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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* and microinvertebrates (< 2 mm). In total 52 water samples were collected from fire hydrants at 31 different locations, and two elevated tanks (6,000 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 2-9 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. Comparisons with data from samples collected in 1988-89 showed that the distribution pattern of *A. aquaticus* had not changed considerably over 20 years. Centrifugal pumps have separated the distribution zones during the whole period and may have functioned as physical barriers in the distribution systems, which large invertebrates such as *A. aquaticus* could not 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 being 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 the study described for the first time that the correlation between presence of living *A. aquaticus* and sediment volume is not simple but rather expressed by a minimum sediment value of approximately

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 distribution companies 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.

Key words: invertebrates, microbial quality, distribution system, cast iron, water storage tank

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), *Salmonella livingstone* (Levy et al. 1984) 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 solely 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 it has therefore been suggested that pipe material may influence the occurrence of invertebrates (van Lieverloo et al. 2002). 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 diminishes the amount of invertebrates in the system (Levy 1990, van Lieverloo et al. 1998). The water consumption in Odense has dropped approximately 40 % since 1990 with a similar tendency nationwide, which enhances the risk of high sedimentation rates in water pipes constructed for higher flows.

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 pers. obs.) due to its size, which makes it visible to the naked eye. Another nuisance is discoloration of the water by the faeces of *A. aquaticus* (pellets). 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 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 in Odense, Denmark supplies approximately 150,000 people, via a distribution system with 1,000 km of pipes and a total pipe volume of 40,000 m^3. The supply company distributes slightly more than 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 pipes are PVC pipes (46%) or PE/PEM pipes (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 11 pressure zones of which zones 1-8 were sampled. Although connected, the pressure varies in 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 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

104 Water samples from pipes were collected by flushing from above ground fire hydrants. Before each
105 sampling, the part of the hydrant above the water main was flushed with tap water to remove
106 terrestrial animals living in the water free part of the hydrant. Clean water (10-20 L) was poured
107 into the hydrant and pumped out through a drainage valve with a manual pump. For sampling a
108 flowmeter and a fire hose were attached to the hydrant and the water was flushed directly into
109 transparent single-use plastic bags in 1 m^3 containers. The flowmeter was cleansed after each
110 sampling and a fresh pre-rinsed fire hose was used at each site. No water was discharged by pre-
111 flushing in order to be able to detect invertebrates inhabiting dead ends by the hydrants.
112 At each site samples were obtained by flushing 1 m^3 at maximum obtainable flow
113 (turbulent flow). The sampled volume was measured per time unit in order to calculate the flow
114 velocity, and the Reynolds numbers were reported. Samples were obtained from 31 locations. To
115 avoid public interest, filtration on the street at the sampling point was not used but all samples were
116 transported to the waterworks and slowly filtered (5-10 L/min) successively through two nets with
117 mesh sizes of 500 and 100 µm. To avoid contamination from one sample to another the nets were
118 cleansed with tap water at high flow.
119 Reproducibility was investigated at three locations where sampling was repeated one
120 or two times with varying time intervals (Table 3).
121 Three water tanks: one 700 m^3 clean water tank of a waterworks and two elevated tanks (elevated
122 tank 1 containing 36,000 m^3 and elevated tank 2 containing 6,000 m^3) were emptied and the floors
123 were carefully inspected. In the elevated tank l, 20 random samples (each covering 0.35 m^2) were
124 taken on the floor in half of the tank. In the other half of the tank the flush channel (30 m^2) in the
125 length of the tank was sampled by sucking up the animals with 10 ml pipettes.
126 *Asellus aquaticus* was easily visible in the 500 µm net samples, while 1-5 ml sediment per
127 sample from 100 µm net samples and samples from clean water tanks were examined by stereo

128 microscopy with a Protec digital camera (16x11.3x0.63-4.0 magnification). Invertebrates were
129 identified, counted and measured (head to tail).
130 In order to investigate whether the occurrence of invertebrates in the drinking water
131 supply was nationwide, additional samples were taken from four large Danish water supply systems
132 during March - December 2009. Three times three samples were obtained from cast iron pipes
133 (Aarhus Water Ltd, Aalborg Supply, Water Ltd and TRE-FOR Water Ltd) by flushing and one
134 sample was collected by visual inspection in an empty elevated tank (Copenhagen Energy Ltd).
135
136 *2.3. Validation of sampling from pipes*
137 Prior to the main sampling rounds, sampling efficiency was studied at varying flow velocities, with
138 swabbing applied, with cut out pieces of pipes and filtration with various mesh sizes. Up to three
139 samples were taken at low laminar flow (Reynolds numbers < 2,100) as well as up to three samples
140 at maximum obtainable flow (turbulent flow, Reynolds numbers > 2,100) at each locality. After
141 sampling, 150 meters of plastic pipe were swabbed with a foam sponge and finally two meters of
142 pipe were cut out for visual inspection. Swabbing was not possible in cast iron pipes due to scaling
143 but two meters of pipe were cut out for visual inspection. Four mesh sizes were tested for filtration
144 of the water samples (500, 100, 20 and 10 µm).
145
146 *2.4. Analyses*
147 **Bacterial analyses**: Biofilm samples were collected from the inner pipe surfaces of the cut out pipe
148 pieces by scraping of biofilm from 10 cm^2 with a cotton bud. Three scrapes were taken from the
149 plastic pipe (one before and two after swabbing with a sponge). Three samples were taken from two
150 pieces of one meter cast iron pipes (one from the end, one from the middle and one from a vent).
151 Each cotton bud was kept cold in 10 ml sterile water until 50 µl of the suspension was spread on

R_2A and 1 ml was spread on yeast extract agar plates within 24 hours 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. Sediment samples from the 36,000 m^3 elevated tank 1 were investigated for bacterial numbers by R_2A colony count 20° C, yeast colony count 22° C and 1 – 5 *Asellus aquaticus* per sample at randomly chosen samplings were crushed with a mortar and analysed for *Escherichia 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.

Iron and Manganese: Sediment from the elevated tank 1 was analysed for content of iron and manganese by absorption flame spectrometry after acid digestion with 14M HNO_3 and filtration (DS259 2003).

Turbidity: After settling for a minimum of two hours, 5 liters of sample were transferred to a plastic container. Following 5 sec. of shaking, turbidity was measured in triplicates on a Hach 2100N Laboratory Turbidimeter. Repeated measurements were made on all samples when only 200 L of water sample remained in the 1 m^3 container. Turbidity readings on the initial water were in accordance with the repeated measurements.

Sediment volume: Sediment remaining in the 100 and 500 µm filters and sediment scraped from the 1 m^3 plastic bags were stored in 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

176 Sampling at different flow rates revealed that only microscopic invertebrates and oligochaete
177 worms were flushed out at laminar flow (Reynolds numbers < 2,100). Highly turbulent flow
178 (Reynolds numbers >25,000) was necessary to flush out *Asellus aquaticus*. When a pipe was
179 swabbed with a sponge following sampling, it was revealed that even after flushing at highly
180 turbulent flow both *A. aquaticus* and microscopic invertebrates were still present in the pipes. In a
181 previous study with flushing at 1.0 m/s, the removal efficiencies of different invertebrate groups
182 varied between 30 % and 75 % assuming a complete removal by extensive cleaning (high velocity
183 flushing and swabbing with 3 consecutive swabs) after sampling. Mains couplings though, proved
184 to be hide-outs for *A. aquaticus* out of reach for practical sampling methods (van Lieverloo et al.
185 2004). In the present study additional invertebrates were not found in the cut out piece of plastic
186 pipe nor in the cast iron pipe but this may be due to the time consuming process of cutting the pipes
187 during which the animals may escape.
188 In studies operating with fixed flows (e.g. van Lieverloo et al. 2004), the sampling procedure is only
189 applicable on pipes within a certain interval of pipe diameters since flow velocities depend on the
190 main diameters. In this study pipes with diameters from 63 to 500 mm were sampled. In order to
191 apply the method to all pipe sizes a novel approach using Reynolds numbers was adopted, which
192 allows for expressing the actual turbulence that the invertebrates experience while the pipes are
193 being flushed.
194 The 10 µm mesh clogged instantly, and the 20 µm mesh net clogged frequently and were only used
195 in the methodology studies. Van Lieverloo et al. (2004) found that 100 µm nets retained 53 – 100 %
196 of the taxa with copepod larvae and nematodes being the hardest to retain. A 20 µm mesh could be
197 used to obtain greater accuracy on the quantification on microinvertebrates but for the purpose of
198 this study, processing of more samples was favored. After implementation of the methodology, all
199 subsequent sampling was done at maximum obtainable flow. Sampling size of 1 m^3 was chosen as

200 the standard sample size due to prioritisation of the quantity of sampling localities, though this
201 volume is most likely to be too small to identify all positive samples. This is in accordance with a 2-
202 year survey in the Netherlands, where a sample volume of 1 m^3 was recommended due to
203 applicability (van Lieverloo et al. 2004). The low filtration rate of 5-10 L/min minimised injuring
204 the invertebrates but damage during sampling may had led to an underestimation of the number of
205 samples containing living *A. aquaticus*.
206 Random sampling in the first half of the 36,000 m^3 elevated tank 1 yielded only one *A.*
207 *aquaticus* in total from 20 random samples covering a total area of 7 m^2. *A. aquaticus* was not
208 randomly distributed on the floor of the tank but gathered in remaining pools of water. In the second
209 half of the tank >200 *A. aquaticus* were sampled from an area of 30 m^2 in the flush channel cutting
210 transversely through the tank with remaining water. The optimal sampling method in tanks was
211 inspection of the entire floor, which was done in the 700 m^3 and the 6,000 m^3 tanks. When size does
212 not allow this method samples should be collected from flush channels and similar low lying areas
213 with water remaining.
214
215 *3.2. Reproducibility of flushing pipes*
216 Three locations were sampled two or three times (Table 3). At site 1, no *Asellus aquaticus* was
217 found during the first sampling, though 3 m^3 were flushed out at highly turbulent flow (Reynolds
218 number: 100,000, flow: 1.1 m/s). Microscopy of the flushed out sediment revealed a high number of
219 *A. aquaticus* pellets. When sampling at the same site approximately one year later, two *A. aquaticus*
220 were caught in 1 m^3 of flushed out water, which indicates that *A. aquaticus* was present or had been
221 present recently at site 1 during the first sampling and that the population size remained low over
222 time. At the sites 9 and 15, *A. aquaticus* were caught at all samplings at higher as well as lower
223 numbers per m^3 than at the previous sampling. At a sampling conducted less than two months after

224 the first sampling at site 9 the caught number of *A. aquaticus* was raised from 9/m^3 to 16/m^3, hence
225 there was no indication of *A. aquaticus* being removed from the location on a long term scale by
226 sampling at maximum obtainable flow (Reynolds number of 84,000).
227
228 *3.3. Occurrence of invertebrates in pipes and clean water tanks*
229 Invertebrates within the phyla: arthropoda, annelida (worms) and plathyhelminthes (flatworms)
230 were found in the drinking water distribution system (Fig. 1). The observed invertebrates are all
231 commonly found in drinking water distribution systems (Evins 2004, van Lieverloo et al. 2002). A
232 land slug was observed on the wall of a clean water tank. The water louse, *Asellus aquaticus*, was
233 found at 55 % of the investigated sampling points, while 94 % of the samples contained animals
234 when microscopic invertebrates (< 2 mm) and annelida were included. The highest concentrations
235 of microinvertebrates observed were 9000 specimens/m^3 sample with an average of 800
236 specimens/m^3 sample. Levels of 0-959 invertebrates/m^3 in drinking water leaving the water works
237 were measured in a German groundwater based supply (DVGW 1997). The concentration of *A.*
238 *aquaticus* in the positive samples varied between 1 and 14 specimens/m^3 with an average of 4/m^3.
239 This is slightly higher than observed in the German survey, where 1-10 *A. aquaticus*/m^3 with an
240 average of 2/m^3 were observed. Compared to observations decades ago these concentrations are
241 relatively low, e.g. another survey from Germany reports concentrations of *A. aquaticus* of 5-30
242 specimens/m^3 (Schwarz et al. 1966).
243 *A. aquaticus* varied in size from 2 to 9 mm, which is small compared to *A. aquaticus*
244 from fresh water ponds, which can reach 20 mm. *A. aquaticus* sampled in this study were brown
245 with small eyes (Fig. 1). Characteristic *A. aquaticus* pellets (DVGW 1997, Walker 1983) were
246 observed in many sediment samples (Fig. 2) and could be used as an indication of the presence of *A.*
247 *aquaticus* populations.

248 The highest occurrence of *A. aquaticus* in the clean water tanks was found in the
249 36,000 m^3 elevated tank 1. The average of *A. aquaticus* in the flush channel in half the tank was
250 7/m^2. In the elevated tank 2 of 6,000 m^3, an equivalent of 0.1 *A. aquaticus*/m^2 was found on the
251 floor of the tank. *A. aquaticus*, annelida and microinvertebrates were found in both elevated tanks
252 but not in the clean water tank of the waterworks, and the water supply company had never
253 observed *A. aquaticus* nor their trails (Fig. 2) during previous controls in clean water tanks of any
254 waterworks. In a German drinking water supply system, partially supplied by ground water, *A.*
255 *aquaticus* was also found in 50 % of the samples from the distribution system, while no *A.*
256 *aquaticus* could be found at the waterworks (DVGW 1997).
257 Both of the investigated elevated tanks contained a layer of fine grained sediment.
258 There was no sediment in the 700 m^3 clean water tank at the waterworks and the bacterial
259 concentration in the water in this tank was 23 CFU/ml water (HPC 22° C). The sediment from the
260 elevated tank 1 had a high content of iron (5 mg/g wet weight), manganese (1 mg/g wet weight) and
261 bacteria (76,000 +/- 2,700 pg ATP/ml wet sediment and 140,000 CFU/ml wet sediment by HPC 22°
262 C. ATP measures of water leaving the two elevated tanks before and after the periods of sampling
263 were low, varying between 1 and 6 pg ATP/ml (Corfitzen and Albrechtsen 2010).
264 Samples taken from four additional large Danish distribution companies, nationwide,
265 showed the presence of invertebrates in all investigated systems. *A. aquaticus* was found in three of
266 four systems.
267
268 *3.4. Distribution between pressure zones*
269 Pressure zone 1 with the elevated tank 1 contained the majority of the caught *Asellus aquaticus* (68
270 % positive samples in zone 1, Fig. 3), while microinvertebrates were present in all parts of the
271 investigated distribution system (94 % positive samples) (Fig. 4). 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. 3).

Samples from 1988-89 covering 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. 3). Hence, the distribution of living and dead *A. aquaticus* in the samples from 2008-09 was consistent with the samples from 1988-89 ($p = 1.000$, Fisher's exact probability test for 2x2 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 are 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 Small and Graves (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 occurrence of *A. aquaticus* was independent on the season of the year. In nature, *A. aquaticus* breed between February and October (Gledhill et al. 1993), while this was not the case in the investigated drinking water distribution system since we found juvenile *A. aquaticus* all year round. *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 this study survived in culture (10° C, darkness, on sediment collected from water pipes and on maple leaves) for up to 2½ years.

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 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 may also exist in the pipes. The presence of both juvenile and adult *A. aquaticus* (2-9 mm) in tanks as well as in pipes 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 *Asellus aquaticus* to survive and establish breeding populations. The number of living *A. aquaticus* was not directly correlated to the sediment volume in the samples (Pearson's test for correlation), however the vast majority of samples with living *A. aquaticus* contained a substantial volume of sediment (typically more than 100 ml sediment/m^3 sample) (Fig. 5).

All samples were collected at highly turbulent flows (Reynolds numbers > 24,000). At these velocities, sediment volume was not correlated to flushing flow velocities or Reynolds numbers (R-values below 0.22), hence the correlation 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 correlations have been made 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 *Asellus aquaticus* (100 % positive samples versus 45 % positive samples) ($p = 0.018$, Fisher's exact probability test for 2x2 tables) (Fig. 6). Five 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*. This indicates that cast iron pipes provide an environment suitable for breeding populations of *A. aquaticus* while *A. aquaticus* caught from plastic pipes are mainly single living specimens or dead specimens, which may have been transported passively through by the water flow. The average concentration of *A. aquaticus* was also 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. 6).

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.

There may be several factors involved in making cast iron pipes a preferable habitat for *A. aquaticus*: They provided many hiding places due to corrosion and scaling. More food, e.g. from iron-oxidising and nitrite-oxidising bacteria may be 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 pos*itive 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 correlated to the specific period of the construction of the system in 1970 than the pipe age itself.

3.7. Turbidity

The abundance of *Asellus 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 sediment in spite of days of settling of the samples. Hence, since turbidity does not

simply reflect the amount of sediment, turbidity cannot 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 *Escherichia coli* or other coliform bacteria detected at any sampling location or in the analyses of crushed *Asellus 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 exceeded at two locations. The two exceedings were measured after sampling at the two sites, where pipes had been cut out and were most likely generated by the pipe work. 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 *Asellus 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* was 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 > 24,000) and preferably swabbing was necessary to sample *Asellus aquaticus* from drinking water pipes, but swabbing injured the animals
- Juvenile and adult invertebrates (*A. aquaticus* or microinvertebrates) were present in 94 % of the samples, both in the distribution system in pipes and in the 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 number of living *A. aquaticus* in the samples was not directly correlated to sediment volume in samples but the vast majority of samples that were positive with living *A. aquaticus* contained a substantial volume of sediment (approximately 100 ml sediment/m^3 sample)
- The microbial quality of the investigated drinking water distribution system was high and without correlation to the presence of *A. aquaticus*

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

Zone	*Area [km²]*	*Pipes [km]*	*Resident pop. #*	*Revenue water [m³]*	*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	1,624	83,474	99	0	1	1	0
5	16	22	1,557	79,535	96	0	4	1	0
6	7	8	281	11,040	93	0	7	1	0
7	4	12	1,805	84,525	100	0	0	1	0
8	2	5	208	9,616	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

Table 3. Repeated samplings

Sample locations	Dates	1st sampling		2nd sampling		3rd sampling	
		Asellus/m³	sediment vol. [ml/m³]	*Asellus*/m³	sediment vol. [ml/m³]	*Asellus*/m³	sediment vol. [ml/m³]
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	-	-

Figure 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.

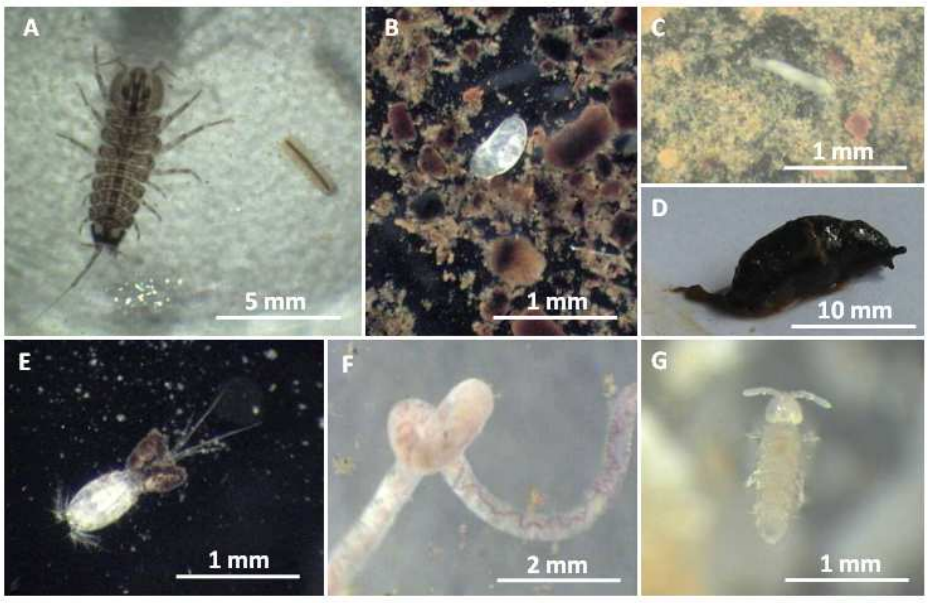
Figure 2. Traces of *Asellus aquaticus.* A) Trails on sediment in empty elevated tank. B) Pellets (faeces). The characteristic transverse fissure is seen on some pellets. Photos: S.C.B. Christensen.

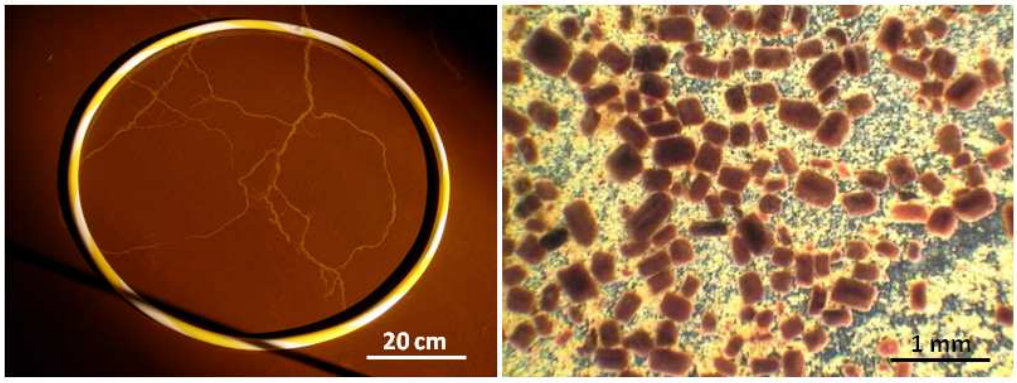
Figure 3. Distribution of *Asellus aquaticus* in pressure zones 1-8. The distribution of living and dead *A. aquaticus* in the samples from 2008-09 was consistent with the samples from 1988-89 (p = 1.000, Fisher's exact probability test for 2x2 tables). The elevated water tanks in zones 1 and 2 contained *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 aria 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.

Figure 4. Samples containing invertebrates in distribution zone 1 and distribution zones 2-8.

Figure 5. Numbers of living *Asellus aquaticus* and the connection to sediment volume per sample. Pointed bars show values above 2500 ml sediment or above two *A. aquaticus*/m^3 sample. The proportion of *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%) (p = 0.008, Fisher's exact probability test for 2x2 tables)."

Figure 6. 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) (p= 0.018, Fisher's exact probability test for 2x2 tables). 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 (p = 0.037, Mann-Whitney U-test).
Replicate samplings are removed. Dead *A. aquaticus* may be present in samples with living *A. aquaticus*.

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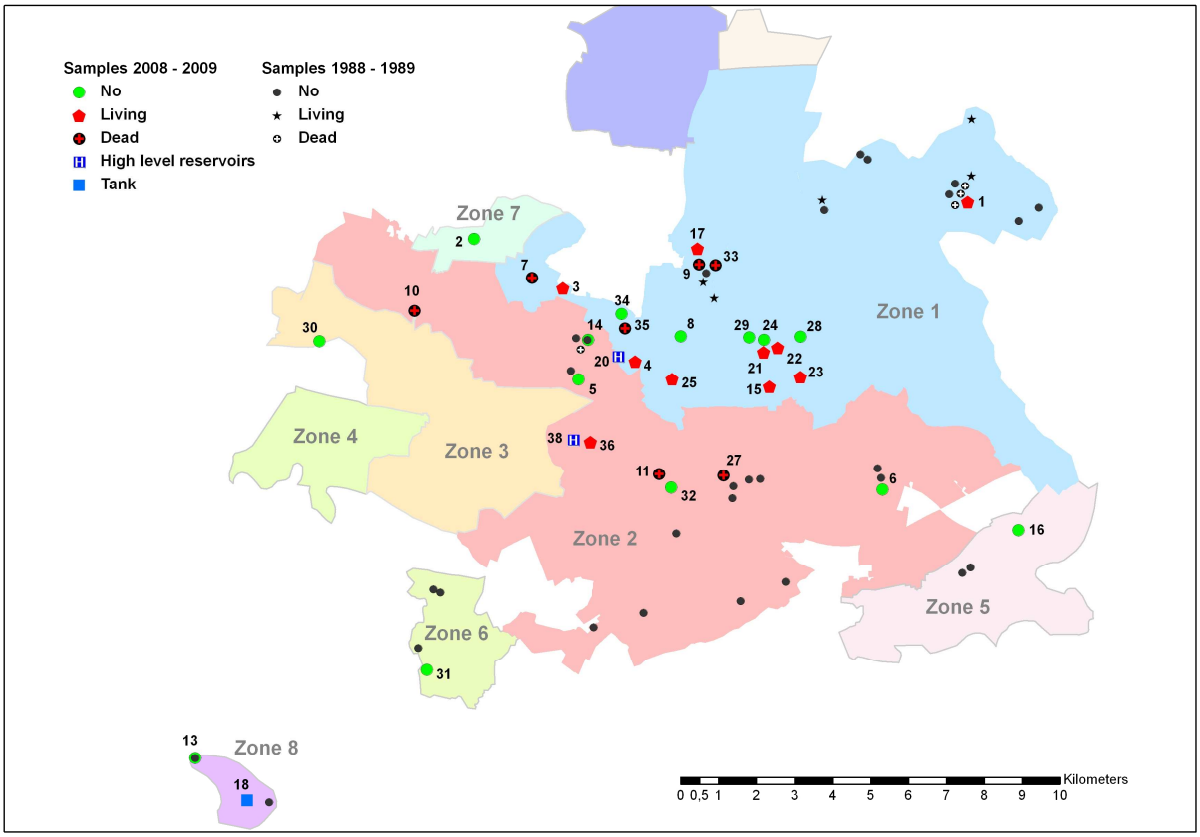
1 mm

525


Samples 2008 - 2009
No
Living
Dead
High level reservoirs
Tank
Samples 1988 - 1989
No
Living
Dead
Zone 1
Zone 2
Zone 3
Zone 4
Zone 5
Zone 6
Zone 7
Zone 8
Kilometers
0 0,5 1 2 3 4 5 6 7 8 9 10

526
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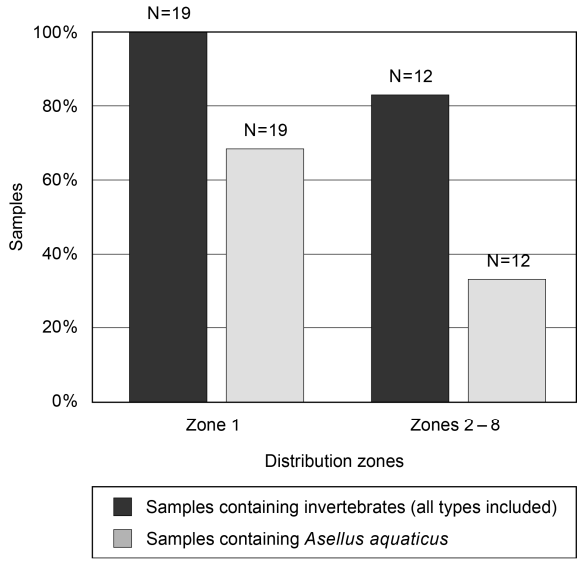

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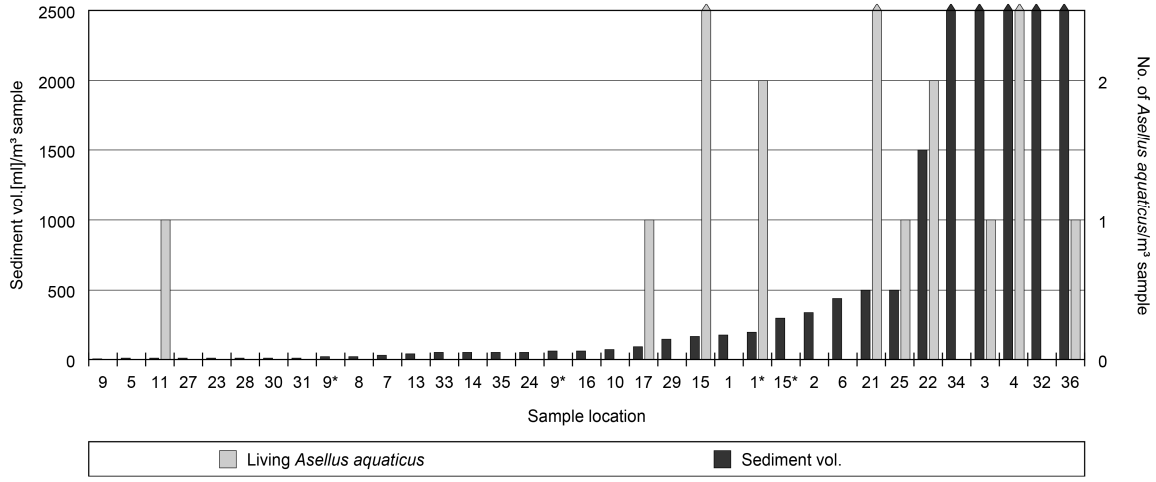

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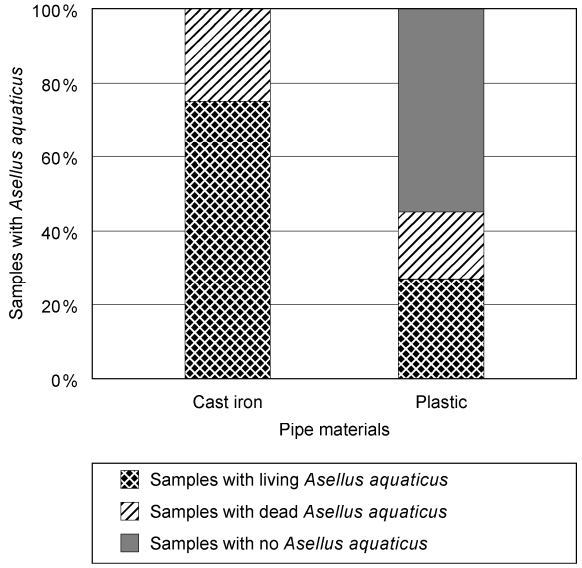

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Supplementary material

Sampling data, invertebrate concentrations and bacterial values

Sample location	Sampling date	Pressure zone	Pipe material	Age 2010 [years]	Pipe diameter [mm]	Pipe vol./m [L]	Flow velocity [m/s]	Flow rate [l/min]	Reynolds number	Sediment volume [ml]	Turbidity [NTU]	Living *A. aquaticus*/m^3 sample	Dead *A. aquaticus*/m^3 sample	Microinvertebrates [#/ml sediment]	HPC (Kim22) [CFU/ml]		HPC (Kim37) [CFU/ml]		Coliform37 [MPN/ml]	
															Before sampling	After sampling	Before sampling	After sampling	Before sampling	After sampling
22eri	16.03.09	1	CI	81	80	5.03	0.50	150	39789	1500	31	2	0	1	2	1	<1	<1	<1	<1
32dal	10.06.09	2	CI	80	200	31.4	0.31	590	108461	5000	50	0	0	0	10	<1	1	<1	<1	<1
17 hav	15.12.08	1	CI	79	100	7.85	1.06	500	106103	90	15	1	13	0	<1	<1	<1	<1	<1	<1
21øst	16.03.09	1	CI	76	100	7.85	0.51	240	50930	500	58	4	1	1	2	1	<1	<1	<1	<1
35 møl	10.06.09	1	CI	73	150	17.7	0.24	250	35368	50	68	0	1	5	8	<1	<1	<1	<1	<1
9 hav	23.10.08	1	CI	72	100	7.85	0.72	340	72150	5	40	0	9	0	<1	<1	<1	<1	<1	<1
9 rep	05.12.08	1	CI	72	100	7.85	0.85	400	84883	60	23	0	16	0	<1	<1	<1	<1	<1	<1
9 rep	10.06.09	1	CI	72	100	7.85	0.85	400	84883	20	16	0	5	0.5	3	5	<1	<1	<1	<1
25 hed	16.03.09	1	CI	71	100	7.85	0.53	250	53052	500	86	1	5	1	1	3	<1	1	<1	<1
27 mar	16.03.09	2	CI	70	150	17.7	0.50	530	74980	<10	25	0	1	2	4	4	2	<1	<1	<1
4 got	25.06.08	1	CI	67	100	7.85	0,66	310	66000	4000	16	3	0	0	3	210	<1	<1	<1	<1
15 vis	15.12.08	1	CI	63	100	7.85	1.17	550	116714	170	23	9	0	0	9	<1	<1	<1	<1	<1
15 rep	16.03.09	1	CI	63	100	7.85	0.57	270	57296	300	13	0	3	1	2	1	<1	<1	<1	<1
23la ri	16.03.09	1	PVC	52	100	7.85	0.74	350	74272	10	5	1	2	1	2	1	<1	<1	<1	<1
5 roes	11.06.08	1	BON	49	500	196	0.05	540	23000	<10	35	0	0	0	4	130	<1	1	<1	<1
14 brø	15.12.08	2	PVC	47	90	6.36	1.44	550	129682	<50	97	0	0	5	18	40	<1	5	<1	<1
10 kal	23.10.08	2	PVC	45	110	9.5	0.99	520	100316	70	54	0	1	2	17	45	5	<1	<1	<1
33gøt	10.06.09	1	PVC	43	110	9.5	1.09	620	119607	Na	Na	0	1	Na	3	5	<1	<1	<1	<1
34 hav	10.06.09	1	PVC	41	90	3.36	1.36	520	122608	3000	90	0	0	1.5	8	<1	<1	<1	<1	<1
1 dalt	07.01.08	1	PVC	40	90	6.36	1.1	420	99030	180	31	0	0	1	5	<1	<1	3	<1	<1
1 (rep)	24.03.09	1	PVC	40	90	6.36	0.89	340	80167	200	26	2	0	1	<1	4	5	2	<1	<1
7 spa	23.10.08	1	PVC	40	90	6.36	1.21	460	108461	30	39	0	1	1	27	20	3	<1	<1	<1
3 stef	11.06.08	1	PVC	39	110	9.5	1	570	110000	3000	18	1	1	3	4	220	<1	<1	<1	<1
13 nev	15.12.08	8	PVC	34	90	6.36	0.71	270	63662	40	4	0	0	1	5	<1	4	<1	<1	<1
36 mik	10.06.09	2	PVC	33	90	6.36	1.47	560	132040	6100	60	1	0	1	10	<1	1	<1	<1	<1
6 vægt	23.10.08	2	PVC	32	90	6.36	1.31	500	117893	440	84	0	0	1	12	<1	1	<1	<1	<1
16 bir	15.12.08	5	PVC	29	90	6.36	2.1	800	188628	60	13	0	0	3	4	10	<1	<1	<1	<1
2 sand	14.01.08	7	PEM	20	200	31.4	2	750	400000	340	2	0	0	4	<1	<1	<1	<1	<1	<1
24 chr	24.03.09	1	PEM	20	110	9.5	0.44	250	48229	50	16	0	0	1	2	1	<1	<1	<1	<1
28 ejb	24.03.09	1	PE	20	110	9.5	0.75	430	82953	10	11	0	0	1	2	1	<1	<1	<1	<1
30 dyr	24.03.09	3	PVC	18	75	4.42	0.87	230	65077	10	172	0	0	1	1	13	<1	1	<1	<1
8 ørst	23.10.08	1	PE	12	90	6.36	1.18	450	106103	20	Na	0	0	1	<1	<1	<1	<1	<1	<1
11 bir	23.10.08	2	PE	10	63	3.12	2.89	540	181891	<10	26	0	1	1	1	10	<1	1	<1	<1
31 bry	24.03.09	6	PE	9	90	6.36	0.39	150	35368	10	10	0	0	0	13	19	<1	<1	<1	<1
29geo	24.03.09	1	PE	8	90	6.36	1.15	440	103745	150	17	0	0	2	2	1	<1	<1	<1	<1

The samples are sorted primarily by pipe material and secondly by age. CI: cast iron, BON: bonna (concrete) PVC: poly vinyl chloride, PE: polyethylene, PEM; polyethylene medium density.