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IMPACTS OF HYDROPEAKING ON BETHIC MACROINVERTEBRATE DRIFT – COMPARING DIFFERENT SUBSTRATES

Graduation thesis

Zagreb, 2017

This thesis had been conducted at the University of Natural Resources and Life Sciences (BOKU), in Vienna, Austria, in collaboration with the Faculty of Science, University of Zagreb, Croatia, under the guidance of Ass. prof. dr. Wolfram Graf (BOKU) and Prof. dr. sc. Zlatko Mihaljević (PMF). The thesis was submitted for evaluation to Division of Biology, Faculty of Science, University of Zagreb in order to acquire the academic title Master of Ecology and Environmental Protection.

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Graduation Thesis

Impacts of hydropeaking on benthic macroinvertebrate drift – comparing different substrates

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Human activity constantly influences the living conditions of benthic communities, where hydropower plants act as big stressors. Sudden discharge of water into a stream can change many flow characteristics like water velocity and depth, which changes shear stress. Mutual influence of those characteristics on macroinvertebrates during hydropeaking is still not fully understood. However, the link between hydropeaking and drift of macroinvertebrates is undisputable, although the final effect depends on taxa involved. Sediment roughness is also one of the characteristics that influence shear stress, and consequently affect benthic invertebrates. Therefore, this thesis deals with the effect of substrate roughness on drift of different macroinvertebrate taxa in hydropeaking conditions. Based on given results, substrate roughness has not proved to be a major influence source of increased drift in hydropeaking events. Significant influence of sediment roughness on increased drift has only been observed in some taxa like Chironomidae, Elmidae, Ephemerellidae and Nemouridae in hydropeaking events, whereas in Chironomidae and Ephemerellidae in base flow. However, our results confirmed the connection between hydropeaking and increased drift with significant differences in drift rates in hydropeaking and base flow conditions.

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Utjecaj naglog porasta vodostaja uslijed rada hidroelektrana na drift makrozoobentosa

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Ljudi svojim aktivnostima neprestano utječu na životne uvjete zajednica bentičkih beskralježnjaka, pogotovo hidroelektranama koje u pravilu izazivaju veliki stres. Nagle promjene protoka vode mogu utjecati na brzinu strujanja i dubinu vode, što mijenja pritisak protoka vode na korito tekućice (shear stres). Uzajamni utjecaj karakteristika protoka vode na beskralježnjake pri pojavi bujičnih valova još uvijek nije u potpunosti shvaćen. Unatoč tome, postoji neosporna veza između pojava bujičnih valova i drifta beskralježnjaka. Hrapavost podloge je jedan od parametara koji utječu na „shear stres“, te time i na bentičke beskralježnjake. Predmet i glavni interes ovog rada bio je istražiti utjecaj hrapavosti površine na drift makrozoobentosa pri pojavi bujičnih valova. Dobiveni rezultati nisu pokazali značajan utjecaj hrapavost podloge na pojačani drift pri pojavi bujičnih valova. Statistički značajan utjecaj hrapavosti podloge na pojačan drift makrozoobentosa primijećen je samo kod nekih svojti kao što su Chironomidae, Elmidae, Ephemerellidae i Nemouridae pri pojavi bujičnih valova, dok kod Chironomidae i Ephemerellidae statistički značajan utjecaj hrapavosti podloge na pojačan drift zabilježen je pri bazičnom protoku vode. Naši rezultati su potvrdili vezu između pojave bujičnih valova i pojačanog drifta beskralježnjaka sa statističkim značajnom razlikom u stopi drifta pri pojačanom i bazičnom protoku vode.

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Index

CONTENTS

1 Introduction 1
1.1 Hydropeaking 1
1.1.1 What is hydropeaking 1
1.1.2 Changes in flow regimes 2
1.1.3 Abiotic and biotic effects of hydropeaking 2
1.1.4 General effects on stream biota 3
1.2 Drift 3
1.3 Hydropeaking and drift 5
1.3.1 Adaptations and species-specific traits 5
1.4 Substrate 6
1.4.1 Substrate preference of macroinvertebrates 6
1.4.2 Sediment roughness 7
1.4.3 Shear stress and the channel bed 8
1.4.4 Hydropeaking induced drift depending on sediment roughness 9
1.5 Knowledge deficiencies as the rationale for this thesis 10
1.6 Study aims 11
2 Materials and Methods 13
2.1 Study area 13
2.2 Sampling of donor populations 14
2.3 Experimental setup 14
2.4 Level of identification of macroinvertebrates 17
2.5 Statistical analyses 17
3 Results 18
3.1 Initial results 18
3.2 Differences between control and treatment 19
3.2.1 General level 19
3.2.2 Effect on selected taxa 21
3.2.2.1 On Order level 21
3.2.2.2 On Family level 27
3.3 Differences between sediments 29
3.3.1 General level 29
3.3.2 Effect on selected taxa 30

3.3.2.1 Order level 30
3.3.2.2 Family level 32
3.4 Effect of hydropeaking on size classes in drift 39
3.4.1 Differences between control and treatment 39
3.4.2 Differences between sediments 43
4 Discussion 46
4.1 Initial results 46
4.2 Differences between control and treatment both together and in different sediment types 46
4.2.1 General level 46
4.2.2 Effects on selected taxa 47
4.3 Effect of hydropeaking on size classes in drift 50
4.4 Experiment imperfections 50
5 Conclusion 52
6 References 53
7 Curriculum vitae 69

1 INTRODUCTION

Throughout history, big cities were built in close proximity to rivers and lakes since ancient as well as modern communities have always depended on fresh water systems to survive, so people started trying to control them. Control of streams and rivers has been vital to technological innovation and human population growth. However, there have always been concurrent negative tendencies of water demand growth. (Smith 1971, World Commission on Dams - WCD 2000).

Nowadays it has been estimated that an average person spends 50 litres of water per day for basic human water requirements, such as drinking, sanitation, bathing and food preparation. Therefore, around 3800 km^3 of fresh water is withdrawn from the world's lakes, rivers and aquifers every year. The volume extracted has dubled from 50 years ago (World Commission on Dams - WCD 2000).

After humans had found their way to tailor water for their basic needs, irrigation and creating drinking water reservoirs, the next step was harnessing the power of moving water for commerce and industry. This has resulted from population growth and lifestyle changes, which have increased our demand for energy and its production, contributing to development of storage hydropower plants. At that point, the influence they would have on the environment was neglected. Changes in the natural flow regime caused by plant discharge have showed negative tendencies and therefore require careful consideration.

1.1 HYDROPEAKING

1.1.1 What is hydropeaking

In order to meet peak electricity demand, some hydropower stations alter their discharge several times a day. Those alterations in discharge are called hydropeaking. Hydropeaking is sometimes defined just as a sudden increase in discharge of hypolimnic water from hydropower plants, which raises water depth, water velocity and shear stress (Ward and Stanford, 1979; Cushman, 1985; Bratrich et al., 2004; Bruno et al., 2013). Shear stress is defined as the force of moving water against the channel bed.

Hydropeaking is a complex phenomenon because several variables are associated with sudden changes in flow characteristics: bottom shear stress, depth, cross-section width, velocity, amount and composition of suspended matter, water temperature and quality (Ward and Stanford, 1979; Cushman, 1985; Moog, 1993; Webb and Walling, 1993; Fette et al., 2007; Olden and Naiman, 2010; Bruno et al., 2013).

1.1.2 Changes in flow regimes

Streamflow quantity and timing are critical components of water supply, water quality, and ecological integrity of river systems. Streamflow is the key driving variable for downstream aquatic ecosystems. The natural flow regime depends on geomorphological, climatic and environmental characteristics of a river. It is defined by five critical components: magnitude of discharge, frequency of flow occurrence, duration of high or low flow conditions, flow timing or predictability, and flow change rate (Poff et al. 1997). In case of naturally occurring floods as well as in hydropeaking events, timing, duration and frequency are all critical for the survival of stable communities of plants and animals living downstream. River flow patterns are influenced by seasonal contrasts in rainfall or melt-water contributions. Storage hydropower peaking plants can disrupt the whole flow regime by altering the hydrological characteristics of downstream flow, including magnitude, duration, timing, rate of change (upramping and downramping rate) as well as frequency of changes in flow (Céréghino and Lavandier, 1998, Marty et al., 2009). They can influence seasonal and daily fluctuations to differ greatly from natural flow levels (Poff, 1997; WCD, 2000).

1.1.3 Abiotic and biotic effects of hydropeaking

Hydropeaking causes physical and chemical changes in the receiving stream (Cushman, 1985). These heavy fluctuations of the water level mostly alter the shoreline by rapid and repeating flooding and drying of these zones (Cushman, 1985; Moog, 1993; Schmutz et al., 2013). This often leads to stranding of animals.

Another occurrence connected to discharge from hydropower plants is thermopeaking. Thermopeaking is the change of thermal conditions, most commonly caused by the release of hypolimnic water from the bottom of reservoirs. It decreases water temperature in summer and

increases it in winter (Hilsenhoff, 1971; Ward and Stanford, 1979; Raddum, 1985; Moog, 1993 Maiolini et al. 2007; Carolli et al., 2012; Bruno et al. 2013). Thermal wave usually occurs shortly after the increase of discharge (Toffolon et al., 2010), which is an additional stressor for river biota (Bruno et al., 2013).

There have been separate experiments on the effects of hydropeaking on macroinvertebrates, as well as thermopeaking. Finally, the effects of hydro- and thermopeaking were observed simultaneously, showing the greatest impact as a result. This combination is also the only one in natural field situations, where hydropower plants are present (Bruno et al., 2013).

In addition, modified water qualities (temperature, oxygen and nutrients, loss of system dynamics, and loss of the ability to maintain continuity of an ecosystem) result in ecologically modified river systems. Modifying the ecosystem also changes the biochemical cycle in the natural riverine system.

1.1.4 General effects on stream biota

Fluctuations in discharge and the corresponding potential changes in flow forces can have dramatic effects on lotic organisms and community structure (Welcomme, 1985), especially during spates.

Hydropeaking can affect macroinvertebrates directly by inducing faunal drift (Troelstrup and Hergenrader, 1990). It also influences them indirectly by modifying available food resources (Bohle, 1978; Anderson and Cummins, 1979; Moog and Janecek, 1991) and making changes in species composition, density, biomass and migration patterns (Moog, 1993; Blinn et al., 1995; Céréghino and Lavandier, 1998; Céréghino, 2002; Bruno et al., 2010).

1.2 DRIFT

Drift is the downstream transport of aquatic organisms in the current. It was discovered accidentally by investigating the drift of terrestrial insects that had fallen into a stream (Needham, 1928). Drifting is only a temporary event in the life of numerous members of bottom fauna (Waters, 1972), but the cause and timing of drifting events differ. Even though it is a normal everyday occurrence in lotic systems, it is usually connected to floods. Drift varies daily as well as sesonally.

Invertebrate drift often exhibits a distinct diel periodicity (Tanaka, 1960; Müller, 1963; Waters, 1972). From observations that many species exhibit drift periodicity throughout their life cycle, it would seem most likely that foraging is the main factor in periodic behaviour. It is not certain that circadian rhythms and some other external factors affect periodicity, but it seems likely that endogenous locomotory rhythms are present to some degree. They are also connected to some environmental agents like light intensity (Waters, 1972). Most of the animals found in drift are active at night. Those nocturnal drifters show a distinctive pattern. The most common one consists of two peaks: one just after sunset, and a smaller one just before sunrise (Müller 1965). This latter pattern was documented in *Baetis*, Simuliidae, Turbellaria and Chironomidae (Waters, 1972; Cowell and Carew, 1976). Many species simply show an unorganized drift increase at night, without a well-defined pattern (Brittain and Eikland, 1988). In the presence of fish chemicals, some animals are active only at night, for example *Rhyacophila*, whereas no periodicity was detected without the presence of fish chemicals (Huhta et al, 1999).

Regarding seasonal varieties, in temperate regions drift is usually the lowest in winter (McLay, 1968; Clifford, 1972a). In tropical and sub-tropical streams, annual variations are less apparent or even non-existent (Hynes, 1975). In mountain regions, drift is similar during most months, showing a slight increase towards the end of the rainy season (Turcotte and Harper, 1982).

In addition to daily and seasonal changes, different densities in drift have been documented throughout a life cycle or different size classes of invertebrates (Waters, 1972; Statzner, 1984; Cellot, 1989). Moreover, drift rate changes among taxa (Elliot, 1967 and 1971; Statzner and Holm, 1982; Brittain and Eikland, 1988; Waringer, 1989; Oldmeadow et al., 2010). Particular taxa, for example some Ephemeroptera, Plecoptera, Trichoptera, Chironomidae and Simuliidae, due to their frequent drifting represent common drift features (Bishop and Hynes 1969; Brittain and Eikeland 1988; Imbert and Perry, 2000).

Another division of drift is based on its cause, grouping it into behavioural or active, constant or passive and catastrophic drift (Waters, 1972). Firstly, behavioural drift is the drift that occurs at night, or some other consistent period of the day, resulting from a behaviour pattern characteristic for certain species (Waters, 1972). It is a voluntary drift (Huhta, Muotka and Tikkanen, 2000; Miyasaka and Nakano, 2001) and it happens indirectly as a result of animal activity, such as avoiding predators (Müller, 1974). Constant drift is defined as the continuous stream of representatives of all species in low numbers and occurring at all times (Waters, 1972). It is also called background drift, and it happens due to accidental dislodgement from

the substrate. Catastrophic drift results from the physical disturbance of the bottom fauna, usually by flood events and consequent bottom scouring, but also by other factors such as drought, high temperature, anchor ice, pollution, and insecticides (Waters, 1972). It is involuntary and accidental, with high and low discharge variations that are often caused by man-made water regulations (Minshall and Winger, 1968; Gore, 1977). Gibbins et al. (2007) argue that without information on the survival or fecundity of animals entrained and carried downstream, it is not possible to say whether a given episode of drift is actually catastrophic for invertebrate populations. Thus, defining catastrophic drift simply as a marked change from the background rate, triggered by bed instability, does not deal with the ecological consequences of displacement. Until the population consequences of drift are fully understood, the term 'mass drift' is suggested to be used to describe large increases in drift associated with periods of increased discharge (hydropeaking).

Furthermore, certain authors mention another classification with distributional drift as a method of dispersal, distribution and an inherent part of the life cycle of numerous species (Müller, 1973; Minshall and Petersen 1985; Moser and Minshall, 1996; Matthaei et al., 1997).

It is seldom possible to categorize drift precisely into all these types. There are numerous classifications, as well as frequently contradicting views on how to divide and distinguish different types of drift (Elliot, 1967; Bogatov, 1988; Poff et al., 1991; Gibbins et al., 2007). Nevertheless, it is clear that macroinvertebrates drift not only naturally, but also as a result of humans and man-made influences like hydropeaking.

1.3 HYDROPEAKING AND DRIFT

1.3.1 Adaptations and species-specific traits

A common anthropogenic cause of increased drift is hydropeaking. Hydropeaking does not necessarily reduce species diversity (Moog, 1993; Céréghino, 2002) since some species are adapted to high flow velocities. A trait is defined as a characteristic that reflects a specie's adaptation to its environment. (Menezes, 2010) Traits are usually divided into two categories: biological traits (for example life cycle or physiological and behavioural characteristics such as maximum body size, lifespan, feeding and reproductive strategies, mobility, etc.) and ecological traits (related to habitat preferences, like pH and temperature tolerances, tolerance to organic pollution, biogeographic distribution, etc.) (Menezes, 2010).

A clear example of morphological adaptation is Ephemerellidae and Heptageniidae with their robust forms and flat or cylindrical body shapes (Moog, 1993; Céréghino, 2002). Another example is Rhyacophila, which has claws to grab onto the sediment (Céréghino, 2002). A representative case of biological adaptation is the high fecundity of stream invertebrates. For example, mayflies have 500 to 3000 eggs (Brittain, 1982) and *Baetis rhodani* up to 4,500 eggs (Elliott and Humpesch, 1980). Furthermore, there is an upstream movement of female imagoes to lay eggs, compensating the downstream drift in aquatic stages, which is called the colonization cycle (Müller, 1954, 1982). Moreover, it has been reported that the drift of *Baetis* nymphs decreases with increasing current velocities (Corkum et al., 1977; Bird and Hynes, 1981). This can be explained by finding protection from the current within the substrate (Lehmkuhl and Andersen, 1972). Leptophlebiidae and Leuctridae (Moog, 1993) represent another example of behavioural adaptation. Nymphs of the stonefly *Peltoperla maria* leave the leaf packs and enter the inorganic substrate during increasing flows, which makes them less vulnerable to catastrophic drift (Elwood and Cushman, 1975). This might also account for their rapid recolonization after floods.

Although hydropeaking is caused by human influence, different macroinvertebrates have developed various traits in order to adapt to the challenges of high flow velocity. Substrate preference plays an important role in this adaptation as well.

1.4 SUBSTRATE

1.4.1 Substrate preference of macroinvertebrates

Invertebrate density and taxonomic richness both increase as substrate size increases from sand to cobbles (Minshall 1984; Jowett and Richardson 1990). Current velocity required to disturb a substrate particle increases with particle diameter above the silt-sand range (Carson and Griffiths 1987; Ashworth and Ferguson 1989). Hence, larger particles provide a more stable habitat for invertebrates. Smaller interstitial spaces of gravel beds are expected to retain finer paniculate organic matter (Parker 1989), which may account for the greatest abundance of detrital-feeding oligochaetes. Simultaneously increased water turbulence and bed roughness are expected to reduce the thickness of boundary layer. This is a low-velocity region that exists just above stone surfaces (Smith 1975; Davis 1986). It increases the exchange of dissolved gases, nutrients, and organic matter between bulk flow, stone surfaces and interstitial water influencing invertebrates (Statzner 1981). Thus, at a given current velocity, coarser substrates

are expected to provide a more suitable habitat for invertebrates with high oxygen requirements, such as some Plecoptera, Ephemeroptera, and Trichoptera (Nebeker 1972; Wiley and Kohler 1980).

On the one hand, areas of larger substrates have a greater bed stability and exchange of water between the bed and water column, so it is expected that filter-feeding invertebrates will favour these habitats. On the other hand, rougher substrates cause greater small-scale variability in stone-surface current velocities (Davis 1986), whereas gravel-boulder-sized beds provide a more complex three-dimensional habitat than silty, sandy or bedrock areas (Minshall 1984). Both result in a greater range of microhabitats and more refuges from predation by other invertebrates, fish (Brusven and Rose 1981), and from scouring during high flows (Williams and Hynes 1974; Cowie 1980). Habitat diversity and the presence of refuges are expected to contribute to greater taxonomic richness, invertebrate biomass and densities in rivers with gravel when compared to cobble/boulder dominated beds (John M. Quinn and Christopher W. Hickey 1990).

In a nutshell, different macroinvertebrates express their preferences of substrate size depending on their feeding mode, water quality requirements, as well as biotic interactions.

1.4.2 Sediment roughness

Bed roughness develops due to stream surface relief at the base of a flowing fluid, exerting a frictional effect on the flow in the stream. Bed roughness can be described as smooth or rough, depending on whether sediment particles go through the viscous sublayer at the base of the flow or not. Roughness generally increases with increasing particle size. Grain roughness refers to the shear forces created by sediment particles at the flow boundary. Grain roughness can be the dominant component of the bed roughness when streambeds consist of gravel or cobbles (Singh et al, 2011).

Bed roughness influences properties of average flow, turbulence, flow resistance and bed particle motion. In addition, the effect of bed roughness is one of the key problems in understanding near-bed processes important for benthic organisms (Nikora et al, 1998).

Benthic macroinvertebrates use sheltered locations under rocks and in the interstitium to avoid extreme hydraulic conditions (Lancaster and Hildrew, 1993; Boulton et al., 1998; Matthaei, Peacock and Townsend, 1999) and predators (Hildrew and Townsend, 1977). Even though substratum can provide refuge from hydraulic stress, near-bed flow forces still determine the

presence or absence of invertebrates in a specific hydraulic environment. They have to leave refuge in order to feed, which makes them subjected to stronger hydraulic forces (Statzner, 1981).

Near-bed hydraulic parameters that are important for benthic macroinvertebrates can be calculated from a combination of substratum roughness, mean velocity, depth and kinematic viscosity. These parameters can also be measured directly from shear stress and velocity, substrate particle size or heterogeneity. They are good predictors of benthic invertebrate distribution (Statzner et al., 1988). Therefore, any hydrological change (like hydropeaking) that leads to an increase in shear stress or other parameters, potentially reduces the availability of suitable microhabitat for some species, but can also increase it for other species (Gore, 2001).

1.4.3 Shear stress and the channel bed

Various studies have shown that shear stress is one of the major factors that influence micro distribution patterns of benthic invertebrates (Statzner et al., 1988; Peckarsky et al., 1990; Lancaster and Hildrew, 1993; Möbes Hansen and Waringer, 1998). Energy required by macroinvertebrates to withstand flow and resist detachment from the bed is more related to the shearing forces of water than its velocity (Statzner and Borchardt, 1994).

Shear stress starts bed-load movement and sediment transport. When the drag force of flowing water against a rock is greater than the gravitational force holding it in its place, the rock begins to move. Lavelle and Mofjeld (1987) claim that the range of bed shear stresses for weak particle motion is indefinitely wide, and that because of that there is a conceptual flaw in the assumption that a definite threshold condition can be defined. It is true that the weaker the flow, the smaller the number of bed particles that are moved by the flow, per unit time and per unit area of the bed, but the lower limit for any particle motion is indefinite (Lavelle and Mofjeld, 1987; John Southard, 2006). It has been observed that the critical shear stress for initiation of particle movement on a rippled bed is greater than for that on a plane bed, although the mean velocity of flow is lower This can be explained by the fact that ripples create form resistance, which contributes to most of the measured average bottom shear stress (John Southard, 2006).

Bed characteristics depend on main flow variables like depth, velocity and sediment size. These hydraulic variables are, however, in turn strongly dependent on bed configuration and its roughness (Leo van Rijn, 2017). Coarse sediments can withstand higher shear stress than finer sediments before being moved downstream. (Table 1).

Table 1. Critical shear stress by particle-size classification for determining approximate condition for sediment mobility at 20 °C. (Modified from Berenbrock, C., & Tranmer, A.W., (2008). Simulation of flow, sediment transport, and sediment mobility of the Lower Coeur d'Alene River, Idaho. U.S. Geological Survey Scientific Investigations Report 2008–5093, 43.).

Particle classification name	**Ranges of particle diameters in mm**	**Critical bed shear stress** (N/m^2)
Coarse cobble	128 – 256	112 – 223
Fine cobble	64 – 128	53.8 – 112
Very coarse gravel	32 – 64	25.9 – 53.8
Coarse gravel	16 – 32	12.2 – 25.9

Both near-bottom flow forces, expressed as shear stress, and the physical characteristics of the habitat, in terms of refugial space, are of great importance during periods of hydraulic disturbance (Borchardt, 1993). Increased abundance of refugial space reduced the amount of loss in populations of tested species and resulted in mitigated impacts of critical flow forces.

1.4.4 Hydropeaking induced drift depending on sediment roughness

By changing the water depth and velocity, hydropeaking changes the shear stress (Statzner et all., 1988). Vertical changes in water velocity produce shear forces that are parallel to the bed. These shear forces acting on the bed of a channel generate shear stress, which initiates bed load movement.

Once the entrainment threshold for small material is reached, any larger material present is being agitated while still not experiencing downstream motion (Schumm and Stevens, 1973; Carling, Kelsey and Glaister, 1992; Garcia et al., 2007). This agitation may result in invertebrates losing their hold and being thrown into the water column. In these circumstances,

drift may result from a combination of sediment transport (finer material) and agitation (larger material).

Even though mass sediment movement is an obvious mechanism that could trigger catastrophic drift, there are no published field data relating the rates of sediment loss from specific places on the streambed to drift losses from these locations. It thus remains unclear precisely how much sediment loss is needed before the drift becomes catastrophic, or whether the sheer agitation of the sediment is sufficient to start it.

Borchardt (1993) states that in lowland rivers with little refugial space 'catastrophic drift' events caused by hydraulic disturbance can be expected before significant substrate movement begins.

In general, substrate roughness elements disrupt flow causing turbulence, eddy formation, and lowered velocities. In some microhabitats, areas behind and between roughness elements can experience a considerable reduction in velocities, depending upon both height and spacing of the roughness elements. Additionally, roughness elements can cause an overall reduction in shearing forces which may establish favorable microhabitats for organisms requiring velocity refuges (Way, 1995). In rivers or streams with a bottom of coarse inorganic material, interstitial spaces within the stream bed serve as important refugia during such critical hydraulic periods (Schwoerbel,1964; Tilzer, 1968), even under sudden changes of discharge (Borchardt and Statzner, 1990). However, in lowland running waters the stream bed often consists of fine inorganic material with very narrow interstices within the substrata. Therefore, this habitat offers little refugial space to most benthic macroinvertebrates while other habitat structures, such as woody debris, fulfil this function (Borchardt, 1993).

1.5 KNOWLEDGE DEFICIENCIES AS THE RATIONALE FOR THIS THESIS

In the beginning of 20th century, research was focused more on the physics than biology of stream invertebrates. Fluid mechanics along with technological innovations helped to integrate all the above elements in stream ecology with the aim to stress that flow adaptations are much more complicated than previously thought. Even though stream ecologists have been addressing this issue for over a century now, they are still far from understanding how stream invertebrates are adapted to the many different flow conditions they face during their life. The near-bottom flows they withstand are extremely complex and create such diverse constraints that the adaptation to all of them is physically impossible (Statzner and Holm, 1989; Statzner, 2008). Nevertheless, scientists are still trying to collect all the data they have on different

adaptations and traits, and implement them to biomonitoring and environmental protection (Poff et al., 2010; Menezes, 2010; Culp et al., 2011).

Despite the fact that we know how fish react to hydropeaking and what measures we can take to protect them, it still has not been clarified how macroinvertebrates respond to it. Therefore, a better understanding of changes in interconnected parameters is needed. Moreover, aiming to protect macroinvertebrates first would prove to be a better approach, since it would shelter the entire ecosystem (Gore, 2001). In order to explain community composition, it is very important to understand how invertebrate species respond to near-bed hydraulic conditions. In the last few decades, a lot of attention has been given to complex hydraulic interactions near substratum and how they influence benthic invertebrates (Statzner et al., 1988; Quinn and Hickey, 1994; Statzner and Borchardt, 1994; Collier et al., 1995; Lancaster and Mole, 1999). However, roughness of substratum represents an area that still calls for more detailed research (Mérigoux and Dolédec, 2004).

1.6 STUDY AIMS

The aim of this study was to test hydropeaking as a stressor and sediment roughness as the factor that might influence the effects of the assessed stressor.

Research question 1: Is the hydropeaking-induced drift influenced by the different roughness of the bottom?

Research question 2: Are there species-specific drift patterns related to bottom roughness?

Research question 3: Does bottom roughness affect size classes of drifted organisms?

Based on the literature research the following hypotheses have been proposed:

Hypothesis 1

Drift will be lower in coarse substrates because animals will find shelter easier there, and shear stress will be lower.

Hypothesis 2

Drifted taxa will differ on different sediment types because they live on different substrata and in diverse conditions, so they have developed different adaptations as well.

Hypothesis 3

We will find mostly smaller sizes of different taxa in the drift, because smaller specimens can hide in the interstitium more easily. However, they are more numerous than larger specimens are, so it is expected they could enter the drift purely by chance. Furthermore, they might drift on purpose as a way of dispersal and colonization of new habitats.

2 MATERIALS AND METHODS

2.1 STUDY AREA

The experiments were conducted at a recently established facility called HyTEC (Hydromorphological and Temperature Experimental Channels). The experimental facility is located in Lunz am See in Lower Austria, approximately 600 m downstream of Lake Lunz beside the stream Unterer Lunzer Seebach, which is a natural drain of the lake. HyTEC consists of two large channels (40 m length, 6 m width) fed with nutrient-poor lake water taken at different depths to vary water temperature (Fig. 1).

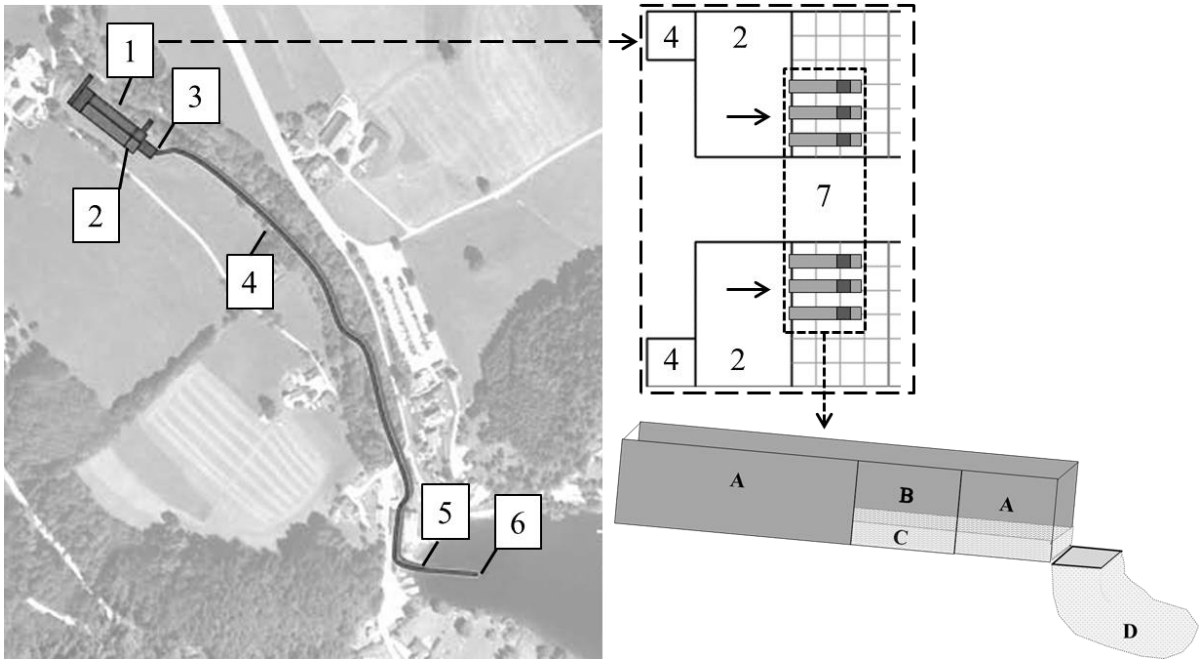


Figure 1. Schematic view of the HYTEC experimental channels, Lunz am See, lower Austria; (1.) experimental channels, (2.) mixing basins, (3.) measuring station, (4.) pipelines, (5.) intake surface water, (6.) intake deep water, (7.) mesocosms, (A) flow homogenisation area, (B) experiment area, (C) sediment, (D) exchangeable drift net.

One pipeline transports water from the depth of 10 meters and the other pipeline from the upper layer of the lake at 0.75-meter depth. The water from these channels is discharged into the stream Unterer Lunzer Seebach. Peak flows of up to 600 l/s can be produced several times a day in order to mimic hydropeaking and extreme floods. Water temperature variations of approximately 4-7°C between base flow and peak flow can be achieved. Time, duration and

intensity of a peak can be controlled manually or automatically by programming a fixed experimental setup. Furthermore, the amount of water taken from the upper or lower pipe can be chosen, which allows controlling the temperature in the experiments to a certain extent.

To measure drift of macroinvertebrates, experimental boxes called mesocosms were installed at the inflow of the flumes. Each mesocosm has a test area of 0.25 m². An additional start-up length of approximately 150 cm was installed to homogenize flow conditions (Fig. 1). At the end of each mesocosm, a buffer of 50 cm was installed to lower the flow on the outlet. The mesocosms were filled with sediments up to 15 cm. Three mesocosms were put in each channel. An exchangeable drift net was installed at the end of the experimental unit to catch drifted individuals.

2.2 SAMPLING OF DONOR POPULATIONS

Benthic invertebrates for the experiments were sampled from the nearby stream Bodingbach. The sediment extracted from the stream Ybbs was first cleaned of specimens and used to fill the mesocosms. This proved to be an advantage enabling a quick transport and storage of the animals and the sediment.

The water temperature of the stream Bodingbach was measured 8.9°C and the water during the experiments was measured to be between 8.6 and 9.3°C, so the animals did not have to deal with major temperature changes when being inoculated into mesocosms. The stream is a second-order stream (Strahler order) and it is 750 m to 1000 m above sea level. Its mean yearly area precipitation is 1.765 mm (Hydrologischer Atlas Österreichs, 2007).

For each mesocosm, four samples were taken with a 25×25 cm hand net, whereas two samples were placed near the shore, and two in-stream. The samples were stored in buckets and carefully transferred into the test zone of the mesocosms. It would have been too time-consuming to count or sort the taxa in advance, and therefore the samples used for the experiments vary in taxa abundances.

2.3 EXPERIMENTAL SETUP

Both experimental channels (with three mesocosms per channel) were used for each experimental arrangement, whereas one channel served as the treatment and the other as the

control channel. Each experimental arrangement was repeated twice and the control and the treatment channel were exchanged randomly. This way 6 treatment and 6 control replicates were gained for each experimental setup. The thing that varied in the arrangements was the sediment composition and there were three different variations. The first one was a mixture of akal (0.2 – 2 cm) and microlithal (2 – 6.3 cm), the second one had akal and microlithal, and the last one was a mixture of microlithal and mesolithal (6.3 – 20 cm).

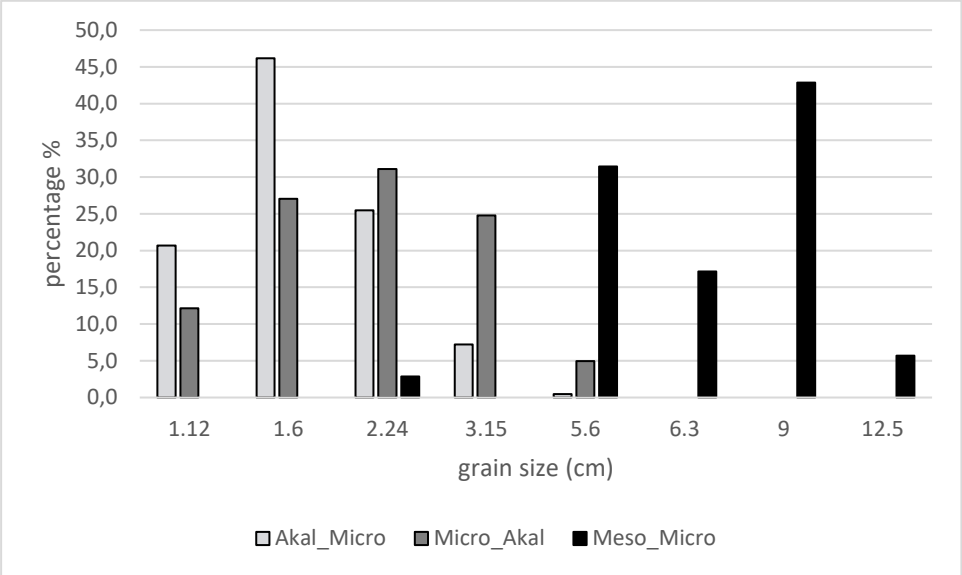


Figure 2. Grain size distribution

Table 2. Sediment mixtures used in the experiment, shown in percentages

	Akal	Microlithal	Mesolithal	type
SEDIMENT 1	39%	61%	0%	Medium
SEDIMENT 2	0%	34%	66%	Coarse
SEDIMENT 3	67%	33%	0%	Fine

Each experiment consisted of six phases (Fig. 3). First the sampled animals were taken out of the buckets and put into the mesocosms filled with sediement. After inoculation of the animals, they were left for 60-minutes to adapt (AT) at a base flow of 2 L per mesocosm. That particular discharge was chosen due to preliminary tests and observations. Preliminary tests also showed that initial drift is high in the first 10 minutes and stabilizes after 20 minutes. After the adaptation time, the base flow was elevated to 20 L/s for each mesocosm by an up ramping phase (UR) with a water level rise of 1cm/min (total 5.6 min.). The peak flow lasted for 20 minutes. The drift net was changed after 10 minutes (P1) and then after 20 minutes (P2),

followed by a down ramping phase (DR) of 5, 6 min where the base flow dropped from 20 L to 2 L per mesocosm. After the discharge had returned to base flow, the experiment was finished. The remaining (not drifted) animals were removed from the mesocosms by increasing the discharge again, and by swirling the sediment to flush the animals out. The control channel was always running with 6 L (2 L per mesocosm) and was fed with water from both the upper and lower pipe, 50% of each. After each phase, the drift net was exchanged and the animals were stored in separate, labelled containers with formalin, waiting to be identified in the laboratory.

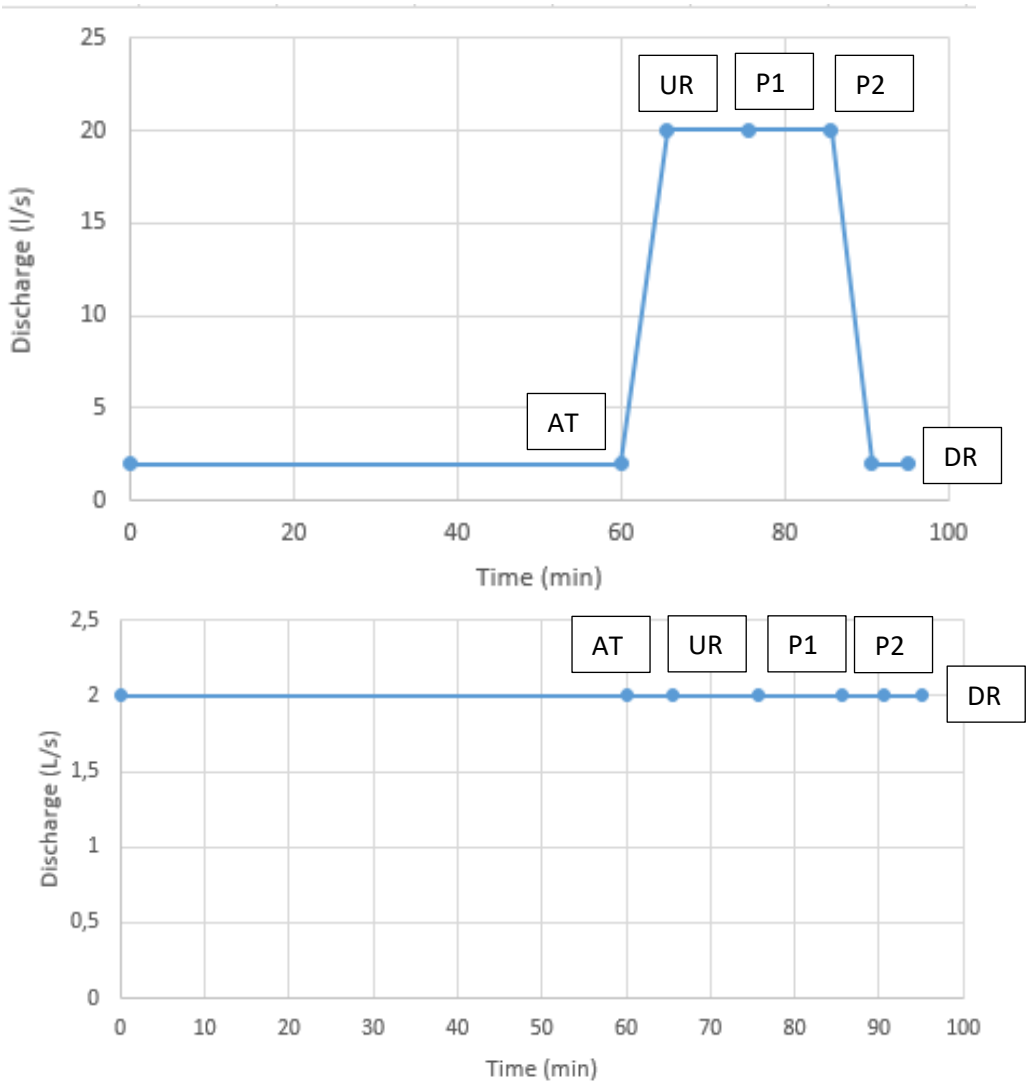


Figure 3. Discharge [l/s] curve during the phases (AT: adaptation time, UR: upramping, P: peak flow, DR: down ramping) at treatment and control settings

2.4 LEVEL OF IDENTIFICATION OF MACROINVERTEBRATES

Due to the three different setups **t**hat were each conducted twice, with 6 treatment and 6 control mesocosms containing 6 phases each, there were 216 samples all together in the end (3 × 2 × 6 × 6 = 216). The samples were isolated and the taxa identified. They were identified to genus, or where possible to species level. Some taxa from the Diptera like Chironomidae, was identified only to family level. In addition, Oligochaeta and Turbellaria were left at that higher level of identification. The taxa were also categorized into four size classes (Table 3.) based on data from prior experiments at the HyTEC facility. After that, the data was organized in Excel for statistical analysis.

Table 3. Size classes of the macroinvertebrates

Size class A	0 – 2 mm
Size class B	2 – 5 mm
Size class C	5 – 8 mm
Size class D	8 + mm

2.5 STATISTICAL ANALYSES

Since not all experimental units had the same number of macroinvertebrates, drift rates were used for better comparison. The drift rate was calculated for each taxon by dividing the sum of all the drifted individuals (sum UR, P1, P2 and DR) by the overall sum of all the individuals that had participated in the experiment (sum UR, P1, P2, DR and REST). The drift rate was calculated on taxon, genus, family and order level, as well as for the different size classes and phases of the experiment. Since not all the animals were identified to species level, the overall comparisons were done at family level drift rates. All the families that were not present in at least three experimental units (mesocosms) and with the abundance ≥10 were excluded from further analysis. Families Baetidae, Chironomidae, Elmidae, Ephemerellidae, Heptageniidae, Leptophlebiidae, Leuctridae and Nemouridae fulfilled those conditions. To determine whether hydropeaking had influence on drift rate, Mann-Whitney U-test was used to compare control and treatment in each sediment type. A Kruskal-Wallis test was used to determine differences

in control or treatment samples in different sediments. Multiple comparisons of mean ranks for all groups was used as a post-hoc test to determine between which sediments was the difference more significant. Those tests were done in Statistica 13.1 software.

A general comparison of all the drifted and non-drifted individuals from all taxa combined resulted in only one drift rate for control and one for treatment, drift rates for all six replicates of control and treatment had to be calculated separately in order to be able to compare the drift rates in Statistica.

3 RESULTS

3.1 INITIAL RESULTS

After the whole isolation and identification of the samples, the total number of individuals that had participated in this experiment in all the phases (AT, UR, P1, P2, DR and REST) amounted to 45,474 (23,332 in control, 22,142 in treatment). 29,013 macroinvertebrate individuals (14,075 in control and 14,938 in treatment) participated in the analysis, because all the individuals from phase AT (16,461) had been excluded. The purpose of this whole phase was for the animals to adapt to the new environment (mesocosm) before the hydropeaking began. In the end, a total of 12 orders, 54 families, 86 genera, and 107 taxa were found.

In all the units, Diptera, Ephemeroptera and Plecoptera were the most numerous orders (Fig. 4). The percentages of orders in control and treatment in one sediment are quite similar, whereas the comparison of different sediment types shows distinct discrepancy. Therefore, to get more accurate results, drift rates were used for the comparison instead of the absolute drift numbers.

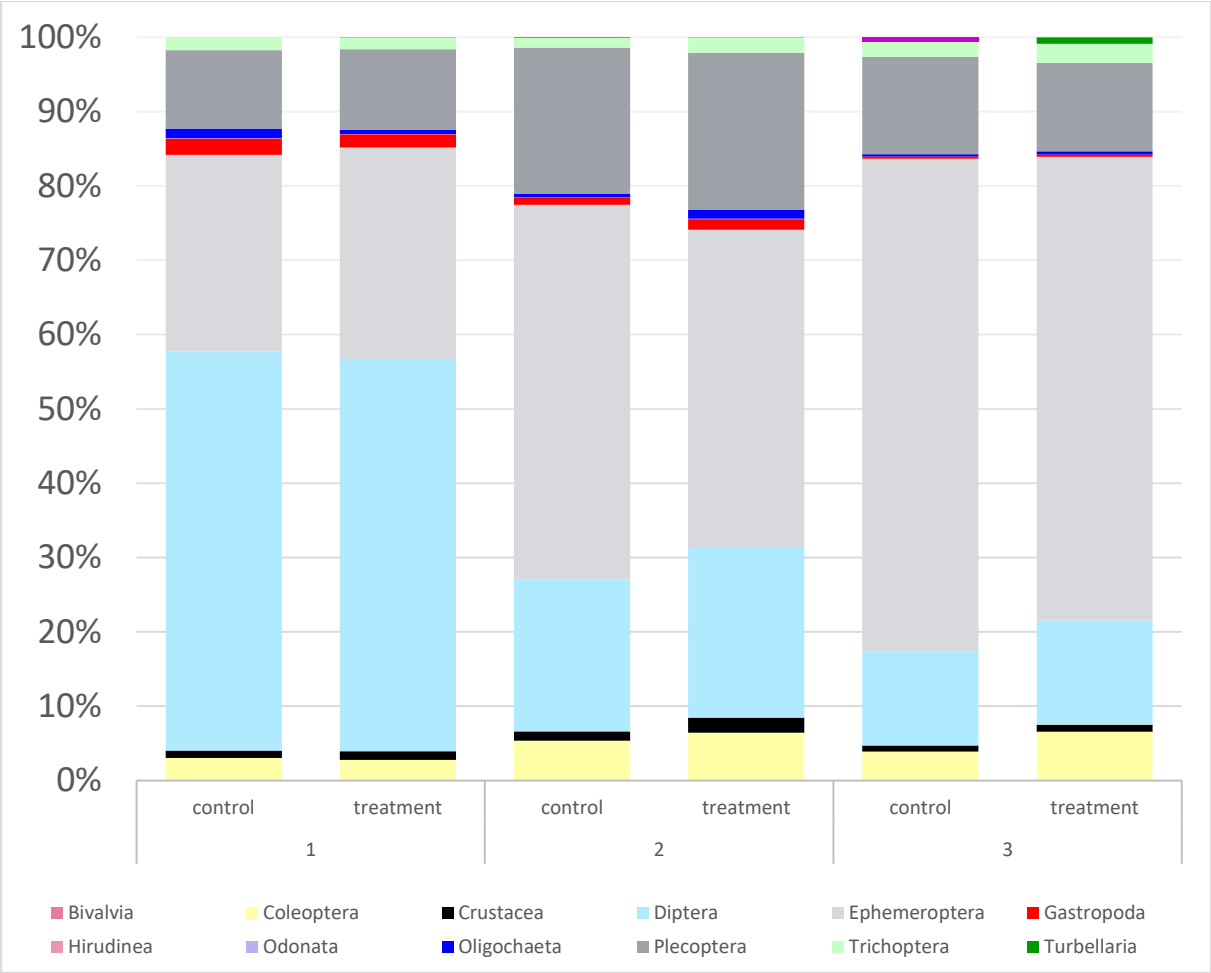


Figure 4. Macroinvertebrate assemblage composition in all 216 samples, from controls and treatments in three different sediment types (1, 2, 3 – as described in Table 1)

3.2 DIFFERENCES BETWEEN CONTROL AND TREATMENT

3.2.1 General level

Firstly, to determine whether hydropeaking increased the drift in treatment, compared to control that had only base flow, differences between these conditions were tested by making a general comparison of all the drifted and non-drifted individuals from all taxa combined. (Fig. 5.) Test results showed a significant difference (p = 0.000). The difference of drift rates in control and treatment units is visible from Fig 6.

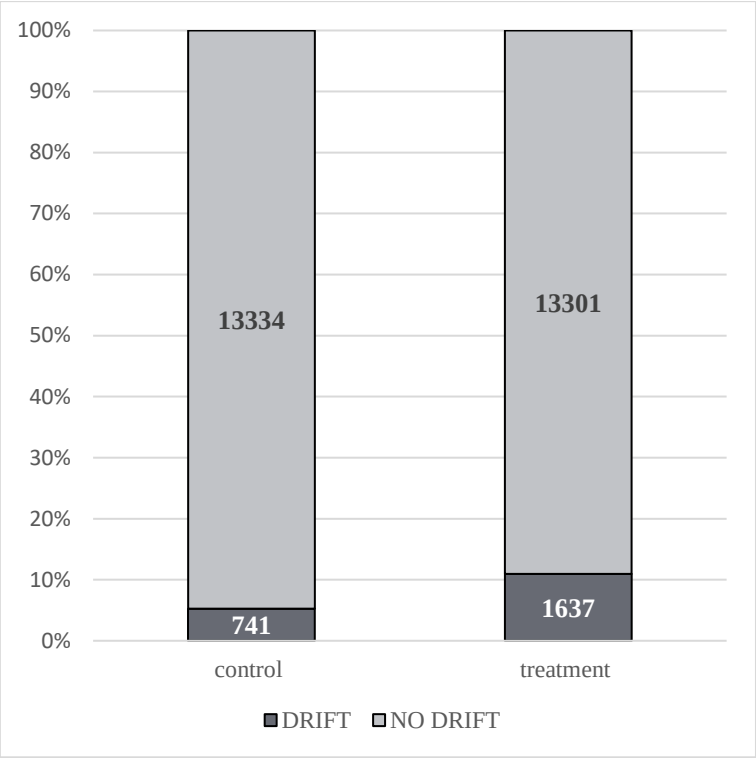


Figure 5. Comparison of control and treatment ratio of drifted and non-drifted individuals based on total macroinvertebrate individuals

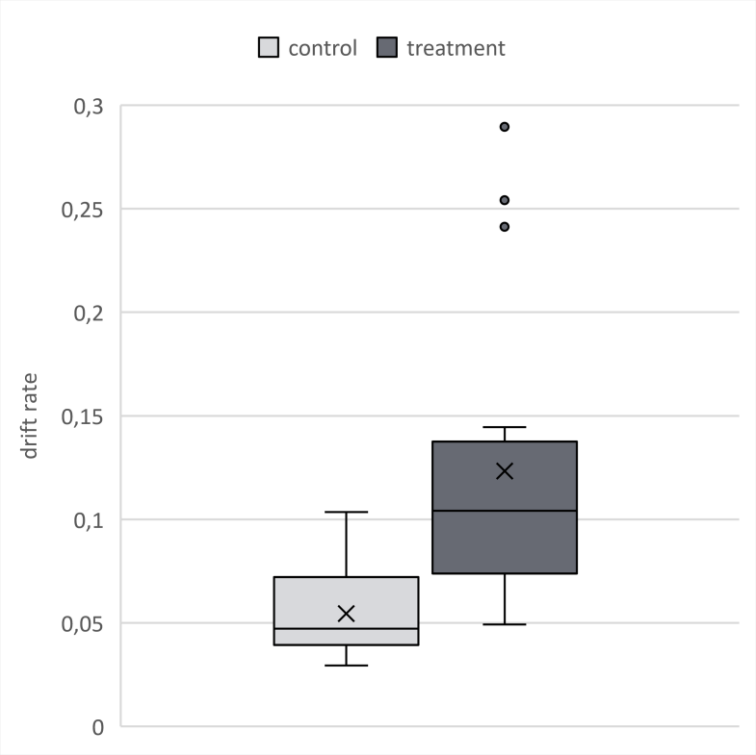


Figure 6. Comparison of control and treatment based on drift rates calculated from six replicates of each from the total of macroinvertebrate individuals

Drift rates from the six replicates were also tested for differences between control and treatment in each sediment type. They were all significant; sediment 1 and 3 had the same *p*-value (p = 0.013), and sediment 2 had the lowest *p*-value (p = 0.005).

3.2.2 Effect of hydropeaking on drift of selected taxa

In addition to finding the differences at general level, we wanted to test if hydropeaking increased drift of specific taxa in treatment units. For this, we did not use actual numbers of drifted individuals but drift rates since the drift rates show a more accurate state of the effects of hydropeaking.

3.2.2.1 Order level

First, the testing was done at order level, since most orders were present in all experimental units in abundances over 10 so those orders were included in the analysis and therefore the results should show a more exact picture of hydropeaking effects (Fig 7).

The only order that showed significant differences between control and treatment in every sediment type was Diptera. Coleoptera and Crustacea had the significant p value in sediment 3 and Ephemeroptera in sediments 2 and 3. (Table 4).

Table 4. Mann-Whitney U Test results - comparing control and treatment in each sediment type separately using order level drift rates

Order	Sediment 1	Sediment 2	Sediment 3
	p-value	*p*-value	*p*-value
Coleoptera	0.936	0.378	0.016
Crustacea	0.936	0.689	0.037
Diptera	0.031	0.031	0.031
Ephemeroptera	0.298	0.008	0.045
Plecoptera	0.471	0.093	0.575

Trichoptera	0.810	0.109	0.200

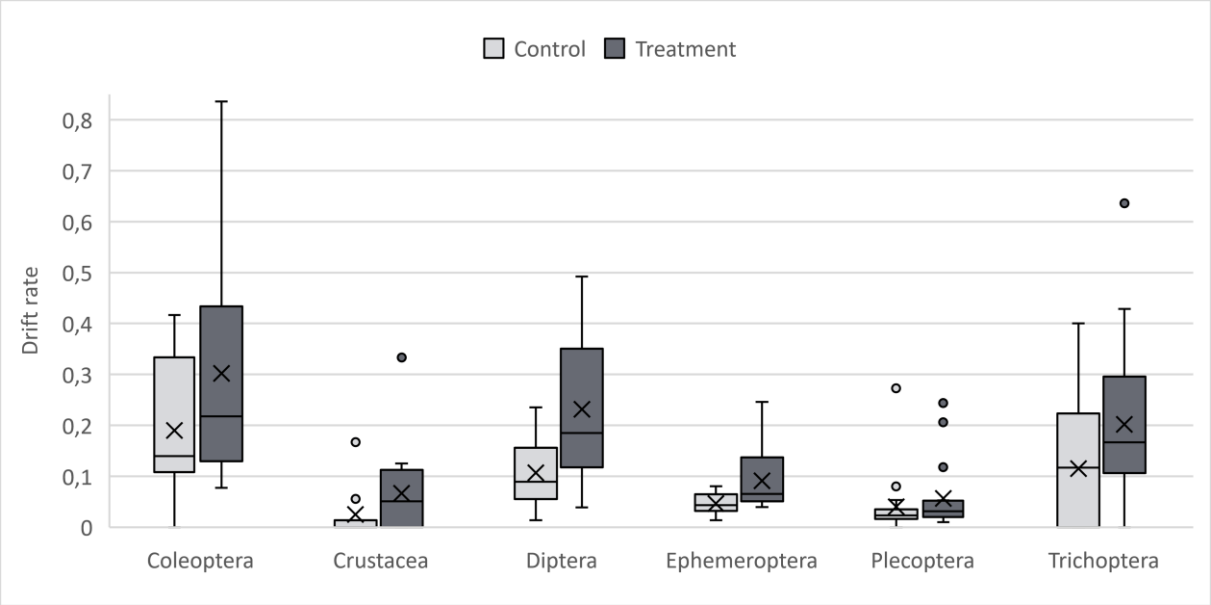


Figure 7. Comparison of drift rates between control and treatment at order level

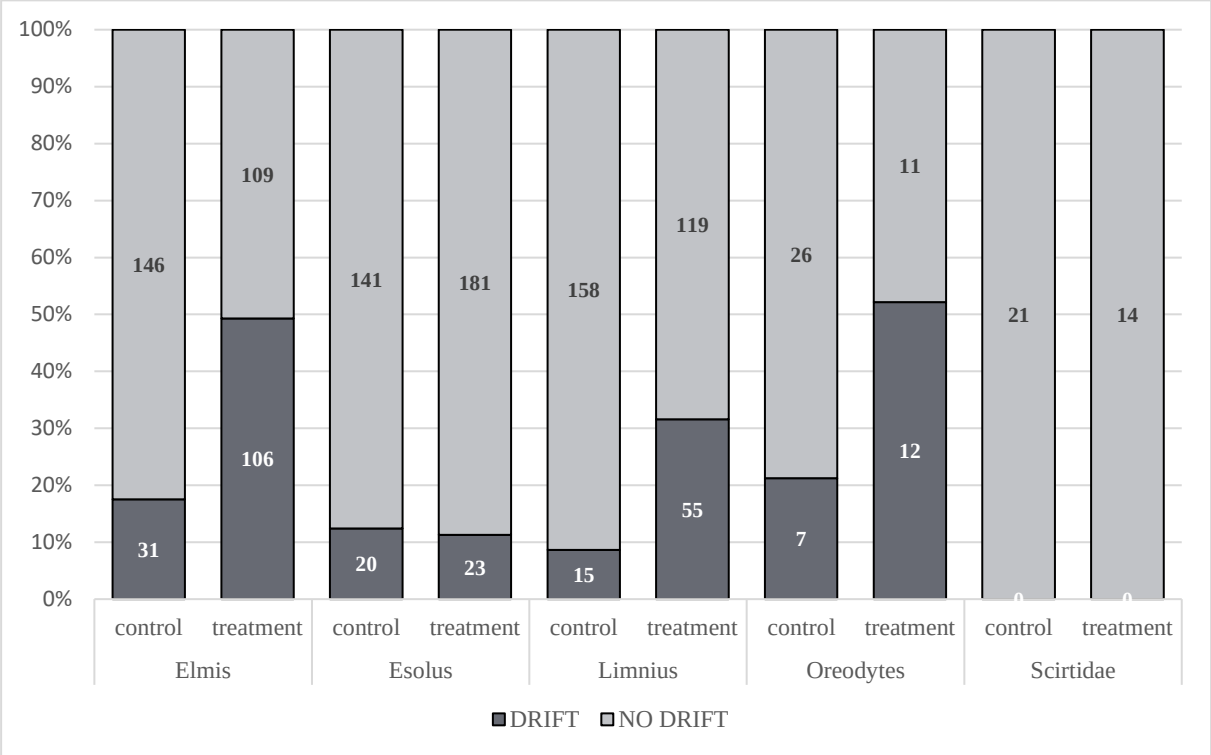
Table 5. Number of cases in which an order had a drift rate – maximumm possible 6 × 3 = 18 (participated in the analyses – see materials and methods)

Order	Setting	Number of cases
Coleoptera	Control	14
	Treatment	18
Crustacea	Control	8
	Treatment	10
Diptera	Control	18
	Treatment	18
Ephemeroptera	Control	17
	Treatment	18
Plecoptera	Control	17
	Treatment	18
Trichoptera	Control	11
	Treatment	15

To find out which genera are responsible for the differences in drift at order level, the relations of macroinvertebrates in drifted fraction and non-drifted fraction were displayed in figure 8.

The ones with abundance under 10 individuals have been left out. The displayed graphs show that in order Coleoptera, genera from the family Elmidae are the most abundant ones. In order Diptera the most abundant one is definitely Chironomidae. Plecoptera has high numbers of genus *Leuctra* and family Nemouridae. Order Ephemeroptera has the biggest abundance mainly of genera *Baetis* and *Serratella*. In order Trichoptera the most abundant genus is *Allogamus*, although the abundances in the entire order are not high.

a)



b)

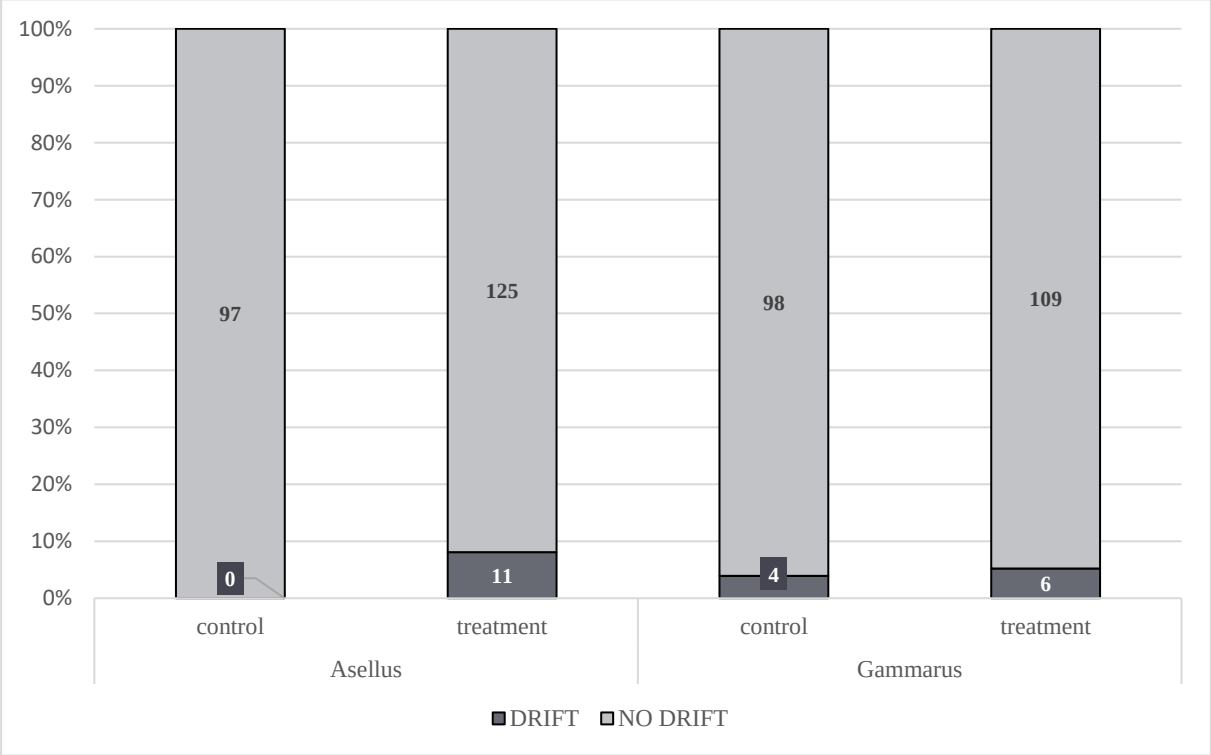
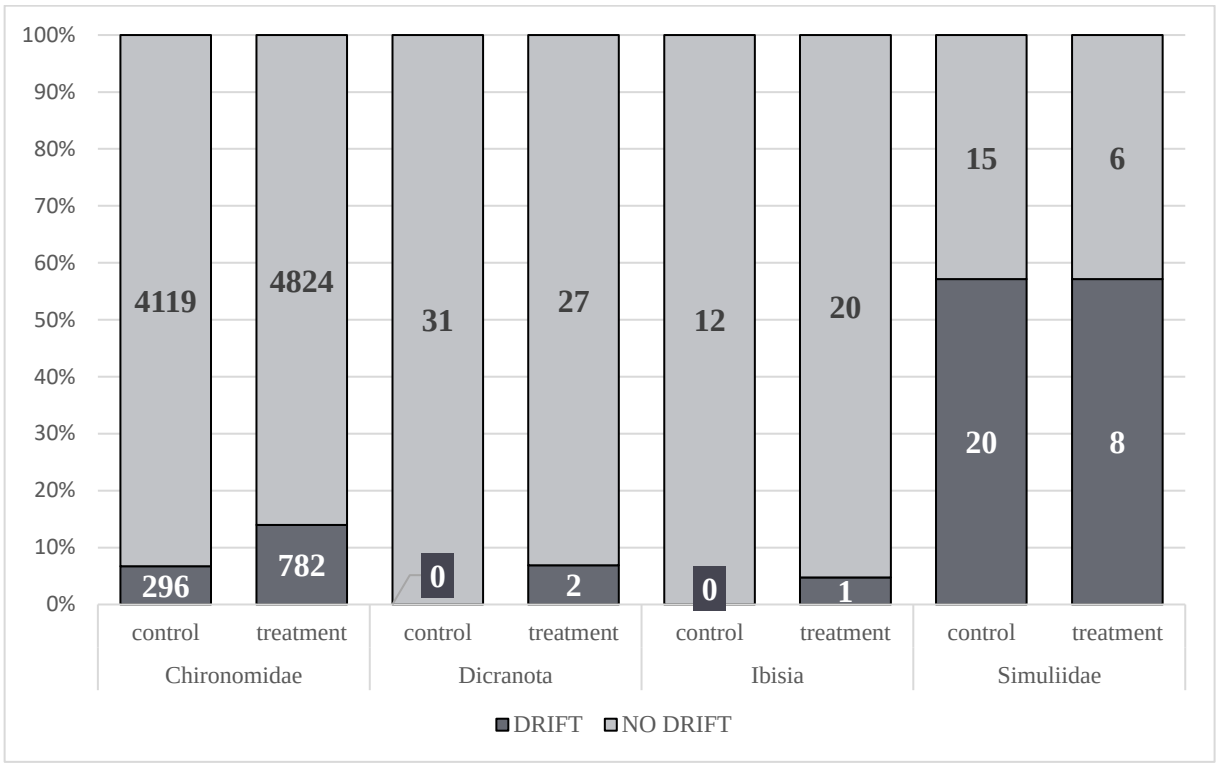


Figure 8. Ratio of drifted and non-drifted individuals in most numerous genera in selected orders: a) Coleoptera b) Crustacea

c)



d)

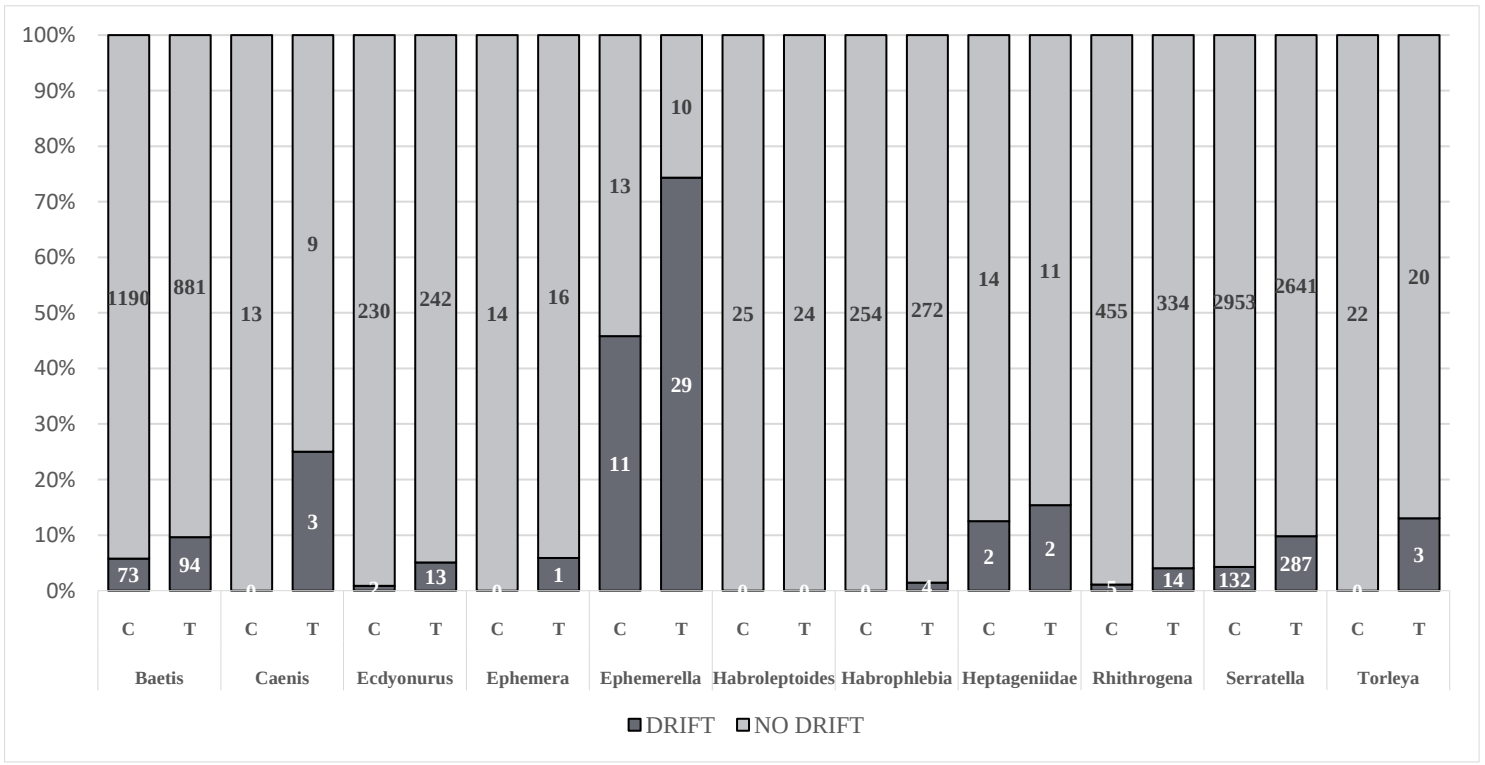
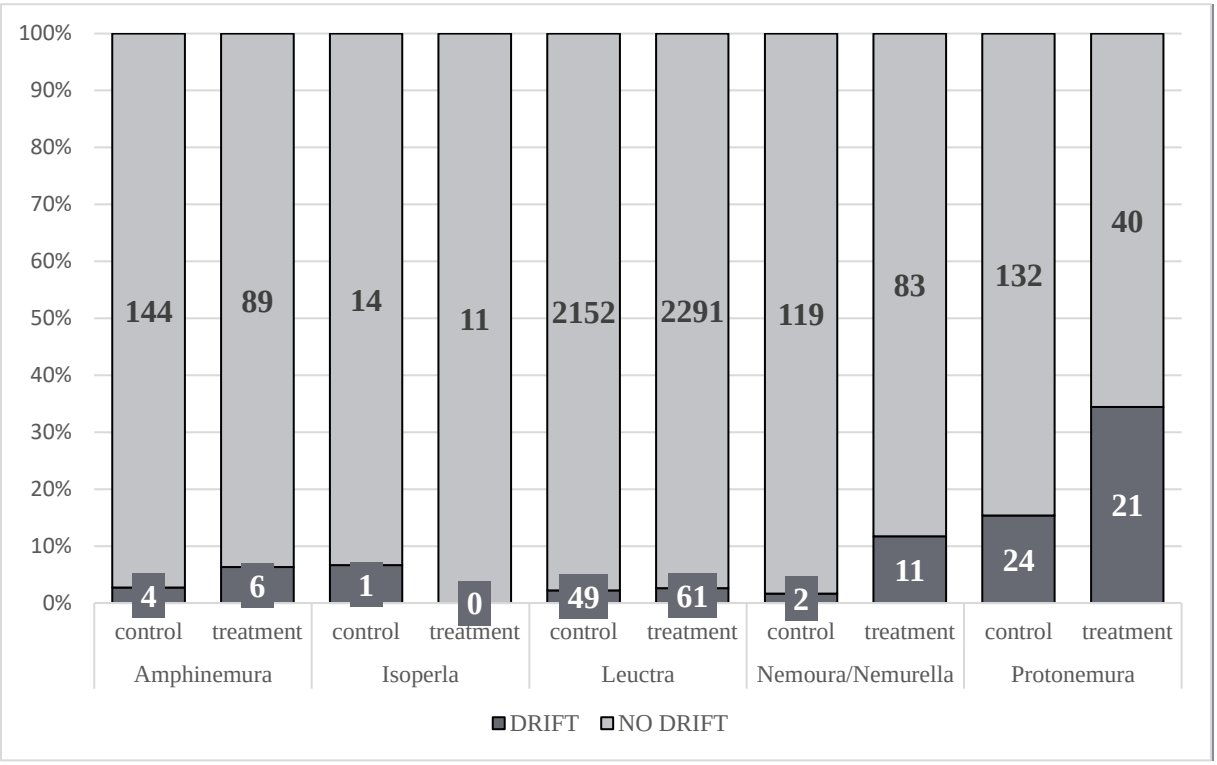


Figure 8. Ratio of drifted and non-drifted individuals in most numerous genera in selected orders:

c) Diptera d) Ephemeropter

e)



f)

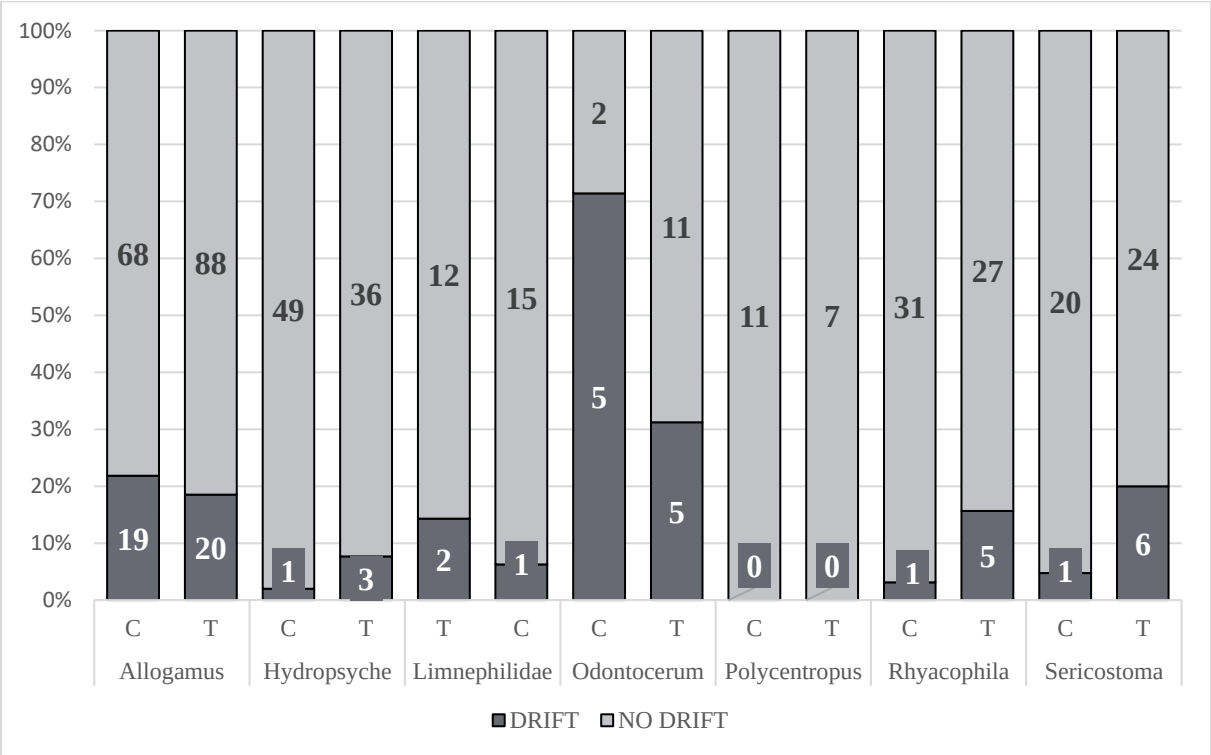


Figure 8. Ratio of drifted and non-drifted individuals in most numerous genera in selected orders: e) Plecoptera f) Tricjoptera

3.2.2.2 Family level

Secondly, the testing was done at family level, because it was expected that lower taxonomic levels would show more specific hydropeaking effects. Macroinvertebrate families that did not occur in at least three units in one sediment, with abundance per unit of under 10 individuals, were excluded from further analysis. Finally, only 11 families were left over from the initial 54. The drift rates of those families are shown in figure 9. to see the difference between control and treatment.

The only family that showed a significant difference between control and treatment in all three sediments was Chironomidae. Nemouridae had the significant difference in sediment 1, Ephemerellidae and Leucride in sediment 2 and the families Elmidae, Ephemerellidae and Heptageniidae in sediment 3 (Table 6.). The other families from table 6 did not show a significant difference between control and treatment in any of the sediment types.

Table 6. Mann-Whitney U Test results - comparing control and treatment in each sediment type separately using family level drift rates

Family	Sediment 1	Sediment 2	Sediment 3
	p-value	*p*-value	*p*-value
Baetidae	0.575	0.810	0.093
Chironomidae	0.031	0.031	0.031
Elmidae	0.689	0.200	0.020
Ephemerellidae	0.936	0.013	0.045
Heptageniidae	0.093	0.128	0.045
Leptophlebiidae	0.936	0.378	0.378
Leuctirdae	0.689	0.020	0.873
Nemouridae	0.031	0.298	0.128

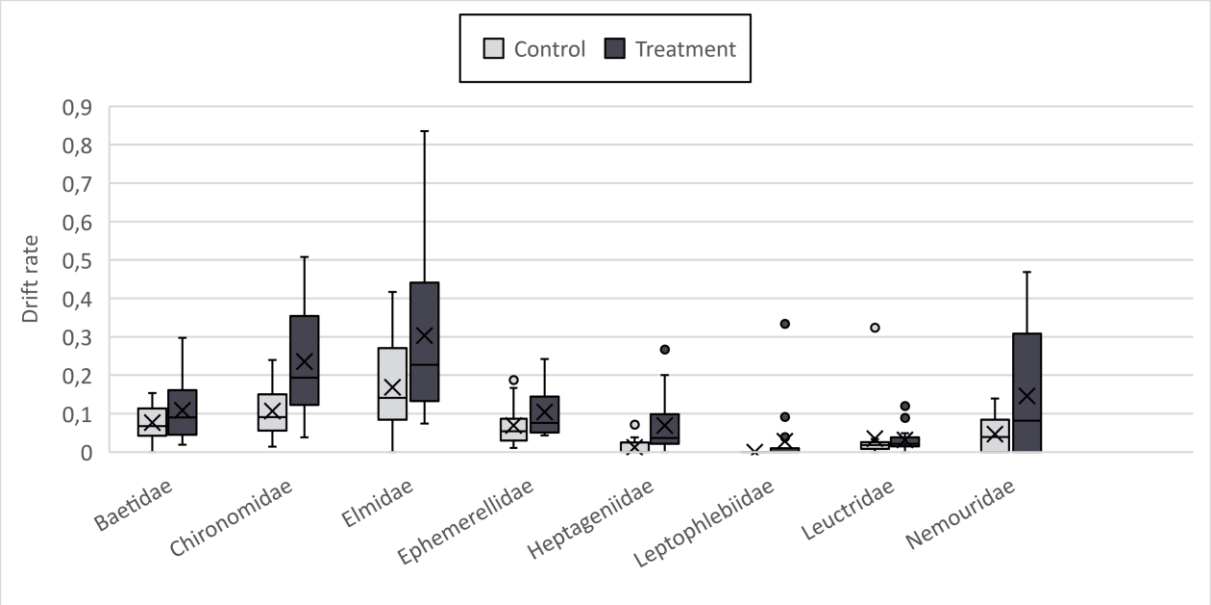


Figure 9. Comparison of control and treatment drift rates of selected families

Table 7. Number of cases in which a family had a drift rate (participated in the analyses – see materials and methods)

Family	setting	Number of cases
Baetidae	Control	16
	Treatment	18
Chironomidae	Control	18
	Treatment	18
Elmidae	Control	14
	Treatment	18
Ephemerellidae	Control	16
	Treatment	18
Heptageniidae	Control	15
	Treatment	17
Leptophlebiidae	Control	9
	Treatment	11
Leuctridae	Control	16
	Treatment	18
Nemouridae	Control	13
	Treatment	9

3.3 DIFFERENCES BETWEEN SEDIMENTS

3.3.1 General level

Since some taxa have different preferences to sediment types and different adaptations to staying in those sediments (not drifting away), differences in drift rates were expected on different sediments. As there were three different substrate compositions tested, in order to determine which sediment would show higher or lower drift rates for each taxon, their drift rates were tested separately in order to find out if there were any differences among controls as well as in treatments in those sediment types. If any differences occurred, this could show some new taxa sediment preferences or confirm some already reported.

The comparison of drift rates from six replicates indeed showed a significant difference when comparing controls in different sediment types ($p = 0.028$), whereas the treatments in different sediment types had borderline significant difference ($p = 0.051$). The post-hoc test showed a significant difference between sediments 1 and 3 in control ($p = 0.033$) and all the other p values were not significant. The graphical display of the drift rate differences is shown in Fig. 11.

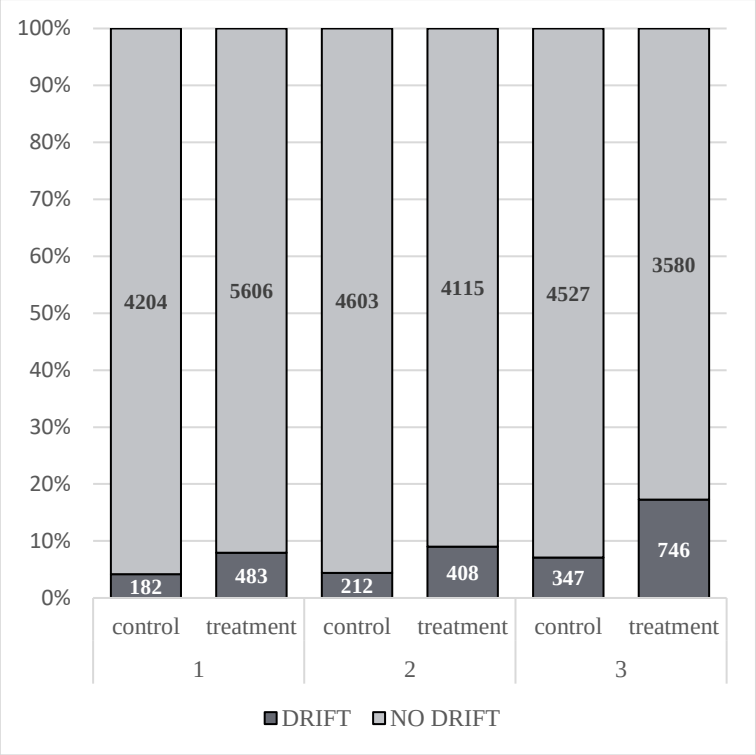


Figure 10. Comparison of drifted and non-drifted ratio of total abundance numbers in each of the sediment types in control and in treatment

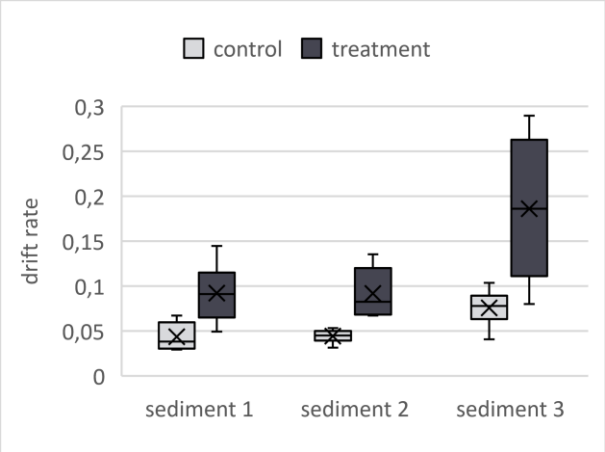


Figure 11. Comparison of control and treatment drift rates in different sediment types using total abundance numbers

3.3.2 Effect on selected taxa

To determine if the same differences as at general level would be found at lower taxonomic levels, drift rates were tested in control on different sediment types as well as the treatment on those sediments. The tests were made on order and family level for the same reasons already stated in 4.2.1

3.3.2.1 Order level

Diptera had significant differences between sediment types in control and treatment, as well as Coleoptera and Plecoptera. Ephemeroptera had a *p*-value under 0.05 when testing the differences between controls (Table 8). The post-hoc test revealed that in control, the difference between sediments 1 and 2 was the significant one in Coleoptera and between 1 and 3 in Diptera. The treatments show a significant difference between sediment 2 and 3 in Coleoptera, Plecoptera- For Diptera the difference is significant again between sediments 1 and 3 (Table 8).

Table 8. Kruskal-Wallis test and post hoc test – comparing differences in drift rates on order level between sediments for control and treatment separately

Order	Control			
	Kruskal-Wallis	Post-hoc		
	p-value	1-2	2-3	1-3
Coleoptera	0.028	0.026	0.237	1.000
Crustacea	0.587	1.000	1.000	1.000
Diptera	0.001	0.155	0.223	0.001
Ephemeroptera	0.047	0.060	0.175	1.000
Plecoptera	0.045	0.198	0.052	0.198
Trichoptera	0.583	1.000	0.991	1.000

Order	Treatment			
	Kruskal-Wallis	Post-hoc		
	p-value	1-2	2-3	1-3
Coleoptera	0.029	0.838	0.024	0.351
Crustacea	0.088	1.000	0.136	0.351
Diptera	0.015	0.198	0.913	0.012
Ephemeroptera	0.150	0.991	0.155	0.991
Plecoptera	0.025	1.000	0.045	0.069
Trichoptera	0.346	1.000	1.000	0.456

3.3.2.2 Family level

When testing the difference in control between sediment types, Ephemerellidae had a *p*-value ≤0.05 and Nemouridae was borderline. Elmidae, Ephemerellidae and Nemouridae had a significant difference in treatments between different sediments. All the other combinations of families and sediment types had a *p*-value larger than 0.05 (Table 9). The post-hoc test shows that in control, the significant difference in Ephemerellidae was between sediments 1 and 2 and in Chironomidae between sediments 1 and 3. In treatment the significant difference between sediments 2 and 3 was in Elmidae and Nemouridae and between sediments 1 and 3 in Chironomidae (Table 9).

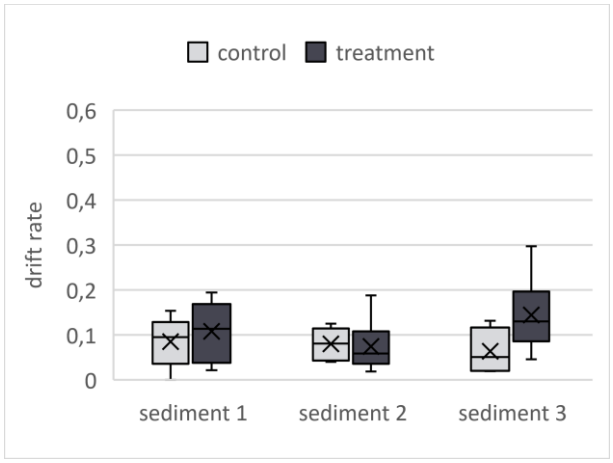
Table 9. Kruskal-Wallis test and post hoc test – comparing differences in drift rates on family level between sediments for control and treatment separately

Family	Control			
	Kruskal-Wallis	Post-hoc		
	p-value	1-2	2-3	1-3
Baetidae	0.777	1.000	1.000	1.000
Chironomidae	0.001	0.155	0.223	0.001
Elmidae	0.129	0.136	1.000	0.641
Ephemerellidae	0.007	0.005	0.351	0.351
Heptageniidae	0.762	1.000	1.000	1.000
Leptophlebiidae	1.000	1.000	1.000	1.000
Leuctridae	0.521	1.000	0.768	1.000
Nemouridae	0.058	1.000	0.120	0.136

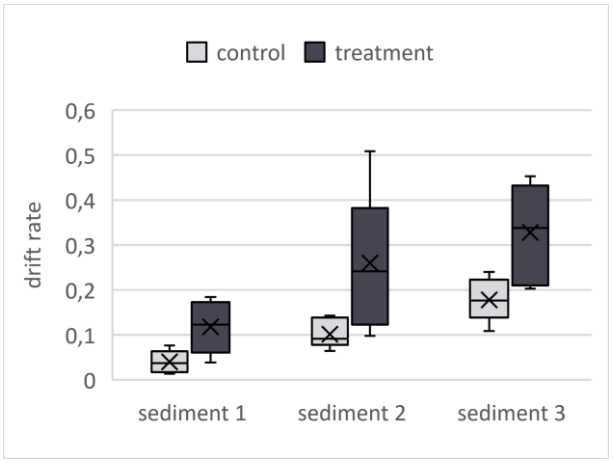
Family	Treatment			
	Kruskal-Wallis	Post-hoc		
	p-value	1-2	2-3	1-3
Baetidae	0.291	1.000	0.351	1.000
Chironomidae	0.015	0.198	0.913	0.012
Elmidae	0.045	0.838	0.039	0.479
Ephemerellidae	0.032	0.069	0.069	1.000
Heptageniidae	0.483	1.000	0.703	1.000
Leptophlebiidae	0.291	1.000	1.000	0.838
Leuctridae	0.153	0.370	1.000	0.223
Nemouridae	0.005	0.069	0.006	1.000

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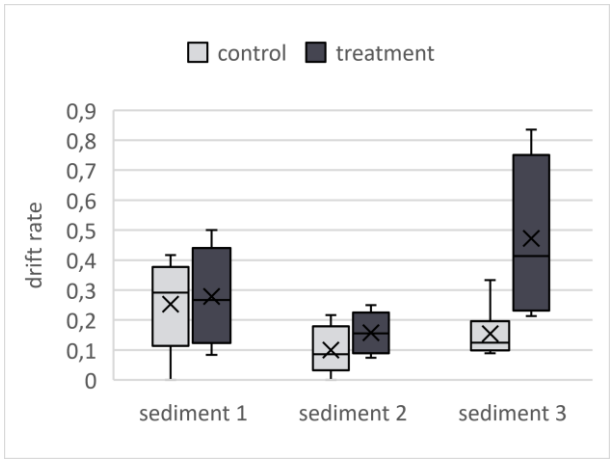
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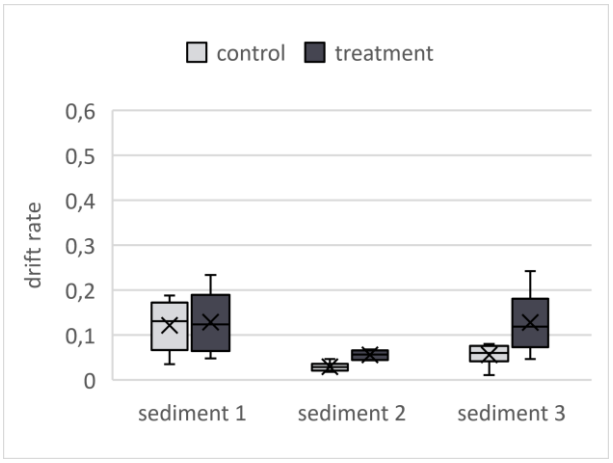
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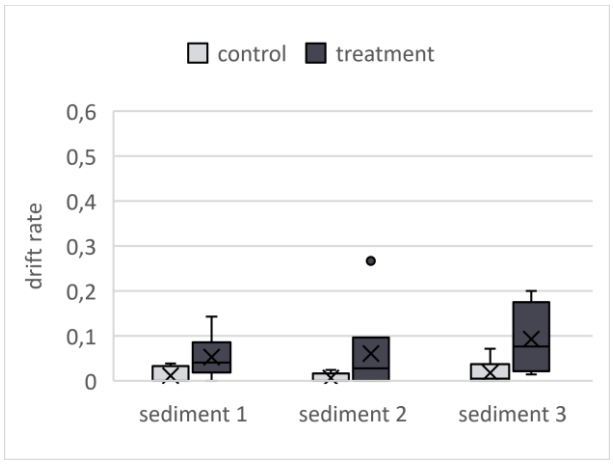
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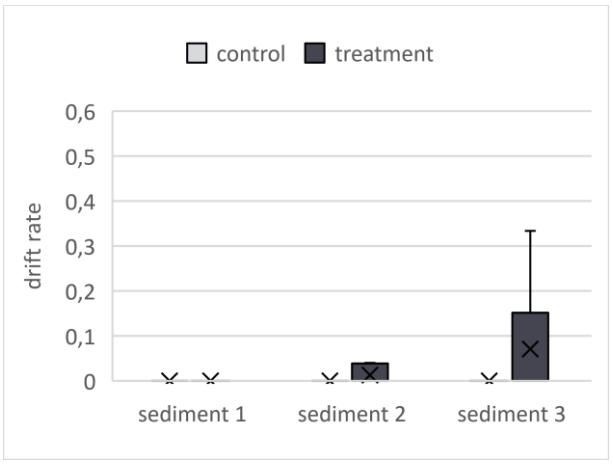
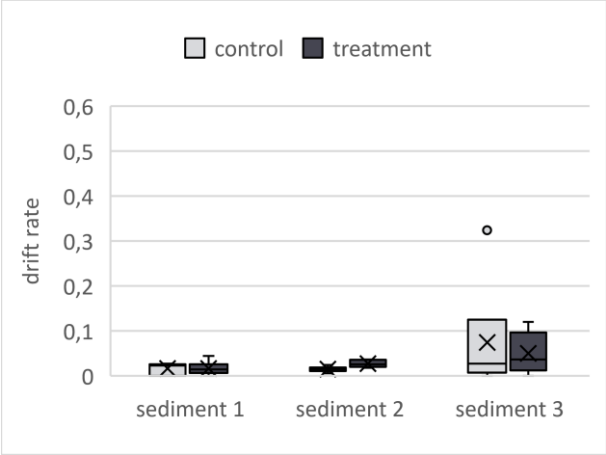


Figure 12. Relationships of control and treatment in different sediment types in each of the 11 families that had participated in statistical analysis: a) Baetidae b) Chironomidae c) Elmidae d) Ephemerellidae e) Heptageniidae f) Leptophlebiidae

g)



h)

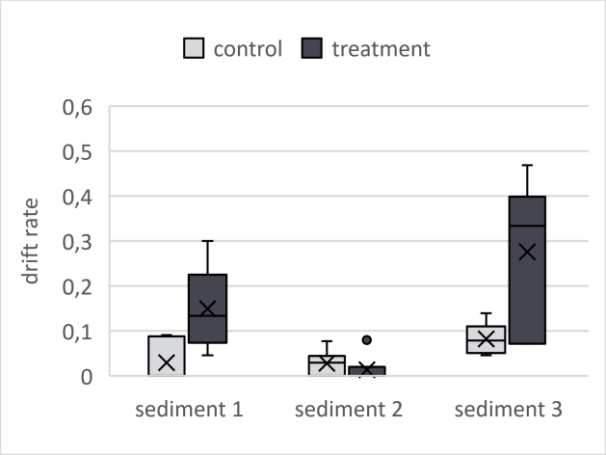
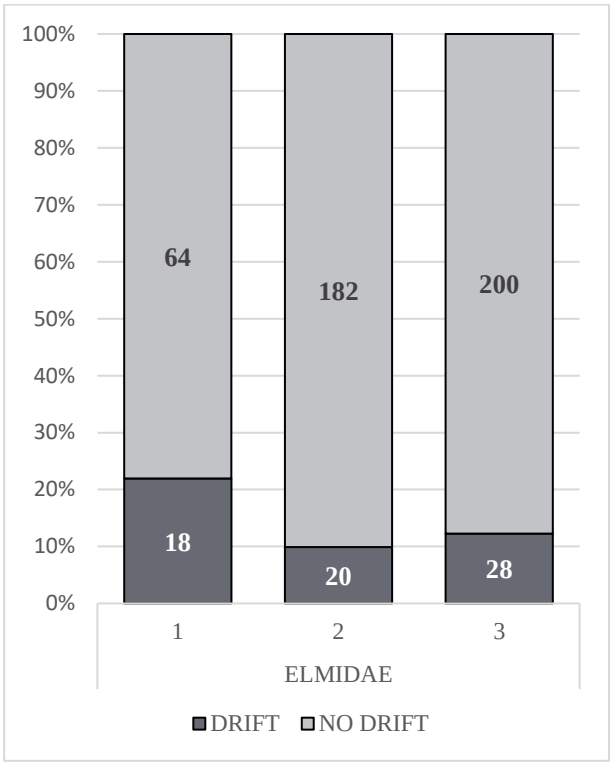
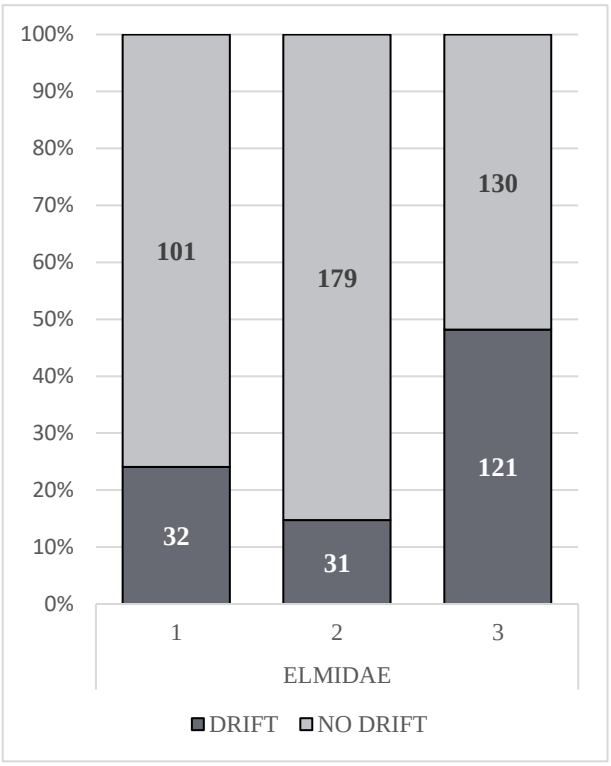


Figure 12. Relationships of control and treatment in different sediment types in each of the 11 families that had participated in statistical analysis: g) Leuctridae h) Nemouridae

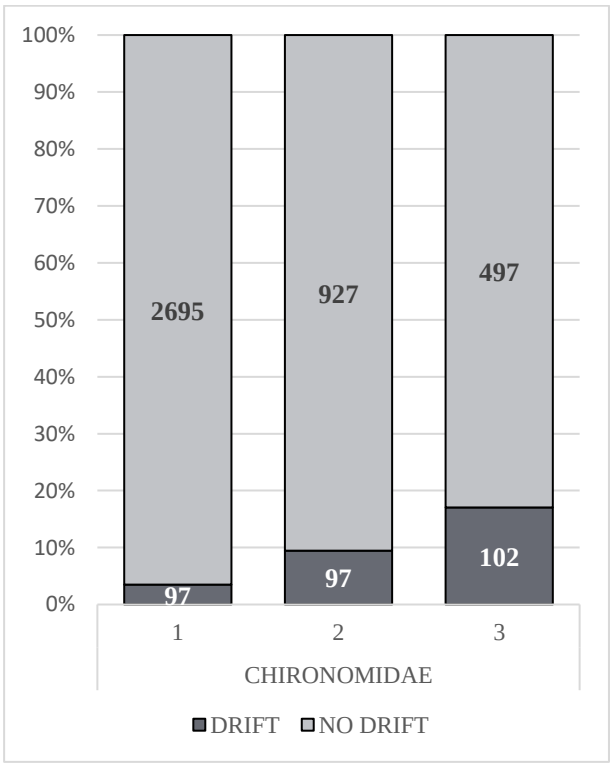
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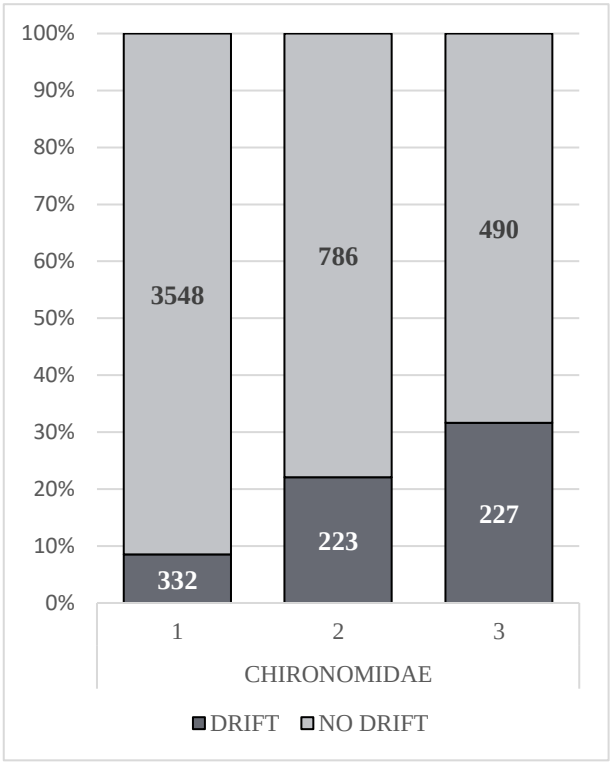
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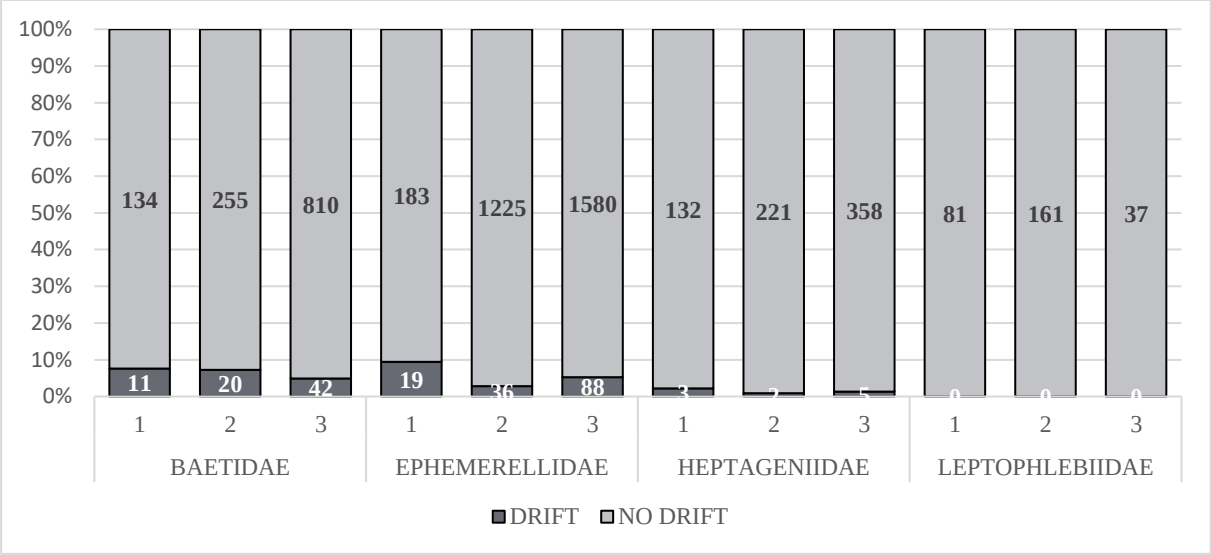
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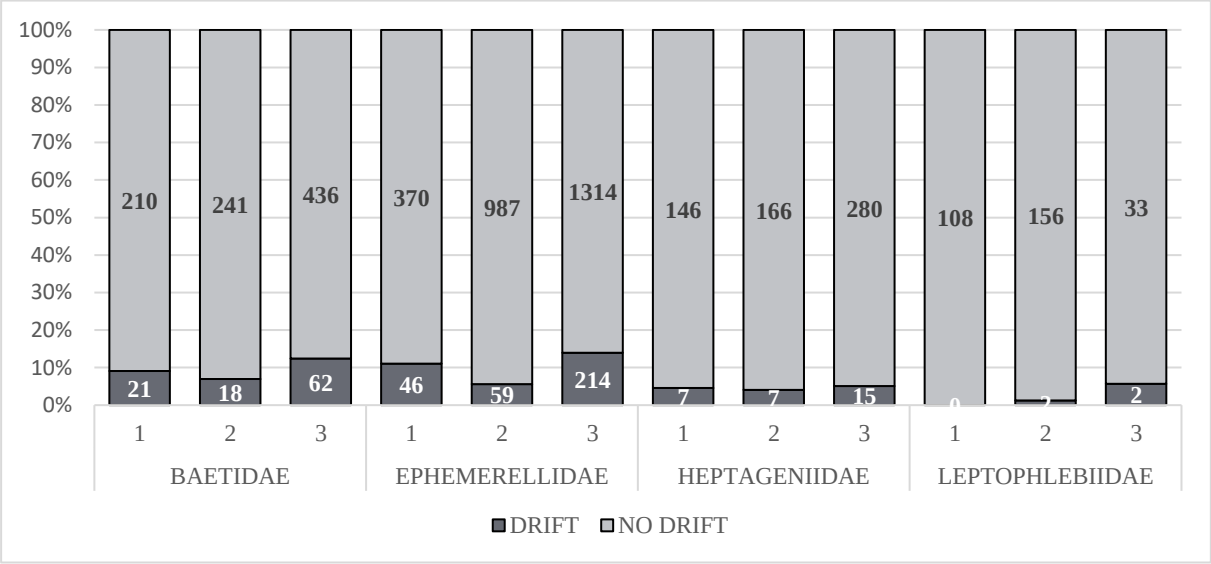
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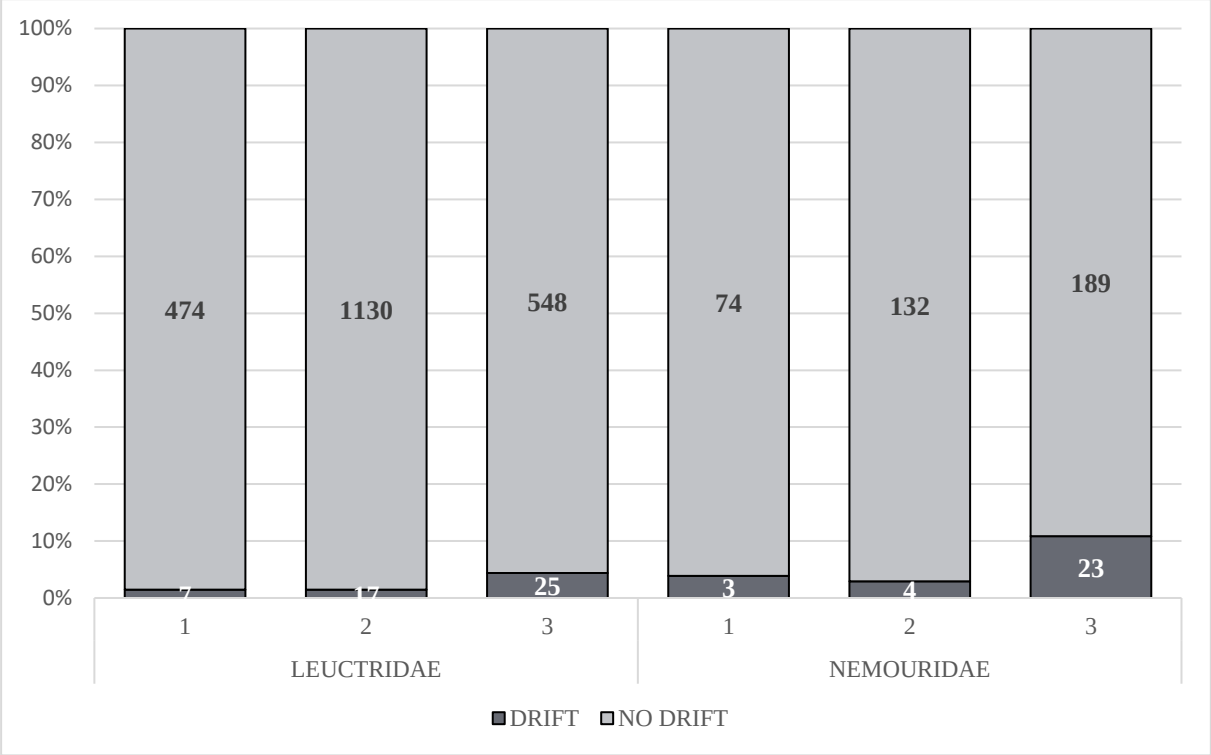
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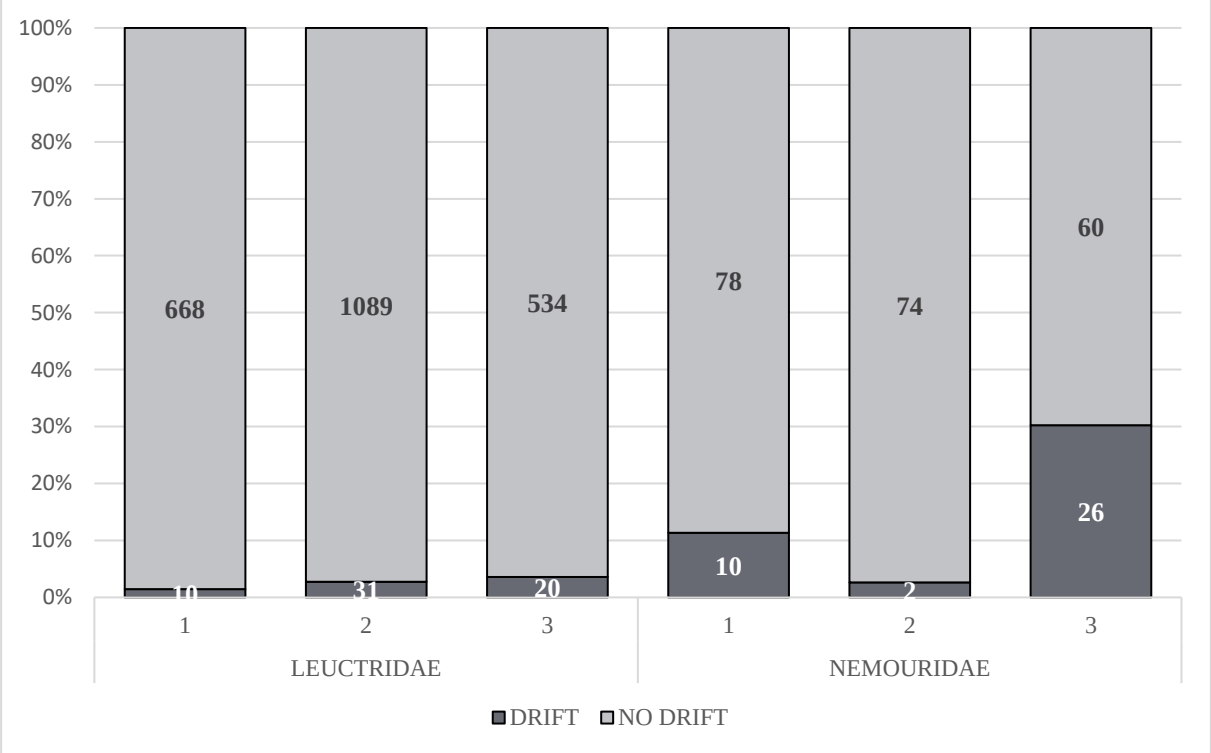


Figure 13. Drifted and non-drifted ratio of families in selected orders: a) Coleoptera control b) Coleoptera treatment c) Diptera control d) Diptera treatment e) Ephemeroptera control f) Ephemeroptera treatment g) Plecoptera control h) Plecoptera treatment

3.4 EFFECT OF HYDROPEAKING ON SIZE CLASSES IN DRIFT

3.4.1 Differences between control and treatment

To see if hydropeaking had any effect on size classes of a taxon in drift. drift rates of different size classes at order level were tested, since lower taxonomic levels were not abundant enough. Those drift rates are shown in Fig 15 and table 9 show that none of the differences between control and treatment in any size class are significant. Moreover, even in some orders, the abundance of certain size classes was too small in some experimental units, and therefore they are not shown in Figure 14 (a, b). Only a few differences between control and treatment in each sediment were significant: Coleoptera in size class B in the sediment type 3, Diptera in size class B in all sediment types as well as in size class C in sediment 1 and 3, and finally Ephemeroptera in size class C in sediment type 3 (Table 11).

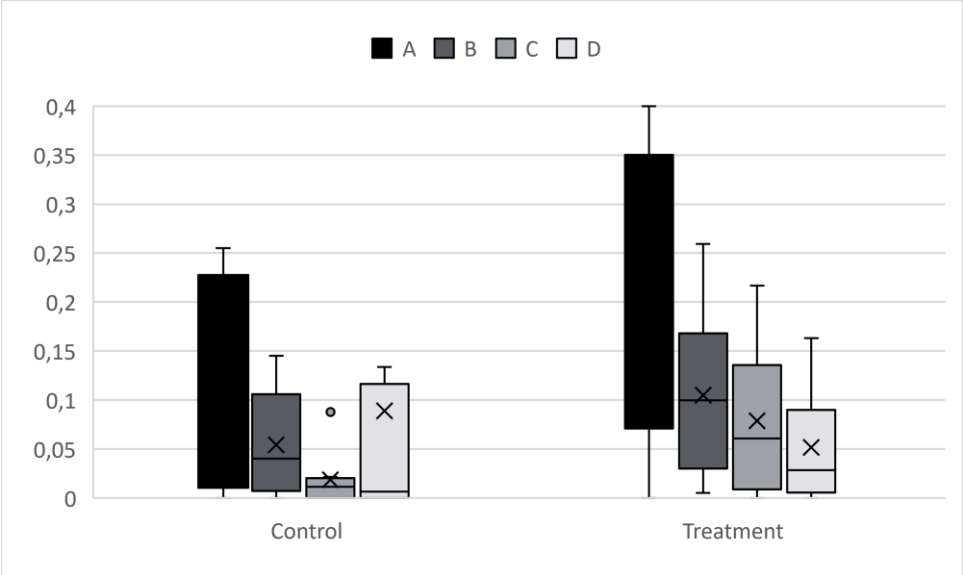
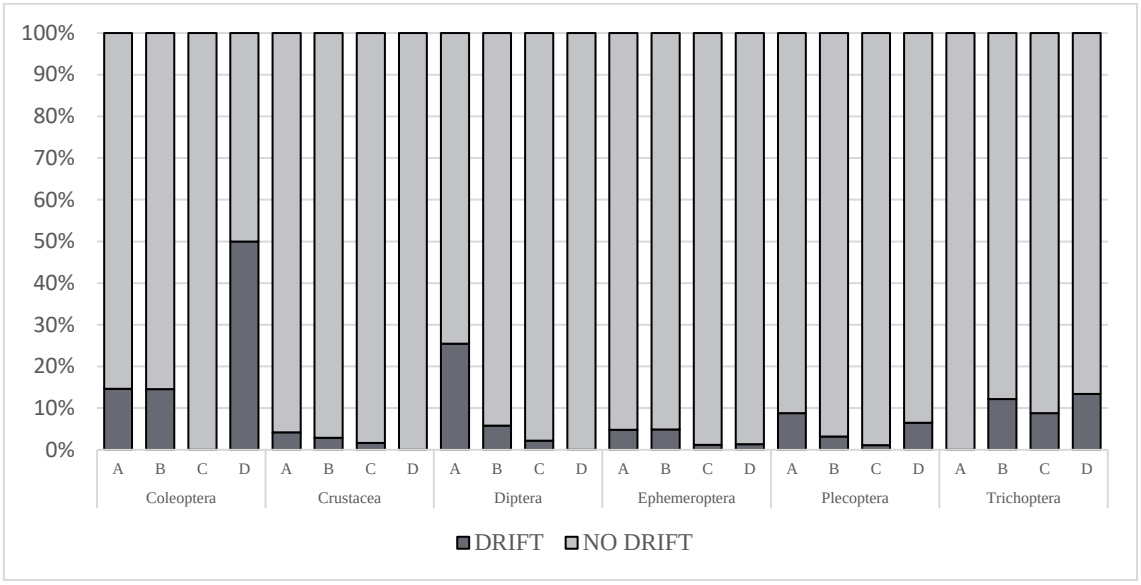


Figure 15. Comparison of control and treatment drift rates on order level size classes of total macroinvertebrate individuals (A = 0 – 2 cm; B = 2 – 5 cm; C = 5 – 8 cm; D = 8+ cm)

Table 10. Mann-Whitney U Test – comparing control and treatment units on drift rates of total abundance of each size class of macroinvertebrates (A = 0 – 2 cm; B = 2 – 5 cm; C = 5 – 8 cm; D = 8+ cm)

Size class	*p*-value
A	0.318
B	0.270
C	0.103
D	0.563

a)



b)

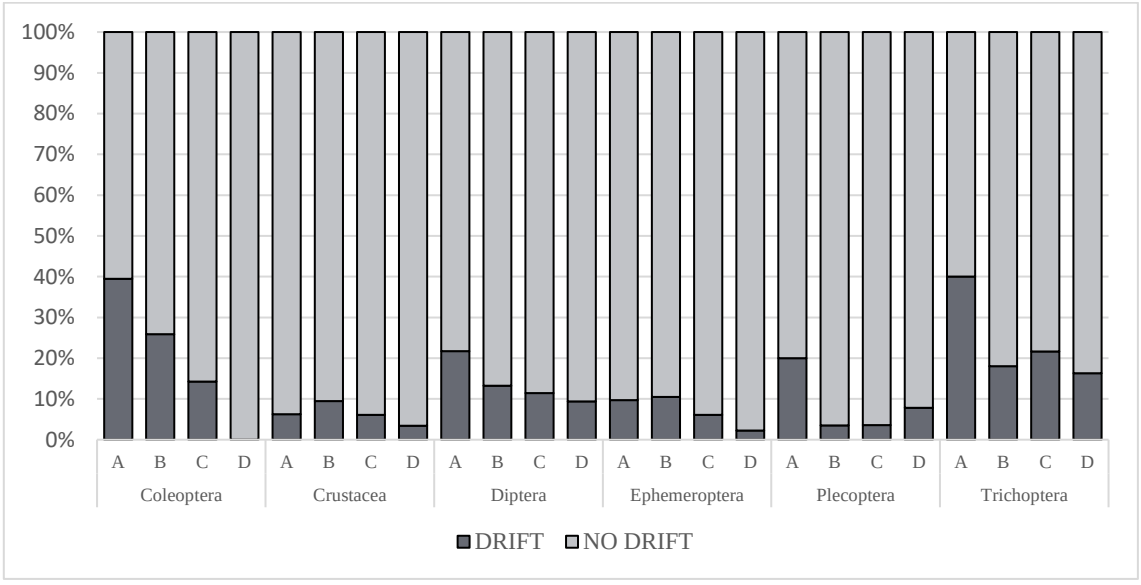


Figure 15. Drifted and non-drifted ratio in each size class (A = 0 – 2 cm; B = 2 – 5 cm; C = 5 – 8 cm; D = 8+ cm) of selected orders of macroinvertebrates in a) control b) treatment

Table 11. Mann-Whitney U Test results - comparing control and treatment in each sediment type separately using order level drift rates of each size class

Order	Sediment 1	Sediment 2	Sediment 3
	p-value	*p*-value	*p*-value
Coleoptera a	0.936	0.927	0.200
Coleoptera b	0.810	0.927	0.020
Coleoptera c	0.936	0.689	0.936
Coleoptera d	0.689	0.337	0.936
Crustacea a	0.689	0.173	0.689
Crustacea b	1.000	0.810	0.066
Crustacea c	1.000	0.936	0.689
Crustacea d	0.689	0.936	0.689
Diptera a	0.873	0.298	0.378
Diptera b	0.031	0.045	0.013
Diptera c	0.008	0.078	0.031
Diptera d	0.378	0.689	0.378
Ephemeroptera a	0.936	0.093	0.093
Ephemeroptera b	0.230	0.093	0.173
Ephemeroptera c	0.873	0.093	0.008
Ephemeroptera d	0.378	0.378	0.378
Plecoptera a	0.689	0.689	0.128
Plecoptera b	0.337	0.575	0.575
Plecoptera c	0.173	0.378	0.689
Plecoptera d	0,810	1.000	0.689
Trichoptera a	0.936	0.378	0.936
Trichoptera b	1.000	1.000	0.262
Trichoptera c	0.378	0.173	1.000
Trichoptera d	0.749	0.689	0.631

3.4.2 Differences between sediments

When testing the differences between sediments, Coleoptera in size class A, Diptera in size class B, Ephemeroptera in size class A and Trichoptera in size class C were significantly different in control units. Diptera and Plecoptera in size classes B were significant in treatment units along with Coleoptera size class B and Ephemeroptera and Plecoptera size class A, which were borderline significant (Table 12).

The post-hoc test showed that in control the difference between sediment 1 and 2 was significant in Coleoptera and Ephemeroptera in size class A and between sediment 1 and 3 in Dipera B. In treatment, the difference between size class 1 and 3 was significant in Diptera and Plecoptera of the size class B (Table 12).

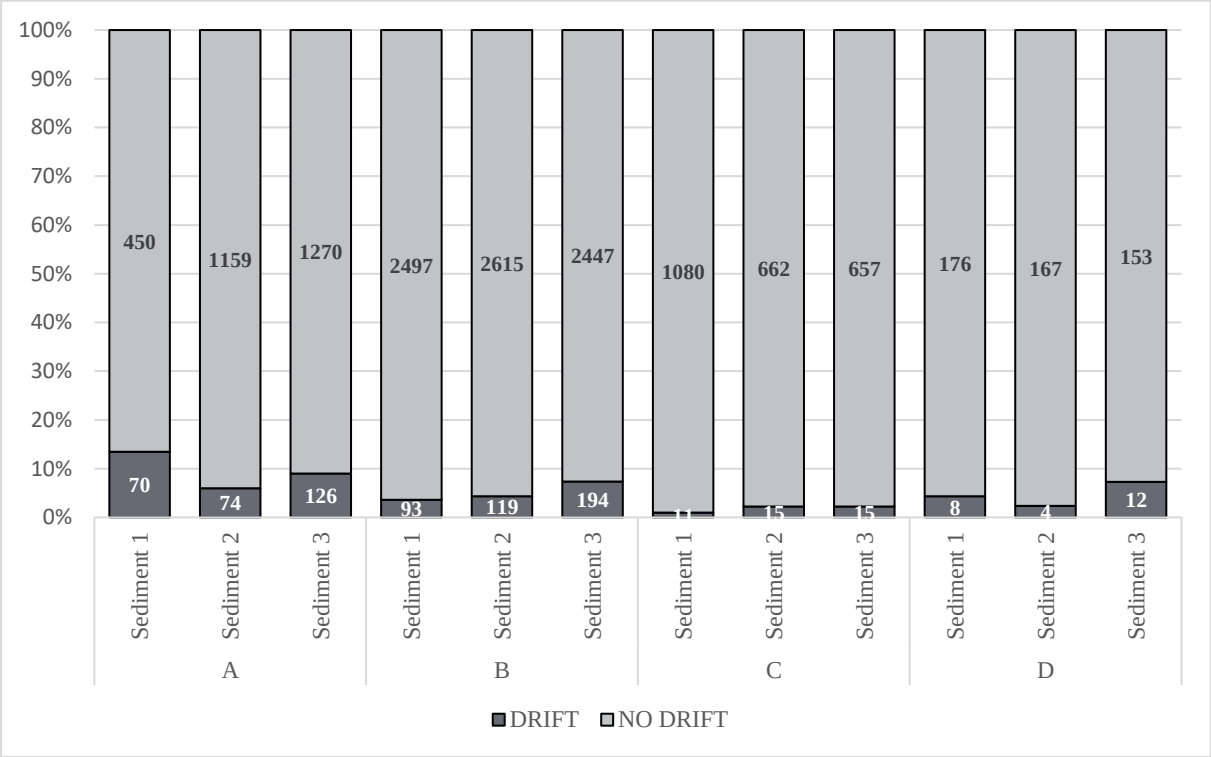
When testing for differences between sediment types in control and treatment of each size class the *p*-value was not signifficant ($p = 0.367$ for all), even though Fig. 16 suggests otherwise.

Table 12. Kruskal-Wallis test and post hoc test - comparing differences in drift rates on order level for each size class between sediments for control and treatment separately

Order	Control			
	Kruskal-Wallis	Post-hoc		
	p-value	1-2	2-3	1-3
Coleoptera a	0.022	0.028	1.000	0.105
Coleoptera b	0.581	0.913	1.000	1.000
Coleoptera c	1.000	1.000	1.000	1.000
Coleoptera d	0.368	1.000	1.000	1.000
Crustacea a	0.368	1.000	1.000	1.000
Crustacea b	0.586	1.000	1.000	1.000
Crustacea c	0.368	1.000	1.000	1.000
Crustacea d	1.000	1.000	1.000	1.000
Diptera a	0.076	1.000	0.390	0.080
Diptera b	0.002	0.155	0.314	0.001
Diptera c	0.840	1.000	1.000	1.000
Diptera d	1.000	1.000	1.000	1.000
Ephemeroptera a	0.007	0.005	0.351	0.351
Ephemeroptera b	0.473	1.000	0.803	0.951
Ephemeroptera c	0.272	0.479	1.000	1.000
Ephemeroptera d	0.120	1.000	0.991	0.991
Plecoptera a	0.120	1.000	0.991	0.991
Plecoptera b	0.113	1.000	0.120	0.529
Plecoptera c	0.291	1.000	1.000	0.838
Plecoptera d	0.586	1.000	1.000	1.000
Trichoptera a	1.000	1.000	1.000	1.000
Trichoptera b	0.986	1.000	1.000	1.000
Trichoptera c	0.035	1.000	0.433	0.433
Trichoptera d	0.954	1.000	1.000	1.000

Order	Treatment			
	Kruskal-Wallis	Post-hoc		
	p-value	1-2	2-3	1-3
Coleoptera a	0.071	0.086	0.266	1.000
Coleoptera b	0.054	1.000	0.052	0.390
Coleoptera c	0.368	1.000	1.000	1.000
Coleoptera d	1.000	1.000	1.000	1.000
Crustacea a	0.368	1.000	1.000	1.000
Crustacea b	0.070	1.000	0.433	0.155
Crustacea c	0.986	1.000	1.000	1.000
Crustacea d	0.588	1.000	1.000	1.000
Diptera a	0.130	0.136	1.000	0.735
Diptera b	0.016	0.281	0.703	0.012
Diptera c	0.716	1.000	1.000	1.000
Diptera d	0.694	1.000	1.000	1.000
Ephemeroptera a	0.050	0.080	0.136	1.000
Ephemeroptera b	0.423	1.000	0.768	0.768
Ephemeroptera c	0.222	1.000	0.583	0.314
Ephemeroptera d	0.291	1.000	1.000	0.838
Plecoptera a	0.055	1.000	0.175	0.237
Plecoptera b	0.031	1.000	0.105	0.045
Plecoptera c	0.967	1.000	1.000	1.000
Plecoptera d	0.804	1.000	1.000	1.000
Trichoptera a	0.119	0.991	0.991	1.000
Trichoptera b	0.205	1.000	0.703	0.641
Trichoptera c	0.668	1.000	1.000	1.000
Trichoptera d	0.528	0.838	1.000	1.000

a)



b)

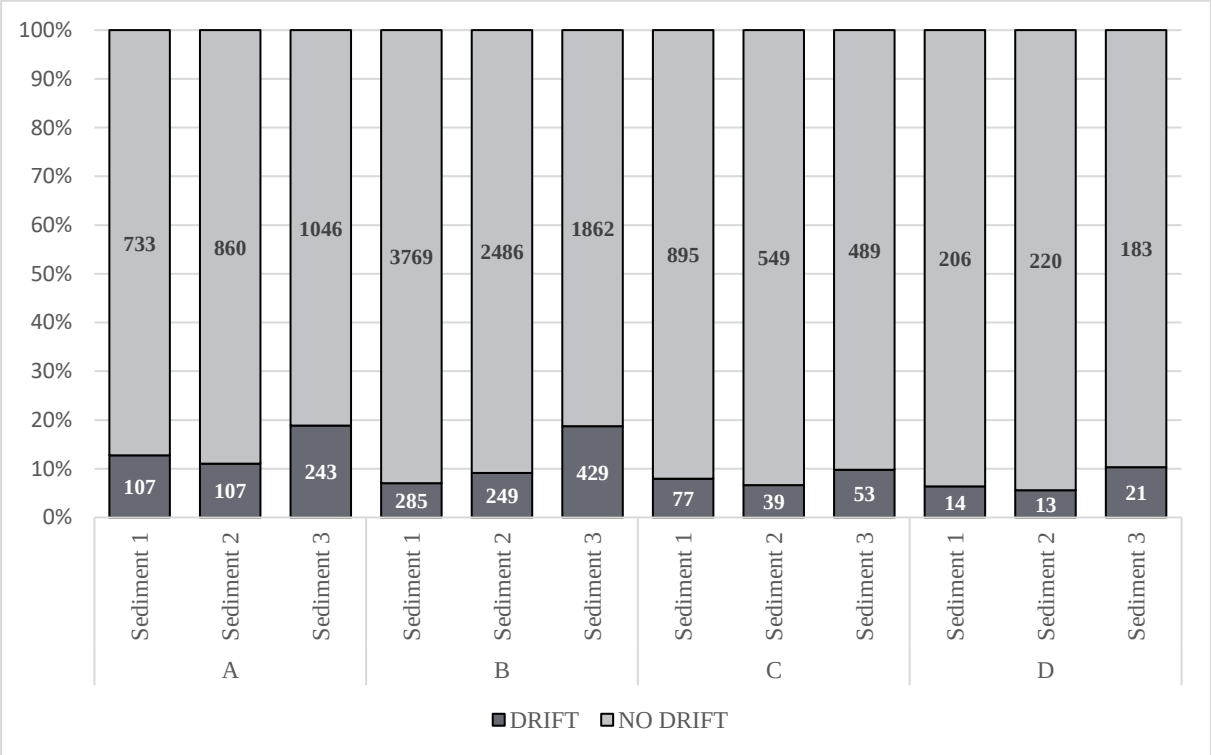


Figure 16. Ratio of drifted and non-drifted individuals of different size classes on different sediment types: a) control b) treatment

4 DISCUSSION

4.1 INITIAL RESULTS

Despite the fact that four samples were taken from several locations on the river Bodingbach for each experimental unit, it seems that the sampling locations were not similar enough. The assumption was that those locations would have a similar taxa composition and abundance since two samples were taken near the shore, and two in-stream so if the microhabitat was similar so should the taxa. However, this did not prove to be the case. That was also the reason why the abundances were transformed into drift rate to begin with, in order to make the data comparable. Nevertheless, the macroinvertebrate distribution in experimental units must have affected the results but it still remains unknown to which proportion.

4.2 DIFFERENCES BETWEEN CONTROL AND TREATMENT BOTH TOGETHER AND IN DIFFERENT SEDIMENT TYPES

4.2.1 General level

Since control and treatment samples showed a significant difference in drift rates within every sediment type, it could be that the hydropeaking increases the drift rates regardless of sediment type.

Expectations that the treatments would differ on different sediment types and the controls would not because there was no hydropeaking were not proved. There was no need for an increased drift in controls, no matter what the sediment type was, since there was no hydropeaking. The controls have a lower *p*-value so their difference among sediment types is higher than among treatments. Significant difference between medium and fine substrate in control might indicate a habitat preference of drifted animals since no increased flow could have made them drift.

4.2.2 Effects on selected taxa

Within order Coleoptera, the family Elmidae with all of its genera was the only one abundant enough to account for the differences in sediment types. Since both order and family only showed a significant difference in sediment with fine particles, we would assume they prefer finer substrate particles. Nevertheless, the differences in order were significant in both control and treatment. It is consequently rather difficult to estimate the impact of hydropeaking. Order Diptera with family Chironomidae displayed the same differences. The order as well as the family showed a significant difference in all sediment types, as well as in control and treatment alike. Therefore, we would connect all the differences to the family itself.

Crustacea only had two families, Asselidae and Gammaridae. Since Asselidae were the most abundant in coarse substrate and Gammaridae in fine substrate, the assumption is Gammaridae were the ones that made the differences shown in order Crustacea. Gammarid crustaceans commonly occur in small stream benthos, and are often observed to exhibit high drift rates along with stream insects. It has been assumed that they swim upstream to compensate for drift. They have been observed to undertake significant up-stream movements after being displaced by floods (Minekley, 1964; Waters, 1972). Since Crustacea show a significant difference only on sediment type 3., the assumption is they prefer habitats with lower particle sizes. This is also supported by Rees's findings (1972), namely that in the field *Gammarus* the preferred substrates are those with a particle size of 1.6-3.2 cm. It should also be considered that neither Asselidae nor Gammaridae had had high enough abundances in every experimental unit so it could have altered the Crustacea results.

Ephemeroptera larvae are found in a variety of locations including lakes, wetlands, streams, and rivers, but they are most common and diverse in lotic habitats (Bouchard, 2004). Ephemeroptera was the most abundant order, but it did not have any significant differences between sediments in treatment. Interestingly enough, in control they did. Since they did not show a significant difference in sediment with medium particles, this sediment could suit them so much that they do not even feel the effects of hydropeaking.

Plecoptera exhibited differences between sediment types in treatment as well as in control, so we cannot conclude that hydropeaking had increased the drift, sice they also drifted a lot in control with base flow. None of the sediment types showed a significant difference between control and treatment, which is not a surprise considering their usual habitats are rocky, stony or gravel substrata. Leuctridae and Nemouridae were the most abundant families in that order.

Trichoptera exploit a diversity of microhabitats thanks to the many ways silk is used to construct retreats, nets, and cases. Their silk probably accounts for the success of the order as a whole (Mackay and Wiggins 1979, Wiggins 1996). Even though not all members have this adaptation to increased flow, it might explain why they had no significant differences in any of the analysis.

Some families are known to be good swimmers like Baetidae (Hefti et al. 1989, Weninger 1968), so the hydropeaking flow might not have been strong enough to make them drift more. *Baetis*, the most frequent genus in the family Baetidae in this experiment, is usually very abundant in drift. They are also very good swimmers and they have a streamlined body (Poff, 2006), so they might not have entered the drift in higher numbers when the hydropeaking occurred. Therefore, they showed no significant difference in any of the analyses.

Family Chironomidae is described as the most susceptible of all drifting invertebrate groups to spate (Anderson and Lehmkuhl 1968; Jones 1951; Richardson 1928), since the larvae, once dislodged and in the water column, are incapable of a rapid return to the substrate (Elliott 1971b; Elliott and Bagenal1972). Expectations that they would be present in drift in high numbers were confirmed. Poff (2006) claims they are abundant, if not dominant in drift samples. Even though they form a large proportion of the drift, their numbers are usually small in comparison with the numbers left in sediments (Davies, 1976), which corresponds to the number of not drifted Chironomides in this experiment. Nevertheless, drift is usually not a big problem for the Chironomidae, because larvae compensate for downstream drift by means of non-random positive directed behaviour against a current called rheotaxis (Bishop and Hynes 1969b; Elliot 1971c).

Members of the family Elmidae are usually common in drift, maybe because they cannot swim. Moreover, they are not streamlined and are smaller than 9mm in size (Poff, 2006). Adults can be found crawling on stones and wooden debris in the riffle zones of freshwater streams. Some occur in the depositional zones of streams, on softer sediments, and some are amphibious and feed along the streams banks. Larvae are strictly aquatic, but otherwise share the same habitats as adult ones. (Brown, 1991; McCafferty, 1983; White and Brigham, 1996). This might explain why they drifted more in sediment with fine particles; which might not have been fine enough for Elmidae, especially combined with increased flow. The significant difference in treatment units proves that hydropeaking caused the increased drift of the family.

Even though family Ephemerellidae is found in all sizes of flowing streams on different types of substrates where there is reduced flow, the only sediment they did not show significant

difference on was the substrate with medium size particles. Therefore, we could conclude that the mixture of sediment particles did not suit the family members, possibly due to the fact they are often found on leaf packs, and the smaller particles might not have held the leaves in place.

Some Heptageniidae larvae are poor swimmers, so they should have been present in drift in greater numbers in hydropeaking treatments. However, they have an adaptation to high flow with their flattened bodies, which allow them to cling to rocks (Macadam, 2004). Mayflies of the family Heptageniidae are widespread in streams with abundant firm substrate, often found on cobbles, submersed vegetation or logs and leaf packs. Since they only showed a significant difference on finer substrate, the assumption would be that this sediment type does not suite them because it is not firm enough, or that in this experiment something yet unknown had made them drift more on this sediment mixture with increased flow.

Leptophlebiidae larvae occur in a variety of habitats including lakes, ponds, and swift and slow flowing streams alike. They are found on rocks and gravel, leaf packs, and submerged roots (Bouchard, 2004). Since they did not show a significant difference in any of the sediment types, or even when comparing control and treatment units, we assume they tolerate a wide range of particle substrate sizes, or have developed some very effective adaptations to hydropeaking (Bouchard, 2004).

Leuctridae are vulnerable to dislodgement. A relative abundance decrease occurs in patches of high mean flow while their typical microhabitats are low-flow microenvironments. They have a gill- less, long thin body shape, which makes them well adapted to life in interstitial spaces between stones, where they feed on fine particles of detritus and algae (Lancaster and Waldron, 2001). They probably rarely encounter high shear stress (Lancaster and Belyea, 2006). Nevertheless, they showed a significant difference only on coarser substrate. Since they tend to hide from increased flow in the substrate, we assume the interstitium in treatment units of that sediment type was accidentally clogged with algae or fine sediment from the lake, which made them drift more on that sediment.

Nemouridae are most frequently encountered in leaf packs and in riparian debris in fast water with a coarse substrate (Key to Aquatic Macroinvertebrates in Utah, n.d.) so the fact that they showed a significant difference in medium sized substrate could be explained by that sediment being too fine for them. They also displayed a significant difference when comparing treatments between sediment types, so the conclusion would be that the hydropeaking made them drift differently on different substrates even though they live in fast waters.

4.3 EFFECT OF HYDROPEAKING ON SIZE CLASSES IN DRIFT

It was not able to detect clear effects of hydropeaking on size classes in drift. Animals in some size classes were just not abundant enough even at order level, which could have altered the final results. Some species never outgrow some size classes, so they do not occur in other size classes like for example Caenidae that grow up to 8mm, so they do not occur in size class D (Bouchard, 2004). Some belong to a different size class only during some seasons (life cycles) and therefore not all size classes of a taxon were present when sampled. Not all size classes of an order were present in the same abundance. That is why it would be hard to make any assumptions and draw any conclusions from the results of the size class analysis, even though there have been reports from the field that specific size distributions in relationship to flow are caused more or less directly by flow forces (Statzner 1981, 1989; Statzner and Bittner 1983).

4.4 EXPERIMENT IMPERFECTIONS

There is an infinite number of factors and possible problems that need to be considered when conducting any experiment. They cannot all be addressed. This experiment certainly had some imperfections. However, it showed some shortcomings that could be prevented in future experiments, like higher abundance per experimental unit as well as more experimental units to get more confident results. Another improvement would be a more homogenous sampling in order for the data to be more comparable. The algae that came with the water from the lake could also have been a factor that increased or decreased the drift by clogging the interstitium or by tangling individuals. This could be solved using bars or nets that would stop the strings of algae before they reach the mesocosms. Due to all these reasons, it is hard to draw definite conclusions. Nevertheless, our experiments have shown some results with significant differences so those can be a starting point for new experiments.

To summarise:

Research question 1: Is the hydropeaking-induced drift influenced by the different roughness of the bottom? Hypothesis that the drift will be lower in coarse substrates because animals will find shelter easier there, and the shear stress will be lower proved to be true.

Research question 2: Are there species-specific drift patterns related to bottom roughness? Hypothesis that the drifted taxa will differ on different sediment types because they live on different substrata and in diverse conditions, so they have developed different adaptations as well, also proved to be true. To what extent and the reasons why still have to be explained.

Research question 3: Does bottom roughness affect size classes of drifted organisms? Hypothesis that we will find mostly smaller sizes of different taxa in the drift, because smaller specimens can hide in the interstitium more easily. However, the fact that they are more numerous than larger specimens, it is expected they could enter the drift purely by chance, was also taken into consideration. Furthermore, the fact that they might drift on purpose as a way of dispersal and colonization of new habitats was also considered. This hypothesis could not be confirmed or rejected because only a few orders showed a significant difference in some size classes.

5 CONCLUSION

- Significant differences have been found between control and treatment experimental units when testing total drift numbers, which shows that hydropeaking increases drift regardless of sediment type.
- Significant differences have also been found between control and treatment units in different sediment types, which shows that substrate roughness affects drift rates.
- Some taxa exhibited significant differences in drift rates on different sediment types but the reasons for those differences are still not determined.
- The differences between sediment types when testing different size classes have not been found neither in control nor treatment units.
- Other factors were tested in similar experiments in this facility. Some of them, like temperature, showed a greater impact on drift. Therefore, we would conclude that the sediment type has a minor effect on the drift in comparison with other factors. More studies are required in order to test the connections between sediment types and increased drift resulting from hydropeaking.

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7 CURRICULUM VITAE

PERSONAL INFORMATION

Una Hadžiomerović

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Sex Female | Date of birth 10/02/1992 | Nationality Croatian

WORK EXPERIENCE

26/05/2017–Present

Guest relation and guest experience agent

EX-ALTO, Zagreb (Hrvatska)

Zavrtnica 17/1, 10000 Zagreb (Croatia)
http://www.ex-alto.hr/?lang=en

Business or sector experiential marketing & tourism services

15/06/2015-01/09/2015
15/07/2014-01/09/2014
04/07/2013-01/09/2013
01/07/2012–01/09/2012
06/07/2011–20/08/2011
23/07/2010–01/09/2010

Tourist entertainer

EX-ALTO
Zavrtnica 17/1, 10000 Zagreb (Croatia)
http://www.ex-alto.hr/?lang=en

Business or sector experiential marketing & tourism services

2010–Present

Promotor, Moderator, Hostess

EX-ALTO
Zavrtnica 17/1, 10000 Zagreb (Croatia)
http://www.ex-alto.hr/?lang=en

Business or sector experiential marketing & tourism services

EDUCATION AND TRAINING

2014–Present

Obtaining Master's degree in Biology (ECOLOGY AND NATURE PRESERVATION, MODULE FRESH WATER)

Faculty of Science of the University of Zagreb – Department of Biology Horvatovac 102A, 10000 Zagreb (Croatia)
https://www.pmf.unizg.hr/en

15/02/2016–15/07/2016

Erasmus plus internship

BOKU (Universität für Bodenkultur Wien) - Department für Wasser-Atmosphäre-

Umwelt (WAU) -Institut für Hydrobiologie, Gewässermanagement (IHG) – (Department of Water - Atmosphere - Environment (WAU) - Institute of Hydrobiology and Aquatic Ecosystem Management (IHG)) Gregor-Mendel-Straße 33
1180 Beč (Austrija)
Tel. +43/1/47654 - 0

2014 Erasmus student exchange – summer semester
UPM (Universidad Politehnica de Madrid) – ETSI Agronomos
Campus Ciudad Universitaria
Avenida Complutense 3
28040 Madrid, Spain
91 336 56 00
http://www.upm.es/internacional

2013 Internship – summer semester
Faculty of Science of the University of Zagreb - Division of Zoology

2010–2014 univ.bacc.biol. (Bachelor degree in Biology)
Faculty of Science of the University of Zagreb – Department of Biology
Horvatovac 102A, 10000 Zagreb (Croatia)
https://www.pmf.unizg.hr/en

2006–2010 High School II. gimnazija
4, Križanićeva, 10000 Zagreb (Croatia)
http://www.gimnazija-druga-zg.skole.hr/

PERSONAL SKILLS

Mother tongue(s): Croatian

Other language(s)

	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken interaction	Spoken production	
English	C2	C2	C1	C1	C1
German	C1	C1	B2	B2	B2
	DSD B2/C1				
Spanish	A2	A2	A2	A2	A2
	Programa de lenguas para la inernacionalizacion, nivel A2 del MCERL				

Levels: A1/A2: Basic user - B1/B2: Independent user - C1/C2: Proficient user
Common European Framework of Reference for Languages

Communication skills	good communication skills gained through seven years working as a touristic entertainer (teamwork, moderation, promotion)
Computer skills	Microsoft Office™
Other skills	DNA extraction electrophoresis PCR phase contrast microscopy epifluorescence microscopy dance aqua-aerobics

ADDITIONAL INFORMATION

Conferences	ICEB (International Conference on Evolution and Behaviour) 27. – 29.11.2015 ISCES'15 (International Student Conference on Environmental Sciences) 13. – 15.11.2016 ICEB (International Conference on Evolution and Behaviour) 17. - 19.10. 2014
Memberships	Aegee (**A**ssociation des**É**tats**G**énéraux des**É**tudiants de l'**E**urope)
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