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Distribution of *Asellus aquaticus* and microinvertebrates in a non-chlorinated drinking water supply system – Effects of pipe material and sedimentation

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ABSTRACT

Danish drinking water supplies based on ground water without chlorination were investigated for the presence of the water louse, *Asellus aquaticus*, microinvertebrates (<2 mm) and annelida. In total, 52 water samples were collected from fire hydrants at 31 locations, and two elevated tanks (6000 and 36,000 m^3) as well as one clean water tank at a waterworks (700 m^3) were inspected. Several types of invertebrates from the phyla: arthropoda, annelida (worms), plathyhelminthes (flatworms) and mollusca (snails) were found. Invertebrates were found at 94% of the sampling sites in the piped system with *A. aquaticus* present at 55% of the sampling sites. Populations of *A. aquaticus* were present in the two investigated elevated tanks but not in the clean water tank at a waterworks. Both adult and juvenile *A. aquaticus* (length of 2–10 mm) were found in tanks as well as in pipes. *A. aquaticus* was found only in samples collected from two of seven investigated distribution zones (zone 1 and 2), each supplied directly by one of the two investigated elevated tanks containing *A. aquaticus*. Microinvertebrates were distributed throughout all zones. The distribution pattern of *A. aquaticus* had not changed considerably over 20 years when compared to data from samples collected in 1988–89. Centrifugal pumps have separated the distribution zones during the whole period and may have functioned as physical barriers in the distribution systems, preventing large invertebrates such as *A. aquaticus* to pass alive. Another factor characterising zone 1 and 2 was the presence of cast iron pipes. The frequency of *A. aquaticus* was significantly higher in cast iron pipes than in plastic pipes. *A. aquaticus* caught from plastic pipes were mainly single living specimens or dead specimens, which may have been transported passively trough by the water flow, while cast iron pipes provided an environment suitable for relatively large populations of *A. aquaticus*. Sediment volume for each sample was measured and our study described for the first time a clear connection between sediment volume and living *A. aquaticus* since living *A. aquaticus* were nearly only found in samples with sediment contents higher than 100 ml/m^3 sample. Presence of *A. aquaticus* was not correlated to turbidity of the water. Measurements by ATP, heterotrophic plate counting and Colilert® showed that the microbial quality of the water was high at all locations with or without animals. Four other large Danish drinking water supplies were additionally sampled (nine pipe samples and one elevated tank), and invertebrates were found in all systems, three of four containing *A. aquaticus*, indicating a nationwide occurrence.

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1. Introduction

Invertebrate animals are present in drinking water distribution systems worldwide. In tropical and subtropical countries, some species of invertebrates can act as secondary hosts for parasites and thereby pose a serious health risk to consumers (Evins, 2004). In temperate areas, the presence of the animals is largely regarded as an aesthetic problem (van Lieverloo et al., 2002). However, previous studies have shown that invertebrates such as crustaceans and nematodes can harbour bacterial pathogens and potential pathogens e.g. *Escherichia coli* (indicator organism for faecal contamination) (Bichai et al., 2009; Levy et al., 1984), *Salmonella wichita* (Smerda et al., 1971) and *Campylobacter jejuni* (Schallenberg et al., 2005) and may play a role in the survival of these organisms in drinking water systems. The Danish water supply systems are based on ground water without chlorination, which may increase the risks of growth of bacteria and biofilm formation in the water pipes (Martiny et al., 2003) that may serve as a food supply for animals in the system. The absence of hygienic barriers between waterworks and consumers in terms of chlorination increases the focus on any potential carrier of pathogens such as e.g. invertebrates.

The abundance of invertebrates in distributed drinking water is a source of consumer complaints and the supply companies highly desire to control the invertebrate abundance. Well established sampling methods have been developed in the Netherlands to assess the abundance of most invertebrate taxa in distribution systems, and a two-year survey has confirmed the wide abundance of invertebrates (van Lieverloo et al., 2004). However, studies on the controlling parameters for the distribution of invertebrates on full scale distribution systems are still lacking. In order to obtain and distribute biostable drinking water, biostable materials are needed. van der Kooij et al. (1999), and van Lieverloo et al. (2002) therefore suggested that pipe material may influence the occurrence of invertebrates. This hypothesis has not been tested on a full scale distribution system, nor has the correlation to sedimentation in the pipes and turbidity of the water. van Lieverloo et al. (2002) suggest that multiplication of invertebrates in distribution systems depends on the presence of biofilms and sediment and it is known that keeping the pipes clean by e.g. flushing reduces the amount of invertebrates in the system (Levy, 1990; van Lieverloo et al., 1998). The risk of high sedimentation rates may be enhanced in water pipes constructed for higher flows than the actual flow due to e.g. reduction of water consumption.

The water louse, *Asellus aquaticus*, is present in water distribution systems globally (Australian Government, 2004; Gauthier et al., 1999; Gray, 1999), which often causes consumer complaints (Walker, 1983 and unpublished results) due to its size, which makes it visible to the naked eye. Another nuisance is discolouration of the water by the faeces (pellets) of *A. aquaticus*. A survey from the Netherlands showed that though *A. aquaticus* was not the most abundant of invertebrates present in water distribution systems, most of the invertebrate biomass (86%) was formed by *A. aquaticus* (van Lieverloo et al., 1998).

The aims of this study were, a) to implement methods to examine the distribution of invertebrates in a drinking water system with special emphasis on *A. aquaticus*, b) to investigate the spatial distribution of *A. aquaticus* in different pressure zones and c) to identify factors influencing or being influenced by the presence of *A. aquaticus* with special emphasis on pipe materials, sedimentation, turbidity and microbial water quality.

2. Material and methods

2.1. Locations

The investigated water supply system, VCS Denmark, in Odense, Denmark supplies approximately 150,000 people via a distribution system with 1000 km of pipes and a total pipe volume of 40,000 m^3. The supply company distributes about 10 million m^3 per year with an average flow velocity in the pipes of 0–0.5 m/s. Hence the average residence time is two days but varies from 1 to 14 days. The majority of the pipes are of PVC (polyvinyl chloride) (46%) or PE (polyethylene) (33%), while 20% of the pipes are concrete, asbestos cement or ductile iron pipes (Table 1). The remaining cast iron pipes (1%) are currently being replaced by plastic pipes. The supply system is divided into eleven pressure zones of which seven zones were sampled (Table 1). Although connected, the pressure varies between the different zones, which are separated by centrifugal pumps. The supply network is constructed after a finger principle, which means that it is branched and has a unidirectional flow, hence terminating at the consumers. The transmission network on the other hand is designed as a ring system in order to obtain security of supply. The raw

Table 1 – Characteristics and number of sampling sites in the various distribution zones.

Zone	Area [km^2]	Pipes [km]	Resident population #	Revenue water [m^3]	Pipe material [%]			Samples taken #	
					Plastic	Cast iron	Other	Plastic	Cast iron
1	78	463	93,567	5,971,911	74	2	24	11	8
2	78	383	54,467	2,871,174	81	1	18	5	2
3	23	43	1624	83,474	99	0	1	1	0
5	16	22	1557	79,535	96	0	4	1	0
6	7	8	281	11,040	93	0	7	1	0
7	4	12	1805	84,525	100	0	0	1	0
8	2	5	208	9616	100	0	0	1	0
Total	208	936	153,509	9,111,275	79.2	1.4	19.4	21	10

Table 2 – Main water quality parameters of the supply system in Odense, Denmark.

Water quality parameter	Measured values in Odense	Danish guideline values
Oxygen	9.0–9.3 mg/l	Min. 5 mg/l
NVOC	1.3–2.0 mg/l	Max. 4 mg/l
Temperature	5–16 °C	Max. 12 °C (recommended)
Conductivity	57–79 mS/m	Min. 30 mS/m (recommended)
Total hardness	14–21 H degrees	5–30 H degrees (recommended)
pH	7.4–7.6	7.0–8.5
Iron	<0.01–0.02 mg/l	Max. 0.1 mg/l
Manganese	<0.005 mg/l	Max. 0.02 mg/l
Ammonium	<0.01–0.06 mg/l	Max. 0.05 mg/l

water is ground water treated only by aeration/stripping and biological rapid sand filtration, and distributed without the use of chlorination. Main water quality parameters are presented in Table 2.

2.2. Sampling from pipes and in clean water tanks

Water samples from pipes were collected by flushing from above ground fire hydrants. Before each sampling, the part of the hydrant above the water main was flushed with tap water to remove terrestrial animals living in the water free part of the hydrant. Clean water (10–20 l) was poured into the hydrant and pumped out through a drainage valve with a manual pump. For sampling, a flowmeter and a fire hose were attached to the hydrant and the water was flushed directly into transparent single-use plastic bags in 1 m^3 containers. The flowmeter was cleansed after each sampling and a fresh pre-rinsed fire hose was used at each site. No water was discharged by pre-flushing in order to be able to detect invertebrates inhabiting dead ends by the hydrants.

At each site, samples were obtained by flushing 1 m^3, corresponding to approximately 5–300 m of pipes, at maximum obtainable flow (turbulent flow). In 80% of the samples, main diameters were between 80 and 110 mm, which corresponds to 100–200 m of flushed pipes. The sampled volume was measured per time unit in order to calculate the flow velocity, and the Reynolds numbers, expressing the turbulence, were reported. Samples were obtained from 31 locations. To avoid public interest, the samples were not filtered in the street at the sampling point but were transported to the waterworks and slowly filtered (5–10 l/min) successively through two nets with mesh sizes of 500 and 100 μm. To avoid contamination from one sample to another the nets were cleansed with tap water at high flow.

Reproducibility was investigated at three locations where sampling was repeated one or two times with varying time intervals (Table 3).

Three water tanks: one 700 m^3 clean water tank of a waterworks and two elevated tanks (elevated tank 1 containing 2 × 18,000 m^3 and elevated tank 2 containing 6000 m^3) were emptied and the floors were carefully inspected. In the elevated tank l, 20 random samples (each covering 0.35 m^2) were taken on the floor in half of the tank. In the other half of the tank the flush channel (30 m^2) in the length of the tank was inspected and the animals were sampled by 10 ml pipettes.

A. aquaticus was easily visible in the 500 μm net samples, while 1–5 ml sediment per sample from 100 μm net samples and samples from clean water tanks were examined by stereo microscopy with a Protec digital camera (16 × 11.3 × 0.63–4.0 magnification). Invertebrates were identified, counted and measured (head to tail, rounded to nearest millimetre).

To investigate whether the occurrence of invertebrates in the drinking water supply was nationwide, additional samples were taken from four large Danish water supply systems during March–December 2009. Three times three samples were obtained from cast iron pipes (Aarhus Water Ltd., Aalborg Supply, Water Ltd. and TRE-FOR Water Ltd.) by flushing and one sample was collected by visual inspection in an empty elevated tank (Copenhagen Energy Ltd.).

2.3. Validation of sampling from pipes

Prior to the main sampling rounds, sampling efficiency was studied at varying flow velocities and with swabbing applied. Samples were filtered with nets of various mesh sizes and pieces of pipes were cut out. Up to three samples were taken at low laminar flow (Reynolds numbers < 2100) as well as up to three samples at maximum obtainable flow (turbulent flow, Reynolds numbers > 2100) at each locality. After sampling, 150 m of plastic pipe were swabbed with a foam sponge and finally 2 m of pipe were cut out for visual inspection. Swabbing was not possible in cast iron pipes due to scaling but 2 m of pipe were cut out for visual inspection. Four mesh sizes were tested for filtration of the water samples (500, 100, 20 and 10 μm).

2.4. Analyses

2.4.1. Bacterial analyses

Biofilm samples were collected from the inner pipe surfaces of the cut out pipe pieces by scraping of biofilm from 10 cm^2 with a cotton bud. Three scrapes were taken from the plastic pipe (one before and two after swabbing with a sponge). Three samples were taken from two pieces of 1 m cast iron pipes (one from the end, one from the middle and one from a vent). Each cotton bud was kept cold in 10 ml sterile water until 50 μl of the suspension was spread on each R_2A agar plate (triplicates) and 1 ml was spread on each yeast extract agar plates (triplicates) within 24 h and incubated 14 days at 20 °C and 22 °C. Regular bacterial control measurements by HPC (heterotrophic plate counts) on yeast extract agar at 22 °C and 37 °C as well as Colilert® on the supply system were conducted by Eurofins Environment Ltd., Vejen, Denmark. Sediment samples from the 36,000 m^3 elevated tank 1 were investigated for bacterial numbers by R_2A colony count 20 °C and yeast colony count 22 °C and 1–5 *A. aquaticus* per sample at randomly chosen samplings were crushed with a mortar and analysed for *E. coli* and other coliform bacteria by Colilert®. ATP measurements on the sediment were conducted on an Advance Coupe (Celsis, Landgraaf, The Netherlands) with a Celsis kit.

Table 3 – *Asellus aquaticus* and sediment volume at repeated samplings.

Sample locations	Dates	1st Sampling		2nd Sampling		3rd Sampling	
		A. aquaticus/m^3	Sediment vol. [ml/m^3]	*A. aquaticus*/m^3	Sediment vol. [ml/m^3]	*A. aquaticus*/m^3	Sediment vol. [ml/m^3]
1	07.01.08 + 24.03.09	0	180	2	200	–	–
9	23.10.08 + 15.12.08 + 10.06.09	9	5	16	60	5	20
15	15.12.08 + 16.03.09	9	170	3	300	–	–

2.4.2. *Iron and Manganese*

Sediment from the elevated tank 1 was dried at 105 °C (18 h) and analysed for content of iron and manganese on a Varian Vista MPX Axial View Inductively Coupled Plasma (ICP) OES after acid digestion with 7 M HNO_3, after 80 °C sand bath (4 h) and 0.45 μm filtration (Fe: 259,940 nm, Mn: 293,931 nm).

2.4.3. *Turbidity*

After settling for a minimum of 2 h, 5 L of sample were transferred to a plastic container. Following 5 s of shaking, turbidity was measured in triplicates on a Hach 2100N Laboratory Turbidimeter. A second 5 L sample was taken from each 1 m^3 container when only 200 L of water sample remained in the container. Turbidity readings on the initial water were in accordance with the repeated measurements.

2.4.4. *Sediment volume*

Sediment remaining in the 100 and 500 μm filters and sediment scraped from the 1 m^3 plastic bags were stored in graduated glass bottles. After sedimentation for a minimum of seven days, the total sediment volume of all three fractions was measured.

Statistical analyses were performed using R software (R Development Core Team, 2010).

3. Results and discussion

3.1. *Validation of sampling methodology*

Sampling at different flow rates revealed that only microscopic invertebrates and oligochaete worms were flushed out at laminar flow (Reynolds numbers < 2100). Highly turbulent flow (Reynolds numbers > 25,000) was necessary to flush out *A. aquaticus*. When a pipe was swabbed with a sponge following sampling at highly turbulent flow both *A. aquaticus* and microscopic invertebrates could still be found in the pipes. Additional invertebrates were not found in the cut out piece of plastic pipe nor in the cast iron pipe but the animals may have escaped while the pipes were being cut. Complete removal of terrestrial animals from the fire hydrants could not be validated. However, gill-bearing animals such as *A. aquaticus* would not originate from the water free part of the hydrants. In a previous study with flushing at 1.0 m/s, the removal efficiencies of different invertebrate groups varied between 30% and 75% assuming a complete removal by extensive cleaning (high velocity flushing and swabbing with 3 consecutive swabs) after sampling. Mains couplings though, proved to be hide-outs for *A. aquaticus* out of reach for practical sampling methods (van Lieverloo et al., 2004).

In studies operating with fixed flows of typically 1.0 m/s (e.g. van Lieverloo et al., 2004), the sampling procedure is only applicable on pipes within a certain interval of pipe diameters since flow velocities depend on the main diameters. In the present study pipes with diameters from 63 to 500 mm were sampled. To apply the method to all pipe sizes, a novel approach using Reynolds numbers was adopted, which allows for expressing the actual turbulence exerted on the invertebrates while flushing. Reynolds numbers for cast iron pipes will always be theoretical though, since the actual inner pipe diameter or roughness is not known due to corrosion and scaling.

The 10 μm mesh clogged instantly, and the 20 μm mesh clogged frequently and were only used in the methodology studies. van Lieverloo et al. (2004) found that 100 μm nets retained 53–100% of the taxa with copepod larvae and nematodes being the hardest to retain. A 20 μm mesh could be used to obtain greater accuracy on the quantification on microinvertebrates but for the purpose of our study, processing of more samples was favoured. After implementation of the methodology, all subsequent sampling was done at maximum obtainable flow. Sampling size of 1 m^3 was chosen as the standard sample size due to prioritisation of the quantity of sampling localities, though this volume is most likely to be too small to identify all positive samples. This sampling volume is in accordance with a 2-year survey in the Netherlands, where a sample volume of 1 m^3 was recommended due to applicability (van Lieverloo et al., 2004). The low filtration rate of 5–10 L/min minimised injuring the invertebrates but damage during sampling may have led to an underestimation of the number of samples containing living *A. aquaticus*.

Random sampling in the first half of the 36,000 m^3 elevated tank 1 yielded only one *A. aquaticus* in total from 20 random samples covering a total area of 7 m^2. Obviously, *A. aquaticus* was not randomly distributed on the floor of the tank but gathered in remaining pools of water. In the second half of the tank >200 *A. aquaticus* were sampled from an area of 30 m^2 in the flush channel with remaining water, cutting transversely through the tank. The optimal sampling method in tanks was inspection of the entire floor, which was done in the 700 m^3 and the 6000 m^3 tanks. When tank size does not allow this method, samples should be collected from flush channels and similar low lying areas with water remaining.

3.2. *Reproducibility of flushing pipes*

Three locations were sampled two or three times (Table 3). At site 1, no *A. aquaticus* was found during the first sampling,

although 3 m^3 were flushed out at highly turbulent flow (Reynolds number: 100,000, flow: 1.1 m/s). Microscopy of the flushed out sediment revealed a high number of *A. aquaticus* pellets. When sampling at the same site approximately one year later, two *A. aquaticus* were caught in 1 m^3 of flushed out water. Hence, *A. aquaticus* was present or had been present recently at site 1 during the first sampling and the population size remained relatively low over time. At the sites 9 and 15, *A. aquaticus* were caught at all samplings at higher as well as lower numbers per m^3 than at the previous sampling. At a sampling conducted less than two months after the first sampling at site 9 the caught number of *A. aquaticus* was raised from 9/m^3 to 16/m^3, hence there was no indication of *A. aquaticus* being removed from the location on a long term scale by sampling at maximum obtainable flow (Reynolds number of 84,000).

3.3. *Occurrence of invertebrates in pipes and clean water tanks*

Invertebrates within the phyla: arthropoda, annelida (worms) and plathyhelminthes (flatworms) were found in the drinking water distribution system (Fig. 1). The observed invertebrates are all commonly found in drinking water distribution systems (Evins, 2004; van Lieverloo et al., 2002). A land slug was observed on the wall of a clean water tank. The water louse, *A. aquaticus*, was found at 55% of the investigated sampling points, while 94% of the samples contained animals when microscopic invertebrates (<2 mm) and annelida were included. The highest concentrations of microinvertebrates observed in this study were 9000 specimens/m^3 sample with an average of 800 specimens/m^3 sample. Levels of 0–959 invertebrates/m^3 in drinking water leaving the waterworks were measured in a German ground water based supply (DVGW, 1997), while samples from pipes contained a rough mean of 1000 specimens/m^3 in a national Dutch survey in 1993–95 with maximum values reaching more than 10,000 specimens/m^3 (van Lieverloo et al., 2002).

The concentrations of *A. aquaticus* in the positive samples of our study varied between 1 and 14 specimens/m^3 with an average of 4/m^3. This is slightly higher than observed in the German survey with 1–10 *A. aquaticus*/m^3 and an average of 2/m^3 (DVGW, 1997). Compared to observations decades ago these concentrations are relatively low, e.g. another survey from Germany reports concentrations of *A. aquaticus* of 5–30 specimens/m^3 (Schwarz et al., 1966), while data from 1948–96 compiled by van Lieverloo et al. (2002) reported of means from 10 to 100 *A. aquaticus*/m^3 in flushing water.

A. aquaticus varied in length from 2 to 10 mm (Fig. 2), which is small compared to *A. aquaticus* from fresh water ponds reaching up to 20 mm. The average size in pipe samples was 4.3 mm (standard deviation 1.4 mm) and in the elevated clean water tanks 6.3 mm (standard deviation 1.2 mm). The abundance of large animals in the tanks may be caused by the stable environment with sufficient bacteria-rich sediment in the elevated clean water tanks. Part of the observed size difference was presumably an effect of the different sampling techniques since the nets retained all sizes of *A. aquaticus* while small specimens were easily ignored when tanks were visually inspected. Besides the small size, transparency of the juvenile *A. aquaticus* made them difficult to observe in the tanks. *A. aquaticus* sampled in this study were brown (adults) with small eyes (Fig. 1). Characteristic *A. aquaticus* pellets

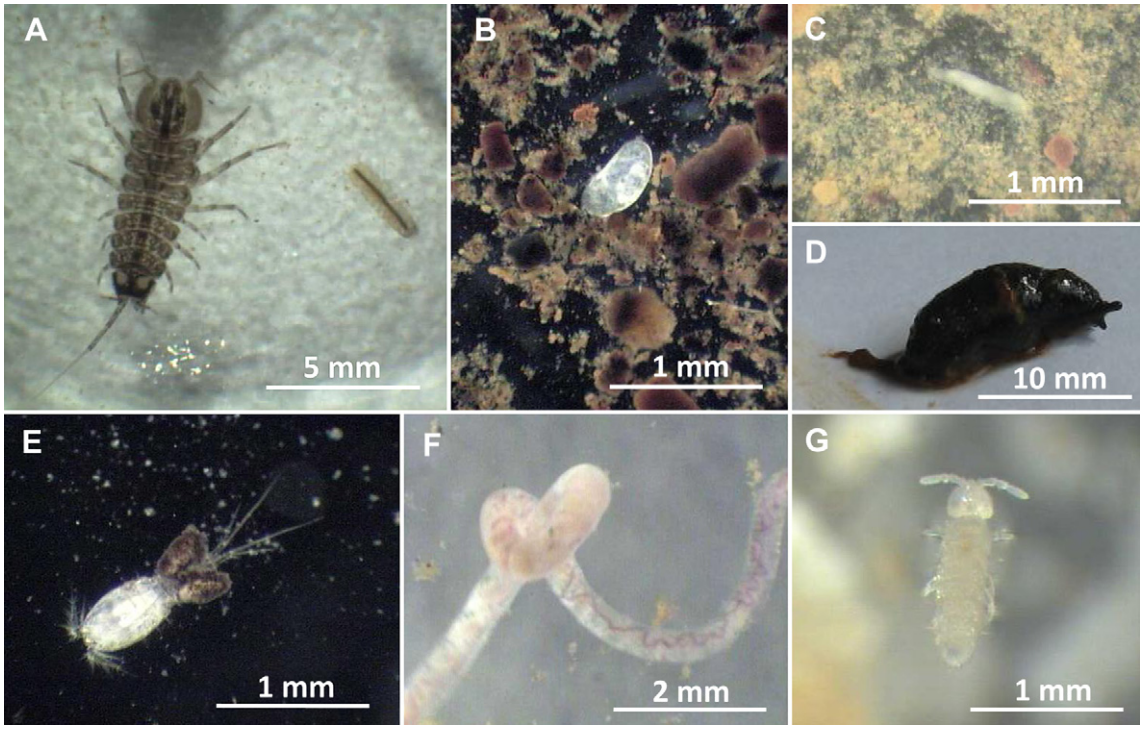

Fig. 1 – A) Adult and juvenile *Asellus aquaticus* (Malacostraca) B) Seed schrimp (Ostracoda) C) Flatworm (Turbellaria) D) Land slug from a clean water tank E) *Cyclops* sp. (Maxillopoda) F) *Tubifex* sp. (Clitellata) G) Springtail (Entognatha). Photos: S.C.B. Christensen.

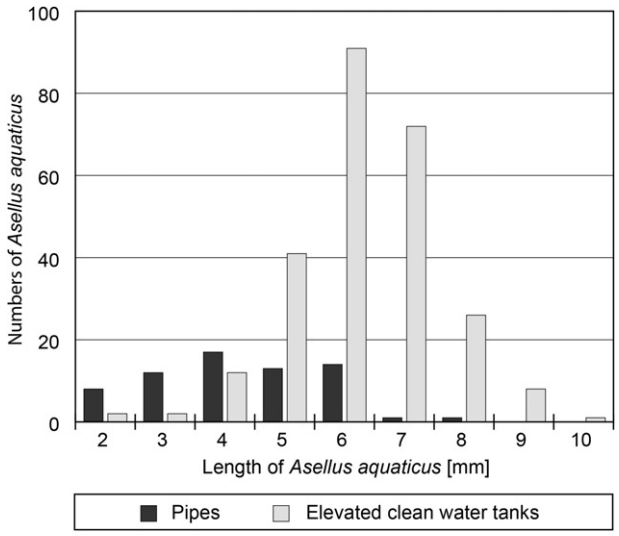

Fig. 2 – Size distribution of *Asellus aquaticus* from all pipe samples and from three sampling rounds in a 36,000 m³ elevated clean water tank.

(DVGW, 1997; Walker, 1983) were observed in many sediment samples (Fig. 3) and could be used as an indication of the presence of *A. aquaticus* populations.

The highest occurrence of *A. aquaticus* in the clean water tanks was found in the 36,000 m³ elevated tank 1 with an average of 7/m² in the flush channel. In the elevated tank 2 of 6000 m³, an equivalent of 0.1 *A. aquaticus*/m² was found on the floor of the tank. *A. aquaticus*, annelida and microinvertebrates were found in both elevated tanks but not in the clean water tank of the waterworks. The water supply company has never observed *A. aquaticus* nor their trails (Fig. 3) during previous controls in clean water tanks of any waterworks. In a German drinking water supply system, partially supplied by ground water, *A. aquaticus* was also found in 50% of the samples from the distribution system, while no *A. aquaticus* could be found at the waterworks (DVGW, 1997).

Both of the investigated elevated tanks contained a layer of fine grained sediment. There was no sediment in the 700 m³ clean water tank at the waterworks and the bacterial concentration in the water of this tank was 23 CFU/ml water (HPC 22 °C). The sediment from the elevated tank 1 had a high content of iron (220 mg/g dry weight), manganese (150 mg/g dry weight) and bacteria (76,000 ± 2700 pg ATP/ml wet sediment and 140,000 CFU/ml wet sediment by HPC 22 °C). ATP measurements of water leaving the two elevated tanks before and after the periods of sampling were low, varying between 1 and 6 pg ATP/ml (Corfitzen and Albrechtsen, 2010).

Samples taken from four additional large Danish distribution companies, nationwide, showed the presence of invertebrates in all investigated systems. *A. aquaticus* was found in three of four systems.

3.4. *Distribution between pressure zones*

Pressure zone 1 with the elevated tank 1 contained the majority of the caught *A. aquaticus* (68% positive samples in zone 1, Fig. 4), while microinvertebrates were present in all parts of the investigated distribution system (94% positive samples) (Fig. 5). Pressure zone 2 with the elevated tank 2 had a few *A. aquaticus* positive samples, with only one living *A. aquaticus* and only an average of 1 specimen per positive sample. No *A. aquaticus* were caught in the remaining zones; zone 3 – zone 8 (Fig. 4).

Samples from 1988–89 from the same area showed a similar distribution pattern: 46% of the samples in zone 1 were positive of *A. aquaticus* while only 5% of the samples in zones 2–8 were positive and only containing dead *A. aquaticus* (Fig. 4). Hence, the distribution of *A. aquaticus* in the samples from 2008–09 was not different from the distribution in the samples from 1988–89 ($p = 1.000$, Fisher's exact probability test for 2 × 2 tables). This indicates that the populations are quite stable once established or that newly entered specimens have similar habitat preferences as prior populations. Previous studies conclude that the establishment of breeding populations is responsible for the greatest number of invertebrates in distribution systems (Evins, 2004). DVGW (1997) pointed at a pipe leakage 30 years prior to the investigations as the way of entry for *A. aquaticus*, and Smalls and Greaves (1968) identified species in several distribution systems in the 1960s that according to Evins (2004) had not been recorded from natural water since the 1920s.

The repeated samplings (Table 3) showed that the season of the year did not affect the occurrence of *A. aquaticus*. In nature, *A. aquaticus* breed between February and October (Gledhill et al., 1993), while we found juvenile *A. aquaticus* all year round in the investigated drinking water distribution

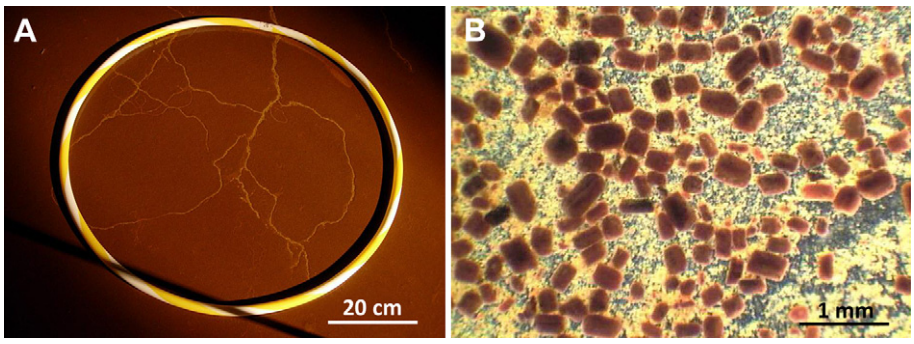

Fig. 3 – Traces of *Asellus aquaticus*. A) Trails on sediment in empty elevated tank. B) Pellets (faeces). The characteristic transverse fissure is seen on some pellets. Photos: S.C.B. Christensen.

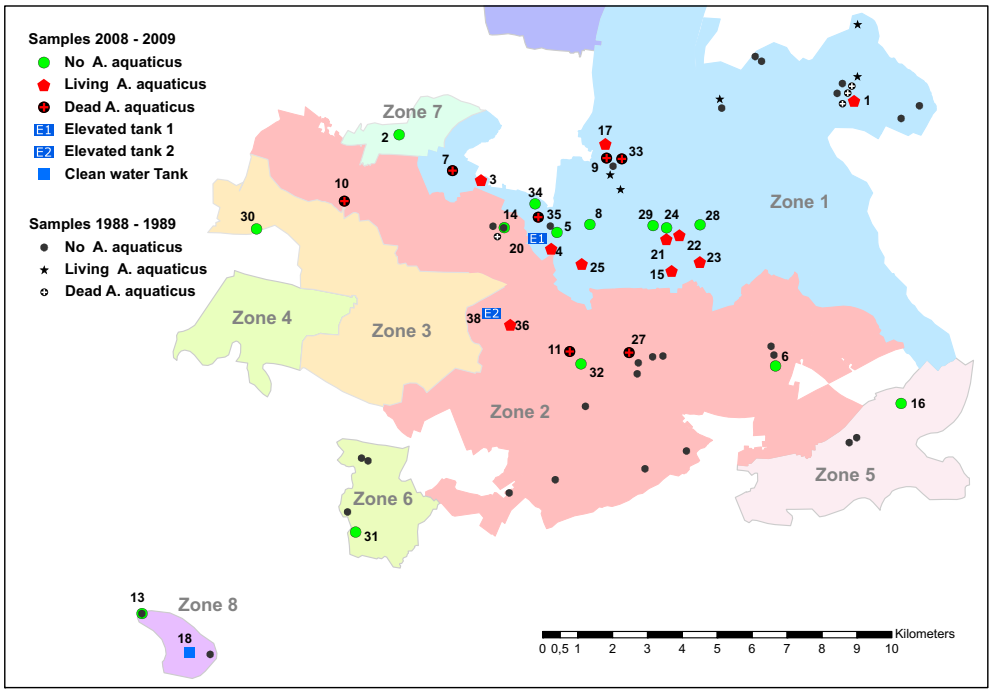

Fig. 4 – Distribution of *Asellus aquaticus* in pressure zones 1–8. The distribution of *A. aquaticus* in the samples from 2008–09 was not different from the distribution in samples from 1988–89. The elevated water tanks in zones 1 and 2 contained *A. aquaticus*, while none was observed in the clean water tank in zone 8. Living *A. aquaticus* were observed in zone 1 covering a wide area while living *A. aquaticus* in zone 2 was found at only one sampling location. No *A. aquaticus* was observed in zones 3–8. Numbers refer to sampling locations.

system. *A. aquaticus* is known to adapt to changing environments over a small spatiotemporal scale (Hargeby et al., 2004). Our observations showed that populations in the drinking water system were able to increase their life span since natural populations in northern Europe are recorded a life span of up to 1 year (Gledhill et al., 1993) while the *A. aquaticus* collected in our study survived in culture (10 °C, darkness, on sediment collected from water pipes and on maple leaves) for up to 2½ years.

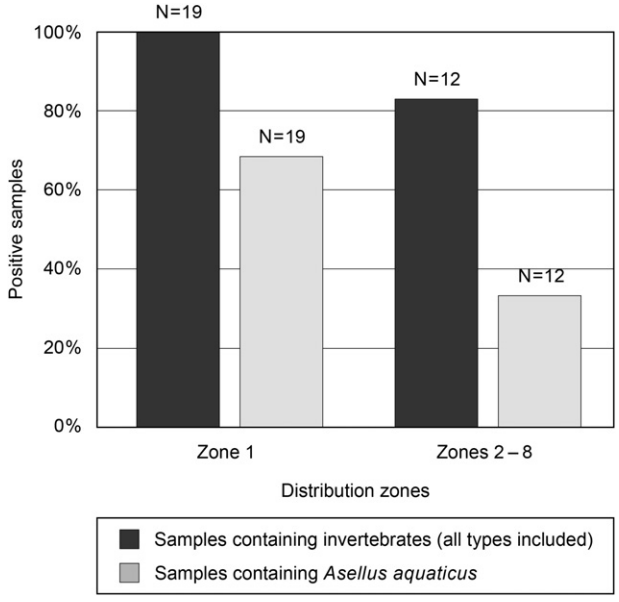

Fig. 5 – Samples containing invertebrates in distribution zone 1 and distribution zones 2–8.

Zone 1 contained above 70% of the cast iron pipes of the system (Table 2) and was furthermore the earliest constructed zone (starting in the 19th century), which would provide plenty of time for the populations of *A. aquaticus* to establish. Zone 2 contained the remaining cast iron pipes (Table 2). It is likely that *A. aquaticus* over time has entered the distribution system in other zones than zone 1 and 2 but have not been able to establish breeding populations. Since zone 1 hosted a larger percentage of both cast iron pipes and *A. aquaticus* than zone 2, pipe material may have the greatest impact on the distribution of *A. aquaticus*. Previous literature states that a species like *A. aquaticus* is recruited into the system infrequently and in small numbers but reach high numbers by successful establishment and breeding (Smalls and Greaves, 1968). Alternatively, the elevated tanks in zone 1 and 2 may have functioned as sources for *A. aquaticus* but since the 36,000 m^3 elevated tank 1 has been emptied, chlorinated and hosed down one year prior to sampling breeding populations are also likely to exist in the pipes. The presence of both juvenile and adult *A. aquaticus* in tanks as well as in pipes (Fig. 2) supports the presence of breeding populations in both systems. Finally, a factor which could inhibit migration between zones was the centrifugal pumps, which separated the zones, and may have functioned as physical barriers by killing larger invertebrates with the fast rotating blades.

3.5. Sedimentation

The availability of food plays a great part in the ability of *A. aquaticus* to survive and establish breeding populations. Sediments in pipes and clean water tanks contain e.g. bacteria and protozoa and may function as a food source for *A. aquaticus* (Gauthier et al., 1999). This study showed that the vast majority of samples with living *A. aquaticus* contained a substantial volume of sediment (more than 100 ml/m^3 sample) (Fig. 6). In 53% of the samples containing >100 ml sediment/m^3 sample, living *A. aquaticus* were observed, while this was significantly lower (10%) in samples containing < 100 ml sediment/m^3 sample (Fisher's exact probability test for 2 × 2 tables, $p = 0.008$). However, the number of living *A. aquaticus* was not directly correlated to the sediment volume in the samples (Pearson's test for correlation). Dead *A. aquaticus* were equally distributed in samples containing low and high sediment volumes. This may be because dead specimens lose their grip instantly and are easily transported to neighbouring parts of the system or because *A. aquaticus* living in areas with low sediment volumes are less fit and more easily killed during sampling.

All samples were collected at highly turbulent flows (Reynolds numbers > 25,000). At these velocities, sediment volume was not correlated to flushing flow velocities or Reynolds numbers (*R*-values below 0.22), hence the relationship between sediment volume and *A. aquaticus* positive samples cannot be explained by higher catchment rates due to more efficient flushing. Regular flushing of pipe systems can reduce the occurrence of *A. aquaticus* (van Lieverloo et al., 1998) but, to our knowledge, no quantitative relationship has been shown before. Repeated sampling at three localities showed that sediment volume varied from sampling to sampling and neither the sediment nor *A. aquaticus* were eliminated by sampling at maximum obtainable flow (Table 3). Flushing larger water volumes than 1 m^3 at maximum obtainable flow may reduce the sediment to values below the threshold of approximately 100 ml sediment/m^3 sample, where living *A. aquaticus* was found to occur, and hence reduce their occurrence.

3.6. Pipe materials

To investigate the importance of pipe materials we compared samples from cast iron and plastic pipes in zone 1. Although present in both pipe types, significantly more samples from cast iron pipes than from plastic pipes contained *A. aquaticus* (100% positive samples versus 45% positive samples) ($p = 0.018$, Fisher's exact probability test for 2 × 2 tables) (Fig. 7). Furthermore, the average concentration of *A. aquaticus* was higher in cast iron pipes (6 specimens/m^3) than in plastic pipes (1.6 specimen/m^3) ($p = 0.037$, Mann–Whitney *U*-test) (Fig. 7).

Five of the samples were taken at localities within a 300 m radius with the same source of water supplying all five points. Three of the sampled pipes were plastic pipes and the remaining two were cast iron pipes. Only the cast iron pipes contained *A. aquaticus*, which indicates that cast iron pipes provide an environment suitable for populations of *A. aquaticus*. *A. aquaticus* caught from plastic pipes elsewhere in the system were mainly single living specimens or dead specimens, which may have been transported passively through by the water flow. The dead specimens could also be an indication of less fit *A. aquaticus*, which were easily killed during sampling.

There was no difference between the median value nor the mean value of sediment/m^3 sample in cast iron and plastic pipes on a 5% level of significance (Mann–Whitney *U*-test and a *t*-test with log transformation of the data), hence the amount of sediment was similar in the two pipe types. High sediment volumes (>100 ml sediment/m^3 sample) were obtained from plastic pipes in 45% of the samples but only 40% of the fraction with high sediment volumes contained *A. aquaticus*. Therefore the pipe type itself had a large influence on the occurrence of *A. aquaticus*, which was not just caused by one pipe type accumulating more sediment than the other.

Several factors may be involved in making cast iron pipes a preferable habitat for *A. aquaticus*: They provided many hiding places due to corrosion and scaling, and more food, e.g. from iron-oxidising and nitrite-oxidising bacteria may be

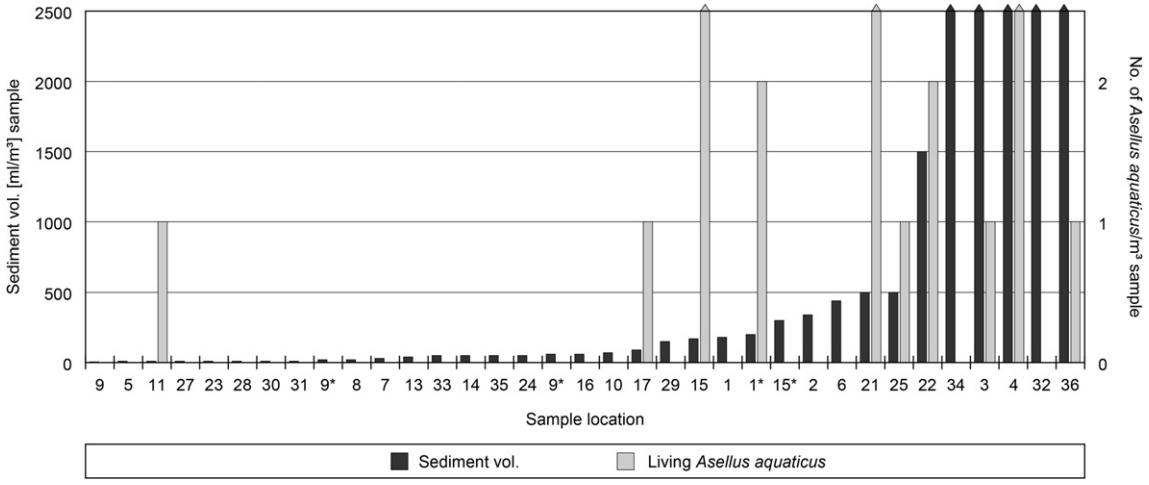

Fig. 6 – Numbers of living *Asellus aquaticus* and the relation to sediment volume per sample. Pointed bars show values above 2500 ml sediment or above two *A. aquaticus*/m^3 sample. The proportion of living *A. aquaticus* in samples containing >100 ml sediment/m^3 sample (53%) was significantly higher than in samples containing < 100 ml sediment/m^3 sample (10%). * shows repeated samplings.

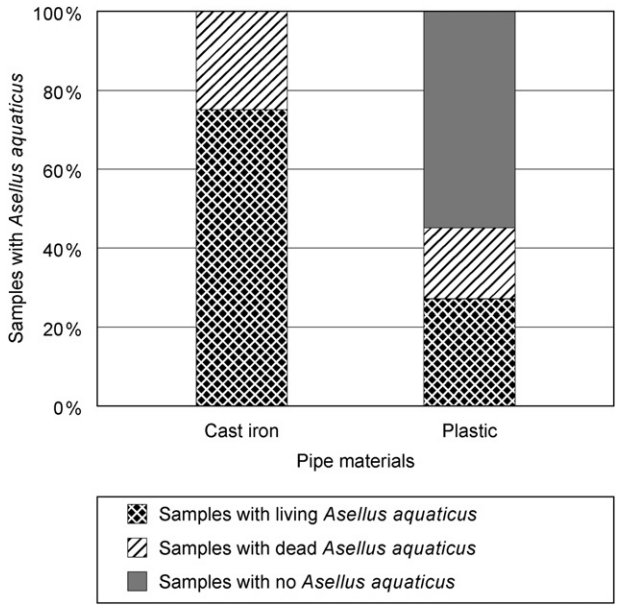

Fig. 7 – The distribution of samples with living *Asellus aquaticus* and dead *A. aquaticus* from 8 cast iron pipes and 11 plastic pipes from zone 1. *A. aquaticus* was present in a significantly higher number of samples from cast iron pipes than plastic pipes (100% positive samples versus 45% positive samples). There was a significantly higher concentration of *A. aquaticus* in cast iron pipes 6.0/m^3 than in plastic pipes 1.6/m^3. Replicate samplings are removed. Dead *A. aquaticus* may be present in samples with living *A. aquaticus*.

available in cast iron pipes (Martiny et al. 2005). Finally, the cast iron pipes were old pipes (up to 90 years) providing an undisturbed environment. Since all cast iron pipes were more than 62 years old at the time of sampling, there was no basis for studying the effects of pipe age of cast iron pipes. For plastic pipes, the samples taken in 2008–09 containing *A. aquaticus* were all but one from pipes older than 32 years. In the 1988–89 samples all *A. aquaticus* positive samples were from pipes, which were 17–19 years old at the time of sampling. The common characteristics of these positive samples were that the pipes originated from around 1970. Hence, it may merely be due to factors related to the specific period of the construction of the system in 1970 than the pipe age itself.

3.7. *Turbidity*

The occurrence of *A. aquaticus* did not correlate with turbidity. This was probably because high turbidity values were often measured due to red iron or black manganese colloidal particles, which did not settle though given several days. Hence, since turbidity did not simply reflect the amount of sediment, turbidity could not be used for prediction of the presence of *A. aquaticus*.

3.8. *Microbial water quality*

Over the two years of sampling heterotrophic plate counts (HPC 37 °C) did not exceed 5 CFU/ml at any control measurement at the sampling points. Neither were any *E. coli* nor other coliform bacteria detected at any sampling location or in the analyses of crushed *A. aquaticus*. This is contrary to land slugs intruding clean water tanks, which have been observed to cause measurable concentrations of coliform bacteria (unpublished results).

Scrapes from biofilm (not sediment) in the cut out pieces of pipes showed low levels of heterotrophic bacteria (below an average of 190 CFU/cm^2, HPC 22 °C) in cast iron as well as plastic pipes. At 80% of the sampling locations, bacterial numbers measured prior to and after sampling did not exceed 10 CFU/ml (HPC 22 °C). The Danish guideline value of 200 CFU/ml (HPC 22 °C for water at the consumers tap) was only exceeded at two locations, both related to cutting out pipes and most likely generated by the pipe work. At these two sites, bacterial concentrations increased from 3 CFU/ml before sampling to 210 CFU/ml after sampling and from 4 CFU/ml before sampling to 220 CFU/ml after sampling. There was no correlation between the distribution of *A. aquaticus* and heterotrophic bacteria based on the regular control measurements, and the microbial quality of the water in the distribution system was good in the investigated zones over the two years of sampling, including locations where *A. aquaticus* were caught repeatedly.

4. Conclusions

In conclusion, this first investigation of invertebrate occurrence in a Danish drinking water distribution system showed that:

- Flushing at highly turbulent flow (Reynolds numbers > 25,000) and preferably swabbing was necessary to sample *A. aquaticus* from drinking water pipes
- Juvenile and adult invertebrates (*A. aquaticus*, microinvertebrates or annelida) were present in 94% of the samples, both in the distribution system in pipes and in the elevated clean water tanks
- Microinvertebrates were present in all parts of the distribution system, while the occurrence of *A. aquaticus* was influenced by the location in the distribution system (percentage of cast iron pipes, separation by centrifugal pumps)
- Data from 1988 – 89 samples showed that the distribution pattern of *A. aquaticus* had not changed considerably over 20 years
- Microinvertebrates were present in cast iron as well as plastic pipes
- *A. aquaticus* was present mainly in cast iron pipes and in higher concentrations than in plastic pipes
- The vast majority of samples with living *A. aquaticus* contained a substantial volume of sediment (more than 100 ml/m^3 sample) however, the number of living *A. aquaticus* in the samples was not directly correlated to sediment volume in the samples
- The microbial quality of the investigated drinking water distribution system was high and without correlation to the presence of *A. aquaticus*

5. Perspective

Despite various attempts over time, total removal of invertebrates from drinking water supply systems have shown close to impossible. A great nuisance to consumers is caused by larger animals like *A. aquaticus*. The knowledge obtained from this study can be applied to control the presence of *A. aquaticus* by replacing cast iron pipes with plastic pipes in areas with high concentrations of *A. aquaticus*. Sediment threshold values in supply system can be used to determine a feasible level of cleaning of the pipes in order to control *A. aquaticus* populations.

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Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.watres.2011.03.039.

REFERENCES

Australian Government, 2004. Australian Drinking Water Guidelines 6, National Water Quality Management Strategy chap. 5.

Bichai, F., Barbeau, B., Payment, P., 2009. Protection against UV disinfection of *E. coli* bacteria and *B. subtilis* spores ingested by *C. elegans* nematodes. Water Res. 43, 3397–3406.

Corfitzen, C.B., Albrechtsen, H.J., 2010. Microbiological Investigations of the Effects of UV Treatment in the Supply System of Water Center South, Phase 2 (In Danish: Mikrobiologiske undersøgelser af effekten af UV-belysning i VandCenter Syds ledningsnet, fase 2). DTU Environment, Technical University of Denmark.

DVGW, 1997. Animals in Water Supply Systems (In German: Tierische Organismen in Wasserversorgungsanlagen). DVGW Regelwerk, Technische Mitteilung, Hinweis W 271.

Evins, C., 2004. Small animals in drinking water distribution systems. In: Safe Piped Water: Managing Microbial Water Quality in Piped Distribution Systems. World Health Organization, IWA Publishing, London, pp. 101–120.

Gauthier, V., Gérard, B., Portal, J.M., Block, J.C., Gatel, D., 1999. Organic matter as loose deposits in a drinking water distribution system. Water Res. 33, 1014–1026.

Gledhill, T., Sutcliffe, D.W., Williams, W.D., 1993. British Freshwater Crustacea Malacostraca: A Key with Ecological Notes, vol. 52. Freshwater Biological Association, Scientific Publication no. 173.

Gray, N.F., 1999. Water Technology an Introduction for Scientists and Engineers. Elsevier, pp. 548.

Hargeby, A., Johansson, J., Ahnesjö, J., 2004. Habitat specific pigmentation in a freshwater isopod – adaptive evolution over a small spatiotemporal scale. Evolution 58, 81–94.

Levy, R.V., Cheetham, R.D., Davis, J., Winer, G., Hart, F.L., 1984. Novel method for studying the public health significance of macroinvertebrates occurring in potable water. Appl. Environ. Microbiol. 47, 889–894.

Levy, R.V., 1990. Invertebrates and associated bacteria in drinking water distribution lines. In: McFeters, G.A. (Ed.), Drinking Water Microbiology. Springer-Verlag, pp. 224–238.

Martiny, A.C., Albrechtsen, H.J., Arvin, E., Molin, S., 2005. Identification of bacteria in biofilm and bulk water samples from a non-chlorinated model drinking water distribution system: detection of a large nitrite-oxidizing population associated with *Nitrospira* spp. Appl. Environ. Microbiol. 71, 8611–8617.

Martiny, A.C., Jørgensen, T.M., Albrechtsen, H.J., Arvin, E., Molin, S., 2003. Long-term succession of structure and diversity of a biofilm formed in a model drinking water distribution system. Appl. Environ. Microbiol. 69 (11), 6899–6907.

R Development Core Team, 2010. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0. http://www.R-project.org/.

Schallenberg, M., Bremer, P.J., Henkel, S., Launhardt, A., Burns, C.W., 2005. Survival of *Campylobacter jejuni* in water: effect of grazing by the freshwater crustacean *Daphnia carinata* (Cladocera). Appl. Environ. Microbiol. 71 (9), 5085–5088.

Schwarz, H., Kucera, R., Klapper, H., Kramer, W., Schuster, W., 1966. Erfahrungen bei der Bekämpfung von *Asellus aquaticus* in den Wasserversorgungsanlagen der Stadt Magdeburg. Fortschritte der Wasserchemie und ihrer Grenzgebiete 4, 96–127 (in German).

Smalls, I.C., Greaves, G.F., 1968. A survey of animals in distribution systems. Water Treat. Exam. 17, 150–186.

Smerda, S.M., Jensen, H.J., Anderson, A.W., 1971. Escape of Salmonellae from Chlorination during Ingestion by *Pristionchus Lheritieri* (Nematoda: Diplogasterinae).

van der Kooij, D., van Lieverloo, J.H.M., Schellart, J.A., Hiemstra, P., 1999. Distributing drinking water without disinfectant: highest achievement or height of folly? J. Water SRT Aqua 48, 31–37.

van Lieverloo, J.H.M., Bosboom, D.W., Bakker, G.L., Brouwer, A.J., Voogt, R., De Roos, J.E.M., 2004. Sampling and quantifying invertebrates from drinking water distribution mains. Water Res. 38, 1101–1112.

van Lieverloo, J.H.M., van der Kooij, D., Hoogenboezem, W., 2002. Invertebrates and protozoans (free-living) in drinking water distribution systems. In: Bitton, G. (Ed.), Encyclopedia of Environmental Microbiology. Wiley, New York, pp. 1718–1733.

van Lieverloo, J.H.M., van Buuren, R., Veenendaal, G., van der Kooij, D., 1998. Controlling invertebrates in distribution systems with zero or low disinfectant residual. Water Supply 16, 199–204.

Walker, A.P., 1983. The microscopy of consumer complaints. J. Inst. Water Eng. Sci. 37, 200–214.