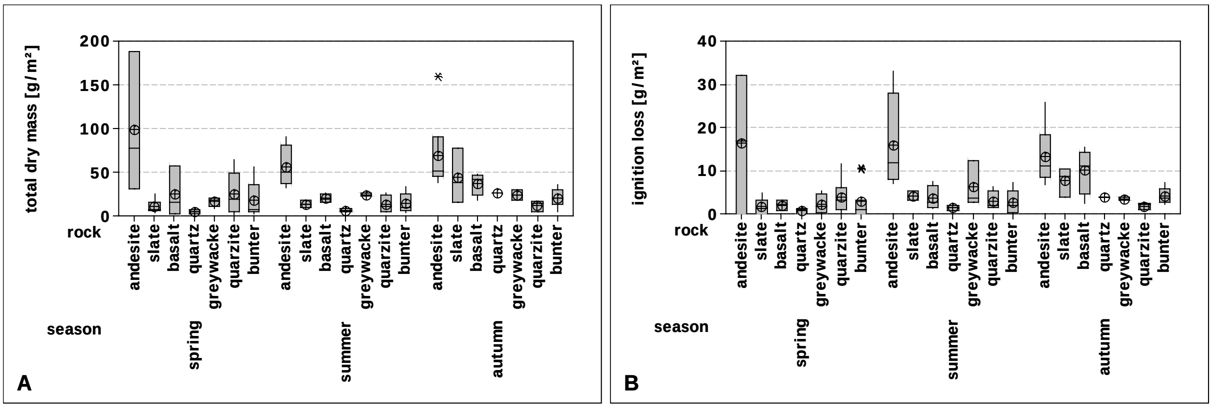

Fig. 3.4. (A) Dry mass of the biofilm per stone area on different rocks. (B) Dry mass of ignition loss per stone area on different rocks. Figures show box plots with median line, interquartile range box, wisker bars and outlier symbols.

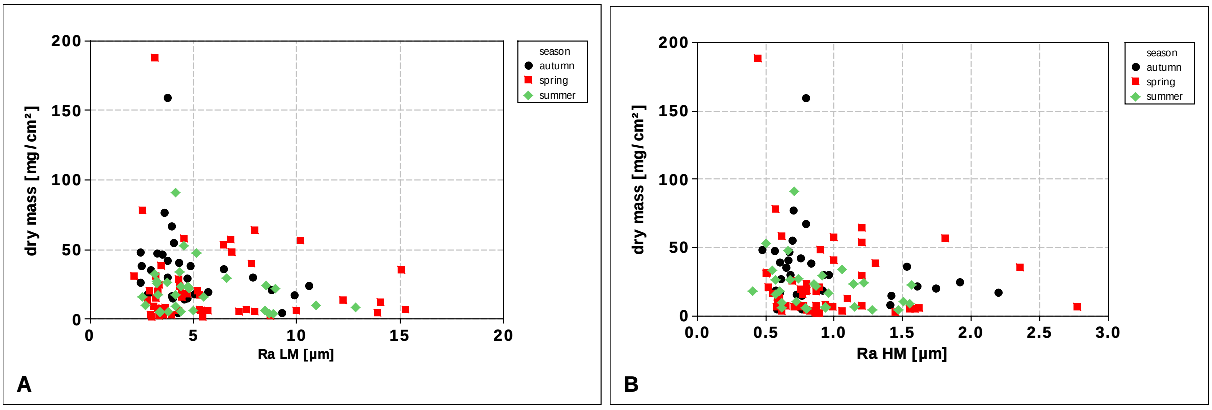

Fig. 3.5. Scatterplots for (A) dry mass of biofilm per stone area in relation to Ra of coarse roughness, and (B) dry mass of biofilm per stone area in relation to Ra of fine roughness. Abbreviations: Ra LM, roughness parameter Ra determined at lower magnification; Ra HM, Ra determined at higher magnification.

Ignition loss showed a large variation on the stones of 0.0-73 %. No significant differences in the composition of the total coverage of the stones were observed between the seasons. Higher total dry mass often occurred together with a low share of ignition of 36 % or less (Appendix 5). Highest dry mass of the total coverage of the stones and ignition loss were found on smooth andesite stones, but other stones with low Ra values were covered only by few or almost no biofilm. For all other rocks the dry mass of the total coverage and ignition loss varied in the same range. Both biofilm characters showed no significant correlation to the Ra values of both magnifications for all seasons (Fig. 3.5A and B).

3.4.3 Distribution of macrozoobenthos organisms

From the determined animal groups, caddis larvae (Trichoptera) had the highest taxa number in the streams Kyll, Nahe and Wiedand contributed 52 % to all collected individuals. Further, higher taxa and individual numbers were found in Ephemeroptera (10 %), Coleoptera (7 %) and Mollusca (5 %). Also Crustacea (13 %) and Diptera (9 %) contributed to a high amount to the collected animals on the stones but only a few species were present (or at least determined in the case of Diptera). Plecoptera were almost not present in the stone fauna of the investigated running waters.

In the streams Kyll, Nahe and Wied between 20 and 44 taxa were registered during the investigated seasons (Appendix 6). With exception of the stream Nahe, taxa and individual numbers were lowest in spring and highest in autumn. The differences between seasons were significant for both taxa number (Kruskall Wallis test: H=60.11, d.f.=2, P<0.001) and total individual number (Kruskall Wallis test: H=24.51, d.f.=2, P<0.001). Therefore, the distribution of the macrozoobenthos on the stones will be considered separately for the seasons. Moreover, compared with the mentioned streams the diversity was lower in the two large rivers (taxa number: river Rhine: 7; river Elbe: 16; Kruskall Wallis test: H=41.27, d.f.=4, P<0.001, Fisher post hoc test). In the river Rhine also the individual number was significantly diminished compared with the other waters (Kruskall Wallis test: H=14.98, d.f.=4, P=0.005, Fischer post hoc test). In the following only these waters were included in further analyses where the selected taxa were present.

Number of taxa

The taxa number differed for the investigated rocks, and the order of the rocks on the basis of taxa number changed depending on the season (Fig 3.6A). Significant differences of the taxa number between the rocks were found in spring and summer. In spring taxa number was significantly higher on andesite than on all other rocks except greywacke and significantly smaller on quartz gravel compared with all rocks except slate (one way ANOVA: d.f.=6, SS=280.92, MS=46.82, F=5.27, P=0.001, Fisher post hoc test). In summer taxa number was significantly smaller on bunter than on all other rocks (one way ANOVA: d.f.=6, SS=223.13, MS=37.19, F=5.02, P=0.002, Fisher post hoc test). In autumn differences between rocks were not significant (one way ANOVA: d.f.=6, SS=171.9, MS=28.6, F=1.27, P=0.311). However, bunter and slate showed relatively high taxa numbers compared to the other rocks and therefore showed almost the opposite tendency as in spring and summer.

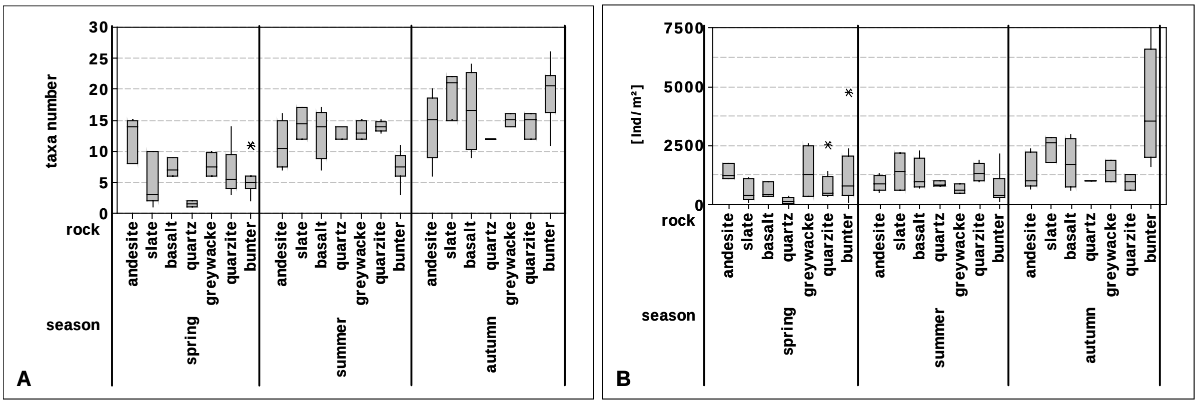

Fig. 3.6. (A) Taxa number of the rocks in different seasons, and (B) total individual number of the rocks in different seasons. AB fehlen, Figures show box plots with median line, interquartile range box, wisker bars and outlier symbols.

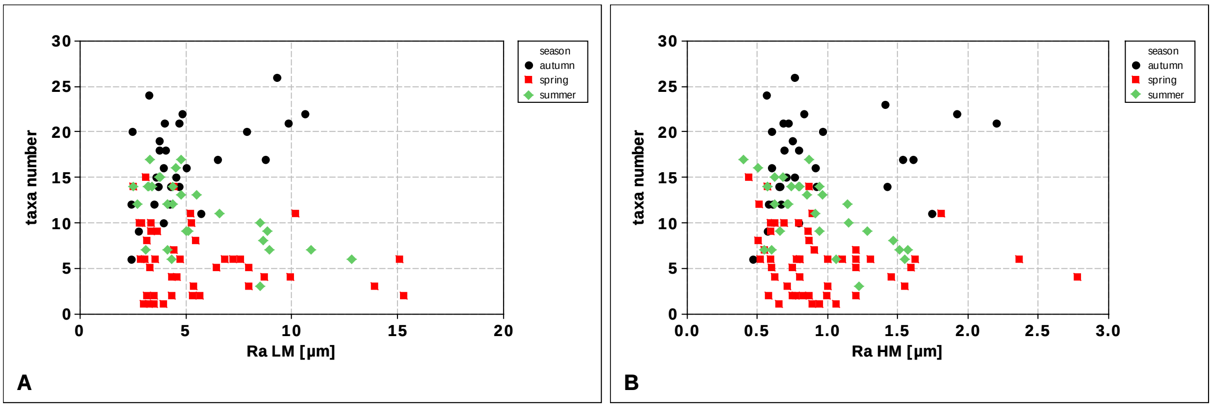

Fig. 3.7. (A) Scatterplot for taxa number in relation to roughness evaluated at lower magnification, and (B) Scatterplot for taxa number in relation to roughness evaluated at higher magnification. Abbreviations: Ra LM, roughness parameter Ra determined at lower magnification; Ra HM, Ra determined at higher magnification.

The higher taxa numbers in autumn compared with spring and summer can be seen in the scatter plots against the roughness evaluated at lower magnification (Fig. 3.7A, Appendix 7). The Spearman's rank-order correlation showed a significant negative association between Ra_{LM} and taxa number in summer season (r=-0.53, d.f.=30, P=0.003) and a significant positive association between Ra_{LM} and taxa number in autumn (r=0.45, d.f.=30, P=0.014). In spring, a significant negative association between Ra_{LM} and taxa number was determined too, if the data of the large rivers Rhine and Elbe are excluded (Spearman's rank-order correlation:

r=-0.55, d.f.=30, P=0.002). The exclusion of the rivers Rhine and Elbe seems appropriate due to the different conditions in these waters. So differences in taxa number might be dominated by other factors and are not comparable with the investigated streams Kyll, Wied and Nahe. For the roughness measurements of higher magnification, only in summer a significant association between Ra_{HM} and taxa number was calculated by Spearman's rank-order correlation (r=-0.56, d.f.=30, P=0.001). Further, Spearman's rank-order correlation advices an association between the number of taxa and the biofilm and sedimentation characters in spring (total coverage: r=0.43, d.f.=30, P=0.017, ignition loss per stone area: r=0.34, d.f.=30, P=0.015).

Number of individuals

The number of total individuals varied significantly between the different rocks in spring and autumn, while in summer no significant differences were present (Kruskal Wallis test: H=8.29, d.f.=6, P=0.217). However, the individual number of the different rocks changed depending on the season (Fig. 3.6B). In spring, individual numbers were significantly higher on bunter and greywacke compared with quartz gravel (Kruskal Wallis test: H=15.08, d.f.=6, P=0.020, Fisher post hoc test), while individual numbers in autumn were significantly higher on bunter compared with andesite and quartzite (Kruskal Wallis test: H=14.56, d.f.=6, P=0.024, Fisher post hoc test).

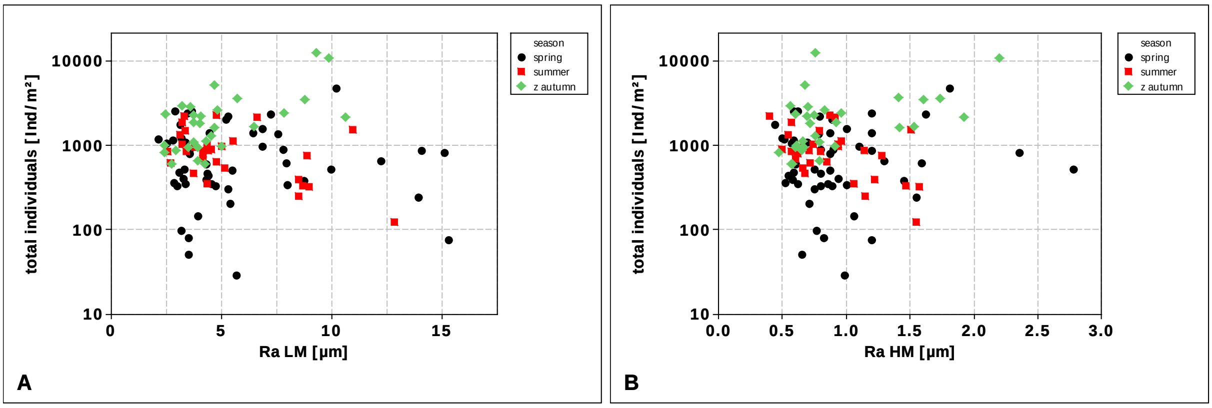

Fig. 3.8. (A) Scatterplot for total individual number per stone area in relation to roughness evaluated at lower magnifcation, and (B) Scatterplot for total individual number per stone area in relation to roughness evaluated at higher magnification. Abbreviations: Ra LM, roughness parameter Ra determined at lower magnification; Ra HM, Ra determined at higher magnification.

The scatterplots of the total individual number against Ra showed a different correlation depending on the seasons for roughness of both magnifications (Fig. 3.8). In autumn, the total individual number per stone increased with the Ra_{LM} (Spearman's rank-order correlation: r=-0.50, d.f.=30, P=0.005), while in spring and summer the Spearman's rank-order correlation indicated no significant trends. Ra_{HM} showed again a positive association in autumn (Spearman’s rank-order correlation: r=0.47, d.f.=30, P=0.008) and no trend for spring, but a significant negative association between the total individual number and Ra_{HM} in summer (Spearman’s rank-order correlation: r=-0.40, d.f.=30, P=0.030). Highest individual numbers were recognized in the stream Kyll on Ra of about 10 µm for the low magnification setting (Appendix 9), while they occurred over a large range of Ra (0.75-2.2 µm) for the higher magnification setting (Appendix 10). Low individual numbers were found on smooth and rough stones equally in both magnifications. A correlation of the individual number and ignition loss per stone area was observed in spring (r=0.35, d.f.=30, P=0.013) and summer (r=0.38, d.f.=30, P=0.037).

Baetis sp. (Ephemeroptera)

In our investigations *Baetis sp.* occurred in the streams Kyll, Nahe and Wied (Appendix 11 and 12). The claws of *Baetis* bear seven teeth on the inner margin and have regular indentations in the posterio-ventral part (Fig. 3.9A). Further, the claws are equipped with two long sensilla on the ventral side of the tip with which they might be able to sense irregularities in the surface texture. On the proximal side of the claw the unguitractor can be seen and additional sensilla are present at the posterio-ventral edge of the tarsus. The diameter of the claw tip was about 5 µm in the 6.5 mm long specimen shown in fig. 3.9A. *Baetis sp.* larvae had a length (mean ± S.D.) of 5.0 ±1.2 mm in spring and 4.3 ± 0.9 in summer. In autumn, *Baetis* larval size differed among the investigated streams (Wied: 5.5 ± 1.1 mm, Kyll: 3.5 ± 1.9 mm, Nahe: 2.2 ± 0.5 mm).

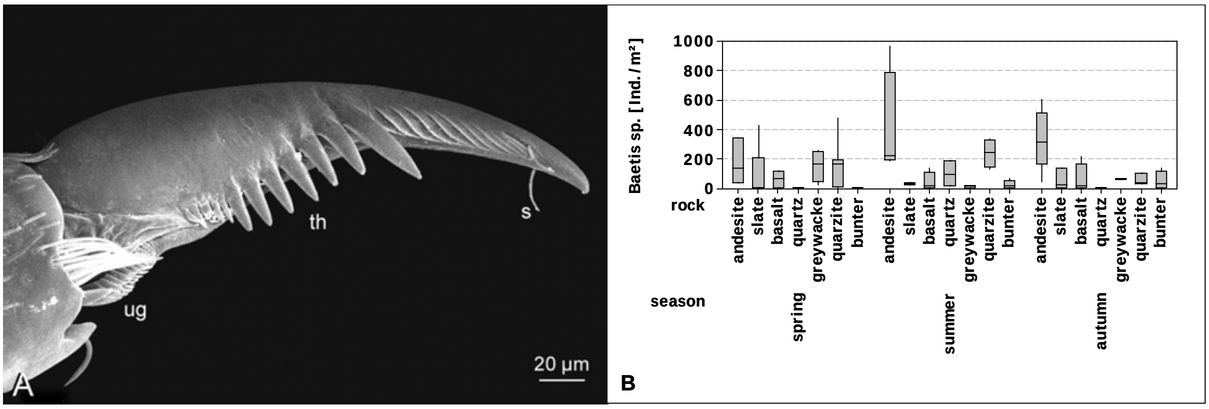

Fig. 3.9. (A) Claw of *Baetis vardarensis*, Abbreviations: s, sensilla, th, theeth, ug, unguitractor. (B) Individual number of *Baetis sp.* (Ephemeroptera) larvae on the rocks in different seasons. Figures show box plots with median line, interquartile range box, wisker bars and outlier symbols.

The abundance of *Baetis sp*. larvae varied between the different rocks (Fig. 3.9B). In spring, abundances of *Baetis sp*. were significantly higher on andesite, greywacke and quartzite compared with bunter and quartz gravel (Kruskal Wallis test: H=17.89, d.f.=6, P=0.007, Fisher post hoc test). In summer, significantly higher individual numbers of *Baetis sp*. occurred on andesite than on all other rocks except quarzite, the latter was also significantly higher inhabited than bunter (Kruskal Wallis test: H=17.82, d.f.=6, P=0.007, Fisher post hoc test). Further, the statistical analyses gave a hint on significantly higher individual numbers of *Baetis sp*. in autumn on andesite than on the other rocks. The results of the Kruskal Wallis test (H=11.96, d.f.=6, P=0.063) were in the range of consideration while Fisher post hoc test showed significant differences for andesite compared with all other stones.

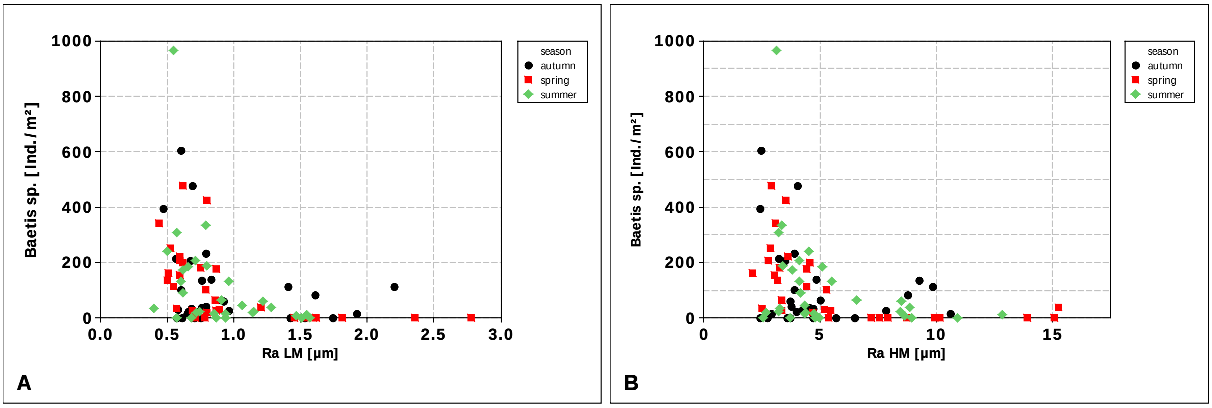

Fig. 3.10. (A) Scatterplot for individual number of *Baetis sp*. larvae per stone area and roughness evaluated at lower magnification, and (B) Scatterplot for individual number of *Baetis sp*. larvae per stone area and roughness evaluated at higher magnification. Abbreviations: Ra LM, roughness parameter Ra determined at lower magnification; Ra HM, Ra determined at higher magnification.

The scatter plots of the individual number of *Baetis sp*. against Ra show that highest densities of *Baetis* were found on low Ra values for both magnifications (Fig. 3.10). This trend can be observed in all seasons but especially in autumn many stones with low Ra showed low individual densities too. The Spearman's rank-order correlation calculated for spring a significant negative association between the individual number of *Baetis sp*. and Ra (Ra_{LM}: r=-0.75, d.f.=30, P<0.001; Ra_{HM}: r=-0.70, d.f.=30, P<0.001). Moreover, a significant negative association between *Baetis* abundance and Ra_{HM} was indicated in summer (Spearman's rank-order correlation: r=-0.51, d.f.=30, P=0.004). There was no significant correlation of *Baetis sp*. abundance with the dry mass of ignition loss or total biofilm/ sediment coverage of the stones.

Elmis sp. (Coleoptera)

Elmis larvae were regularly present in the streams Kyll, Nahe and Wied (Appendix 13 and 14). The claws have a diameter of about 8 µm and a long setae on the ventral side (Fig. 3.11A). On the ventral side of the tarsus, two additional setae are present. Teeth or other structures are absent. *Elmis sp.* larvae were of 2.6 ± 0.4 mm in spring, 3.0 ± 0.9 in summer and 4.0 ± 0.8 mm long in autumn (mean ± S.D.).

Only a few larvae of *Elmis sp.* were collected on the stones in spring while comparably higher individual numbers occurred in summer and autumn (Fig. 3.11B). The individual numbers of *Elmis sp.* larvae showed no significant differences in spring (Kruskal Wallis test: H=1.49, d.f.=6, P=0.960) and summer (Kruskal Wallis test: H=5.83, d.f.=6, P=0.443). But in autumn the beetle inhabited bunter in significantly higher densities than andesite, greywacke and slate (Kruskal Wallis test: H=13.41, d.f.=6, P=0.037, Fisher post hoc test).

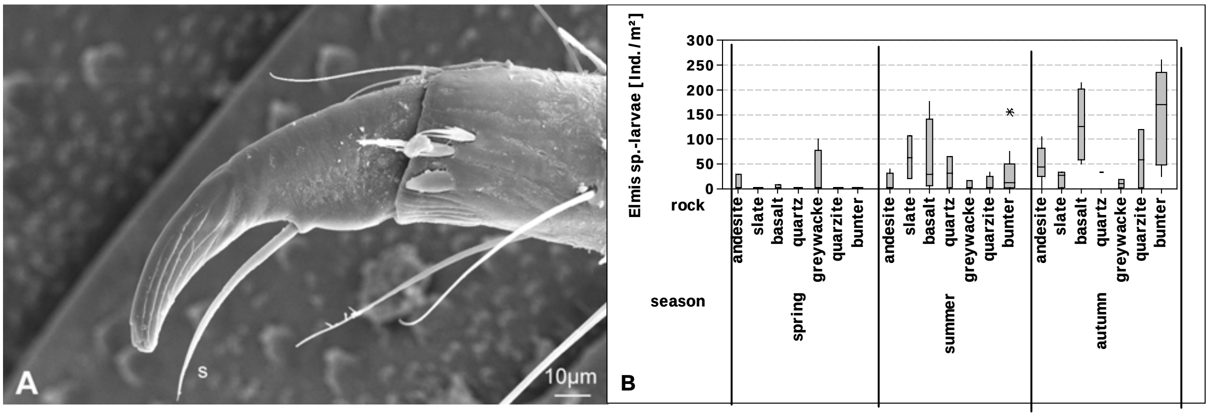

Fig. 3.11. (A) Claw of *Elmis cf. aena* larvae. Abbreviations: s, sensillum. (B) individual numbers of *Elmis sp.* larvae (Coleoptera) of the rocks in different seasons. Figures show box plots with median line, inter-quartile range box, wisker bars and outlier symbols.

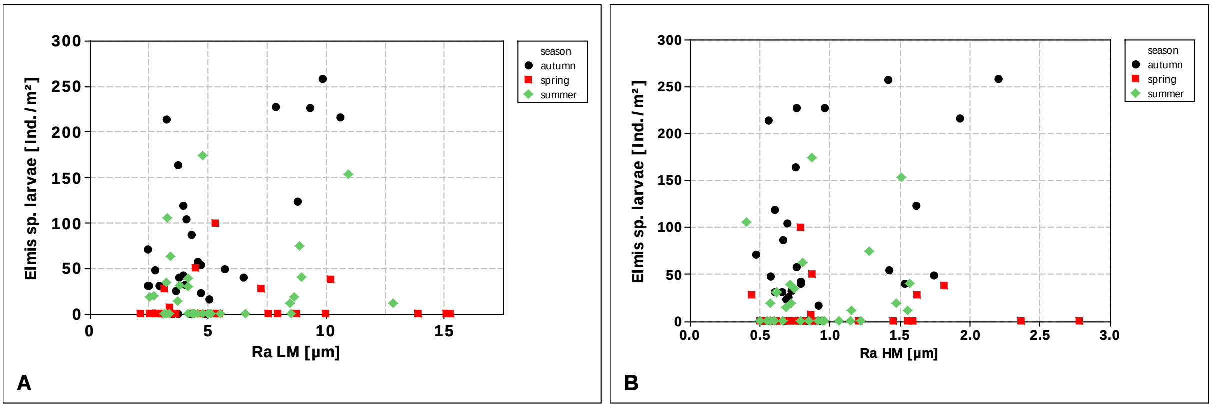

Fig. 3.12. (A) Scatterplot for individual number of *Elmis sp.* larvae and roughness measured at lower magnification, and (B) Scatterplot for individual number of *Elmis sp.* larvae and roughness measured at higher.

Highest *Elmis* densities were recognized in autumn at Ra_{LM} values of about 10 µm (Fig. 3.12A). In autumn, the Spearman's rank-order correlation showed a significant positive association between the individual number of *Elmis* larvae and Ra_{LM} (r=0.38, d.f.=30, P=0.041), while in spring and summer no significant trends were observed. Further, Ra_{HM} and sedimentation/biofilm characters showed no association to the individual number of *Elmis*.

Chironomidae (Diptera)

In our study Chironomid larvae inhabited all investigated running waters with exception of the river Rhine (Appendix 15 and 16). The larvae have circlets of hooks on their posterior and anterior prolegs. The single hooks of these circlets are very sharp and the tip measured just about 3 µm in diameter. A picture of the circlets of the posterior prolegs had already been shown in Chapter 2 (Fig. 2.6). In contrast, to the hooks of the posterior prolegs the hooks of the anterior prolegs bear sharp teeth on their inner margin (Fig. 3.13A).

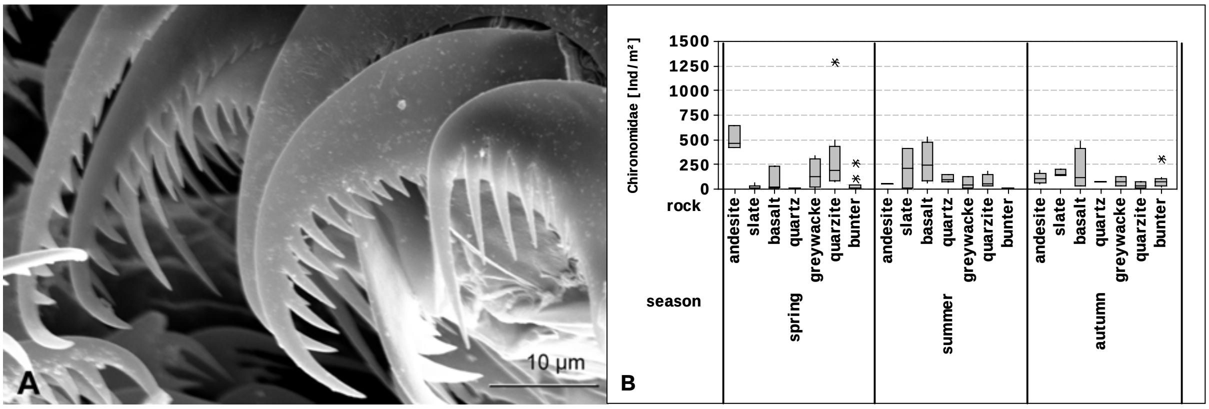

Fig. 3.13. (A) Circlet of hooks on a anterior proleg of a Chironomidae larva, and (B) total individual number of Chironomid larvae in different seasons. Figures show box plots with median line, interquartile range box, wisker bars and outlier symbols.

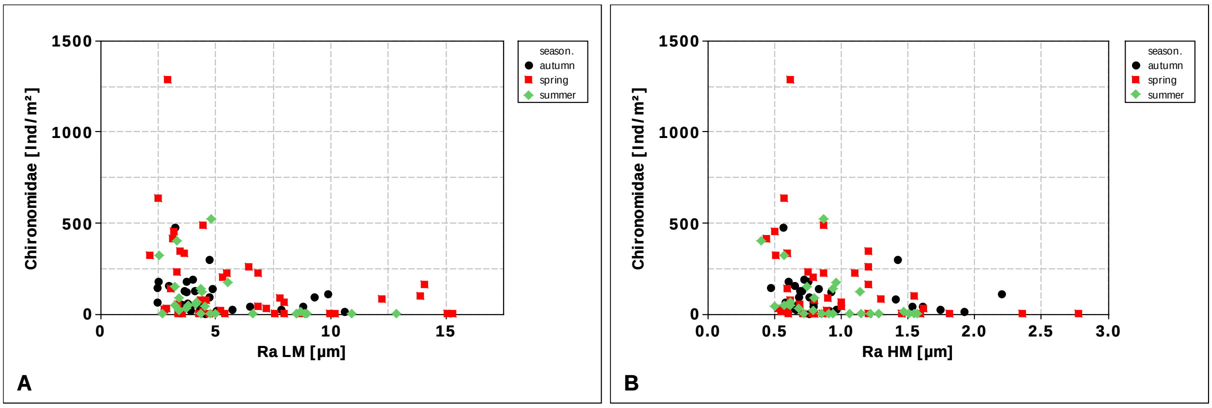

Fig. 3.14. (A) Scatterplot for individual number of Chironomid larvae per stone area and roughness measured at lower magnification, and (B) Scatterplot for individual number of Chironomid larvae per stone area and roughness measured at higher magnification.

The abundance of Chironomid larvae was different for the investigated rocks (Fig. 3.13B) but trends varied in-between the seasons. In spring the densities of the Chironomidae were significantly higher on andesite compared with all other rocks except quartzite. Further, the individual number of Chironomidae was significantly higher on quartzite compared with bunter and slate (Kruskal Wallis test: H=16.15, d.f.=5, P=0.006, Fisher post hoc test). In summer, Chironomidae were significantly higher on basalt compared with greywacke, quartzite and bunter. The latter was significantly higher inhabited than slate too (Kruskal Wallis test: H=16.96, d.f.=6, P=0.009, Fisher post hoc test). In contrast, individual numbers of Chironomidae showed no significant differences between the investigated rocks in autumn (Kruskal Wallis test: H=7.66, d.f.=6, P=0.264).

The scatter plots showed a decrease of the individual number of Chironomidae with increasing surface roughness of the stones (Fig. 3.14). A significant negative association between the individual number and Ra of roughness determined at both magnifications was calculated by the Spearman’s rank-order correlation for spring (Ra_{LM}: r=-0.60, d.f.=30, P<0.001; Ra_{HM}: r=-0.57, d.f.=30, P=0.001) and summer (Ra_{LM}: r=-0.54, d.f.=30, P<0.004; Ra_{HM}: r=-0.55, d.f.=30, P=0.003). In contrast, in autumn no trend for the Chironomidae was observed in relation to Ra (both magnifications). The individual number of Chironomidae positively correlated with the dry mass of biofilm in autumn (r=0.40, d.f.=30, P=0.027).

Rhyacophila sp. (Trichoptera)

Rhyacophila sp. was present in the streams Kyll, Nahe and Wied. The larvae have strong claws. Claws of the second and third legs have an extension on the inner margin (Fig. 3.15A) which might be movable and enable the claw to function as a clamp.

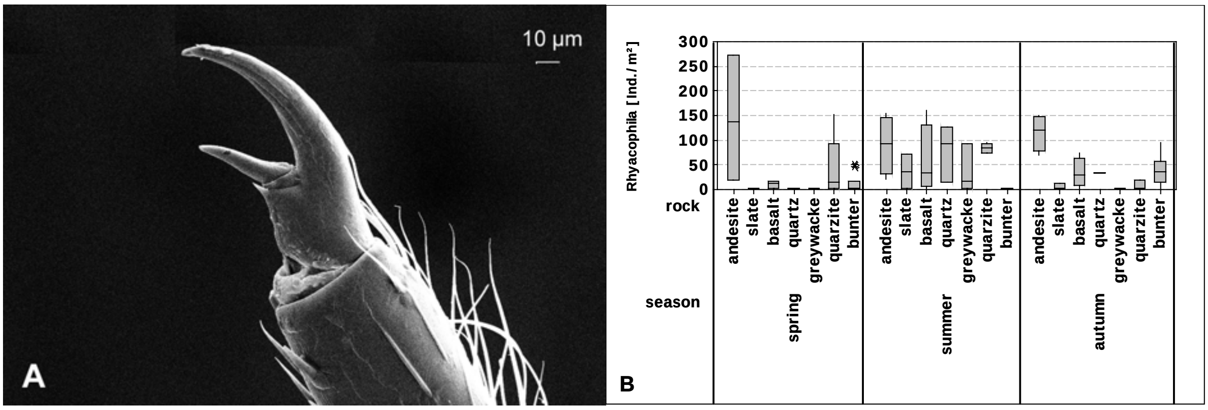

Fig. 3.15. (A) Claw of second leg of *Rhyacophila sp.* (dorsalis group), and (B) total individual number of *Rhyacophila sp.* larvae in different seasons. Figures show box plots with median line, interquartile range box, wisker bars and outlier symbols.

In contrast, claws of the first leg pair have no such structure but a long setae on the ventral side. The tip of the claw has a diameter of about 4 µm. Furthermore, *Rhyacophila* larvae have additional claws like grapples on their posterior prolegs which have already been shown in Chapter 2 (Fig. 2.4B).

The individual numbers of *Rhyacophila sp.* were significantly different between the investigated rocks (Fig. 3.15B). In spring, the individual numbers of *Rhyacophila sp.* were significantly higher on andesite than on all other rocks (Kruskal Wallis test: H=14.04, d.f.=6, P=0.015, Fischer post hoc). In summer, bunter showed significantly lower *Rhyacophila* abundance compared with andesite, basalt, quartz gravel and quartzite (Kruskal Wallis test: H=16.48, d.f.=6, P=0.011, Fisher post hoc). Also in autumn, *Rhyacophila* inhabited andesite in significantly higher densities than all other rocks (Kruskal Wallis test: H=19.29, d.f.=6, P=0.004, Fisher post hoc test).

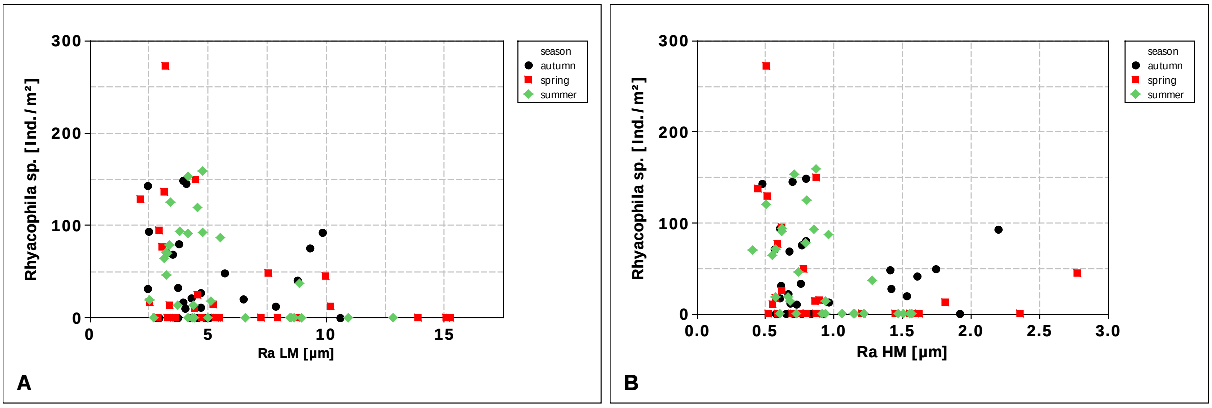

Fig. 3.16. (A) Scatterplot for individual number of *Rhyacophila sp*. larvae and roughness measured at lower magnification, and (B) Scatterplot for individual number of *Rhyacophila sp*. larvae and roughness measured at higher magnification.

The highest densities of *Rhyacophila sp.* larvae were found on Ra_{LM} values about 2.5 and 5.0 µm (Appendix 17, Fig. 3.16A). Spearman's rank-order correlation calculated a significant negative association between *Rhyacophila sp.* abundance and both Ra determined at both magnifications in spring (Ra_{LM}: r=-0.37, d.f.=30, P=0.042; Ra_{HM}: r=-0.37, d.f.=30, P=0.047) and summer (Ra_{LM}: r=-0.39, d.f.=30, P=0.033; Ra_{HM}: r=-0.54, d.f.=30, P=0.002). However, a stronger positive correlation was determined by Spearman's rank-order correlation for the abundance of *Rhyacophila sp.* compared with the sum of the prey animals *Baetis* and Chironomidae in all seasons (spring: r=0.415, d.f.=30, P=0.023; summer: r=0.68, d.f.=30, P<0.001, autumn: r= r=0.56, d.f.=30, P=0.001).

Ancylus fluviatilis (Mollusca)

Ancylus fluviatilis inhabited the streams Kyll, Nahe and Wied as well as the river Elbe (Appendix 19 and 20).

This mollusc did not show a preference for some of the investigated rocks in spring and summer (Fig. 3.17). In autumn *Ancylus fluviatilis* showed higher densities on slate compared with andesite and quartzite. Further, basalt was better inhabited by *Ancylus fluviatilis* than andesite (Kruskal Wallis test: H=12.29, d.f.=6, P=0.046, Fisher post hoc test).

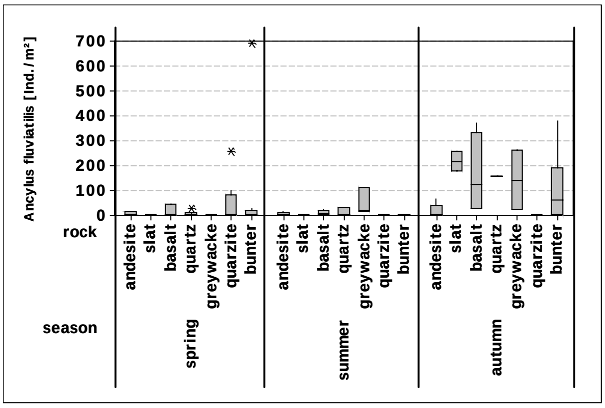

Fig. 3.17. Individual number of *Ancylus fluviatilis* on the rocks in different seasons. Figures show box plots with median line, interquartile range box, wisker bars and outlier symbols.

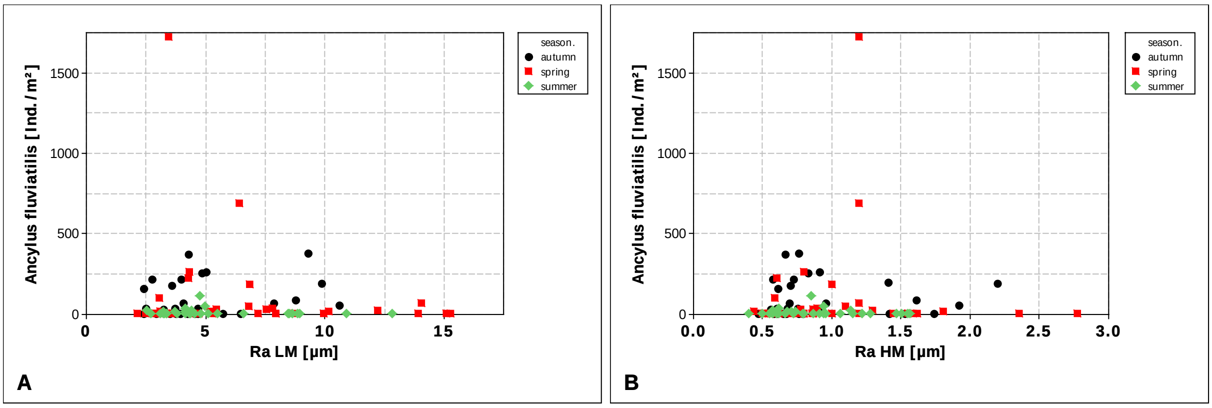

Fig. 3.18. (A) Scatterplot for individual number of *Ancylus fluviatilis* per stone area and roughness measured at lower magnification, and (B) Scatterplot for individual number of *Ancylus fluviatilis* per stone area and roughness measured at higher magnification.

Highest individual numbers of *Ancylus fluviatilis* were observed on Ra_{LM} of 3-7 µm and on Ra_{HM} of about 1.2 µm (Fig. 3.18). A significant association between the individual number of *Ancylus fluviatilis* and Ra was not indicated by the Spearman's rank-order. Moreover, *Ancylus fluviatilis* showed no correlation with the dry mass of total coverage of stones or ignition loss.

3.5 Discussion

3.5.1 Surface roughness of the stones

As we could show, the surface roughness of stones in running waters can show a large variation. In our study the average surface roughness of the smoothest and the roughest stones varied about a factor of 6 (Ra_{LM}) respectively 7 (Ra_{HM}). Significant differences of surface roughness were found between different rocks but also between stones within the same rock. The extreme variations within bunter (sand stone) found in our study can be an explanation for the different classification of sand stone in literature regarding smooth (Downes et al. 2000a) and rough (Casey and Clifford 1989). Significant differences of surface roughness within the same rock were found for all investigated rocks which shows that surface texture of rocks in general is very heterogeneous. This might be caused by different genesis processes and materials. For example, the original sand material could have been of different grain size prior to the formation of sand stone. Moreover, strong heterogeneity can be observed even on the same stone. This might be caused by the same reasons as the differences within the same rock. Sometimes fractions and pieces of different rock types can be found in the same stone. Consequently, if surface roughness is the matter of interest a simple distinction between rocks as described in some literature (e.g. Erman and Erman 1984, Downes et al 2000a) can cause inaccuracies if the stones of the chosen rock show large heterogeneity as in our results. The heterogeneity in surface texture of the rocks might contribute to the sometimes contra dictionary results regarding the colonization of macrozoobenthos organisms in literature (s. introduction). Here a direct measurement of the surface roughness of each stone results in an enhanced accuracy. Further, a quantitative description of surface roughness would enable the comparison of different investigations at least, if the same method and setting are used. In this study the roughness measurements at lower magnification achieved good and practicable results. In this setting relatively quick measurements were possible and only few technical problems occurred. In contrast, roughness measurements at higher magnification were very time intensive and problematic due to many erroneous measurements. The latter required a lot of additional replications and is not appropriate for many samples. The profilometric technique itself has already been tested for a lot of other technical applications and can be bought readily.

3.5.2 Sediment and biofilm coverage of the stones

The original stone surface in waters is covered by biofilm (e.g. periphyton, bacteria, fungi) and mineral particles due to siltation. The organic biofilm effects the distribution of macrozoobenthos organisms on the stones due to its importance as food resource for grazers (Hart 1978, Mc Auliffe 1983, Allan 1995). The coverage of biofilm and sediment particles changes the surface properties of the stone and therefore might affect the attachment of the animals. In this study dry mass of the total coverage as well as its organic part were significantly higher on smooth andesite stones than on stones of other rocks. However, many other stones with low Ra values were covered only by little or almost no biofilm and a significant association between biofilm density and surface roughness was not present. Therefore, not the surface roughness of andesite might be the determining parameter for increased biofilm mass but other properties may be important as for instance chemical components. In other studies epilithon was more abundant on rough than on smooth substrates (Sanson et al. 1995). Whitthon (1975) suggested that the rough sandstone surface is favourable for the colonization of algae whereas smooth surfaces are colonized more slowly. Also the time factor was not investigated in our study, biofilm densities give no indication for better conditions on rough substrates at all.

In this study the coverage of biofilm and sediment particles on the stones showed a large heterogeneity of both biofilm abundance and contents. On some stones almost no ingition loss was measured which indicates a very low organic content. In these cases the high share of mineral contents on the coverage shows a strong influence of siltation.

3.5.3 Role of the surface roughness for the distribution of macrozoobenthos organisms

Species richness and the total individual density showed significant differences for the investigated rocks but the observed trends were not the same in-between the seasons. In spring and summer, a direct comparison with the measured surface roughness Ra_{LM} of the stones indicated decreasing taxa numbers with increasing roughness. These results are in contradiction to most literature reporting higher species richness on rough substrates compared with smooth ones (Clifford, Gotceita and Casey 1989, Downes et al 2000A, Downes et al. 2000B) or no effect (Erman and Erman 1984). In contrast to the taxa number, in spring and summer no significant trends for individual number and surface roughness were present in our study. But in autumn significantly higher taxa numbers and individual densities were collected on rough substrates compared with smooth ones. This trend is conform with most literature mentioned above. Moreover, increased individual numbers on rough stones were also reported from Erman and Erman 1984, Clifford, Gotceita and Casey 1989. The varying trends of taxa number and total individual density during the different seasons suggest that the specific species and taxa show different associations to surface roughness. This explanation is supported by the results of Clifford, Gotceita and Casey (1989) who describe higher abundance of e.g. Chironomidae on rough substrates compared with smooth substrates and the contrary behaviour for e.g. some Heptageniid larvae. Boyero (2003) describes a varying influence of surface texture for different taxa too. A different association of single taxa and species could explain the sometimes contra dictionary results in literature regarding the influence of surface roughness on species richness and densities. However, we assume that the sampling method itself might cause some inaccuracy in our study as well as in most of the mentioned literature. When the stones are lifted, not only animals from the stones surface might be swept into the net behind, but also animals inhabiting areas close to the stone (e.g. leaves, debris or gravel under the stone).

In spring the significant association between dry mass of the total biofilm/sediment coverage and taxa number as well as between ignition loss per stone area and taxa number indicates an influence of the amount of biofilm on the presence of some taxa. However, the biofilm itself is reduced by grazers so that the amount of biofilm is difficult to evaluate. The reality might often be much more complex than a direct correlation of measured biofilm characters and taxa distribution.

We assume that beside present food resources the attachment strategy might influence the distribution of the taxa on the stones under natural conditions in running waters. In the following the distribution of the selected species is discussed summarized in groups regarding their attachment strategy:

Taxa clinging to the substrate by claws only

The larvae of both selected taxa *Baetis sp*. and *Elmis sp.* attach to the stones surface only by claws but their distribution in relation to surface roughness was different.

Larvae of *Elmis sp.* are grazers in running water inhabiting stones and moss in the current (Braukmann 1987, Moog 1995). The larvae cannot swim and hold to the surface with their strong claws (Chinery 1987). In our study *Elmis sp.* preferred rough bunter and its densities increased significantly with surface roughness evaluated at lower magnification at least in autumn when larvae were largest. These results support the assumption that the larvae can attach better to rough surfaces where the claws find more and larger surface irregularities to grasp to. Nevertheless, a few *Elmis* larvae were collected from stones with low Ra values of both magnifications. This indicates that the larvae are able to cling to smooth stones even if this might not be very favourable. A reason for this can be the crevices usually found on stones independent from their average surface roughness. Moreover, the distribution of *Elmis* larvae showed no clear trend in spring and summer. This might be caused by smaller claw tips due to their smaller size.

Baetis sp. is a common inhabitant of streams which can cling to the stones surface even in the current and scrap periphyton from the surface. In contrast to *Elmis* larvae, *Baetis sp.* preferred smooth andesite stones and showed a significant negative association between surface roughness and individual density, at least in two seasons. The latter results were surprising because we would have expected the contrary distribution of *Baetis* in regard to surface roughness due to the same reasons like explained for *Elmis* larvae. Further, our results stay in contrast to the study of Clifford, Gotceita and Casey (1989) describing significantly increased numbers of *Baetis sp*. on rough tiles compared with smooth ones.

An important factor influencing the attachment to the substrate might be the morphology of the claw. The claws of *Elmis* are strong but plump and have a relatively broad tip diameter up to 8 µm (Fig. 3.11A). In comparison, the claws of *Baetis* have a small tip diameter (5 µm and less) (Fig. 3.9A). Moreover, they are equipped with long small teeth and small indentations on the inner margin. Both structures might be able to interlock with very fine surface irregularities and increase friction with the substrate. This special claw morphology might improve the ability of *Baetis* to cling to smooth surfaces, but it does not explain the preference for them. Another important factor for the distribution can be the presence of food resources. However, both taxa are known to scrape periphyton and detritus from the stones surface in running waters (Merrit and Cummins 1996, Klausnitzer 1984), but their density did not correlate with biofilm characters in our study. Nevertheless, the covering biofilm and mineral particles might have an influence on the attachment ability of the animals. Moreover, it is conspicuous that highest *Baetis* densities occurred on andesite stones which also had highest dry mass of biofilm. A direct association might be disturbed by grazing effects of *Baetis* larvae itself. Another possible reason for the preference of *Baetis* for smooth substrates would be the avoidance of predators but the important predator *Rhyacophila sp.* was present on smooth substrates in higher numbers too and therefore this assumption cannot be uphold.

Taxa with two or more coexistent attachment strategies

The investigated taxa which attach by different coexistent strategies either showed a preference for smooth substrates or no association to surface roughness.

Chironomidae secrete silk to build their larval tubes and attach themselves in order to resist water current (Tönjes 1989). In our study Chironomid larvae showed highest densities on smooth substrates and a significant decrease with increasing Ra of both magnifications, at least in spring and summer. Chironomidae use circlets of hooks in addition with secretion for attachment to the substrate. While the hooks should need a certain kind of surface roughness secretion should be able to adapt to all kinds of surface profiles. Moreover, larvae might not even have direct contact with the substrate during their stay in their larval tubes. Therefore, it can be expected that Chironomid larvae are not much affected in their distribution by the surface roughness. However, this explains why Chironomidae can attach to smooth surfaces but it does not explain the preference for them found in our study. Further, the preference for smooth substrates over rough ones is contrary to the results of Clifford, Gotceita and Casey (1989). We assume that Chironomidae can attach to both smooth and rough substrates very well but their distribution is determined by other factors as e.g. food resource. However, no correlation between Chironomid density and biofilm characters was found in this study but unfortunately this taxon comprises many species which can have different diet (Nilssen 1997).

The free living caddies larvae *Rhyacophila sp.* showed a preference for smooth stones as well. This is somehow surprising because claws and claw like grapples seem to be the dominating attachment devices. Under normal conditions the larvae are sprawled on the rocks with both the thoracic and the abdominal claws engaged (Hynes 1970). Nevertheless, these larvae are obviously able to inhabit smooth stones very well. The small claw tip diameter might contribute to interlocking with very small surface irregularities, but also the reported ability of *Rhyacophila* to secure against current with a secreted thread (Sauer 1988) might be important here. *Rhyacophila sp.* is a common predator in swift running waters (Adlmanneder 1983, Burgmeister 1992). Therefore, *Rhyacophila* had good reasons to move on smooth stones because the prey animals *Baetis* and Chironomidae were most abundant there. The density of *Rhyacophila* was significantly associated with the sum of *Baetis* and Chironomid larvae in all seasons.

Another attachment strategy has the mollusc *Ancylus fluviatilis*. According to Hynes (1970) the broad feet of gastropods are rather similar to true suckers. Further, the soft flexible periostracum round the edges of limpet shells fits closely to the surface irregularities and therefore increase marginal contact (Hynes 1970). This species inhabited stones with all kinds of surface roughness but did not show an association to the surface roughness. These results are in accordance with our expectations because the broad flexible feed covered by secretion and the soft flexible periostracum of this mollusc should be able to adapt to the surface profile very well. Therefore, the surface profile should not have a strong influence on the attachment ability of *Ancylus fluviatilis* as long as the texture is not going to extremes.

3.6 Conclusions and outlook

This study gives an overview about the range of surface roughness occurring on different rocks in natural running waters. Moreover, the distribution of macrozoobenthos organisms show that many species occur on stones over a large range of surface roughness. The results indicate that under natural conditions often the presence of food resources or other factors might influence the distribution of the macrozoobenthos more strongly than the original surface roughness of the stones. This might be due to special adaptations of the attachment devices (claws with additional teeth, secretion e.g.) that enable them to cling to relatively smooth surfaces. Independently from average surface roughness crevices on the stones should be important for the attachment to smooth stones too. Moreover, the coverage of the original stone surface by sediment and biofilm might also have an effect on attachment. However, statistical analyses in this study were affected by the unexpected large heterogeneity of surface roughness within the same rock, the large heterogeneity of the coverage of the stones by biofilm and sediments as well as the varying trends of the seasons. For a deeper understanding of the interplay of attachment devices and surface texture, additional experiments with more defined conditions are required. Further, an optimized method in a way that excludes the sampling of animals which have contact with other surfaces close to the collected stone would improve accuracy of the results in accordance to surface roughness.

Nevertheless, the used profilometric technique is suitable for the characterization of the surface roughness of stones in running waters in the setting of lower magnification. In contrast, measurements at higher magnification were very problematic and did not yield many additional results. Consequently, the used profilometric technique in the setting of lower magnification can be recommended for further investigations. Nevertheless, the sometimes large heterogeneity of surface roughness on the same stone indicates that the accuracy of roughness measurements on natural stones it limited. So, if a homogeneous surface texture is needed (e.g. for statistical reasons or in order to gain deeper understanding of the interplay of surface texture and attachment devices), the application of artificial substrates seems much more appropriate.

Chapter 4

New insights into a life in current: Do the gill lamellae of *Epeorus assimilis* and *Iron alpicola* larvae (Heptageniidae) function as a sucker or as friction pads?

Abstract. - *Epeorus assimilis* and *Iron alpicola* larvae inhabit swift running waters where they scrape algae from the stones. Previous authors suggested that the gill lamellae are modified to a sucker as an adaptation to withstand currents. Video observations showed that the gill lamellae stay tilted in strong currents. Moerover, larvae attached to the surface without any problem even if single gill lamellae were missing and therefore no negative pressure could be developed. Consequently, gill lamellae cannot have a sucker function. SEM analysis revealed areas with spike-shaped microtrichia on the abdominal sternites and setose pads ventrally on the gill lamellae. These setose pads looked very similar to those described in some terrestrial insects. Setae of *I. alpicola* showed a similar size, but a higher density than those of *E. assimilis*, what might cause a greater adhesive strength and could be an adaptation to the swifter currents in which these species lives.

Keywords: Ephemeroptera, underwater attachment, attachment devices, sucker, attachment pads, flow velocity

Contents

4.1 Introduction

Adaptations to current of aquatic insects living in torrential habitats such as attachment devices were consistently investigated since the beginning of the last century (e.g. Steinmann 1907; Dodds and Hisaw 1924; Hora 1930; Wesenberg-Lund 1943; Ambühl 1959; Ruttner 1962; Hynes 1970; Smith and Dartnall 1980; Statzner and Holmes 1982). By-and-by, the results and ideas of the observations entered the fundamental literature and limnological text books (e.g. Ward 1992; Allan 1995; Merritt and Cummins 1996; Wetzel, 2001). However, the function of some structures described as attachment devices has not been observed in detail.

Many larvae of Heptageniidae are typical inhabitants of swift running waters. Heptageniids with sucker-like gill pads are represented among others by *Epeorus assimilis* Eaton, 1885 and *Iron alpicola* Eaton, 1871. The larvae of both species live in habitats with high flow velocities where they graze algae and biofilm from surfaces of the stones. *E. assimilis* larvae tolerate near bottom velocities up to 0.4 m/s without any problems (Ditsche-Kuru, personal observations in laboratory flume). This is a velocity in the upper range of bottom velocities usually measured in mountain streams (Statzner 1988). The larvae dwell on exposed places and therefore need morphological adaptations to current. A very well known adaptation is the dorso-ventral flattening of the body which is also described for both species (e.g. Haybach and Malzacher 2002).

The flat body was once assumed to use the boundary layer of reduced flow velocity (Ambühl 1959). However, compared with other aquatic macroinvertebrates the larvae are relatively tall. The height of the boundary layer, on the other hand, mostly measures just fractions of a millimetre (Nachtigall 1982), and decreases with increasing current velocity (Vogel 1996). Statzner and Holm (1982) showed with laser doppler anometry for larvae of the related genus *Ecdyonurus*, which have a similar body shape like *Epeorus*, that they are influenced by flow. Therefore, a strong influence of flow on the larvae of *E. assimilis* and *I. alpicola* can be expected because they prefer habitats with even higher flow velocities than *Ecdyonurus*. It can even be expected that *E. assimilis* and *I. alpicola* need further morphological adaptations to cope with these flow forces. For both species strong laterally directed legs and gill lamellae which are modified to form a kind of sucker are described in addition to the dorso-ventral flattening (Dodds and Hisaw 1924; Wesenberg-Lund 1943; Ruttner 1962; Uhlmann and Horn 2000; Bauernfeind and Humpesch 2001; Haybach and Malzacher 2002; Staniczek 2003). Ruttner (1962) wrote that *I. alpicola* is able to attach to the substrate by using its gill lamellae to form a sucker apparatus and he assumed that for this purpose the gill lamellae are arranged like roofing tiles covering the entire ventral side. However, it has not yet been explained in detail how this “gill-sucker apparatus” works. In contrast, Hora described as early as in 1930 a spinous pad on the gill lamella of *Epeorus sp.* from the Himalaya. Further, he wrote that the gill lamellae form a complete seal at the sides, which only allows the water to flow in through the gap between the first pair of lamellae and can leave through the gap between the posterior gills. Hora was intensively cited in Hynes (1970) who called these mechanisms “friction pads” and “marginal contact”. The description in Hynes might have inspired Meritt and Cummins (1996, p. 44) to write ‘Several aquatic insects have structures that simulate the action of suckers. The enlarged gills of some mayflies (e.g. *Epeorus*…) function as a friction pad…’ Wichard et al. (1995) show a SEM-picture with specialized attachment structures on the ventral side of the gill lamella of an *Epeorus* larva, but give no further information about the way these devices function. The inconsistencies in recent literature lead us to following questions:

- Do the gill lamellae of *Epeorus* and *Iron* larvae really function as a “sucker apparatus”?
- Are there microstructures on the gill lamellae of *Epeorus* and *Iron* larvae like those already identified by Hora 1930?
- Are there further structures on the ventral side of the larvae which could support attachment to the substrate?

4.2 Materials and methods

4.2.1 Material

For our investigations *E. assimilis* larvae were collected in the low mountain river Wied near Koblenz (Germany). After collecting the larvae from the stones they were transported alive to the laboratory flume in a box cooled by freeze packs. Maximum transport time was about 3 hours. For SEM, specimens were fixed in 70 % ethanol according to Wetzel, Leuchs and Koop (2005). The total body lengths as well as width and length of all femora of ten specimens of *E. assimilis* were measured. Because *I. alpicola* is a protected species in Germany

(Bundesamt für Naturschutz 1998), no live animals could be used. Instead, biometric data were collected from three larval specimens of *I. alpicola* fixed in 70 % ethanol, which were kindly provided by the Senckenberg Research Institute and Natural History Museum, Gelhausen, Germany.

4.2.2 Laboratory flume

E. assimilis larvae were observed in an artificial stream flume made of Plexiglas. A paddle wheel driven by an electric motor forced the water into a circular flow (batch-system). The water temperature was kept at constant 10 ± 0.1°C by means of a thermostat (5000 W, Phoenix 2, Thermo Haake), a heat exchanger, and a thermal sensor. The system was fed with a mixture of stream and tap water (1:3) to a water level of 15 cm in height. The larvae were investigated at different bottom velocities up to 0.4 m/s. To reduce variability in water velocity, a special array of bent plates was installed in the flume to divert water around the corners. By varying the distances between the single plates, in- and outflows were regulated in a way that flow velocity was almost the same over the whole width of the flume. The bottom of the flume was covered with cobbles (slate and red sandstone) of different sizes (range of diameters 10-20 cm). Some stones were covered with periphyton in order to feed the larvae.

4.2.3 Videoscopy

The movement and locomotion of *E. assimilis* larvae were recorded in the flume using a videoscope (Iplex II, Olympus, Hamburg, Germany). Its small diameter of 6 mm allowed observations between cobbles and even at the lower side of the stones. Selected video sequences taped at 25 fps were evaluated and dismantled in single pictures using SIS picture analysing software EIS (Olympus, Münster, Germany). The magnification of the object depends on its distance to the video camera. Because the distance between object and video camera varies during recording, the magnification could not be determined directly. Therefore, the average length of body or femur of larvae of the same species and larval stage was used for calibration when a scale was needed for reference (Frutiger 1998). Based on this calibration, the near bottom flow velocity was calculated from the length of lines of single particles and bubbles on the pictures.

4.2.4 Scanning electron microscopy (SEM)

For preparation, selected specimens of last instar larvae were dehydrated in an increasing series of Ethanol and subsequently placed in a mixture (50:50) of HMDS (1,1,1,3,3,3-Hexamethyldisilasan) and Isopropanol followed by immersion in pure HMDS, for a time of 10 min each. Due to its vapour pressure, HMDS evaporates so slowly that no surface tension develops to impair the cells. Changes in size and shape lie in a similar range as in the commonly used critical point drying (Jacob 2004). After drying, specimens were attached to a needle using a two-component glue and sputter-coated with gold (Blazer Union SCD 034; Blazer Wiesbaden, Germany). The needle with the larva was clamped in a special sample holder made of aluminium. The latter was built according to Wichard et al. (1995) but in reduced height, with expanded diameter and a laterally positioned screw in order to allow improved flexibility in our SEM. Samples were examined with a LEO 1450 (Leica-Zeiss, Oberkochen, Germany) scanning electron microscope at 15 kV.

4.3 Results

4.3.1 Body posture

As observed for several Heptageniidae (e.g. Gonser 1990) the larvae of *E. assimilis* usually arrange their bodies in a specific position to the flow with the head against the current (Fig. 4.1A). The anterior part of the head, the thorax, and the abdomen were in close contact with the substrate (Fig. 4.1B, 4.2A). In this typical resting position, the larvae were observed to remain for longer periods on the same place in the midst of the current.

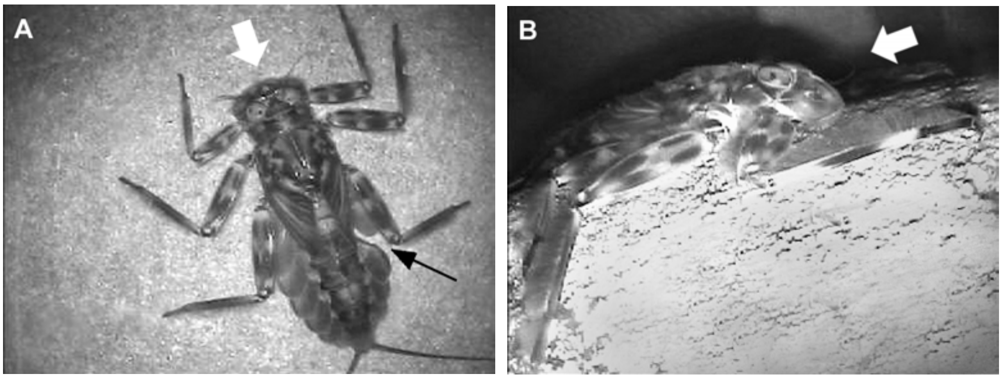

Fig. 4.1. An *Epeorus assimilis* larvae resting on the surface of a stone in swift currents: (a) dorsal view, (b) lateral view. White arrows show the direction of the flow. Black arrow shows the position of the missing gill lamella.

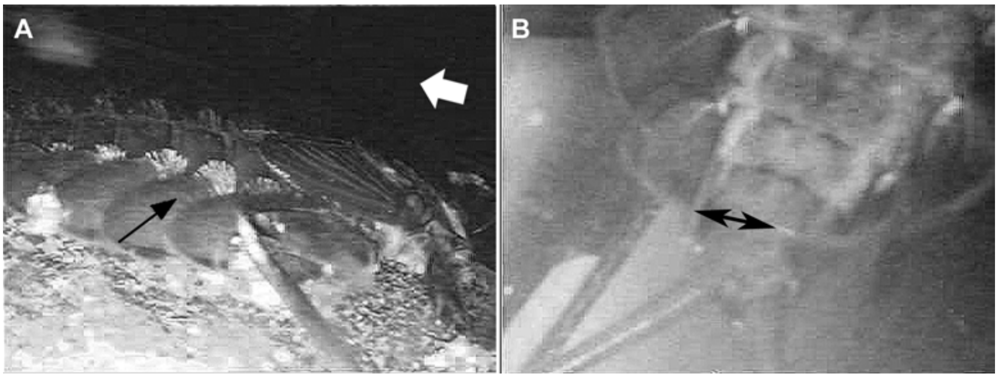

Fig. 4.2. (A) Gill lamellae of *E. assimilis* larvae remain tilted in swift currents. The filamentous part of the gill inserts ventrally of the lamellae and reaches dorsally over the lamallae (black arrow). (B) Ventral view on a larva through a smooth plexiglass plane, there is a gap between the last pair of gill lamellae (black arrow). White arrows show the direction of the flow.

In this resting position, the gill lamellae were tilted in all observed larvae. The anterior part of the gill lamellae were in direct contact with the substrate while the lamella itself formed an angle of about 45° to the substrate (Fig. 4.2A). The video recordings showed that the gill lamellae remained tilted against the flow even at higher flow velocities (bottom velocities up to 0.4 m/s, N=9). Gill lamellae overlapped each other in such a way that the front of each gill lamella was covered by the next one (Fig. 4.1A). The first gill lamellae are located below the hind femora. In most cases little gaps were identified in the video pictures in-between the gill lamellae. The observation of gaps had been tested against the theoretical assumption of no gaps and showed a significant difference (Chi-square test: Chi-Sq=14.0, d.f.=2, P=0.001; N=9). In all observed cases (N=9) the filamentous parts of the gill inserted ventrally of the lamellae and reached dorsally over the lamellae (Fig. 4.2A), and therefore prevented tight connection of the gill lamellae to its environment. In two cases it was possible to tape the ventral side of the abdomen through a normal Plexiglass plane. These videos show a gap between the last gill lamellae (Fig. 4.2B). Further on, it was accidentally observed in four cases that larvae with missing single gill lamellae had no problems in staying attached to the substrate during high flow velocities.

In resting position, the legs were directed laterally (Fig. 4.1A) and all femora were tilted against the flow (Fig. 4.1B). Femora and tibiae usually formed approximately a right angle (Fig. 4.1A). The fore femora stayed anterior-ventrally in close contact with the substrate surface, while the middle and hind femora usually did not have direct contact with the substrate (Fi. 4.1B). The distances of the femora to the substrate became longer with increasing posterior position of the legs.

4.3.2 Structures on the ventral side of the gill lamellae

SEM showed specialized structures on the ventral side of all gill lamellae of the two species *E. assimilis* and *I. alpicola* (Fig. 4.3A, B*).* They are located on the thickened rim of each gill lamella. Higher magnification revealed that these structures consist of a large number of tiny protuberances each set within a socket, thus resembling a seta (McIver 1975, Gorb 2001).

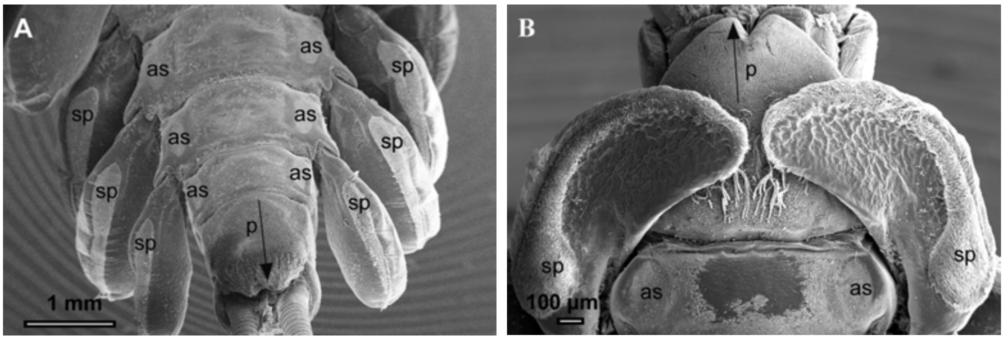

Fig. 4.3. Ventral view on the last abdominal segments and the gill lamellae of (A) *E. assimilis* and (B) *I. alpicola*. Abbreviations: sp, setose pads on the ventral side of the gill lamellae; as, areas with spiky michrotricha; p, posterior.

The single seta was bent at its tip and was about 2 µm wide at the terminal end (Fig. 4.4A, B). While the size of the setae was almost the same in both species, the density of setae was significantly higher in *I. alpicola* (5.2 ± 0.8 setae/100 µm², mean ± SD, n=10) than in *E. assmilis* (1.6 ± 0.2 setae/100 µm², mean ± SD, n=10) (t=14,3, P<0,001, d.f.= 10, t-test). Most setae of *E. assimils* and *I. alpicola* were positioned in such a way that the setal shaft was directed antero-ventrally, whereas the tip of the seta was directed more or less in a postero-ventral direction. Other setae, especially those in the outer parts of the pads, standed in varying directions. In both species, the first gill lamella, which differs in shape and size from the rest, had smaller setose pads.

The distal ends of all pads bore long setae (Fig. 4.4C, D) which had the same diameter as the other setae (about 2 µm). These long setae were positioned all around the first gill lamellae and on the lateral parts of the second to seventh gill lamellae. These lateral parts were not covered by the gill lamellae in front of it. The number of long setae was also higher in *I. alpicola (*about 30 hairs/100 µm) than in *E. assimilis* (about 19 hairs/100 µm). While these long setae sat in one or two lines around the setae of *E. assimils* they stood in many lines on the gill lamellae of *I. alpicola*.

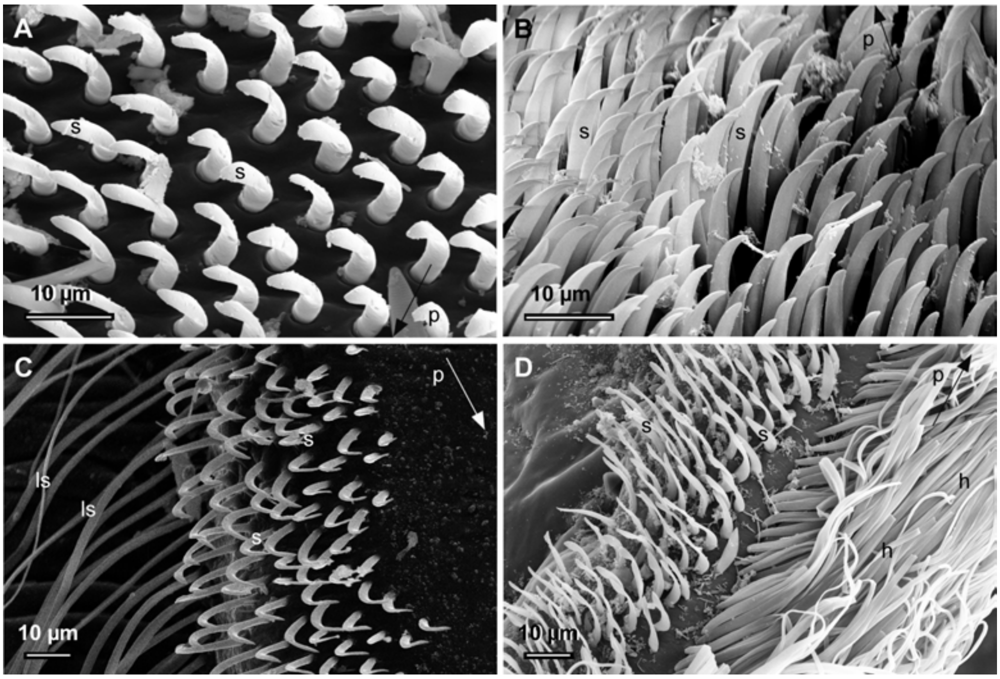

Fig. 4.4. Gill lamellae have ventrally special attachment devices, which consists of a high number of setae. The density of setae on the pads of (A) *E. assimilis* is lower than on the setose pads of (B) *I. alpicola.* Setae on the lateral part of the gill lamellae are of different shape and bordered by a fring of long setae. (C) In *E. asimilis*, this fringe consists of only one or two rows of hairs, wheras in (D)*I. alpicola* it is formed by many rows.. Abbreviations: s, setae; h, long hairs.

4.3.3 Further attachment devices on the abdominal sterna

SEM revealed that larvae of both species possess further specialized structures on the ventral side of the abdomen. These areas were located laterally on the sternites (Fig. 4.3) and consist of microtrichia that were more or less directed in postero-distal direction to the substrate (Fig. 4.5A, B). The tip of each microtrichia was very sharp and measured less than 200 nm in width in both species. Also the basis (about 2 µm in width) and the length of these spikes (4-5 µm) were similar in size in both species. However, the basis of the microtrichia of *E. assimilis* protruded more than in *I. alpicola*.

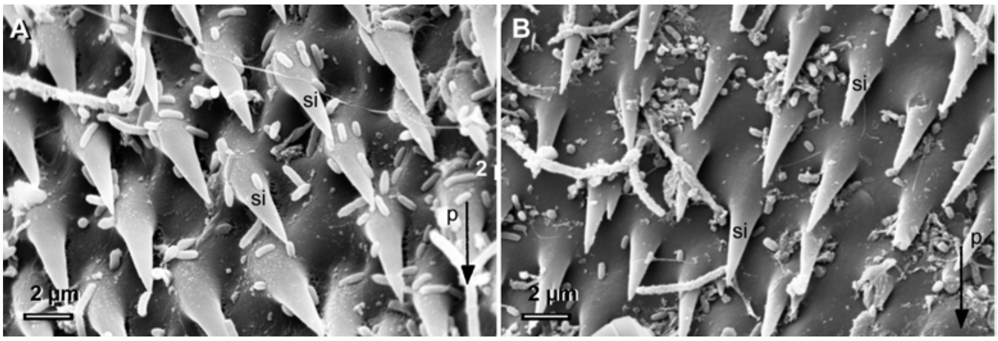

Fig. 4.5. Microtrichia on lateral areas on the abdominal sternites of larval *E. assimilis* (A) and *I. alpicola* (B). Abbreviations: si, spiky microtrichia; p, posterior.

The density of microtrichia in both species was similar: 6.8 ± 0.8 spikes/100 µm² (mean ± SD, n=10) for *E. assimilis* and 7.1 ± 1.0 spikes/100µm² (mean ± SD, n=10) for *I. alpicola* (t=0,67, P=0,514, d.f.=18). The spiked areas on the sternites of *E. assimilis* were about 200 µm wide and 500 µm long. The spiked areas of *I. alpicola* was somewhat smaller with 170 µm in width and 350 µm in length.

4.4 Discussion

Previous interpretations of the function of gill lamellae in *Epeorus* and *Iron* are contradictory (sucker, friction pads and marginal contact). Our results derived from SEM and video observation confirm some assumptions while others have been disproved. Nevertheless, they also show that the adaptations to high currents of *Epeorus sp.* and *Iron sp.* are obviously much more complex than assumed before.

4.4.1 Do the gill lamellae have a sucker function?

The assumption that the gill lamellae work as a kind of sucker cannot be upheld. A sucker attaches to the substrate by developing negative pressure under the sucker cup. For the development of negative pressure a tight contact at the borders of the sucker is necessary. Otherwise no negative pressure can be built up. Video recordings of *E. assimilis* larvae showed that only the anterior part of the gill lamellae is in close contact with the substrate. In addition, little gaps were visible in-between most gill lamellae, and the last gill pair even showed a large medial gap. Moreover, larvae easily stayed attached to the substrate even if single gill lamellae were missing. Consequently, neither single gill lamellae nor the whole gill apparatus are able to develop a tight contact with the substrate and the gill lamellae can, therefore, not function as a sucker as assumed by previous authors (e. g. Wesenberg-Lund 1943, Ruttner 1962; Bauernfeind and Humpesch 2001; Haybach and Malzacher 2002, Staniczek 2003).

Hora (1930) noticed that ‘the thickened portion of the gill lamellae of *Epeorus* larvae is closely applied to the substrate, while the upper free portion is kept in a rapid to-and-fro motion’. He hypothesized that the movements of the upper portions of the gill lamellae have the purpose of expelling leakage water. Our results agree with Hora (1930) so far that just the anterior part of the gill lamellae stays in close contact with the substrate while the posterior part does not. However, our video observations did not show an active rapid movement of the upper part of the gill lamellae. An active movement of the gill lamellae further stays in contrast to the described immobility of the gill lamellae of *Epeorus sp.* (e.g. Ambühl 1959, Bäumer et al. 2000). Our videos showed that the filamentous part of the gills reaches over the lamellae and Hora might have seen their movements due to the water flow.

No video observations were possible for *I. alpicola.* However, since its gill lamellae are arranged in a similar way as in *E. assimilis,* we assume that it has a similar function. The gill lamellae overlap each other like roof tiles, even if they are somewhat larger than those of *E. assimilis*. The front gill lamella of *I. alpicola* is much larger than that of *E. assimilis* and may meet ventro-medially. Morisi, Battegazzore and Fenoglio (2003) interpreted this as an adaptation of *I. alpicola* to faster currents compared with *E. assimilis.* Referring to Hynes (1970) the authors assumed an increased area of marginal contact of the animal with the substrate and thus a reduced possibility of current underneath the larval body. The marginal contact is not complete on the lateral sides due to the observed gaps between the gill lamellae in the case of *E. assimilis*. Nevertheless, the marginal contact, especially in anterior direction might be an important factor, although we assume that another mechanism is also important. Tilted body parts are pressed to the substrate by the water current. This can be tested in a simple experiment with a tilted panel which stays in its position even at high currents in a flume. A similar effect was already discussed by Steinmann (1907) in connection with dorso-ventral flattening of the body. With exception of the front gills, which are usually covered by the hind legs, all gill lamellae are tilted just like the femora. It was already assumed by Dodds and Hisaw (1924) and Gonser (1990) that in the larvae of Heptageniidae the positioning of the femora plays an important role in stabilising their spatial position.

4.4.2 Setae on the ventral side of the gill lamellae

In both species setose pads had been found on the ventral edge of the gill lamellae. This is the part of the gill lamellae which stayed in close contact with the substrate. The density of setae was much higher in the pads of *I. alpicola* than in that of *E. assimilis*.

Other hairy structures in aquatic insects (e.g. *Dicercomyzon, Drunella doddsi*) had also been associated with attachment (Hynes 1970). However, these hairy structures seem to be of different shape. For example, the hairs of *Drunella doddsi* are very soft and branched (Ditsche-Kuru, personal observation by means of SEM analyses). In contrast, setose pads of *Epeorus* and *Iron* seem to have a high elasticity and look very similar to those known from several terrestrial insects as well as from lizards and spiders, where these “hairy” pads play an important role in attachment (Gorb 2001; Arzt et al. 2003; Kesel et al. 2004; Autumn in Smith and Callow 2006). Terrestrial setose attachment pads are well known for their extraordinary abilities like high attachment force, fast detachment, and directionality. The hairy surface guarantees a maximum contact area with diverse substrates regardless of their micro sculpture (Gorb and Beutel 2001), and even allows attachment on totally smooth surfaces. The mechanism of (terrestrial) attachment is described as a combination of molecular interactions and capillary attractive forces mediated by secretion or purely van der Waals interaction (Autumn et. al. 2000; Arzt et al. 2003 according to Stork 1980). The setae of *E. assimilis* show high similarity in shape and size to the tarsal setae of *Forficula auricularia* (Dermaptera) shown in Beutel and Gorb (2001). However, there is an important difference regarding the orientation of the setae. In the setose pads of *E. assimilis* and *I. alpicola* most tips are bent in postero-ventral direction. This is almost the opposite direction as in terrestrial setose attachment pads in relation to the acting force. Thus the orientation of the setae supports the interpretation that setose gill areas are just friction pads due to interlocking effects as described in Hora (1930). Our SEM investigations of the ventral side of the gill lamellae confirm Hora’s observation that *E. assimilis* and *I. alpicola* have pads with tiny protuberances on the ventral side of their gill lamellae. However, not all of these protuberances have a sharp hook-like curved apical portion as described by Hora (1930). Many of them have a blunt tip and are just slightly bent (Fig. 4A, B). Furthermore, these protuberances are not spines but setae. Spines are multicellular processes without differentiation of cells while setae are multicellular processes originating from special differentiated cells (trichogen, tormogen and often also sensory cells) (Gorb 2001 according to different authors). Moreover, the blunt shape of the majority of the seta tips let us doubt that they just function by hooking to the surface irregularities of the substrate, and the investigation of relevant attachment mechanisms of the setae of *E. assimilis* and *I. alpicola* is a matter of further research.

In terrestrial insects setose attachment pads with a higher density of setae as well as with smaller setae tips result in a higher adhesive strength (Arzt et al. 2003). In aquatic environments an animal exposed to higher flow velocities needs to develop stronger attachment forces because higher flow velocities cause higher detachment forces. Consequently, we hypothesize that the significantly higher density of the setae of *I. alpicola* is an adaptation to the higher flow velocities of the preferred habitat of *I. alpicola*.

In contrast to the terrestrial setose attachment pads *E. assimilis* and *I. alpicola* had a fringe of long setae on the distal side of their setose pads. It is known that long hairs (setae) can protect the part lying behind from flow forces, as they do in front of the head plate or mouth parts where they avoid that food is swept away (Gonser 1990). Thus we assume that the fringe of long setae are important to protect the short setae from being rinsed by the flowing water, which otherwise could be able to interrupt the contact between setae and substrate.

4.4.3 Sternal microtrichia

SEM revealed further specialized devices on the abdominal sterna of the larvae of *E. assimilis* and *I. alpicola.* We assume that these areas of sternal microtrichia increase the friction with the substrate. Thus, if the surface of the substrate shows a micro-roughness in a corresponding range, the pressure of the animal in caudal-ventral direction due to tilted body parts will cause an anchoring of the spiky microtrichia to the substrate. Consequently, the areas with microtrichia might improve attachment of the larvae, especially on substrates with a corresponding micro-roughness.

4.4.4 Cooperation of the different attachment devices

In addition to the described setose pads on the gill lamellae and the areas with spiky michrotrichia on the sternits, Heptageniid larvae, like all clingers, also have strong claws on their laterally directed legs. The different attachment devices may offer advantages on substrates with different surface properties. However, not much has been said so far about the question why these mayfly larvae need several attachment devices. There are two basic explanations which are not completely excluding each other: (1) The overall attachment force of the animal is increased due to the cumulated resistance of all attachment structures. (2) The different attachment structures mainly work on corresponding substrate properties. Thus one attachment structure for instance might increase the attachment force on a certain substrate where another attachment structure does not work or has only little effect. An example for the latter can be found in some adult Ephemeroptera, which possess a claw plus a claw pad (Beutel and Gorb 2001). While claws need a certain surface roughness for attachment, and therefore only work on relatively rough surface, the claw pads have the ability to adapt to the profile of a surface and therefore can attach to smooth substrates.

Furthermore, a comparison of the sizes of the different attachment devices of *E. assimilis* larvae indicates that they work best on different surface roughness. The diameter of the claw tip is about 6 µm (Ditsche-Kuru, personal observations). Therefore, in order to hook to the substrate, a corresponding roughness with hollows of clearly more than 6 µm in width is required. In contrast, the tips of the setae measured just 2 µm in width and might work well on smoother substrates. The spikes on the lateral part of the sternites, finally, had the finest tips

(0.2 µm in width) and therefore might attach best on another micro roughness. In addition to the original surface structure of the hard substrates the periphyton covers the surface structure. Moreover, the functioning of the several attachment devices may also be influenced by other properties of algae and biofilm (e.g. chemical components, elasticity).

We hypothesize that the attachment structures of *E. assimilis* and *I. alpicola* attach on different surface properties (e.g. roughness and structure, elasticity). Nevertheless, there might be overlapping ranges of surface properties for the different attachment devices which offer an additional advantage on special substrates.

Chapter 5

Underwater attachment in current: The role of gill lamella surfaces of the mayfly larvae *Epeorus assimilis* in the attachment to substrates of different roughness

Abstract. - Attachment devices of *Epeorus assimilis* larvae are located ventrally on the part of the gill lamella contacting the substrate. To test the role of these structures in underwater attachment in strong currents, we measured friction forces generated by the gill lamellae on solid substrates with different surface roughness. Setose pads have been also investigated in scanning electron microscope.

Our results showed that the pads significantly contribute to friction force generated on smooth and some rough substrates. Interestingly, the gill lamellae showed significant effect on smooth and very rough substrates, but not on a certain kind of intermediate roughness. The contribution of pads to the friction coefficient was lower as expected which may be caused by less large contact area between the pads and substrate than under natural conditions (changes in material properties, lack of the active control of body positioning of the larva). The friction coefficient of the gill lamellae with the substrate depended on the surface roughness of the substrate and on the pulling direction. Our results indicate that interlocking between insect surface protuberances and substrate irregularities as well as molecular adhesion contribute to friction.

Keywords: Ephemeroptera, underwater attachment, attachment devices, sucker, attachment pads, flow velocity

Contents

5.1 Introduction

Larvae of the mayfly *Epeorus assimilis* are typical grazers in swift running waters (Minshall 1967, Wellnitz et al. 2001). In order to graze algae, the larvae crawl on the stones surface, which is the most current-exposed place in running waters for ground living animals. Previous authors assumed that animals can hide inside of the boundary layer of stones surfaces (Ambühl, 1959), whereas Statzner and Holm (1982) showed later that macrozoobenthic organisms have to cope with flow forces. To withstand these forces, larvae bear a number of morphological adaptations, such as dorsal-ventral flattening of the body, strong claws (Fig. 5.1B) and strong laterally directed legs (e.g. Haybach and Malzacher 2002). Our recent results (Chapter 4) show that larvae have additional structures with probable attachment function: setose pads on the ventral edge of gill lamellae and areas with spiky acanthae on abdominal sternits (Fig. 5.1C, D). In strong currents, larvae always orientate themselves to face the flow. It has been assumed that the specialized body shape and body posture of the larvae in current deflects water in such a way that a part of the drag force is used to press the animal against the substrate (Dodds and Hisaw 1924, Gonser 1990). Probably this pressure is used from the larvae to generate contact between setae and substrate. The gill lamellae, overlapping each other similar to roof tiles, look like a suction cup, and give the larva a very typical appearance. However, our results described in Chapter 4 do not agree with the assumption of some former authors (e.g. Wesenberg-Lund 1943, Ruttner 1962, Bauernfeind and Humpesch 2001, Staniczek 2003) that gill lamellae of *E. assimilis* work like a sucker because gill lamellae do not form a complete seal. Contrary to the majority of related species, which are able to ventilate using gill lamellae, the lamellae of *E. assimilis* have been previously described as unmovable (e.g. Ambühl 1959, Baeumer et al. 2000).

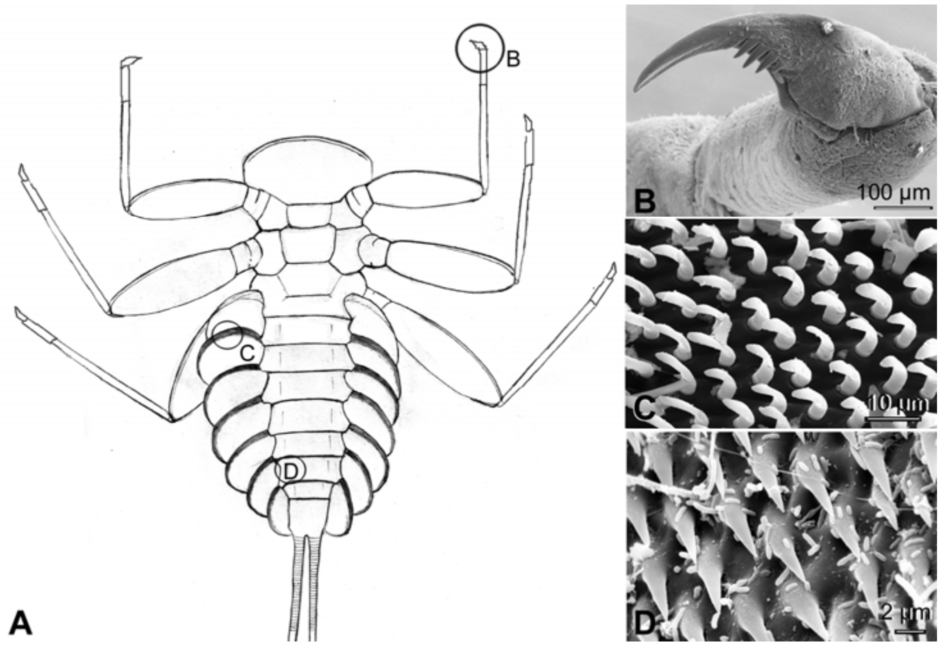

Fig. 5.1. Attachment devices of *E. assimilis* larvae: (A) ventral view of an *E. assimilis* larva, (B) claw of the first leg, (C) setae of the pads on the ventral side of the gill lamellae, (D) areas with spiky acanthae on the lateral parts of the abdominal sternits.

Conspicuouly, the setose pads of gill lamellae in the lotic *E. assimilis* larvae look somewhat similar to the setose attachment pads of some terrestrial insects (e.g. *Forficula auricularia*, Dermaptera, Haas and Gorb 2004). In terrestrial insects, lizards and spiders, setose or "hairy" pads play an important role in attachment (Autumn et al. 2000, Gorb 2001, Gorb et al., 2002, Kesel et al. 2004). They are known for their high attachment force, fast attachment/detachment ability and force directionality (Autumn 2006). Due to the high adaptability of setose surfaces to the micro sculpture of the substrate, a maximum real contact area with the substrate is guaranteed (Gorb and Beutel 2001). Attachment force in such systems is described to be a combination of molecular interactions (van der Waals forces) and capillary attractive forces mediated by the pad secretion in insects (Stork 1980, Langer et al. 2004). In lizards, the attachment system mainly rely on van der Waals interactions (Autumn et. al. 2000), but wetting phenomena can additionally contribute to the generation of attractive forces (Huber et al., 2005). For setose pads of gill lamellae of *E. assimilis* larvae, no experiments were previously made, in order to clarify their attachment mechanism. Using light microscope, these structures have been initially described as spines with sharp tips for an unknown *Epeorus* species from Himalaya (Hora 1930). Recent scanning electron microscopy study demonstrated blunt tips and socketed bases in these structures (Chapter 4). Setose attachment pads from terrestrial insects are known to have an excellent ability to adhere to smooth surfaces (Gorb and Beutel 2001). For *Epeorus* species, the ability to move on smooth glass in running water was previously mentioned (Ambühl 1959). Furthermore, *E. assimils* larvae were observed on smooth Plexiglas surfaces (Ditsche-Kuru, personal observations). The question arises, whether do setose pads contribute to the ability of larvae to attach on smooth surfaces. Furthermore, experiments with flies, beetles, and geckos show that the roughness plays an important role in attachment of terrestrial animals (Gorb 2001, Dai et al. 2002, Huber et al. 2007). We assume that the different attachment structures of *Epeorus* larvae may provide an adaptation to attachment on substrates with different surface profile. For the setose pads of gill lamellae of *E. assimilis* larvae neither their effect on attachment force nor the influence of surface roughness on attachment is known.

This study was undertaken to understand function of setose pads on gill lamellae of *E. assimilis*. By friction measurements, videotaping, and scanning electron microscopy (SEM) the following questions were asked:

- Do gill lamellae increase friction force with substrate?
- Does such an effect depend on the surface roughness?
- Do gill lamella structures show direction dependency of the attachment force?
- Are the gill lamellae unmovable?

5.2 Materials and methods

5.2.1 Animals

Epeorus assimilis Eaton, 1885 (Heptageniidae) larvae were collected in small rivers located in the Thuringian Forest, Germany and kindly provided by the Museum Natural History of the city of Gotha. After collecting, several larvae were transported alive to the laboratory flume in a cooled box. Specimens for friction experiments were obtained in 70 % Ethanol.

5.2.2 Measurement of friction force

For friction experiments, larvae fixated in 70% Ethanol and rehydrated in water for at least 3 h were used. Afterwards, all legs were cut off, and the larva was positioned on a slide with the ventral side down. Gill lamellae were dorsally fixed by means of wax drops in such a way that the ventral side of the larva and its gill lamellae have contact to the support.

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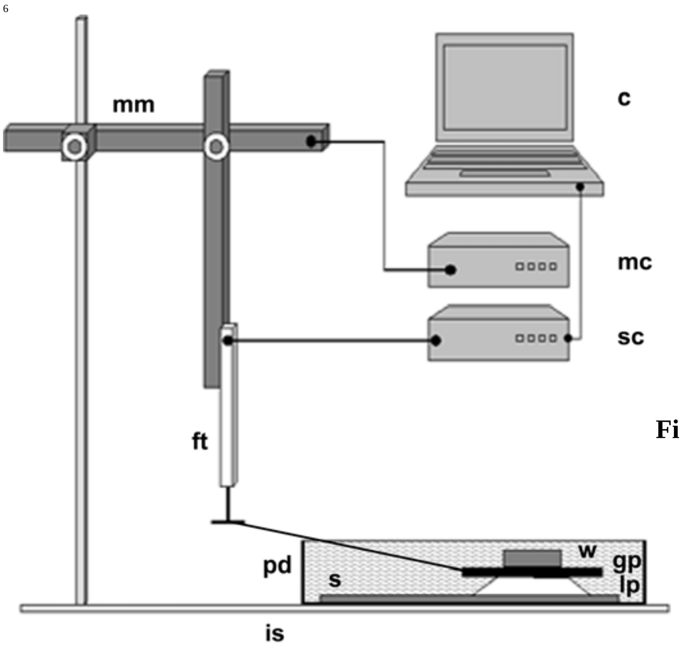


Fig. 5.2. Experimental design for force measurements: mm, motorized micromanipulator; ft, force transducer; c, computer; mc, motor control; sc, sensor control; is, immobile stage; w, weight; gp, glass plane; lp, larval preparation; s, substratum; pd, petri dish.

The experimental design is shown in the Fig. 5.2. The larva preparation was glued dorsally to a small glass plate by means of double-sided adhesive tape and the complete preparation was then positioned on the substrate. The substrate was mounted on the ground of a Petri dish which was connected with the immobile stage and filled with tap water. The glass plate with the larva preparation was connected with a force transducer by means of a human hair. The distance between plane and force transducer was 5.5-5.8 cm. Human hairs were renewed twice in the course of experiments. The angle between the hair and the direction of the pull was determined, in order to calculate applied forces. Friction measurements were carried out in a combination of constant movements of a motorized micromanipulator and the force monitoring using a load cell force transducer (100 g capacity, Biopac Systems Ltd., Santa Barbara, CA, USA). For this purpose, the force transducer was mounted on the micromanipulator (MS314, M/W, Märzhäuser, Wetzlar, Germany). The micromanipulator was moved with a constant velocity of 100 µm/s in horizontal direction. During movement, friction force was monitored for different loads. Average attachment force was extracted after data processing (Fig. 5.3).

Friction force has been measured for three different normal loads: (W1) weight of the larval preparation plus glass plane; (W2) similar to W1 with one added weight; (W3) similar to W1 with 2 added weights. The normal load of different weights was determined for all preparations under water by means of the force transducer. Furthermore, four substrate types were tested: (S1) glass as reference for smooth surface structure; (S2) polishing paper with a nominal asperity size of 1 µm; (S3) polishing paper with a nominal asperity size of 12 µm, and (S4) polishing paper 400 P.

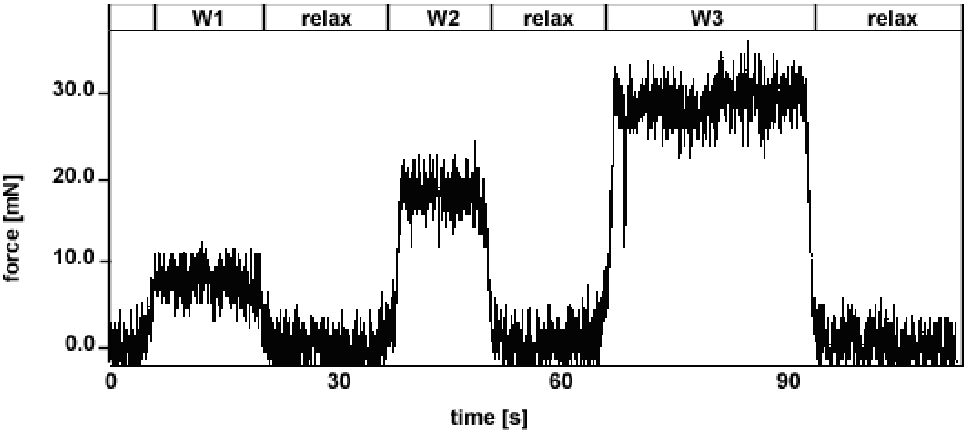


Fig.5.3. Example of a typical friction force curve generated by the ventral body side of *E. assimilis* under different loads (W1-W3). In relaxing phases the larval preparation was not moved while in W-phases the preparation was drawn over the substrate. Friction force has been measured for three different normal loads: (W1) weight of the larval preparation plus glass plane; (W2) similar to W1with one added weight; (W3) similar to W1 with 2 added weights.

The height of the water level relatively to the surface of the substrate was kept the same for all substrates. Measurements have been performed in posterior and anterior directions of the animal. First, all measurements were made with larval preparations with gill lamellae. Afterwards, gill lamellae have been removed and measurements have been repeated for all factor combinations. For each factor combination, 5 larvae were measured with 7 repetitions.

5.2.3 Determination of surface parameters

All four substrate types were measured by means of white light interferometer (FRT MicroProf, CHR 150N high resolution optical sensor, Fries Research & Technology, Bergisch Gladbach, Germany) to determine surface topography. The determination of surface roughness was made at two different magnifications in order to detect different scales of surface roughness. At the lower magnification an area of 1000 x 1000 µm (Pixel size: 10 µm^2) was measured while at higher magnification an area of 100 x 100 µm (Pixel size: 1 µm^2) was scanned. Each substrate and setting were measured for ten different areas on the substrate surface. 3D profiles and analyses of several roughness parameters were made using software FRT-Mark III. The wavelength (λc) defines the limit between roughness and waviness and therefore influences the value of roughness parameters. According to Volk (2005), the wavelength (λc) was defined as the sixth part of the length of the measured profiles. Therefore, the wavelength was 166.7 µm in the setting of lower magnification and 16.7 µm in the setting of higher magnification.

5.2.4 Gill movement experiments

The laboratory flume used for gill movement experiments is described in detail in Chapter 4. In the flume, larvae were recorded by means of a videoscope (Iplex II, Olympus, Hamburg, Germany, 25 fps). Selected video-sequences were evaluated and digitised in single pictures using SIS Bildanalysesoftware EIS (Olympus, Münster, Germany). Larvae, attached to a Plexiglas plane, which was installed inside of the flume, were taped from the ventral side.

5.2.5 Scanning electron microscopy (SEM)

For SEM, selected specimens were dehydrated in an increasing series of ethanol (80 %, 90 % and 99 %- ethanol, 10 min in each). Subsequently, larvae were critical point dried, mounted on holders and sputter-coated with gold-palladium (6 nm). Samples were examined in the scanning electron microscope Hitachi S-4800 SEM (Tokyo, Japan) at accelerating voltage of 3 kV.

5.2.6 Data analyses

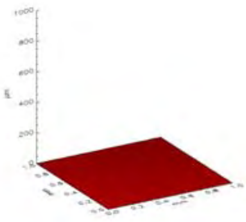
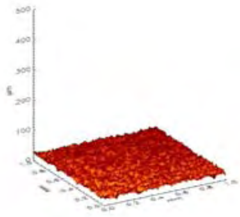
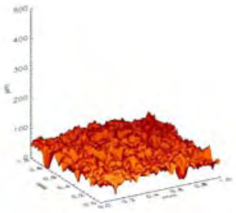
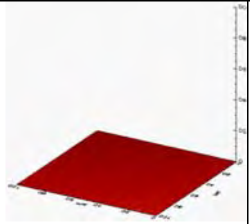
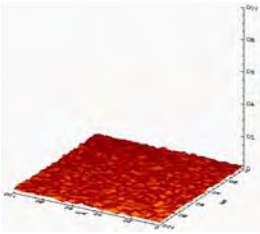
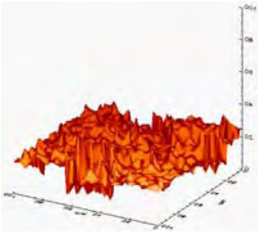
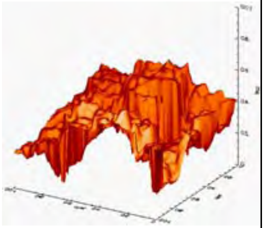
The friction coefficients of the ventral side of the larvae associated for the different conditions (substrates, weights, before and after removal of the gill lamellae) were tested for normal distribution (Anderson-Darling Normality test) and equal variances (Levene’s test). As premises were fulfilled (normally distributed and homogeneous variances) the paired t-test was applied because the same larvae were tested under different conditions.

5.3 Results

5.3.1 Surface structure of the substrates

Roughness parameters and 3D-profiles reflected the different surface topography of the four investigated substrates. Roughness measurements at lower magnification showed increasing values of both roughness parameters, the arithmetic roughness average (Ra) and average maximum height of the profile (Rz), for the substrates S2-S4 (Tab. 5.1). Both roughness parameters determined for S2-S4 were in the range of the natural roughness of stream stones. However, also higher roughness parameters were found on natural stone surfaces (see Chapter 2). Measurement of the surface topography of S1 was not possible at the setting of lower magnification. However, 3D-profiles and low roughness, obtained at the setting of higher magnification as well as SEM-pictures (Fig. 5.3), demonstrate the smoothness of S1.

Tab. 5.1. *Parameters of surface roughness (mean ± S.D., N=10) and 3-D profile of the tested substrates (S1-S4) at two different magnifications.*

Substratum	**S1**	**S2**	**S3**	**S4**
A) ***roughness at lower magnification*** **3-D Profile** Area: 1mm x 1mm z-range: 1 mm	measurement not possible			
Ra$_{(\lambda=167\ \mu m)}$	--	0.56 ± 0.03 µm	3.33 ± 0.09 µm	6.25 ± 0.23 µm
Rz$_{(\lambda=167\ \mu m)}$	--	5.65 ± 0.72 µm	36.03 ± 1.30 µm	59.50 ± 3.53 µm
B) ***roughness at higher magnification*** **3-D Profile** Area: 100 µm x 100 µm z-range: 100 µm				
Ra$_{(\lambda=16.7\ \mu m)}$	0.05 ± 0.01 µm	0.33 ± 0.02 µm	2.18 ± 0.30 µm	2.19 ± 0.52 µm
Rz$_{(\lambda=16.7\ \mu m)}$	0.55 ± 0.18 µm	2.86 ± 0.23 µm	34.61 ± 8.47 µm	32.90 ± 13.29 µm

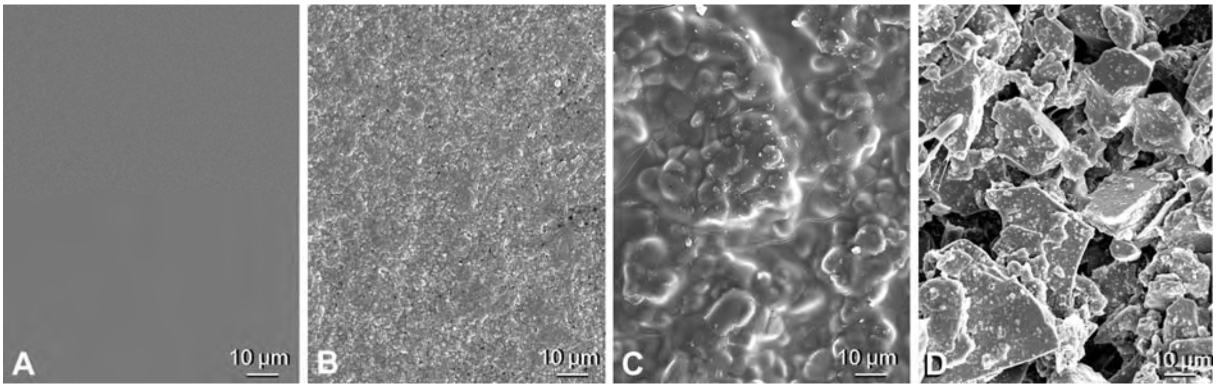


Fig.5.3. Investigated substrates: (A) smooth glass, substrate S1, (B) replications of polishing papers with a nominal asperity size of 1 µm, substrate S2, (C) replications of polishing papers with a nominal asperity size of 12 µm, substrate S3, (D) replications of normal polishing papers 400P, substrate S4.

The roughness parameters evaluated at higher magnification increased for the substrates S1-S3, while substrate S4 had roughness almost similar to that of S3. SEM-pictures showed that the roughness of S4 is much coarser as of S3. Moreover, the shape of texture was very different for both substrates. The roughness of the investigated substrates S2-S4 measured at higher magnification was approximately in the range of the common natural roughness of stones found in running waters (Chapter 2).

5.3.2 Friction properties of the ventral body side

Friction properties of the ventral side of *E. assimilis* larvae were different on the investigated substrates. The lowest values were measured on S3 and the highest ones on S4 and S2 (Fig. 5). With increasing normal force, the friction force increased on all substrates (Fig. 5A-C). The friction coefficient (Fig. 5D-F) decreased with an increasing normal force. Beside the real friction force the drag of the larval preparation and the glass plane contributed to the measured values. However, due to the very low velocity of 100 µm/s it can be estimated that drag must be lower than 10^{-6} mN and therefore can be neglected. Moreover, the mistake caused by drag must be the same for all measurements so that a relative comparison is possible anyway. Differences between substrata S2 versus S3, and S3 versus S4 were significant for all loads (Tab. 5.2).

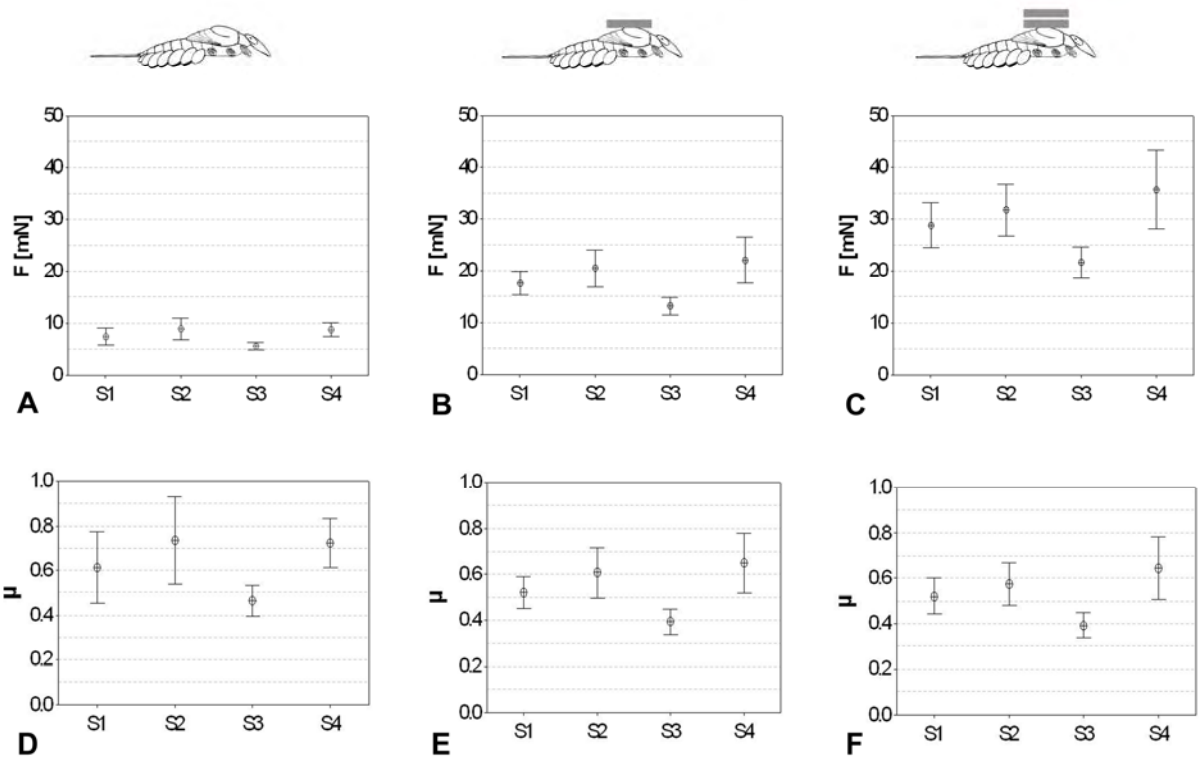


Fig. 5.5. Friction force F (A-C) and friction coefficient µ (D-F) of the ventral side of *E. assimilis* measured on four different substrates under different normal forces: (A, D) without added weight – normal force 11.9-12.6 mN, (B, E) with one added weight – normal force 33.5-34.1 mN, (C, F) with two added weights – normal force 55.1-55.8 mN. Bars are 95 % confidence intervals for mean. Abbreviations: S1-S4, substrates.

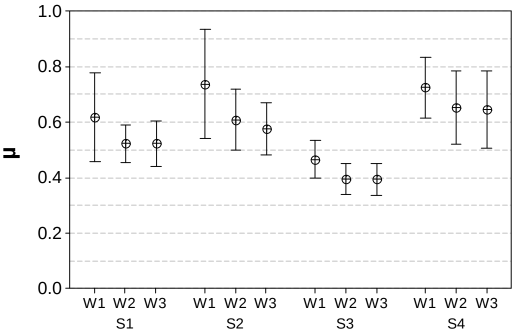


Fig. 5.6. Friction coefficient of the ventral side of *E. assimilis* measured under different normal forces: without added weight (W1) – normal force 11.9-12.6 mN, with one added weight (W2)– normal force 33.5-34.1 mN, with two added weights– normal force 55.1-55.8 mN. Bars are 95 % confidence interval for mean. Abbreviations: S1-S4, substrates.

Tab. 5.2. *Results of the paired t-test for friction coefficient of larvae on different substrates (S1-S4)*

Comparison	d.f.	t	P
Friction coefficient			
W1			
S1 vs. S2	4	2.67	0.056
S2 vs. S3	4	4.99	0.008
S3 vs. S4	4	5.48	0.005
W2			
S1 vs. S2	4	3.12	0.036
S2 vs. S3	4	7.90	0.001
S3 vs. S4	4	5.75	0.005
W3			
S1 vs. S2	4	2.08	0.106
S2 vs. S3	4	8.34	0.001
S3 vs. S4	4	5.93	0.004

W1-W3, different weights, S1-S4, substrates d.f., degrees of freedom; t, t-value, P, probability value.

Tab. 5.3. *Results of the paired t-test for friction coefficient for different normal forces*

Comparison	d.f	t	P
Friction coefficient on S1			
W1 vs. W2	4	2.68	0.057
W1 vs. W3	4	3.06	0.038
W2 vs. W3	4	0.09	0.929
Friction coefficient on S2			
W1 vs. W2	4	4.02	0.016
W1 vs. W3	4	4.26	0.013
W2 vs. W3	4	5.26	0.006
Friction coefficient on S3			
W1 vs. W2	4	9.47	0.001
W1 vs. W3	4	5.15	0.001
W2 vs. W3	4	0.10	0.913
Friction coefficient on S4			
W1 vs. W2	4	2.97	0.041
W1 vs. W3	4	2.67	0.056
W2 vs. W3	4	0.93	0.406

W1, without added weight; W2, with one added weight; W3, with two added weights; d.f., degrees of freedom; t, t-value, P, probability value.

Tab. 5.4. *Results of the paired t-test for friction coefficient on ventral side in larvae with and without gill lamellae - pull in posterior direction*

Comparison	d.f	t	P
With GL vs without GL			
W1			
S1	4	3.20	0.033
S2	4	4.48	0.011
S3	4	1.27	0.297
S4	4	5.61	0.005
W2			
S1	4	5.80	0.004
S2	4	6.79	0.002
S3	4	0.11	0.917
S4	4	4.22	0.013
W3			
S1	4	4.39	0.012
S2	4	10.00	0.001
S3	4	0.02	0.986
S4	4	3.38	0.028

GL, gill lamellae; W1-W3, different weights, S1-S4, substrates, d.f., degrees of freedom; t, t-value, P, probability value.

Tab. 5.5. *Results of the paired t-test for friction coefficient of ventral body side in larvae with and without gill lamellae*

Comparison	d.f	t	P
Δ μ posterior pull vs. Δ μ anterior pull			
W1			
S1	4	0.92	0.408
S2	4	2.53	0.065
S3	4	0.44	0.686
S4	4	2.69	0.052
W2			
S1	4	0.09	0.932
S2	4	3.98	0.016
S3	4	0.12	0.912
S4	4	3.00	0.040
W3			
S1	4	0.29	0.783
S2	4	3.68	0.021
S3	4	0.70	0.524
S4	4	2.62	0.059

GL, gill lamellae; W1-W3, different weights, S1-S4, Substrates; d.f., degrees of freedom; t, t-value, P, probability value.

The highest frictional coefficients were determined for the lowest normal forces on all substrates (Fig. 5.6). It decreased significantly with an added weight (S2, S3, S4; Tab. 5.3). Between one or two added weights, there was no difference in frictional coefficient for S1, S3, and S4 substrates. Only on S2, there was a significant decrease of the friction coefficient after adding the second weight.

5.3.3 Effects of gill lamellae on friction in posterior direction

A significant contribution of the gill lamellae to the friction coefficient (µ) was found on three (S1, S2 and S4) of four tested substrates (Tab. 5.4). The contribution was highest on S2 and almost the same on S1 and S4 (Fig. 5.7A). Only S3 showed no change of the friction coefficient after removing gill lamellae. The contribution of gill lamellae to friction is determined by calculation of the difference of friction force (F) (or friction coefficient) measured in larvae with and without gill lamellae pulled in posterior direction.

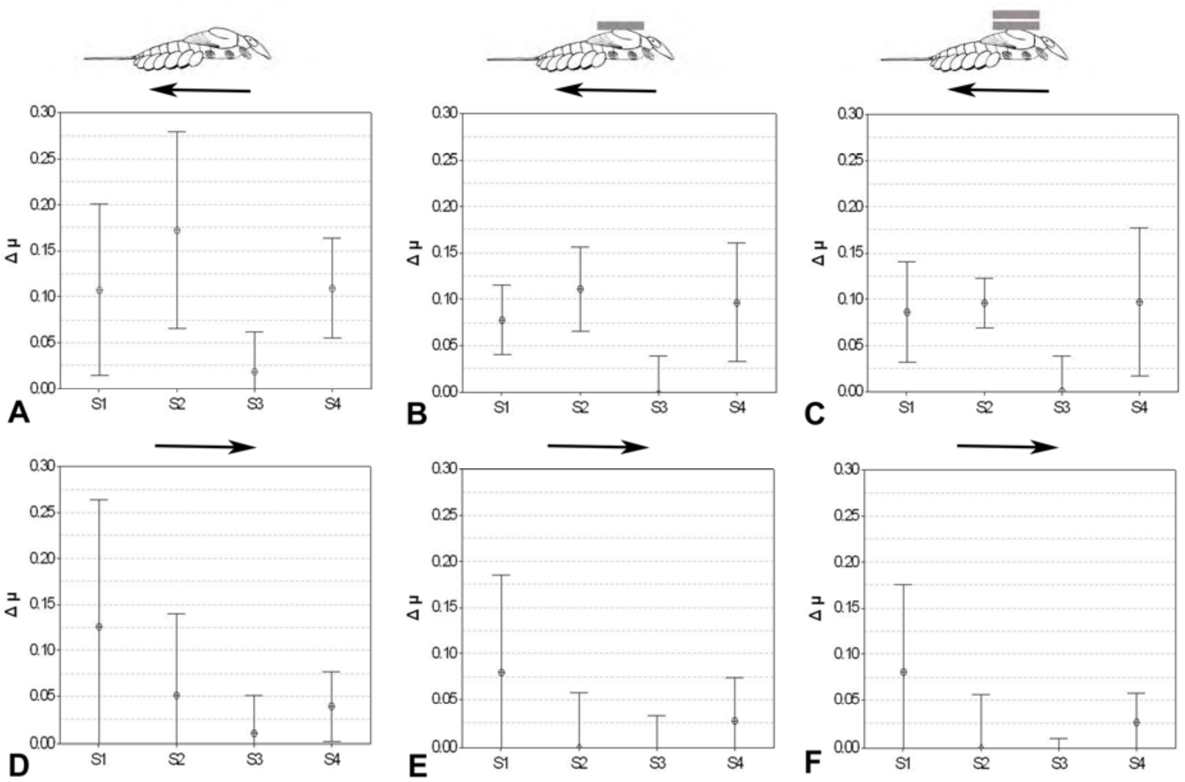


Fig. 5.7. Effect of the gill lamellae of *E. assimilis* to the friction coefficient µ. Graphs show differences between the friction coefficients in larvae with and without gill lamellae. Larvae pulled in posterior (A-C) and anterior direction (D-F): without added weights (A, D), with one added weight (B, E) and with two added weights (C, F). Bars are 95 % confidence intervals for mean. Abbreviations: S1-S4, substrates.

The friction coefficient ($\mu = F_{friction}/F_{normal}$) allows comparison of the friction at different normal forces. Due to the individuality of samples, normal forces have been determined for each single preparation. The range of the normal force was 11.9-12.6 mN without added weight (W1), 33.5-34.1 mN with one weight added (W2) and 55.1-55.8 mN with two weights added (W3). The reduction of the friction coefficient after removing gill lamellae was 18 % on average on S1, 23 % on S2, and 15 % on S4 without additional weights. The contribution of gill lamellae was significant on the substrates S1, S2, and S4 for all weights, while no difference was found on S3 (Tab. 5.4). The reduction of the friction coefficient with an increasing normal force was 17 % on average on S1, 17 % on S2, and 15 % on S4.

The difference of the friction force (Δ F) was 1.29 ± 0.90 on S1, 2.10 ± 1.03 on S2, 0.22 ± 0.41 on S3 and 1.29 ± 0.55 on S4 (mean ± S.D., N=5) at the lowest weight (W1). Δ F increased with increasing normal force (F_{normal}). The friction force was up to four times higher after adding two weights (W3) than for W1 (W3: 4.82 ± 2.46 on S1, 5.38 ± 1.20 on S2, 0.01 ± 1.75 on S3 and 5.39 ± 3.66 on S4, mean ± S.D., N=5).

5.3.4 Effects of gill lamella on friction in anterior direction

When the larvae were pulled in anterior direction, Δ F and Δ μ were highest on S1 (Fig. 7D, E, F). Both values were in the same range similar to the data obtained from pulling in posterior direction so that no directionality was found on S1. While Δ μ showed no significant differences between anterior and posterior pull on S1, Δ μ measured in anterior direction on S2 and S4 was lower than that measured in posterior direction.

However, the difference on S2 and S4 was significant only for some tested normal forces while it was in the range of consideration for others (Tab. 5.5). On S3, differences in Δ μ between both directions of pull were not significant, which means that no contribution of the gill lamella was found on both directions. The described effects were found more or less clearly for all normal forces (W1-W3).

5.3.5 Morphology of setose pads on gill lamellae

Investigations of gill lamellae in SEM showed that the setae on the pads were of different shape (Fig. 5.8A). Four different types of seta were distinguished. The largest part of the pad was covered by type 2 setae (ST2). The basis of each seta was just slightly tilted, whereas the setal tip was bent (Fig. 5.8B, D, G, H). ST2 were 21.3 ± 1.7 µm long (mean ± S.D., N= 15). The tip was more or less oriented in posterior direction. On the anterior proximal edge, type 1 setae (ST1) were found. These setae were 18.3 µm ± 1.8 µm (mean ± S.D., N= 8) long with a blunt tip and were strongly bent at the basis (Fig. 5.8C). The third type of setae (ST3) was located on the lateral distal edge of the pad. In contrast to the previous setal types, these setae had sharp tips and were hook-shaped (Fig. 5.8E, F). Moreover, setal tips of ST3 type of setae were oriented more or less in the proximal direction. Distally, the pads bore long setae (ST4) which were surrounding the ST 2 and 3 (Fig. 5.8E). The latter setae were located just on that part of gill lamella, which was not covered by the anterior overlapping gill plate (Fig 5.8A).

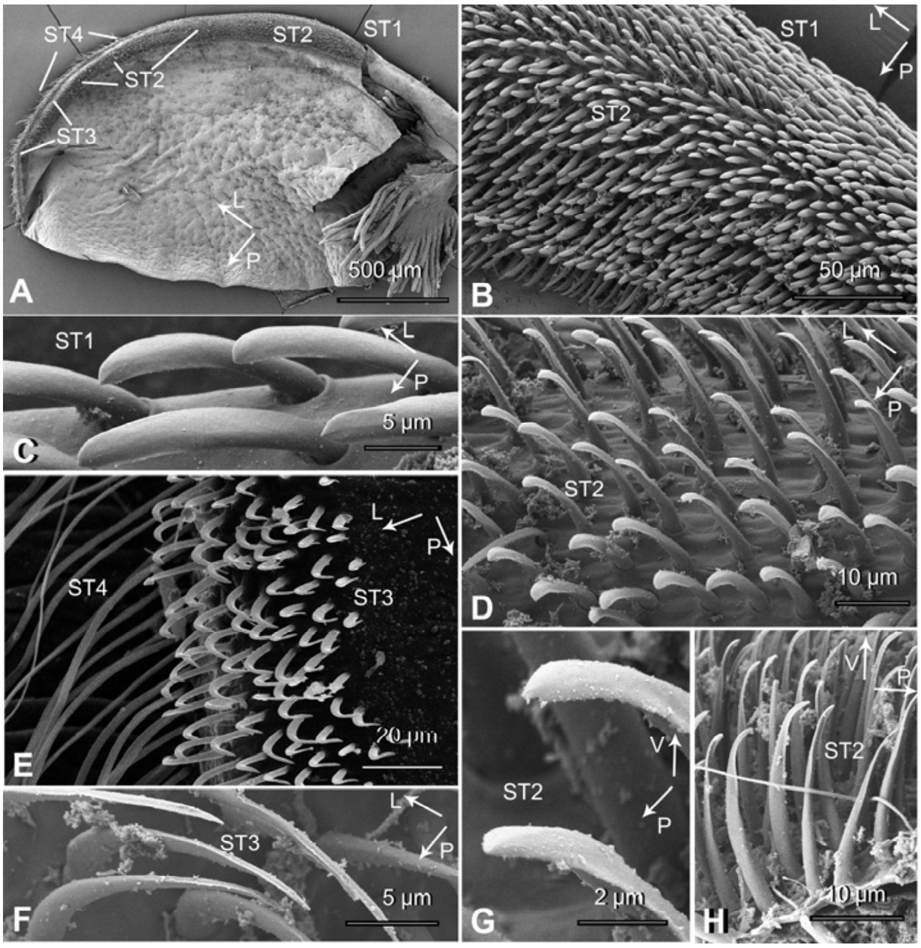


Fig. 5.8. Setose pads of gill lamellae consist of different types of setae. (A) overview of a whole gill lamella, (B) anterior proximal part of the pad with ST1 and ST2, (C) ST1 is blunt, (D) ST2 are the abundant setal type, (E) lateral distal part of the pad with ST3 and ST4, long hairs, (F), ST4 have a hooked shape with a sharp tip, (G, H) ST2 are bent on the blunt tip and relatively long, Abbreviations: ST1, type 1 setae; ST2, type 2 setae; ST3, type 3 setae; ST4, type 4 setae; p, posterior; l, lateral; v, ventral.

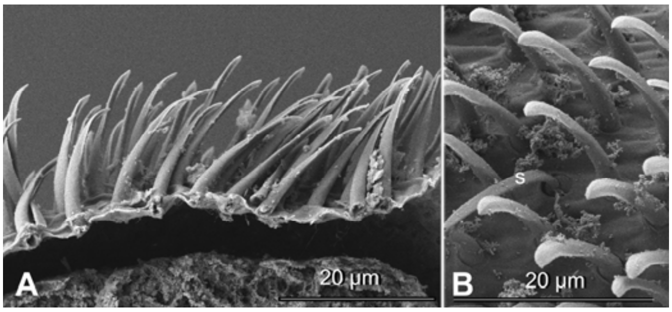


Fig. 5.9. (A) Cut through a setose pad on a gill lamella, (B) in-between the setae are smaller sensilla, Abbreviations: s, sensillum.

All setae arose from the ventral cuticle of the gill lamella (Fig. 5.9A). The setae were hollow at least at their bases. Between setae smaller hair sensilla were found (Fig. 5.9B).

5.3.6 Mobility of gill lamellae

We succeeded in taping the movements of two single gill lamellae from the ventral side. Videos were recorded through a Plexiglas plate inside of the flume. Movements of the lamellae can clearly be seen in the video. The angle of the lamella deflection relatively to the corresponding abdominal segment was determined from single video frames, in order to measure these movements (Fig. 5.10). The angle of the fourth gill lamella changed from 162° to 147° within 0.64 s. After a time period of 1.72 s, the third lamella moved from an angle of 172° to 155° within 0.16 s. The video showed that, in both cases, the contact of setose pads of the gill lamellae with the support has been broken.

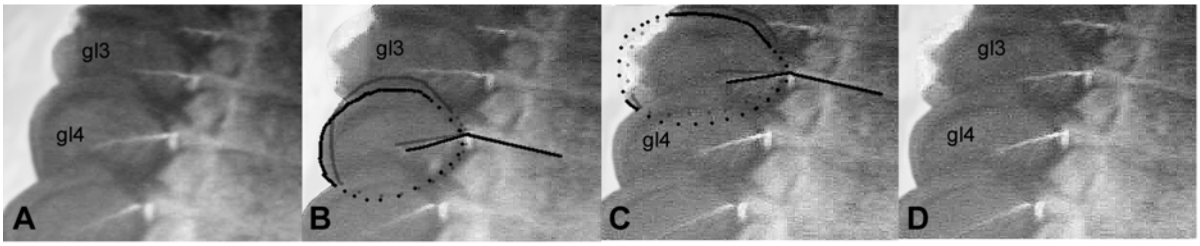


Fig. 5.10. Separate movements of two gill lamellae of *E. assimilis* recorded by means of videoscope IPLEX II (Olympus), ventral view through a Plexiglas plane in a flume: (A) position of the gill lamellae before movement (0.0 s), (B) gill lamella 4 has moved after 0.64 s, the angle changed from 162˚ to 147˚, (C) gill lamella 3 and 4 showed after 2.36 s almost the same position like in B, after 2.52 s gill lamella 3 has moved, the angle changed from 173˚ to 155˚, (D) position of the gill lamellae after movement (2.52 s). Abbreviation: gl, gill lamella.

5.4 Discussion

Our measurements of the friction force showed that the setose pads on the gill lamellae had a significant contribution to attachment on different substrates. The friction coefficient of the gill lamellae depended on the surface roughness of the substrate and on the pulling direction. Our results showed that interlocking effects are mainly responsible for friction generation on the rough substrates, whereas molecular interactions (adhesion) contribute to friction on smooth substrates. However, the contribution of the gill lamellae to the friction coefficient ($\Delta \mu$) was lower than expected. Possible reasons are changes in material properties of the cuticle due to the dehydration/rehydration and the lack of the active control of body positioning by the larva. Perhaps both factors have caused less perfect contact between the setose pads and the substrate than under natural conditions. Below we discuss these aspects in detail.

5.4.1 Mobility of the gill lamellae

In spite of previously accepted statement that the gill lamellae in *Epeorus* species are immobile (Ambühl 1959, Baeumer 2000), our videos of the ventral side of the larvae have demonstrated that the gill lamellae are at least slightly moveable. Species from the closely related genus *Ecdyonurus* are well known for their ability to oscillate with the gill lamellae (Estham 1936). This mechanism was previously not known from *E. assimilis*, which live in swift running and usually well aerated water. The movements observed in *E. assimilis* had very low amplitude and were not visible by naked eye observation.

Nevertheless, the movements might be important for positioning of lamellae during attachment and detachment. Our video recordings show that the lamellae moved in posterior direction and detached themselves from the substrate. Shortly afterwards, the detached larva started walking. Consequently, the slight movement of the gill lamellae might be important for detachment. It can be assumed that slight movements of the gill lamellae can perform a better adaptation to the support too if needed.

5.4.2 Surface roughness and friction

A considerable contribution to friction by the gill lamellae was measured on the smooth substrate (S1) and on some rough substrates (S2, S4) as well. A significant effect of the lamellae was detected only on substrates of a slight roughness (S2) or of a strong roughness (S4). Interestingly, the gill lamellae showed no effect on friction on the substrate with an intermediate roughness (S3). The highest contribution of the gill lamellae to friction was found on S2. While on the rough substrates, setae may interlock with surface asperities, the ability to attach to smooth glass (S1) let us assume that this cannot be the only attachment mechanism. On the smooth substrate, the setae with the blunt tips (ST2 and ST3) may increase the area of real contact with the substrate and might therefore increase the contribution of molecular forces (adhesion). The spaces between the setae might cause water escape from contact regions between setal tips and substrate and by this contribute to formation of solid-solid close contacts responsible for generation of molecular forces.

On the substrate S2, additional interlocking effects with the surface irregularities might be assumed to explain the increased friction force compared to that measured on the smooth substrate (Fig. 5.11). On the substrate with the coarsest roughness (S4), two different mechanisms might contribute to friction. The setae may interlock with the surface irregularities of the fine scale of roughness (much smaller than the seta size) similar to the effects detected on the substrate S2. Additionally, there might be an interlocking between entire setae and roughness larger or comparable with the seta size. It was surprising to see almost no contribution of the gill lamellae to friction on the substrate with an intermediate roughness (S3). We assume that due to the grooves too less setae have contact with the substrate to cause a significant contribution to friction. Also the rounded shape of the substrate tips (Fig. 5.3C) might offer the setae not much possibilities to interlock.

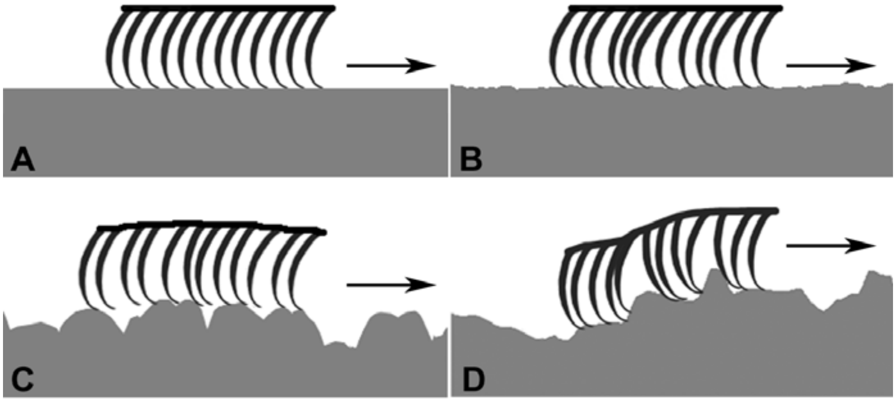


Fig. 5.11. Diagram of the hypothetical interactions between setae (ST2) and tested substrates: (A) on smooth surface of S1-substrate all setal tips are in contact with the support, (B) on the slight roughness of S2-substrate setae-tips are in contact with the substrate too and additionally may interlock with substrate irregularities, (C) on the increased roughness of S3-substrate having a micro topography with deep grooves only few setae may have intimate contact with the substrate, (D) on the increased roughness of S4-substrate setae interlock with the surface irregularities. Additionally, there might be an interlocking between entire setae and roughness larger or comparable with the seta size.

The gill lamellae are filled with liquid inside and, therefore, in combination with rather thin cuticle are able to adapt to the coarse roughness (or waviness) of the substrate. Additionally, setae of the type ST3 with their sharp tips may contribute to interlocking with rough substrates. Such interlocking devices on the gill lamellae of an unknown *Epeorus* species from the Himalaya were discussed by Hora (1930), as those increasing friction between the animal and the substrate. He has concluded that the “spinous pads” are marvellous friction devices. Our results support this function of the pads of the gill lamellae for rough substrates.

Furthermore, it has to be taken into account that the functioning of setose pads might work in combination with the other attachment structures. We assumed that different attachment structures of *E. assimilis* might provide an advantage for larvae in attachment to substrates with different surface properties. There might be an overlapping range of surface properties for different attachment devices specialized to attach on particular substrates. For example, insect claws are adapted for interlocking with rather coarse surface roughness only (Dai et al. 2002), whereas the gill lamellae increased friction force not only on rough but also on smooth substrates as well as on substrates with slight surface roughness. The natural substrate of *Epeorus* larvae are stones, and even on smooth stones crevices can be found that might enable the claws to hook on. So under natural conditions, even on smooth surfaces the effect of the gill lamellae might be additional to the claws. These effect of the gill lamellae to friction force might bring an advantage for *Epeorus*. In this connection it is interesting that Clifford, Gotceita and Casey (1989) describe significantly higher abundance for *Epeorus sp.* on smooth substrates compared to rough ones while for most species the contrary distribution in relation to surface roughness was observed.

On the other hand, rather high friction on smooth substrates might be a side effect. Stones under natural conditions under water are normally not totally smooth, but rather covered with algae and biofilm. Biofilms in running waters usually have a smooth and slippery surface. One may hypothesize that the setose pads of the gill lamellae represent an adaptation to attachment on biofilms. An example of the use of the biofilm for attachment can be found in plants which inhabit waterfalls. Different Prodosteamaceae species develop adhesive hairs which stick to cyanobacteria threads and biofilm matrix (Jäger-Zürn and Grubert 2000). In their habitats, these plants withstand enormous tensile stress caused by action of running water.

5.4.3 Pulling direction and friction

Most cuticle protuberances, described in this work, are directed in posterior direction, and directionality in friction was found on rough substrates while on smooth substrates no directionality was shown. On the rough substrates (S2, S4), the gill lamellae contributed to friction in the direction of the shear force (posterior) caused by water current, while, in anterior direction, such a contribution was much less expressed. This can be explained by the interlocking of the setae, located on gill lamellae, with the surface irregularities. However, on both substrates still some insignificant effects of the gill lamellae on friction are found. Possible reasons for this are the contribution of molecular forces to friction, as well as an adaptation of the insect cuticle to the coarse roughness on S4.

5.4.4 Drag forces caused by water current and friction

The average friction force was between 5.6 mN and 9.4 mN for the different substrates (W1). The friction of the larvae has to antagonise the drag force caused by flow. It has to be taken into account that in living insects the claws contribute to friction too. Moreover, the friction force is influenced by normal force. Consequently, a comparison with the values measured on living larvae is difficult. Nevertheless, the measured friction force of the whole ventral side (without claws!) exceeds drag forces determined on living *E. assimilis* larvae by Weißenberger et al. (1991). The highest measured drag forces were about 4.5 mN for living larvae, which however is not the maximum drag. Drag increases with flow velocity and in the experiments of Weißenberger et al. larvae stayed attached to the substrate without problems. So higher drag forces have to be expected at higher flow velocities.

Chapter 6

Which surface roughness does the claw need to cling to the substrate? – Investigations on the running water mayfly larvae *Epeorus assimilis* (Heptageniidae, Ephemeroptera)

Abstract. - Tarsal claws are common attachment organs in arthropods. With their help animals interlock with the surface irregularities of the substrates. Most insects have two tarsal claws, but larvae of some aquatic insects as Ephemeroptera and Trichoptera bear just one claw on their tarsus. For a terrestrial beetle with two tarsal claws the required surface texture was identified, but the range of surface roughness corresponding with single claws is not known so far. Basing on attachment experiments with replications of defined surface roughness, white light profilometry, videotaping and scanning electron microscopy (SEM), we investigated the required conditions of the surface texture needed by the claws of the mayfly *Epeorus* to grasp on. Different roughness parameters and magnifications for roughness measurement have been tested.

The results demonstrate that the choice of an appropriate magnification is very important. For the claws of *Epeorus assimilis,* a magnification resulting in a wavelength of about thirty times the diameter of the claw tip achieved good results. Common technical roughness parameters like Ra, Rq or Rz allow a sufficient differentiation between the tested substrates in most cases. In some cases additional parameters (Rk, Rpk, Rvk) were helpful to characterize the shape of the surface texture. *Epeorus assimilis* larvae needed a minimum surface roughness of the substrate of Ra about 6.0 µm in order to cling to. When the surface roughness was smaller the larvae left either by passive drifting or even by active swimming. This was surprising, because *Epeorus assimilis* larvae had already been observed to attach to smooth surfaces. Due to the sterile substrates in this experiment we assume that the coverage of the biofilm influences the attachment of the larvae considerably.

Keywords: surface texture, roughness, claw, attachment, substrate properties, biomechanics, flow velocity, drift, reattachment, friction

Contents

6.1 Introduction

Claws are the most common attachment devices in aquatic macrozoobenthos and probably in arthropods at all. They occur in most orders which appear usually in freshwater habitats like crustacea, arachnea and insecta. The claws are used for attachment and locomotion, as well in lotic as in lenitic habitats. In running waters, most arthropods have well developed tarsal claws by which they hold on to the roughness of the surface of the stones (Hynes 1970). In some investigations of macrozoobenthos organisms it has been qualitatively distinguished between smooth and rough stones. Often higher individual numbers of several taxa, which cling to the surface by means of claws (e.g. several mayflies and stoneflies), were found on rough substrates than on smooth ones (e.g. Erman and Erman 1984, Boyero 2003, Downes et al. 2000b). Moreover, from a terrestrial beetle with two claws on the pretarsus it is known that friction forces of the tarsal system are determined by the surface roughness and the diameter of the claw tip (Dai et al. 2002). Now, many aquatic animals have only one tarsal claw. For example Ephemeropteran species bear a single claw at the unsegmented tarsus (Nilsson 1996).

In the present study we investigated the mayfly larvae *Epeorus assimilis.* this mayfly inhabits swift currents (Bauernfeind 1990, 1995) and is like all Heptageniid larvae a typical clinger.

Due to their habitat, clingers have to cope with higher flow forces than other macrozoobenthos organisms. Therefore, they developed special adaptations like long, curved tarsal claws (Merrit and Cummins 1996). Beside their strong laterally directed legs (e.g. Haybach and Malzacher 2002) and strong claws Epeorus larvae have additional attachment structures: setose pads on the ventral edge of the gill lamellae and areas with spiky acanthae on the abdominal sternits (Chapter 4). We assumed that the different attachment structures of *Epeorus* larvae may offer an advantage on substrates with different surface properties. The setose pads increased friction force not only on rough substrates but also on substrates with smooth or slightly rough surfaces (Chapter 5). Furthermore, for *Epeorus* the ability to move on smooth glass planes in a running water aquarium was mentioned by Ambühl (1959) and personally observed on smooth plexiglass surfaces (Ditsche-Kuru, personal observations in a laboratory flume, *E. assimilis*). Consequently, one goal of this work was to study the effect of surface roughness on the attachment of living *Epeorus* larvae with all their attachment devices. On the other hand, a better knowledge of the required roughness for the claws to cling on is needed in order to gain deeper understanding of the interaction of different attachment structures.

It is known that drifting larvae first get contact to the substrate with the claws when reattaching (Gonser 1990). Basing on this knowledge, reattachment experiments with drifting larvae have been performed in order to distinguish the effect of the roughness on the claw from the influence of the other attachment devices. Furthermore, we tested different ways to quantify the roughness of the substrates. According to DIN 4760, roughness is defined as shape variations of 3rd to 5th order for technical surfaces. The technology of roughness measurement and determination provides a number of roughness parameters which describe different roughness properties (e.g. Volk 2005). In our work, two different orders of roughness (magnifications) and selected roughness parameters (Appendix 1) have been tested regarding their suitability of describing the relevant roughness properties for the interlocking of the claw to the surface irregularities. By means of attachment experiments with replica of different roughness, white light profilometry, videotaping and scanning electron microscopy (SEM) we address the following questions:

- Which surface roughness of the substrate needs the claw to cling on?
- Which roughness orders and parameters describe the relevant microtopography best in relation to the needs of the claw on surfaces irregularities?
- How is the influence of the surface roughness of the substrate on attachment of living *Epeorus* larvae including all attachment devices?

6.2 Materials and methods

6.2.1 Animal material

Last instars of living *E. assimilis* larvae were collected in streams in the Thuringian Forest close to the village Thambach-Dietharz, Germany. After collecting, the larvae were transported alive to the laboratory flume in a box cooled by freeze packs. The maximum transport time was about 4 hours. Some specimens were fixed in 70 %- Ethanol for SEM.

6.2.2 Scanning electron microscopy (SEM)

For preparation, selected specimens were dehydrated in an increasing series of ethanol (80 %, 90 % and 99 %- ethanol, each 10 min) and subsequently dried in a mixture (50:50) of HMDS (1,1,1,3,3,3-Hexamethyldisilasan, Merk-Schuhardt, Hohenbrunn) and Isopropanol (Carl Roth GmbH & CoKG, Karlsruhe) and afterwards in pure HMDS (each 10 min). After drying, larvae were glued to a needle, sputter-coated with gold (Blazer Union SCD 034; Blazer Wiesbaden, Germany) and clamped to a special sample holder according to Wichard et al. (1995) and modified as described in Chapter 4. Samples were examined in the scanning electron microscope LEO 1450 (Leica-Zeiss, Oberkochem, Germany) at 15 KV.

6.2.3 Attachment experiments in a laboratory flume

Attachment experiments have been made in an artificial stream flume, which is described in detail in Chapter 4. For the experiments with living larvae, four different types of artificial substrates were used. Glass (S1) was chosen as smooth reference and supplemented by the three replications of different surface roughness. Therefore, special polishing paper with a nominal asperity size of 1 µm (S2), 12 µm (S3) and normal polishing paper 400 P (S4) were moulded by means of a dental wax (President Light Body, Coltene Whaledent Lagenau, Germany) and Epoxy resin with hardener (Epoxidharz L and Härter S, Conrad electronics, Hirschau, Germany) according to Koch et al. (2008).

The substrates were arranged in a line of increasing roughness (Fig. 6.1). At the end of this line, a net was installed in order to catch the animals for next experiments. This experimental apparatus was fixated inside of the flume shortly before the experiments have been performed. Two different kinds of experiments have been carried out to investigate the influence of surface roughness on (A) reattachment of drifting larvae and (B) attachment of sitting larvae.

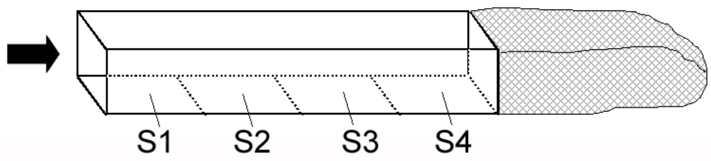


Fig. 6.1.Experimental design: Replica of defined roughness were arranged in a line and glued on a plexiglass frame. At the end of this frame a net was installed in order to catch the animals. This apparatus was fixated inside of an artificial flume. Abbreviations: S1, substrate type 1; S2, substrate type 2; S3, substrate type 3; S4, substrate type 4.

Some of the experiments were taped on video by means of the videoscope Iplex II (Olympus, Hamburg, Germany). Later, selected video-sequences were evaluated and dismantled in single pictures using SIS Bildanalysesoftware EIS (Olympus, Münster, Germany). The length of the total body and the first femura were determined for each specimen before starting with the experiments. Using these values for calibration it was possible to determine the bottom flow velocity from the length of lines of single particles and bubbles on the single pictures of the video (see Chapter 4).

Reattachment of drifting larvae

In order to determine which roughness is needed for reattachment of drifting larvae specimens were released in front of each type of substrate shortly over the surface. It was observed if the larva was able to attach to the substrate behind the starting point. Experiments were repeated with 10 animals for each substrate. Investigations have been carried out for two different settings of the paddle wheel in order to generate different flow velocities.

Attachment of sitting larvae

The aim of this experiment was to investigate the influence of the surface roughness of the substrate on attachment of living *E. assimilis* larvae. Specimens were placed on the substrate while the flow velocity was zero. Then the rotational speed of the paddle wheel was enhanced until the animal left the substrate or the maximum speed of the flume was reached.

6.2.4 Attachment experiments with claw preparations

The following investigations with claw preparations were made in order to get information about the range of roughness the claw can cling to. Therefore, the legs of dead larvae were cut between femur and trochanter. These legs were positioned in the natural way of the larvae. The claw tip was in contact with the substrate and the medial end of the femur was clamped in a forceps. This claw preparations were moved over seven different polishing papers for a maximal length of 20 mm. The distance the claw moved until it hooked to the substrate was measured. The experiments were repeated with eight parallels for each substrate.

The experiments with claw preparations were performed on the original polishing papers of different size: special polishing paper with a nominal asperity size of 1 µm (SII) and 12 µm (SIII) as well as normal polishing paper 1000 P (SIV), 400 P (SV), 280 P (SVI), 100 P (SVII) and 40 P (SVIII).

6.2.5 Determination of roughness parameters

All substrates were measured by means of white light interferrometer FRT MicroProf (CHR 150N high resolution optical sensor, Fries Research & Technology, Bergisch Gladbach, Germany) in order to determine surface topography and roughness. It was measured with two different magnifications in order to detect different orders of surface irregularities. At the lower magnification an area of 1000 µm x 1000 µm (Pixel size: 10 μm^2) was measured while at higher magnification an area of 100 µm x 100 µm (Pixel size: 1 μm^2) was scanned. Each substrate and magnification was measured at ten different areas of the substrate. Analyses of the different roughness parameters were made by using software FRT-Mark III. According to Volk (2005), the wavelength (λc) was defined as the sixth part of the length of the measured profiles. Therefore, the wavelength was 166.7 µm in the setting of lower magnification and 16.7 µm in the setting of higher magnification. The wavelength (λc) defines the limit between roughness and waviness (lower order of surface irregularity) and therefore influences the value of the roughness parameters.

6.2.6 Data analyses

Reattachment and attachment of living larvae to the various substrates were investigated by the Chi-squared test of association. This test is appropriate for observations which can be assigned to two or more categories in two variables (Dytham 1999). In the attachment experiments the velocity classes had to be combined in order to fulfil test premises.

The length of pull of the claw preparation on the investigated polishing papers as well as the roughness parameters of all substrates were tested for normal distribution (Anderson-Darling Normality test) and equal variances (Levene's test). Because premises for ANOVA (normally distributed, homogeneous variances) were not fulfilled, the non-parametric Kruskall Wallis test was applied. For pair wise comparison also the Mann-Whitney test was accomplished.

6.3 Results

6.3.1 Morphology and positioning of the claws

The claws of all legs had the same design in *E. assimilis*. The strong and curved claws were equipped with sensilla in the front part (Fig. 6.2C). These sensilla might help them to detect surface irregularities on the substrates. In the middle part the claw bore four teeth (Fig. 6.2A) of different length which might help to increase friction with the substrate in some cases.

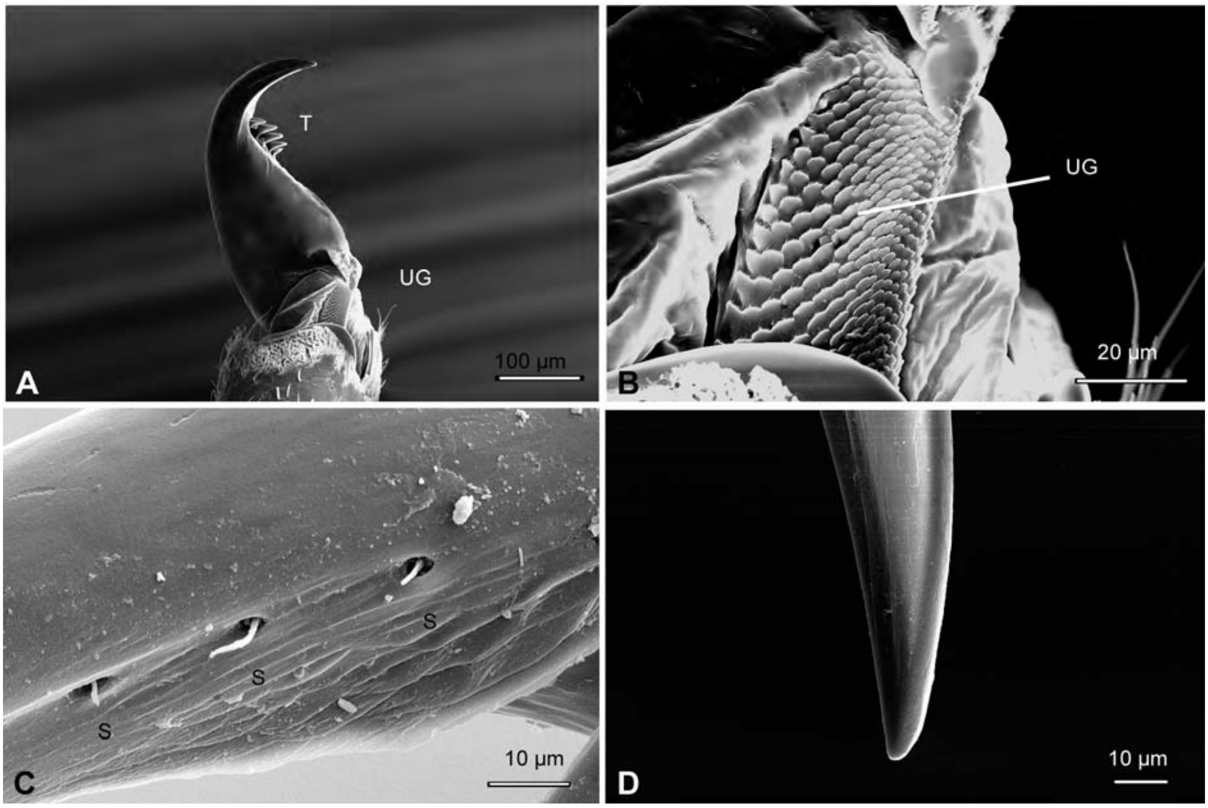


Fig. 6.2. Morphology of the claw: (A) lateral view on the claw, (B) unguitractor plate with distally directed microtrichia, (C) sensilla on the distal part of the claw, (D) tip of the claw; T-teeth, UG-unguitractor, S-sensilla

The diameter of the claw tip (Fig. 6.2D) was 6.2 ± 2.6 µm (mean ± S.D., N=3) and the radius of the outer distal claw was 94.7 ± 5.0 µm (mean ± S.D., N=3). The distance between the claw tip and the ventral-proximal edge of the claw was 331 ± 42 µm (mean ± S.D., N=3). The unguitractor plate of *E. assimilis* can be seen in Fig. 6.2B due to the unnatural stretching of the claw. It consists of regular distally arranged microtrichia. Fig. 6.3C showed that the claw was strongly adducted when it hooked to the substrate.

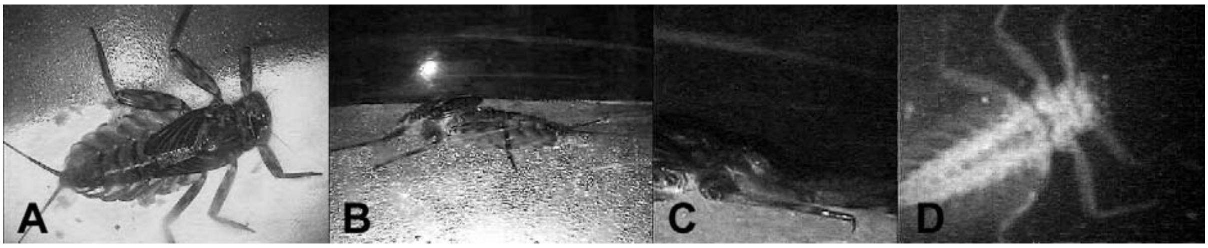


Fig. 6.3. *E. assimilis* clinging to substrate S4; (A) dorsal view, (B) lateral view, (C) claw clinging to the substrate, (D) larvae on plexiglass wall, ventral view.

6.3.2 Reattachment of drifting larvae on substrates with different surface roughness

Drifting *E. assimilis* larvae reattached to substrate S4 which had a relatively high roughness (Fig. 6.4). Reattachment on smooth glass (S1) or the other replica (S2, S3) was not observed at all (Fig. 6.4). In 20-30 % of the experiments on the different substrates larvae managed to cling to the frame of the substrates. However, this is not the same like clinging to the substrate because crevices between the artificial substrates and the wall provide other surface properties than the substrate itself.

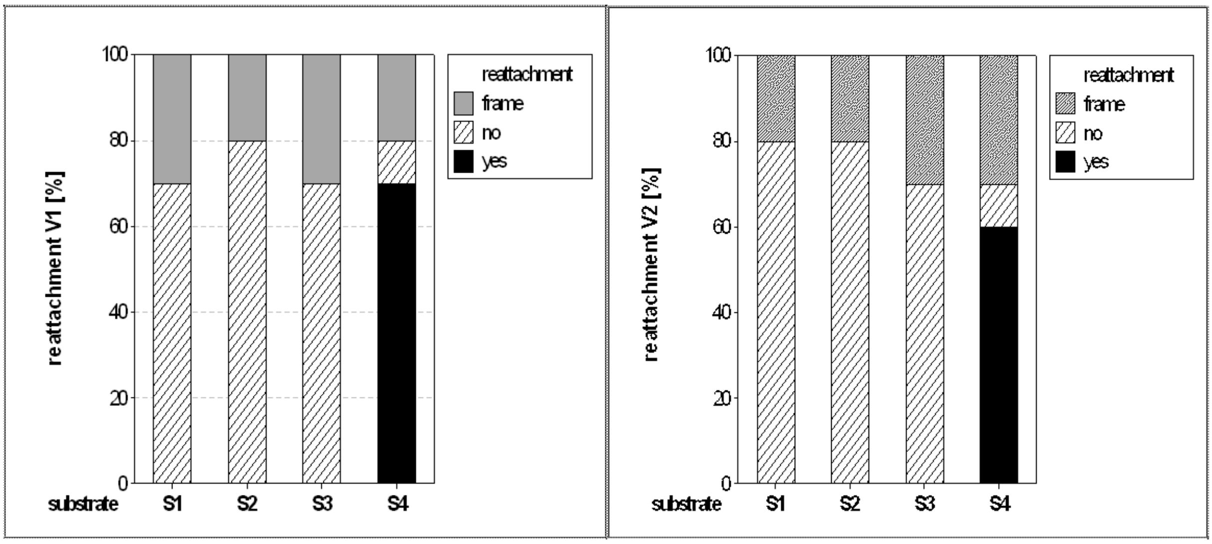


Fig. 6.4. Reattachment of drifting *E. assimilis* larvae on substrates with different surface roughness, (A) at a flow velocity of 10-20 cm/s and (B) at a flow velocity of 25-40 cm/s.

Results of reattachment were almost the same for both tested settings of the paddle wheel. For the first setting, flow velocities directly above the larvae were 10-20 cm/s while for the second setting flow velocities between 25 and 40 cm/s were determined. At lower flow velocity (Fig. 6.4A) 70 % of the larvae reattached on the substrate with the highest roughness (S4) while at higher flow velocity 60 % reattached to S4 (Fig. 6.4B). The reattachment was significantly different on the tested substrates for both flow velocities (Chi-square test of association: V1; Chi-Sq =26.75, d.f.=6, P=0.000; V2; Chi-Sq=24.07, d.f.=6, P=0.00).

6.3.3 Attachment of sitting larvae on substrates with different surface roughness

At higher flow velocities, larvae just stayed on substrate S4 (Fig. 6.5). In 90 % of the experiments larvae attached till the maximum flow velocity of the flume (> 40 cm/s) was reached, which might not be the maximum flow velocity animals are able to endure. The attachment to the tested substrates was significantly different (Chi-square test of association: Chi-Sq=40.00, d.f.=3, P=0.000; velocity classes had to be combined in order to fulfil test premises). On the substrates S1, S2 and S3, larvae either left immediately after being placed there or directly with enhancement of the flow velocity (Fig. 6.5). Actually, in many cases the larvae left the substrate not only passively due to the flow forces. Mostly, the larvae started leaving by active swimming on the substrates (Fig. 6.6).

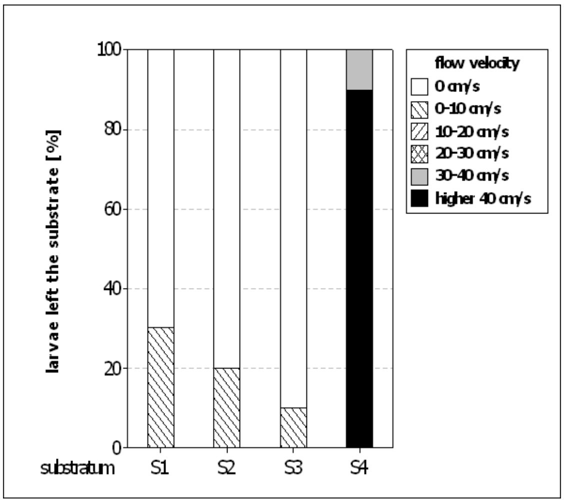


Fig. 6.5. Attachment of sitting *E. assimilis* larvae on substrates with different surface roughness. It was observed up to which maximal flow velocity the specimens stayed on the substrate.

	leg 1					**leg 2**					**leg 3**				
	anterior ◄				► posterior	anterior ◄				► posterior	anterior ◄				► posterior
0.00 s	▨							▨							▨
0.08 s			▨						▨					▨	
0.16 s		▨							▨				▨		
0.24 s	▨						▨							▨	
0.32 s	▨							▨							▨

Fig. 6.6. *E. assimilis* larvae leaving the substrate (S2) by active „swimming-like movements on the substrate“ at very low flow velocity (about 1-2 cm/s), A-E show leg position in the dismantled pictures of the video, the table below demonstrates the movement pattern of the legs.

6.3.4 Attachment experiments with claw preparations on polishing paper

The surface texture significantly affected the length of pull till the claw hooked to the substrate. On two relatively smooth substrates (SI, SII), the claws were not able to hook to the surface irregularities over the whole tested distance. On the three rougher substrates SIII, SIV and SV, the claws hooked to the surface roughness usually within less than 0.5 mm (Fig. 6.7).

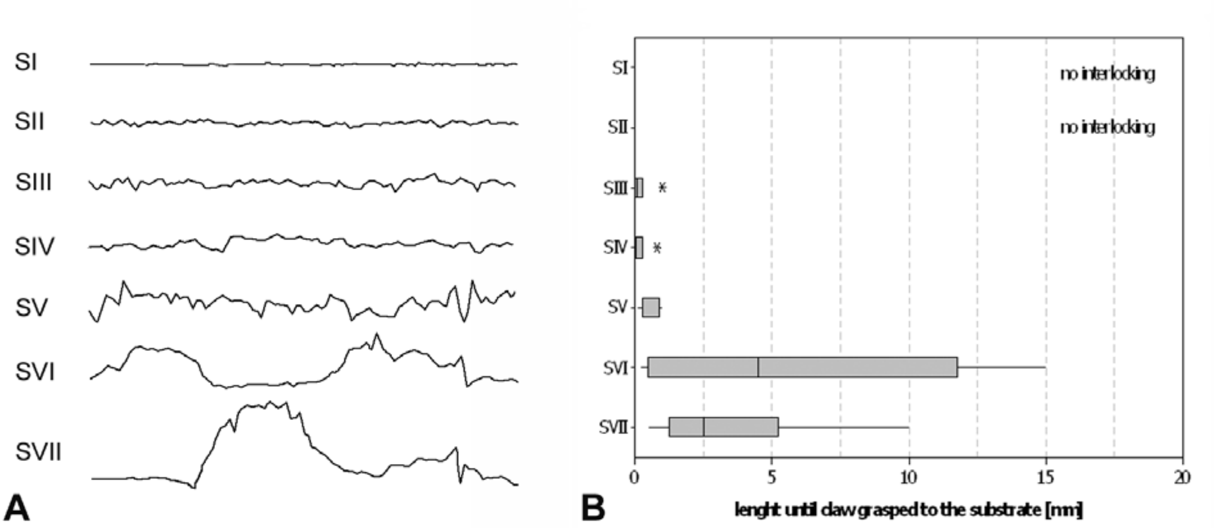


Fig. 6.7. Pull of claw preparations over substrates of different surface texture, (A) profiles of the substrates, (B) length of pull over the investigated substrates until the claw clings on to the substrate.

Compared with the latter ones the substrates SVI and SVII cause an increased length of pull. These relatively rough substrates have a different surface texture compared with the second group. It can be distinguished between three groups: no hooking to the substrate (SI, SII), fast hooking to the substrate (SIII, SIV, SV) and delayed hooking to the substrate (SVI, SVII). On substrates of the same group no significant differences of the length of pull were found whereas substrates of different groups were significantly different in all cases (Tab. 6.1).

Tab. 6.1. ***Results of the Kruskal-Wallis test for differences of the pull length until the claw clings on the substrates***

Comparison	d.f	H	P
All substrates	6	46	<0.001
SI versus SII	1	0.18	0.674
SI versus SIII	1	11.29	<0.001
SI versus SIV	1	11.29	<0.001
SI versus SV	1	11.29	<0.001
SI versus SVI	1	11.29	<0.001
SI versus SVII	1	11.29	<0.001
SII versus SIII	1	11.29	<0.001
SII versus SIV	1	11.29	<0.001
SII versus SV	1	11.29	<0.001
SII versus SVI	1	10.94	<0.001
SII versus SVII	1	11.29	<0.001
SIII vs SIV vs SV	2	4.06	0.131
SIII versus SVI	1	7.75	0.005
SIII versus SVII	1	10.26	<0.001
SIV versus SVI	1	6.35	0.012
SIV versus SVII	1	10.06	<0.001
SV versus SVI	1	3.98	0.046
SV versus SVII	1	8.96	0.003
SVI versus SVII	1	0.14	0.713

d.f., degrees of freedom; H, test statistic; P, probability value.

Tab. 6.2. ***Results of the Kruskal-Wallis test for differences of the roughness parameters Ra and Rz evaluated at lower magnification- tested substrates***

Comparison	d.f	H	P
Ra			
S2 versus S3	4	14.29	0.000
S2 versus S4	4	15.00	0.000
S3 versus S4	4	15.00	0.000
Rz			
S2 versus S3	4	14.29	0.000
S2 versus S4	4	15.00	0.000
S3 versus S4	4	15.00	0.000

d.f., degrees of freedom; H, test statistic; P, probability value.

Tab. 6.3. ***Results of the Kruskal-Wallis test for differences of the roughness parameters Ra and Rz evaluated at higher magnification- tested substrates***

Comparison	d.f	H	P
Ra			
S1 versus S2	1	14.29	0.000
S1 versus S3	4	14.29	0.000
S1 versus S4	4	14.29	0.000
S2 versus S3	4	14.29	0.000
S2 versus S4	4	14.29	0.000
S3 versus S4	4	0.46	0.496
Rz			
S1 versus S2	1	14.29	0.000
S1 versus S3	4	14.29	0.000
S1 versus S4	4	14.29	0.000
S2 versus S3	4	14.29	0.000
S2 versus S4	4	14.29	0.000
S3 versus S4	4	3.57	0.059

d.f., degrees of freedom; H, test statistic; P, probability value.

6.3.5 Surface structure of replica

The 3-D-profiles reflect the different surface topography of the investigated substrates in the experiments with living larvae (Fig. 6.8). The roughness evaluated at lower magnification showed increasing values of all roughness parameters for the substrates S2-S4 (Appendix 21). The differences between Ra and Rz were significant for all substrate pairs (Tab. 6.2).

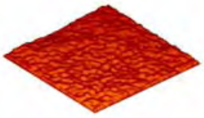
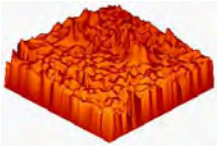
Substrate	S1	S2	S3	S4
A) *roughness at lower magnification* **3-D Profile** Area: 1000 µm x 1000 µm z-range: 1000 µm λ=167 µm	measurement not possible			
B) *roughness at higher magnification* **3-D Profile** Area: 100 µm x 100 µm z-range: 100 µm λ=16.7 µm				

Fig. 6.8. 3-D profile of the tested replica measured by two different sensors by means of white light interferrometer FRT-MicroProf.

The increasing surface roughness of S2-S4 was clearly visible in the 3D-profiles (Fig. 6.8). Measurements of the surface topography of S1 were not possible at the setting of lower magnification because the white light of the sensor was not continuously reflected by the glass surface. The roughness evaluated at lower magnification of the substrates S2-S4 was also in the range of the natural roughness of stones, but also higher roughness was found on natural stones surfaces (see Chapter 3).

The 3D-profiles clearly showed increasing roughness evaluated at higher magnification of the substrates S1-S3 whereas there were not many differences between S3 and S4. The common roughness parameters arithmetic roughness average (Ra) and the average maximum height of the profile (Rz) clearly reflected this impression (Appendix 22). While there were no significant differences between S3 and S4 the differences between all other substrate pairs were highly significant (Tab. 6.3). Furthermore, all other roughness parameters without differentiation between peaks and valleys reflected these results very well (Appendix 22). The substrates S2-S4 showed a roughness evaluated at higher magnification in the range of the usual natural roughness of stones found in running waters (Chapter 3).

6.3.6 Surface structure of polishing paper

The roughness of the polishing papers used for the investigations with the claw preparation showed different trends of roughness parameters (Appendix 23). While most roughness parameters, which were only determined at lower magnification, increased from SI to SVII in average, such common parameters like Ra and Rq did not show such a trend for SV-SVII (Fig. 6.9A, Appendix 23).

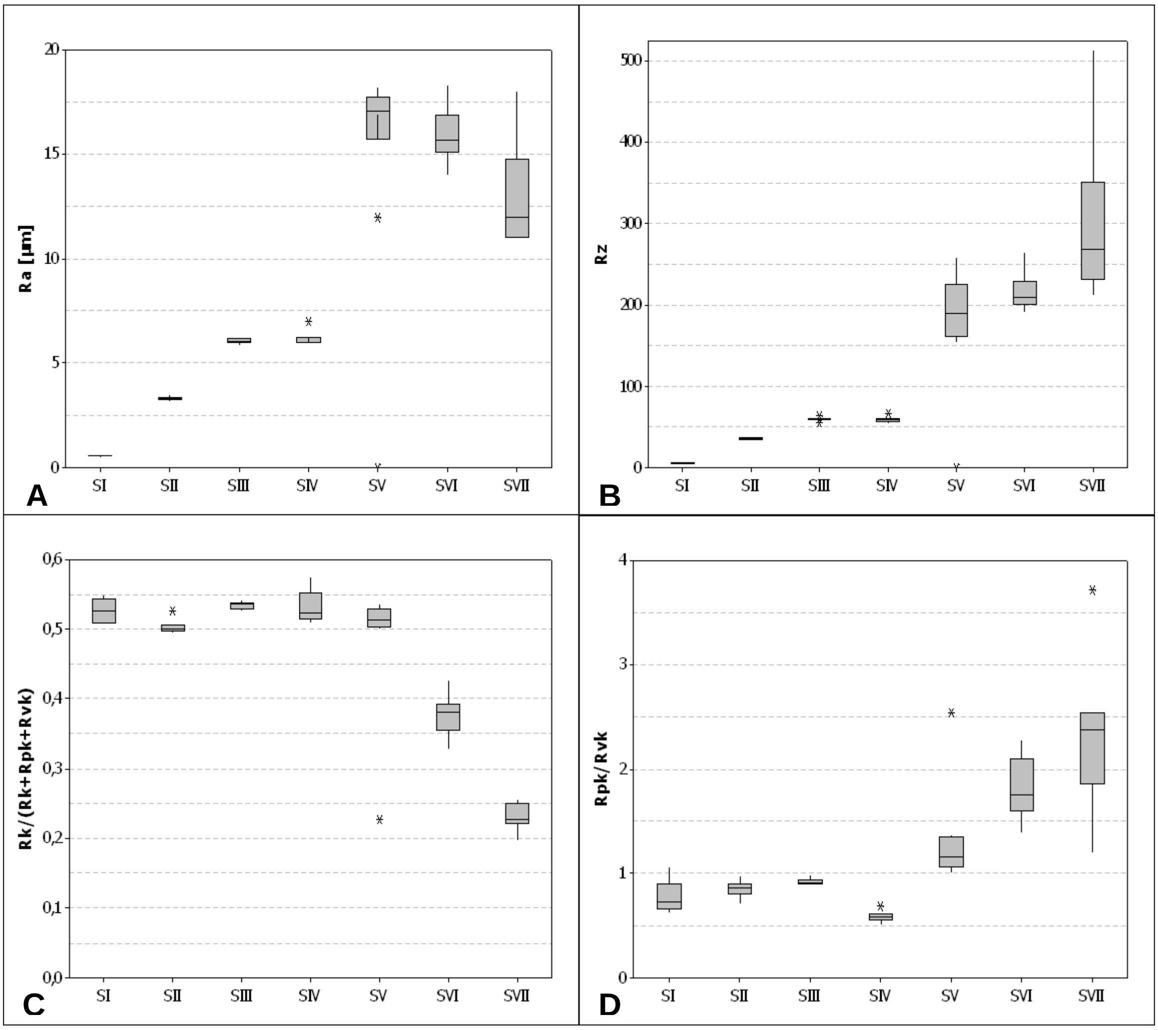


Fig. 6.9. Selected roughness parameters of the substrates investigated with the claw preparation, (A) Roughness Average Ra, (B) Average maximum height of the profile Rz, (C) core roughness ratio RK, (D) peak-vally proportion RP.

The interlocking of the claw with the surface texture changed between SII and SIII as well as between SV and SVI. Consequently, the changes in surface roughness between the mentioned substrates are of main concern. Ra and Rz increased significantly between SII and SIII but there were no significant differences between SV and SVI for both parameters (Tab. 6.4). The profiles of the polishing paper (Fig. 6.7) demonstrated a different surface texture of SV and

SVI. Therefore, roughness parameters which usually are used to describe the shape of the profile (Appendix 1) have been checked.

Tab. 6.4. ***Results of the Mann-Whitney test for differences of substrates used for experiments with the claw preparation***

Comparison	d.f.	W	P
Ra			
SI versus SII	1	55	0.0002
SII versus SIII	1	55	0.0002
SIII versus SIV	1	91	0.3075
SIV versus SV	1	65	0.0028
SV versus SVI	1	125	0.1405
SVI versus SVII	1	73	0.0173
Rz			
SI versus SII	1	55	0.0002
SII versus SIII	1	55	0.0002
SIII versus SIV	1	112	0.6232
SIV versus SV	1	65	0.0028
SV versus SVI	1	85	0.1405
SVI versus SVII	1	148	0.0013
Rp/Rv			
SI versus SII	1	92	0.447
SII versus SIII	1	69	0.0073
SIII versus SIV	1	60	0.0008
SIV versus SV	1	65	0.0028
SV versus SVI	1	119	0.3075
SVI versus SVII	1	134	0.0312
Rk/Rz			
SI versus SII	1	135	0.0257
SII versus SIII	1	55	0.0002
SIII versus SIV	1	106	0.9698
SIV versus SV	1	137	0.0173
SV versus SVI	1	145	0.0028
SVI versus SVII	1	55	0.0002
Rk/(Rk+Rpk+Rvk)			
SI versus SII	1	150	0.0008
SII versus SIII	1	55	0.0002
SIII versus SIV	1	89	0.2413
SIV versus SV	1	118	0.153
SV versus SVI	1	125	0.0048
SVI versus SVII	1	45	0.0003
Rpk/Rvk			
SI versus SII	1	85	0.1405
SII versus SIII	1	74	0.0211
SIII versus SIV	1	55	0.0002
SIV versus SV	1	55	0.0003
SV versus SVI	1	55	0.0048
SVI versus SVII	1	116	0.0373

d.f., degrees of freedom; W, test statistic; P, probability value.

The core roughness depth Rk in relation to Rz decreased significantly from SIV-SVII. But Rp (Average Maximum Profile Peak Height) in relation to Rv (Average Maximum Profile Valley Depth) did not show significant differences between SV and SVI. Therefore, we had to look for other parameters to describe the differences of the profiles in a better way. The Abbott-Firestone curve is the cumulative probability density function of the surface profile’s height and a way to describe the shape of the profile. The roughness parameters Rk, Rpk and Rvk are determined out of the Abbot-Firestone curve and give information about the shape of the profile. These parameters were used to calculate the relation of peaks and valleys to each other, which we call in the following text the peak-valley proportion:

$$RPk = Rpk/Rvk \qquad (1)$$

This value (RPk) shows a significant increase between SV and SVI (Fig. 6.9, Tab. 6.1).

To determine the relation of the core roughness depth (Rk) to a dimension for the height of the profile (without extreme values) a second parameter was calculated:

$$RK = Rk/(Rk+Rpk+Rvk) \qquad (2)$$

This parameter we label core roughness ratio. It describes almost the same circumstance like Rk/Rz but has the same basis as RPk (Abbott-Firestone curve with reduced height of peaks and valleys). RK decreased significantly between SV and SVI (Fig. 6.9, Tab. 6.1).

6.4 Discussion

With replication and white light profilometry two new techniques in limnology have been used in order to investigate the surface roughness of the substrate needed by the claw to hook to. My means of these techniques we were able to describe the relevant magnification in relation to the claw size and characterize the substrates by means of common technical roughness parameters.

6.4.1 Reattachment of drifting larvae on different surface roughness

The reattachment of *E. assimilis* larvae out of the drift was significantly different on the four tested substrates. Reattachment was observed only on the comparably rough substrate S4, which provided a surface texture that interplays with the needs of the claw for reattachment of the drifting larvae. Fig. 6.10 shows the hypothetical interaction between the claw and the tested substrates. It is obvious that on the smooth (S1) and relatively smooth (S2) substrates the claw cannot find surface irregularities to interlock with. Furthermore, the surface irregularities of S3 were too small for the claw to interlock with. The claw generally clings to the surface by creating friction forces. According to Dai et al. (2002) friction forces generated by the claw depend on the size of the surface roughness and the diameter of the claw tip. If the claw cannot grasp to surface irregularities only the friction properties between the claw tip and the substrate particle determine friction force. For reattachment of drifting larvae, these low friction forces were not sufficient. On S4 substrate the claw can interlock with the deeper surface irregularities. If the claw can grasp surface irregularities a high degree of attachment is generated due to mechanical interlocking with substrate texture (Dai et al. 2002). Interestingly, the claw system of the terrestrial beetle *Pachnoda marginata* causes relevant friction forces on a substrate similar to S4 and very small friction forces on a substrate similar to S2. The claws of *Pachnoda marginata*, however, are somewhat taller (tip about 9 µm) compared with that of *Epeorus assimilis* (tip diameter about 6 µm) and therefore not directly comparable.

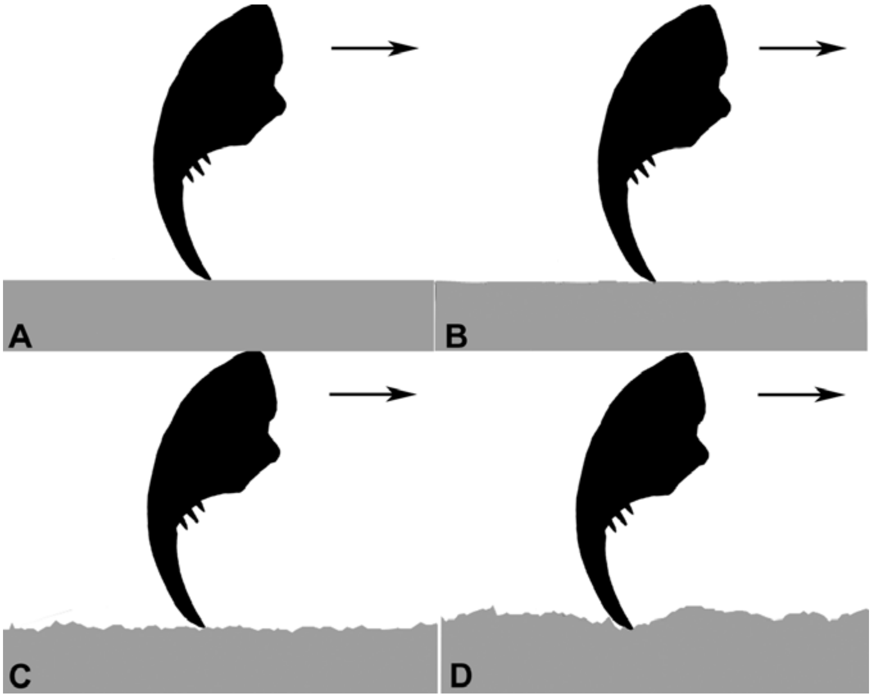


Fig. 6.10. Diagram of the hypothetical interactions between claw and tested substrates: (A) on a smooth surface of S1 the claw has no irregularities to cling on, (B) on the slight roughness of S2 the irregularities are to small to cling on, (C) on the intermediate roughness of S3 the irregularities are to small to cling on too, (D) only on S4 a substrate with coarser roughness the irregularities are high enough for the claw to cling on, length of substrate: 100 µm, claw in the same scale.

Walton (1978) observed in some cases a significantly delayed reattachment of the mayfly species *Stenacron interpunctatum* on sterile stones compared with stones with epilithic food resource. However, in our experiments all substrates were sterile and therefore comparable. An influence of the material itself can be excluded due to the identical material of the substrates S2, S3 and S4. Subsequently, the surface texture was the only parameter that varied for the mentioned substrates.

In insects the claw is generally connected with tendons to a sclerotised plate called the unguitractor (Goel 1972) which is an energy saving device, anchoring the claw in the grasping position due to a frictional system (Conde-Boytel et al. 1989, Seifert and Heinzeller 1989). The unguitractor plate has a denticle-like structure of distally directed microtrichia. The anterior part of the (final) tarsomere bears a wall invagination, that forms an additional corresponding plate like structure facing the unguitractor. This structure bears proximally directed microtrichia (Gorb 2001). At the unguitractor the claw-flexor-muscle is inserted, which is responsible for claw-bending. The return of the claw to an extended position is passive, due to resilin filled cuticle areas at the base (Gorb 1996). Fig. 6.2 shows that the claws of *Epeorus* were equipped with an unguitractor plate. Therefore, it can be concluded that the claws of *Epeorus* do not have to effort energy for grasping to the substrate after the unguitractor of the claw interlocked at end position.

6.4.2 Roughness parameters describing the surface texture in relation to the claw

Our results show that the choice of magnification for roughness measurement is very important for the interpretation of the interplay of the claw with the surface texture. In most cases common roughness parameters like Ra, Rz or Rq provide sufficient information about the surface roughness, but in some cases additional parameters are necessary. In our experiments, the lower magnification showed good results (measured area: 1 mm x 1 mm, Pixel size: 10 µm²). The profile has been filtered by a wavelength (167 µm) of about thirty times the diameter of the claw tip (6.2 µm).

Tab. 6.2. ***Results of the Mann-Whitney test for differences of roughness parameters evalutated at lower magnification - between S3 and S4***

Comparison	d.f	W	P
Rmax	1	55	0.0002
Rmax25	1	55	0.0002
Rz25	1	55	0.0002
Rq	1	55	0.0002
Rt	1	55	0.0002
Rp	1	63	0.0017
Rv	1	55	0.0002
Rk	1	55	0.0002
Rpk	1	72	0.0140
Rvk	1	55	0.0002
Rp/(Rp+Rv)	1	114	0.5205
Rk/Rz	1	145	0.0028
Rk/(Rk+Rpk+Rvk)	1	154	0.0002
Rpk/Rvk	1	116	0.274

d.f., degrees of freedom; W, test statistic; P, probability value.

In contrast, we found no significant difference of the roughness parameters between substrate S3 and S4 at a higher magnification. Nevertheless, the latter substrates showed a highly significant influence on reattachment and attachment of the larvae. Consequently, the higher magnification did not describe the relevant surface roughness. In contrast to the roughness evaluated at higher magnification the important roughness parameters Ra and Rz of the roughness evaluated at lower magnification were significantly different for all tested substrates. In the experiments with living larvae only substrate S4 provided a surface roughness that enabled the claw to hook on. S4 had the highest roughness parameters of the investigated substrates. A further inspection showed that all tested roughness parameters were significantly different for S3 and S4 (Tab. 6.2). Consequently, in this experimental case all roughness parameters reflected the differences of surface roughness very well. The commonly used roughness parameters Ra, Rz or Rq (e.g. Dai et al. 2002, Huber et. al. 2007) supply good results in these cases. Nevertheless, it should be kept in mind that the different roughness parameters describe different roughness properties or at least variations of the latter ones.

The minimum surface roughness required from the claw to cling to can be determined from our results (mean: Ra=6.8 µm, $Rz_{(DIN)}$=103.8 µm, Rq=10.2 µm). The minimum roughness Ra was in the range of the diameter of the claw tip (6.2 µm). Nevertheless, the upper range of roughness supporting the claw was not clear after experiments with living larvae. Therefore, additional experiments with claw preparations were performed on seven substrates with different surface texture. On a surface roughness up to Ra 3.3 µm and Rz 36.0 µm (SI, SII) the claw was not able to hook to the substrate. For both substrates Ra was considerably below the diameter of the claw tip. In contrast, almost immediate grasping to the surface irregularities was observed on substrates with Ra of 6.1-15.2 µm and Rz of 59-180 µm (SIII, SIV, SV). For these substrates Ra was in the range of the diameter of the claw tip or above. Rz was clearly below the inner length of the claw (about 330 µm). Furthermore, all these polishing papers had a RK between 0.5 and 0.6. This means that the main part of the surface profiles height reaches over a relatively wide range. Under these circumstances Ra and Rz describe the substrate properties well.

However, the Ra of the substrates causing delayed interlocking (SVI, SVII) of the claw showed no significant differences to SV. While Rz of SVII was in the range of the inner length of the claw and significantly higher than in the other substrates, Rz of SVI was not different to SV. Further inspection of the roughness parameters showed that SVI and SVII had significantly smaller RK values indicating that the main part of the surface profiles height reached over a relatively small amplitude. Moreover, the peak-valley proportion RPk showed a significant increase of SVI and SVII. The relatively high peaks were dominating over the comparably small valleys. So RK and RPk described the different shape of the profile well which can be seen in the profiles in Fig. 6.9C, D. The delayed interlocking on these substrates can be explained by the special shape of the surface texture. The single claw of *E. assimils* slid over the relatively smooth basis of the profile and sometimes moved laterally around a particle of the substrates before it interlocked. In the case of interlocking the teeth in the middle part of the claw might have had an important contribution. These results seem to be in contrast to Dai et al. (2002) who found a saturation of friction force with increased texture roughness. In fact, it is not a contradiction, because the tested beetle has two claws on the pretarsus while *Epeorus* just bears a single claw. The two claw system might avoid sliding of the claws around a particle.

6.4.3 Interplay of different attachment devices on varying surface roughness

The attachment ability of *E. assimilis* larvae was significantly different on the tested substrates. At higher flow velocities the larvae were only able to attach to the substrate with coarse roughness (S4). All other tested substrates (S1, S2 and S3) were left by the larvae either immediately after placement or with slightly increasing flow velocity. In many cases they left these substrates actively by swimming. Obviously, the surface properties of S1, S2 and S3 did not harmonize with the attachment abilities of the larvae. Consequently, the larvae only attached to the same substrate like the drifting larvae did when reattaching. It can be concluded that the larvae were not able to attach without hooking the claws to the substrate.

At first, these results are surprising because attachment to smooth substrates have been described by Ambühl (1959) and also personally observed (Ditsche-Kuru, personal observations in a laboratory flume). However, there might be an important difference in these observations in comparison to our experiments described here. Our intention was to investigate the influence of the surface roughness. Therefore, the substrates were put into the flume shortly before the experiments have been performed. Consequently, almost no biofilm covered the substrates in our experiments. In contrast to these conditions the attachment of *E. assimils* larvae on the plexiglass wall was observed after a longer operation period of the flume. Therefore, the surface of the smooth plexiglass plane should have been covered with biofilm. The same might have been the case for Ambühl's observation. We assume that the coverage with biofilm plays an important role regarding the attachment to originally smooth substrates.

Interestingly, it has been shown that the setose pads on the ventral side of the gill lamellae increase friction force not only on rough but also on smooth and slightly rough substrates (Chapter 5). A significant contribution to friction force was found on substrates with an almost similar surface texture like S1, S2 and S4. However, without the attachment of at least one claw larvae were not able to withstand flow forces on smooth surfaces without biofilm. The larvae are probably not able to position in an appropriate way to flow and substrate without fixation of at least one claw. Actually, during experiments we repeatedly observed larvae sitting on the smooth plexiglass of the side walls clinging with only one claw to a crevice between substrate and wall (Fig. 4D). Moreover, natural substrates in running waters are usually not totally smooth (Chapter 3) and the increased friction force on smooth substrates like glass might be a side effect as already assumed in Chapter 5. The setose pads might be an adaptation to attach to the biofilm covering the stones in the natural habitat. In this case additional surface properties like e.g. elasticity should be relevant for the attachment force beside roughness. Furthermore, the attachment ability of the claws might increase due to a covering biofilm on smooth substrates. However, these questions have to be investigated in some additional work.

Chapter 7

General Discussion

Contents

The objective of this study was to gain deeper understanding of the influence of surface topography and roughness of solid substrates on the attachment ability of the torrential stone fauna. In order to evaluate the effect of surface roughness under natural conditions, field studies in several running waters have been undertaken. Moreover, laboratory experiments have been performed under defined conditions in order to understand the interplay of attachment devices and surface texture.

7.1 Surface roughness of stones in running waters and its influence on the distribution of macrozoobenthos

In the first part of this thesis the range of surface roughness of stones occurring in several running waters with different geological background has been quantified by profilometric technique. A large variation of the surface roughness of the stones was found and the roughest stone was about six times rougher than the smoothest one with regard to the average roughness “Ra”. All investigated rocks showed significant differences of surface roughness between stones of the same rock. In some cases strong heterogeneity was observed even on the same stone. The surface texture of rocks seems to be very heterogeneous in general which might be caused by different genesis processes and materials. Consequently, a simple distinction between different kinds of rocks as described in some literature (e.g. Erman and Erman

1984, Downes et al 2000a) can cause considerable inaccuracy if surface roughness is the matter of interest provided that the stones of the investigated rocks show the large heterogeneity as in our results. In most studies there has only been qualitatively distinguished between smooth and rough rocks and, therefore, the variation of roughness cannot be proven. It has to be taken into account that the heterogeneity in surface texture of the rocks might contribute to the sometimes contradictory results regarding the colonization of macrozoobenthos organisms as described in literature for e.g. taxa richness (higher on rough substrates: Clifford, Gotceita and Casey 1989, Downes et al. 2000a, Downes et al. 2000b; no effect: Erman and Erman 1984) or individual numbers (higher on rough substrates: Erman and Erman 1984; no effect: Downes et al. 2000a). However, even in two studies using a quantitative method of roughness measurement contrary results regarding the association between individual numbers and surface roughness were found for some taxa on artificial substrates (Clifford, Gotceita and Casey 1989) and stones (Casey and Clifford 1989). These studies quantified the surface roughness of the stones by means of a stylus-type roughness meter. This specially constructed roughness meter measures roughness that might be a degree rougher than microscopic roughness. This seems to be a roughness evaluated at a relatively low magnification. Roughness can occur in different orders, but it is unknown which roughness order or magnification is relevant for the macrozoobenthos. It is also possible that not only one but more roughness orders are relevant for the different animals with respect to kind and size of their attachment devices. Therefore, in the present study roughness has been evaluated at two different magnifications. Nevertheless, in this examination the distribution of selected macrozoobenthos taxa on the stones with respect to surfaces roughness was not always conclusive, too. Depending on their attachment devices animals were assumed to be found on stones with the respective surface roughness but only the beetle larvae *Elmis sp.* and the snail *Ancylus fluviatilis* indicated such an association for the stones. *Elmis sp.* larvae attach to the substrate only by their strong but blunt claws. The density of this species increased significantly with surface roughness at least in autumn when larvae were tallest. On the other hand *Ancylus fluviatilis* did not show an association to the surface roughness. This snail attaches by its broad flexible feet covered with secretion. Its feed and the soft flexible periostracum should be able to adapt to the surface profile very well. However, the distribution of other species (*Baetis*, *Rhyacophila*, Chironomidae) was contrary to our expectations and was obviously dominated by other factors as e.g. food resources. Interestingly, even animals with claws were able to cling to smooth stones. Only a few individuals of *Elmis* larvae were found on smooth stones. Nevertheless, their presence shows that the larvae were able to cling to smooth stones although this might not have been very favourable. The mayfly larvae *Baetis sp.* even preferred smooth andesite stones and showed a significant negative association between surface roughness and individual density. Also *Baetis* larvae cling to the stones surface only by means of their claws but SEM-pictures revealed very fine structures on the claws (teeth and indentations) which might help the animal to grasp to very tiny surface asperities. However, exactly the contrary trend of *Baetis* larvae regarding the substrate roughness was observed by Clifford, Gotceita and Casey (1989) describing significantly increased numbers of *Baetis sp*. on rough tiles compared with smooth ones. Moreover, in most taxa the trends varied in the course of the different seasons which might be influenced by the varying size of most species due to their development stage or differences in other factors. Nevertheless, even if other factors as food resources should have influenced the distribution of the macrozoobenthos, the specific taxa had to be able to attach to the surface texture in order to maintain their position. Independent of the average surface roughness crevices which were often found on the surface of the stones could be a reason for the not always conclusive results regarding the association of taxa distribution and surface roughness. Another important factor influencing the attachment might be the biofilm and sediment coverage of stones. Obviously, attachment mechanisms of the animals to the substrates are complex and many factors which might influence the mechanisms vary under natural conditions.

7.2 The role of the gill lamellae in attachment of *Epeorus* and *Iron* larvae

Previous authors (e. g. Wesenberg-Lund 1943, Ruttner 1962; Bauernfeind and Humpesch 2001; Haybach and Malzacher 2002, Staniczek 2003) assumed that the gill lamellae of *Epeorus* and *Iron* are modified to a sucker as an adaptation to withstand currents. A sucker attaches to the substrate by developing negative pressure underneath the sucker cup. Therefore, the gill lamellae have to form a complete seal at the borders in order to function as a sucker cup.

Our video recordings of *Epeorus assimilis* showed that the gill lamellae stayed tilted in strong currents and little gaps were visible in-between most gill lamellae. The last gill pair even showed a large medial gap. Moreover, larvae attached to the surface without any problem even if single gill lamellae were missing and therefore no negative pressure could be maintained. Consequently, gill lamellae cannot seal the cavity underneath and therefore cannot function as a real hydraulic sucker. Our SEM analysis revealed setose pads ventrally on the gill lamellae. These setose pads looked very similar to those described in some terrestrial insects. The hairy surface of these terrestrial pads guarantees a maximum contact area with diverse substrates regardless of their micro sculpture (Gorb and Beutel 2001), and even allows attachment on totally smooth surfaces. Nevertheless, in the setose pads of *E. assimilis* and *I. alpicola* most tips are bent in postero-ventral direction. This is almost the contrary direction of the tips of terrestrial setose attachment pads in relation to the acting force.

These gill lamella structures have been already remarked by Hora (1930) who described them as spines with a sharp hook-like curved apical portion. SEM pictures showed that many of these protuberances had a blunt tip and were just slightly bent. Furthermore, these protuberances were not spines but setae each set within a socket, thus resembling a seta (McIver 1975, Gorb 2001). Setae of *I. alpicola* showed a similar size, but a higher density than those of *E. assimilis,* what might cause a greater adhesive strength and could be an adaptation to the swifter currents in which these species lives.

7.3 Interplay of roughness and selected attachment devices

In order to gain deeper understanding of the interplay of attachment devices and surface texture experiments were performed under defined laboratory conditions. Two kinds of attachment devices, claws and setose pads, were intensively investigated on substrates of different kinds of surface roughness (glass and replications of polishing paper).

Our results showed that the single tarsal claws of *E. assimilis* need relatively rough substrates in order to grasp on. In our experiments with living larvae on several substrates of different surface roughness attachment was only observed on the roughest substrate (S4). Additional experiments with claw preparations showed that a minimum roughness of 6 µm (Ra estimated at lower magnification) was necessary for the claw to cling on. When the roughness was smaller the claw was not able to grasp to the surface irregularities and the larvae left either by passive drifting or even by active swimming. In some cases an unfavourable shape of the surface caused delayed interlocking even at roughness values of 6 µm and more. On these substrates, the single claw of *E. assimilis* slid over the relatively smooth basis of the profile and sometimes moved laterally around a particle of the substrates before it interlocked. Such effect was not observed in the terrestrial beetle *Pachnoda marginata* bearing two claws on the pretarsus. In this beetle the friction force showed a saturation curve with increasing roughness (Dai, Gorb and Schwarz 2002). This effect can be caused by the two claw system of the beetle avoiding the claws to slide around a particle. Moreover, the distribution of animals with claws on the stones in running water indicated that the size and shape of the claw influence the ability to attach to surfaces of different roughness. So far almost nothing is known about the influence of additional teeth and indentations on attachment to different surface texture. Therefore, the determined surface roughness needed by the claws of *E. assimilis* indicates the range of surface roughness but a direct transfer to all animals with claws seems not appropriate.

The setose pads on the gill lamellae of *E. assimilis* contributed significantly to friction force generated on smooth and some rough substrates. Significant effects were measured on three of four tested substrates: glass (S1) and replications with surface roughness of 0.5 µm (S2) and 6.3 µm (S4) (Ra estimated at low magnification). Interestingly, no effect on friction was found on a certain kind of intermediate roughness (S3: 3.3 µm Ra estimated at low magnification). We assume that the special shape with deep grooves and rounded tips is responsible for minimizing friction on this substrate. Our results indicate that interlocking between setae and substrate irregularities are mainly responsible for friction generation on rough substrates whereas molecular adhesion contributes to friction particularly on smooth substrates.

7.4 Interplay of different attachment devices

The species of main interest was the running water mayfly *Epeorus assimilis* which is equipped with different kinds of attachment devices: claws, setose pads on the gill lamellae and spiky acanthae on the abdominal sterna. The question arises why an animal develops different kinds of attachment devices. Two possibilities are imaginable: (1) The total attachment force of the animal is increased due to the cumulated resistance of all attachment structures. (2) The different attachment structures just work at corresponding substrate properties. This means that one attachment structure for instance might increase the attachment force on a certain substrate whereas another attachment structure does not work or has only little effect. Different attachment devices for smooth and rough substrates are described for several terrestrial insects (e.g beetles, bees). Also many adult Ephemeroptera bear a claw and a claw pad (Beutel and Gorb 2001). While claws need a certain surface roughness for attachment the claw pads have the ability to adapt to the profile of a surface and therefore can attach to smooth substrates. We assumed that such supplementary interplay of different attachment devices might also be the case in *Epeorus* larvae. Our results showed that the claws of *E. assimilis* need relatively rough substrates in order to hook to. In the experiments living larvae were only able to attach to the roughest substrate (S4). On the other hand, the setose pads on the gill lamellae of *E. assimilis* had significant effects not only on rough but also on smooth substrates and those of slight surface roughness. Consequently, the setose pads bring supplementary effects on smooth surfaces where claws do not function. On rough substrates setose pads showed a significant effect too. On these substrates the friction force of claws and setose pads will sum up. So both mentioned possibilities of the interplay of different attachment devices are relevant for *Epeorus assimilis*. In this context it is interesting that Clifford, Gotceita and Casey (1989) described significantly higher abundance for *Epeorus sp.* on smooth substrates compared with rough ones while for most species the contrary distribution in relation to surface roughness was observed. However, stones are the natural substrate of *Epeorus* larvae and their surface texture is not as homogeneous as that of the artificial substrates in our experiments. Our investigations showed that even on smooth stones crevices can be found that might enable the claws to hook to. Therefore, the gill lamellae might increase attachment force in addition to the claws on smooth stones.

7.5 Influence of biofilm on attachment

Nevertheless, living *Epeorus* larvae were not able to attach to smooth surfaces and substrates of slight roughness in our experiments with steril artificial substrates. This was surprising because it has already been observed that *Epeorus* larvae can attach to smooth glass and plexiglass (Ambühl 1960, Ditsche-Kuru personal observation). The important difference between these observations might be that the latter was made on plexiglass walls after a longer operation period of the flume. Therefore, the surface of the smooth plexiglass plane should have been covered with a layer of biofilm. We hypothesize that the biofilm coverage relevantly influences the attachment ability of the larvae. It is imaginable that the biofilm coverage decreases friction forces on rough substrates because they become more slippery. On the other hand, the biofilm coverage might increase friction force on smooth substrates because the biofilm could offer the claws increased resistance due to its higher viscosity compared with the original solid surface. Moreover, it is possible that the rather high friction on smooth solid substrates is a side effect. As mentioned above stones in running waters are normally not totally smooth but rather covered with algae and biofilm. Biofilms in running waters usually have a smooth and slippery surface. The setose pads of the gill lamellae might be an adaptation to attach on biofilms. An example for attachment to biofilm can be found in Prodosteamaceae species inhabiting waterfalls. These plants develop adhesive hairs which stick to cyanobacteria threads and biofilm matrix (Jäger-Zürn and Grubert 2000) and can withstand enormous tensile stress caused by action of running water. In some species as for instant Simuliidae (Kiel 1996) and *Dreissena* (Wainman et al. 1996) biofilm showed no influence on attachment. Neverthelss, the effect on attachment seems to depend on the kind of the attachment device.

7.6 Determination of surface roughness

In our experiments, the surface roughness of all substrates has been evaluated at two different magnifications. Only one of these two applied settings turned out to be suitable for further investigations dealing with surface roughness. Roughness measurements of the stones surface estimated at higher magnification did not bring additional results compared with the roughness measurements evaluated at lower magnification with regard to the distribution of macrozoobenthos. Moreover, in attachment experiments with claws and setose pads the roughness estimated at higher magnification was not the relevant one. Further, the roughness measurements at higher magnification were very problematic in practice. They were very time intensive and problematic due to many erroneous measurements which made necessary a lot of additional replications. Therefore, roughness measurements on stones at higher magnification seem to be not appropriate for many samples. In contrast, roughness measurements evaluated at lower magnification can be suggested for further investigations. In this setting, measurements were much quicker accomplished and only few erroneous measurements occurred. The profilometric technique itself has already been tested for a lot of other technical applications and can readily be bought.

Unfortunately, *Epeorus* larvae were not found on the stones investigated with regard to surface roughness. Therefore, direct comparison of the influence between roughness parameters determined on stones and replications was not possible.

7.7 Conclusions and outlook

First steps to understand the interplay of surface roughness and attachment devices have been undertaken. The range of surface roughness of natural stones commonly occurring in running waters has been quantified. An appropriate method to estimate surface roughness has been described. Setose pads on the gill lamellae of *Epeorus* and *Iron* larvae have been revealed. The required surface roughness of claws and setose pads have been determined and relevant mechanisms have been identified. Nevertheless, the more interesting answers were found the more new questions arose. Adaptations to flow and the interplay of attachment devices and surface texture turned out to be very complex. Next steps to an increased understanding could be the investigation of the influence of surface roughness on other attachment devices as well as on claws of different shape and size. The adaptability of the roughness parameters determined on homogeneous replica of polishing papers on heterogeneous stones surfaces should be tested. Moreover, attention should be directed to the influence of biofilm on attachment.

Summary

Petra Ditsche-Kuru (2009), *Influence of the surface roughness of hard substrates on the attachment of selected running water macrozoobenthos.* Dissertation, Mathematisch-Naturwissenschaftliche Fakultät, Rheinische Friedrich-Wilhelms-Universität Bonn. 166 pp.

Flowing water can develop an immense force pushing on animals inhabiting the surface of stones in running waters. These animals have developed attachment devices supporting them to maintain their position against current. Some of these attachment devices (e.g. claws, suckers) have been described to require a certain kind of surface roughness. In this thesis, the interplay of attachment devices and surface texture of the hard bottom substrates in running waters was investigated in detail for selected animals of the torrential fauna using scanning electron microscopy, videotaping, attachment experiments, friction measurements, white light profilometry and replication techniques. Moreover, a quantitative description of the range of surface roughness of stones commonly occurring in running waters is given first time.

The surface roughness of stones occurring in several streams with different geological backgrounds was quanitified by means of profilometric technique. The roughest stone was about six times rougher than the smoothest one in terms of the average roughness “Ra”. The surface roughness of the stones showed a significant variability within the same rock for all examined rocks (andesite, slate, basalt, quartz gravel, greywacke, quarzite, bunter sandstone). These results indicate that simple qualitative differentiation between the roughness of rocks as performed in some former studies can cause considerable inaccuracy.

Depending on their attachment devices, animals were expected to be found on certain stones that have the appropriate surface roughness. However, only *Elmis* larvae and *Ancylus fluviatilis* indicated such an association to the stones. In other cases (*Baetis*, *Rhyacophila*, Chironomidae) the distribution was contrary to these expectations and was dominated by other factors such as food resources. Even animals with claws were able to cling to smooth stones on which at least a few individuals occurred. A possible reason can be the crevices often found on the surface of the stones irrespective of their average surface roughness. Another factor influencing attachment might be the coverage of biofilm and sediments on stones.

To gain deeper understanding of the interplay of attachment devices and surface structure intensive studies were performed under defined laboratory conditions. The species of main interest was the running water mayfly *Epeorus assimilis* equipped with different kinds of attachment devices. In addition to strong claws SEM analysis revealed areas with spike-shaped microtrichia on the abdominal sternits and setose pads located on that part of the gill lamella which stays in contact with the substrate. These setose pads look very similar to the setose attachment pads of terrestrial insects which are known for their extraordinary attachment abilities. Moreover, we found the same attachment structures in the closely related species *Iron alpicola*. The setae of this species have a similar size but a significantly higher density than those of *E. assimils* what might cause a greater adhesive strength and could be an adaptation to the swifter currents in which *I. alpicola* live. Previous authors suggested that gill lamellae of both species are modified to a sucker as an adaptation to withstand currents but our video observations demonstrated that gill lamellae do not perform a tight contact with their support and therefore no negative pressure can be uphold.

Friction experiments with *E. assimils* larvae showed that the setose pads on the gill lamellae significantly contribute to the friction force on smooth and some rough substrates. The friction coefficient of the gill lamellae depended on the surface roughness of the substrate and the pulling direction. The results indicate that interlocking effects are mainly responsible for friction generation on rough substrates while molecular adhesion contributes to friction on smooth substrates. However, in additional experiments living larvae were not able to attach to smooth glass or surfaces of slight roughness. This was surprising because it had been observed previously that living *Epeorus* larvae can attach to smooth surfaces. Due to the sterile substrates in these experiments, we assume that the coverage of biofilm considerably influences the attachment conditions. On sterile substrates larvae attached only to the rough surfaces. The claws grasped to substrates with a surface roughness “Ra” higher than 6 µm, but some unfavourable textures caused delayed interlocking. One of the two applied profilometric settings turned out to be relevant for the distribution of macrozoobenthos and could also handle large amounts of samples. Therefore, this array can be recommended for further investigations dealing with surface roughness.

We had assumed that different attachment devices of the same animal may offer advantages on substrates with certain surface properties. The investigated attachment devices of *Epeorus* larvae showed a different interplay depending on the surface roughness. On smooth substrates, where claws can only grasp on a few surface asperities, the setose pads provided an additional advantage while on rough substrates a cummulated resistance was observed.

Zusammenfassung

Petra Ditsche-Kuru (2009), ***Einfluss der Oberflächenrauheit von Hartsubstraten auf die Anhaftung von Makroinvertebraten in Fließgewässern.*** Dissertation, Mathematisch-Naturwissenschaftliche Fakultät, Rheinische Friedrich-Wilhelms-Universität Bonn. 166 S.

In Fließgewässern lebende Tiere haben eine Vielzahl von Anpassungen entwickelt, um mit den teilweise enormen Strömungskräften umzugehen. Zu solchen Strömungsanpassungen gehören die verschiedenen Haftorgane, welche das Tier unterstützen seine Position in der Strömung zu halten. Von einigen dieser Haftorgane (Krallen, Saugnäpfe) wird beschrieben, dass sie eine spezielle Oberflächenrauheit benötigen. Gegenstand dieser Doktorarbeit ist das Zusammenspiel von tierischen Haftorganen und der Oberflächenstruktur der Hartsubstrate, welches für ausgewählte Makroinvertebraten mittels Rasterelektronenmikroskopie, Videoaufnahmen, Weißlichtprofilometrie, Kraftmessungen, Abformungstechnik und Anhaftungsexperimenten untersucht wurde. Weiterhin wurde erstmalig die Amplitude der Oberflächenrauheit von üblicherweise in Fließgewässern vorkommenden Gesteinen quantifiziert.

Die Oberflächenrauheit von Steinen aus verschiedenen Gewässern mit unterschiedlichem geologischen Einzugsgebiet wurde mit Hilfe von profilometrischen Messungen quantifiziert. Bezogen auf den arithmetrischen Mittenrauhwert Ra war der raueste Stein etwa sechs mal rauher als der glatteste. Innerhalb aller näher untersuchten Gesteinsarten (Andesit, Schiefer, Basalt, Quarz, Grauwacke, Quarzite, Buntsandstein) zeigte die Rauheit der einzelnen Steine signifikante Unterschiede. Folglich kann eine einfache qualitative Unterscheidung zwischen der Rauheit der Gesteinsarten, wie sie in verschiedenen Studien vorgenommen wurde, zu beachtlichen Ungenauigkeiten führen.

Ursprünglich hatten wir erwartet, die Taxa in Abhängigkeit von der Art ihrer Haftorgane auf Steinen bestimmter Oberflächenrauheit zu finden. Ein solcher Zusammenhang wurde jedoch nur für *Elmis* Larven und *Ancylus fluviatilis* gefunden. Die Verteilung anderer Taxa (*Baetis*, *Rhyacophila*, Chironomidae) war dagegen genau umgekehrt wie erwartet. Hier waren offenbar andere Faktoren, wie z. B. die Futterverfügbarkeit, dominierend. Sogar Taxa, die sich nur mit Krallen festhalten, wurden zumindest mit wenigen Exemplaren auf glatten Steinen gefunden. Eine Erklärung dafür könnten die Bedeckung der Steine mit Biofilm und

Sedimentablagerungen sowie Ritzen auf den Steinen sein, welche sich meist unabhängig von der mittleren Oberflächenrauheit auf den Steinen befinden.

Da unter natürlichen Bedingungen zu viele mögliche Einflussfaktoren variierten, um das Zusammenspiel von Haftorganen und Oberflächenrauheit zu verstehen, wurden intensive Studien für ausgewählte Haftstrukturen unter definierten Laborbedingungen durchgeführt. Das Hauptuntersuchungsobjekt war die Eintagsfliege *Epeorus assimilis*, welche in schnell strömenden Bereichen lebt und verschiedene Haftstrukturen aufweist. Neben den kräftigen Krallen zeigten rasterelektronenmikroskopische Aufnahmen Felder mit spitzen Acanthae auf den Sterniten sowie mit Setae bestandene Haftkissen auf der Unterseite der Kiemenblättchen. Letztere ähneln in ihrem Erscheinungsbild stark den Haftstrukturen terrestrischer Insekten, welche für ihre außergewöhnlichen Fähigkeiten bekannt sind. Die gleichen Haftstrukturen wurden auch bei der verwandten Art *Iron alpicola* gefunden. Die Setae dieser Art haben die gleiche Größe wie bei *Epeorus*, die Dichte war dagegen signifikant höher. Letzteres könnte eine größere Haftkraft bedingen, welche eine Anpassung an die höhere Fließgeschwindigkeit im Habitat dieser Eintagsfliege sein könnte. In verschiedener Literatur wird angenommen, dass die Kiemenblättchen beider Arten einen Saugnapf bilden um der Strömung zu widerstehen. Unsere Videoaufnahmen zeigten jedoch, dass die Kiemenblättchen keinen dichten Randkontakt zum Untergrund ausbilden und somit kein Unterdruck aufrecht erhalten werden kann. Messungen der Reibungskraft zeigten einen signifikanten Effekt der Haftkissen auf den Kiemenblättchen auf glatten und einigen rauen Substraten. Der Reibungskoeffizient der Kiemenblättchen hing von der Oberflächenrauheit und Zugrichtung ab. Diese Ergebnisse weisen darauf hin, dass sowohl Verklammerungseffekte mit Oberflächenunregelmäßigkeiten als auch molekulare Adhäsion eine Rolle bei der Anhaftung spielen. In weiteren Experimenten waren lebende Larven jedoch nicht in der Lage, sich auf glatten Substraten zu halten. Dies war überraschend, da *Epeorus* Larven bereits auf glatten Oberflächen beobachtet worden waren. Da die in diesen Versuchen verwendeten Substrate ohne Biofilmbewuchs waren, nehmen wir an, dass der Biofilm einen bedeutenden Einfluss auf die Haftbedingungen hat. Auf diesen sterilen Substraten hielten sich die Larven nur auf den rauesten Substraten. Die Krallen benötigten eine Oberflächenrauheit von minimal 6 µm (Ra). Eine von zwei Einstellungen zur Rauheitsmessung erwies sich als empfehlenswert für weitere Untersuchungen.

Das Zusammenspiel der beiden näher untersuchten Haftorgane der *Epeorus* Larven war in Abhängigkeit von der Oberflächenrauheit verschieden. Auf glatten Substraten, wo die Krallen nur in wenigen Ritzen einhaken können, bewirken die Haftkissen einen zusätzlichen Vorteil während sich auf rauen Substraten die Haftkräfte beider Haftstrukturen aufsummieren.

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Appendix 1

Survey of selected surface roughness and profile parameters

Parameter	Name	Description	Comment (according to Volk. 2005)	Filtered or unfiltered profile	Differentiation between peaks and valleys	Horizontal or vertical characteristic	Standard
Ra	Roughness Average	Arithmetic average of the absolute values of the roughness profile ordinates.	Relativly unsensitive to the different shapes of the surface, e.g. to single peaks and valleys	F	no	H	DIN EN ISO 4287; standard roughness parameter
Rz	Average maximum height of the profile	Average distance between the highest peak and lowest valley in each sampling length	Sensitive to the different shapes of the surface, e.g. to single peaks and grooves	F	no	H	DIN EN ISO 4287; often used roughness parameter
Rmax (Rz1max)	Maximum Roughness Depth	Highest distance between the highest peak and lowest valley in each sampling length	Sensitive to the different different shapes of the surface, e.g. to single peaks and grooves	F	no	H	Rz1max in DIN EN ISO 4287
Rq (= RMS)	Root Mean Square Roughness	Average of the square of the absolute values of the roughness profile ordinates.	Similar to Ra. but a bit more sensitive to single peks and grooves	F	no	H	DIN EN ISO 4287; priviously often used roughness parameter
Pt	Maximum Heights of the profile	Distance between the highest peak and the lowest valley of the whole profile	Rt is the only commonly used parameter of the non-filtered profile	UF	no	H	DIN EN ISO 4287
Rp	Average Maximum Profile Peak Heigth	Average distance between the highest peak and the center line of the profile in each sampling length	The relation of Rp and Rv gives some information about the profile shape; if Rp/Rv is < 0.5 the profile is notched. if Rp/Rv is > 0.5 the profile is peaked	F	peak	H	DIN EN ISO 4287
Rv	Average Maximum Profile Vally Depth	Average distance between the deepest valley and the center line of the profile in each sampling length	The relation of Rp and Rv gives some information about the shape of the profile	F	valley	H	DIN EN ISO 4287

Parameter	**Name**	**Description**	**Comment (according to Volk. 2005)**	**Filtered or unfiltered profile**	**Differentiation between peaks and valleys**	**Horizontal or vertical characteristic**	**Standard**
RPc (=D)	Profile peak density			F	peak	V	SEP 1940
RSm	Mean spacing of profile irregularities			F	valley	V	DIN EN ISO 4287
Rk	core roughness depth	Middle part of the Abbott-Firestone Curve*, Rk contains the region of highest increase of the material	The relation of Rk to Rz gives information abaut the surface texture, if Rk is relatively smal compared to Rz the surface should be plateu like	F	Middle part	Combined parameter	DIN EN ISO 13565
Rpk	reduced peak height	(reduced) upper part of the Abbott-Firestone Curve*, which gives information about the material part of the peaks	Rpk, Rk and Rvk together give a good survey of heights and destribution of the material part of the profile	F	peak	Combined parameter	DIN EN ISO 13565
Rvk	reduced valley depth	(reduced) lower part of the Abbott-Firestone Curve*, which gives information about the material part of the valleys	Rpk, Rk and Rvk together give a good survey of heights and destribution of the material part of the profile	F	valley	Combined parameter	DIN EN ISO 13565

* The Abbott-Firestone curve was first described by Abbott and Firestone (1933). Mathematically it is the cumulative probability density function of the surface profile's height and can be calculated by integrating the profile trace (Stachowiak and Bachelor. 2001).

** The amplitude desity curve is a grafical synthesis of the distribution of the ordinate heigh over the whole profile's height (Volk. 2005).

Appendix 2

Characters of the investigated stones

water	season	stone-nr.	rock	Ra - lower magnification mean ± S.D. [µm]	Ra - higher magnification mean ± S.D. [µm]	surface area [m²]	drymass biofilm [g/m²]	ignition loss [%]	individual number [Ind./m²]	taxa number [taxa/stone]
stream Kyll	spring	K10	bunter	7.6 ± 1.5	0.8 ± 0.2	0.041	6.6	12.50	1 378	6
stream Kyll	spring	K11	bunter	7.2 ± 0.7	1.6 ± 0.5	0.036	5.6	56.25	2 346	6
stream Kyll	spring	K12	bunter	10.0 ± 1.4	2.8 ± 0.9	0.067	5.8	16.00	519	4
stream Kyll	spring	K13	bunter	8.7 ± 1.2	1.5 ± 0.6	0.021	2.3	0.00	375	4
stream Kyll	spring	K14	bunter	7.9 ± 0.9	1.6 ± 0.5	0.029	4.9	0.00	630	5
stream Kyll	spring	K15	bunter	5.2 ± 0.8	0.9 ± 0.4	0.067	20.5	51.72	2 036	11
stream Kyll	spring	K16	bunter	15.1 ± 2.3	2.4 ± 1.1	0.031	35.2	0.00	807	6
stream Kyll	spring	K17	bunter	13.9 ± 1.5	1.6 ± 0.5	0.021	4.4	0.00	242	3
stream Kyll	spring	K18	bunter	15.3 ± 2.7	1.2 ± 0.4	0.027	6.6	40.00	74	2
stream Kyll	spring	K19	bunter	10.2 ± 1.7	1.8 ± 1.2	0.080	56.5	18.18	4 738	11
stream Kyll	summer	K20	bunter	6.6 ± 1.9	0.9 ± 0.3	0.016	29	20.00	2 101	11
stream Kyll	summer	K21	bunter	4.3 ± 0.8	1.1 ± 0.4	0.023	33.7	8.82	342	6
stream Kyll	summer	K22	bunter	10.9 ± 1.7	1.5 ± 0.3	0.026	10.1	50.00	1 549	7
stream Kyll	summer	K23	bunter	8.5 ± 2.2	1.2 ± 0.2	0.088	6	9.52	250	10
stream Kyll	summer	K24	bunter	9.0 ± 1.5	1.6 ± 0.5	0.025	21.8	0.00	323	7
stream Kyll	summer	K25	bunter	8.5 ± 1.4	1.2 ± 0.4	0.033	23.7	30.00	389	3
stream Kyll	summer	K26	bunter	8.7 ± 2.0	1.5 ± 0.5	0.108	3.8	6.67	334	8
stream Kyll	summer	K27	bunter	12.8 ± 2.1	1.6 ± 0.4	0.091	8.3	9.30	121	6
stream Kyll	summer	K28	bunter	8.9 ± 0.5	1.3 ± 0.5	0.027	4	0.00	735	9
stream Kyll	summer	K29	bunter	5.0 ± 0.7	0.9 ± 0.3	0.023	6.1	50.00	954	9
stream Kyll	autumn	K30	bunter	5.7 ± 0.8	1.7 ± 0.7	0.041	19.6	18.33	3 582	11

water	season	stone-nr.	rock	Ra - lower magnification mean ± S.D. [µm]	Ra - higher magnification mean ± S.D. [µm]	surface area [m²]	drymass biofilm [g/m²]	ignition loss [%]	individual number [Ind./m²]	taxa number [taxa/stone]
stream Kyll	autumn	K31	bunter	4.7 ± 0.8	0.7 ± 0.1	0.086	29.5	18.75	5 143	21
stream Kyll	autumn	K32	bunter	9.9 ± 2.1	2.2 ± 1.0	0.054	17.1	15.79	10 808	21
stream Kyll	autumn	K33	bunter	k.M. ± k.M.	1.4 ± 0.5	0.062	7.4	29.63	3 626	23
stream Kyll	autumn	K34	bunter	4.7 ± 1.1	1.4 ± 0.7	0.037	14.8	16.67	1 644	14
stream Kyll	autumn	K35	bunter	6.5 ± 1.4	1.5 ± 0.5	0.050	36.1	20.00	1 654	17
stream Kyll	autumn	K36	bunter	7.8 ± 1.3	1.0 ± 0.4	0.079	30.1	9.39	2 433	20
stream Kyll	autumn	K37	bunter	10.6 ± 1.2	1.9 ± 1.5	0.074	24.4	16.11	2 131	22
stream Kyll	autumn	K38	bunter	9.3 ± 1.1	0.8 ± 0.2	0.066	4.8	72.00	12 297	26
stream Kyll	autumn	K39	bunter	8.8 ± 1.6	1.6 ± 0.6	0.073	21.2	31.20	3 479	17
stream Nahe	spring	N14	andesite	2.5 ± 1.0	0.6 ± 0.1	0.058	77.9	0.00	1 078	14
stream Nahe	spring	N15	andesite	3.1 ± 1.0	0.4 ± 0.1	0.073	188.2	17.08	1 745	15
stream Nahe	spring	N16	quarzite	5.5 ± 1.5	0.9 ± 0.2	0.036	1.7	0.00	503	8
stream Nahe	spring	N17	quarzite	3.1 ± 0.5	0.6 ± 0.2	0.052	9.1	72.73	484	6
stream Nahe	spring	N18	quarzite	4.4 ± 1.0	0.9 ± 0.3	0.080	17.9	23.89	1 389	14
stream Nahe	spring	N19	quarzite	2.9 ± 0.5	0.6 ± 0.1	0.021	3.3	53.85	2 483	10
stream Nahe	spring	N20	andesite	3.2 ± 1.0	0.5 ± 0.1	0.022	30.9	54.39	1 225	8
stream Nahe	spring	N21	quarzite	4.6 ± 1.4	0.6 ± 0.2	0.040	58.1	20.00	350	4
stream Nahe	spring	N22	melaphyre	2.1 ± 0.9	0.5 ± 0.1	0.062	31.1	67.50	1 171	12
stream Nahe	spring	N23	quarzite	3.3 ± 1.6	0.8 ± 0.2	0.039	19.3	4.05	514	5
stream Nahe	summer	N24	quarzite	3.2 ± 0.5	0.6 ± 0.2	0.042	25.7	24.36	1 842	14
stream Nahe	summer	N25	andesite	3.1 ± 0.6	0.6 ± 0.1	0.031	32.6	33.33	1 308	7
stream Nahe	summer	N26	quarzite	3.8 ± 0.8	0.6 ± 0.3	0.064	5.3	40.00	937	15
stream Nahe	summer	N27	quarz gravel	3.4 ± 1.0	0.8 ± 0.3	0.048	4.8	15.38	830	14
stream Nahe	summer	N28	andesite	4.1 ± 1.0	0.7 ± 0.2	0.078	91	36.36	874	12

				Ra - lower magnification	Ra - higher magnification	surface area	drymass biofilm	ignition loss	individual number	taxa number
water	season	stone-nr.	rock	mean ± S.D. [µm]	mean ± S.D. [µm]	[m²]	[g/m²]	[%]	[Ind./m²]	[taxa/stone]
stream Nahe	summer	N29	andesite	5.1 ± 2.5	0.7 ± 0.2	0.054	47.7	14.71	536	9
stream Nahe	summer	N30	quarz gravel	4.2 ± 1.7	0.6 ± 0.1	0.033	8.9	21.43	795	12
stream Nahe	summer	N31	quarzite	5.5 ± 1.0	1.0 ± 0.3	0.023	15.8	9.09	1 110	13
stream Nahe	summer	N32	quarzite	3.4 ± 1.3	0.8 ± 0.3	0.051	5.5	33.33	1 462	14
stream Nahe	summer	N33	andesite	4.5 ± 2.0	0.5 ± 0.2	0.050	52.3	24.00	901	16
stream Nahe	autumn	N34	andesite	2.5 ± 0.4	0.6 ± 0.2	0.096	38.6	23.48	2 334	20
stream Nahe	autumn	N35	quarzite	4.3 ± 2.8	0.6 ± 0.2	0.064	4.4	47.06	593	12
stream Nahe	autumn	N36	andesite	3.7 ± 1.9	0.8 ± 0.2	0.050	159.5	7.93	1 074	18
stream Nahe	autumn	N37	andesite	2.4 ± 0.5	0.5 ± 0.1	0.028	47.8	20.00	815	6
stream Nahe	autumn	N38	andesite	3.5 ± 0.7	0.7 ± 0.2	0.058	46.9	55.00	945	12
stream Nahe	autumn	N39	quarzite	3.9 ± 1.0	0.6 ± 0.2	0.059	16.7	14.43	946	16
stream Nahe	autumn	N40	andesite	4.1 ± 1.1	0.7 ± 0.1	0.048	54.9	29.03	2 194	18
stream Nahe	autumn	N41	quarzite	4.5 ± 1.6	0.8 ± 0.2	0.052	14.5	6.12	1 278	15
stream Nahe	autumn	N42	andesite	3.9 ± 0.8	0.8 ± 0.3	0.047	67	10.00	657	10
stream Nahe	autumn	N43	melaphyre	2.9 ± 0.8	0.7 ± 0.2	0.064	35	65.38	863	14
River Rhine	spring	R1	quarz gravel	3.5 ± 0.8	0.8 ± 0.2	0.076	3.2	20.00	79	2
River Rhine	spring	R10	quarz gravel	5.3 ± 0.9	0.8 ± 0.1	0.020	6.4	0.00	295	2
River Rhine	spring	R2	quarz gravel	3.5 ± 0.9	0.7 ± 0.2	0.040	7.2	15.00	50	1
River Rhine	spring	R3	quarz gravel	5.7 ± 2.1	1.0 ± 0.2	0.070	6.3	16.67	29	2
River Rhine	spring	R4	slate	3.2 ± 0.8	0.8 ± 0.2	0.083	15.2	1.22	97	2
River Rhine	spring	R5	quarz gravel	3.9 ± 1.6	1.1 ± 0.4	0.028	3.2	50.00	142	1
River Rhine	spring	R6	tuff	3.5 ± 0.6	0.9 ± 0.3	0.025	7.2	0.00	796	2
River Rhine	spring	R7	slate	4.3 ± 1.5	0.6 ± 0.1	0.028	6.2	0.00	399	2
River Rhine	spring	R8	quarz gravel	3.0 ± 0.6	0.9 ± 0.3	0.024	1.6	0.00	327	1

				Ra - lower magnification	Ra - higher magnification	surface area	drymass biofilm	ignition loss	individual number	taxa number
water	season	stone-nr.	rock	mean ± S.D. [µm]	mean ± S.D. [µm]	[m²]	[g/m²]	[%]	[Ind./m²]	[taxa/stone]
River Rhine	spring	R9	slate	3.3 ± 0.7	0.9 ± 0.3	0.015	7.3	66.67	387	1
stream Wied	spring	W13	slate	3.4 ± 0.8	0.7 ± 0.1	0.040	25.1	8.54	1 092	10
stream Wied	spring	W14	slate	5.4 ± 1.3	0.7 ± 0.1	0.064	6.1	50.00	202	3
stream Wied	spring	W15	greywacke	3.6 ± 2.1	0.6 ± 0.1	0.009	8.1	66.67	2 672	9
stream Wied	spring	W16	slate	2.8 ± 0.4	0.6 ± 0.1	0.039	13.8	8.33	1 146	10
stream Wied	spring	W17	greywacke	5.3 ± 0.5	0.8 ± 0.1	0.010	17.6	0.00	2 282	10
stream Wied	spring	W18	basalt	3.3 ± 1.3	0.9 ± 0.3	0.142	2.2	32.26	352	9
stream Wied	spring	W19	greywacke	4.7 ± 1.1	0.8 ± 0.3	0.051	18.2	12.50	334	6
stream Wied	spring	W20	greywacke	2.9 ± 0.5	0.5 ± 0.1	0.075	20.6	5.56	361	6
stream Wied	spring	W21	basalt	4.4 ± 1.6	0.6 ± 0.1	0.097	16	13.33	434	7
stream Wied	spring	W22	slate	3.5 ± 0.7	0.8 ± 0.1	0.026	3.8	0.00	879	6
stream Wied	summer	W23	basalt	2.5 ± 0.4	0.6 ± 0.1	0.053	15.6	8.33	854	14
stream Wied	summer	W24	slate	3.3 ± 0.9	0.4 ± 0.1	0.057	17.6	18.31	2 200	17
stream Wied	summer	W25	basalt	4.8 ± 1.4	0.9 ± 0.4	0.063	21.6	35.00	2 257	17
stream Wied	summer	W26	quarz gravel	4.4 ± 1.3	0.9 ± 0.3	0.074	5.6	25.93	1 008	14
stream Wied	summer	W27	basalt	3.2 ± 0.7	0.7 ± 0.2	0.087	26.7	6.35	1 037	14
stream Wied	summer	W28	greywacke	4.8 ± 1.1	0.9 ± 0.2	0.065	23.2	15.38	630	13
stream Wied	summer	W29	greywacke	4.4 ± 0.9	1.1 ± 0.5	0.058	23	53.85	859	12
stream Wied	summer	W30	slate	2.7 ± 0.7	0.7 ± 0.3	0.052	9.6	54.17	615	12
stream Wied	summer	W31	basalt	4.1 ± 1.3	0.6 ± 0.2	0.038	17.4	20.59	686	7
stream Wied	summer	W32	greywacke	3.7 ± 1.3	0.7 ± 0.1	0.072	26	9.86	456	15
stream Wied	autumn	W33	basalt	3.2 ± 0.7	0.6 ± 0.1	0.042	47.4	32.31	2 952	24
stream Wied	autumn	W34	basalt	3.7 ± 1.3	0.8 ± 0.2	0.061	42.2	26.52	2 277	19
stream Wied	autumn	W35	slate	3.6 ± 0.8	0.7 ± 0.2	0.040	76.9	13.50	2 830	15

water	season	stone-nr.	rock	Ra - lower magnification mean ± S.D. [µm]	Ra - higher magnification mean ± S.D. [µm]	surface area [m²]	drymass biofilm [g/m²]	ignition loss [%]	individual number [Ind./m²]	taxa number [taxa/stone]
stream Wied	autumn	W36	slate	4.0 ± 0.8	0.7 ± 0.2	0.094	15.4	55.70	1 787	21
stream Wied	autumn	W37	greywacke	5.0 ± 2.6	0.9 ± 0.2	0.062	18.4	16.47	968	16
stream Wied	autumn	W38	greywacke	3.7 ± 0.6	0.9 ± 0.2	0.050	29.8	12.86	1 896	14
stream Wied	autumn	W39	slate	4.8 ± 1.2	0.8 ± 0.2	0.051	38.4	10.20	2 612	22
stream Wied	autumn	W40	quarz gravel	2.4 ± 0.6	0.6 ± 0.2	0.032	26.5	14.49	999	12
stream Wied	autumn	W41	basalt	4.3 ± 1.2	0.7 ± 0.2	0.046	40.4	27.37	1 135	14
stream Wied	autumn	W42	basalt	2.8 ± 0.7	0.6 ± 0.1	0.042	18.2	13.79	600	9
River Elbe	spring	E1	quarzite	4.3 ± 0.5	0.8 ± 0.2	0.039	23.1	5.54	514	5
River Elbe	spring	E2	pegmatite	7.8 ± 4.0	0.9 ± 0.4	0.057	40.1	7.05	914	8
River Elbe	spring	E3	quarzite	8.0 ± 1.4	1.0 ± 0.3	0.065	64	5.50	340	3
River Elbe	spring	E4	bunter	6.4 ± 2.6	1.2 ± 0.4	0.058	53.3	5.34	1 436	6
River Elbe	spring	E5	metamorphite	3.5 ± 1.8	1.2 ± 0.6	0.058	38.4	13.49	2 427	8
River Elbe	spring	E6	gneiss	12.2 ± 4.9	1.3 ± 0.4	0.051	13.2	5.20	641	6
River Elbe	spring	E7	lydite	4.3 ± 2.3	0.6 ± 0.1	0.081	28.9	10.30	594	5
River Elbe	spring	E8	pegmatite	14.1 ± 6.0	1.2 ± 0.3	0.111	11.9	5.80	862	6
River Elbe	spring	E9	basalt	6.8 ± 1.2	1.1 ± 0.5	0.071	57.2	6.95	976	6
River Elbe	spring	E10	granite	6.8 ± 2.0	1.0 ± 0.6	0.049	48.2	4.23	1 579	6

Appendix 3

Parameter of surface roughness (mean ± S.D.) of the different rocks – roughness estimated at lower magnification (measured by the white light interferrometer FRT-MircoProf.; H2 sensor; Area: 1000 x 1000 µm, Pixel: 10 µm^2)

Parameter		**andesite**	**slate**	**basalt**	**quartz gravel**	**greywacke**	**quartzite**	**bunter sandstone**
		N=13	N=12	N=11	N=10	N=9	N=15	N=30
Ra	µm	3.52 ± 0.83	3.68 ± 0.81	3.93 ± 1.19	3.92 ± 1.00	4.23 ± 0.80	4.30 ± 1.29	8.61 ± 2.92
Rz$_{(DIN)}$	µm	34.96 ± 9.41	36.19 ± 7.81	38.72 ± 12.77	40.52 ± 8.16	37.48 ± 6.18	44.59 ± 14.66	79.66 ± 31.72
Rq (=Rms)	µm	4.79 ± 1.21	4.97 ± 1.05	5.29 ± 1.71	5.42 ± 1.34	5.56 ± 1.06	5.91 ± 1.84	11.40 ± 4.05
RK*		0.46 ± 0.03	0.47 ± 0.05	0.47 ± 0.02	0.45 ± 0.04	0.51 ± 0.03	0.45 ± 0.03	0.50 ± 0.05
RPk**		0.85 ± 0.17	0.89 ± 0.24	0.85 ± 0.20	0.69 ± 0.10	0.88 ± 0.09	0.76 ± 0.16	0.99 ± 0.19

* RK=Rk/(Rk+Rvk+Rpk), ** RPK= Rpk/Rvk

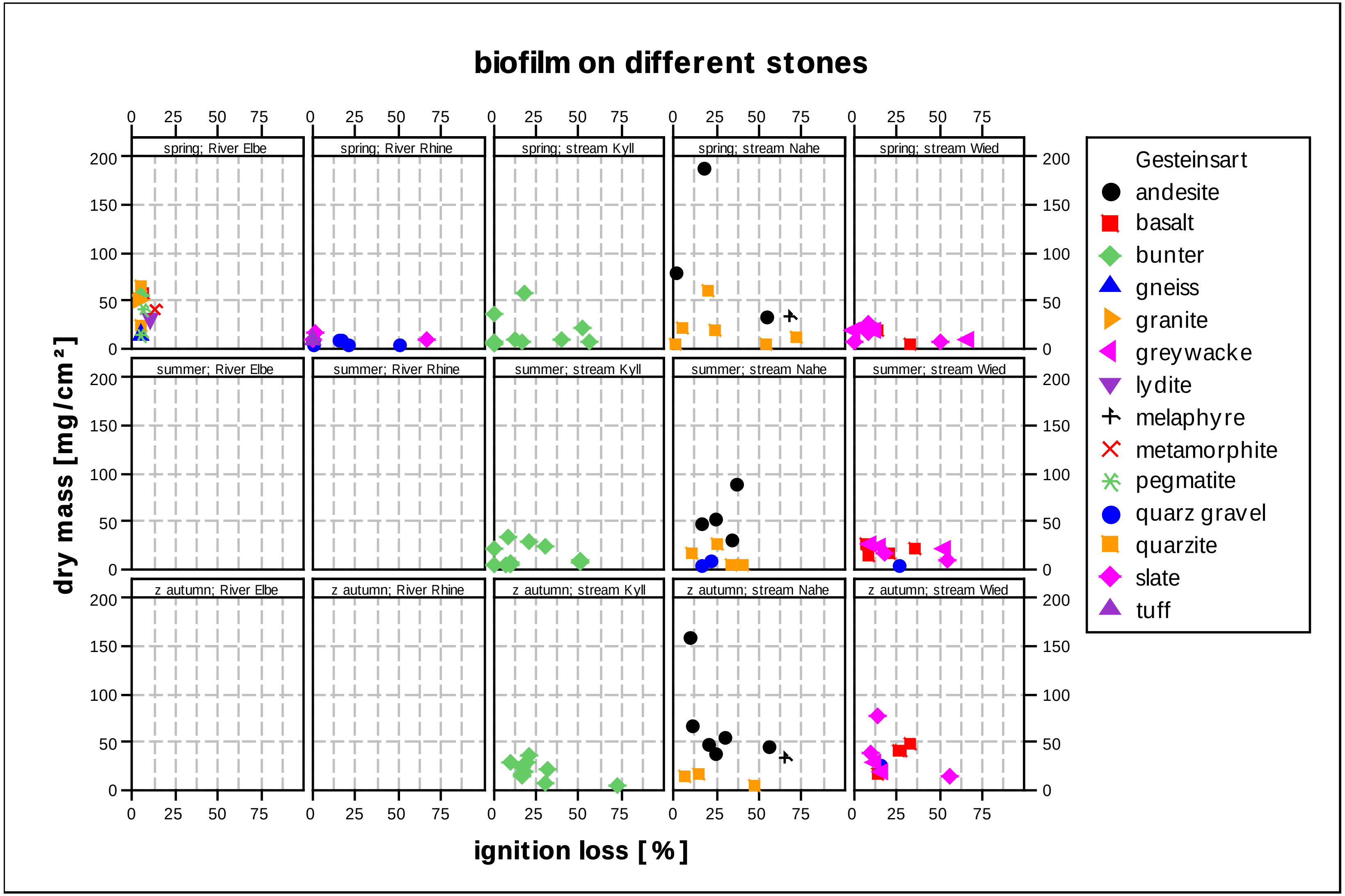
Appendix 4

Parameter of surface roughness (mean ± S.D.) of the different rocks – roughness estimated at higher magnification (measured by the white light interferrometer FRT-MircoProf.; H0 sensor; Area: 100 x 100 µm, Pixel: 1 µm²)

Parameter		**andesite**	**slate**	**basalt**	**quartz gravel**	**greywacke**	**quartzite**	**bunter sandstone**
		N=13	N=12	N=11	N=10	N=9	N=15	N=31
Ra	µm	0.61 ± 0.11	0.70 ± 0.14	0.71 ± 0.18	0.81 ± 0.16	0.80 ± 0.19	0.73 ± 0.14	1.42 ± 0.47
$Rz_{(DIN)}$	µm	6.84 ± 1.82	9.43 ± 3.23	9.05 ± 3.92	10.79 ± 2.91	11.19 ± 4.56	10.19 ± 4.64	22.43 ± 9.79
Rq (=Rms)	µm	0.84 ± 0.18	1.04 ± 0.23	1.04 ± 0.33	1.18 ± 0.27	1.22 ± 0.35	1.07 ± 0.28	2.33 ± 0.88
RK		0.44 ± 0.03	0.44 ± 0.03	0.43 ± 0.03	0.42 ± 0.03	0.42 ± 0.04	0.43 ± 0.03	0.34 ± 0.04
RPk		0.96 ± 0.18	0.84 ± 0.08	0.93 ± 0.17	0.79 ± 0.10	0.88 ± 0.11	0.85 ± 0.11	0.85 ± 0.08

* RK=Rk/(Rk+Rvk+Rpk), ** RPK= Rpk/Rvk

Appendix 5



biofilm on different stones
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
0
25
50
75
0
50
100
150
200
dry mass [mg/cm²]
ignition loss [%]
Gesteinsart
andesite
basalt
bunter
gneiss
granite
greywacke
lydite
melaphyre
metamorphite
pegmatite
quarz gravel
quarzite
slate
tuff

Appendix 6

Macrozoobenthos taxa on the stones in the investigated waters and seasons

Ind./m^2 stone area	Wied spr	Wied sum	Wied aut	Kyll spr	Kyll sum	Kyll aut	Nahe spr	Nahe sum	Nahe aut	Rhein spr	Elbe spr
Spongillidae											
non det.		1,6 ±5,0									
Bryozoa											
non det.											1,7 ±5,5
Nematoda											
non det.		1,4 ±4,4	16,1 ±22,9		1,1 ±3,6	3,2 ±10,2				12,5 ±39,5	
Turbellaria											
Dugesia lugubris/polychra				8,1 ±17,5				13,4 ±44,5	1,6 ±4,9		
Dugesia gonochephala			2,4 ±7,5			5,6 ±11,8	1,6 ±5,1				
Dugesia tigrina			5,7 ±12,2					36,4 ±120,9	26,3 ±48,5		
Polycelis nigra/tenuis								3,8 ±12,7			
non det.		1,6 ±5,0	2,0 ±6,3	2,8 ±8,8		2,7 ±8,5	5,2 ±16,4		1,6 ±4,9		
Mollusca											
Ancylus fluviatilis		16,9 ±32,9	171,7 ±116,2	5,2 ±8,9	4,4 ±13,0	99,2 ±121,0	13,8 ±30,4	13,5 ±31,9	9,4 ±21,1	2,6 ±8,3	323,6 ±533,0
Radix sp.											
Theodoxus fluviatilis			5,7 ±12,1	8,8 ±27,7	8,4 ±17,6	68,5 ±131,3	2,6 ±8,1				
Bithynia tentaculata											15,1 ±23,8
Gyraulus albus											
Curbicula fluminea										5,4 ±11,3	
Potamopyrgus jenkinsi			2,2 ±6,9		47,2 ±69,5	12,5 ±23,6					
Gastropoda non det.			2,0 ±6,2								
Pisidium sp.				1,3 ±4,0							
Sphaerium sp.							2,5 ±7,9	7,9 ±26,2			
Oligochaeta			25,2 ±45,0		10,3 ±16,8	5,1 ±12,3	22,4 ±49,3	16,5 ±39,2	1,7 ±5,5		
non det.											

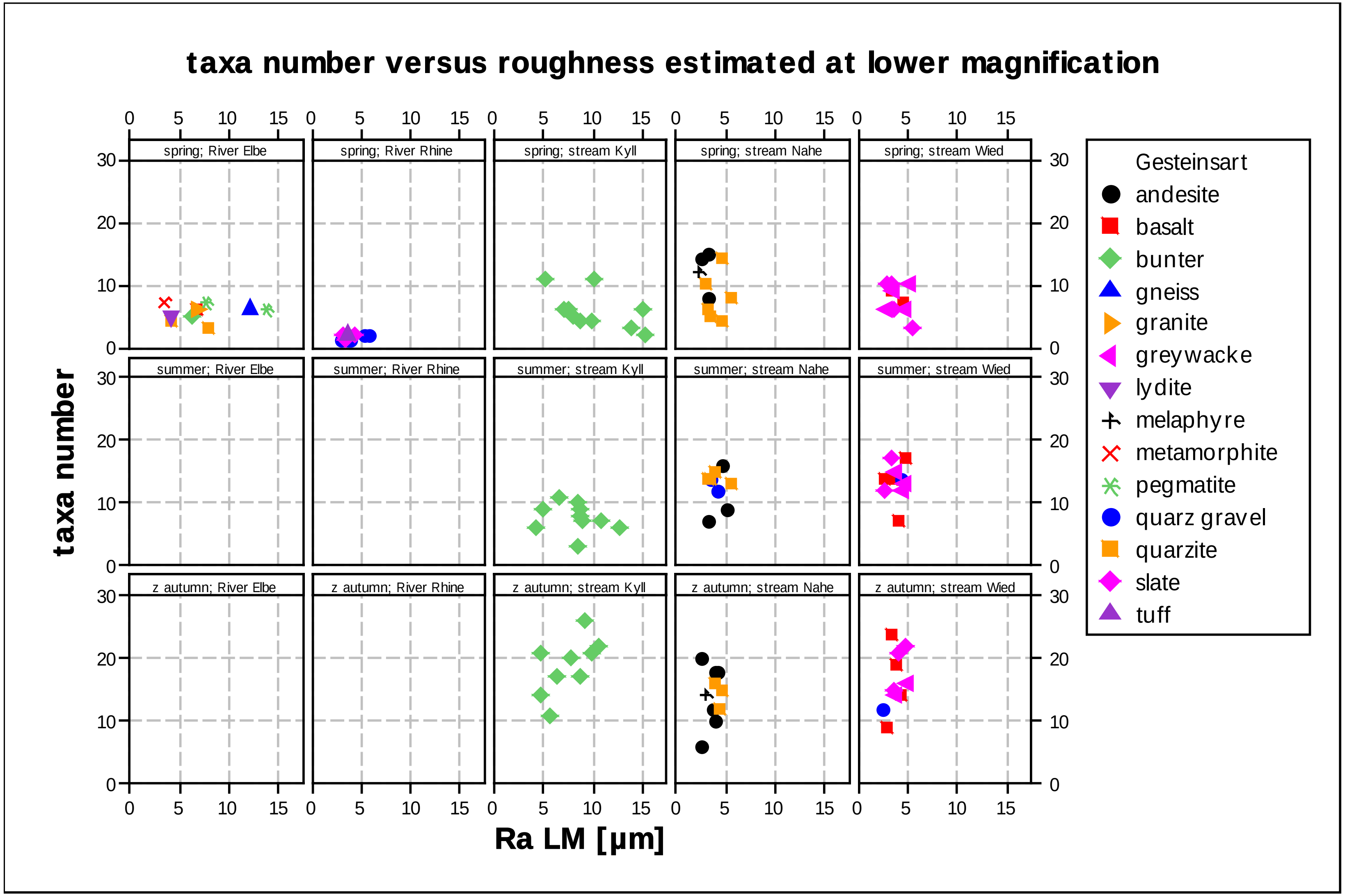
Ind./m² stone area	Wied spr	Wied sum	Wied aut	Kyll spr	Kyll sum	Kyll aut	Nahe spr	Nahe sum	Nahe aut	Rhein spr	Elbe spr
Hirudinea								1,9 ±6,4			
Glossiphonia complanata											
Erpobdella octoculata			1,6 ±5,1			6,6 ±17,1	13,8 ±20,1	1,8 ±6,0			
non det.		1,1 ±3,6									
Arachnida											
Aranaea				2,4 ±7,7							
Acari		9,6 ±13,4	46,8 ±48,9		27,1 ±31,8		5,0 ±15,8	7,9 ±11,7	9,6 ±25,2		
Crustacea											
Echinogammarus berilloni				187,3 ±396,9	463,7 ±430,5	456,3 ±341,9					
Gammarus pulex	31,4 ±50,6	13,9 ±28,5	20,0 ±44,2				43,6 ±107,3	52,9 ±51,4	64,0 ±139,3		
Dikerogammarus villosus										34,4 ±66,3	336,2 ±329,3
Corophium curvispinum										1,2 ±3,8	
Jaera sarsi										213,6 ±231,4	116,6 ±100,1
Heteroptera											
Aphelocheirus aestivalis		1,4 ±4,4	16,1 ±28,1								
non det.		3,5 ±7,3							1, 41 ±3,3		
Coleoptera											
Imagines											
Elmis sp.	10,7 ±31,5	1,6 ±5,0	9,7 ±17,8	7,2 ±15,1	6,3 ±19,8	32,8 ±39,1	20,8 ±40,2	18,3 ±25,9	13,0 ±14,9		
Elmis cf. aenea											
Stenelmis sp.	0,7 ±2,2	1,4 ±4,3					1,6 ±5,1	3,6 ±8,0			
Stenelmis cf. canaliculata											
Ouilimnius tuberculatus		1,5 ±4,9	2,5 ±7,9		29,8 ±78,2	25,1 ±27,3	1,7 ±5,5	6,1 ±10,9	9,2 ±12,3		
Limnius perrisi			4,6 ±7,8			3,8 ±8,4		1,8 ±6,0			
Esolus cf. angustatus				16,7 ±18,5	35,3 ±77,2	145,2 ±167,0					
Hydraena gracilis			2,0 ±6,3					1,9 ±6,3	1,6 ±4,9		
Larven											
Elmis sp.	10,7 ±31,5	36,6 ±58,0	61,7 ±72,6	6,5 ±14,0	30,9 ±49,2	147,8 ±99,1	7,7 ±17,2	14,8 ±22,1	49,7 ±39,5		
Ouilimnius tuberculatus					4,4 ±13,8	1,4 ±4,3			5,8 ±13,1		
Esolus sp.		1,6 ±5,0	1,6 ±5,2			27,4 ±36,2					
Limnius sp.											

Ind./m² stone area	Wied spr	Wied sum	Wied aut	Kyll spr	Kyll sum	Kyll aut	Nahe spr	Nahe sum	Nahe aut	Rhein spr	Elbe spr
Stenelmis sp.					27,4 ±38,5	30,6 ±44,6	6,5 ±20,4	32,9 ±37,8	3,9 ±8,3		
Orectochilus villosus		15,9 ±33,7	56,9 ±31,8		4,0 ±12,7	4,7 ±7,7	8,9 ±18,8	1,4 ±4,7	11,8 ±15,5		
sonstige											
<u>Larven</u>											
Elmis sp.	10,7 ±31,5	36,6 ±58,0	61,7 ±72,6	6,5 ±14,0	30,9 ±49,2	147,8 ±99,1	7,7 ±17,2	14,8 ±22,1	49,7 ±39,5		
Ouilimnius tuberculatus					4,4 ±13,8	1,4 ±4,3			5,8 ±13,1		
Esolus sp.		1,6 ±5,0	1,6 ±5,2			27,4 ±36,2					
Limnius sp.											
Stenelmis sp.					27,4 ±38,5	30,6 ±44,6	6,5 ±20,4	32,9 ±37,8	3,9 ±8,3		
Orectochilus villosus		15,9 ±33,7	56,9 ±31,8		4,0 ±12,7	4,7 ±7,7	8,9 ±18,8	1,4 ±4,7	11,8 ±15,5		
sonstige											
Ephemeroptera											
Caenis sp.	10,0 ±31,6						3,4 ±10,9				
Ecdyonurus sp.	42,8 ±59,9	12,1 ±11,7	8,7 ±18,4								
Heptagenia cf. sulphurea			6,9 ±18,5		1,1 ±3,5	1,5 ±4,8					
Heptagenia flava											9,9 ±12,2
Baetis rhodani						3,7 ±11,7	5,6 ±17,6				
Baetis fuscatus						1,3 ±4,0					
Baetis sp.	142,5 ±133,1	23,8 ±39,7	35,2 ±43,9	3,7 ±11,7	24,7 ±24,7	19,1 ±35,6	183,6 ±135,2	244,8 ±218,0	18,5 ±25,4		
Baetis sp. juv		1,7 ±5,5	16,7 ±52,7	3,0 ±9,4		31,3 ±48,0	5,1 ±16,2	11,7 ±38,9	195,9 ±208,1		
Seratella ignita	303,1 ±347,7	8,3 ±11,4	1,6 ±5,2								
Torleya mayor			2,0 ±6,2		15,4 ±23,4	92,9 ±92,5	1,3 ±4,0		5,9 ±13,2		
Potamantus luteus		1,4 ±4,3	5,9 ±18,6								
Habroleptoides confusca							1,7 ±5,5				
Ephemera danica					6,3 ±19,8	1,4 ±4,3					
non det.							4,8 ±15,1				
Plecoptera											
Leuctra geniculata		1,6 ±5,0									
Leuctra sp.		1,6 ±5,0									
Trichoptera											
Psychomyia pusilla	1,6 ±4,9	341,0 ±262,9	385,5 ±432,3	6,3 ±15,3	4,35 ± 13,7	98,5 ±83,9	2,7 ±8,7	9,2 ±19,3	110,0 ±147,1		69,8 ±88,8
Polycentropus flavomaculatus	7,6 ±16,9	30,3 ±29,3	51,0 ±51,3		2,27 ± 7,19		6,8 ±16,6	55,4 ±54,4	24,1 ±32,4		
Rhyacophila sp.			3,3 ±10,4			2,0 ±6,3	27,6 ±38,9	9,0 ±16,1	22,0 ±30,8		

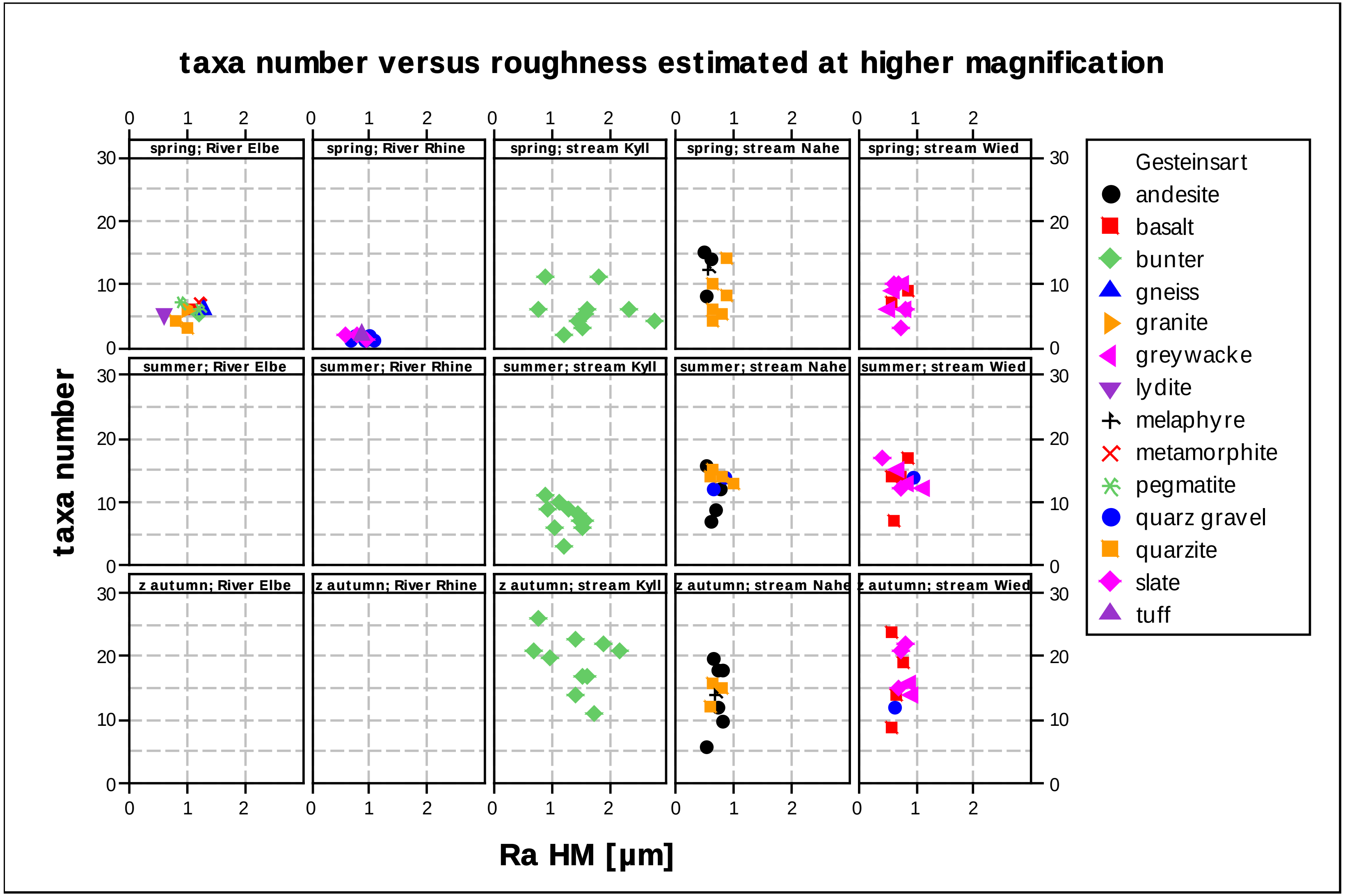
Ind./m² stone area	Wied spr	Wied sum	Wied aut	Kyll spr	Kyll sum	Kyll aut	Nahe spr	Nahe sum	Nahe aut	Rhein spr	Elbe spr
Rhyacophila sensu str.Gr.	2,4 ±5,2	18,3 ±24,2	13,5 ±23,2	1,3 ±4,0	3,7 ± 11,7	35,8 ±31,5	62,7 ±84,8	65,5 ±46,2	47,7 ±49,7		
Rhyacophila dorsalis Gr.		23,0 ±30,3						7,6 ±25,1			
Rhyacophila cf.fasciata				10,8 ±19,5							
Chneumatopsyche lepida	83,4 ±155,1	85,6 ±86,4	311,2 ±325,8	13,7 ±18,2		138,1 ±167,2	48,4 ±69,5	80,9 ±113,8	73,2 ±128,8		
Hydropsyche siltalai	12,5 ±39,5		36,7 ±50,2	17,3 ±20,6		20,8 ±29,9	37,9 ±33,1	13,9 ±41,2	71,4 ±110,7		
Hydropsyche guttata						2,0 ±6,3		10,0 ±18,6	33,2 ±29,7		
Hydropsyche pellucidula/ incognita	26,5 ±43,5	69,0 ±53,1	139,1 ±74,5	3,0 ±6,3		16,2 ±23,4	1,9 ±6,1	74,3 ±87,3	42,5 ±83,1		
Hydropsyche incognita	29,2 ±38,9	36,8 ±25,5	114,8 ±68,3			10,2 ±16,2	7,3 ±15,9	72,7 ±79,2	130,7 ±113,8		1,8 ±5,5
Hydropsyche contubernalis											12,3 ±27,5
Hydropsyche sp.	22,0 ±35,3	34,9 ±58,8	35,3 ±39,5	9,7 ±17,7		8,9 ±23,9	81,7 ±93,4	87,5 ±135,5	75,1 ±146,9		
Hydroptila sp.			1,1 ±3,4			36,6 ±36,8			7,9 ±18,7		
Sericostoma flavicorne/schneideri			2,4 ±7,5			1,6 ±5,1					
Limnephilidae					6,25 ± 19,8						
Limnephilus lunatus											
Brachycentrus subnubilus	52,0 ±138,9	40,8 ±51,8	21,5 ±35,2								
Brachycentrus maculatum				1004 ±1412	10,6 ± 22,8	3080 ±3470					
Oecetis notata			2,5 ±7,9								
Ceraclea annulicornis									6,3 ±19,8		
Lasiocephala basalis			2,4 ±7,5	1,3 ±4,0	17,2 ± 38,1	3,4 ±7,2					
sonstige		43,4 ±58,0	9,5 ±30,1		13,2 ± 26,2	9,3 ±15,3		2,2 ±7,2	4,1 ±8,6		
Diptera											
Nematocera											
Tipula sp.							4,8 ±15,1				
Limoniidae											
Antocha sp.					5,1 ±12,7	108,9 ±57,9	1,3 ±4,0	2,2 ±7,2	4,1 ±8,6		
Chiromidae	1,0 ±3,3										
Tanypodinae	63,6 ±112,8	173,4 ±182,0	135,4 ±136,0	13,8 ±30,1	0,9 ±2,9	81,7 ±83,0	426,2 ±347,6	42,9 ±52,0	83,5 ±61,8	3,6 ±11,3	133,6 ±110,3
Simuliidae	79,6 ±72,4		1,1 ±3,4					6,5 ±14,5	3,1 ±9,9		
Ceratopogonidae							9,1 ±28,7		2,0 ±6,3		
Brachycera											
Atherix ibis		2,9 ±6,2	3,1 ±9,9								
Hemerodrominae							1,4 ±4,3				
non det.			2,4 ±7,5			3,2 ±10,2	12,4 ±18,1				8,6 ±16,6

nd./m² stone area	Wied spr	Wied sum	Wied aut	Kyll spr	Kyll sum	Kyll aut	Nahe spr	Nahe sum	Nahe aut	Rhein spr	Elbe spr
Odonata											
Zygoptera											
Platycnemis pennipes							1,7 ±5,5				
Anisoptera							±0,0				
Onchogomphus forcipatus							1,4 ±4,3				
Sonstige	24,3 ±49,6	3,1 ±6,6		5,6 ±17,6		3,2 ±10,2					
taxa number	**20**	**32**	**44**	**21**	**25**	**38**	**36**	**32**	**32**	**7**	**16**
total individuals	**978** ±1.424	**1.095** ±1.205	**1.849** ±2.042	**1.360** ±2.126	836 ±1.124	**4.928** ±5.435	**1.134** ±1.402	**1.065** ±1.479	**1.203** ±1.647	**280** ±379	**1.045** ±1.269

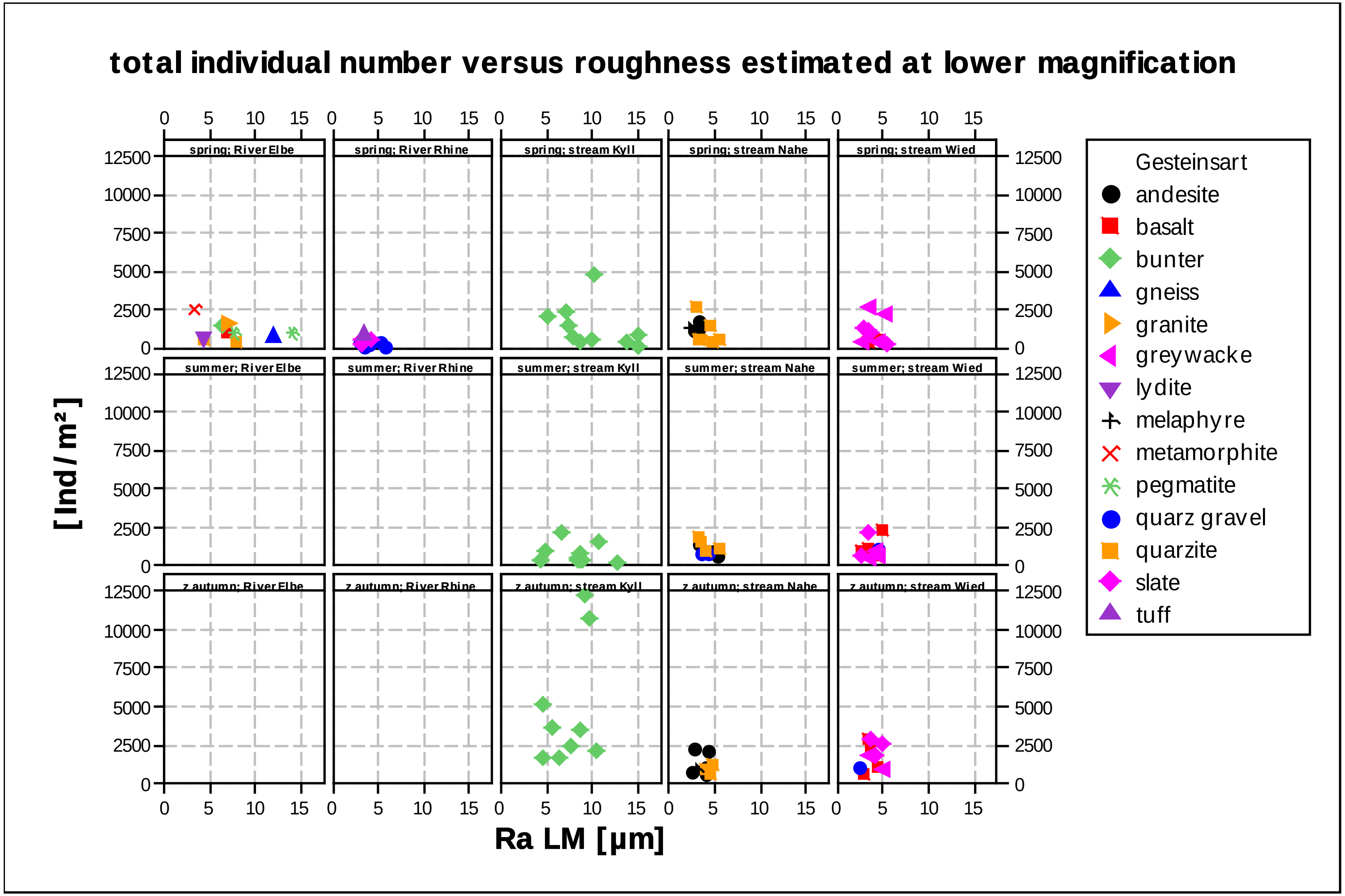
Appendix 7


taxa number versus roughness estimated at lower magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
taxa number
Ra LM [µm]
0
5
10
15
0
10
20
30
Gesteinsart
andesite
basalt
bunter
gneiss
granite
greywacke
lydite
melaphyre
metamorphite
pegmatite
quarz gravel
quarzite
slate
tuff

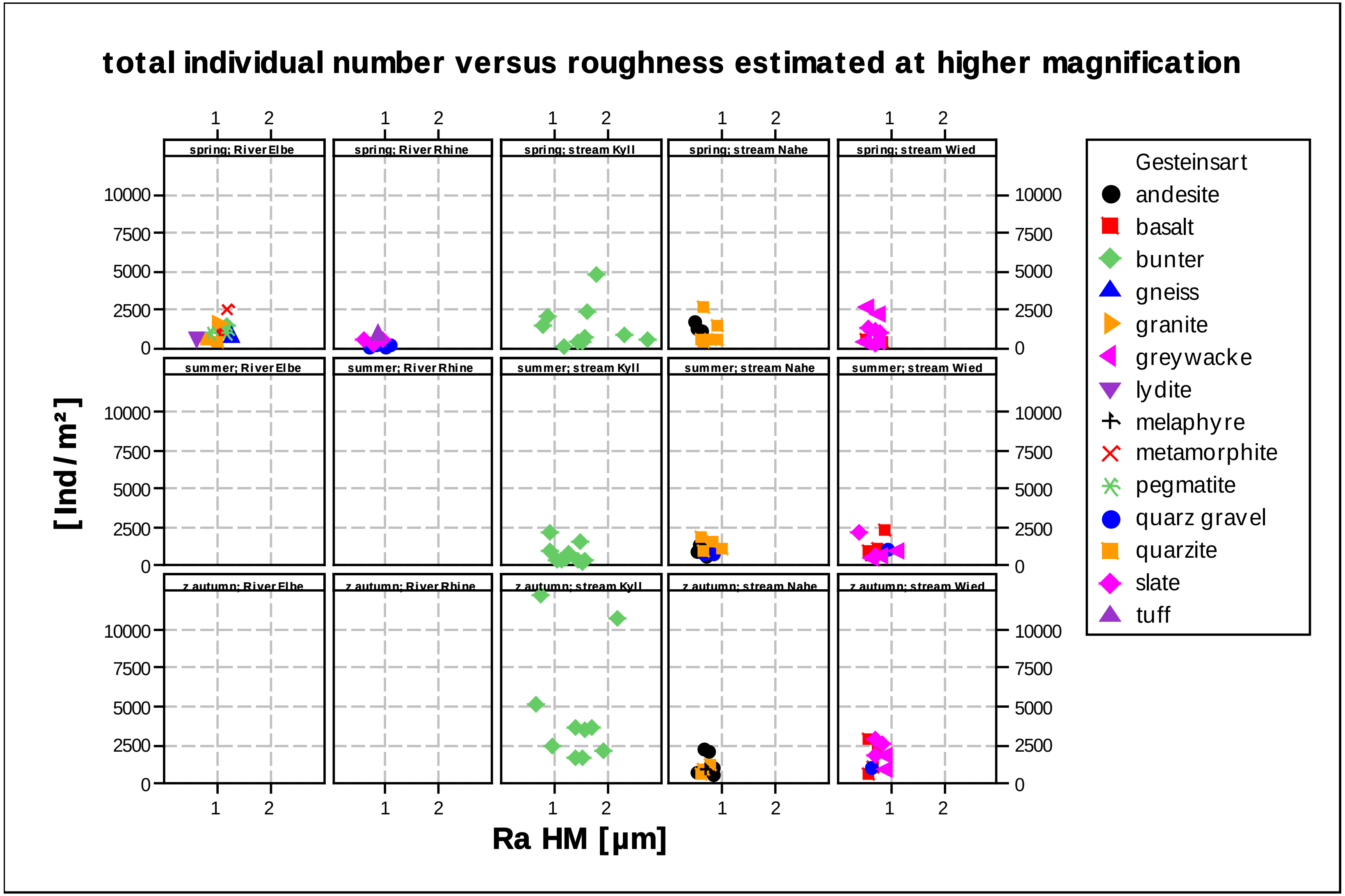
Appendix 8


taxa number versus roughness estimated at higher magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
taxa number
Ra HM [µm]
0
1
2
0
10
20
30
Gesteinsart
andesite
basalt
bunter
gneiss
granite
greywacke
lydite
melaphyre
metamorphite
pegmatite
quarz gravel
quarzite
slate
tuff

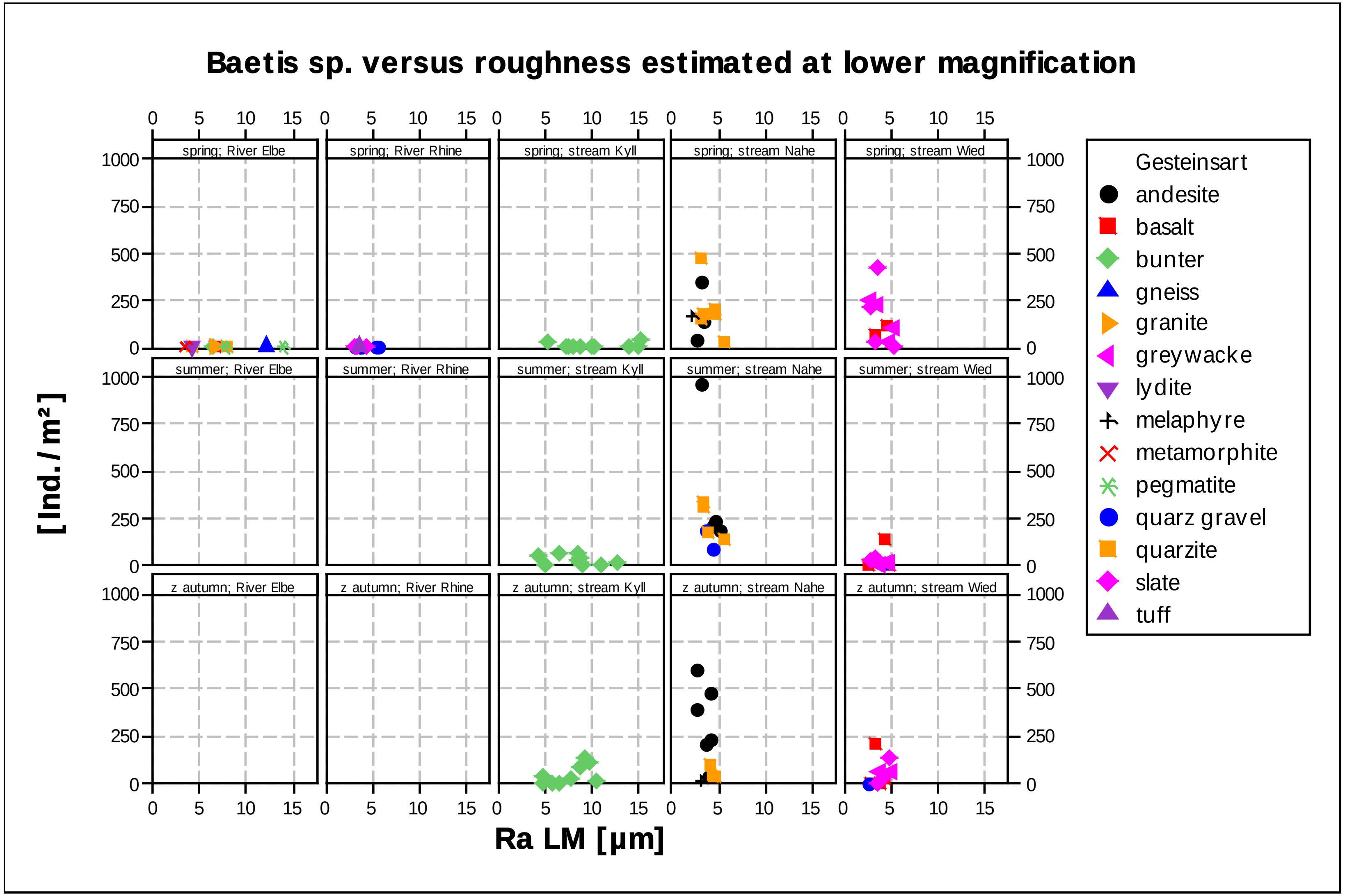
Appendix 9


total individual number versus roughness estimated at lower magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
[Ind / m²]
0
2500
5000
7500
10000
12500
Ra LM [µm]
0
5
10
15
Gesteinsart
● andesite
■ basalt
◆ bunter
▲ gneiss
▶ granite
◀ greywacke
▼ lydite
+ melaphyre
✕ metamorphite
✳ pegmatite
● quarz gravel
■ quarzite
◆ slate
▲ tuff

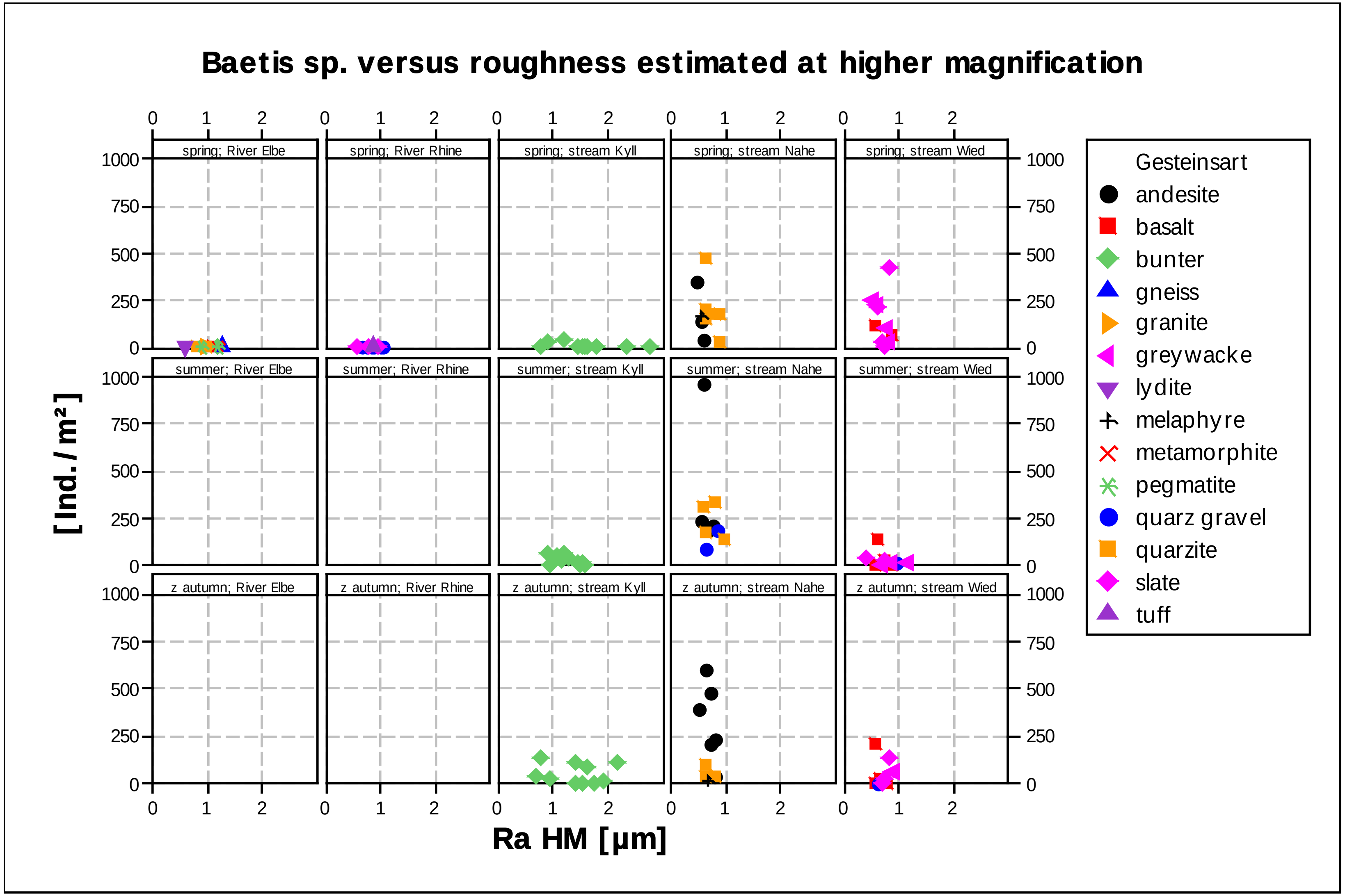
Appendix 10


total individual number versus roughness estimated at higher magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
0
2500
5000
7500
10000
1
2
[Ind / m²]
Ra HM [µm]
Gesteinsart
andesite
basalt
bunter
gneiss
granite
greywacke
lydite
melaphyre
metamorphite
pegmatite
quarz gravel
quarzite
slate
tuff

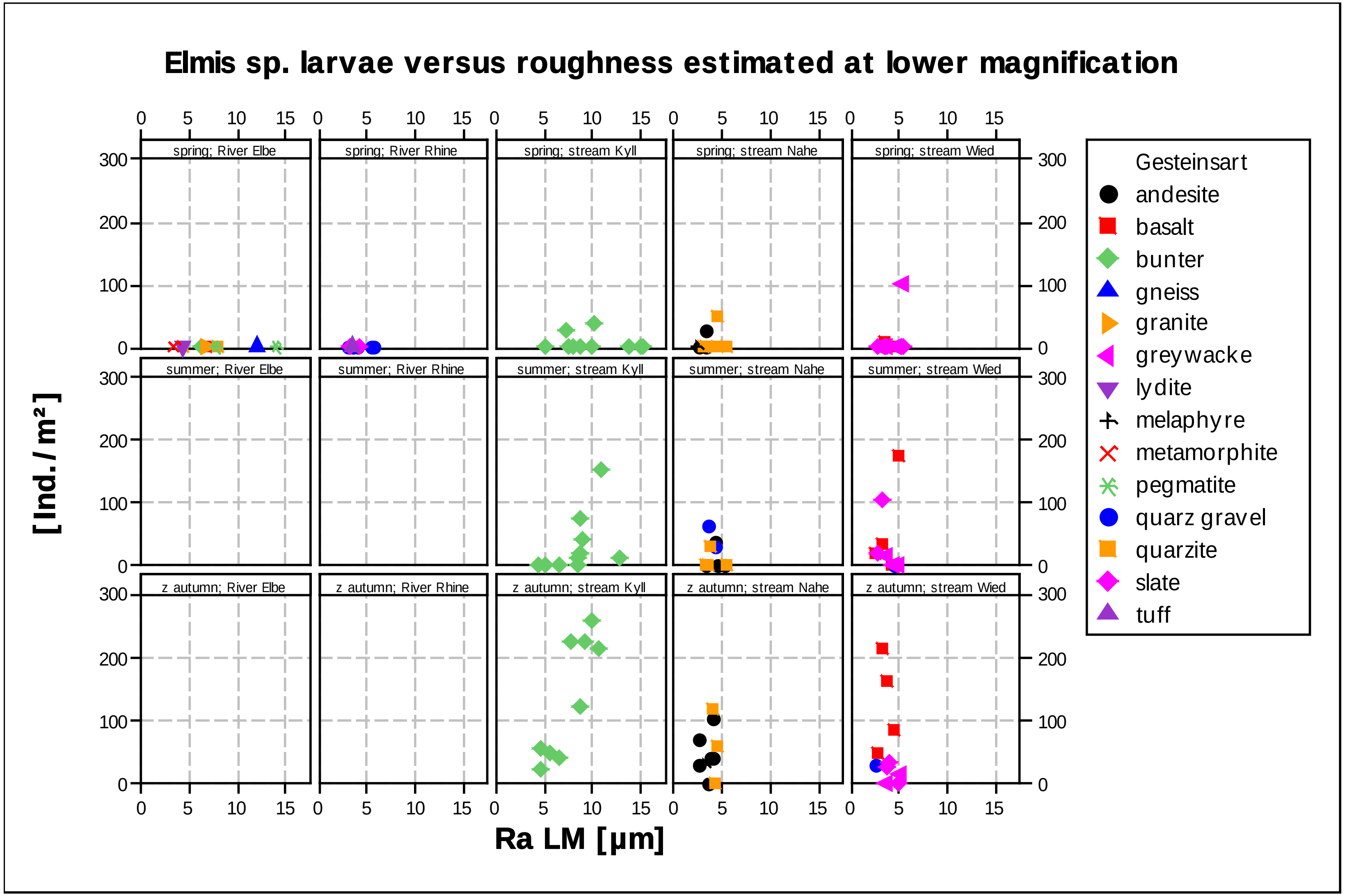
Appendix 11


Baetis sp. versus roughness estimated at lower magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
0
5
10
15
0
250
500
750
1000
[Ind./m²]
Ra LM [µm]
Gesteinsart
andesite
basalt
bunter
gneiss
granite
greywacke
lydite
melaphyre
metamorphite
pegmatite
quarz gravel
quarzite
slate
tuff

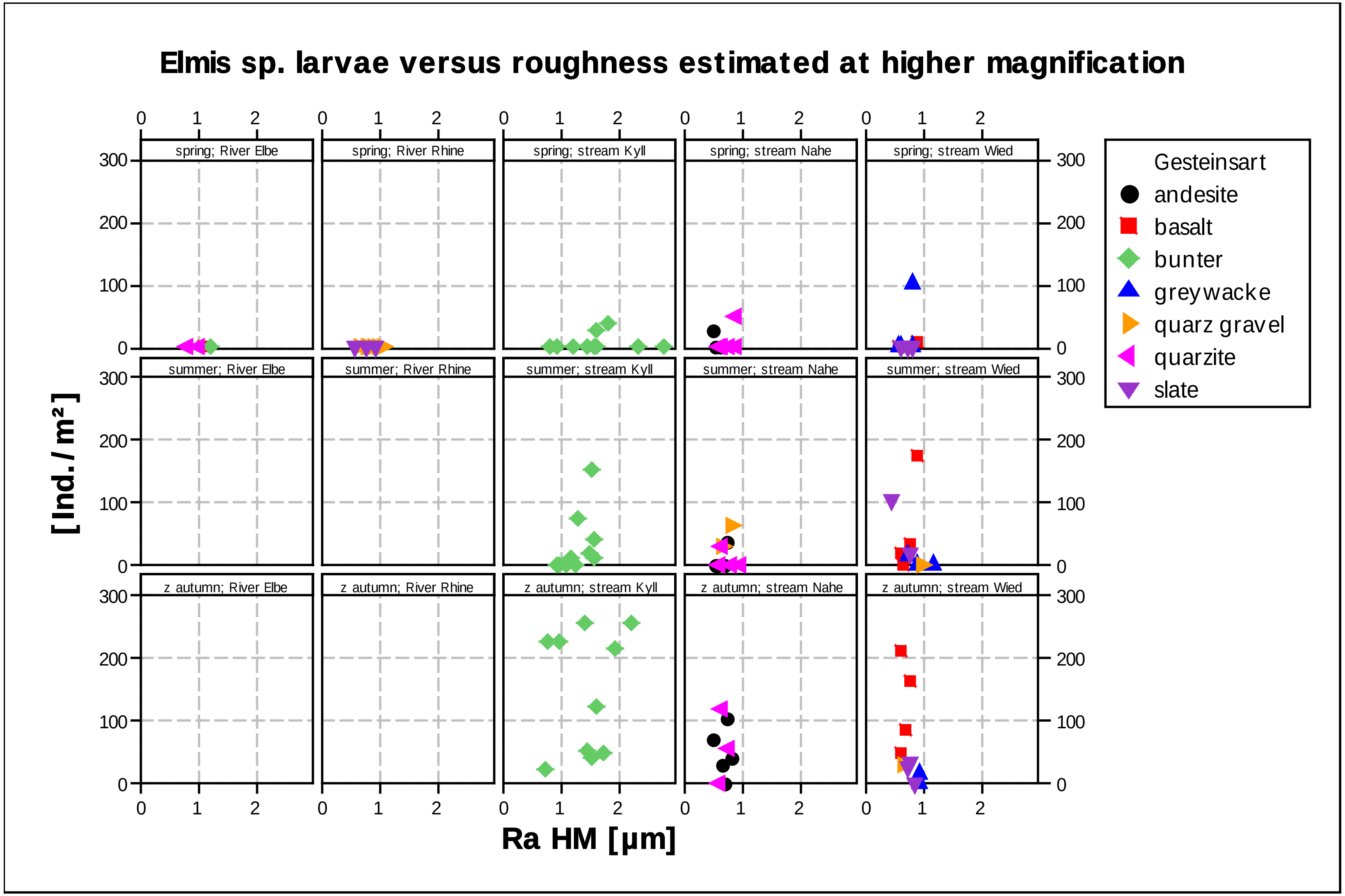
Appendix 12


Baetis sp. versus roughness estimated at higher magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
[Ind./m²]
Ra HM [µm]
0
1
2
0
250
500
750
1000
Gesteinsart
andesite
basalt
bunter
gneiss
granite
greywacke
lydite
melaphyre
metamorphite
pegmatite
quarz gravel
quarzite
slate
tuff

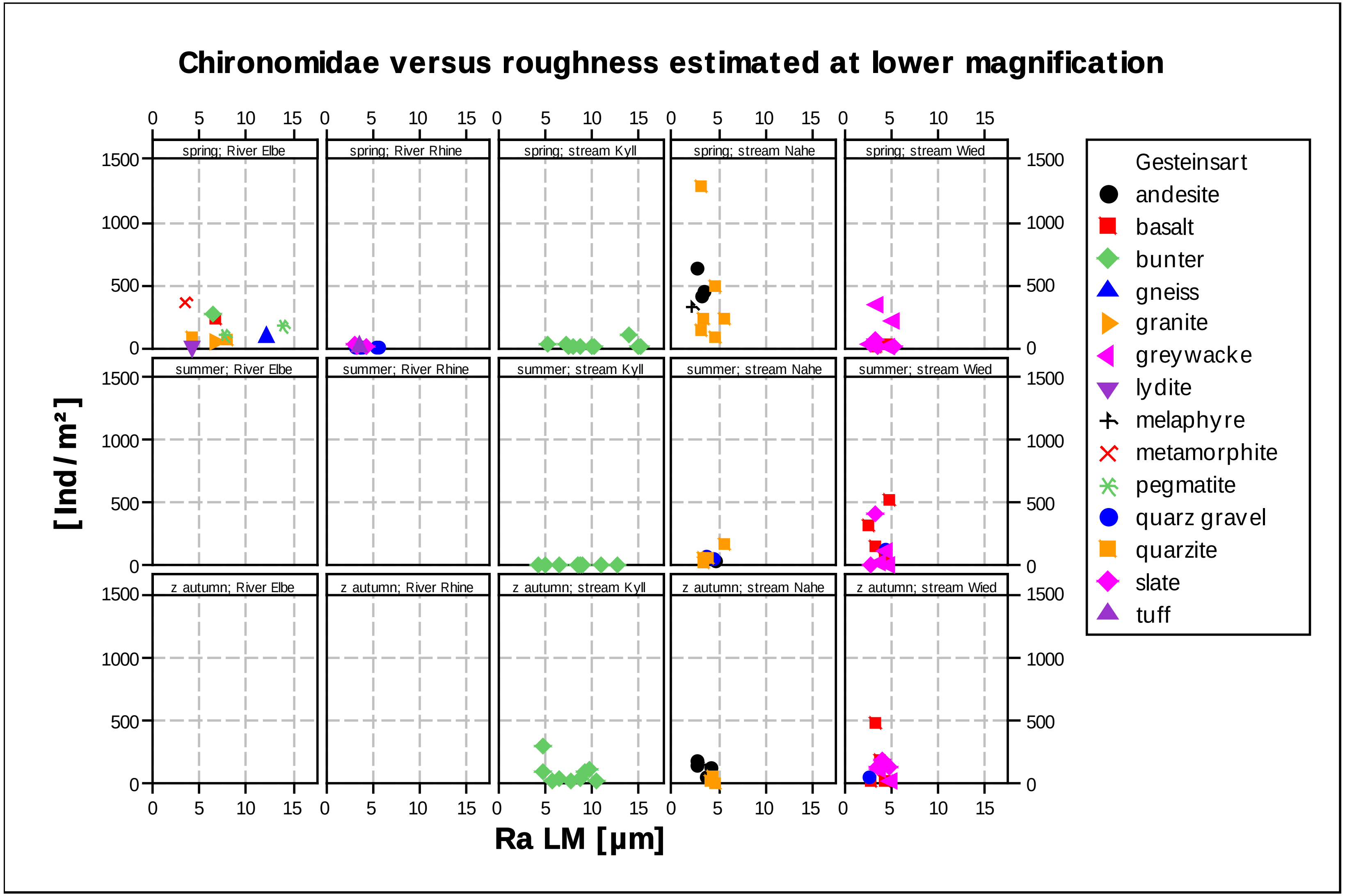
Appendix 13


Elmis sp. larvae versus roughness estimated at lower magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
[Ind. / m²]
Ra LM [µm]
0
5
10
15
0
100
200
300
Gesteinsart
andesite
basalt
bunter
gneiss
granite
greywacke
lydite
melaphyre
metamorphite
pegmatite
quarz gravel
quarzite
slate
tuff

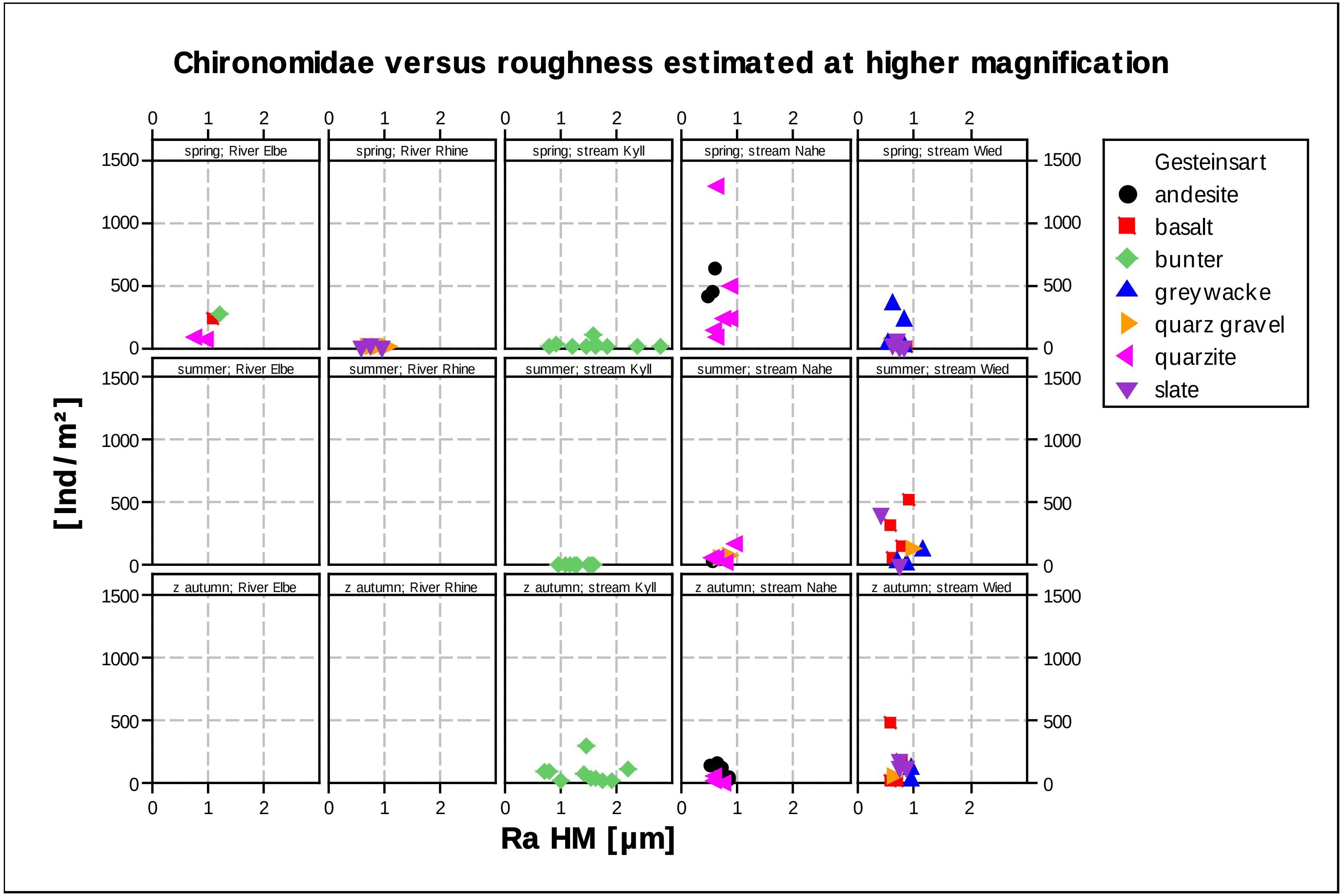
Appendix 14


Elmis sp. larvae versus roughness estimated at higher magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
0
1
2
0
100
200
300
[Ind./m²]
Ra HM [µm]
Gesteinsart
andesite
basalt
bunter
greywacke
quarz gravel
quarzite
slate

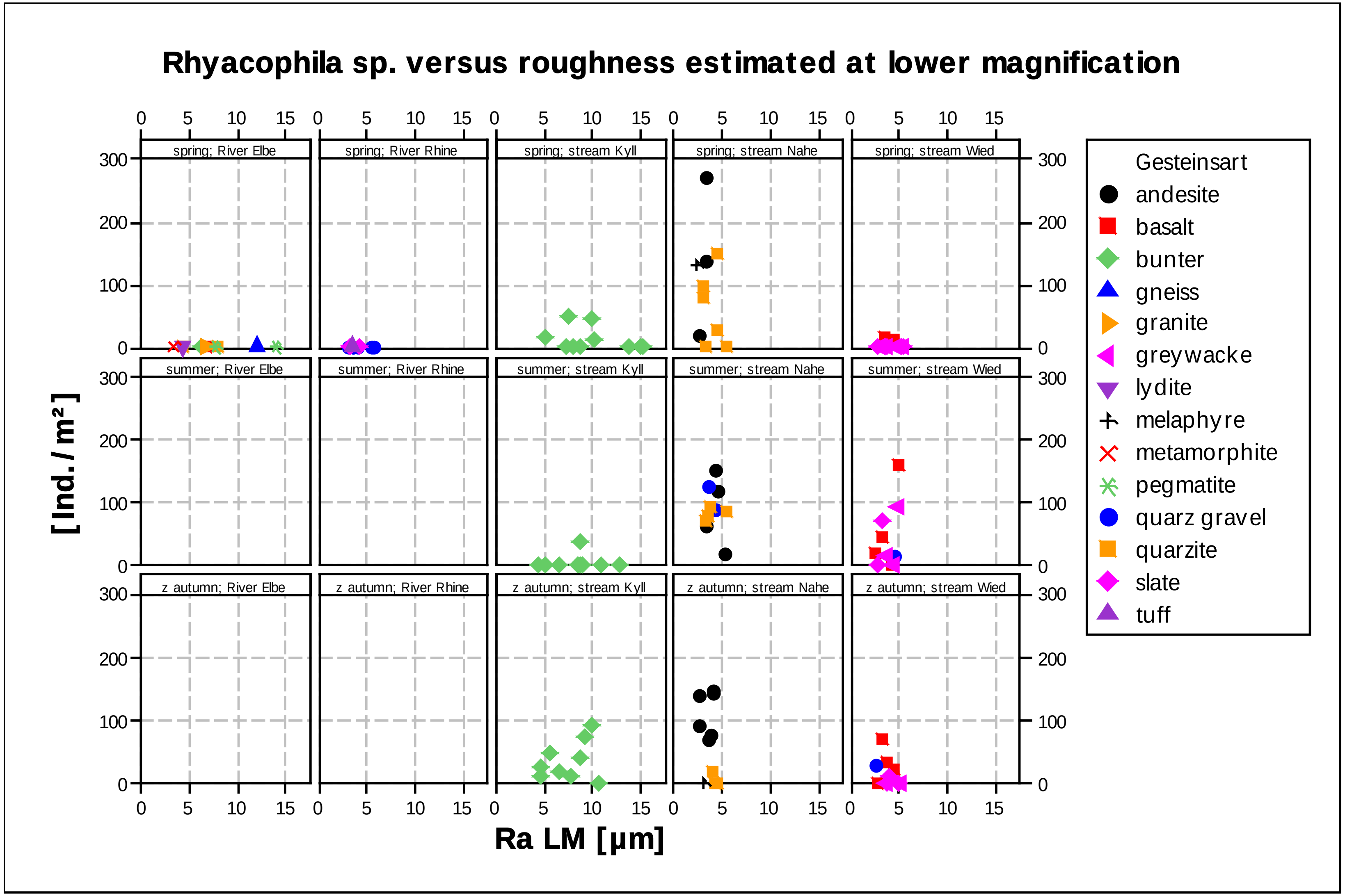
Appendix 15


Chironomidae versus roughness estimated at lower magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
0
5
10
15
0
500
1000
1500
[Ind / m²]
Ra LM [µm]
Gesteinsart
andesite
basalt
bunter
gneiss
granite
greywacke
lydite
melaphyre
metamorphite
pegmatite
quarz gravel
quarzite
slate
tuff

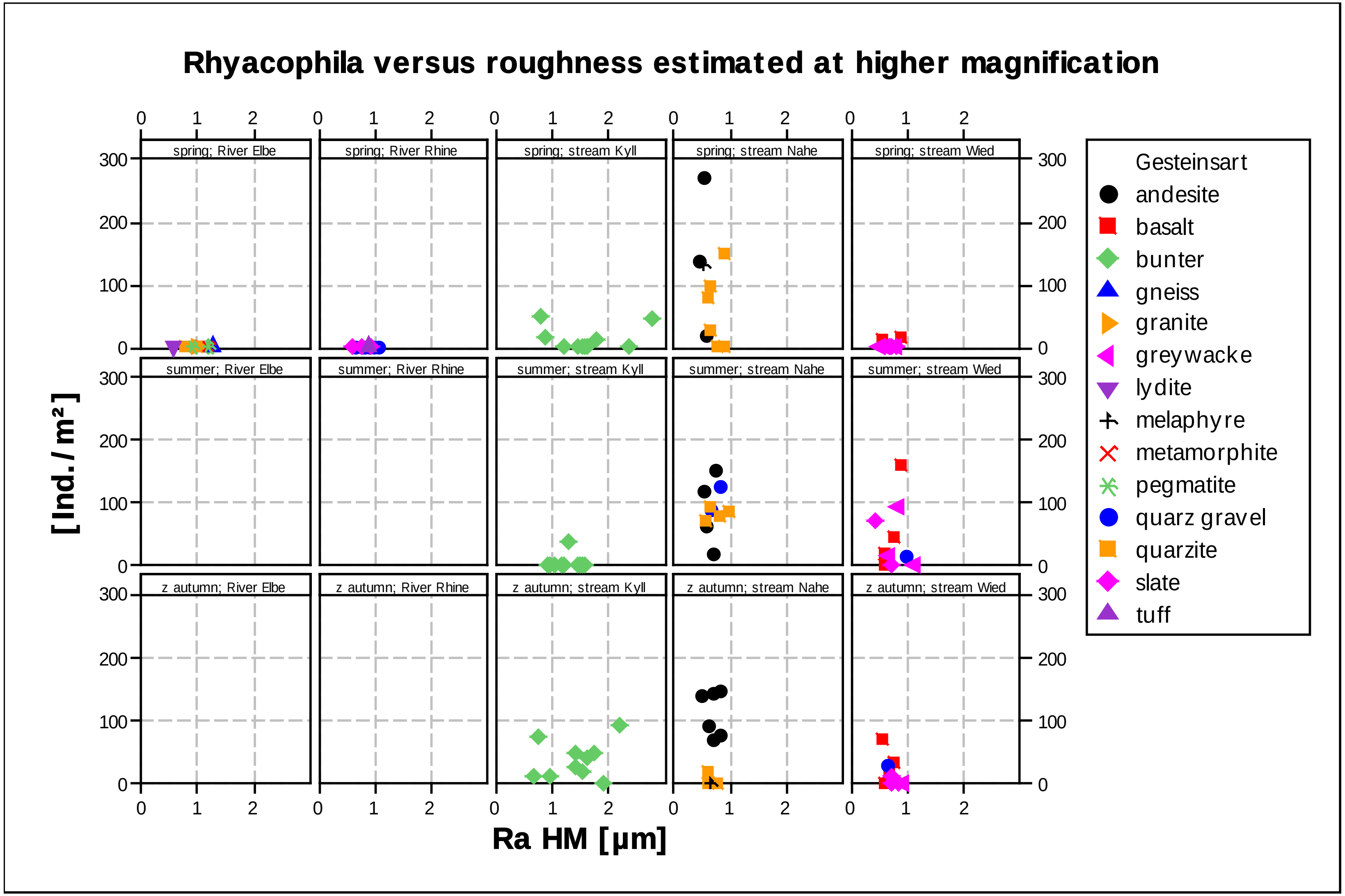
Appendix 16


Chironomidae versus roughness estimated at higher magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
0
1
2
0
500
1000
1500
[Ind / m²]
Ra HM [µm]
Gesteinsart
andesite
basalt
bunter
greywacke
quarz gravel
quarzite
slate

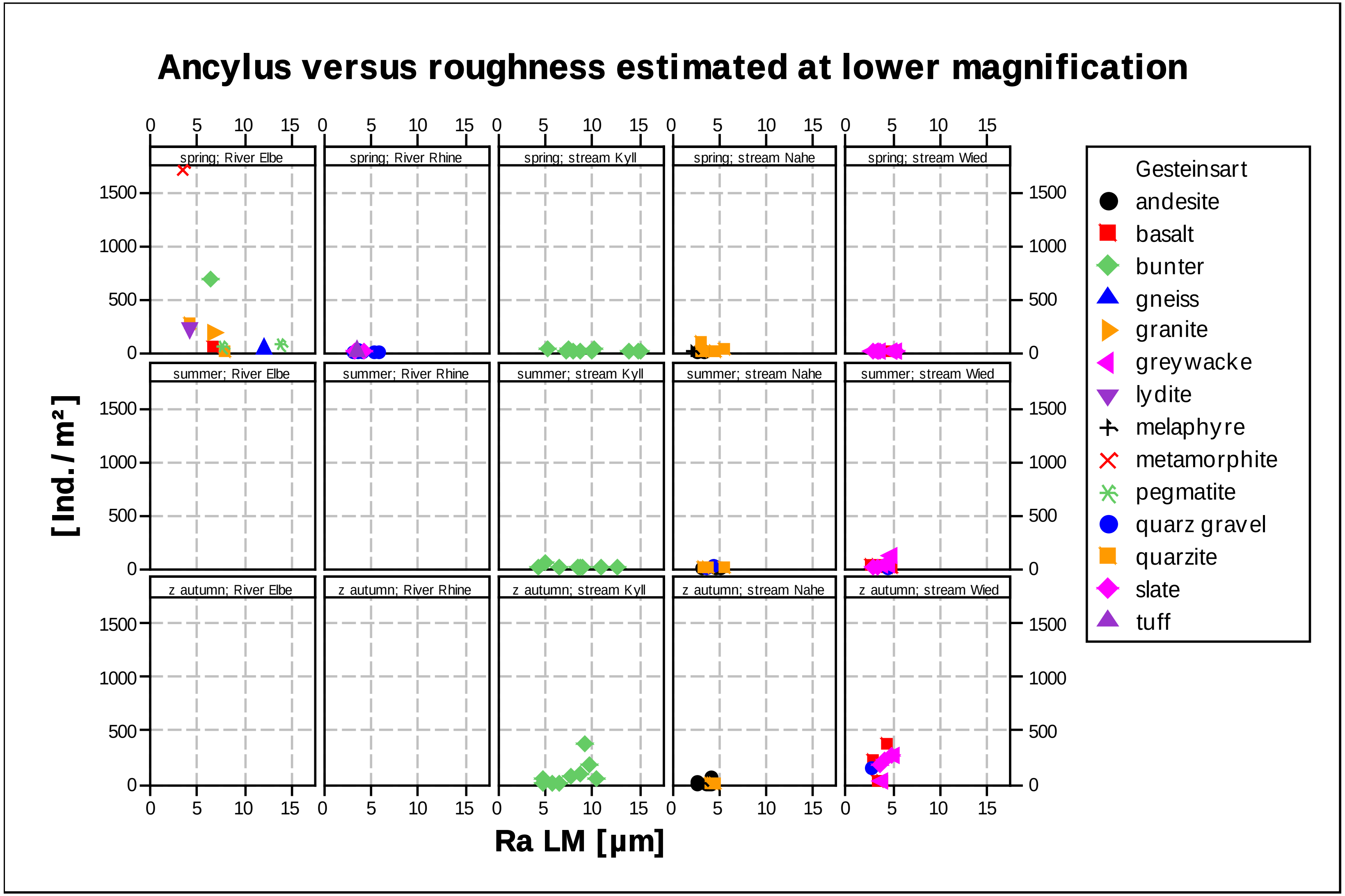
Appendix 17


Rhyacophila sp. versus roughness estimated at lower magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
0
5
10
15
0
100
200
300
[Ind. / m²]
Ra LM [µm]
Gesteinsart
andesite
basalt
bunter
gneiss
granite
greywacke
lydite
melaphyre
metamorphite
pegmatite
quarz gravel
quarzite
slate
tuff

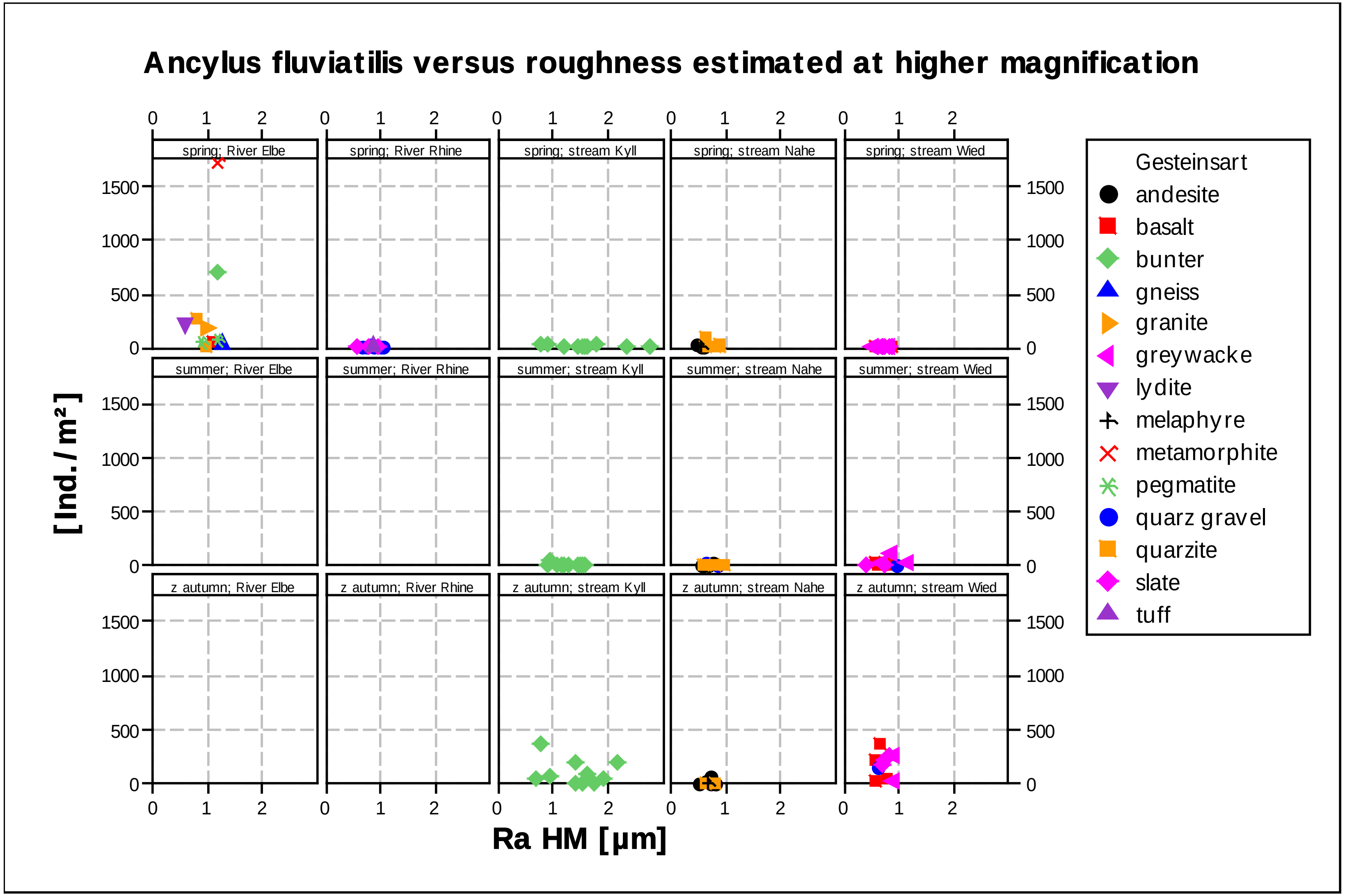
Appendix 18


Rhyacophila versus roughness estimated at higher magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
[Ind. / m²]
Ra HM [µm]
0
1
2
0
100
200
300
Gesteinsart
andesite
basalt
bunter
gneiss
granite
greywacke
lydite
melaphyre
metamorphite
pegmatite
quarz gravel
quarzite
slate
tuff

Appendix 19


Ancylus versus roughness estimated at lower magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
0
5
10
15
0
500
1000
1500
[Ind./m²]
Ra LM [µm]
Gesteinsart
andesite
basalt
bunter
gneiss
granite
greywacke
lydite
melaphyre
metamorphite
pegmatite
quarz gravel
quarzite
slate
tuff

Appendix 20


Ancylus fluviatilis versus roughness estimated at higher magnification
spring; River Elbe
spring; River Rhine
spring; stream Kyll
spring; stream Nahe
spring; stream Wied
summer; River Elbe
summer; River Rhine
summer; stream Kyll
summer; stream Nahe
summer; stream Wied
z autumn; River Elbe
z autumn; River Rhine
z autumn; stream Kyll
z autumn; stream Nahe
z autumn; stream Wied
0
1
2
0
500
1000
1500
[Ind. / m²]
Ra HM [µm]
Gesteinsart
andesite
basalt
bunter
gneiss
granite
greywacke
lydite
melaphyre
metamorphite
pegmatite
quarz gravel
quarzite
slate
tuff

Appendix 21

Parameter of surface roughness (mean ± S.D.. N=10) of the tested replica – roughness estimated at lower magnification (measured by the white light interferrometer FRT-MircoProf.; H2 sensor; Area: 100 x 100 µm, Pixel: 10 µm²)

Parameter		**S1**	**S2**	**S3**	**S4**
Ra	µm	--	0.77 ± 0.09	4.44 ± 0.09	6.83 ± 0.85
$Rz_{(DIN)}$	µm	--	7.30 ± 0.87	51.91 ± 3.40	103.82 ± 25.79
Rz25	µm		6.04 ± 0.70	40.03 ± 1.56	60.47 ± 11.28
Rmax	µm		8.10 ± 1.07	61.53 ± 5.68	146.45 ± 46.00
Rmax25	µm		9.49 ± 4.29	55.59 ± 5.45	143.09 ± 47.48
Rq (=Rms)	µm		0.98 ± 0.12	5.83 ± 0.13	10.16 ± 1.98
Rt	µm		8.62 ± 0.99	64.70 ± 7.01	150.75 ± 43.37
Rp	µm		3.94 ± 0.62	37.91 ± 5.02	83.76 ± 24.19
Rv	µm		4.67 ± 0.69	26.78 ± 4.70	66.99 ± 27.02
Rk	µm		2.48 ± 0.27	13.35 ± 0.30	19.17 ± 1.51
Rpk	µm		1.03 ± 0.11	8.53 ± 0.54	17.78 ± 7.66
Rvk	µm		1.22 ± 0.18	5.03 ± 0.23	11.86 ± 2.79
Rp/(Rp+Rv)			0.46 ± 0.05	0.59 ± 0.05	0.56 ± 0.09
Rk/Rz			0.34 ± 0.01	0.26 ± 0.02	0.19 ± 0.04
Rk/(Rk+Rpk+Rvk)			0.52 ± 0.01	0.50 ± 0.01	0.41 ± 0.07
Rpk/Rvk			0.86 ± 0.12	1.70 ± 0.15	1.48 ± 0.54

Appendix 22

Parameter of surface roughness (mean ± S.D.. N=10) of the tested replica – roughness estimated at higher magnification (measured by the white light interferrometer FRT-MircoProf.; H0 sensor; Area: 100 µm x 100 µm, Pixel: 1 µm^2)

Parameter		S1	S2	S3	S4
Ra	µm	0.06 ± 0.01	0.35 ± 0.01	1.74 ± 0.20	1.77 ± 0.31
Rz$_{(DIN)}$	µm	0.53 ± 0.17	2.99 ± 0.20	29.75 ± 3.70	25.62 ± 5.30
Rz25	µm	0.40 ± 0.05	2.42 ± 0.10	17.88 ± 3.07	15.01 ± 2.73
Rmax	µm	0.83 ± 0.64	3.47 ± 0.51	41.96 ± 9.54	35.91 ± 7.27
Rmax25	µm	0.81± 0.61	3.34 ± 0.52	40.05 ± 9.36	35.47 ± 7.32
Rq (=Rms)	µm	0.07± 0.01	0.44 ± 0.01	2.73 ± 0.85	2.69 ± 0.53
Rt	µm	0.85± 0.63	3.52 ± 0.50	43.16 ± 9.18	37.24 ± 7.65
Rp	µm	0.38 ± 0.27	1.79 ± 0.24	20.56 ± 8.99	17.34 ± 3.69
Rv	µm	0.47± 0.41	1.73 ± 0.33	22.60 ± 7.45	19.90 ± 5.14
Rk	µm	0.18 ± 0.01	1.14 ± 0.02	3.70 ± 0.33	4.29 ± 0.78
Rpk	µm	0.10± 0.02	0.44 ± 0.04	4.42 ± 0.81	3.99 ± 0.90
Rvk	µm	0.09 ± 0.02	0.43 ± 0.02	5.38 ± 0.97	4.68 ± 1.36
Rp/(Rp+Rv)		0.48 ± 0.09	0.51 ± 0.04	0.47 ± 0.12	0.47 ± 0.07
Rk/Rz		0.32 ± 0.07	0.38 ± 0.02	0.13 ± 0.01	0.17 ± 0.03
Rk/(Rk+Rpk+Rvk)		0.50 ± 0.04	0.57 ± 0.01	0.28 ± 0.02	0.34 ± 0.05
Rpk/Rvk		1.14 ± 0.13	1.02 ± 0.11	0.83 ± 0.12	0.88 ± 0.17

Appendix 23

Parameter of surface roughness (mean ± S.D.. N=10) of the tested substrates – roughness estimated at lower magnification (measured by the white light interferrometer FRT-MircoProf.; Area: 1000 x 1000 µm, Pixel: 10 µm^2)

Parameter		**SI**	**SII**	**SIII**	**SIV**	**SV**	**SVI**	**SVII**
		Pp 1 µm. original of S2	Pp 12 µm. original of S3	Pp 1000P	Pp 400P. original of S4	Pp 280	Pp 100P	Pp 40P
Ra	µm	0.56 ± 0.03	3.33 ± 0.09	6.08 ± 0.12	6.25 ± 0.23	15.16 ± 5.60	15.87 ± 1.25	13.00 ± 2.53
Rz$_{(DIN)}$	µm	5.65 ± 0.72	36.03 ± 1.30	58.99 ± 1.93	59.50 ± 1.93	179.51 ± 70.69	215.88 ± 12.00	300.8 ± 94.8
Rz25	µm	4.38 ± 0.28	28.41 ± 0.67	47.61 ± 0.87	46.35 ± 1.33	124.34 ± 44.40	138.92 ± 12.00	143.2 ± 35.7
Rmax	µm	7.59 ± 2.48	40.34 ± 1.90	64.44 ± 3.77	68.29 ± 6.06	252.29 ± 130.44	269.41 ± 51.94	444.7 ± 155.5
Rmax25	µm	7.35 ± 2..25	38.72 ± 2.36	61.71 ± 4.35	64.34 ± 5.17	240.87 ± 122.07	246.17 ± 42.81	418.3 ± 157.7
Rq (=Rms)	µm	0.71 ± 0.03	4.34 ± 0.12	7.78 ± 0.13	8.02 ± 0.28	20.32 ± 7.15	22.32 ± 1.88	23.3 ± 5.7
Rt	µm	7.91± 2.36	43.68 ± 3.17	67.88 ± 4.69	71.37 ± 7.47	264.74 ± 134.78	297.13 ± 53.93	512.1 ± 172.5
Rp	µm	3.43 ± 1.91	19.29 ± 1.46	33.94 ± 3.40	29.51 ± 4.15	161.31 ± 108.10	148.00 ± 21.08	310.6 ± 87.32
Rv	µm	4.49 ± 1.69	24.39 ± 2.93	33.94 ± 3.82	41.86 ± 4.87	103.34 ± 61.11	149.13 ± 49.53	201.60 ± 120.5
Rk	µm	1.82 ± 0.10	10.29 ± 0.34	19.24 ± 0.52	19.61 ± 1.05	45.75 ± 19.32	37.61 ± 3.73	21.8± 3.33
Rpk	µm	0.71 ± 0.08	4.67 ± 0.19	8.03 ± 0.17	6.31 ± 0.42	26.75 ± 12.10	39.75 ± 4.41	51.2 ± 11.7
Rvk	µm	0.92 ± 0.10	5.48 ± 0.39	8.68 ± 0.26	10.83 ± 0.94	20.82 ± 7.58	22.29 ± 3.66	23.4 ± 9.4
Rp/(Rp-Rv)		0.43 ± 0.13	0.44 ± 0.03	0.50 ± 0.04	0.41 ± 0.03	0.34 ± 0.23	0.51 ± 0.08	0.63 ± 0.13
Rk/Rz		0.33 ± 0.04	0.29 ± 0.01	0.33 ± 0.02	0.33 ± 0.03	0.27 ± 0.07	0.18 ± 0.02	0.08 ± 0.01
Rk/(Rk+Rpk +Rvk)		0.53 ± 0.02	0.50 ± 0.01	0.54 ± 0.01	0.53 ± 0.02	0.49 ± 0.09	0.38 ± 0.03	0.23 ± 0.02
Rpk/Rvk		0.79 ± 0.16	0.86 ± 0.08	0.93 ± 0.04	0.59 ± 0.05	1.32 ± 0.48	1.82 ± 0.29	2.32 ± 0.69