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Water Lice and Other Macroinvertebrates in Drinking Water Pipes: Diversity, Abundance and Health Risk

Günter Gunkel [1,2,*], Ute Michels [2,3] and Michael Scheideler [2,4]

[1] Department of Water Quality Control, Berlin University of Technology, 10623 Berlin, Germany
[2] INWERT Institute for Biological Drinking Water Quality, 45721 Haltern am See, Germany; utemichels@aqualytis.com (U.M.); ms@scheideler.com (M.S.)
[3] AquaLytis, 15745 Wildau, Germany
[4] Scheideler Dienstleistungen, 45721 Haltern am See, Germany
* Correspondence: guenter.gunkel@water-quality-control.de

Abstract: Activities to ensure and maintain water quality in drinking water networks, including flushing, are presented after standardized hydrant sampling combined with a stainless-steel low pressure–high flow rate (NDHF) filter and a 100 µm mesh size was used to separate pipe inhabitants. A databank of more than 1000 hydrant samples in European lowland areas was developed and used to analyze the diversity and abundance of macroinvertebrates in drinking water networks. Load classes for water louse (*Asellus aquaticus*) and oligochaetes are given with three evaluation classes: normal colonization, increased colonization, and mass development. The response of *Asellus aquaticus* in drinking water networks to environmental conditions are presented as are their growth and reproduction, promotion of a third generation by climate change effects, food limitations, and the composition and stability of their feces. Finally, the health risks posed by dead water lice and water lice feces with bacterial regrowth and the promotion of microbe development on house filters are analyzed.

Keywords: drinking water quality; drinking water pipes; biological stability; *Asellus aquaticus*; oligochaete; climate change effect; biofouling

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Citation: Gunkel, G.; Michels, U.; Scheideler, M. Water Lice and Other Macroinvertebrates in Drinking Water Pipes: Diversity, Abundance and Health Risk. *Water* **2021**, *13*, 276. https://doi.org/10.3390/w13030276

Academic Editor: Avi Ostfeld
Received: 23 December 2020
Accepted: 20 January 2021
Published: 24 January 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Drinking water quality and acceptance by consumers is highly important to human welfare, and strategies for providing drinkable tap water are associated with sustainable environmental policy. These strategies include the protection of raw water, advanced drinking water treatment, and good maintenance of piping networks. House filters with <100 µm mesh size retain many invertebrates but have a risk of microbial contamination [1]. Alternatives such as end of pipe technologies with nano-filters are not acceptable for water consumers due to environmental eco-balances.

Control and evaluation of drinking water quality is accomplished by physical-chemical criteria and, increasingly, by hygienic-pathogen criteria. However, the ongoing occurrence of small invertebrates in drinking water networks and their effects on water quality have been a topic of discussion over the last ten years [1–3]. Water quality is influenced by the development of biofilms embedded with harmful bacteria, as well as species diversity, abundance, biomass, and deposition of water lice feces pellets. Accordingly, large increases in invertebrate abundance leads to increases in crude and fine particulate organic matter (POM; this means living animals and their feces, and dead animals) and increases in microbe abundance [4,5]. Indeed, this influences the biological stability of drinking water [6,7] as well as biological drinking water quality [8].

The biological stability of drinking water describes the processes that occur in distribution networks and impact drinking water quality; accordingly, the goal of biological stability is minimum change in water quality during transport processes within distribution networks. In 2006, the World Health Organization (WHO) pointed out the importance of biological stability of drinking water in the context of microbiological safety. Specifically, they stated that water entering distribution systems should be ideally biologically stable [9,10]. A comprehensive assessment of biological stability is given by [7]. Biological stability is a complex, multifactorial process that encompasses interactions between water temperature, ingredients, materials, the structure of pipes, biofilm formation and colonization by invertebrates. Key parameters for the evaluation of the biological stability of drinking water are assimilable organic carbon (AOC) and biodegradable dissolved organic carbon (BDOC). Current research approaches include studies of the development of biofilms in pipe networks [11], functions of invertebrates in pipe networks [3,12], microbiology [5,13], and optimization of flushing methods [14,15].

The concept of "biological drinking water quality" describes colonization of drinking water networks by invertebrates, the food web and interactions with microorganisms of the biofilm (bacteria and fungi), and the dissolved and particulate organic constituents of water (DOC, particulate organic carbon POC) as food resources for the invertebrates [12]. POC is formed by the introduction of materials from water treatment plants, water lice feces and iron/manganese oxidizing bacteria. This organic material together with iron and carbonate precipitation and sand form the deposits in the pipes (Figure 1).

Figure 1. Drinking water pipe (normal diameter 200) gray cast iron pipe as habitat for invertebrates, typical are incrustations and biofilm coating.

Groundwater used as drinking water generally possesses low levels of nutrients; however, some groundwater inhabitants are only occasionally flushed out into drinking water treatment systems, after which they can migrate into drinking water distribution systems. These groundwater organisms are characterized by high sensitivity to water heating, are adapted to low nutrient levels and have small growth rates [16,17]. Thus, drinking water quality problems associated with such organisms are rare. In contrast, surface water and bank filtration water possess higher nutrient levels and therefore pose a greater risk of migration of surface water organisms into water treatment and distribution systems [18–20]. In temperate climate zones, many freshwater invertebrates are characterized by high tolerance to environmental conditions, fast growth (e.g., several generations per year) and high fertility. When these invertebrates enter drinking water distribution systems, they can survive and reproduce in the pipes, which leads to impacts on drinking water quality [21].

Most species found in drinking water systems are typical freshwater organisms that do not occur in raw water; thus, other mechanisms for their spread must be considered [3]. Introduction of freshwater macroinvertebrates can occur via sand filter outflow but only a few (small) animals can pass through a sand filter [22]. More important routes of entry seem to be via maintenance, construction of drinking water pipes, leakage of pipes without enough pressure to avoid the introduction of raw water and through mobile hydrant standpipes. Conversely, the introduction of midge larvae (chironomids) into drinking water networks occurs primarily via entry of adult animals through incompletely sealed drinking water tanks.

Overall, the frequency of macroinvertebrate introduction into drinking water networks is rare and a secondary problem. The main problem associated with these organisms is their propagation within the pipes.

The occurrence of invertebrates in drinking water networks has been known for at least 100 years, when reported that small water lice (*Asellus aquaticus*) and freshwater shrimps (*Gammarus pulex*) entered homes via their taps [23]. In 2004, the WHO suggested that the presence of animals in drinking water systems may affect the microbiological quality of water. The WHO also mentioned the significance of invertebrates and the risk of hosting parasites, but data regarding these issues are currently insufficient. A similar evaluation was conducted in Germany [20] in a Technical Report that reported that "the occurrence (of invertebrates) reduces the enjoyment capability and leads to disgust feeling by many consumers", and the "need for action is always given, when increased abundance of invertebrates are visible".

Macroinvertebrates, which normally occur in low numbers, but are visible with the naked eye, also have an esthetic impact on drinking water quality. Although the occurrence of macrofauna in tap water generally does not pose a direct health risk, their presence leads to a strict refusion of the water and may have indirect harmful effects such as microbial regrowth.

Adverse effects of the consumption of piped drinking water by humans generally occurs as a result of bacteria such as *Pseudomonas* spp. [24], *Escherichia coli* [25] and *Legionella pneumophila* [26], all of which are linked with invertebrates in drinking water networks. One mechanism for such adverse effects is microbial colonization of the guts of invertebrates [27–29]. In such cases, harmful microbes are protected from chlorination of drinking water [28].

Therefore, this study investigated the diversity and abundance of macroinvertebrates in drinking water networks, focusing on the life cycle of *Asellus aquaticus* and their reproduction, as well as the health risks they pose with the goal of developing guidelines/limiting values.

2. Methods

Studies were conducted to investigate methods for control of water lice populations in drinking water pipes, including (1) development of an effective flushing method with the CO_2 flushing method [30], (2) investigation of the impact of water lice and their feces pellets on drinking water quality, mainly the microbial contamination by feces and dead animals on house filters [1], and (3) monitoring invertebrates in drinking water pipes by hydrant sampling using a standard method with 1 m^3 sample volume, a flushing rate of 1 m s^{-1} and unidirectional separation of the pipe section, and the outflow [31].

2.1. Laboratory Experiments

Laboratory experiments were conducted to analyze the stability of *Asellus aquaticus* feces pellets exposed on glass frit filters of 12 mm diameter filled with water up to 5 cm, and three replicates for successive sampling of the feces. Briefly, filters were subjected to flow-through of Berlin tap water at a low flow rate for a period of up 6 weeks, after which samples were taken for scanning electron microscope (SEM) analyses. Berlin tap water has the following characteristics: non-chlorination, pH = 7.4–7.6, conductivity = 600–790 µS cm^{-1}, temperature = 11.7–14.6 °C, Ca = 2.2–2.5 mmol L^{-1}, TOC = 4.3–5.6 mg L^{-1}.

To study the growth of bacteria in the presence of dead water lice and water lice feces, a series of commercial house filters (Honeywell F 67S) were installed in the Berlin laboratory building of the Berlin University of Technology and subjected to continuous tap water flow-through. The house filters consisted of 100 µm stainless steel filters without nano silver coatings and a valve for cleaning/sampling. Four trial approaches were carried out, flow-through of tap water as reference (>1 L min^{-1}), flow-through of tap water with a cleaned house filter, stagnant period after 2 months filter use, stagnant period after addition of 5 dead water lice. Samples were taken after 1, 3, 7 and 10 days. Bacterial growth was evaluated at 22 °C and reported as colony forming units (cfu) according to the German Standard DIN 5667-3. Sampling and analyses were carried out by the State Laboratory Berlin-Brandenburg (LLBB) State Institute, Berlin.

Leaching of *Asellus aquaticus* feces was studied using liquid chromatography coupled with organic carbon detection (liquid chromatography–organic carbon detection, LC-OCD) [32] to detect high molecular weight compounds such as polysaccharides (biopolymers), humic substances, and low molecular weight compounds (acids and neutral substances).

2.2. Sampling of Invertebrates

Drinking water networks located in the European lowland were analyzed using a standardized sampling procedure in which hydrants with a 1 m^3 water flow (flushing rate 1 m s^{-1}; [20] that was unidirectional and provided by a separated pipe section were sampled. Studied pipes were of ND 80–150 mm, and 1 m^3 hydrant sampling corresponded to 200 m to 60 m pipe length. All pipe types were sampled, but no significant effects of pipe material on invertebrate settlement was observed. Other parameters such as pipe age, flow rate dynamic (day/night cycle), pipe course, accumulation of detritus, and water temperature seems to be much more important for population regulation, but these data were not available.

Hydrant sampling comprising more than 1000 samples was conducted in 2009–2018. Samples were collected from conspicuous and non-conspicuous drinking water networks as well as from drinking water monitoring networks for several years.

At total of 157 different hydrants were sampled in 13 different drinking water networks (12 in Germany, 1 in the Netherlands) corresponding to about 25 different drinking water supply systems (one drinking water network can consist of several drinking water treatment plants which feed into the network). Water resources were mostly groundwater. Chlorination of drinking water was not done.

A further classification of the sampled pipe sections could not be conducted because many of the related parameters such as intensity of pipe maintenance, position of the hydrants (on side/top of the pipe), incrustations and intensity of biofilm, and variations in flow rate and direction among else caused by firefighting were not known.

A low-pressure high flow-through stainless-steel filter was used to separate the invertebrates without causing damage. The mesh size was 100 µm for macroinvertebrates and 25 µm for the detection of all pipe invertebrates. The capacity of the filter was 100 m^3 h^{-1}, which enabled a high flow rate during sampling. The final filtrate volume was restricted to less than 300 mL [31].

The number of invertebrates were calculated as empirical p-quantile statistics with the 10%-percentile (=10% of the samples), median (=50% of the samples) and 90%-percentile (=90% of the samples). Significance analysis of the co-relationships was done by using the Pearson function.

2.3. Invertebrates Analyses

Macroinvertebrates, which are distinguishable with the naked eye, are classified as >2 mm in size. In this study, macroinvertebrates were determined under optical magnification (Olympus SZX 16 and SZ 40, Olympus Hamburg, Germany), measured and counted. Systematic analysis was generally conducted to the species level (but not for oligochaetes, simuliides and springtails). For water lice, the size, sex, molting stages of females, and egg and embryo number were recorded. The results were used to build up a databank with 1039 data sets that was used to evaluate invertebrate abundance.

Analyses by scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) were used to evaluate the composition of *Asellus aquaticus* feces (Hitachi S 2700 electron microscope, IDFix hard and software from SAMx, Hitachi Krefeld, Germany). For analyses, samples were sputtered with gold or carbon.

2.4. Calculation of Load Classes

Only positive hydrant samples were used for calculation of load classes, and the percentage of positive samples was used to describe the occurrence probability. The abundance of water lice and oligochaetes with increasing density was given by an exponential curve that could be logarithmically normalized to a sigmoid curve. The load classes were given by dividing the log abundance into three sections of similar size, normal, increased and mass development, without considering a few outliers (<1.5%). In practice, the load classes covered a range of 4 to 5 potencies of ten or 0.1 to 1000 water lice m^{-3}, that is, 0.1–10,000 oligochaetes m^{-3}.

3. Results

3.1. Diversity and Abundance of Macroinvertebrates

Macroinvertebrates were found in almost all hydrant samples, with a median abundance of 16 ind. m^{-3} and 10- and 90-percentiles of 2 and 135 ind. m^{-3}, respectively. The highest density of macroinvertebrates was 4,764 ind. m^{-3}. Water lice and oligochaetes were most common and abundant, while other groups were rare and sporadic in drinking water networks (Table 1).

Table 1. Size range and occurrence of macroinvertebrates in drinking water networks in the European lowlands. Data base = 1039 hydrant samplings with 100 µm filtration.

Animal Groups and Species	Size Range (Length) (mm)	Occurrence Probability (%)	n (Positive Samples)	Median (Ind. m^{-3})	Percentile (10%) (Ind. m^{-3})	Percentile (90%) (Ind. m^{-3})	Maximum (Ind. m^{-3})
Macroinvertebrates (total)	>2	96.5	1003	15.9	2.0	135	4764
Isopods Water louse (*Asellus aquaticus*)	0.5–11	79.3	824	15.6	1.0	61	869
Cave water louse (*Proasellus cavaticus*) **	1–6	sporadic	34	8.0	1.0	34	89
Amphipoda Freshwater amphipod (*Niphargus aquilex*)	0.4–6.5	2.4	20	1.9	0.9	14.6	40
Midges Simuliide, adults	1–4	sporadic					
Chironomide, larvae *Paratanytarsus grimmii*	5	rare	66 *	27	1.9	154	1834
Chironomide, adults, larvae (*Limnophyes asquamatus*)	2	sporadic					
Oligochaete earthworms (Oligochaeta)	0.5–40	74.9	778	6.0	1.0	92.3	4723

Table 1. *Cont.*

Animal Groups and Species	Size Range (Length) (mm)	Occurrence Probability (%)	n (Positive Samples)	Median (Ind. m^{-3})	Percentile (10%) (Ind. m^{-3})	Percentile (90%) (Ind. m^{-3})	Maximum (Ind. m^{-3})
Springtails (Collembola)	1–5	sporadic					
Snails Nautilus ramshorn (*Gyraulus crista)*	1–2	rare	12 *	6			1599
Bladder snail *(Physella acuta)*	1.5–5	rare					
Bryozoa (*Plumatella* spec.)	20	rare					

* = occurrence in only one drinking water network; value, size range and mean size are based on the drinking water pipe populations.
** drinking water network located in Southern Germany.

In European lowland drinking water networks, 11 different representatives of macroinvertebrates were found. Most of these were surface water organisms, with cave water lice (*Proasellus cavaticus*) and the freshwater amphipode (*Niphargus aquilex*) being the only representatives of obligate groundwater fauna (Table 1).

Water lice, *Asellus aquaticus* were found to be the dominant macroinvertebrate species in drinking water networks (Figure 2), and presented with a frequency of 79% and a mean abundance of 16 ind. m^{-3}. It was evident that these organisms had a high reproduction potential as indicated by the 90-percentile level of 61 ind. m^{-3} and maximum of 869 ind. m^{-3}. Water louse grow up to 2 cm in freshwater, are omnivorous, and have an extremely high reproduction rate (see below).

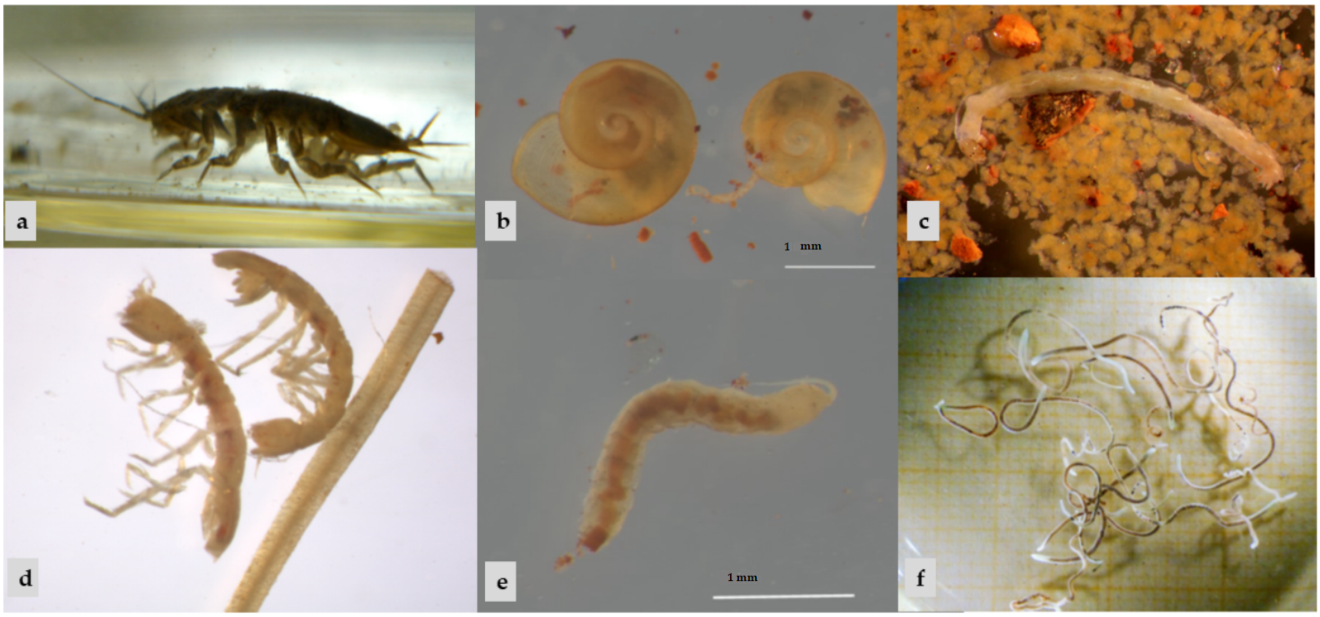

Figure 2. Macroinvertebrates in drinking water networks. (**a**): water lice (*Asellus aquaticus*), (**b**): Nautilus ramshorn (*Gyraulus crista*), (**c**): Chironomid larvae (*Paratanytarus grimmii)*, (**d**): cave water louse (*Proasellus cavaticus*), (**e**): Oligochaete (*Stylaria lacustris*), (**f**): Oligochaete (*Lumbriculus variegatus*). Fotos© U. Michaels, G. Gunkel.

Two species occurred only sporadically in the studied area, the cave water louse, *Proasellus cavaticus*, and the freshwater amphipod, *Niphargus aquilex* (Figure 2). *Proasellus cavaticus* feeds on organic deposits and grows to 5–8 mm in natural environments. However, we observed high population densities in one drinking water network in which a mean of 8 ind. m^{-3} and maximum of 89 ind. m^{-3} was observed. *Niphargus aquilex* occurs naturally in groundwater interstices and in caves. This organism is predatory, but also eats detritus and biofilms. The size of this organism can reach 30 mm and it appears to propagate in drinking water networks based on the observed mean density of 1.9 ind. m^{-3} and a maximum density of 40 ind. m^{-3}.

Midges (chironomids) occur sporadically as adults and in larval stages in drinking water networks. A few species multiply parthenogenetically and can occur in large quantities in drinking networks, even if only a few flying females enter drinking water tanks (e.g., *Limnophyes asquamatus*). Another harmful chironomid is *Paratanytarsus grimmii* (Figure 2), which can undergo parthenogenetic propagation without reaching the flying adult phase; thus, its entire life cycle can occur within a drinking water network. To date, only a few occurrences of *Paratanytarsus grimmii* larvae have been reported, but their densities can reach up to 1834 ind. m^{-3}. *Paratanytarsus grimmii* is one of the organisms leading to the so-called biofouling [33].

Earthworms (Oligochaeta) are frequent inhabitants of drinking water networks (74.9% probability), and consist of species that are hard to distinguish. These organisms are detritus feeders with a body size that can reach up to 85 mm in drinking water networks, although smaller species are more frequent. Consumer complaints usually result from the presence of larger forms, e.g., the sludge worm (*Tubifex tubifex*, up to 85 mm) or Lumbriculidae (*Stylodrilus heringianus*, up to 40 mm). In the present study, these organisms were found to have a mean abundance of 6 ind. m^{-3} and a 90-percentile level of 92 ind. m^{-3} with a maximum of 4723 ind. m^{-3}, indicating the high importance of these species. Thus, species determination to the genus or sub-family level should be conducted routinely for organisms found in drinking water systems.

Snails were found in only two drinking water networks, but they were present at high densities. Specifically, the Nautilus ramshorn (*Gyraulus crista*, Figure 2), a plate snail, and the bladder snail (*Physella acuta*) were found. *Gyraulus crista* has a small size of up to 3 mm, while *Physella acuta* reaches 5 mm. These organisms are typical grazers that feed on biofilm. Although they are representatives of pulmonary snails, they can live permanently under water due to their small size, skin respiration, and hemoglobin in the blood. Currently, little is known about the development of snails in drinking water networks, but data suggests they can reach high population densities. For example, 1599 *Gyraulus crista* were observed in a one m^3 hydrant sample.

Spring tails (Collembola) are small animals of 1–5 mm in length that feed on organic deposits and live in moist soil and water surfaces. In this study, they were rarely found in drinking water networks.

Bryozoa (*Plumatella* spec.) were found in one drinking water network; these are colony forming organisms that can form colonies of several centimeters and *Plumatella* is a filtering organism. Propagation occurs via free swimming larvae. Bryozoa also lead to biofouling.

It should be noted that many of the invertebrates found living in drinking water networks did not reach the maximum body size of surface water organisms (Table 1), which could have been due to suboptimal living conditions.

3.2. Co-Relationships of Macroinvertebrates

The co-relationships of the most abundant macroinvertebrates, water lice and oligochaete, were studied for one drinking water network for which sufficient data were available. These data clearly indicated that there was no co-relationship (Figure 3).

The habitat requirements of species differ, and oligochaetes are found at higher abundance in end pipe sections (which have smaller diameters). Other pipe characteristics, such as diameter and pipe material, were not found to significantly impact water lice abundance.

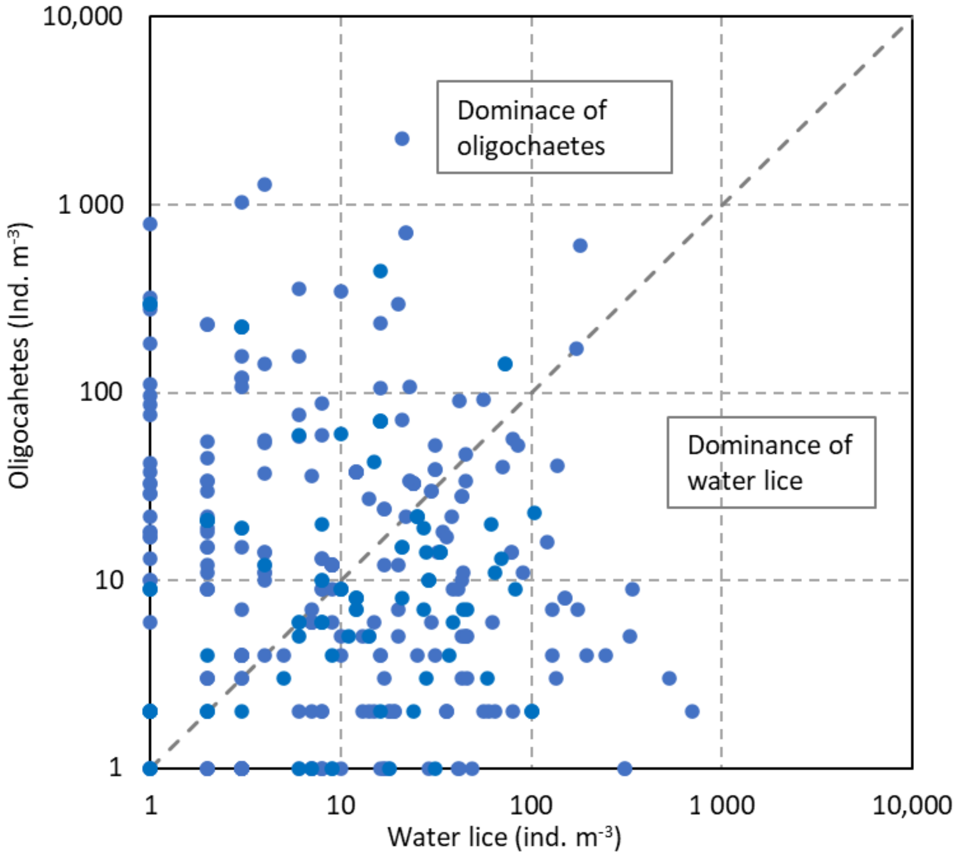

Figure 3. Test of co-relationship of water lice and oligochaetes in a drinking water network. The abundances of standard hydrant sampling for several years are shown (r^2 = 0.004).

3.3. Load Classes of Macroinvertebrates

The occurrence of water lice can be described as a distribution curve with increasing abundance (Figure 4). In this study, hydrant samples were divided into three load classes with increasing log abundance. Overall, 20.7% of the hydrant samples were free of water lice, and an additional 16.7% had only a small amount of <2 ind. m^{-3}; therefore, this area can be defined as subject to normal colonization. Overall, 37.4% of the hydrant samples were assigned to this range.

Increased colonization with a frequency of 2–20 ind. m^{-3} was classified as the control range. However, due to the increased abundance of water lice, these pipe sections need regular and real monitoring. In addition, measures to reduce the incidence of macroinvertebrates should be considered and, where appropriate, implemented. Overall, 40.8% of the hydrant samples fell into this category.

Levels of 20 to >200 ind. m^{-3} were classified as mass development. The maximum density observed in this study was 869 ind. m^{-3}. In cases of mass abundance of water lice, there is an urgent need for action. Specifically, this means the development of water lice must be regularly investigated, and measures must be implemented to reduce colonization. In addition, microbiological-hygienic deterioration of water quality cannot be excluded in such areas. Overall, 21.8% of the hydrant samples were assigned to this load class.

The aquatic oligochaetes had similar load class limits. The normal colonization of aquatic oligochaetes was <1 ind. m^{-3}, the control range was 1–30 ind. m^{-3}, and mass development was 30 to >400 ind. m^{-3} (Figure 4). Some extremes of oligochaetes abundance occurred, with a maximum of 4723 ind. m^{-3} being observed.

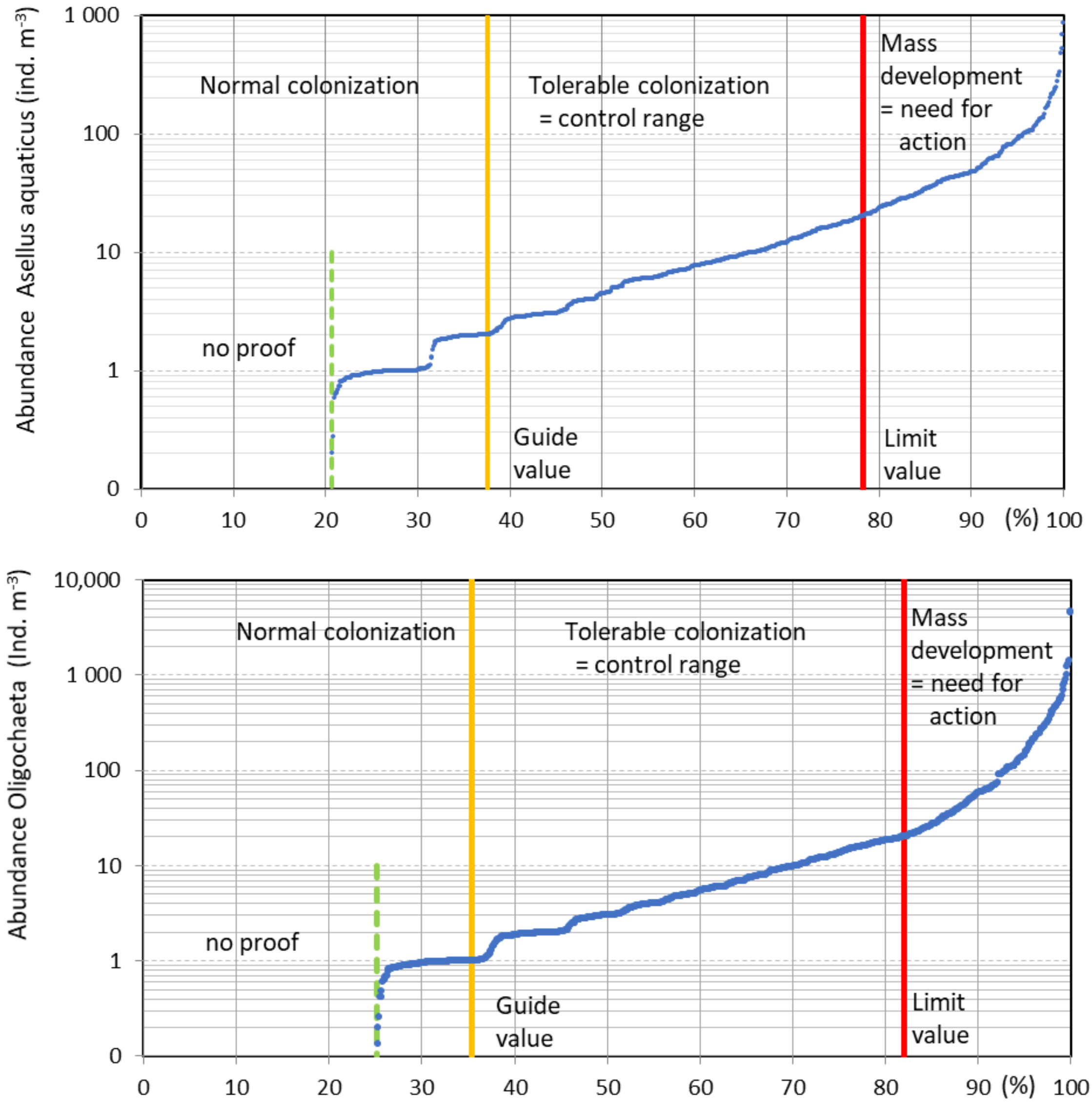

Figure 4. Abundance of water lice (**above**) and aquatic oligochaetes (**below**) in drinking water networks. Data base = 1039 hydrant samples; classification was done with three empirical load classes (see text).

Based on our data pool, load classes for the total number of macroinvertebrates during normal colonization was <3 ind. m^{-3}, while the control range was 1–35 ind. m^{-3}, and mass development wad indicated by 35 to >500 ind. m^{-3}, with some extremes of >>500 ind. m^{-3} occurring. However, it must be pointed out that evaluation at species level is necessary, especially for the development of control strategies.

3.4. Reproduction of *Asellus aquaticus*

Water lice reproduction is characterized by brood care; females carry eggs and after hatching, the embryos remain on the ventral site until they reach a size of 2 mm. In the study area, reproduction started with the large winter form in February/March, when the water temperature was >7 °C, at which time about 40 embryos (= the mean of the winter generation) were observed; the number of eggs and embryos depended on the size of the female, with a maximum of 78 embryos being observed. The development of eggs and embryos occurs in 300 d° (day degrees, the product of number of days and water temperature), which was about 50 d [12,34,35]. In general, water lice die after breeding.

Studies of water lice population dynamics in drinking water networks have revealed the formation of three generations per year. The first generation (F1) is characterized by juveniles with a size of 2 mm in March and adults with a size of >4–5 mm in July (= summer form, Figure 5).

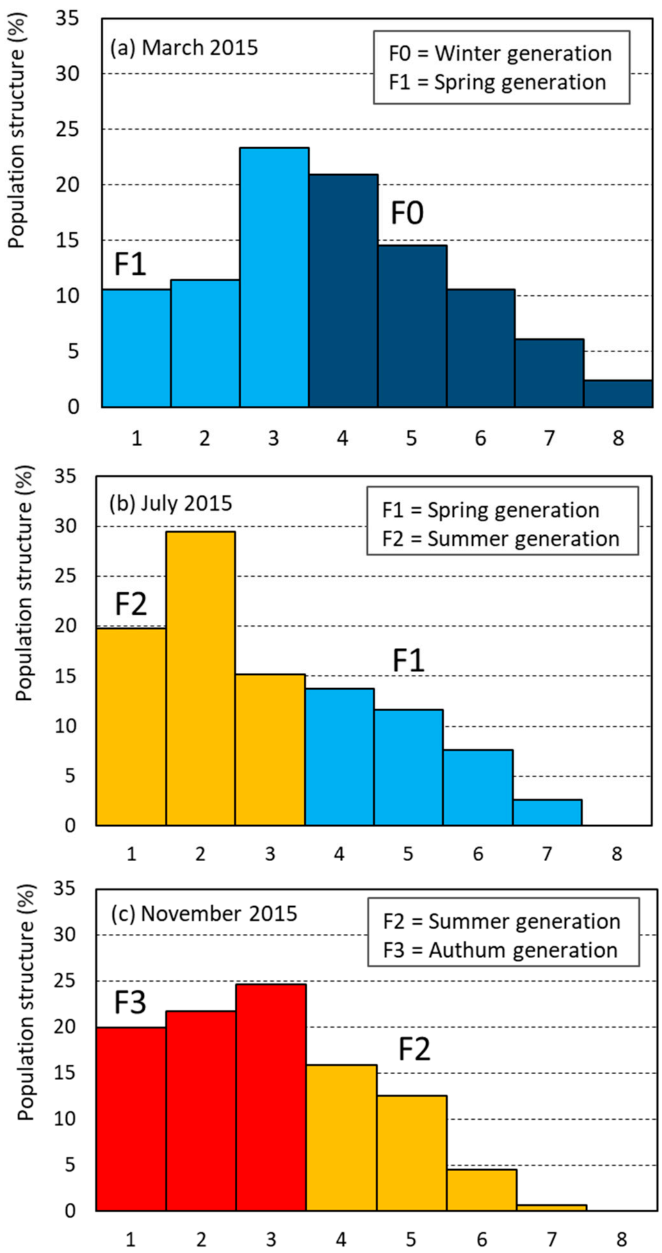

Figure 5. Population analysis of *Asellus aquaticus* in a drinking water network located in the European lowland in (**a**) March, (**b**) July and (**c**) November 2015. Shown are the abundance of water lice size classes from 1 to 8 mm; 1 and 2 mm represent embryonic stages, 3 mm represents the juvenile stage and >4 mm represents the adults (data of one drinking water network located in Northern Germany).

A second generation develops in July with reproduction of the F1 generation. This generation is characterized by smaller organisms with about 15 embryos (= mean of the summer generation, F2). Development of the eggs and embryos for F2 occurs in about 20 d. This F2 generation growth to adults in autumn, which reproduce and build up the third generation (F3).

The occurrence of the third generation depends on water temperature, which is generally increasing due to climate change effects on ground water and soil temperature. A mean drinking water temperature increase of 1.9 °C in 13 years was registered at a particle filter in one of the studied drinking water networks.

3.5. *Impact of Asellus aquaticus on Drinking Water Quality*

Macroinvertebrates do not lead to a direct health impact to drinking water consumers, but they indicate a decrease in water quality and an aesthetic impairment.

Additionally, some indirect effects are of significance; mainly, the promotion of bacterial growth in the case of dead animals and feces in water pipes and on house filters. The occurrence of dead water lice on house filters leads to a significant increase in total bacteria. For example, when water was stagnant, maximum bacterial growth up to 3500 cfu mL^{-1} occurred within 3 days, clearly exceeding the German guideline value of 100 cfu mL^{-1} (Figure 6). In stagnant water without dead animals on the house filter (but with water lice colonization of the pipes), up to 288 cfu mL^{-1} were registered. Flow-through conditions as control led to a colony number of only 44 cfu mL^{-1}.

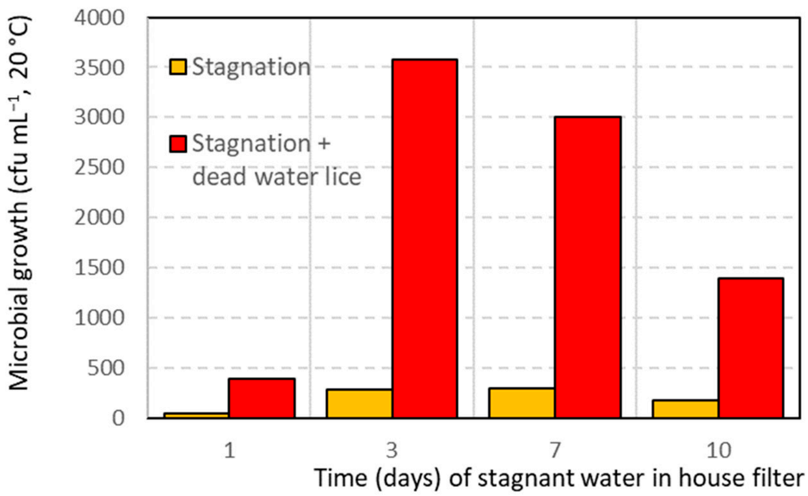

Figure 6. Microbial contamination of house filters with stagnant water without dead water lice (orange) and stagnant water with 5 dead water lice (red) as colony forming units (cfu).

The feces of water lice occur as pellets and are very stable in water due to a protein cover. In cases of intensive colonization with *Asellus aquaticus*, a very high amount of deposit is formed by feces pellets (Figure 7). Moreover, flow-through experiments with feces did not show any significant destruction within 6 weeks (Figure 8), indicating feces accumulates within drinking water pipes over a period of many weeks and can represent most of the deposits.

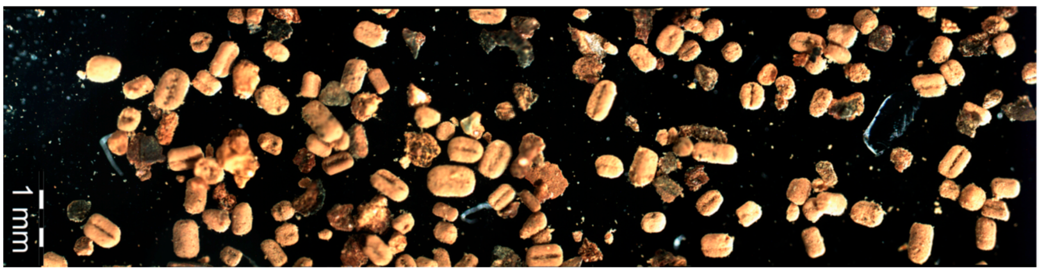

Figure 7. Light microscopy of flushed out drinking water pipe deposits. The deposits are formed by feces pellets of *Asellus aquaticus*.

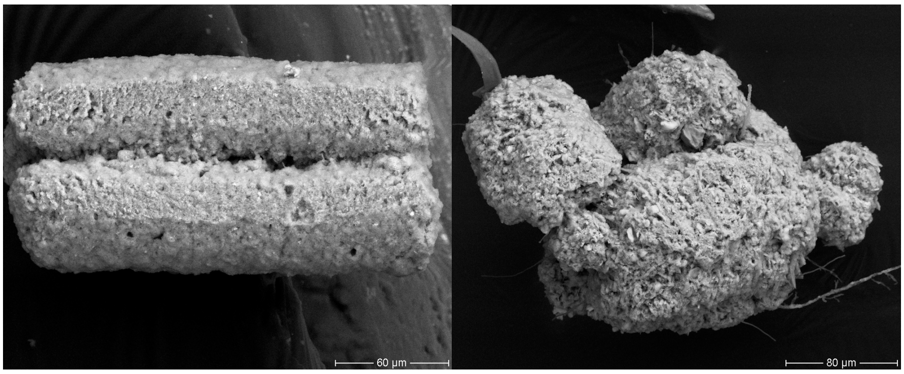

Figure 8. SEM photos of feces pellets of *Asellus aquaticus*. **Left:** fresh released *Asellus aquaticus* feces, **right:** feces pellets after 42 days in a laboratory tap water flow-through experimental device.

Asellus aquaticus feces have a very high carbon content, which is a sign of a low food conversion and influences microbial growth. The mean composition of the feces was found to be 39.8% C, 9.3% SiO_2, 3.5% CaO, 0.3% MnO and 44.9% FeO based on SEM-EDS analyses as the mean of five drinking water networks. It should be noted that there was significant variation in the feces composition due to changes in DOC and POC supply as well as biofilm and deposit sources.

Leaching experiments investigating feces pellets using LC-OCD analyses clearly demonstrated that leaching is a long-lasting process that occurs for about 3–4 weeks (Figure 9). During the experiments, biopolymers (polysaccharides) were continuously released from the feces, as were humic acids, although they occurred in smaller concentrations. Low molecular weight acids and neutral substances were not registered.

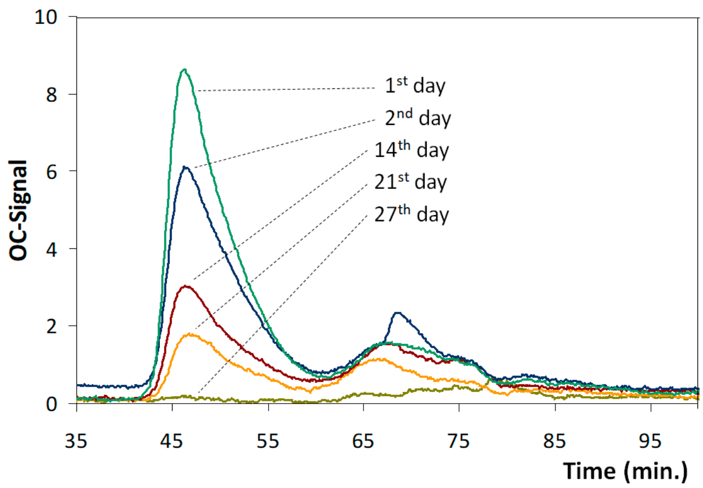

Figure 9. Leaching of *Asellus aquaticus* feces analyzed with liquid chromatography coupled with organic carbon detection (LC-OCD). The first peak (48 min) corresponds to biopolymers (polysaccharides), the second peak (69 min) represents humic acids, low molecular weight acids and neutral substances do not give a signal (>85 min).

4. Discussion

4.1. Macroinvertebrates as Inhabitants of Drinking Water Pipes

An evaluation of the occurrence of small invertebrates in drinking water networks can be applied by dividing the organisms into three classes according to dimension: macro- (>2 mm), meio- (0.1–2 mm) and micro-invertebrates (<0.1 mm), which are comparable to common filter mesh sizes used in drinking water systems. The esthetic impact of drinking water quality is mainly given by macroinvertebrates, which occur in lower numbers but are visible with the naked eye on house filters and in tap water. Some studies of macroinvertebrates in drinking water networks have been conducted, but there is very little information about meio- and micro-invertebrates [2,3,20,31,36,37].

When considering the biological drinking water stability, the growth of invertebrates in water networks must be evaluated. This focuses on biological processes that occur after the clear water outlet from the drinking water treatment system and during transport in pipes to the tap, which can take several days [6,7]. During this transport, microbial growth can occur, mainly in the form of biofilms as well as associated microbes and small invertebrates (e.g., ciliates, rotatories, nematodes). This biomass can serve as food for larger invertebrates such as water lice, oligochaetes, chironomids, and snails.

The diversity of macroinvertebrates in drinking water networks is high, with 11 representatives of invertebrates found in European lowlands, but only two species, water lice and oligochaetes, have a high frequency and build up high population densities. Some other macroinvertebrates are found at low frequency, but have the potential to reach mass development. Some organisms (*Paratanytarsus grimmii*, *Plumatella* spec.) directly lead to a mass development with considerable reduction of the inner pipe diameter by so-named biofouling [33]. Representatives of obligate groundwater fauna, such as *Proasellus cavaticus* prefer cool water and therefore have a low metabolism and small growth rate [16].

Water lice tolerate a wide range of flow rates (<0.7 m s^{-1}), and in cases of high flow rates they can migrate into protected areas (e.g., laminate layer or niches in the pipe system), after which they cling to pipe walls. This behavior prevents the flushing out of water lice, which has led to the development of the CO_2 flushing method [31]

Monitoring *Asellus aquaticus* in drinking water networks [31] has shown that distribution is not homogeneous, and that there are some hot spots in which there are significant differences in abundance and animal size. To date, it has not been possible to identify the key factors responsible for the occurrence of these hot spots. End pipe sections have been found to be the preferred habitat for oligochaetes.

Increases in water temperature due to climate change effects—as well as decreased water consumption due to water saving technologies and demographic development (and therefore oversized water pipes)—facilitate the development of undesired invertebrates in drinking water networks [38]. A key factor influencing this is the number of generations of water lice occurring per year. Indeed, with increased water temperature, a third generation can be found in late autumn and this leads to a significant increase of abundance. We must assume that water temperature affects the development of other invertebrates in a similar way. The abundance of water lice increases with increased temperature, particularly when there is a reduction of days < 7 °C in winter, which results in reproduction beginning earlier.

A third generation (F3) of *Asellus aquaticus* has been observed in Central European lakes [34,39] as well as in European lowland drinking water networks in areas with sufficiently high water temperatures. When this occurs, the first generation (F1) is characterized by juveniles in March and adults in July, while a second generation (F2) develops in July. This second generation consists of smaller organisms that reproduce in September/November (F3), resulting in a strong population increase. Reproduction of F3 occurs in February/March of the following year. An approximation of the *Asellus aquaticus* number is as follows F0 = 2 organisms, F1 = 40 organisms, F2 = 300 organisms, and F3 $\geq$ 1000 organisms.

The effects of water chemistry on the co-relationship of invertebrates were not evaluated, but our investigation indicated that the occurrence of water lice is not limited by food. This is because the typical range of DOC (>2 mg L^{-1}) and fine POC from water treatment plants is sufficient for mass development of pipe inhabitants [12]. Accordingly, the limitation of water lice development based on reducing biofilms as a food resource reduction will not be a successful strategy. Indeed, growth experiments have shown that limitation of Asellus aquaticus occurs at about 0.2 mg L^{-1} BDOC (biodegradable DOC), which corresponds to about 1–2 mg L^{-1} DOC [40].

The available food can be calculated as follows: *Asellus aquaticus* (of 3 mm length) possess a wet weight of 1.3 mg and have a median food intake of about 2% d^{-1} (wet weight/wet weight), or 0.03 mg d^{-1}. Larger *Asellus aquaticus* of 8 mm possess a wet weight of 6.3 mg, which leads to a daily food intake of 0.13 mg d^{-1} [12,41]. The biomass of biofilm is about 10^6 (10^4–10^8) cells cm^{-2}, which is about 0.3 g m^{-3} pipe volume [7]. Thus, 10 water lice of 3 mm per m^3 consume about 1% d^{-1} of the biofilm, while larger water lice consume about 4% d^{-1}. This predation rate is lower than the regrowth rate of biofilms, indicating that normal population densities of water lice do not significantly influence the growth of biofilms and are therefore not limited by biofilm biomass. Although there may be some limitation in cases of mass development, other food resources are still available.

4.2. Macroinvertebrates as Hosts for Harmful Bacteria

Macroinvertebrates in drinking water networks can have indirect adverse impacts including: (i) the regrowth of microbes on house filters with accumulated dead animals or feces in cases of stagnant water, (ii) support of microbial regrowth in pipe sections with deposition of dead animals and feces, (iii) protection of harmful microbes against disinfection in the gut of macroinvertebrates, and (iv) introduction of harmful microbes in drinking water networks (e.g., adult chironomids enter water reservoirs).

The linkage of harmful bacteria and the occurrence of macroinvertebrates in drinking water networks have been intensively investigated, with most studies focusing on microbial colonization of the gut [24,29,42]. Moreover, the guts of macroinvertebrates other than those found in this study have been found to host harmful bacteria. Accordingly, it is important to differentiate microbes that (i) proliferate actively in the intestines of organisms while causing no damage to the host organism (= colonists) from (ii) those that multiply and cause damage to the host organism (= intestine parasites), and (iii) those that do not multiply and are transported passively (= intestinal visitors). Moreover, any risk assessments of bacteria hosted by invertebrates in drinking water systems must consider protection against UV and chlorine disinfection in the gut. Indeed, several studies have reported that survival of bacteria increases up to 50-fold against disinfection measures when they are present within invertebrates [43,44].

Feeding experiments in surface waters have demonstrated that *Asellus aquaticus* is a selective feeder with some preference for fungal species [45]. In drinking water networks, water lice primarily feed on pipe depositions such as bacteria, fungi and detritus. Experiments with *Asellus aquaticus* confirmed that selective feeding occurs with a preference for *Cladosporium herbarum* followed by *Marmoricola* sp. and then *Aquabacterium commune* [46]. PCA analyses of feces revealed that harmful bacteria such as *Pseudomonas hydrophila*, *P. putida*, *Aeromonas* spec., *Enterobacter* spec. and *Mycobacterium* spec. occur in the guts of *Asellus aquaticus* and *Proasellus cavaticus*. Even under long-term starvation conditions (6 weeks), *Pseudomonas* sp., *Enterobacter* sp. and *Aeromonas* sp. are the dominant bacteria in the guts of water lice [46], indicating that these bacteria are inhabitants of the gut and therefore proliferate in water lice. *Asellus aquaticus* takes up *E. coli* from water and sediment as well, but these microbes do not remain for a long time and the overall number of *E. coli* in the guts of *Asellus aquaticus* seems to be low [42].

Chironomids may host *Vibrio cholerae* and *Aeromonas* that become part of its intestinal microbiome, while others will just pass through the intestine as visitors and are depleted [47]. Meio- and micro-invertebrates in drinking water networks are also well known to act as bacterial reservoirs. For example, amyloid transport of *Legionella pneumophila* and *E. coli* [44], and nematode transport of *E. coli* [25,44] have been documented.

Additionally, studies of the feces of *Asellus aquaticus* have indicated that the feces leach organic acids, among other, biopolymers, which seems to support regrowth of bacteria [4,5].

Chironomid egg masses may also serve as a reservoir for *Vibrio cholera* and *Aeromonas* spp. [48], and bacteria in such reservoirs are protected from disinfecting agents [49]. Moreover, chironomids pose a high risk for drinking water contamination because they can enter systems via aerial transfer and through reservoirs that are not completely closed [24,49,50].

4.3. Proposed Activities for Biological Water Quality Control and Maintenance

Macroinvertebrates in drinking water networks can reach very high population densities; therefore, advanced network flushing strategies are needed to limit invertebrate development. This can be done by flushing drinking water pipes with clear water to avoid the input of invertebrates, or with advanced flushing methods such as ice pigging, impulse flushing or CO_2 flushing [31,51,52].

Further research is needed to analyze and quantify the drivers of invertebrate development; however, our results revealed that none of the macroinvertebrate species has an indicator function. Accordingly, guidance values must be developed and monitored at the species level, and areal heterogeneity as well as hot spots of settlement become highly significant [10,20].

The support that invertebrates provide for the survival and regrowth of microbes indicates the significance of these organisms to water quality and human health, as well as the need for continuous and quantitative monitoring of invertebrates. Invertebrate occurrence is one of the key parameters influencing microbial regrowth following treatment in addition to raw water quality, e.g., given by microbial growth, development of harmful bacteria as biome of invertebrates and jelly egg masses as substrate for microbe development [5,47,48].

Increasing efforts are necessary to ensure good water quality and minimize the risk of microbial contamination. Such efforts should include (i) optimization of water treatment processes, (ii) regular pipe flushing, (iii) monitoring of invertebrate colonization and evaluation of abundance, and (iv) in cases of mass development of invertebrates, advanced pipe-cleaning technologies.

Author Contributions: All authors contributed to the work presented in this paper and participated on project management. G.G. managed the ZIM research funding project, and U.M. managed the ProInno II research funding project. G.G. directed the experimental laboratory research and evaluated the data of field research, U.M. directed the biological laboratory analyses, and M.S. directed the construction of research equipment within the ZIM and ProInno II projects and provided the drinking water network flushings. As specific contribution G.G. designed the article content and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The German Federal Ministry for Economic Affairs and Energy funded the research by the ZIM program (KF 2058602 RH9) and the ProInno II program (KF 2488501 RH9).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: This work was supported by the German Federal Ministry for Economic Affairs and Energy (ZIM and ProInno II funding). In addition, we thank the project staff, D. Tietze and J. Kosmol as well as the State Institute Berlin-Brandenburg (LLBB) for microbial analyses and International Science Editing for language revision.

Conflicts of Interest: The authors declare no conflict of interest.

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