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DTU Environment
Department of Environmental Engineering

PhD Thesis
September 2011

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The thesis will be available as a pdf-file for downloading from the homepage of the department: www.env.dtu.dk

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Preface

This thesis presents the outcome of a PhD project carried out at DTU Environment, Technical University of Denmark and VCS Denmark. The project was supervised by Professor Hans-Jørgen Albrechtsen and Professor Erik Arvin from DTU Environment and Erling Nissen from VCS Denmark. Henrik Juul (VCS Denmark) was external supervisor until he was stationed abroad. The PhD project was funded by VCS Denmark, DTU Environment and The Danish Research Agency through the UrbanWaterTech Graduate School.

The thesis is based on three scientific journal papers

I. Christensen, S.C.B., Nissen, E., Arvin, E. & Albrechtsen, H.-J. (2011) Distribution of *Asellus aquaticus* and microinvertebrates in a non-chlorinated drinking water supply system - effects of pipe material and sedimentation. *Water Research*, 45(10), 3215-3224

II. Christensen, S.C.B., Nissen, E., Arvin, E. & Albrechtsen, H.-J. Influence of *Asellus aquaticus* on the indicator organisms *Escherichia coli* and *Klebsiella pneumoniae* and the pathogen *Campylobacter jejuni* in drinking water (Submitted manuscript)

III. Christensen, S.C.B., Arvin, E., Nissen, E. & Albrechtsen, H.-J. *Asellus aquaticus* as a potential carrier of *Escherichia coli* and other coliform bacteria into drinking water distribution systems (Submitted manuscript)

The papers will be referred to as roman numerals (e.g. Christensen et al. I). They are not included in this www-version but can be obtained from the library at DTU Environment, Department of Environmental Engineering, Technical University of Denmark, Miljoevej, building 113, DK-2800 Kgs. Lyngby, Denmark, library@env.dtu.dk.

During my PhD I have presented results at international conferences, which resulted in the following conference proceedings:

Christensen, S.C.B, Nissen, E., Arvin, E. & Albrechtsen, H.-J. (2010) *Invertebrate animals in Danish drinking water distribution networks*. Proceedings from the 7th Nordic Drinking Water Conference, 7.-9. June 2010, Copenhagen: 93-96. Awarded: Best presentation.

Christensen, S.C.B, Arvin, E. & Albrechtsen, H.-J. (2009) *Invertebrate animals in a Danish drinking water distribution network*. 15th Health Related Water Microbiology Symposium 31 May - 6 June 2009, Naxos, Greece. Proceedings: 335-336.

Christensen, S., Juul, H., Arvin, E. & Albrechtsen, H.-J. (2008) *Invertebrate animals in Danish drinking water distribution networks*. In: IWA World Water Congress and Exhibition, 7-12 September 2008, Vienna. Proceedings. CD-ROM, International Water Association, London, UK.

In addition, the PhD project resulted in Danish conference and seminar contributions, a fact sheet on invertebrate occurrence in drinking water systems, distributed among Danish water utilities as press material and a Danish journal paper which led to more than 50 features in national news media:

Christensen, S.C.B, Hansen, H.L. & Albrechtsen, H.-J. (2010). *Biologi i ledningsnettet* (*Biology in the distribution network*). danskVAND, 1/10: 22-23.

June 2011
Sarah Christensen

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Thanks to all the people at VCS Denmark (VandCenter Syd) especially Arne Svendsen for his great engagement and support, Finn Mollerup and Steen Jakobsen for their involvement and great help and Anders Bækgaard for daring to launch investigations in sensitive issues. I owe a deep felt thank to all the great people working with me in the field and in the stockroom and many hours in dark, cold water tanks, as well as the people providing data and answering all my questions. Copenhagen Energy Ltd, Aarhus Water Ltd, Aalborg Supply, Water Ltd and TRE-FOR Water Ltd are acknowledged for their participation in the project by allowing us to investigate their systems. Thanks to Walter Brüsch, Line Fredslund Volkers, Carsten Suhr Jacobsen and Per Rosenberg from GEUS for an interesting collaboration.

The department and all my fellow PhDs and Post Docs have provided an inspiring atmosphere and pleasant work place. I thank Óluva and Charlotte for always being big-hearted, helpful and knowledgeable and Arnaud, Martin and Sanin for always lending a helping hand and sharing their expertise. Thanks to all the lab technicians, graphic designers, librarians and administrative forces who always made an extra effort as well as Birgit and Susanne at the reception and Anne Harsting for keeping all practicalities under control with a smile. I have enjoyed working with Lisbet who has been doing graphical work on all parts of this thesis and who is extremely helpful. My present and former officemates have been an important part of my time as a PhD student by always lending an ear and making good experiences even better.

Funding from VCS Denmark, DTU Environment and The Danish Research Agency through the UrbanWaterTech Graduate School is greatly acknowledged.

My family and friends have been very supportive especially both my closest and extended family regarding entertainment of Eigil and Alvin during my long work days and weekends.

Last but most important I am eternally grateful to Knud for making it possible for me to put so many hours of work into this project and still have an incredibly happy family. Thanks to Eigil & Alvin for filling my life with joy. I am thankful that all three, besides showing their endless personal support, have taken a very active part in my work.

Summary

Few if any drinking water distribution systems worldwide are completely free of invertebrate animals and presumably it has been that way since the very first distribution system was constructed. Invertebrates visible to the naked eye cause consumer complaints and are considered a sign of bad hygiene. Whereas invertebrates in drinking water are known to host parasites in tropical countries they are largely regarded an aesthetical problem in temperate countries. Publications on invertebrate distribution in Danish systems have been completely absent and while reports from various countries have described the occurrence of invertebrates in drinking water there have been a knowledge gap concerning a quantitative approach to the controlling parameters of their distribution and occurrence.

This thesis describes the distribution and controlling parameters of invertebrates with special emphasis on the largest of the regularly occurring invertebrates in temperate regions, *Asellus aquaticus*, which is also a cause of consumer complaints. The main controlling parameters of the occurrence of *A. aquaticus,* studied in a non-chlorinated distribution system, were the pipe material and sediment volume in the pipes. Cast iron pipes and a substantial sediment volume (>100 ml/m^3 sample) supported relatively large concentrations of *A. aquaticus* (up to 14/m^3). Microscopic invertebrates were present in almost all samples regardless the sediment volume and pipe material.

Whether invertebrates are solely an aesthetic problem or also affect the microbial water quality is a matter of great interest. The few studies on the influence of the invertebrates on microbial water quality have shown opposite tendencies for different invertebrate-bacteria relations, thus some crustaceans graze on pathogenic bacteria while other crustaceans and nematodes protect bacteria from treatment processes. The influence of *A. aquaticus* has never previously been investigated.

Investigations in this PhD project revealed that presence of *A. aquaticus* did not influence microbial water quality measurably in full scale distribution systems. The influence of *A. aquaticus* on survival of indicator and pathogenic bacteria was studied in laboratory experiments, and no effects on bacterial concentrations could be measured for the faecal indicators and opportunistic pathogens *Escherichia coli* and *Klebsiella pneumoniae* nor for the pathogen *Campylobacter jejuni*.

Invertebrates enter drinking water systems through various routes e.g. through deficiencies in e.g. tanks, pipes, valves and fittings due to bursts or maintenance works. Some invertebrates pass treatment processes from ground water or surface water supplies while other routes may include back-siphonage of waste water or surface water via unprotected connections or cross connections.

Since *A. aquaticus* is known to enter drinking water distribution systems through deficiencies in the systems, the risk of transport of faecal contaminations into drinking water supply systems by intruding *A. aquaticus* was assessed. *E. coli* and other coliform bacteria were associated with *A. aquaticus* from fresh water environments such as lakes and ponds. However, incoming water and sediment were found to pose a larger risk of faecal contamination of the supply systems than transport by *A. aquaticus*.

Previous and currently applied methods for removal of invertebrates from distribution systems are discussed and suggestions of control strategies are given, based on the results obtained in this study in order to obtain or maintain an acceptable level of invertebrates in drinking water systems.

Dansk resumé

I al den tid der har eksisteret drikkevands-distributionssystemer, har der levet invertebrater (hvirvelløse dyr) i vandet. Kun få, hvis overhovet nogle, drikkevandssystemer på verdensplan er fri for disse dyr, som fører til forbrugerklager og bliver opfattet som et tegn på ringe hygiejne i systemerne. I tropiske dele af verden kan invertebrater i drikkevandet fungere som mellemværter for humane parasitter, hvorimod de i tempererede lande hovedsageligt bliver opfattet som et æstetisk problem. Der er aldrig blevet publiceret undersøgelser angående forekomst af invertebrater i danske drikkevandssystemer, og på trods af at der eksisterer talrige publikationer om invertebrater i drikkevand på verdensplan har disse ikke haft en kvantitativ tilgang til at identificere hvilke parametre, der er styrende for dyrenes forekomst og distribution i systemerne.

Denne ph.d.-afhandling beskriver distributionen af invertebrater i drikkevandssystemer samt parametre, der er kontrollerende for deres forekomst. Fokus er specielt på den største af de almindeligt forekommende invertebrater i tempererede områder, vandbænkebideren *Asellus aquaticus*, som ofte er årsag til forbrugerklager. De vigtigste parametre for forekomsten af *A. aquaticus* i et ukloret system var rørmateriale og mængden af drikkevandssediment i rørene. Støbejernsrør og et sedimentvolumen over 100 ml/m^3 prøve dannede basis for relativt store *A. aquaticus* populationer (op til 14/m^3). Mikroskopiske invertebrater var derimod til stede i stort set alle prøver uafhængigt af rørmateriale og sedimentvolumen.

Det er af stor betydning, hvorvidt invertebrater udelukkende medfører æstetiske problemer eller også påvirker den mikrobielle vandkvalitet. De få studier der eksisterer angående invertebrates indflydelse på den mikrobielle vandkvalitet har givet forskelligrettede resultater, alt efter hvilke invertebrater og bakterier der er blevet studeret. Således græsser nogle krebsdyr på bakterier og begrænser derved deres antal, mens andre beskytter bakterier mod vandrensningsprocesser såsom kloring og UV-behandling. Det er aldrig tidligere undersøgt, hvorvidt *A. aquaticus* påvirker den mikrobielle kvalitet af drikkevand.

Dette ph.d. projekt har vist, at tilstedeværelsen af *A. aquaticus* ikke påvirkede den mikrobielle vandkvalitet målbart i et større dansk distributionssystem. Derudover blev der udført laboratorieforsøg for at studere, hvorvidt tilstedeværelsen af *A. aquaticus* påvirker indikatorbakterier og pathogener.

Overlevelsen af de fækale indikatorer og opportunistiske pathogener *Escherichia coli*, *Klebsiella pneumoniae* and *Campylobacter jejuni* i drikkevand blev ikke påvirket af *A. aquaticus*' tilstedeværelse.

Invertebrater kommer ind i drikkevandssystemer ad mange forskellige veje såsom gennem utætheder i rentvandsbeholdere, rør, ventiler og fittings ved brud eller under reparationsarbejde. Nogle invertebratgrupper kan passere behandlingstrinnet i vandværker og på denne måde komme fra grund- eller overfladevand, mens de mere sjældne ruter er ved tilbageløb af spildevand eller overfladevand p.g.a. fejlinstallationer og manglende kontraventiler.

A. Aquaticus kommer hovedsageligt ind i drikkevandssystemer via utætheder i systemet, så vi udførte derfor en vurdering af risikoen for fækal forurening ved indtrængen af disse dyr. *E. coli* og andre coliforme bakterier levede associeret med *A. aquaticus* i overfladevandsmiljøer såsom damme og søer. Koncentrationerne af associerede bakterier var dog lave, og vand og sediment, der kan komme ind i systemet sammen med *A. aquaticus*, udgør således en større risiko for fækal mikrobiel forurening end ved transport med *A. aquaticus*.

I afhandlingen diskuteres nye og forhenværende metoder til fjernelse af invertebrater i drikkevandssystemer, og strategier til at kontrollere niveauet af invertebrater på et acceptabelt niveau vil blive foreslået på grundlag af resultaterne fra dette studie.

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1. Introduction

Invertebrate (spineless) animals have probably been present in drinking water distribution systems since the time of the first simple systems. When water resources were unprotected or even open, all kinds of animals could be found in the drinking water but with increased quality of storage and distribution of drinking water, the ways of entry and growth conditions were diminished and invertebrate concentrations decreased (van Lieverloo et al. 2002). In 1827 an anonymous pamphlet informed about invertebrates in Thames river water distributed for domestic use (van Lieverloo et al. 2002) and in the 1850s the study of organisms in drinking was recognized as having practical sanitary value. Initially only droplets of water or sediment were examined but in the 1880s, filtration was applied before analysis (Whipple 1899).

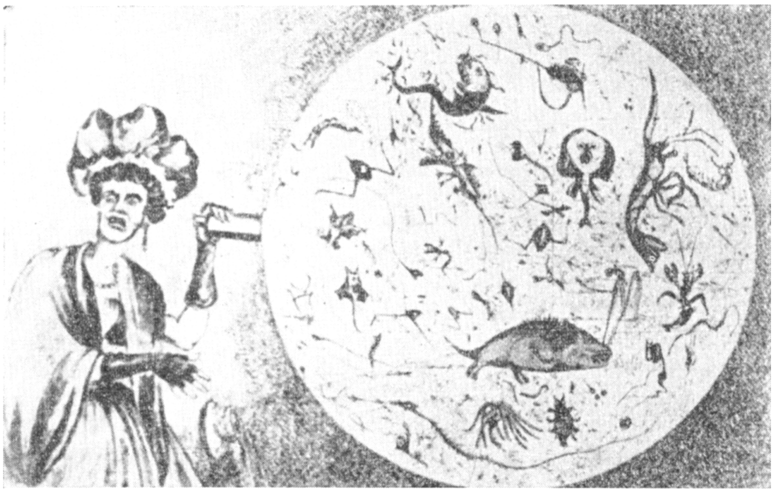

Figure 1-1. Woman losing her tea cup when she realises what is living in the drinking water. Caricature from 1827 about the water quality of drinking water supplied by the Thames (Berger 1966).

The density and diversity of invertebrates vary widely from heavy infestations of breeding populations to single invertebrates only living part of their life cycle in the aquatic environment (Evins 2004). Reports of invertebrates in drinking water are global, and the World Health Organization (WHO) concludes that few if any drinking water systems worldwide are free of animals. In tropical regions the invertebrates may act as intermediate hosts for parasites such as *Dranunculus medinensis* (guinea worm). In temperate regions the problems are mainly aesthetic and typically caused by larger invertebrates such as the water louse, *Asellus aquaticus*, and annelids (worms) which are visible to the naked eye (Evins 2004). Consumer complaints are also caused by secondary effects such as discoloured water or bad odours (Evins 2004). To most people the presence of invertebrates is associated with diminished hygiene and a fear of lack of integrity raising questions about the microbial safety of the water.

Reduced acceptability of the water is a concern for consumers as well as water utilities. Consumers may changes to far more expensive and resource demanding water sources such as bottled water or purification systems, and utilities may fail to comply with the main goal specified in the International Water Association's Bonn Charter (International Water Association 2004), to supply: "*Good safe drinking water that has the trust of consumers*".

Contrary to microbial indicators, monitoring of invertebrates is not required by law anywhere, which probably contributes to the low level of knowledge available on the subject. It is neither economically feasible nor desired to obtain sterile drinking water but knowledge on the controlling parameters for invertebrates as well as their influence on the drinking water quality is essential for evaluating the preventive measures and control strategies that should be applied to maintain the invertebrate communities in drinking water distribution systems on an acceptable level.

1.1. *Asellus aquaticus*

The water louse, *Asellus aquaticus* (isopoda) (Fig. 1-2), occurs in drinking water distribution systems throughout temperate parts of the northern hemisphere (Maltby 1991) and is one of the major causes of consumer complaints when emerging from taps or causing clogged water meters (Christensen et al. I, van Lieverloo et al. 2002, Gray 1999, Walker 1983). With their relatively large size of up to 1 cm (supply system specimens) they often constitute the majority of invertebrate biomass in drinking water (van Lieverloo et al. 1997). At investigations of drinking water pipes in the city of Hamburg, Germany, in the 1880s, hundreds of water lice were found at each examination (Whipple 1899) and in the same period *A. aquaticus* frequently emerged from taps in Rotterdam, the Netherlands (van Lieverloo et al. 2002). *A. aquaticus* is an omnivore/detritivore shredder, which ingests e.g. leaves and sediments with microflora (Rossi et al. 1983). In drinking water systems they ingest the sediments of mainly iron and manganese oxides and bacteria (Christensen et al. I, Barbeau et al. 2005) and their faecal pellets can cause discoloration of the water. The ability of *A. aquaticus* to protect or graze on bacteria in drinking water systems has never been investigated nor has their ability to transport bacteria into drinking water systems.

Figure 1-2. Adult and juvenile *A. aquaticus* from a Danish drinking water system.

1.2. Microbial water quality

Presence of invertebrates has been suggested to affect the microbial quality of the water, but only few studies have been carried out in this field and show different effects with the different animals (e.g. Christensen et al. II, Schallenberg et al. 2005, Levy et al. 1984, Huq et al. 1983). The ecosystems in drinking water distributions are complex with protozoa grazing on bacteria and invertebrates feeding on bacteria, protozoa and other invertebrates (Fig. 1-3). Whether the microbial communities as a whole are reduced or enhanced by presence of invertebrates most likely depend on whether the specific invertebrate mainly digest bacteria or protozoa, but studies on the ecology of drinking water distribution systems are lacking.

Pathogenic bacteria enter drinking water systems when faecal contaminations occur. The World Health Organization (WHO) recommends *E. coli* as an indicator of faecal contamination of drinking water (WHO 2008), and according to the European Council Directive (1998) *E. coli* and other coliform bacteria must be undetectable in 100 ml of drinking water. Besides being faecal indicators, some *E. coli* strains are also highly virulent (e.g. Paton & Paton 1998). Other indicator bacteria from the coliform group are harmless bacteria naturally occurring in the environment while some are also pathogenic (Struve & Krogfelt 2004).

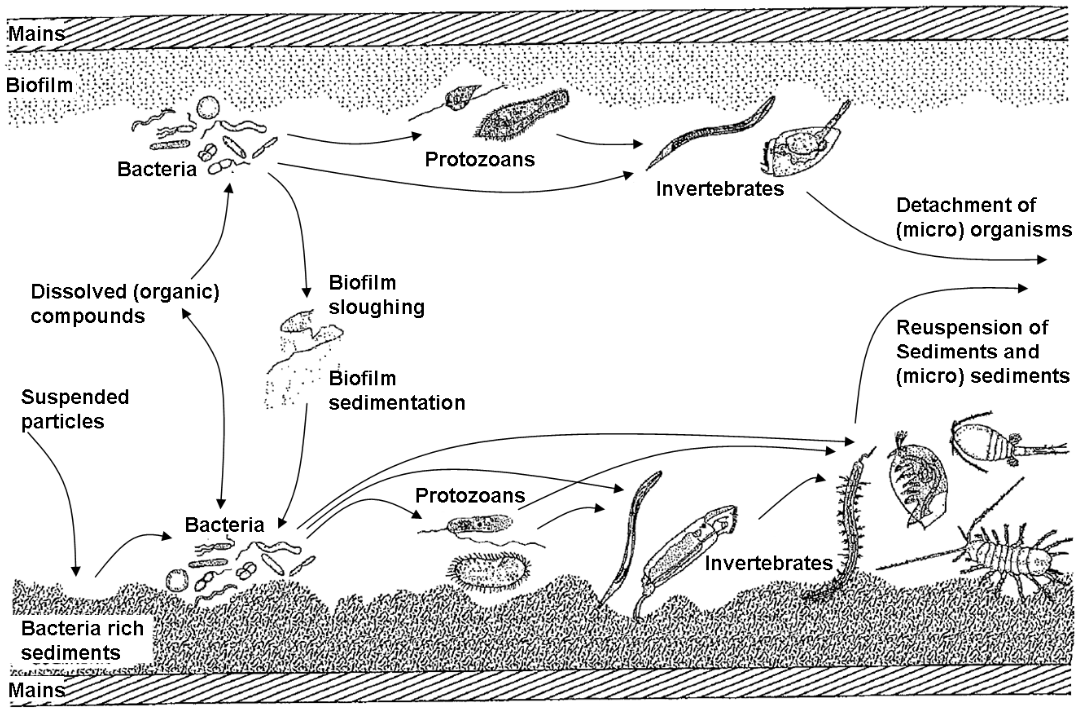

Figure 1-3. Hypothesis on food supply and feeding patterns in a drinking water pipe. Some protozoans and invertebrates feed on organisms within their own group (not shown in the drawing) (slightly modified from van Lieverloo et al. 2002).

In drinking water supply systems based on ground water without chlorination, such as e.g. Danish and Dutch systems, the risk of regrowth of bacteria and biofilm formation in the water pipes may be increased (Martiny et al. 2003) and serve as a food supply for invertebrates. The absence of hygienic barriers between waterworks and consumers increases the focus on invertebrates as potential carriers or regulators of bacterial growth and in particular pathogenic bacteria. In distribution systems with treatment such as UV or chlorination invertebrates provide protection against the treatment (Bichai et al. 2009, Levy et al. 1984).

1.3. Motivation and structure

Surveys throughout the world have identified various invertebrate groups present in the drinking water, yet the correlations to the main controlling parameters for their occurrence have not been substantiated. Paper I presents quantitative information on the distribution of invertebrates and controlling parameters for *A. aquaticus* in full scale distribution systems.

Suggestions that invertebrates may affect the microbial quality of the water have only been investigated in a few instances and never on *A. aquaticus*. Paper I discusses the influence of *A. aquaticus* on the naturally occurring bacteria in drinking water in a full scale distribution system while the papers II and III focus on two aspects of *A. aquaticus*' influence on indicator and pathogenic bacteria in drinking water during contamination cases: Paper II presents laboratory studies on the influence of *A. aquaticus* living in the distribution system on intruding bacterial populations. Paper III discusses whether *A. aquaticus* constitutes a risk of transport of faecal bacteria into drinking water systems when entering a system from a contaminated environment. Paper III is based on field and laboratory experiments.

No widely acceptable methods are currently available to eliminate invertebrates from drinking water systems. This thesis discusses attempts to remove invertebrates and suggests preventive measures and strategies to control the levels of invertebrates in drinking water systems, based on a discussion of ways of entry and controlling parameters.

1.4. Local background for the project

Though widely recognized worldwide there has not been any public awareness of invertebrates in drinking water distribution systems in Denmark. In VCS Denmark (former Odense Water Ltd.) three consumer complaints since the 1950s about *A. aquaticus* emerging from taps resulted in a small survey in 1989. The survey lead to a minute about *A. aquaticus* and *Cyclops* sp. (DMU 1990) and the Danish Environmental Protection Agency was orally informed, which led to a brief comment in a report on errors in technical connections in water distribution systems (Adeler et al. 2003). Another large Danish water utility reported a consumer complaint of a single *A. aquaticus* emerging from a consumer´s tap in 2009 and a utility has tracked coliform bacteria measured in the drinking water to findings of polychaete (bristle) worm colonization of rapid sand filters (Damgaard et al. 2008). Besides the above mentioned cases there have not been any publications or studies on invertebrates in Danish drinking water supply systems.

1.5. Objectives, aims and approaches

The main objectives of this PhD thesis are to investigate the occurrence of invertebrates in drinking water systems globally and to evaluate whether their intrusion and presence influence the drinking water quality.

More specifically the aims are to:

1. evaluate and develop methods to sample invertebrates from drinking water pipes and clean water tanks and to implement and develop procedures further to perform controlled laboratory experiments on invertebrates and bacteria in drinking water

2. study the occurrence of invertebrates in drinking water distribution systems and to determine parameters, which are controlling for their distribution

3. investigate whether invertebrates affect the microbial drinking water quality during regular management, during contamination cases and by transport of bacteria into drinking water systems

4. evaluate previous and current attempts to remove invertebrates from drinking water systems and to suggest preventive measures and strategies to control the levels of invertebrates in drinking water systems

The aims 2 and 3 as well as the laboratory part of aim 1 are approached with special focus on *A. aquaticus*.

Besides literature overview the thesis is based on investigations of full scale Danish non-chlorinated drinking water distribution systems as well as laboratory experiments with drinking water, drinking water sediment and naturally occurring drinking water organisms. Risk estimations are conducted on the basis of data obtained from field samples of surface water environments as well as from laboratory experiments.

2. Methodology of invertebrate sampling in supply systems

No broadly applied sampling method of invertebrates in drinking water exists. The Netherlands and United Kingdom have developed standard methods for sampling in pipes (van Lieverloo et al. 2004, Standing Committee of Analysts 1985) while sampling strategies for other parts of the system such as tanks have only briefly been suggested (Standing Committee of Analysts 1985). In other countries, sampling from pipes also varies with the individual studies (Table 2-1). A suitable method for sampling in piped systems with above ground fire hydrants was developed in this study as well as a protocol for sampling in clean water tanks (Christensen et al. I).

Figure 2-1. A) Sampling from a fire hydrant into containers with single use plastic bags. B) filtration of the samples through a series of nets. Photos: S.C.B. Christensen and E. Nissen

2.1. Flushing from pipes

Common for the vast majority of sampling methods for water pipes is that water is flushed from above or below ground hydrants and filtered. Parameters such as flushing flow rate, volume, mesh size and pre-flushing (discarding the initial flush water) vary. A widely used method is to fit the net into a barrel equipped with outlet valves in order to minimize hydraulic damage to the organisms and special devices have been developed to split the flow when filtering on site (van Lieverloo et al. 2004, Schreiber et al. 1997). If on-site filtration is not possible, sampling can be done in containers with single use plastic bags (Fig. 2-1) (Christensen et al. I). Sample volume and flow are measured with a water meter or flow meter. An overview of methods applied for sampling from pipes is given in Table 2-1.

Table 2-1. Overview of different sampling methodologies applied when sampling drinking water pipes by flushing.

Vol. [m³]	**Flow rate [m/s] or [l/s]**	**Mesh size [µm]**	**Pre-flush**	**Density [invertebrates/m³]**	**Comments**	**References**
1	Max. obtainable flow. Flushing efficiency expressed by Re numbers	(20) 100 500	No	0 – 9000 avg. 800		Christensen et al. I
0.01	NA	5	Yes - few minutes	200-71,000 nematodes avg. NA		Castaldelli et al. 2005
NA	NA	50	NA	NA	Used for electron microscopy	Wolmarans et al. 2005
NA	NA	10 50	NA	NA		Shaddock 2005
1 or 4	1 m/s applicable to pipes of 50-150 mm	30 100 500	Yes - 1 m³	0-10,000 avg. 1000	Standard method in the Netherlands	van Lieverloo et al. 2004, 1998
1	NA	10	NA	52-16,420 avg. 3350		Schreiber et al. 1997
20-50	8 l/s	100	NA	<1		Westphal et al. 1996
NA	NA	63	NA	NA		Levy et al. 1986
2.25	7.5 l/s for 5 minutes Applies to 75-100 mm pipes	142	Yes – open and close as quickly as possible	NA	Standard method in the United Kingdom	Standing Committee of Analysts 1985
Standard vol e.g. 2000-4000	Controlled	142	Yes – 10-20 seconds	NA		Smalls & Greaves 1968

2.1.1. Flow rate

Some studies operate with fixed flows (e.g. van Lieverloo et al. 2004, Westphal 1996), resulting in flow velocities varying with mains diameter, therefore resulting in varying removal percentages. Hence, only microscopic invertebrates and oligochaete worms are flushed out at laminar flow (Reynolds numbers < 2,100), while highly turbulent flow (Reynolds numbers > 25,000) is necessary to flush out invertebrates which adhere to pipe surfaces such as *A. aquaticus* (Christensen et al. I). In studies operating with fixed flow rates of typically 1.0 m/s, the sampling procedure is only applicable on pipes within a certain interval of diameters since flow velocities depend on the pipe diameters (van

Lieverloo et al. 2004). In Christensen et al. (I) diameters of the sampled pipes varied from 63 to 500 mm. To apply the method to all pipe sizes, a novel approach using Reynolds numbers (Re) was adopted. Re is a dimensionless number, which can be used to express whether the flow in a pipe is laminar or turbulent:

$$Re = (V * D_H) / \mu$$

given that: V= mean velocity (m/s), D_H = hydraulic diameter (m), μ = kinematic viscosity (m^2/s)). The kinematic viscosity of water at 20°C is approximated to 10^{-6} m^2/s.

When Re is calculated for each sample, flushing may be done at maximum possible flow rate while the actual turbulence exerted on the invertebrates while flushing can still be expressed. However, in corroded cast iron pipes Re cannot be expressed accurately and the invertebrates may be protected from flushing where turbulence is locally lowered.

2.1.2. Mesh size

Mesh sizes vary greatly within different studies. The smallest applied mesh size was 5 µm in polycarbonate fibre filters, which were dried and subjected to microscopic observations of invertebrates (Castaldelli et al. 2005). The largest applied meshes were 500 µm (Christensen et al. I, van Lieverloo et al. 2004), which were appropriate for quantification of invertebrates visible to the naked eye. A 100 µm filter retained 53-100% of the taxa with copepod larvae and nematodes being the hardest to retain (van Lieverloo et al. 2004). To obtain different size fractions, a series of nets with different mesh sizes (Fig. 2-1) (Christensen et al. I) or split flow devices designed for the purpose (van Lieverloo et al. 2004) facilitate separation of easily quantified invertebrates and microscopic invertebrates that can only be identified by microscopy.

2.1.3. Flushed volume and pre-flushing

The flushed water volume does not vary between different studies to the same extend as other parameters, since 1 m^3 is widely applied. Studies with 3-4 m^3 have been carried out (Christensen et al. I, van Lieverloo et al. 2004) but turned out to be excessively time consuming though yielding more representative data. Whether or not pre-flushing (discarding a varying volume of the initial flush water) is applied is a matter of different focus since pre-flushing gives a representative sample of a pipe section without dead ends included, while no pre-flushing allows animals in dead ends to be included in the samples.

2.2. Other sampling methods for pipes

Other methods can be applied to obtain a higher catchment rate, however these methods are often time consuming and costly compared to flushing:

Swabbing: A foam sponge the size of the pipe is inserted and pushed forward by the water pressure. The method applies mainly to plastic pipes since passage in heavily encrusted pipes is not possible. The method is very efficient and removes invertebrates as well as sediments and some biofilms. However, it is a time consuming and expensive method if pipes need to be dug free and cut open to insert the sponge. van Lieverloo *et al*. (2004) reported that the method is also time consuming due to many pieces of foam in the samples.

Air scouring: Air scouring is conducted by injection of filtered, compressed air into pipes with diameters preferable less than 200 mm. Injection can normally be done via hydrants but demands skilled personnel to obtain the desired dynamics in the water (Vitanage et al. 2004). The method removes more sediment than by flushing and can be applied where high flows are not available but may exacerbate corrosion in iron mains (Evins 2004).

Cutting out pieces of pipes: All types of pipes and also vents can be dug free and cut out of distribution systems. The pipe ends are sealed right after cutting and the pipes are cut open for visual inspection. Adhering invertebrates can then be collected but other invertebrates typically escape with the water. The method is very time consuming and expensive and is mostly applicable as a control.

Traps: Invertebrate traps are built into distribution systems by splitting the flow and leading part of the water through a pipe with an inserted net formed as a fish trap. The systems must be constructed in a way that allows easy asses to empty the net and reinsert it into the system. The trap methods makes it possible to collect invertebrates passing over a long period of time, however if the net is too fine meshed it will clog while a coarse net will discount small invertebrates.

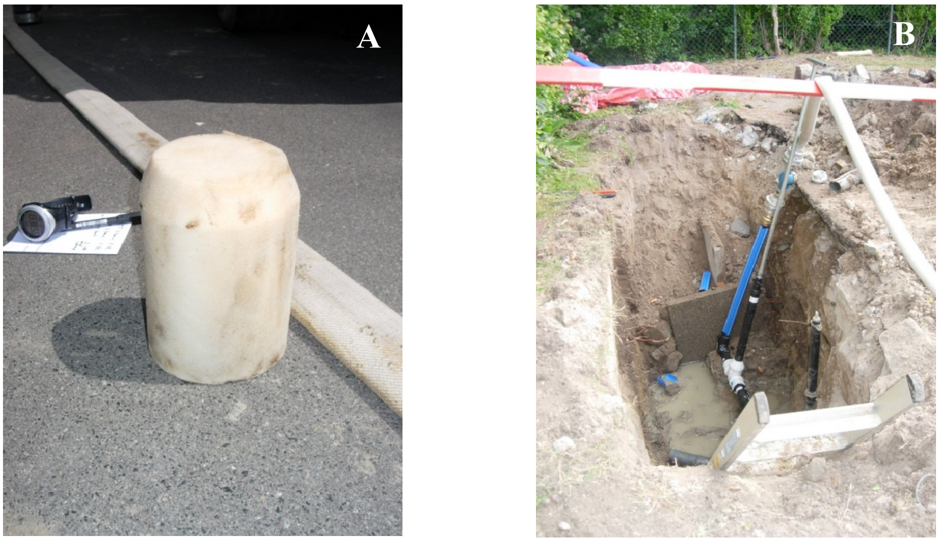

Figure 2-2. A) Foam sponge applied when swabbing pipes. B) Plastic pipe dug free to remove the sponge after swabbing. Photos: S.C.B. Christensen.

2.3. Sampling in clean water tanks

For invertebrates visible to the naked eye, such as *A. aquaticus*, clean water tanks are sampled by emptying the tanks and inspecting the floors carefully (Christensen et al. I). To avoid damaging the animals, approximately 10 cm of water can be left in the tanks. However, this impedes sampling since drinking water sediment on the bottom is resuspended due to the movement of the sampler, making the water murky.

Ideally the entire floor should be inspected, but when tank size and manpower do not allow this method, samples should be collected from flush channels and similar low lying areas with water remaining. The invertebrates are transported to these areas while the tank is being emptied or large invertebrates such as *A. aquaticus* actively move to places with remaining water. Sampling of microscopic invertebrates is also done in remaining water and sediment, preferably by sterile pipettes.

Sampling in or rather inspection of clean water tanks can be done by commercial divers without emptying the tank. They will be able to provide photo documentation on the undisturbed living of the invertebrates. However, a quantitative approach is complicated by the large volume of water present and would be extremely time demanding and expensive.

Figure 2-3. A) Empty clean water tank with reddish-brown sediment on the floor and a flush channel. B) Sampling equipment at the entrance of the tank. Photos: S.C.B. Christensen.

2.4. Sampling before and during treatment processes

2.4.1. Sampling from granular filters

Various methods have been developed for sampling of granular filters. Filter outlet samples can be collected from sand and biologically active carbon filters by sampling from preinstalled taps after the filters and filtering the sample through a net (Castadelli et al. 2005, Madoni et al. 2000, Schreiber et al. 1997). Hijnen et al. (2007) modified the method developed by Anderson (1981) to quantify invertebrates in sand filters. Sand is taken from the filter bed at the end of the operational time and mixed in tap water. Separation of invertebrates from the sediment is done in a $MgSO_4$ solution which is filtered several times before being loaded in a counting chamber for microscopic examination, identification and enumeration of the invertebrates. A method for sampling of Naidids (oligochaete worms) in the filter bed (core sampler) as well as from the effluent water (column trap) was developed by Beaudet et al. (2000), which revealed large differences in concentrations between filter samples and water samples.

Sampling of invertebrates from raw water such as abstraction wells and raw water mains are not discussed in this thesis.

2.5. Summary of pros and cons of different methods

Different sampling methods apply to different needs and one must decide whether a high degree of accurateness or fast handling of many samples should be prioritized. Small mesh sizes increase catchment success but work slower than filtration through larger meshes. Large volumes of water can be filtered on-site but if discretion is needed samples can be collected in single use plastic bags in containers and filtered elsewhere. If uniform sampling conditions are desired, flushing flow rate must vary with varying pipe diameters but this implicates using the lowest common flow. The Reynolds approach developed in Christensen et al. (I) allows for using maximum obtainable flow, which means that flow rates vary but results can be compared. Pre-flushing should be disregarded if samples from dead ends of pipes are required but the risk of sampling terrestrial animals from the water free part of above ground hydrants is lowered by pre-flushing.

According to the above, it is not recommendable to apply a uniform method for all samplings but it is important that each parameter is well considered and reported in detail.

3. Occurrence of invertebrates in drinking water supply systems

Drinking water systems are inhabited by a variety of invertebrate groups with sizes ranging from few micrometers to several centimetres (Fig. 3-1).

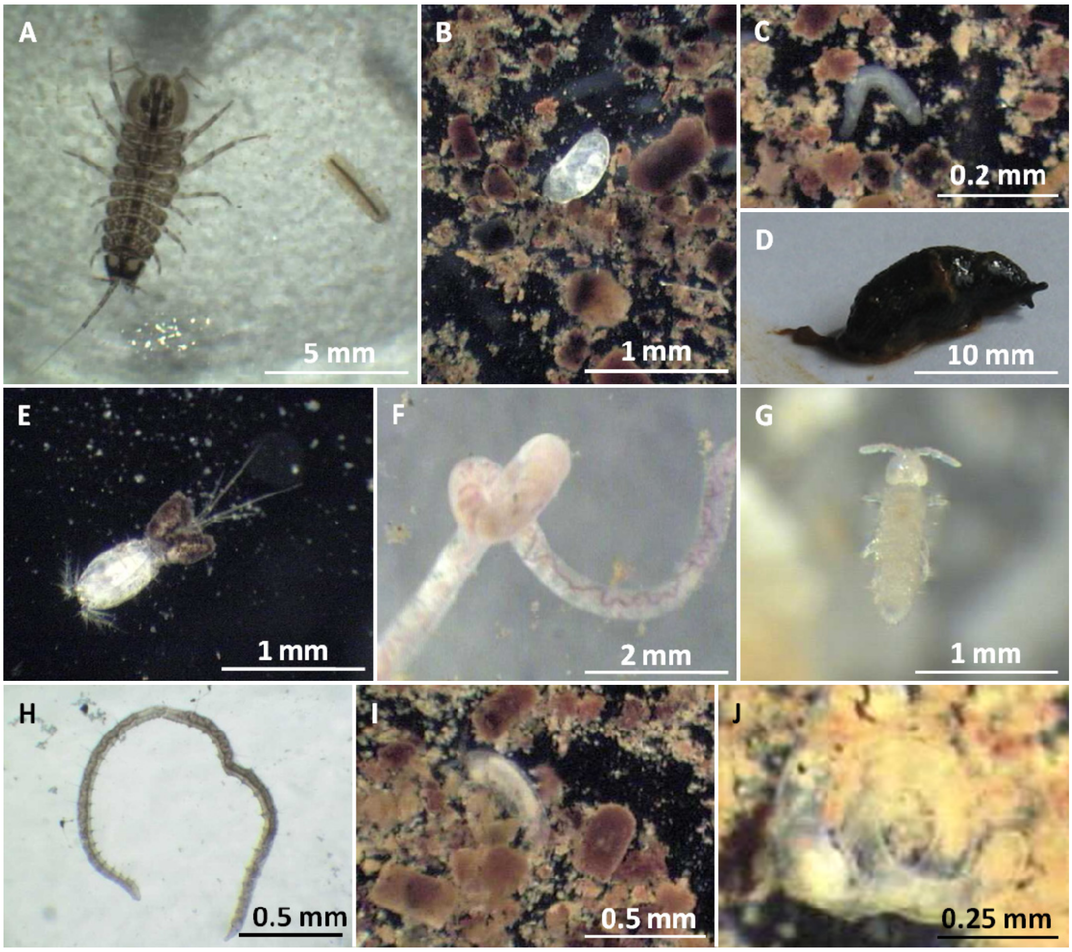

Figure 3-1. Invertebrates sampled in Danish distribution systems. A) Adult and juvenile *Asellus aquaticus* (Malacostraca) B) Seed shrimp (Ostracoda) C) Flatworm (Turbellaria) D) Land slug from a clean water tank E) *Cyclops* sp. (Maxillopoda) F) *Tubifex* sp. (Clitellata) G) Springtail (Entognatha) H) Bristle worm (Polychaeta) I) Amphipod (Malacostraca) J) Roundworm (Nematoda). Photos: S.C.B. Christensen.

In addition to invertebrates, drinking water also host protozoa (e.g. Otterholt and Charnock 2011, Sibille et al. 1998, Valster et al. 2009), which are single celled organisms. They will only be mentioned briefly in chapter 3 and 4 though abundant in drinking water and important for the quality. Microscopic fungi, which are also present in drinking water (Göttlich et al. 2002, Zacheus et al. 2001) will not be discussed, nor will vertebrates such as frogs, eels and sticklebacks, which were common in early distributions but are only occasionally reported in modern distributions. The commonly occurring invertebrate groups in drinking water are shown in Table 3-1.

Table 3-1. Invertebrates reported from drinking water pipes, tanks and filters worldwide. It is not attempted to include all existing reports on invertebrates but a few publications on each invertebrate group representing different sources of water or geography is provided.

Invertebrates	**Concentrations [ind./m^3]**	**Water sources**	**Consumer complaints**	**Countries**	**References**
Turbellaria (flatworms)	NA NA	GWD SWD	No No	Denmark France	Christensen et al. I Poitelon et al. 2009
Rotifera	0-5488 avg. 1360 NA 3 – 5400 avg. 750	GAC effluent - SWD NA BAC effluent - SWD	No No No	Germany South Africa China	Schreiber et al. 1997 Shaddock 2005 Li et al. 2010
Nematoda (roundworms)	NA 2-70 avg. 21 NA 200-71,000 0-10 avg. 1/litre	GWD SWD NA Ground water plant SWD	No No NA NA odours Yes	Denmark Germany South Africa Italy USA	Christensen et al. I Schreiber et al. 1997 Shaddock 2005 Castaldelli et al. 2005 Chang et al. 1960
Gastrotricha	0 – 2884 avg. 170	GAC effluent - SWD	NA	Germany	Schreiber et al. 1997
Tardigrada	NA	GAC effluent - SWD	No	Germany	Schreiber et al. 1997
Oligochaeta (segmented worms)	NA 0-10,000 avg. 100 0 – 20 Naidids in effluent. 0-25,000 in filter surface 0 – 200 avg. 18	GWD GWD (and SWD) SWD BAC effluent	No Yes No No	Denmark Netherlands Canada China	Christensen et al. I van Lieverloo et al. 1998 Beaudet et al. 2000 Li et al. 2010
Gastropoda	Single land slugs on walls NA	Clean water tanks, GWD Clean water tanks and pipes, SWD	No No	Denmark Canada	Christensen et al. I Gauthier et al. 1999
Hydrachnellae (water mites)	0-2000 avg. 80 NA NA	GWD (and SWD) NA SWD	NA No NA	Netherlands South Africa USA	van Lieverloo et al. 1998 Shaddock 2005 Smalls & Greaves 1968
Cladocera (water fleas)	NA 0-92 avg. 7	NA BAC effluent	No No	South Africa China	Shaddock 2005 Li et al. 2010
Ostracoda (seed shrimps)	NA NA	GWD NA	No No	Denmark South Africa	Christensen et al. I Shaddock 2005
Copepoda	NA 0-10,000 avg. 300 NA 0 – 2100 avg. 350	GWD GWD (and SWD) NA BAC effluent	No No NA No	Denmark Netherlands South Africa China	Christensen et al. I van Lieverloo et al. 1998 Shaddock 2005 Li et al. 2010
Asellidae (water lice)	0-14 avg. 4 0-1000 avg. 50 NA 0-10 avg. 2	GWD GWD (and SWD) SWD SWD	Yes Yes NA Yes	Denmark Netherlands Canada Germany	Christensen et al. I van Lieverloo et al. 1998 Gauthier et al. 1999 DVGW 1997
Larvae of chironomidae	0-1000 avg. 5 NA NA	GWD (and SWD) Sand filters (by air) NA	NA No NA	Netherlands USA South Africa	van Lieverloo et al. 1998 Silvey 1955 Shaddock 2005
Bryozoa	NA	NA	No	South Africa	Shaddock 2005
Collembola	NA 0-20 avg. <1	GWD GWD (and SWD)	No No	Denmark Netherlands	Christensen et al. I van Lieverloo et al. 1998

NA = not available, GWD = ground water distribution, SWD = surface water distribution

Most invertebrate groups in drinking water systems are globally distributed, however some groups such as Asellidae are confined to temperate regions (Table 3-1). Concentrations vary from none to more than 70,000 invertebrates from a single group per m^3 (Table 3-1). The highest reported concentrations were of nematodes sampled from a ground water treatment plant in Italy (Castaldelli et al. 2005).

3.1. Aesthetic and ethical implications of the invertebrates

When invertebrate populations are not excessive in numbers, they mostly cause no nuisance to consumers or specific problems for the water utilities. Furthermore the majority of invertebrates present in drinking water distribution systems are microscopic and unless they occur in high concentrations not visible to the naked eye.

Larger visible invertebrates usually remain in the distribution system. However, worms and other visible invertebrates such as *A. aquaticus* and larvae of chironomidae occasionally cause consumer complaints (Table 3-1). They typically block water meters, cause discoloration or bad odours of the water or emerge from taps. Nematodes are the most frequent and abundant colonizers of granular filters of treatment plants (Castaldelli et al. 2005). However, not only invertebrates visible to the naked eye have caused problems and debate in the public. In 2004 Orthodox Jews in New York had to install private water filters or change to bottled water when it was published that microscopic crustaceans, which is not regarded as kosher food were present in the tap water (Berger 2004).

It is highly desired for the water utilities to be able to control the occurrence of invertebrates to be able to supply water of high quality, which not only meets regulatory demands but also has the trust of the consumers.

3.2. Ways of entry

3.2.1. Deficiencies and errors in supply systems

Invertebrates enter distribution systems by various routes (Fig. 3-2). These routes may include structural deficiencies of tanks, reservoirs, pipes, valves and fittings as well as back-siphonage of waste water or surface water via unprotected connections or cross connections. Intrusion of invertebrates may also occur during pipe construction and repair (Fig. 3-3) and incorrect handling of material e.g. underground hydrants. The average number of pipe breaks in Denmark is

1 per 10 km pipe according to the Danish Water and Wastewater Association (DANVA 2010). This is low compared to most other systems, and a globally estimated water loss from distribution systems is estimated 50 % (WHO and UNICEF 2000) while the Danish average loss is 7 % (DANVA 2010) only surpassed by the Netherlands with a water loss below 6 % (Vewin 2011).

Figure 3-2. Potential entry points of invertebrate intrusion into drinking water systems. *A. aquaticus* represents ingress of surface water invertebrates, while the invertebrates at B come from the groundwater and D by air. Graphical design: L. Brusendorff.

3.2.2. Immigration of *A. aquaticus*

Invertebrates like *A. aquaticus* can enter systems in situations where water is still running out (van Lieverloo et al. 2002) and can even crawl short distances without the presence of water (Holland 1956 and personal observations). Rough surfaces, dead ends and blind spaces in main junctions may prevent the invertebrates from being flushed after repair. Boettcher (1935, referred by van

Lieverloo et al. 2002) described that asellids even enter backwash water outlets to enter treatment plants from backwash water reservoirs or surface waters.

Figure 3-3. Bursted pipe awaiting repair. Water is not properly drained from the hole. Photo: H.-J. Albrechtsen.

Cooperation with the Geological Survey of Denmark and Greenland (GEUS) resulted in DNA analyses of *A. aquaticus* populations from i.a. Danish distribution systems, surface water and wells (GEUS 2011). *A. aquaticus* specimens from the investigations reported in Christensen et al. (I) were included in the analyses by GEUS, in which 18S ribosomal DNA (conserved DNA) and CO1 genes (a less conserved gene coding for mitochondrial cytochrome oxidase, an enzyme used in respiration) were analysed by polymerase chain reaction (PCR) and sequencing. 18S DNA sequences confirmed the species identification as *A. aquaticus*, while CO1 was used to differentiate populations within the species. While a small garden pond hosted 3 different *A. aquaticus* populations (20 analysed animals), only one population was identified in a large drinking water distribution system (10 analysed animals) and another large distribution system hosted two different populations (7 analysed animals). Though preliminary, these results indicate that entry of *A. aquaticus* in the investigated systems have only happened on rare occasions, which have then led to establishment of breeding populations.

Studies at several water utilities where e.g. invertebrate traps were inserted revealed that despite large numbers of *A. aquaticus* present in water pipes there was no ingress of *A. aquaticus* from any sources of water supply (Holland 1956). Further studies showed that eggs of *A. aquaticus* only hatch in the brood pouch (egg sac) on the ventral side of the female and not if released into the water prematurely. Based on these studies, Holland (1956) hypothesised that the most likely cause of the heavy infestations of *A. aquaticus* in Coventry, England, was the heavy bombardments of the system during the Second World War, where damaged water mains were awaiting repair for up to months.

3.2.3 Immigration of invertebrates via raw water

Invertebrates may also enter with the raw water, either ground water or surface water, where some are able to pass treatment plants. Slow sand filter have been shown to remove invertebrates to a larger degree than rapid gravity filters (Evins & Greaves 1979) but small motile invertebrates such as rotifers are hardly removed by most treatment plants (Li et al. 2010). However, planktonic invertebrates are better removed by filters than benthic species (Evins and Greaves 1979) and invertebrates such as nematodes, gastrotriches, rotifers and annelids may even settle and develop mainly in sand and GAC filters (Li et al. 2010, Locas et al. 2007, Castaldelli et al. 2005). Hence, the output of invertebrates are often higher in granular activated carbon (GAC) filters (Schreiber et al. 1997) and biologically active carbon (BAC) filters (Li et al. 2010) than in the untreated incoming surface water.

Immigration of invertebrates from ground water depends e.g. of levels of oxygen and the soil structure and hydrology. From coarse sediment layers invertebrates visible to the naked eye may enter but finer sediments only allow low numbers of small invertebrates to pass into the water supplies (van Lieverloo et al. 2002). Coarse sediments or cracks in rocky bottoms will facilitate passage of surface water into groundwater supplies together with flooded abstraction wells (van Lieverloo et al. 2002).

Surface water supplied systems generally contain higher concentrations of invertebrates than systems supplied with ground water (Evins & Greaves 1979). This can be due to both the fact that fewer invertebrates are present in ground water than in surface water and that the organic content of ground water is lower than in surface water (Evins 2004).

Flying insects with aquatic larvae such as chironomids may enter via unprotected service reservoirs but most species are unable to complete their life-cycle in the distribution system (Evins 2004).

3.2.4. Settlement

It is not known which way of entry is the most significant for occurrence of invertebrates but as suggested by Evins and Graves (1979) the invertebrates able to establish breeding populations are most likely to succeed compared to invertebrates without this ability even when entering the systems in large numbers. The initial entry of a species may have been a while ago when water treatment was less effective. DVGW (1997) pointed at a pipe leakage 30 years prior to the investigations as the way of entry for *A. aquaticus*, and Smalls 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.

3.3. Controlling parameters for invertebrates in supply systems

Though it is well established that invertebrates are abundant in drinking water, there still exists a knowledge gap on the controlling parameters for the distribution and abundance of invertebrates in supply systems. In order to support survival and reproduction of invertebrate populations, suitable habitats and a sufficient level of food must be present. However, the correlation between invertebrate occurrence and parameters in distribution systems such as pipe materials, pipe age, amounts of sediments, turbidity of the water, physical barriers, bacterial and protozoa concentrations or season of year had never previously been tested.

In the study of a large Danish distribution system, microinvertebrates were present in all parts of the distribution system in 94 % of all samples while the distribution of *A. aquaticus* varied markedly (Christensen et al. I). *A. aquaticus* reproduce sexually contrary to most other invertebrates in drinking water. Hence, they depend on a certain sustainable population size to form breeding populations. Furthermore as being the highest trophic level in drinking water systems their demands for food are larger than for other invertebrate groups.

A thorough discussion of the controlling parameters for the occurrence of *A. aquaticus* is given in Christensen et al. (I). However, two main parameters are presented here.

3.3.1. Pipe material

A. aquaticus were present in both cast iron and plastic pipes in the investigated non-chlorinated system, but significantly more samples from cast iron than from plastic pipes contained *A. aquaticus* (100 % positive samples versus 45 % positive samples) (Fig. 3-4). 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). *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*. Five samples were taken at locations 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*.

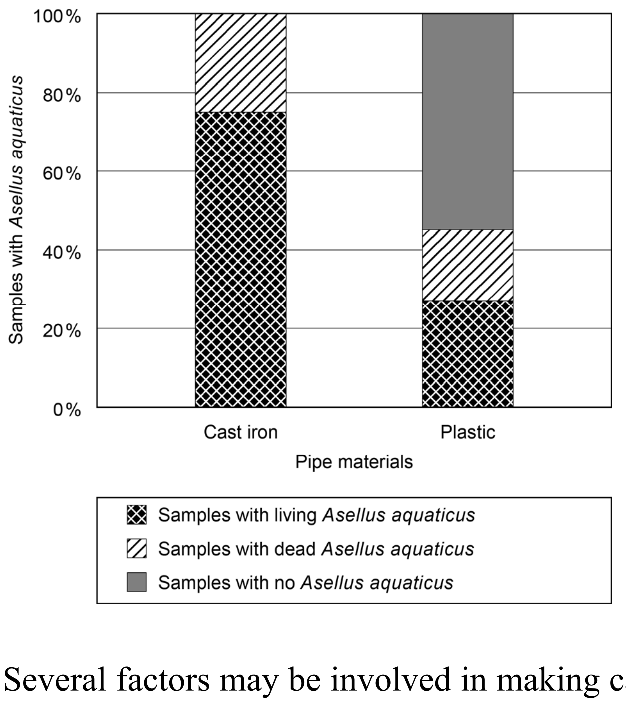

Figure 3-4. Occurrence of *A. aquaticus* in samples flushed from 8 cast iron and 11 plastic pipes in a Danish distribution system. All samples are taken within the same pressure zone (From Christensen et al. I).

Several factors may be involved in making cast iron pipes a preferable habitat for *A. aquaticus*: They provide many hiding places due to corrosion and scaling and more food, e.g. from iron-oxidising and nitrite-oxidising bacteria may be available in cast iron pipes (Martiny et al. 2005). Finally, cast iron pipes are usually old pipes, providing an undisturbed environment.

3.3.2. Drinking water sediment

The volume of sediment retained in the 100 µm mesh from each 1 m^3 sample from the survey (Christensen et al. I) was measured. A clear connection between sediment volume and living *A. aquaticus* was found, since living *A. aquaticus* were nearly only found in samples with sediment contents higher than 100 ml/m^3 sample (Fig. 3-4). However, the number of living *A. aquaticus* was not directly correlated to the sediment volume in the samples.

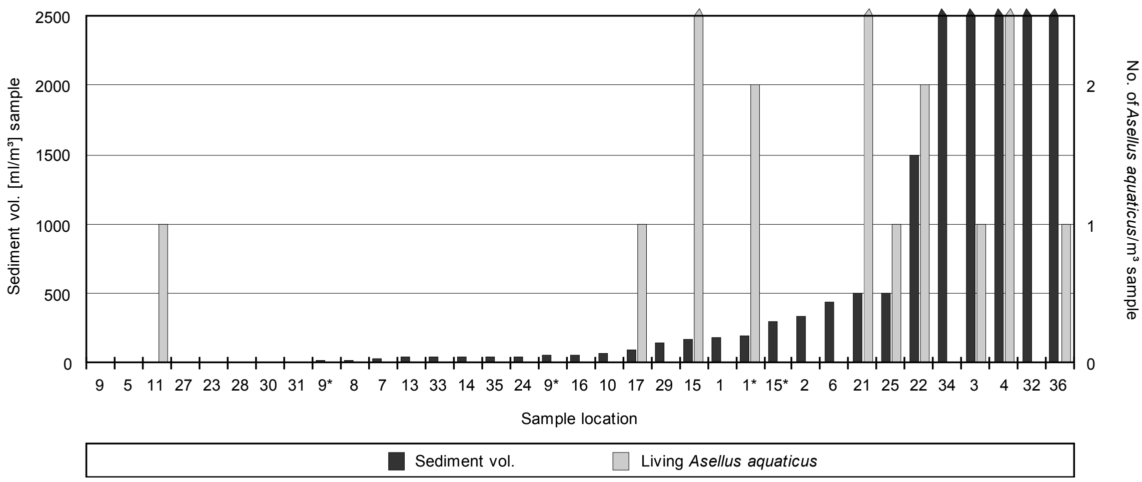

Figure 3-5. Numbers of living *A. aquaticus* and the relation to sediment volume per sample. Pointed bars show values above 2500 ml sediment or above two *A. aquaticus*/m^3 sample. The proportion of living *A. aquaticus* in samples containing > 100 ml sediment/m^3 sample (53%) was significantly higher than in samples containing < 100 ml sediment/m^3 sample (10%).
* shows repeated samplings at the same location (From Christensen et al. I).

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.

Sediments in pipes and clean water tanks contain e.g. bacteria and protozoa and function as a food source for *A. aquaticus* but also as a means of making bacteria and protozoa available to *A. aquaticus* since they are not able to filter the water directly. This was seen during initial studies on *A. aquaticus* and *E. coli*, where *A. aquaticus* were placed in beakers without sediment, which resulted in fighting and even cannibalism. Sediment was added immediately, which terminated the fighting (Crafack et al. 2010). Furthermore observations of *A. aquaticus* in beakers containing drinking water and sediment revealed that they live submerged in the sediment part of the time.

The risk of high sedimentation rates in drinking water systems may be enhanced in water pipes constructed for higher flows than the actual flow due to e.g. consideration of fire fighting demands or due to reduced water consumption. In stagnation zones such as dead ends and sections with generally low flows, the sedimentation rate is normally high like it is in elevated clean water tanks. In clean water tanks at water works we only observed very limited amounts of coarse sediment and no *A. aquaticus* were observed in these tanks. Similar finding was reported by Holland (1956).

3.4. Summary of important factors for invertebrate success in drinking water systems

Invertebrates are distributed in drinking water systems worldwide, with some groups being confined to specific climate zones. The success of invertebrates in drinking water systems is controlled by their rate of entry and mainly by whether the available food and e.g. oxygen concentrations are sufficient to support survival and reproduction. Sexually reproducing invertebrates furthermore depend on a sufficient population size to maintain the population, which implicates a suitable habitat. For the sexually reproducing invertebrate *A. aquaticus* various parameters control its occurrence in distribution systems, e.g. cast iron pipes and substantial amounts of sediment promote the occurrence. Whether the invertebrates cause consumer complaints mainly depend on size, which makes *A. aquaticus* and worms the most widely reported cause of complaints, but even microscopic invertebrates may be a nuisance to consumers and water utilities when abundant in large numbers.

4. Invertebrates and human health

Invertebrates found in drinking water systems in temperate countries are not considered as directly harmful to humans. In tropical or subtropical countries invertebrates may act as intermediate hosts for certain parasites such as the guinea worm and flatworms. However there is no evidence that transmission occurs from piped distribution systems (Evins 2004).

Presence of invertebrates has been suggested to affect the microbial quality of the water since they play a role in the biological equilibrium in drinking water supply systems (Evins 2004, Levy 1986). However, only a limited amount of studies have been carried out on how the presence of invertebrates affect survival of certain indicator or pathogenic bacteria (e.g. Schallenberg et al. 2005, Huq et al. 1983) while no studies have previously quantified their effects of the microbial community as a whole.

Table 4-1 presents available studies on specific bacteria-invertebrate relations, of which nematodes are often reported as carriers of indicators or pathogens in drinking water systems. Furthermore they provide protection against treatment for various bacteria (e.g. Bichai et al. 2009, Smerda et al. 1971). However, Lupi et al. (1995) concluded that nematodes in drinking water do not constitute a large risk for human health since only low numbers of non-pathogenic bacteria (up to 300 CFU/nematode) could be isolated from nematodes both collected from raw water containing pathogens and treated surface water.

Studies of the influence of crustaceans on bacterial survival also reveal different results from almost all studies (Table 4-1). Hence, some crustaceans graze on and thereby reduce pathogenic bacteria (Schallenberg et al. 2005), while others lead to increase of pathogenic bacteria (Huq et al. 1983) or protection of indicator bacteria from chlorination (Levy et al. 1984). Other crustaceans are able to carry indicator and pathogenic bacteria but do not influence their overall survival measurably (Christensen et al. II).

Table 4-1. Influence of invertebrates on bacteria in drinking water.

Invertebrates	Bacteria	Locations	Relations	References
Nematodes				
Pristionchus lheritieri	*Salmonella typhi* *S. wichita*	L	Protection from chlorination and excretion of viable cells	Smerda et al. 1971
Caenorhabditis elegans	*E. coli* *Bacillus subtilis*	L	Protection from UV treatment of bacteria in nematode guts	Bichai et al. 2009
Unidentified nematodes	Enterobacteriaceae and HPC	D	The bacteria were present in the guts of the nematodes	Lupi et al. 1995
Unidentified nematodes	Coliform bacteria	D	Bacteria carried by nematodes and released when nematodes were cut by pressure pumps	Locas et al. 2007
Crustaceans				
Asellus aquaticus	*E. coli* *K. pneumoniae* *C. jejuni*	L	*A. aquaticus* carried all three bacteria but no effects were measured on survival	Christensen et al. II
Hyalella azteca	*E. coli,* *Enterobacter cloacae*	L	Protection from chlorination	Levy et al. 1984
Daphnia carinata	*C. jejuni*	L	Grazing on and thereby elimination of *C. jejuni*	Schallenberg et al. 2005
Copepoda	*V. cholerae*	L	Increase of *V. cholerae* concentrations at 30°C with copepods present	Huq et al. 1984
Asellus aquaticus	*E. coli* Total coliforms HPC	D	No *E. coli* or other coliforms carried by *A. aquaticus.* Its presence had no effects on HPC concentrations	Christensen et al. I

L = laboratory, D = distribution systems

4.1. Effects of *A. aquaticus* on bacteria

4.1.1. Distribution systems

A. aquaticus ingest bacteria rich sediments and constitute a large part of the invertebrate biomass in drinking water systems. We investigated whether their presence had an effect on the microbial quality of the water, over two years of invertebrate sampling in a Danish distribution system. No control measurements at the sampling points exceed 5 CFU/ml (heterotrophic plate counts (HPC), 37°C) including locations where *A. aquaticus* were caught repeatedly, and no correlation between bacterial concentrations and presence of *A. aquaticus* was observed. Neither were any *E. coli* or other coliform bacteria detected at any sampling location or in analyses of crushed *A. aquaticus* (Christensen et al. I). In comparison, just around 3-4 intruding land slugs (Gastropoda) in a clean water tank cause measurable concentrations of coliform bacteria in drinking water systems (unpublished results). Land slugs typically enter clean water tanks through cracks or through lose gaskets at entrances. They live on the water free walls of the tanks but end up in the water when they die or accidentally fall off the walls.

4.1.2. Experiments of association between *A. aquaticus* and bacteria

To investigate how *A. aquaticus* influence bacteria during contamination cases, laboratory experiments were conducted on *A. aquaticus* together with the indicator organisms *E. coli* and *K. pneumoniae* and the pathogen *C. jejuni* in drinking water and drinking water sediment containing naturally occurring bacteria. A detailed discussion is given in Christensen et al. (II) but in brief, all three investigated bacteria became associated with *A. aquaticus* over time as did other heterotrophic bacteria (Fig. 4-1). The total numbers of culturable heterotrophic bacteria associated with *A. aquaticus* in our study were 10^3 times higher than the associated indicators and pathogen. HPC increased over time and reached numbers above 60,000 per dead *A. aquaticus* (18,000 per living) (Fig. 4-1).

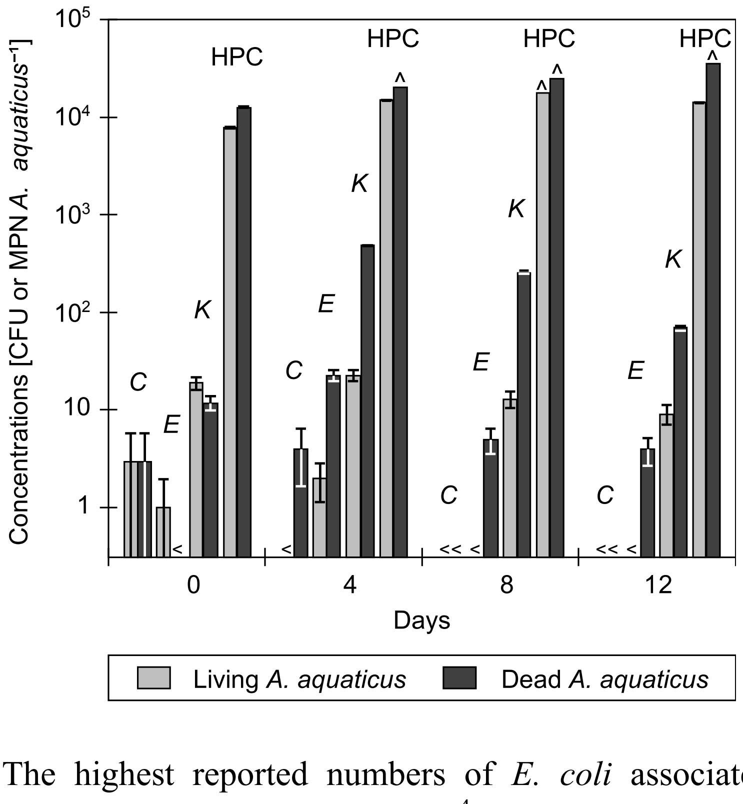

Figure 4-1. Bacteria associated with living and dead *A. aquaticus* over time. Pointed bars show concentrations above the indicated. *C* = *C. jejuni*, *E* = *E. coli*, *K* = *K. pneumoniae*, HPC = heterotrophic plate counts (From Christensen et al. II).

The highest reported numbers of *E. coli* associated with invertebrates were measured on amphipods ($1.6*10^4$ *E. coli*/amphipod) in laboratory experiments in sterile filtered water without competing organisms (Levy et al. 1984). In our studies, concentrations reached maximum 25 (Christensen et al. II) or 3 (Christensen et al. III) *E. coli* per *A. aquaticus*. High concentrations of associated heterotrophic bacteria seem to limit the numbers of associated coliforms. Therefore studies without naturally occurring bacterial populations may risk overestimating the association between invertebrates and pathogens due to lack of competition.

4.1.3. Experiments on effects of *A. aquaticus* on bacterial survival

While the total concentrations of heterotrophic bacteria in the experiments increased over time (Fig. 4-2) concentrations of culturable *E. coli*, *K. pneumoniae* and *C. jejuni* decreased (Fig. 4-3).

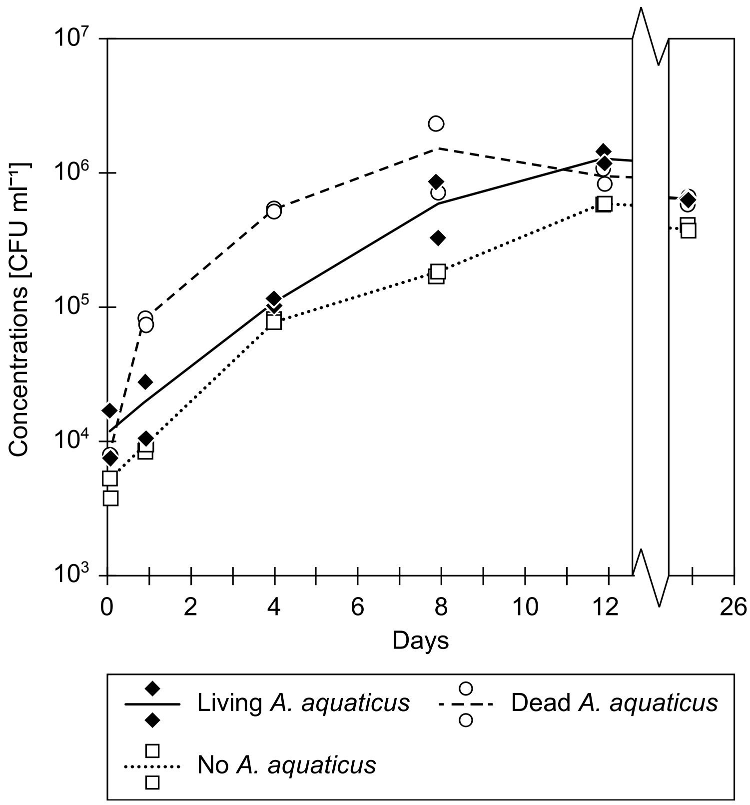

Figure 4-2. Concentrations of heterotrophic bacteria (HPC on R-2A agar, 20°C). Lines show the average of duplicate beakers and points show concentrations in each beaker. Each point presents an average of duplicate or triplicate measurements (From Christensen et al. II).

This contradicts suggestions by van Lieverloo et al. (2002) that invertebrates in drinking water systems may lead to an overall grazing of bacteria, reducing the bacterial competition and giving room for proliferation of pathogens. While the hypothesis may hold for other invertebrate groups, the presence of *A. aquaticus* caused an increase of heterotrophic plate counts (Fig. 4-2) and total cell counts (data not shown) from all beakers, while metabolically active indicators and culturable pathogens decreased (Fig. 4-3). Since the decomposition of dead *A. aquaticus* seems to promote overall bacterial growth (Fig. 4-2) large concentrations of dead *A. aquaticus* may impede the microbial quality of the water. Also presence of living *A. aquaticus* enhanced the concentrations of bacteria, which may be due to removal of protozoa in the sediment by *A. aquaticus* and thereby reduced grazing on the microbial community by protozoa.

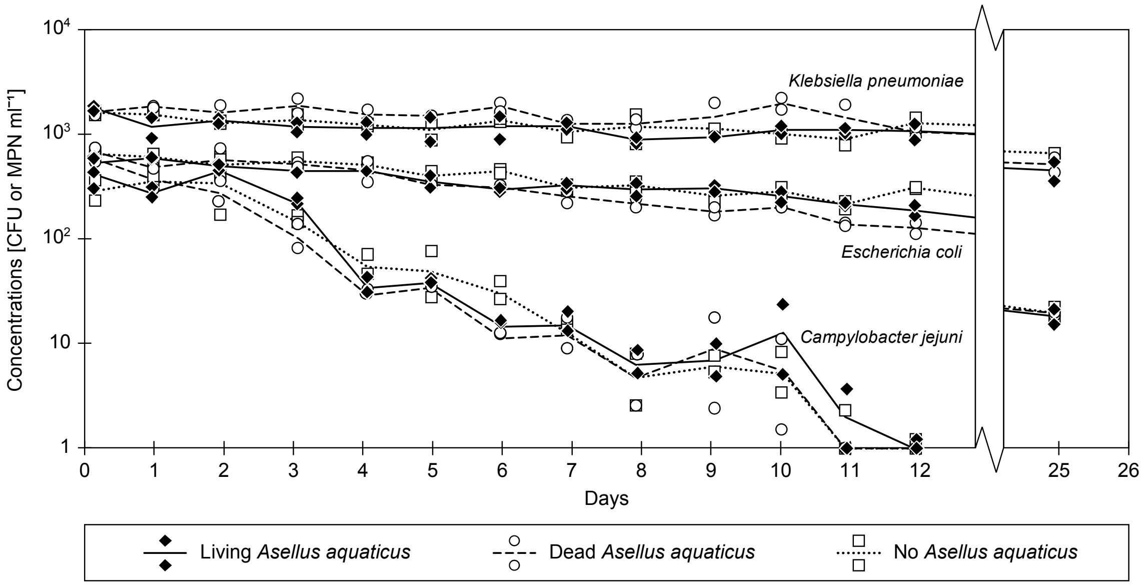

Figure 4-3. Concentrations of culturable *K. pneumoniae*, *E. coli* and *C. jejuni*. Lines show the average of duplicate beakers and points show concentrations in each beaker. Each point presents an average of duplicate or triplicate measurements. Concentrations measured on day 25 fitted to the 1st order decay curves (From Christensen et al. II).

Interestingly, all bacteria were found to be present in higher concentrations in the sediment than in the water phases (Christensen et al. II). After becoming non-detectable in the water phases by culturable methods, bacteria from sediment samples were still culturable, indicating a pool of bacteria not normally considered when drinking water quality is monitored, and which are also available for benthic (bottom dwelling) invertebrates.

4.2. Risk of faecal contamination by intrusion of *A. aquaticus*

Faecal bacteria such as *E. coli* and other coliform bacteria are associated with *A. aquaticus* from surface water samples (Christensen et al. III). When the animals enter a drinking water distribution system through deficiencies in pipes or tanks they may pose a risk of contaminating the water with indicator organisms or pathogens.

This risk of faecal contamination caused by intruding *A. aquaticus* was assessed in Christensen et al. (III) using maximum observed concentrations of *E. coli* and total coliforms associated with *A. aquaticus* in two ponds. Guideline values for *E. coli* were not exceeded in any of the analyzed scenarios, neither did total coliform concentrations exceed guideline values in any scenarios with pipe diameters above 150 mm. Attention should therefore be directed towards smaller pipes such as branch pipes where special hygienic measures must be taken and preferable flushing procedures, others than through consumers' taps, implemented after pipe work. In general, when water and dissolved sediment enter a distribution system along with *A. aquaticus*, bacterial concentrations in the water and sediment pose a larger risk of contamination than *A. aquaticus*. However, 10^5 CFU of associated bacteria per *A. aquaticus* were measured (HPC, R-2A, 20°C) and it should be investigated whether other bacteria, including pathogens, show a higher degree of association with *A. aquaticus* than *E. coli* and other coliform bacteria.

4.3. Summary of invertebrate-bacteria relations

Most invertebrate groups may be present in drinking water distribution systems in high concentrations without causing elevated bacterial concentrations, while others such as land slugs cause measurable levels of coliform bacteria in drinking water systems by intrusion of only few specimens. Laboratory experiments reveal that various bacteria become associated with invertebrates to a varying degree and that different invertebrates either protect, eliminate, promote or do not affect the survival of various bacteria. Presence of *A. aquaticus* did not affect bacterial concentrations in full scale distribution systems nor did it affect the survival of investigated faecal indicators and pathogens in laboratory experiments. However, presence of living as well as dead *A. aquaticus* led to increased concentrations of total heterotrophic bacteria. High concentrations of associated non-pathogenic bacteria prevent high numbers of pathogens from being associated with the invertebrates and in the reported studies with naturally occurring bacterial populations, concentrations of indicators and pathogens associated with *A. aquaticus* were low at all times. *A. aquaticus* should therefore not be regarded as a significant risk of carrying faecal bacteria into drinking water systems even when originating from contaminated water. However, it cannot be ruled out that other bacteria than the investigated, as well as protozoa or vira, may show a higher degree of association with *A. aquaticus*.

5. Control of invertebrates in water supplies

Various attempts have been made over time to remove invertebrates from drinking water systems. The attempts have primarily been levelled at larger invertebrates such as annelids (worms) and crustaceans. The choice of method to control a particular infestation will depend on the present invertebrate groups, whether consumers will tolerate them, their ease of removal and the numbers present (Evins 2004).

5.1. Chemical methods, UV treatment and ozonation

I the past some workers have suggested the use of copper for the control of animals in the water mains, including *A. aquaticus* and *Nais* sp., (oligochaete worm) but the methods were discarded, mainly because of its ability to cause corrosion (Evins 2004, Crabill 1955). Use of the insecticide pyrethrin and a synthetic analogue, permethrin, was found to be effective against a range of arthropods including chironomid larvae, *Gammarus* and *A. aquaticus* (Evins 2004, Oleszkiewicz et al. 2001). However, the concentration effective for controlling invertebrates in distribution systems is highly toxic to fish (Evins 1981) which is a problem not only for end recipients but also for aquacultures and aquaria using the drinking water. Many countries do not allow pesticide treatment of drinking water and its use today is limited. Another insecticide, DDT (dichlordiphenyltrichlorethan), was effectively used to limit invertebrate infestations in the USA From the 1940's until prohibited in 1973 (Levy 1986).

Other chemical approaches include disinfection with chlorine or chloramines. Though traditionally aimed at controlling bacterial growth, chlorine has been used to remove oligochaete worms, leeches (Small & Greaves 1968) and chironomid larvae (Broza et al. 1998). E.g. chloramine concentrations of 2 mg Cl_2/litre caused disintegration of oligochaete worms in a system and the worms were following controlled with doses of 1 mg Cl_2/litre (van Lieverloo et al. 2002). However most invertebrates are not affected by chlorine concentrations normally present in distribution systems and have even proved to protect pathogens from the treatment (Levy et al. 1984).

Nematodes, which are known to be resistant to chlorination (Smerda et al. 1971) have been removed to some degree by chlorination or ozonation in combination with rapid gravity filtration (Evins and Graves 1979). Ozone is highly oxidative and concentrations of 0.8 mg/l for 10 minutes kill even large invertebrates as *A. aquaticus* (Limno-Plan 2001). However, ozonation followed by biofiltration have been found to promote development of invertebrates such as Naidids in the filters (Beaudet et al. 2000).

UV disinfection, which is used for bacterial control, is not effective against invertebrates, rather contrary do invertebrates protect bacteria against the treatment (Bichai et al. 2009).

5.2. Mechanical removal in mains

Pipes are cleaned by flushing, swabbing or airscouring as discussed in chapter 2 on invertebrate sampling methodology. For removal of species that move freely in the water, flushing is relatively efficient, while invertebrates as *A. aquaticus* cling to surfaces and require dislodging by high turbulence or alternative methods. Not only invertebrates but also sediment (Lethola et al. 2004) and to a varying degree biofilm (Vitanage et al. 2004) is removed by these methods. The removal should be systematic so treated mains do not receive water from untreated mains in order to reduce re-colonisation. This can be done via a controlled flow route through the use of valve operation (Vreeburg & Boxall 2007). However, sediment and invertebrates are re-established in the pipes over time and repeated flushing is necessary (Barbeau et al. 2005, van Lieverloo et al. 1998).

Sectional removal of *A. aquaticus* by flushing at maximum obtainable velocity does not remove the animals on a long term scale (Christensen et al. I, van Lieverloo et al. 1997). When locations were sampled repeatedly in Christensen et al (I), *A. aquaticus* were caught at all repeated samplings. Concentrations varied and both higher and lower numbers of *A. aquaticus* than at the previous sampling were obtained. At a sampling conducted less than two months after the first sampling the number of collected *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).

A novel approach for removal of invertebrates in distribution systems is CO_2-scouring (Gunkel et al. 2010). Water supersaturated with CO_2 is led through the system and anesthetises invertebrates so even well adhering animals lose their grip and can be removed following when the system is flushed. Invertebrates such as asellids, copepods, nematodes and bryozoa are removed by this method (Gunkel & Scheideler 2011). However, long term removal efficiency has not yet been investigated nor have possible side effects such as altered microbial communities following treatment.

5.3. Mechanical removal in clean water tanks and filters

Clean water tanks are cleaned by emptying the tank and flushing the floor, and occasionally also chlorinating the tank. In some instances tanks are cleaned by commercial divers, who vacuum the tank for sediment without emptying the tank. Though a tank is totally cleaned, invertebrates still present in the distribution system will be able to enter the tank repeatedly when water from the distribution system is pumped into the tank. Re-colonisation by *A. aquaticus* in a large underground elevated clean water tanks was observed two years after the tank had been flushed and chlorinated (Christensen et al. I). Compared to previous concentrations of more than 200 *A. aquaticus* alone in one flush channel of a 17,000 m^3 tank, no more than 60 *A. aquaticus* were collected two years later though two flush channels and a large area of the floor were inspected intensively (unpublished results). Compared to the first sampling round more water was left in the tank during the second sampling. This impeded the inspection and may have contributed to the lowered catchment rate, however the difference was significant, indicating that despite re-colonisation of *A. aquaticus*, concentrations had not reached pre-treatment level after two years.

Sand filters retain some invertebrates and prevent them from entering drinking water distribution systems. However some invertebrates may colonise filters leading to higher effluent than influent concentrations (Schreiber et al. 1997). Especially nematodes have been a nuisance to water utilities since they are able to re-colonise filters 15 days after backwash (Castaldelli et al. 2005). Decreasing backwash duration and increasing backwash rate lowered the numbers of nematodes together with cleaning of sedimentation tanks by removal of sediment and use strong disinfectant (Castaldelli et al. 2005). Schreiber et al. (1997) on the other hand observed a significant mobilisation of invertebrates during backwash which were following washed out into the distribution system.

Alternative measures have been taken by a Danish water utility (anonymous) where electrodes placed on the filterbed caused oligochaete worms to seek upwards to try to escape the filter, and were thereby easily removed.

Membrane filtration removes all invertebrates so if present in these systems, invertebrates have entered the system by other routes. However membrane filtration is not a method specifically applied to remove invertebrates and does not fall within the scope of this discussion.

5.4. Preventive measures and long term control

Biologically stable drinking water with low assimilable organic carbon (AOC) content (as a rule of thumb below 10 µg C/l) are hypothesised to prevent excessive numbers of invertebrates due to limited bacterial growth (van Lieverloo et al. 1998). Biofilm formation and microbial growth may also be limited by maintaining a disinfectant residual in the distribution system, which may furthermore have an effect on some invertebrates (van Lieverloo et al. 2002). Drinking water sediments accumulate minerals but also organic matter and bacterial biomass (Christensen et al. II, Gauthier et al. 1999) and limitation of sediment accumulation in distribution systems is considered an efficient long term measure for control of especially infestations of benthic invertebrates (van Lieverloo et al. 2002). In disinfected systems the benthic organisms receive little or no exposure to disinfectants because of the high reductive capacity of the organic matter in the sediments (Gauthier et al. 1999).

Besides the discussed removal methods for pipes, which cause disturbances in the systems, Vreeburg & Boxall (2007) suggested a self-cleaning system of the pipes. Self-cleaning is obtained by reaching flow velocities of 0.4 m/s everyday to prevent accumulation of material. This method has not been developed especially for invertebrate removal but would lower the amount sediment and thereby also the available food for invertebrates if successful.

When designing new distribution systems, various measures can be made such as an easy access for insertion and outtake of sponges at essential locations for swabbing the system. If possible, dead ends, oversized pipes and redundant loops should be avoided to minimise stagnation zones.

To prevent large invertebrates, such as *A. aquaticus*, from emerging from consumers' taps, a physical barrier is provided by e.g. water meters with filters at the entry of private properties. Filters directly at the tap will also stop larger invertebrates, however clogging may occur due to dead animals. In tropical countries where also microscopic crustaceans are a potential health risk it is of great importance that fine-meshed filtration close to taps is applied.

5.5. Guideline values

No official guideline values on invertebrate occurrence in drinking water exist. Benchmark values have been proposed in the Netherlands and in East-Flanders (Belgium) based on relationship between consumer complaints and abundance. However, the two sets of values vary greatly e.g. Belgian values for *A. aquaticus* were 10 per 100 m^2 pipe corresponding to $4/m^3$ in a 100 mm pipe, while this value was $30/m^3$ in the Netherlands, where 10 consumer complaints per year were considered as low (van Lieverloo et al. 2002). In 1960 a guideline value for nematodes of approximately 2500 nematodes/m^3 in North American drinking water was suggested (Chang et al. 1960). The comparable guideline value from the Netherlands in 1993 was 300 nematodes/m^3 (van Lieverloo et al. 2002). This shows that tolerance levels are neither uniform nor static and therefore very difficult to establish. To my knowledge no nations currently monitor the drinking water distribution systems regularly for invertebrates.

Certain water utilities, e.g. Thames Water provide guidelines for consumers on how to react when invertebrates are discovered in the drinking water. Recommended procedures are flushing from the taps for 5 minutes and contacting the utility if the problem persists. Whether the increased level of information about the possibility of invertebrate occurrence reassures consumers and prevents complaints is not studied.

5.6. Summary of available control measures

Though no methods so far have proven applicable for total removal of invertebrates in drinking water, various measures to lower concentrations have been identified: For short term control, the rather new approach with CO_2 scouring of the system followed by flushing seem promising in the sense that even well adhering invertebrates are removed without application of persistent or accumulating toxins. Long term measures that have proven efficient include control of food availability by limiting bacterial growth especially in biofilms and sediments. This may be done by providing water with low carbon content and by physical removal of biofilms and sediments. Furthermore, maintaining a disinfection residual in the water may limit bacterial growth. The level of invertebrate concentrations considered as being tolerable varies greatly and so far no official guideline values and regular monitoring of invertebrates are applied for the control of invertebrates in drinking water systems.

6. Conclusions

This thesis provides an overview of invertebrate occurrence in drinking water systems worldwide and their implications on human health. Full scale investigations on Danish supply systems revealed that all investigated systems hosted a variety of invertebrates and supported thereby the assumption of WHO that no drinking water systems globally are expected to be free from invertebrates.

During the study we have developed methods to sample invertebrates from pipes and clean water tanks. Still demands and situations vary from investigation to investigation, which makes different approaches and methods applicable. However, the rationale behind the selected method should be well considered and sufficiently reported. Since *A. aquaticus* is a shredder and not filter feeding as previously investigated crustaceans, a protocol for experimental procedures and analytical methods on the association between bacteria and *A. aquaticus* and their influence on bacterial survival was also developed.

Controlling parameters for the occurrence of microscopic invertebrates is not conspicuous compared to larger and sexually reproducing invertebrates such as *A. aquaticus*. In the investigated systems *A. aquaticus* was controlled by variables such as pipe material and amount of drinking water sediment present and can furthermore be confined by physical barriers such as pumps as seen for nematodes.

The numerous reports that exist on impeded drinking water quality due to presence of invertebrates mainly concern larger invertebrates such as worms and *A. aquaticus*. Besides the aesthetic impairments, odours and blocked meters, the microbial water quality is affected by bacteria associated with and protected by invertebrates. The effects of *A. aquaticus* had never been investigated in spite of its repeated status as cause of consumer complaints. Our studies showed that the observed levels of *A. aquaticus* (up to $14/m^3$) did not impede the microbial quality in a full scale drinking water distribution system, and though *E. coli*, *K. pneumoniae* and *C. jejuni* became associated with *A. aquaticus* in laboratory experiments the presence of *A. aquaticus* did not affect their survival. The low degree of association between *A. aquaticus* and indicators and pathogens leads to the assessment that intruding *A. aquaticus* should not be regarded as a risk of faecal contaminations in drinking water distribution systems.

Despite various attempts to eradicate invertebrates from drinking water systems no methods have been successful up to date. As invertebrates enter drinking water systems by various routes and occur in all parts of distribution systems they are normally able to re-colonise distribution systems after cleaning. We found that *A. aquaticus* can be controlled by replacing cast iron pipes with plastic pipes and lowering the amount of drinking water sediment. However, smaller invertebrates are not influenced by these parameters to the same degree. As well as concerning bacteria and protozoa, invertebrates must be regarded as a natural component of drinking water systems and maintained at a tolerable level, which may be aided by providing biologically stable water with low carbon contents.

Focused efforts should be levelled at invertebrate groups causing consumer complaints and affecting the quality of the water, which were identified in this study as nematodes and certain crustaceans. Since nematodes and large crustaceans are controlled to some degree by cleaning pipes and tanks for bacteria rich sediment and biofilm, this can be done together with physically preventing invertebrates from emerging from consumers' taps by filtration. However, elimination of large invertebrates mainly causing aesthetic problems may pave the way for large populations of organisms causing impeded microbial drinking water quality, therefore regulation may prove more desirable than a total elimination in most cases.

In tropical countries where also microscopic crustaceans are a potential health risk, the demand for fine-meshed filtration of water before reaching the consumers is distinct.

7. Perspectives

The knowledge obtained from this PhD 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*, as well as the identified sediment threshold value of 100 ml/m^3 sample can be used to determine a feasible level of cleaning of the pipes in order to control *A. aquaticus* occurrence. Physical removal of sediment and invertebrates from tanks and pipes by flushing, possibly extended with CO_2 scouring, swabbing or air scouring, can also be used to limit concentrations of *A. aquaticus* and other invertebrates.

Taken as a whole, drinking water sediment was identified as a key factor for non-filter feeding invertebrates such as *A. aquaticus* as well as for bacterial concentrations and should be considered an important parameter for controlling growth in drinking water systems. Microbiological drinking water control samples are only taken from the water phase, however during hydraulic disturbances the bacteria become resuspended and may reach consumers, which further emphasises the importance of sediment removal.

7.1. Significance of the work

This PhD study correlated controlling parameters such as pipe material and sediment volume with the occurrence of *A. aquaticus* in a full scale distribution system as well as correlating presence of *A. aquaticus* with microbial quality parameters of the water.

Though it has been a cause of consumer complaints reported recurrently during the last 100 years there have never previously been any controlled experiments on the influence of *A. aquaticus* and bacterial survival. Hence, methods were developed for the analytical procedures as well as for the experimental set-up in order to investigate systems containing drinking water, drinking water sediment and naturally occurring organisms together with faecal indicators and pathogens.

The results obtained from the experimental work contribute to our understanding of the association between bacteria and invertebrates in drinking water and suggest that high degrees of association between indicators or pathogens and invertebrates previously observed in experiments may be overestimated due to lack of competition from naturally occurring bacteria.

Furthermore this PhD study showed that though *A. aquaticus* is a nuisance to consumers and utilities it does not cause impeded microbial quality of the water when not excessive in numbers. Its presence did not affect survival of two indicators and one pathogenic bacterium in drinking water nor did intrusion of *A. aquaticus* pose a risk of faecal contaminating of distribution systems.

7.2. Future challenges

This study has shed light on how complex ecological niches drinking water systems are. Research in the dynamics of these ecosystems are lacking, and though full scale distribution systems were studied and experimental studies designed with all naturally occurring organisms present the main focus of this PhD study was on a single invertebrate. To get a thorough understanding of the ecology of drinking water systems, microinvertebrates and protozoa should not only be included but also analysed in future studies.

A. aquaticus formed associations with pathogenic bacteria, however in very low numbers compared to other bacteria. To identify the variety of organisms associated with *A. aquaticus* or other invertebrates, future studies would benefit from applying PCR (polymerase chain reaction) and sequencing of well preserved (conserved) genes specific for bacteria such as 16S ribosomal DNA and for analysis of associated eukaryotes (higher organisms) e.g. 18S ribosomal DNA. By these methods it is possible to create a full list of associated bacteria, protozoa, microscopic invertebrates and possibly fungi and thereby reveal invertebrates that should be the matter of special attention in future studies and removal strategies.

To develop guidelines for control strategies of invertebrates in water supplies the results obtained in this study on the importance of sediment concentrations in pipes and tanks could be taken a step further. Controlled studies in which flushing intensities, frequencies and methodologies are varied, should be conducted in different drinking water systems and the re-colonising invertebrates following quantified. In this way, knowledge is obtained on the level of invertebrates that can be expected when applying different degrees of cleaning, making it possible for water utilities to aim their efforts at the desired degree of removal.

Guidelines for optimum frequency and methodology for cleaning of tanks should equally be developed. As for pipes, no regulations of the degree of cleaning or inspection exist for clean water tanks in most countries. Controlled studies would aid water utilities and authorities to aim at a cost-beneficial level of cleaning and control.

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Papers

The following papers are included in the thesis:

I. Christensen, S.C.B., Nissen, E., Arvin, E. & Albrechtsen, H.-J. (2011) Distribution of *Asellus aquaticus* and microinvertebrates in a non-chlorinated drinking water supply system - effects of pipe material and sedimentation. *Water Research*, 45(10), 3215-3224

II. Christensen, S.C.B., Nissen, E., Arvin, E. & Albrechtsen, H.-J. Influence of *Asellus aquaticus* on the indicator organisms *Escherichia coli* and *Klebsiella pneumoniae* and the pathogen *Campylobacter jejuni* in drinking water (Submitted manuscript)

III. Christensen, S.C.B., Arvin, E., Nissen, E. & Albrechtsen, H.-J. *Asellus aquaticus* as a potential carrier of *Escherichia coli* and other coliform bacteria into drinking water distribution systems (Submitted manuscript)

The papers are not included in this www-version but can be obtained from the library at DTU Environment. Please contact library@env.dtu.dk or Department of Environmental Engineering, Technical University of Denmark, Miljoevej, building 113, DK-2800 Kgs. Lyngby, Denmark.

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