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Changes in Water Source Cause Shifts in Invertebrate Biomass, Composition, and Regrowth in a Non-Chlorinated Drinking Water Distribution System

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Abstract: Invertebrates such as *Asellus aquaticus*, halacarid mites, copepods and cladocerans are common in drinking water distribution systems. The Zeeuws-Vlaanderen drinking water distribution system (DWDS) of Evides water company is divided into western and eastern sections, initially supplied with drinking water derived from a eutrophic reservoir (water) and groundwater, respectively. The drinking water derived from eutrophic reservoir water was characterised as less biologically stable than the drinking water from groundwater. Due to groundwater level protection measures, since 2015 the groundwater supply to the eastern section has been gradually replaced with supply from the drinking water treatment plant, which uses eutrophic reservoir water as source water. This change caused increased regrowth conditions, as observed by regulated microbial regrowth indicators (HPC22 and *Aeromonas*), increased invertebrate biomass, and the dominant occurrence of *Asellus aquaticus*, confirming observations in other Evides DWDSs. The results from the western section supplied with the same less biological stable drinking water, however, showed that the occurrence of microbial regrowth, invertebrate biomass and *A. aquaticus* is not only related to the biological stability of the supplied drinking water, but also to the influence of DWDS-specific conditions. The DWDS configuration as well as higher water demands in summer (western section) and/or higher sediment and Fe accumulation in the DWDS (eastern section) are suggested factors affecting regrowth and therefore subjects for further research.

Keywords: invertebrates; *Asellus aquaticus*; biologically stable drinking water; *Aeromonas*; sediment; iron

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1. Introduction

Evides Water Company distributes non-chlorinated drinking water produced from eutrophic reservoir water and groundwater in the south-western region of the Netherlands. This requires the production of biologically stable drinking water with low microbial growth potential to minimise biological activity during storage and distribution. Microbiologically, the safety of the drinking water is secured by enough disinfection capacity and periodic quantitative microbial risk assessment [1]. Too much biological activity or regrowth may result in a number of health-related, aesthetic and technical problems (opportunistic pathogens, taste/odour, visible invertebrates and faecal pellets, and clogging, e.g., of water meters [2–8]). Besides the heterotrophic plate count HPC22, *Aeromonas* is also implemented in Dutch legislation as a more sensitive technical standard for regrowth. In parts of the Evides distribution network, the *Aeromonas* count in drinking water in the warmer period of the year is above the action level, indicating the occurrence of regrowth conditions [9,10]. This has resulted in/led to further research activities to assess the cause of the regrowth, also initiated by current developments in climate change [11].

Since the 1980s, AOC P17/NOX was used as the biological stability parameter; however, for drinking water produced from eutrophic reservoir water, this parameter appeared not predictive for regrowth [9]. To assess the microbial growth potential of drinking water, several new methods have been developed and applied in the Netherlands [4]. The value of these parameters for the drinking water industry can only be assessed by applying them under real world conditions. Evides Water Company used these methods to determine the biological stability of its drinking water and demonstrated a correlation between *Aeromonas* counts and the appearance of invertebrates, e.g., water lice, *Asellus aquaticus*, in the drinking water distribution system (DWDS) [2–11].

In this paper, we describe a case of changing the drinking water source from groundwater to surface water in a drinking water distribution system in order to protect nature and groundwater levels at the Brabantse Wal. This change resulted in the supply of less biologically stable drinking water, as assessed with the new introduced biological stability parameters [4]. The objective of this study was to monitor the effect of this water quality change on (i) the invertebrate biomass and composition, with special attention given to the presence of water lice, *A. aquaticus*, and (ii) on regrowth conditions assessed with HPC22 and *Aeromonas* in the DWDS.

2. Materials and Methods

2.1. Water Treatment and Distribution

Evides Water Company produces drinking water for the southwestern region of The Netherlands. The most southern area, Zeeuws-Vlaanderen, is supplied by two treatment plants, TP#2 and TP#9 (Figure 1) (numbers according to [10]) (Table 1). The surface water treatment plant, TP#2, treats River Meuse water, which after five months of residence in large impoundment reservoirs in the Brabantse Biesbosch is transported over approximately 120 km prior to further treatment. Since March 2017, this eutrophic reservoir water has passed through a local impoundment reservoir with a residence time of four weeks. The water treatment consists of flocculation, flotation, rapid sand filtration, ultraviolet disinfection (since 2014; before 2014, ozone disinfection was used), biological activated carbon filtration, and filtrate disinfection with a low concentration of chlorine dioxide, which was zero after the clear water reservoir in the drinking water supplied to the DWDS. The groundwater treatment plant, TP#9, treats anaerobic groundwater with aeration, filtration and softening.

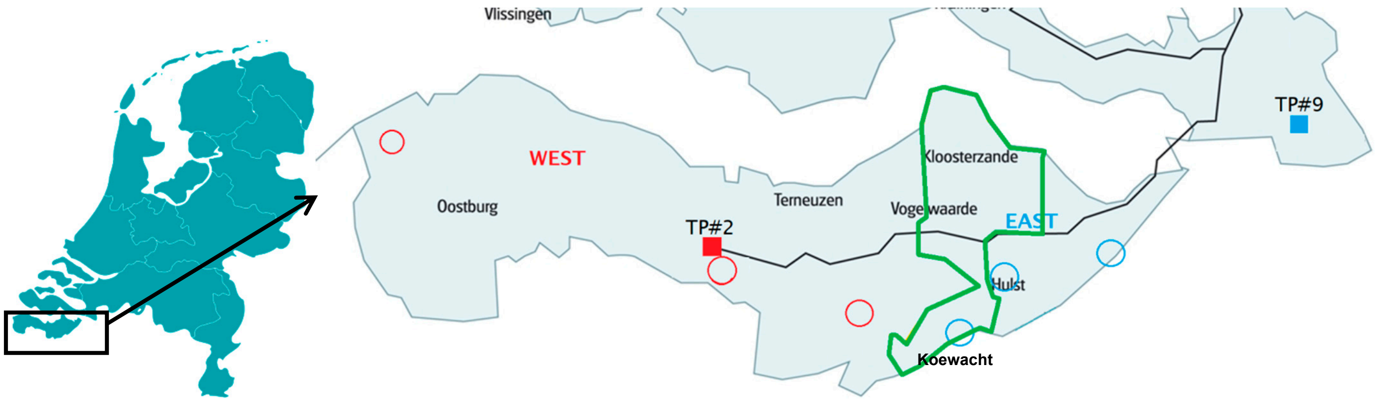

Figure 1. Location of Evides treatment plants (TP#2 and TP#9) and DWDS Zeeuws-Vlaanderen. Location of flush samples in DWDS Zeeuws-Vlaanderen West (○) and East (○). □: indication of the mixing area for TP#9 and TP#2 in the eastern section of Zeeuws-Vlaanderen until 2019.

Table 1. Production volume, pH, metals (Fe, Mn), dissolved organic carbon (DOC), biopolymers (BP LC-OCD), biomass (ATP) and biological stability (growth potential parameters) of the drinking water of TP#2 and TP#9 over the period 2012–2021 (mean values, with standard deviation s.d. and number of samples).

Parameter	TP#2 (Eutrophic Reservoir Water)	TP#9 (Ground Water)
Volume (Mm^3/year)	11.5 [a]	3.8 [a]
pH	8.2 (0.1; 1817)	7.9 (0.1; 907)
Fe (μg/L)	2.0 (2.0; 191)	6.1 (4.0; 800)
Mn (μg/L)	1.7 (2.3; 143)	4.3 (5.0; 799)
DOC (mg C/L)	2.0 (0.3; 172)	1.3 (0.2; 52)
BP LC-OCD (μg C/L)	76 (26; 97) [b]	7.3 (2.3; 7) [d]
Biomass ATP (ng/L)	6 (7; 484)	6 (11; 155)
BPC_{14} (day ng ATP/L)	132 (103; 71) [c]	35 (11; 12) [c]
AOC A3 (μg C/L)	7.8 (4.6; 36) [c]	0.6 (0.3; 7) [d]
AOC P17/NOX (μg C/L)	20.5 (7.8; 141)	12.1 (14.7; 12)

Note: [a]: 2011–2018; [b]: 2015–2021; [c]: 2019–2021; [d]: 2020–2021.

The drinking water from both treatment plants is distributed to two separate DWDS sections, a western (area west of the green line) and an eastern one (area inside the green line and east of it) (Figure 1; Table S1). Both DWDS sections consist of cement-lined iron, asbestos cement, PVC and cast-iron pipes. DWDSs are only cleaned by flushing locally after serious water quality incidents or when unacceptably high turbidity is measured.

2.2. Research Outline: Effect of Change in the Water Supply on Biological Stability and Invertebrates in the DWDS

Since 1989, the western section of the DWDS has been supplied with drinking water from treatment plant TP#2. In the eastern section, however, water quality changed due to a change in water supply. Prior to 2015 (Period 1), the eastern section of the DWDS was supplied from treatment plant TP#9. As a result of this, in drinking water in the area around Kloosterzande-Vogelwaarde-Koewacht (Figure 1, inside the green line), only a very small part was fed from TP#2. From 2015 until 2018 (Period 2), the source of the supplied drinking water in the eastern section within the area around Kloosterzande-Vogelwaarde-Koewacht was dependent on the time of year: in the summer period, TP#9 supplied the eastern section and in winter, TP#2 and TP#9 both supplied the eastern section. Since 2019 (Period 3), the production capacity of TP#2 has been increased to supply both the west and east of Zeeuws-Vlaanderen all year round. Since then, TP#9 only occasionally supplied the eastern part of Zeeuws-Vlaanderen. The rationale/reason for this change in water supply was to reduce groundwater abstraction at TP#9 in order to protect the local groundwater level to sustain nature at the abstracted area on the Brabantse Wal. One of the potential effects of this change could be a decline in microbiological water quality. Drinking water produced from eutrophic reservoir water (TP#2) is less biologically stable than drinking water produced from groundwater, although the microbial regrowth conditions in the western DWDS supplied from TP#2 were not problematic. Nevertheless, changes in the water quality, microbial regrowth and the presence of invertebrates were closely monitored in the eastern section since the supply change in 2015.

The biological stability of the drinking water from both TPs was determined over time. The effect of changing the drinking water supply in the eastern section of the DWDS area on sediment and invertebrate density and invertebrate composition was also monitored. Additionally, microbial regrowth conditions were assessed by monitoring the specific indicators HPC22 and *Aeromonas* (action level of annual geometric mean of 100 CFU/mL and action level of 1000 CFU/100 mL, respectively), according to Dutch water regulations.

In the supplementary information, a sampling scheme of the research is included (Table S2).

2.3. Sediment and Invertebrate Sampling and Analysis

In the eastern and western sections of the DWDS, three locations were selected based on estimated residence time (short, middle, and long; if the maximum residence time was 120 h, short was defined as less than 40 h, middle 40 to 80 h and long as more than 80 h). The locations were sampled once a year.

The DWDS supplied with drinking water derived from surface water are under the influence of a seasonal pattern in temperature and water quality [9]. To enable assessment of trends in invertebrate conditions in the DWDS it is of importance to select a constant period and sample locations for monitoring. The period between mid-August and mid-October is after the period with the highest biological activity, which is in spring and summer due to rising and high temperatures [10]. In the period from 2011 to 2020, every year between mid-September and mid-October, sediment and invertebrates from the mains of the DWDS were sampled at fire hydrants on 100 mm (inner diameter) pipes at a flow of 1.0 m/s (28.3 m^3/h) to determine invertebrate density. Sampling continued until a total volume of at least 1000 L had been filtered. Ninety percent of the flush sample was used for the collection of larger organisms and sediment particles using a plankton net with a mesh size of 500 μm (Hydro-Bios, Kiel, Germany). Smaller invertebrates and particles were collected from the remaining 10% of the flush sample using a cascade of plankton nets with mesh sizes of 500 μm and 100 μm (Hydro-Bios, Kiel, Germany) (Figure 2). The volume of the remaining 10% flow was measured upon filtration.

In 2021, between mid- September and mid-October, invertebrates and sediment were sampled from the mains of the DWDSs at fire hydrants on 100 mm (inner diameter) pipes at a flow of 1.0 m/s (28.3 m^3/h), until a total volume of at least 200 L had been filtered using a 100 μm sieve in a specially constructed mobile sampling lorry (Logisticon, Groot-Ammers, the Netherlands) (Figure 2). In 2022, a similar sampling procedure was followed as in 2011 to 2020, but in 2022 90% of the flush sample was filtered for organisms and sediment, using a plankton net with a mesh size of 100 μm (Hydro-Bios, Kiel, Germany). Again, the volume of the remaining 10% flow was measured upon filtration.

The flush samples (100 μm and 500 μm) were concentrated using plankton gauze with a mesh size of 70 and 250 μm (Hydro-Bios, Kiel, Germany), respectively. The concentrated samples were transferred to square petri dishes (100 mm) with 25 compartments, and the organisms were identified to groups according to [10] and counted using a dissection microscope (Leica MZ APO and M205C, magnification up to 100 times) (Figure S1). The biomass per taxonomic group per m^3 was calculated from density using the average fresh weight per individual, as published by [10]. Sediment volume of the different fractions was measured per mesh size using an Imhoff funnel (Boom, Meppel, The Netherlands), and the total sediment volume (mL per m^3) of the fraction larger than 100 μm was calculated.

The sedimentation of small particles in the distribution pipes of the DWDSs is measured yearly using a specific turbidity test at selected fire hydrants: the turbidity of the drinking water is measured during a flush at a velocity increase of 0.25 m/s over 10 min. The average turbidity is a quantitative measure for the degree of sediment fouling in the selected pipe section and is used to decide on pipe flushing actions.

2.4. Biological Stability and Other Analysis

The drinking water of both treatment plants, TP#2 and TP#9, was sampled regularly between 2011–2020 and analysed for pH, dissolved organic carbon (DOC; Shimadzu TOC-V_{CPN} -analyser and ASI-L auto sampler), iron and manganese. Iron was also measured in flush samples. Iron and manganese were measured after filtration (glass fibre, 0.45 μm) and acidification using an ICP-MS (Thermo Fischer Scientific iCAP RQ ICP-MS with PrepFAST 2 sc-4DX autosample).

Figure 2. Sampling sediment and invertebrates during flushing. Top left: sampling with plankton nets during flushing; Below: the sampling lorry with stainless steel sieves during flushing; Top right: (**A**): stainless steel sieve inside the lorry, (**B**): sieve after sampling, (**C**): concentrated sample of loose deposits, (**D**): microscopic photo of loose deposits, sand particles, empty cochlea of gastropods and parts of isopods.

The biopolymer fraction of the DOC was measured with Liquid Chromatography-Organic Carbon Detection (LC-OCD), in which the biopolymer and the humic substance fraction of the DOC were quantified [12]. This method is strongly correlated with the particle-associated and high-molecular organic carbon (PHMOC) method [9,10,13]. ATP (NEN 7393 [14]) was measured to determine the total bacterial biomass in the drinking water. The biological stability of the drinking waters was determined using the microbial growth potential methods AOC-P17/NOX (easily biodegradable compounds, NEN 6271 [15]), AOC-A3 (biopolymers) (NEN 6271 [15,16]) and the Biomass Production Potential (all biodegradable compounds; cumulative biomass production after 14 days; BPC_{14}) [4].

2.5. Microbial Water Quality of the Distributed Drinking Water: Sampling and Analysis

The microbial water quality in the distribution system is regulated according to Dutch legislation using heterotrophic plate counts (HPC22; NEN 6222 [17]) and *Aeromonas* colony count using membrane filtration (0.45 mm) of 100 mL and incubation of the filter on ampicillin–dextrin agar for 24 ± 2 h at 30 ± 1 °C [18,19], in drinking water (from the treatment plants) and in tap water. As DWDS samples were not collected equidistantly in time, only samples from the summer season (May until September) were used to determine the regrowth of bacteria in the DWDS.

2.6. Statistical Analysis

The log-transformed values of the biomass of invertebrates, the sediment volume and the log-transformed HPC22 and *Aeromonas* counts in different periods were analysed via ANOVA (Jamovi 2021, version 2.3.13). The Games–Howell post-hoc test was used when the ANOVA produced a significant ($p < 0.01$) result. Differences in sediment volume, turbidity and the metals, iron and manganese, between the eastern and western sections of the DWDS were analysed with an independent samples Mann–Whitney U test (Jamovi). Differences with a p-value equal to or smaller than 0.01 were considered to be significant. The results of these statistical tests are provided/indicated in Tables S3 and S4.

3. Results and Discussion

3.1. Water Quality of the Drinking Waters from TP#2 and TP#9

The results show that the pH and iron and manganese concentrations of the drinking water from TP#2 were respectively higher, lower and lower than in the drinking water from TP#9 (Table 1). The organic carbon, biopolymer fraction and growth potential parameters in the drinking water from TP#2 were clearly higher than in the drinking water from TP#9 (Table 1). Drinking water from TP#2 was thus considered to be less biologically stable than the drinking water from TP#9. The biopolymer concentration and biological stability parameters in the drinking water from the surface water plant TP#2 followed a seasonal trend, whereas in the drinking water from the groundwater plant TP#9 these parameters in the drinking water were constant over time.

The supplied drinking water quality in the western section of TP#2 changed moderately since March 2017 due the passage of a local impoundment reservoir. The biological stability parameter biopolymers (BP), as measured using PHMOC ([9]; similar analytical results as measured by LC-OCD [13]), increased from 57.6 ± 24.8 (n = 23) to 76 ± 26 µg C/L (n = 97), which also resulted in higher values for the growth potential parameters as measured using BPC14 (110 ± 40 to 132 ± 103 d/ng/L) and AOC-A3 (4.6 ± 2.8 to 7.8 ± 4.8 µg C/L). However, the increasing supply of drinking water from TP#2 to the eastern section of the DWDS, first only in the mixing zone and eventually across the total area, caused a much larger change in water quality, especially in the biological stability parameters as shown in Table 1. Due to the dynamic and variable supply conditions, this change in water quality was not monitored in the DWDS.

3.2. Sediment and Invertebrates in the DWDS

The larger fraction of the sediment (>100 µm), including invertebrates, varied by one to two logunits between years and areas (Figure S2; Table S3). The sediment data were highly variable, as shown by the high standard deviations, and no systematic differences could be observed between both DWDSs.

In the third period (2019–2022), the invertebrate biomass in the eastern section of the DWDS increased compared to (the levels in) Periods 1 and 2 (Figure 3). In this period, the biomass in the drinking water in the eastern section of the DWDS was more than 30 times higher than in the western section of the DWDS (Figure 3; Table S3). The invertebrate biomass in the western section did not change over time.

From 2019, the invertebrate composition in the eastern section of the DWDS changed strongly (Figure 4). Before 2019, *Asellus aquaticus* was found incidentally and Chydoridae, Harpacticoida and Oligochaeta were the dominant taxa (Figures 4 and S3). Since 2019, the dominant taxon in the eastern section has been water lice (*A. aquaticus*) (more than 90% of the biomass; Figure 4). The colonisation of the DWDS by *A. aquaticus* also resulted in higher biomass (Figure 3) because of its dominant biomass density, as presented in Table S5.

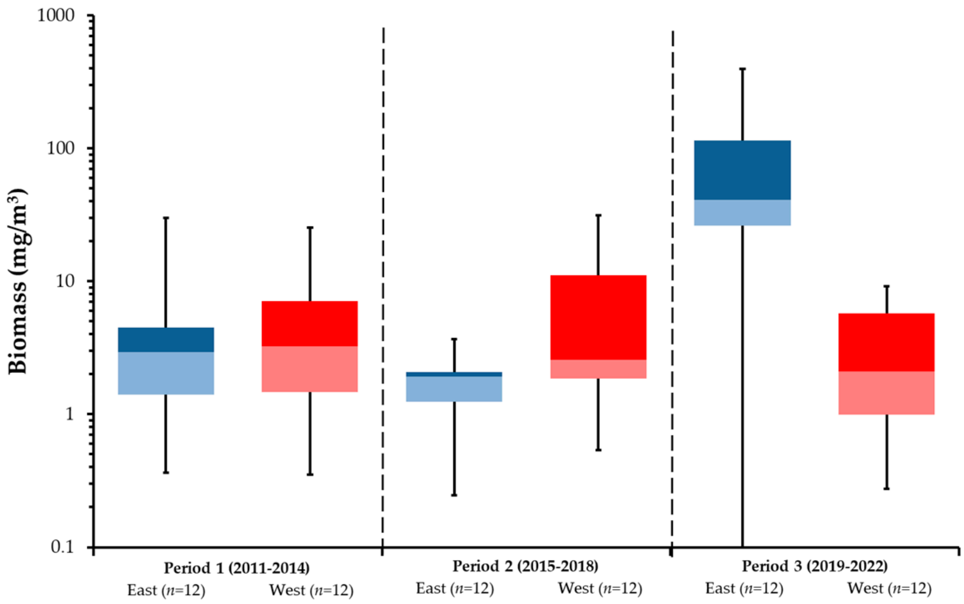

Figure 3. Boxplot of invertebrate biomass (per period) in the eastern (■) and western (■) sections of DWDS Zeeuws-Vlaanderen.

In the western section of the DWDS, the invertebrate biomass was dominated by Chydoridae, Cyclopoida and Harpacticoida in annually variable fractions (Figure 4). Water lice are only occasionally found, with the highest density in 2014. In 2019–2022 an increase in biomass of the Turbellaria and Oligochaeta was observed in this section, but the total invertebrate biomass did not change.

Danish researchers found that *A. aquaticus* is predominantly present in cast-iron pipes, where it has been found at higher concentrations than in plastic pipes [20]. The eastern and western sections of the DWDS are physically separated, which prevents the migration of mobile invertebrates, and in both DWDS sections, cast-iron pipes are only sporadically present. In the eastern section, all sampled pipes were made of asbestos cement (AC) and in the western section the sampled pipes were made of PVC (two) and AC (one) (Table S1). In Period 3, in the eastern section of the DWDS, the density of *A. aquaticus* was clearly higher than in the western section of the DWDS. The difference in material of the transport pipes in the eastern section (steel and PVC) compared to the western section (PVC) may have contributed to the increase in *A. aquaticus* density [20].

3.3. Growth of Bacteria in the DWDS

The HPC22 and *Aeromonas* colony counts per year illustrate the increase in microbiological activity during the periods of change in the water supply to the eastern section of the DWDS (Figure 5). In Period 3, during which 100% of the drinking water supplied to the eastern section was produced by TP#2, *Aeromonas* and HPC22 concentrations both increased significantly (Table S3). The regrowth conditions in Period 3 did not change further (Figure 5). From this observation we conclude that the regrowth conditions applied to the water quality distributed in Period 3 by TP#2 and the local conditions in the eastern section of the DWDS.

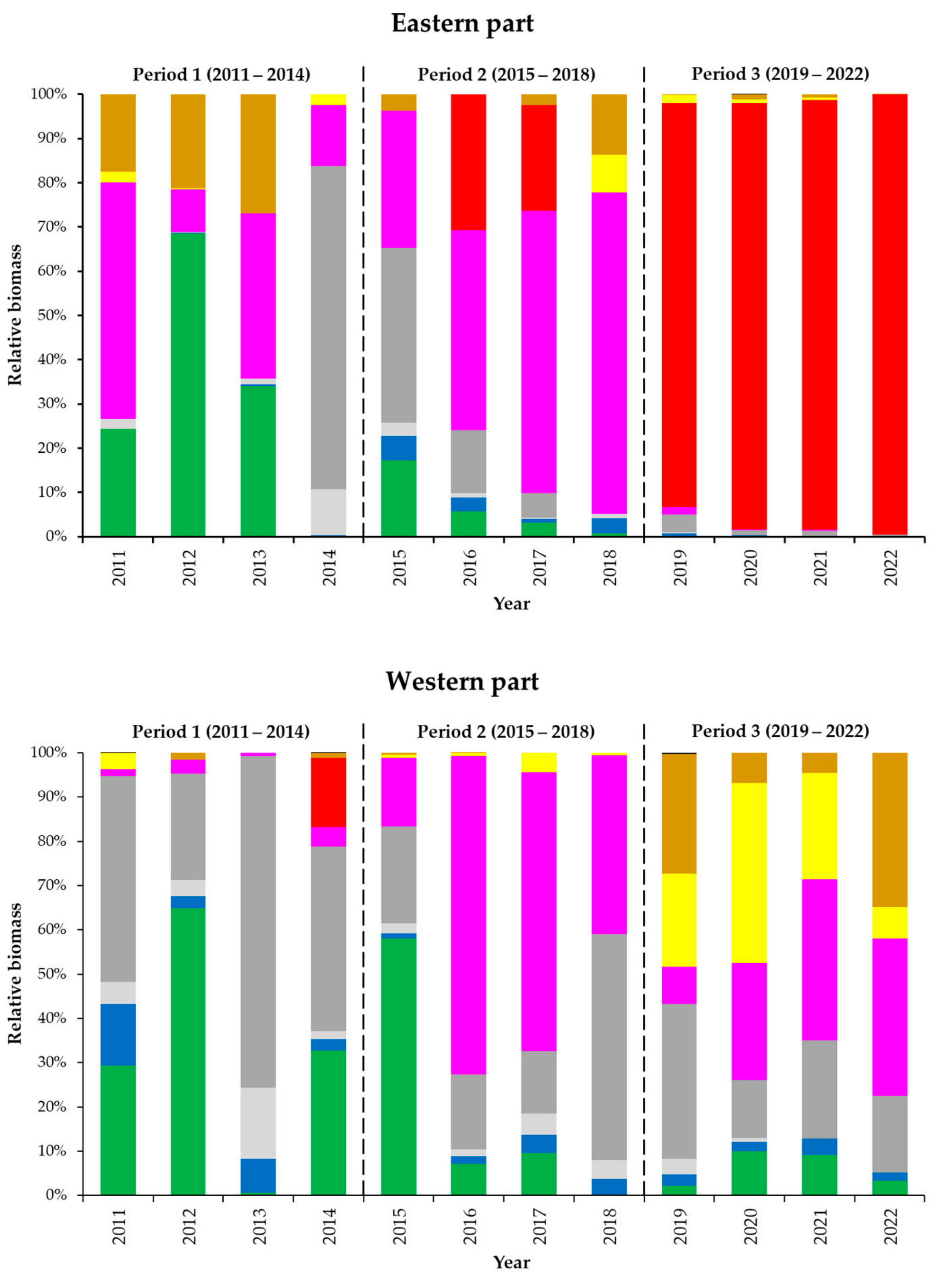

Figure 4. Average composition (in terms of biomass of three samples) of invertebrates per year in the eastern and western sections of DWDS Zeeuws-Vlaanderen. ■: Chydoridae; ■: Halacaridae; ■: Nauplius larvae of copepods; ■: Cyclopoida; ■: Harpacticoida; ■: *Asellus aquaticus*; ■: Turbellaria; ■: Oligochaeta; ■: other (not identified) invertebrates.

The relative exceedances of the action level of 1000 CFU/100 mL for *Aeromonas* in the eastern section increased when relatively more water was supplied to the area from TP#2; in Period 1, there were no exceedances, while in Period 2 and Period 3 respectively 3.6% and 16.7% of the samples exceeded the *Aeromonas* action level. Despite an observed increase in HPC22, this parameter did not exceed the action level in terms of the geometrical mean of annual HPC22 samples of 100 CFU/mL.

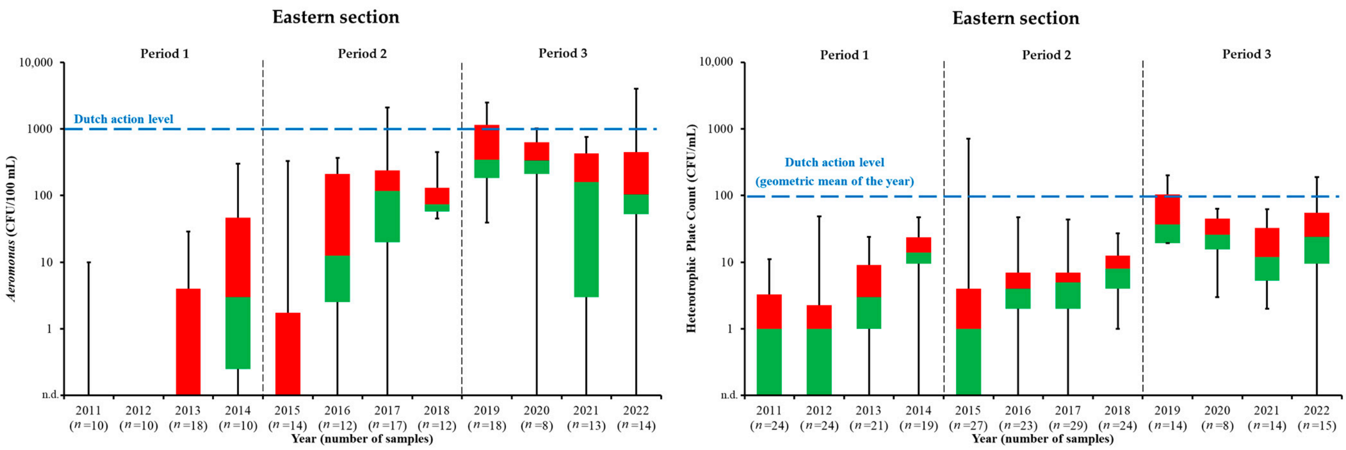

Figure 5. Boxplots of *Aeromonas* (**Left**) and HPC22 (**Right**) in the eastern section of DWDS Zeeuws-Vlaanderen. ■: second quartile; ■: third quartile; T: first and fourth quartile; n.d.: not detected.

In the western section of the DWDS, *Aeromonas* and HPC22 counts were relatively low and more steady (Figure 6). In this section, HPC22 counts showed a small but significant decrease from Period 1 to Periods 2 and 3 (Table S3). In Period 2 *Aeromonas* counts were slightly higher than in Period 3 (Table S3). In the western section of the DWDS, exceedances of the action levels for both *Aeromonas* and HPC22 did not occur in the research period.

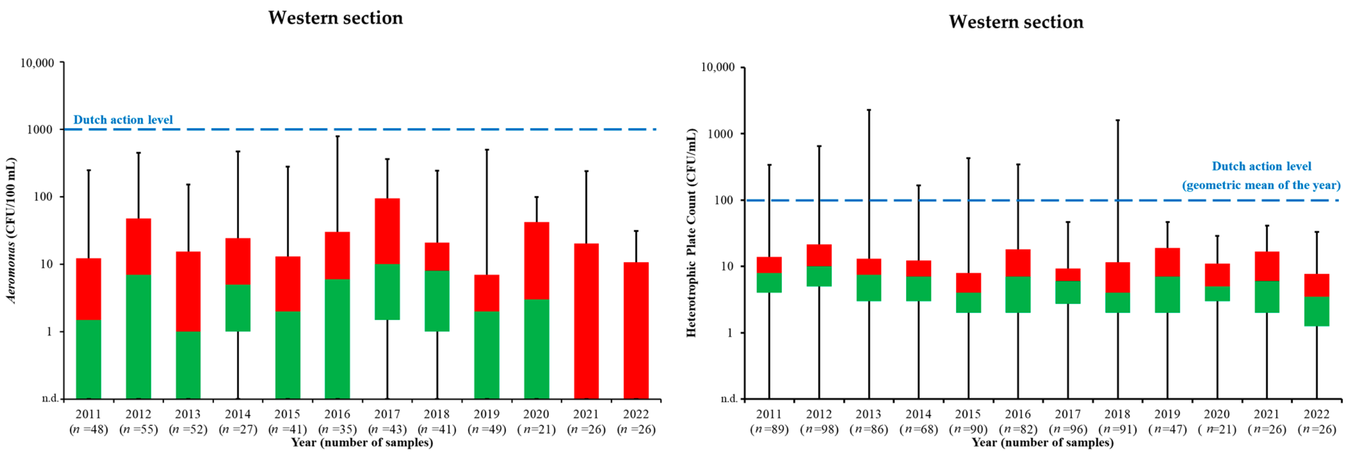

Figure 6. Boxplots of *Aeromonas* (**Left**) and HPC22 (**Right**) in the western section of DWDS Zeeuws-Vlaanderen. ■: second quartile; ■: third quartile; T: first and fourth quartile; n.d.: not detected.

3.4. Possible Causes of the Observed Change in (Micro) Biological Water Quality

The results of invertebrate monitoring in both parts of the DWDS revealed changes in total invertebrate biomass and the occurrence of *A. aquaticus* in the eastern section during the transition period of the water supply from biologically stable drinking water from TP#9 to less biologically stable drinking water from TP#2 (Table 1; Figure 3). Simultaneously, an increase in regrowth indicators, *Aeromonas* and HPC22, was observed in this area (Figure 5). The correlation of *A. aquaticus* density and both microbial parameters with the biological stability of the drinking water was first observed by [9]. Recently, Dutch researchers have found more definitive evidence of the correlation of invertebrate biomass with both microbiological parameters in a large and long-term study [10]. In both studies, the elevated counts of both microbial regrowth indicators, i.e., exceedances of the action level of *Aeromonas* and increased invertebrate biomass with the prominent occurrence of *A. aquaticus*, were observed in DWDSs supplied with less biologically stable drinking water. Evides has initiated actions due to the observed regrowth conditions: (i) more systematic

DWDS flushing and (ii) research to understand the regrowth mechanism of *Aeromonas* [21], as well as optimisation of the biological stability of the drinking water produced from eutrophic reservoirs water by water treatment [13].

However, in our study, we found a rejection of the biological stability-regrowth hypothesis by the results of the western section of DWDS Zeeuws-Vlaanderen. In this area, the supply of less biologically stable drinking water did not result in microbial regrowth as indicated by *Aeromonas* and HPC22, or in invertebrate biomass with a prominent occurrence of *A. aquaticus* (Figures 3 and 6). It is important to identify why the supply of biologically instable drinking water to the DWDS did not result in more regrowth, as this affects the future policies of the drinking water company to improve the biological stability of its drinking water. Differences in the net configuration, water demand and sediment conditions between the two separate DWDS sections (east and west) may explain the differences in hydrobiological and microbiological water quality:

- In summer, due to the presence of numerous tourists (an increase of inhabitants with more than 170% [22]) in the coastal area (peripheric zone of the western DWDS) and consequently high water demand, the flow rates are higher and the retention times in the western section of the DWDS are relatively short (<40 h) compared to the eastern DWDS (>70 h). Moreover, most of the drinking water is consumed in the periphery of this western section of the DWDS, where the tourists take their vacations. In the eastern section of the DWDS there is no such summer peak related to tourism (in 2021, on a yearly basis, 4.0 million overnight stays in the west section (with more than 1.6 million overnight stays in July and August) and 0.25 million overnight stays in the east section) and most of the water is used in the centre of the DWDS instead of at the periphery.
- The sedimentation of small particles in the distribution pipes of the DWDS is measured yearly using a specific turbidity test, as described in Section 2.3. The results from the period 2019–2021 demonstrate that the western section of the DWDS is less sensitive to sediment accumulation than the eastern section (Table S4). This observation is most likely related to the differences in net configuration and water demand between both DWDSs.
- The large transport pipes in the western section of the DWDS are made of PVC, while in the eastern DWDS PVC and steel pipes are both present. The smaller pipes in the DWDS of both areas are made of PVC, asbestos cement, cast iron and PE. Moreover, in the past, TP#9 has supplied drinking water with higher concentrations of iron and manganese (Table 1) in the eastern section of the DWDS. The sediment in flush samples in this area is consequently higher in iron; mean values are 3.45 and 0.85 mg/L in the eastern and western sections of the DWDS, respectively (Table S4). The higher iron concentrations in the sediment of the eastern section compared to the western section of the DWDS are also related to the differences in net configuration and water demand, as argued above. Elevated iron concentrations in the DWDS enhance the accumulation of (biodegradable) organic compounds and biomass [23,24] on the pipe wall and in the sediment. Invertebrates find their food in these niches of the DWDS.

Besides these differences in the configurations of the DWDS, the mix of two water qualities (of which the particles from TP#9 were settled) could be an important factor in the increased biomass observed in the eastern section of the DWDS. Dutch researchers have observed relatively high biomasses in DWDSs to which drinking water from more than one treatment plant was distributed [10].

4. Conclusions

The results of this study show that invertebrate biomass and composition in a DWDS without microbial regrowth changed in response to changes in the biological stability of the supplied drinking water. The change in water supply in a DWDS from biologically stable drinking water produced from groundwater to less biologically stable drinking water derived from eutrophic reservoir water coincided with an increase in invertebrate biomass and an increased abundancy of *Asellus aquaticus*. Additionally, increases in HPC22 and *Aeromonas* bacteria in the distributed drinking water were observed. Both are microbially regulated regrowth parameters in the Netherlands. These observations of undesirable regrowth conditions as a result of the supply of drinking water derived from eutrophic reservoir water agree with the observations from other Evides facilities producing drinking water from the same eutrophic reservoir water source [10]. It confirms the hypothesis that the assessed biological instability of this drinking water is most likely the cause of higher invertebrate biomass densities, the abundance of *A. aquaticus* and the highly regulated microbial parameters in these DWDS [9,10]. However, since the regrowth conditions (occurrence of *A. aquaticus* and high *Aeromonas* counts) are not observed in the western DWDS section, supplied with the same biologically unstable drinking water, we conclude that aside from the biological stability of the drinking water there are additional and currently unidentified factors that contribute to these undesirable regrowth conditions. We found three possible differences between the eastern and western sections that may be of additional significance for regrowth control: infrastructural conditions that limit sediment accumulation (DWDS configuration combined with peak demand in summer), the avoidance of cast-iron pipes, and/or a reduction in the iron load of the DWDS.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w15040732/s1.

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