FACULTY OF BIOSCIENCE ENGINEERING

THE ROLE OF *ASELLUS AQUATICUS* ON ORGANIC MATTER DEGRADATION IN CONSTRUCTED WETLANDS

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Master's Dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Science in Bioscience Engineering: Environmental Technology

Academiejaar: 2016 - 2017

UNIVERSITEIT GENT

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Ghent, June 2017

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Acknowledgements

I would like to thank the people without whom it would not have been possible to write this master thesis. First of all, I would like to give my gratitude to my promotor, prof. Goethals, for his confidence in me, his friendly support and good advices.

I also really appreciate everything that my tutors, Natalia and Wout, have done for me. Natalia, you were a wonderful lab buddy! Thank you for the talks, your kindness and friendship, and the good support and guidance throughout this year. Wout, thank you for your excellent advices, your help with anything I didn't know and your patience with me.

Last but not least, I am very grateful to my parents, for always being there and for their listening ear and pep talks when I needed it.

Contents

Abstract

Throughout the past centuries, constructed wetlands (CWs) for wastewater treatment gained attention since they constitute a low-cost and effective alternative for conventional wastewater treatment systems. Although the prevailing microbial processes are already well-documented within literature, the role of macroinvertebrates in these man-made ecosystems has received little attention so far. The classification of macroinvertebrates into functional feeding groups allows to identify the different feeding mechanisms and the type of food source these macroinvertebrates require. Consequently, due to their different food preferences, each functional feeding group is energetically dependent on the other groups and allochthonous energy input. Presence of these feeding groups can enhance the removal efficiency in CWs, for instance, shredders reduce coarse particulate organic matter (CPOM) into fine particulate organic matter (FPOM) and can, as such, contribute to the removal of CPOM originating from the influent and the reed leaves themselves.

In this study, it was investigated whether the freshwater isopod *Asellus aquaticus* (L.) can survive in the prevailing water conditions within these CWs. 80% of the animals survived in pure influent water collected from a CW treating domestic wastewater, suggesting that *A. aquaticus* has indeed the potential to colonize CWs. Subsequently, the effect of *A. aquaticus* presence was determined by providing *A. aquaticus* with conditioned reed leaves (*Phragmites australis*), thereby mimicking the conditions within a wetland. These feeding tests indicated that *A. aquaticus* can significantly reduce the leaf biomass compared to controls ($p < 0.01$) with CPOM removal rates in the range 0.02-0.07 mg ind^{-1} d^{-1}. Additionally, by feeding on this reed, FPOM is produced at a rate of 0.04-0.06 mg ind^{-1} d^{-1}, including comminuted plant material and feces. In contrast to the significant reduction of CPOM, no significant reduction in biochemical oxygen demand (BOD) and nutrient concentrations was observed when comparing series with and without *A. aquaticus*. Nevertheless, these observations suggest that *A. aquaticus* has the potential to be used within CWs to reduce the CPOM content and thereby to slightly improve the removal efficiency in these natural wastewater treatment systems.

Samenvatting

Rietvelden voor waterzuivering kregen de laatste decennia meer en meer aandacht, aangezien zij een lage-kost en effectief alternatief behelzen voor conventionele waterzuiveringsinstallaties. In tegenstelling tot microbiële processen die al uitgebreid beschreven zijn in de literatuur, is nog niet veel aandacht besteed aan de rol van macro-invertebraten in deze artificiële ecosystemen. De classificatie van macro-invertebraten in functionele voedingsgroepen maakt het mogelijk om verschillende voedingsmechanismen en het type voedingsbron dat macro-invertebraten nodig hebben, te identificeren. Aangezien elke functionele voedingsgroep een andere voedingsgewoonte heeft, is die dus energetisch afhankelijk van de andere groepen en van invoer van allochtone energiebronnen. De aanwezigheid van deze voedingsgroepen kan verwijderingsefficiëntie in rietvelden verbeteren, bv. shredders herleiden een deel van het grof particulair organisch materiaal (GPOM) naar fijn particulair organisch materiaal (FPOM) en kunnen zo bijdragen aan de verwijdering van GPOM uit influent en GPOM bestaande uit de rietbladeren zelf.

In deze studie werd getest of de (zoet)waterpissebed *Asellus aquaticus* (L.) kan overleven in condities zoals die in rietvelden voor waterzuivering voorkomen. 80% van de dieren overleefde in puur influent water uit een rietveld dat huishoudelijk afvalwater zuivert, wat impliceert dat *A. aquaticus* effectief potentieel heeft om deze rietvelden te koloniseren. Vervolgens werd het effect van de aanwezigheid van *A. aquaticus* bepaald door de dieren te voeden met geconditioneerde rietbladeren (*Phragmites australis*), dit om de condities in rietvelden na te bootsen. Deze voedingstesten gaven aan dat *A. aquaticus* de biomassa van bladeren significant kan reduceren in vergelijking met controles ($p < 0.01$), met verwijderingssnelheden van GPOM van 0.02-0.07 mg ind^{-1} d^{-1}. Tevens werd FPOM geproduceerd door de consumptie van GPOM aan een snelheid van 0.04-0.06 mg ind^{-1} d^{-1}, bestaande uit verkleind plant materiaal en fecaliën. In tegenstelling tot de significante reductie van GPOM, werd er geen significante reductie in biologische zuurstofvraag (BZV) en nutriënten concentraties gevonden tussen series met en zonder *A. aquaticus*. Toch blijkt uit observaties dat *A. aquaticus* potentieel heeft om ingezet te worden in rietvelden voor waterzuivering om GPOM te reduceren en hierdoor de verwijderingsefficiëntie in deze natuurlijke waterzuiveringssystemen enigszins te verhogen.

1 Introduction

Constructed wetlands (CWs) for wastewater treatment gain more and more attention, especially in developing countries since they are a low cost and effective alternative for conventional wastewater treatment options. As such, the increased attention and application of these techniques worldwide comes as no surprise. The main pathways through which wastewater is purified in CWs are mineralization via microbial decomposition and nutrient uptake by plants. Animals can colonize these man-made ecosystems, either through the air or the surrounding land or water. Macroinvertebrates, for example, spend part of their life (e.g. immature stages of many insects) or their entire life (e.g. aquatic beetles) in aquatic environments. By their individual behavior and interaction with each other, macroinvertebrates provide key environmental functions in stream ecosystems, e.g. nutrient cycling, decomposition and translocation of materials. Thus, it is highly probable that these animals influence the performance of CWs treating wastewater, both positively or negatively. However, nor the presence of macroinvertebrates, nor the role they play in these ecosystems has been given much attention.

In this study, the role of the freshwater isopod *Asellus aquaticus* (L.) on organic matter degradation in CWs is investigated, although this species is not commonly found in these ecosystems. Plant litter is the main source of both particulate and soluble biochemical oxygen demand (BOD) in the effluent of CWs. Given the fact that *A. aquaticus* shreds decaying leaves for feeding, they could enhance organic matter decomposition and thus reduce BOD concentrations in the treated wastewater. This could be achieved by ingesting part of the leaves on the one hand and comminuting part of the leaves on the other hand, which increases the specific surface area for microbial colonization, fostering more rapid breakdown. In contrast, these animals could have a negative effect on microbial degradation by feeding on the microbiology on the leaf litter. Furthermore, their shredding activity releases fine particulate organic matter (FPOM), which could increase the amount of suspended solids in the effluent.

The aim of this study is to investigate the effect of *A. aquaticus* presence on the degradation of reed leaves (*Phragmites australis*), a macrophyte commonly encountered in CWs, and to determine the feasibility to introduce this macroinvertebrate in CWs. To reach these goals, lab-scale experiments are designed on a mass balance approach.

2 Literature study

2.1 Wetlands

Wetlands are transitional areas between land and water, such as marshes, bogs, swamps, wet meadows, tidal wetlands, floodplains, and riparian wetlands along stream channels (USEPA, 1995). According to the Water Framework Directive, wetlands are “heterogeneous but distinctive ecosystems which develop naturally, or are the product of human activities. Their biogeochemical functions depend notably on a constant or periodic shallow inundation by fresh, brackish or saline water, or saturation at, or near, the surface of the substrate. They are characterized by standing or slowly moving waters. Common features include hydric soils, micro-organisms, hydrophilous and hygrophilous vegetation and fauna which has adapted to chemical and biological processes reflective of periodic or permanent flooding and/or water-logging” (WFD CIS, 2003).

Considering the periodic or permanent flooding, the role of macrophytes in the functioning of the wetland cannot be ignored. When a wetland is flooded, macrophytes provide attachment sites for the microbial community and slow down the flow of water, filtering solid particles out of the water. Given their presence, a cycle of nutrients and organic matter takes place: plants take up nutrients for growth, when they die they become part of the substrate, and a source of carbon, nitrogen and phosphorus to fuel microbial processes. Due to the slow current of the water through the wetland, sediments are allowed to settle and the diverse community of micro-organisms can break down or convert a wide variety of substances (USEPA, 1995). Aerobic breakdown is possible in the direct vicinity of the plant roots, since these excrete oxygen. To minimize the risk of asphyxiation, the roots are provided with oxygen for respiration through aerenchym, supporting long-distance gas transport pathways in the plant. Via these air spaces in the plant roots, oxygen is released in the surroundings of the roots (Jackson and Armstrong, 1999). In addition, roots hold together the sediment surface and thus stabilize it. The aerial plant parts reduce the wind velocity providing a more stable microclimate at the surface of the wetlands. However, this results in less mixing in the water column, and thus less interchange of substances between different water layers. This also holds for dissolved oxygen (DO), which can reach high concentrations near the water surface and low concentrations in the underlying water layers (USEPA, 2000).

Due to the presence of these processes, certain benefits towards society can be identified as being provided by wetlands. For example, wetlands can provide in(i) the improvement of water quality, (ii) cycling of nutrients, (iii) landscape and biodiversity enhancement, (iv) food web support or habitat creation for wildlife, and (v) flood control (USEPA, 1995; WFD CIS, 2003). These benefits have resulted in the development of artificial wetlands to treat wastewater in a natural way, which has nowadays become a viable alternative regarding small-scale wastewater treatment systems. Throughout the last decades, optimization of the prevailing process conditions have improved the treatment efficiency of these systems, from here on referred to as ‘Constructed Wetlands’ (CWs). These man-made systems consist of shallow (usually less than 1 m deep) ponds or channels with aquatic plants, relying on microbial, biological, physical and chemical processes to treat wastewater. They typically have impervious clay or synthetic liners, and engineered structures to

control the flow direction, retention time and water level. CWs have multiple advantages (e.g. low capital costs and energy demands) and limitations (e.g. large area requirement, variable treatment performance), which are listed in Table 1.

Table 1: Advantages and limitations of constructed wetlands relative to conventional wastewater treatment systems (USEPA, 1995)

Advantages	**Limitations**
• Low capital costs	• Large area demand
• Low energy demand	• Microbiology is sensitive to pollutants such as pesticides
• Low operational attention and costs	• Some operational skills required
• Provide effective treatment	• Treatment performance can be less consistent than in conventional treatment and may vary seasonally
• Can tolerate fluctuations in flow	• Can produce odors due to anaerobic conditions
• Landscape and biodiversity enhancement	• Attract mosquitos (disease vector)

Due to their success for treating domestic wastewater from small villages, and/or agricultural and industrial wastewater (e.g. from pulp and paper mills), CWs have been considered in waste management strategies (Cronk, 1996). Most wastewaters only need primary treatment before introduction in the CW (e.g. a sedimentation tank for particle removal), however some also need prior secondary treatment. This can both be a conventional biological treatment (Matamoros *et al.*, 2017; Abira *et al.*, 2005) or a physical treatment (Boets *et al.*, 2011). In these cases, CWs are considered as a tertiary or polishing treatment. In addition, CWs are effective barriers for nutrients entering the environment from e.g. storm water, runoff, compost and landfill leachates and fish pond discharges (USEPA, 1995).

General processes occurring in wetlands are sedimentation of suspended solids, filtration of solids, degradation of biochemical oxygen demand (BOD), uptake of nutrients, oxidation, reduction, adsorption, precipitation and pathogen removal. These comprise both biological and physiochemical processes, and they can differ among different types of CWs. Based on configuration and the related occurring processes, two types of CWs can be identified: Free Water Surface (FWS) CWs and Subsurface Flow (SSF) CWs, which will be discussed below (USEPA, 2000).

FWS CWs are flooded to 10-45 cm above ground (Figure 1), creating anaerobic conditions in the water column and the soil beneath. The vegetation can be a combination of emergent aquatic plants (e.g. cattail, reeds), floating plants (e.g. duckweed), and submerged aquatic plants (e.g. sago pondweed, widgeon grass). The roots provide oxygen in the area immediately surrounding the root hairs, creating micro-environments for aerobic microbial activity in the otherwise anaerobic soil. Algae can be present as well, although macrophytes limit the introduction of light into the water. Nevertheless, excessive algae growth can occur, leading to more varying dissolved oxygen concentrations due to the daily cycle of photosynthesis (O_2 production) and respiration (O_2

depletion) and can block the light for present submerged plants. It can also lead to increased suspended solids concentration of the effluent. Overall, this technology is only appropriate for low strength wastewaters due to the low dissolved oxygen concentration in the water (Tilley *et al.*, 2008; USEPA, 2000).

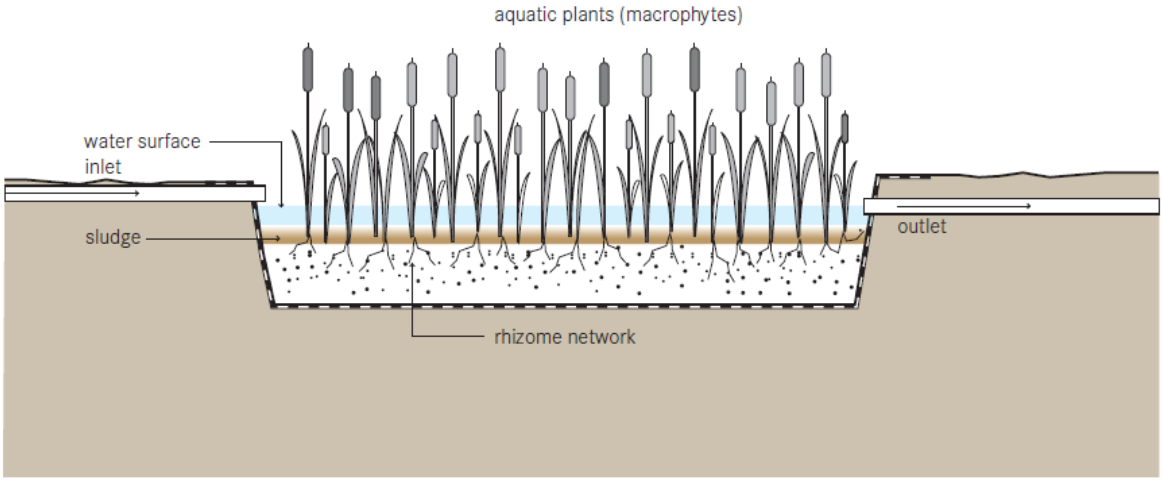

Figure 1: Schematic of a Free Water Surface (FWS) CW (Tilley *et al.*, 2008)

The second type, SSF CWs, can be divided in Horizontal Subsurface Flow (HSSF) and Vertical Subsurface Flow (VSSF) CWs. In a HSSF CW, the water level is kept at 5-15 cm below the surface and the wetland lays in a slope to have horizontal flow. Water level often can be adjusted by the outlet (Figure 2). The coarse gravel at the inlet makes sure that the water is evenly distributed. The smaller media with which the bed is filled, acts as (i) a filter for removing solids, (ii) attachment sites for microbiology and (iii) support for macrophytes. Clogging is a common problem, which can be avoided by installing a primary sedimentation tank or lagoon (cfr. the recommended primary treatment). Also in this type of wetlands, aerobic bacteria get the oxygen through the plant roots (Tilley *et al.*, 2008).

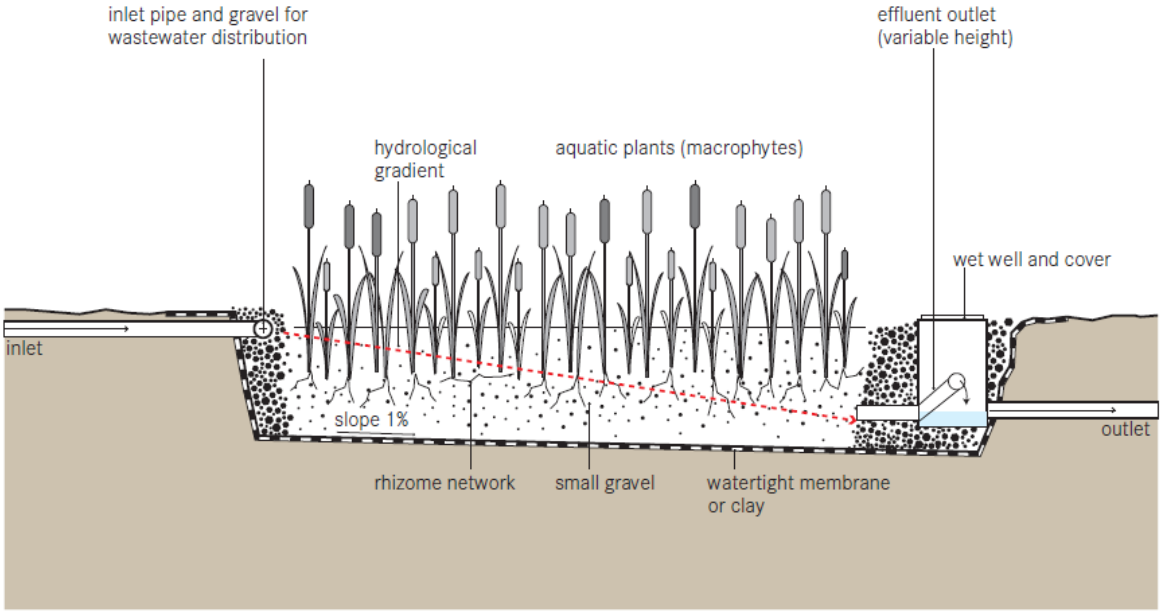

Figure 2: Schematic of a Horizontal Subsurface Flow (HSSF) CW (Tilley *et al.*, 2008)

In contrast to HSSF, water can also flow through the substrate in a vertical pattern, hence VSSF. Water flow can be top-down or bottom-up and can be continuous or intermittent. In general, intermittent top-down flows are applied in VSSFs and are therefore the main focus further on (Figure 3). By dosing the water intermittently, alternately anaerobic and aerobic conditions take place. When the wetland is filled, the bed is saturated and thus anaerobic conditions prevail. As the water percolates through the bed, oxygen is allowed to diffuse through the filter media, into the void spaces. Coarse gravel at the bottom makes sure the water can reach the drainage pipe easily. Clogging can be an issue, but is reported to be less than in HSSF CWs. In both SSF systems, roots maintain the permeability of the filter medium (Tilley *et al.*, 2008).

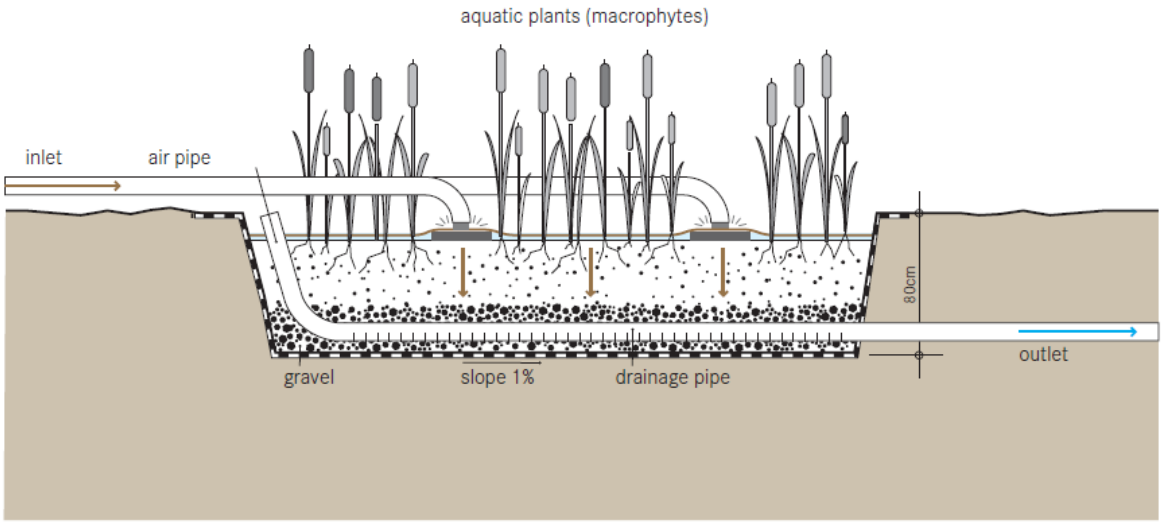

Figure 3: Schematic of a Vertical Subsurface Flow (VSSF) CW (Tilley *et al.*, 2008)

Organic matter removal in CWs occurs both biological as physical. Sources of organic matter in CWs are particulate and soluble organic matter from the influent as well as dead plant litter. Fine particulate influent organic matter can accumulate at the bottom within the plant litter and sediments in case of FWS CWs, or can be intercepted by plant roots or media in SSF CWs and subsequently be colonized by microbial biofilm. The amount of plant litter is dependent on the species and the coverage. Accumulated organic debris degrades at different rates, depending on its composition. Emergent plant material is relatively slowly degraded since it contains high quantities of structural material (cellulose, hemicellulose and lignin). When organic debris enters the water, low molecular weight molecules will leach into the water both under influence of microbial activity and hydrolysis (Westlake *et al.*, 2009). Those soluble substances can in turn be oxidized by microbes under aerobic conditions, given by reaction (1) (Okafor, 2011).

(1) $C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$

If anaerobic conditions prevail, organic matter is converted into fatty acids (e.g. acetic acid) and alcohols (ethanol). Under strict anaerobic conditions, methanogenesis (conversion into CH_4 and CO_2) can occur. The following four steps take place during anaerobic organic matter degradation (The Pennsylvania State University, 2017):

1. Hydrolysis: polymers like proteins, polysaccharides and lipids are broken down to monomers and oligomers such as amino acids and sugars (e.g. glucose, reaction (2))

(2) $C_6H_{10}O_4 + 2\,H_2O \rightarrow C_6H_{12}O_6 + H_2$

2. Acidogenesis: conversion of monomers to alcohols (e.g. ethanol, reaction (3)) and volatile fatty acids (e.g. propionate, reaction (4))

(3) $C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2$

(4) $C_6H_{12}O_6 + 2H_2 \rightarrow 2\ CH_3CH_2COOH + 2\ H_2O$

3. Acetogenesis: conversion of volatile fatty acids to acetate. Possible pathways are given by reactions (5) to (8)

(5) $CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2$

(6) $C_6H_{12}O_6 + 2H_2O \rightarrow 2\ CH_3COOH + 2\ CO_2 + 4H_2$

(7) $CH_3CH_2OH + 2H_2O \rightarrow CH_3COO^- + 2\ H_2 + H^+$

(8) $2\ HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4\ H_2O$

4. Methanogenesis: methane production converting the products of the acetogenesis process. Three key pathways are given by reactions (9) to (11)

(9) $4H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$

(10) $CH_3COOH \rightarrow CH_4 + CO_2$

(11) $4\ CH_3OH + 2\ H_2O \rightarrow 3\ CH_4 + CO_2 + 4\ H_2O$

Organic matter degradation is temperature dependent as it relies on microbial activity, which increases with increasing temperatures up to a certain maximum (Qiu *et al.*, 2005). Thus there is more accumulation of sediment organic matter during winter than in spring and summer, when rapid degradation and release of soluble organic matter take place. Since influent organic matter from domestic wastewaters is usually easily biodegradable, the soluble and particulate organic matter in the effluent mainly comes from decomposing plant litter (USEPA, 2000).

Besides organic matter degradation, CWs also account for nitrogen and phosphorus removal. Processes in nitrogen removal include ammonification (conversion of organic nitrogen into ammonium, most efficiently under aerobic conditions), nitrification (oxidation of ammonium into nitrite and nitrate under aerobic conditions by autotrophs, reactions (12) and (13)) (Okafor, 2011):

(12) $2\ NH_4^+ + 3\ O_2 \rightarrow 2\ NO_2^- + 2\ H_2O + 4H^+$
(13) $2\ NO_2^- + O_2 \rightarrow 2\ NO_3^-$

and denitrification (reduction of nitrate to nitrogen gas under anaerobic conditions by heterotrophs). Denitrification goes through the following forms (Okafor, 2011):

(14) $NO_3^- \ (nitrate) \rightarrow \ NO_2^- \ (nitrite) \rightarrow NO \ (nitric\ oxide) \rightarrow N_2O \ (nitrous\ oxide) \rightarrow N_2 \ (nitrogen\ gas)$

Thus, nitrification in FWS and HSSF CWs can only take place in the area surrounding the plant roots, while nitrification and denitrification take place intermittently in VSSF CWs.

In contrast, phosphorus does not have a volatile stage and is, thus, hard to remove completely from the system. Particulate phosphates can be deposited or attached to biofilms. Soluble phosphates may be sorbed onto biofilms, precipitated or adsorbed onto soil particles. Processes of sorption/desorption are a major pathway for soluble phosphates in wetlands. Insoluble forms can be transformed into soluble inorganic forms by micro-organisms, which are available for plants. As such, most phosphorus stays in the system, attached to the substrate, requiring the renewal of the substrate when it reaches saturation, or as part of microbial or macrophytic biomass. Hence, most CWs are characterized by a limited phosphorus removal efficiency (USEPA, 2000).

Plants also can take up nutrients when they become available (USEPA, 2000). Harvesting plants then exports nutrient out of the system, however this is not routinely done in CWs. In addition, it provides a minor nutrient removal pathway compared to microbial processes as described above (Crites *et al.*, 2006).
In short, CWs are useful in the overall wastewater treatment process by removing carbon, nitrogen and phosphorus by combining the activity of both microorganisms and vegetation. Furthermore, their natural character allows a proper integration into a natural environment and provides a habitat for other organisms like macroinvertebrates and birds, which can have a potential influence on the occurring processes.

2.3 Macroinvertebrates

2.3.1 Definition

Macroinvertebrates are invertebrates that can be seen with the naked eye (Stroud Water Research Center, 2017). Benthic freshwater macroinvertebrates live in or on the sediment, on rocks or vegetation at the bottom of lakes, rivers and streams. They provide key environmental functions such as nutrient cycling, sediment mixing and aeration, decomposition and translocation of materials and energy flow through food webs (Covich *et al.*, 1999; Wallace and Webster, 1996). Examples of freshwater macroinvertebrates include nymphs and adults of many insects(Figure 4 A), worms (Figure 4 B), crustaceans (Figure 4 C), bivalves (Figure 4 D) and snails (Figure 4 E) (Mellanby, 1963).

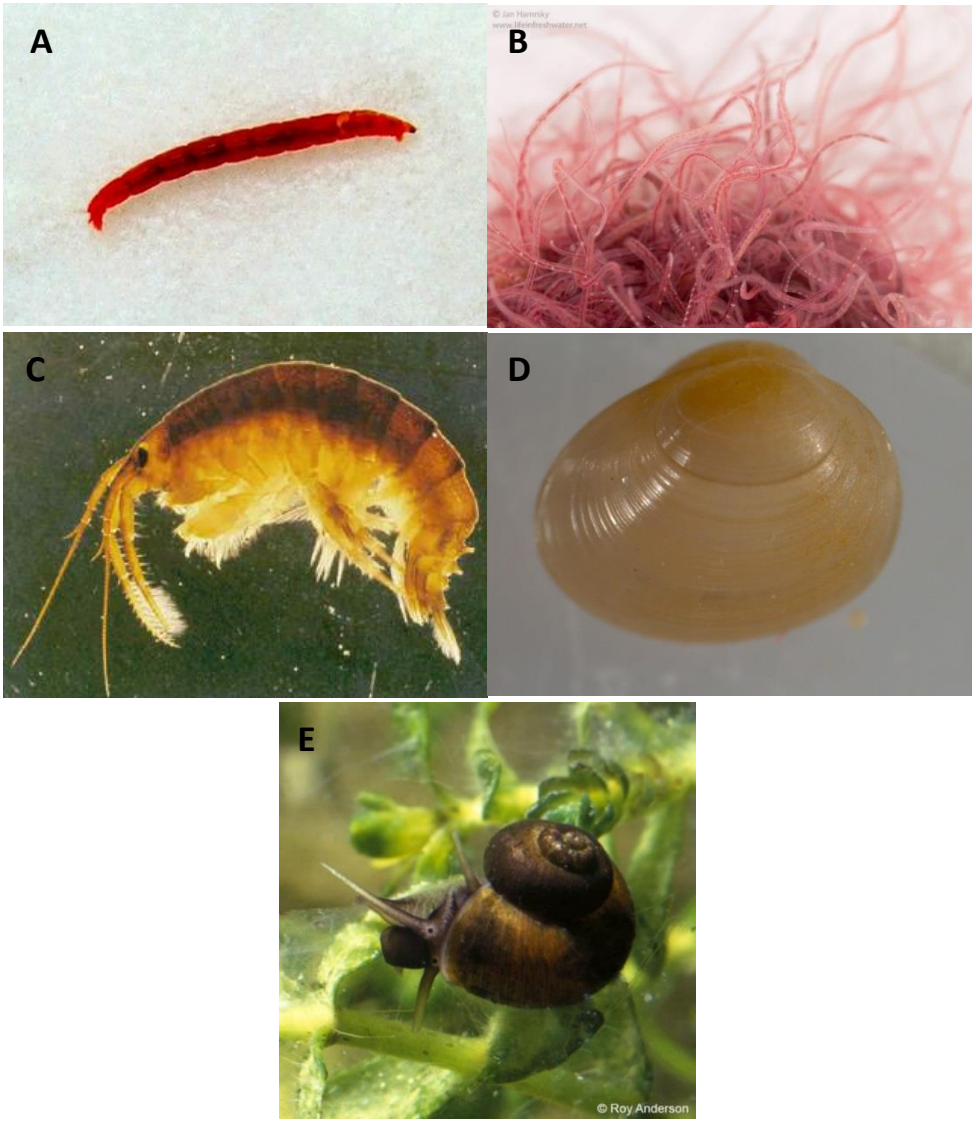

Figure 4: A) Non-biting midge larva from the family Chironomidae (First Nature, 2015). B) *Tubifex tubifex* from the family Tubificidae (Hamrsky, 2015). C) *Gammarus villosus* from the family Gammaridae (NOAA Great Lakes Aquatic Nonindigenous Species Information System (GLANSIS), 2015). D) *Pisidium nitidum* from the family Sphaeriidae (BOLD systems, 2014). E) *Valvata piscinalis* from the family Valvata (Anderson, 2016)

2.3.2 Functional feeding groups

Stream invertebrates have been classified into functional feeding groups according to morpho-behavioural mechanisms for food acquisition. One can distinguish shredders, gatherers (collectors), filterers, scrapers (grazers), piercers and predators (Cummins and Klug, 1979).

Shredders consume coarse particulate organic matter (CPOM, > 1 mm diameter), e.g. decomposing leaf litter, along with the associated microbiology, hereby converting CPOM to fine particulate organic matter (FPOM, < 1 mm and > 0.5 µm) and dissolved organic matter (DOM). They can also feed on living macrophytes or gouge decomposing wood, exposing the reduced organic particles to further microbial colonization and decomposition. Examples of shredders are caddisfly larvae (Trichoptera), the amphipods *Gammarus* spp. and the isopods *Asellus* spp. (Cummins and Klug, 1979; Wallace and Webster, 1996).

Gatherers represent a more diverse feeding group as they feed on deposited detritus FPOM related with the sediment. The particle size range that they can ingest depends on the morphology of the mouth parts. Through their feeding activity, gatherers convert the sediment detritus either into smaller or bigger particles (Cummins and Klug, 1979). *Hexagenia limbata* nymphs (burrowing mayfly, Ephemeroptera) for example aggregate fine particles before ingesting them (Zimmerman *et al.*, 1975). Particles can also be compacted in fecal pellets. Other examples of gatherers are midge larvae (Chironomidae) and Tubificidae (Cummins and Klug, 1979; Wallace and Webster, 1996).

While gatherers feed on FPOM from the sediment, filter feeders capture particles from suspension. Some filter feeders such as bivalves transport organic matter from suspension to the sediment by ingesting minute particles and egesting bigger faecal particles that sink to the bottom. This is an input for the deposit-feeding detritivores, and a retardation of the downstream transport of FPOM in streams (Wallace and Webster, 1996). Filterers make use of the flow velocity to acquire their food, or induce a current themselves, e.g. by cilia around the mouth of bivalves or beating of the legs of *Daphnia* spp. (Mellanby, 1963). Other examples of filterers include net-spinning caddisflies (Trichoptera) and blackfly larvae (Simuliidae) (Cummins and Klug, 1979; Wallace and Webster, 1996).

Macroinvertebrates with mouth parts adapted to scrape (i.e. shear off) periphyton (attached algae, bacteria and other micro-organisms) from surfaces like rocks or vegetation, are classified as scrapers or grazers (Cummins and Klug, 1979; Wallace and Webster, 1996). Snails (Gastropoda) fit in this class, but also the water penny beetle (Coleoptera) and some caddisfly and mayfly larvae (Cummins and Klug, 1979). It has been shown that scrapers can both reduce or enhance algal production. The latter is possible by removing dead or senescent algal cells, which shifts the algal community to more productive species. The grazing also decreases the thickness of the algal film, which allows more light and nutrient penetration (Wallace and Webster, 1996).

In contrast, certain macroinvertebrates are classified as piercers (e.g. Trichoptera: Hydroptilidae), as the larvae can climb on macrophytes, pierce the tissue and suck up the fluids (Cummins and Klug, 1979).

Lastly, predators capture alive prey, and either suck up body contents or engulf the prey directly. Examples are stoneflies (Plecoptera), caddisflies (Trichoptera) and damselfly and dragonfly nymphs (Odonata) (Cummins and Klug, 1979; Wallace and Webster, 1996).

Presence of functional feeding groups and their relative importance in the overall macroinvertebrate community composition is highly linked with energy flow through the system. For instance, forested headwaters receive mainly allochthonous energy as twigs, branches and leaves of the surrounding trees fall (or are blown into) the water. This CPOM is the food source for many shredders, steering the community to be mainly composed of shredder taxa, with minor presence of collectors (both gatherers and filterers) and predators. The degradation of this CPOM to FPOM and DOM by the shredders' activity provides a food source for collector taxa, both locally as downstream. Furthermore, in the downstream section, the river becomes wide enough for allowing the sunlight to reach the water column, supporting the development of macrophytes and algae (i.e. autochthonous energy input). Consequently, less shredders will be present, while scraper and collector taxa have increased in relative contribution. Even more downstream, the river becomes too wide and deep to have sunlight penetrate through the water column and reach the benthic environment. As such, macrophytes will be present in lower amounts, thereby also limiting the presence of scraper species, finally ending up with a collector-dominated macroinvertebrate community. This link between the biotic community, local energy input and upstream locations is described in the River Continuum Concept by Vannote *et al.* (1980) and has been investigated in several rivers (e.g. Damanik-Ambarita *et al.* (2016), Tomanova *et al.* (2006), …). Within this framework, a higher diversity in collector species exist when compared to shredder species, although the latter is of specific importance as it provides the degradation of coarse particulate organic matter into finer particles that can be used by the high variety of collector species. Therefore, providing the optimal conditions for shredders to thrive in is of utmost importance when aiming to reduce the amount of CPOM in a natural way.

In contrast to streams, which are lotic systems (flowing water), CWs are lentic systems (still water). This factor can give rise to a different macroinvertebrate community based on habitat preference, e.g. mosquito larvae and most aquatic beetles like still or slow moving waters, whereas the freshwater shrimp and blackfly larvae prefer running water (Mellanby, 1963). Another difference is that energy input mainly comes from the influent wastewater, and from autochthonous plant litter. Allochthonous plant litter input is much smaller, depending on the surroundings. Still, studies investigating the presence of macroinvertebrates in CWs treating wastewater are limited. For instance, Spieles and Mitsch (2000) investigated a CW treating secondary treated municipal wastewater in the USA. Most abundant taxa were Chironomidae and Gastropoda. Jurado *et al*. (2009) sampled macroinvertebrates in CWs treating farmyard wastewater in Southeast Ireland. In contrast, most abundant taxa there were Coleoptera and Hemiptera. Boets *et al.* (2011) and (Donoso, 2015) studied CWs treating secondary treated liquid fraction of pig manure in Ichtegem and Langemark, Belgium, respectively. Similarly, it was found that Chironomidae, Corixidae and Culicidae, along with Scirtidae, were the most abundant species. An overview of the observed taxa can be found in Table 2. The families Chironomidae, Culicidae, Dytiscidae, Hydrophilidae and Coenagrionidae were found in all four studies. Regarding feeding groups, collectors, scrapers and

predators were found in great quantities in most of the CWs. In contrast, shredders were not found or were existent in minor quantities. Four taxa were found by Spieles and Mitsch (2000), *Asellus* sp. was found by Jurado *et al.* (2009).

This lack of shredder species constitutes a missing link in organic matter cycling in CWs, given the fact that these systems receive autochthonous plant litter each year. Introducing shredders could be beneficial for the system by enhancing decomposition processes of organic material (Boets *et al.*, 2011), and by providing food for other functional feeding groups, for instance collectors. More specifically for Flanders, *A. aquaticus* seems a good candidate because of its broad natural distribution and its tolerance to high nutrient levels (Messiaen *et al.*, 2010).

Table 2: Taxa of macroinvertebrates found in CWs treating a certain type of wastewater. Diptera taxa are non-limiting

Higher classification	Functional feeding group	Family
Bivalvia	Filter feeder/collector	N/A[1]
Coleoptera	Shredder	Chrysomelidae[1]
		Curculionidae[1]
		Haliplidae[1]
	Predator	Dytiscidae[1,2,3,4]
		Gyrinidae[1]
		Noteridae[2]
	Collector	Dryopidae[4]
		Elmidae[1]
		Hydrophilidae[1,2,3,4]
		Scirtidae[1,4]
Diptera	Predator	Ceratopogonidae[1]
	Collector	Chironomidae[1,2,3,4]
		Culicidae[1,2,3,4]
		Phsychodidae[2]
		Simuliidae[1]
	Filter feeder	Dixidae[4]
	Scraper	Ephydridae[4]
Ephemeroptera	Collector	Caenidae[1]
	Scraper	Baetidae[3]
Gastropoda	Scraper	Gastrodontidae[2]
		Lithoglyphidae[4]
		Lymnaeidae[2,3]
		Physidae[1,2]
		Succineidae[2]
Hemiptera	Predator	Gerridae[2]
		Nepidae[2]
		Pleidae[4]
	Piercer/predator	Corixidae[1,3,4]
	Piercer	Hydrometridae[1,2]
		Mesoveliidae[1]
		Naucoridae[1]
		Notonectidae[1,3]
Hirudinea	Predator	Glossiphoniidae[4]
		Hirudinidae[1]
Isopoda	Shredder	Asellidae[2]
Neuroptera	Predator	Sialidae[4]
Odonata	Predator	Aeshnidae[1,2]
		Calopterygidae[1]
		Coenagrionidae[1,2,3,4]
		Lestidae[1]
		Libellulidae[1,3,4]
Oligochaeta	Collector	Lumbricidae[4]
		Tubificidae[1]
Trichoptera	Scraper/predator	Hydroptilidae[1]
	Predator/filter feeder	Polycentropodidae[2]
	Collector/shredder/scraper/predator	Leptoceridae[4]
	Shredder/collector	Limnephilidae[4]

1: Spieles and Mitsch (2000); 2: Jurado *et al.* (2009); 3: Boets *et al.* (2011); 4: Donoso (2015)

2.4 Asellidae

2.4.1 Morphology and physiology

The Family Asellidae belongs to the subphylum Crustacea, of the phylum Arthropoda (Table 3), being characterised by segmented bodies, paired jointed limbs and a chitinous exoskeleton, causing the animals to moult periodically to allow for growth. This subphylum also entails e.g. lobsters, crabs, barnacles and shrimps. Asellidae differ from them by a dorsoventrally flattened body and highly identical limbs, classifying them in the order of Isopoda. Their body is grey-brown containing two pairs of antennae, four pairs of jaws, seven pairs of thoracic limbs and six pairs of abdominal limbs of which the first five are broad plates, being the gills (Figure 5) (Mellanby, 1963). *A. aquaticus and A. meridianus* are the most abundant species in northern Europe (Field Studies Council, 2015). The first has two white spots on the head, whereas the latter has one elongated spot (Fitter and Manual, 1986).

Table 3: Classification of *Asellus* sp. (Mellanby, 1963)

Phylum	Arthropoda
Subphylum	Crustacea
Class	Malacostraca
Order	Isopoda
Family	Asellidae
Genus	*Asellus*

Figure 5: *Asellus aquaticus* (Focusnatura., 2017)

In a study of Ellis (1961) on the life history of *A. intermedius* Forbes (very similar to *A. aquaticus*) in the Houghton Creek, Michigan, the main breeding season of *A. intermedius* was found to be from May to September. In April and May the large, overwintering adults reproduce and then become proportionately much less abundant. By early June, the smaller, overwintering individuals and possibly some of the early spring progeny have matured and bred. Gravid females were found during every month of the year, but the reproductive rate was low in winter. The female carries the eggs and young in a marsupium or brood pouch under the abdomen. The eggs hatch when the young are at a fairly early stage of development, but the young remain in the brood pouch until they have developed further. The number of young in the marsupium is dependent on the size of

the female. Molting and growth of *A. intermedius* were observed through as many as nine instars. Individuals live about one year.

Asellus sp. is very common in ponds or slow-moving streams, crawling or climbing about among weeds (Mellanby, 1963). This shredder feeds on decaying leaves along with the associated microbiology and they also ingest microscopic algae (Moore, 1975). When dead leaves fall into a stream or pond, microorganisms will colonize them and soluble substances such as phenols, amino acids and sugars will leach out (i.e. leaf conditioning). The leaching of the latter two substances will probably give rise to a lower food value of the leaves, while leaching of phenols has a positive effect. Fungi and other micro-organisms can make dead leaves more palatable and more nutritious by two mechanisms: (i) addition of easily digestible compounds to the nutritionally poor leaf substrate, including the microbial cells itself as well as microbial secretions, and (ii) conversion of indigestible leaf substances (e.g. cellulose, hemicellulose and pectin) into digestible compounds by microbial enzymes (Barlocher, 1985).

The importance of fungi in the feeding of this animal has been shown in several studies. Marcus and Willoughby (1978) found that *A. aquaticus* fed with conditioned oak leaves or the fungus *Saprolegnia* attained similar growth rates. However, when fed with the fungus *Lemonniera aquatic*, the animals attained significantly smaller growth rates. These results suggest that different fungi may have different nutritional value for aquatic invertebrates. Rossi and Fano (1979) offered *A. aquaticus* sterilized *Platanus* leaves, eight types of pure fungus cultures and conditioned leaves as control. All the animals refused the sterilized detritus and died within the first 15 days of the experiment. The animals to whom the fungi were offered showed, compared to the controls, survival rates significantly higher for five of the eight fungi offered, similar values for one (*Humicola*), and lower values for two (*Trichoderma* and *Aspergillus niger*) fungi. In contrast to the study of Marcus and Willoughby (1978), the animals fed with pure fungi showed significantly higher growth rates than those observed for the control animals. Fano *et al.* (1982) conducted a similar experiment, though instead of offering pure fungus cultures, they inoculated alder leaf discs with single fungal strains (10 strains in total) and fed them to *Proasellus coxalis*. Also here, sterilized leaves were untouched and the animals died. Leaf discs inoculated with single fungal strains differed in mass loss. Different fungi gave values significantly higher, equal to, or lower than the leaves soaked in stream water. The results indicated the high importance of fungal colonization of detritus in the diet of *Proasellus coxalis* as different fungi differ in their food suitability for aquatic invertebrates. Graça *et al*. (1993a) investigated if *A. aquaticus* preferred unconditioned elm leaf discs, leaf discs inoculated with *Anguillospora longissima* or discs of pure *A. longissima* mycelia. They found that the animals fed primarily on fungal mycelia and virtually ignored the leaf discs, which is in line with the results of Rossi and Fano (1979). In another feeding experiment, *A. aquaticus* preferred conditioned elm leaves over unconditioned leaves (Graca *et al.*, 1993b), which again shows the importance of fungi in the feeding of this animal. Visual examination of the leaf discs suggested that *A. aquaticus* scrapes at the leaf surface rather than biting through the leaf, leaving even the fine veins intact (Graca *et al.*, 1993a).

The role of adult feces in the nutrition of larvae of *A. aquaticus* was examined by Rossi and Vitagliano-Tadini (1978). Larvae reared with *Platanus* leaves and feces from adults showed higher food intake and growth increase than the controls (only plant detritus) (0.12 vs. 0.07 mg ind^{-1} d^{-1} and 0.04 vs. 0.021 mm d^{-1}). Also survival was higher than in the control group (86 % vs. 61 %). Microscopic examination of the feces showed that it is a mixture of finely comminuted plant material and many types of microorganisms, a.o. *Alternaria* and *Fusarium*. Larvae fed with sterilized plant detritus and sterilized feces did not consume the food and were all dead after 30 days. The results indicate that the presence of adult feces has a positive effect on growth and survival of the larvae.

In addition, Asellidae can survive in areas with low oxygen levels, making them tolerant to moderately polluted water (Field Studies Council, 2015). Based on their survival in waters polluted with organic matter and their shredder activity, it is expected that they are performant in wetland systems, helping in the degradation of organic matter.

2.4.2 Impacts

To see the impact of *A. aquaticus* with respect to organic matter degradation and cycling of nutrients in an ecosystem, results of lab-scale studies investigating feeding rate, growth rate and survival of *A. aquaticus* are listed in Table 4. Information is rather scattered though, since only a limited number of studies focuses on these processes of *A. aquaticus* in particular. In addition, measurements of nutrient excretions are shown and the chemical composition of *Asellus* can be seen in Table 5. The latter gives an idea of the nutritional needs of the animal, as well as the recycling of substances when it dies.

Table 4: Feeding rate, growth rate and survival of *A. aquaticus* (DW = dry weight; WW= wet weight)

Substrate	Conditions	Feeding rate	Growth rate	Survival
Conditioned elm leaves[1]	15°C; 5 days	0.12 mg ind^{-1} d^{-1}	N/A	N/A
Conditioned elm leaves[1]	15°C; 3 days	0.26 mg mg $animal^{-1}$ d^{-1}; Feces prod.: 0.17 mg mg $animal^{-1}$ d^{-1}	N/A	N/A
Conditioned elm leaves[1]	Juveniles; 15°C; 60 days	N/A	7.25 % DW d^{-1}	85 %
Conditioned oak leaves[2]	Resp. 2.5 mm and 1 mm in length; 15°C; 49 days	N/A	Resp. 4.85 ± 0.55 % and 6.81 ± 0.86 % WW d^{-1}	Resp. 100 % and 90%
Conditioned *Platanus* leaves[3]	Larvae; 20°C; 30 days	0.07 mg ind^{-1} d^{-1}	Growth increase: 0.62 mm	61 %
Conditioned alder and oak leaves[4]	Adults; 4°C; 30 days	0.038 g g $animal^{-1}$ d^{-1}	Resp. 0.0025 ± 0.0021 and 0.0019 ± 0.007 g g^{-1} d^{-1}	95-100 %

1: Graca *et al.* (1993b), 2: Willoughby and Marcus (1979), 3: Rossi and Vitagliano-Tadini (1978), 4: Bjelke and Herrmann (2005)

Besides the abovementioned traits, activity of *A. aquaticus* is also influenced by meeting their nutritional needs to end up with the required substance composition. Ultimately, these substances will be released and recycled after the death of the organism. Frost and Tuchman (2005) determined the chemical composition of the animals (Table 5) and measured nutrient release rates of *Asellus* after feeding on senesced leaves of *Populus tremuloides* (trembling aspen) for 48 hours at 20°C. Dissolved organic carbon (DOC) release rate was 6 μg C h^{-1} mg DW^{-1}, NH_4 release rate was 0.45 μg NH_4 h^{-1} mg DW^{-1} and soluble reactive phosphorus (SRP) release rate was 0.01 μg P h^{-1} mg DW^{-1}.

Table 5: Chemical composition of Asellus. % C, % N and % P are percentages of dry weight and the elemental ratios are on a mass basis (Frost and Tuchman, 2005)

% C	% N	% P	C:N	C:P	N:P
32.5 ± 1.50	7.20 ± 0.66	1.06 ± 0.03	4.86 ± 0.41	31.7 ± 1.17	6.56 ± 0.38

2.6 Vegetation

As mentioned above, an important part of CWs is the presence of vegetation. In most cases, common reed (*Phragmites australis*) is used as the preferred vegetation type as it is rather robust to freezing and can form dense biomass stands. Still, it follows the seasons by dying off during fall and winter, followed by growth during spring and summer. Consequently, a part of the overall biomass stand will be transferred from the vegetation to the detritus pool when plants die off. This part is, preferably, smaller than the increase in biomass during the growth season, and will, eventually, break down.

The first shoots of *P. australis* emerge in the first half of April and reach a peak density in early July (Mason and Bryant, 1975; Graneli, 1984). Mason and Bryant (1975) found a peak shoot density of 127 ± 7.1 shoots m^{-2} and a peak biomass of 0.9 kg DW m^{-2} in a swamp in England. Similarly, Allirand and Gosse (1995) found production levels from 0.9 to 1.3 kg DW m^{-2} in a French marsh, and in southern Sweden, above- ground biomass is 1 kg DW m^{-2} in August and 0.5 kg DW m^{-2} in winter (Graneli, 1984). From early August, senescence of shoots as well as flowering begins. During autumn, the leaves are shed but the dead shoots remain standing, at least for part of the winter. Many dry shoots even survive for at least two years, protecting the young shoots from frost (Mason and Bryant, 1975; Graneli, 1984).

As soon as plant litter enters the water, it starts to break down into smaller parts, with substances continuously leaching out of the litter material. Mostly, this is the result of a combined working of micro-organisms and macroinvertebrates, though some studies only look into the effect of micro-organism activity on the degradation rate. For instance, small-size litter bags can be put in the water, excluding macroinvertebrates from coming in, while large-size litter bags enables them to enter. Mass losses of *P. australis* litter during decomposition in lakes are shown in Table 6. It can be seen that results vary among studies, but culms are always slower degraded than leaves due to more fiber content in culms (Dinka *et al.*, 2004; Szabó and Dinka, 2008). Most abundant species on the plant litter found by Mason and Bryant (1975) were *A. aquaticus*, ranging from 207 to 2160 ind m^{-2}, *A. meridianus*, ranging from 193 to 840 ind m^{-2}, *Ischnura elegans* (Odonata), ranging from 20 to 500 ind m^{-2} and Chironomidae, ranging from 30 to 10 000 ind m^{-2}.

Table 6: mass loss of *P. australis* litter in lakes during time

Mass loss in small- size litter bags	Mass loss in large- size litter bags
Leaves	
50 % after 242 and 80 % after 628 days[1]	
10 % in first month, 30 % after 506 and 45-51 % after 863 days[2]	10 % in first month, 37-47 % after 506 and 60-85 % after 863 days (significant effect of animals)[2]
	50 % after 185 and 80 % after 245 days[3]
82-90 % after 990 days[4]	
Culms	
50 % after 574 and 59 % after 628 days[1]	
5 % in first month, 15 % after 506 and 23 % after 863 days[2]	5 % in first month, 15 % after 506 and 27 % after 863 days[2]
	50 % after 550 and 80 % after 730 days[3]
39–43 % after 990 days[4]	
Both leaves and stems	
0-20 % in first month and 50 % after 200 days[5]	10-20 % in first month and 50 % after 233 days (no significant effect of animals)[5]

1: Szabó and Dinka (2008), 2: Hietz (1992), 3: Gessner (2000); Szabó and Dinka (2008), 4: Dinka *et al.* (2004), 5: Mason and Bryant (1975)

2.7 Hypothesis and objectives

Plant litter accumulates at the bottom of CWs, which is the main source of effluent BOD in CWs. *A. aquaticus* could significantly reduce the plant litter biomass in CWs given its shredder activity, hereby reducing BOD concentrations in the effluent. Also, by comminuting a big part of the leaves, *A. aquaticus* increases the specific surface area for microbial colonization, fostering more rapid breakdown. On the other hand, these animals could have a negative effect on microbial degradation by feeding on the microbiology on the leaf litter. Furthermore, releasing FPOM by their shredding activity could increase suspended solids in the effluent. In short:

- It is hypothesised that *A. aquaticus* can enhance organic matter decomposition by its shredder activity. This is investigated by measuring (i) the reduction of reed leaves biomass (*Phragmites australis*), at the presence and absence of *A. aquaticus*, and (ii) the variation of BOD concentrations of the water through time.
- Since Asellidae are not commonly found in CWs treating wastewater, the capacity of Asellidae to adapt to CW conditions will be tested to determine the feasibility to introduce them in these ecosystems.

3 Materials and methods

First, an acclimatisation test was performed by checking the survival of *Asellus aquaticus* at different concentrations of wetland water, since these animals are commonly missing in these types of ecosystems. Furthermore, the feeding of *A. aquaticus* on reed leaves (*Phragmites australis*), a macrophyte regularly encountered in CWs, was investigated in lab-scale experiments to determine organic matter degradation by Asellidae. Chemical parameters of the water were measured as well.

3.1 Acclimatization test

To test the feasibility of introducing *A. aquaticus* in CWs for wastewater treatment, a five days acclimatization test was performed. Both influent and effluent water samples were taken from a HSSF CW treating primary domestic wastewater in Sint-Martens-Latem.

Weckpots of 500 mL were used in two replicates for influent and effluent water, each containing five animals. Starting from 100 mL tap water, each day respectively 25, 42, 83 and 250 mL wetland water was added to each weckpot in order to have respectively 20 %, 40 %, 60 % and 80 % wetland water in the weckpots. The fifth day, the surviving animals were brought in new weckpots containing pure wetland water. Simultaneously, another test where animals were brought in pure influent or effluent water without any previous acclimatization was performed to see the impact of this shock on survival. There was only one replicate containing five animals for influent and effluent water each. Temperature (T), dissolved oxygen (DO), conductivity and pH were measured each day for each weckpot, as well as the survival of the animals.

3.2 Preliminary feeding test

Based on the known presence of *A. aquaticus*, five stream locations passing by the cities of Ghent, Lovendegem and Nevele were selected for sampling. The aim was to determine the chemical water conditions in which the macroinvertebrates were present. Temperature (T), pH, dissolved oxygen (DO), conductivity, biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP) and organic matter (OM) were determined for each of the water samples. COD, TN an TP were measured spectrophotometrically (Aquamate, Thermo Electron Corporation; Merck, Spectroquant). Graaf van Hoornestraat in Nevele was selected for collecting water samples and *A. aquaticus*. Water quality parameters can be seen in Table 7. Once in the lab, individuals of *A. aquaticus* were kept in an aquarium and the surface water was filtered through a filter with pore size of 40 µm to remove fine particulate organic matter (FPOM).

Table 7: Water quality parameters of the surface water at Graaf van Hoornestraat, Nevele. OM was below detection limit

Parameter	Value
T (°C)	7.6
DO (mg L^{-1})	4.6
Conductivity (µS cm^{-1})	618
pH	7.3
BOD (mg L^{-1})	2.4
COD (mg L^{-1})	41.3
TN (mg L^{-1})	6.4
TP (mg L^{-1})	1.2

Reed leaves (*P. australis*) were collected from a constructed wetland treating pig manure in Ichtegem and were air dried for 4 weeks at 25°C. Part of the dry leaves was shredded in a mixer to obtain FPOM and part was cut into pieces of 2x5 mm to obtain CPOM. The FPOM was sieved with sieves of 500 µm, 200 µm and 100 µm to obtain FPOM < 100 µm.

The test was performed in weckpots of 1.5 L containing 500 mL of filtered water. The test included blanks (B1) (only filtered river water), controls (C1) (filtered river water and FPOM and CPOM) and pots containing *A. aquaticus* (A1) (filtered river water, FPOM and CPOM and 10 animals), each in 4 replicates (Figure 6 and Figure 7). 85 mg of FPOM and 85 mg of CPOM was added to each C1 and A1 pot as food source. The test was done at 20°C and aeration was provided. To determine the starting point, initial concentrations of BOD, COD, TN, TP and pH, T, conductivity, DO were determined. Recovery of leaf CPOM mass was determined after leaving it for one night in the filtered river water.

To determine the change of the chemical composition of the water as well as the degradation of OM through time, batches of 12 samples in total (Figure 6) were analyzed after 11, 22 and 33 days. Moreover, the survival and weight of the animals was registered at the end of each period.

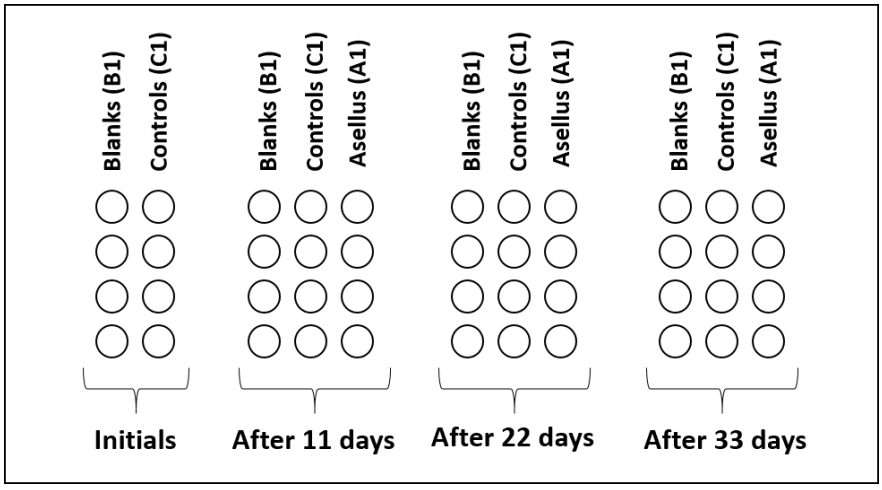

Figure 6: Scheme experimental set up preliminary feeding test

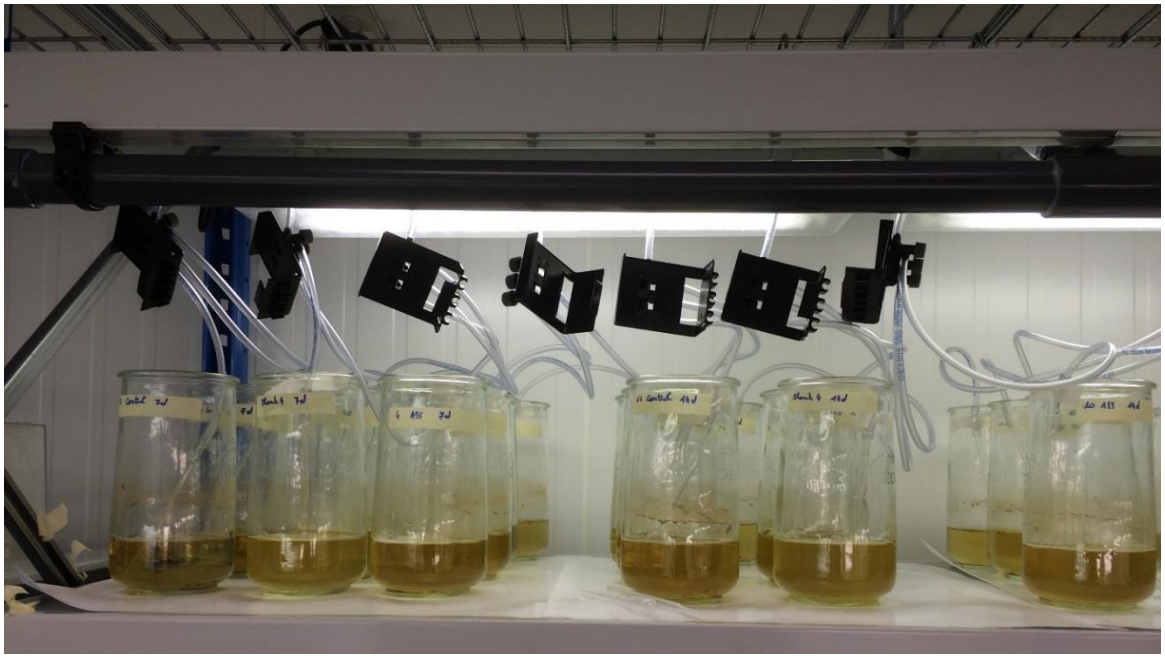

Figure 7: Partial overview of the preliminary feeding test

At the 11th, 22nd, and 33rd day, each weckpot was emptied and properly rinsed. To separate the Asellidae and CPOM from FPOM a sieve of 1 mm was used. Alive and dead Asellidae were counted, rinsed with deionised water and dried at 60°C for 2 days. CPOM was also rinsed and dried in porcelain cups at 100°C for one night. The test water and the rinsing water were then filtered with the same ash free filter paper with a pore size of 4-12 µm using a büchner filter. The test water and the rinsing water were caught separately, and subsequently, chemical analysis of the filtered test water was performed (Figure 8). The ash free filter paper was used for FPOM determination, and for this purpose dried at 100°C for one night as well. Dry weight (DW) of Asellidae, CPOM and FPOM were measured and after that, the organic matter content of CPOM and FPOM was determined by using a muffle oven at 450°C for 2 hours.

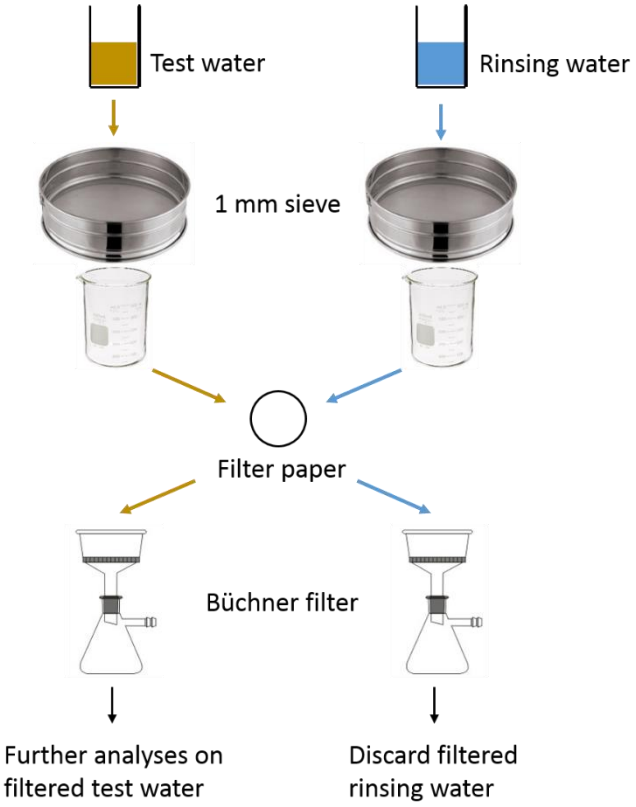

Figure 8: Schematic representation of the followed procedure for the FPOM – CPOM – Asellidae separation and filtration

3.3 Optimized feeding test

A repetition of the feeding test was performed, though specific attention was given to the optimisation of the following parameters. Instead of filtered river water, a standard, synthetic freshwater (moderately hard) described by USEPA (2002) was used, adding 10 % of pond water for microbial colonization. The pond water was first filtered (1,2 µm) to limit algae presence. In addition, an algae inhibitor (Alg Control F) was added to prevent algae growth. Animals and pond water were collected from the same pond.

No FPOM was added as food for the animals in order to be able to collect feces and FPOM production by the shredding activity of the animals. The reed was collected from the same CW, however the leaves were already shed, thus lying in the water. The leaves were rinsed first, then oven dried at 60°C for 5 days, and cut in squares of 1.5 cm x 1.5 cm. After weighing these leaf squares, they were conditioned for two weeks in pond water to allow micro-organisms and fungi to colonize them, resulting in better palatability for the animals. Subsequently, the recovery of leaf mass was determined after this conditioning period.

Plastic cups with 50 mL of test water were used. Blanks (B2) contained only test water, controls (C2) contained test water and one square of reed, and cups containing *A. aquaticus* (A2) contained test water, one square of reed and one *A. aquaticus*. The B2, C2 and A2 cups included 30 replicates

each, and this for each period (Figure 9). Additionally, ten cups without algae inhibitor were added to see the effect of the algae inhibitor on the activity of the animals. Furthermore, five separate blanks, controls and cups containing *A. aquaticus* were added (with algae inhibitor), henceforth called 'separate cups', to measure T, DO, pH and conductivity approximately every two days. Also, dead animals were replaced every two days, and evaporated water was replaced using deionized water. The experiment was performed at 20°C and no aeration was provided.

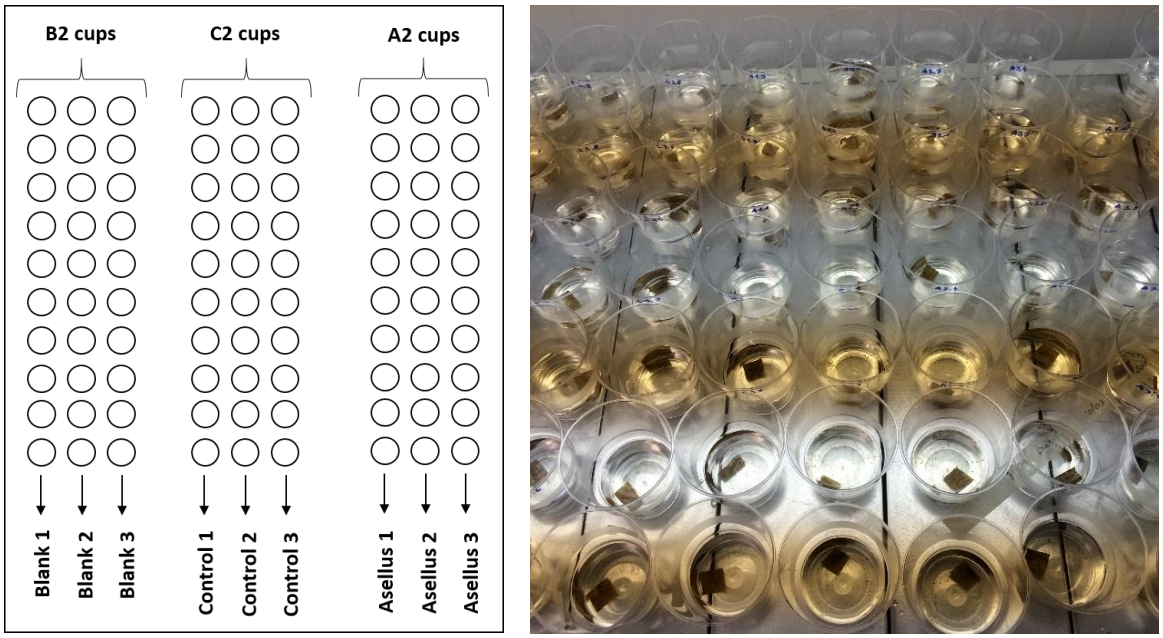

Figure 9: Left: scheme of the experimental set up of the optimized feeding test. This set is repeated for each period. Right: Partial overview of the optimized feeding test

Initial concentrations of BOD, COD, TN, TP and pH, T, conductivity, DO were measured to determine the starting point. After 11, 22 and 33 days, animals and reed squares were rinsed and dried at 60°C for two days. After measuring the dry weight, the organic matter content of the reed squares was determined. At first, the remaining water was filtered with ash free filters with pore size of 4-12 µm to collect FPOM (feces and FPOM coming from the reed). Since the weight of FPOM was too small to be measured, smaller filters with pore size of 1.2 µm were used for the 22 and 33 days periods. For each cup a new filter was used, but the filtrate of all ten replicates was poured together for chemical analysis, resulting in three replicates for each set of B2, C2 and A2 cups (Figure 9 Left). The filters were dried at 60°C for two days as well.

From five cups without algae inhibitor, only the reed squares were analyzed after 22 days, and from the other five cups, reed squares and FPOM were analyzed after 33 days. The separate cups were analyzed after 33 days. Here, chemical analysis (BOD, COD, TN and TP) of the water was performed for each cup separately to see the variability between the cups. Reed squares and FPOM were analyzed as well.

3.5 Statistical tests

Differences in percentage remaining CPOM, FPOM production, BOD, COD and nutrient concentration between C and A for each period in each test were checked for their significance via the Wilcoxon singed ranks test. Differences in percentage remaining CPOM and consumption by Asellidae between periods in each test were also checked with this test. The Mann-Whitney U test was used to check differences in consumption rates between the tests.

4 Results

Only a selection of the obtained results will be discussed in-depth in this section, focusing on survival, conductivity, organic matter and chemical analyses. Other results will be mentioned shortly as no (clear) patterns were observed. Throughout this section, results of the acclimatization test, the preliminary feeding test (B1, C1 and A1) and the optimized feeding test (B2, C2 and A2) will be reported, with B representing blanks, C representing controls and A representing recipients with Asellidae. Reported BOD represent the soluble fraction of BOD since all solids were filtered out of the water before analysis. In addition, if measurements of COD standards were too low/too high, the right amount was added/subtracted from the measured concentrations to obtain a more correct value.

4.1 Acclimatization test

Water composition was different between the influent and effluent water from the wetland in Sint-Martens-Latem. Variables linked with pollution (conductivity, BOD and COD) were lower in the effluent (e.g. 94.7 mg COD L^{-1} versus 121.5 mg COD L^{-1}), while pH was lower in the influent (7.42 versus 7.55). Measured values and their standard deviations are reported in Table 8.

Table 8: Water quality parameters of influent and effluent water of a CW treating domestic wastewater (mean ± SD)

Parameter	Influent	Effluent
T (°C)	14.3 ± 0.2	14.5 ± 0.5
DO (mg L^{-1})	3.2 ± 1.3	0.4 ± 0.0
Conductivity (µS cm^{-1})	1384 ± 8	1278 ± 1
pH	7.42 ± 0.01	7.55 ± 0.01
BOD (mg L^{-1})	34.7 ± 0.8	26.6 ± 2.1
COD (mg L^{-1})	121.5 ± 13.8	94.7 ± 3.2

In general, survival was highest in effluent replicate 1, followed by influent replicate 1, effluent replicate 2 and lastly influent replicate 2. Survival of *A. aquaticus* in the acclimatization tests with influent and effluent water is shown in Figure 10. The survival in pure influent and effluent water resulted in the survival of four out of five animals in pure influent water after six days whereas only two survived in pure effluent water (Figure 11). Throughout the test, conductivities clearly increased from the start till the end, which is depicted in Figure 12 and Figure 13.

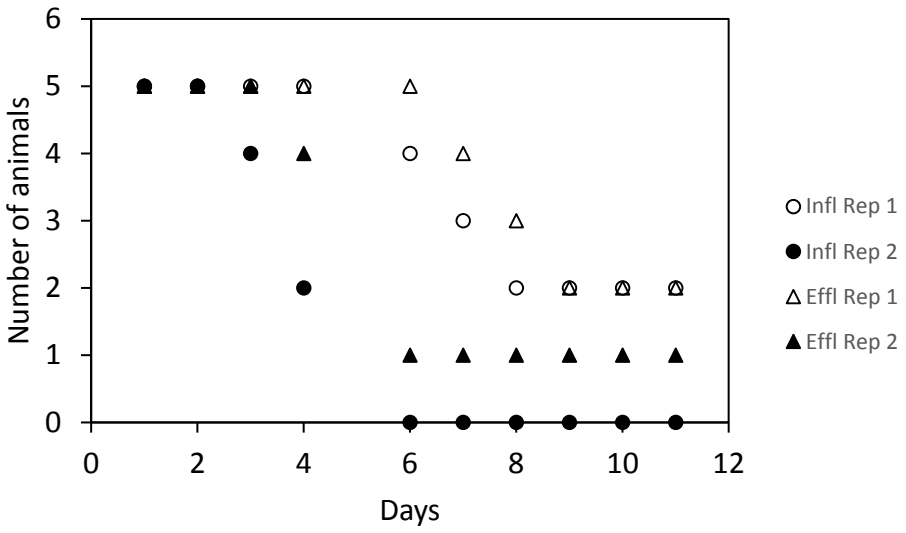

Figure 10: Survival of five *A. aquaticus* animals in the two replicates of the acclimatization test with influent water and effluent water. Day 1 to 4 represents survival at 20 %, 40 %, 60 % and 80 % wetland water, respectively. At day 5, the surviving animals were transferred to 100 % wetland water.

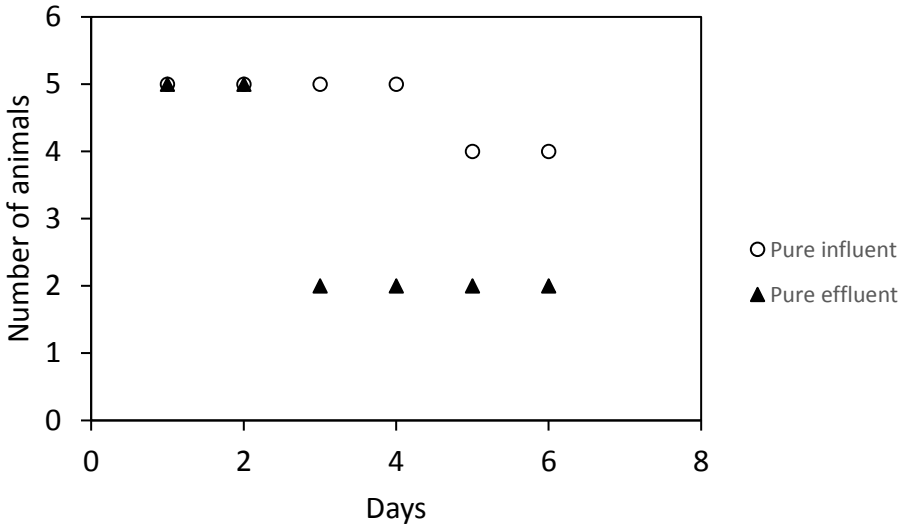

Figure 11: Survival of five *A. aquaticus* animals in pure influent and effluent water. Survival of *A. aquaticus* is lower in pure effluent water, compared to pure influent water.

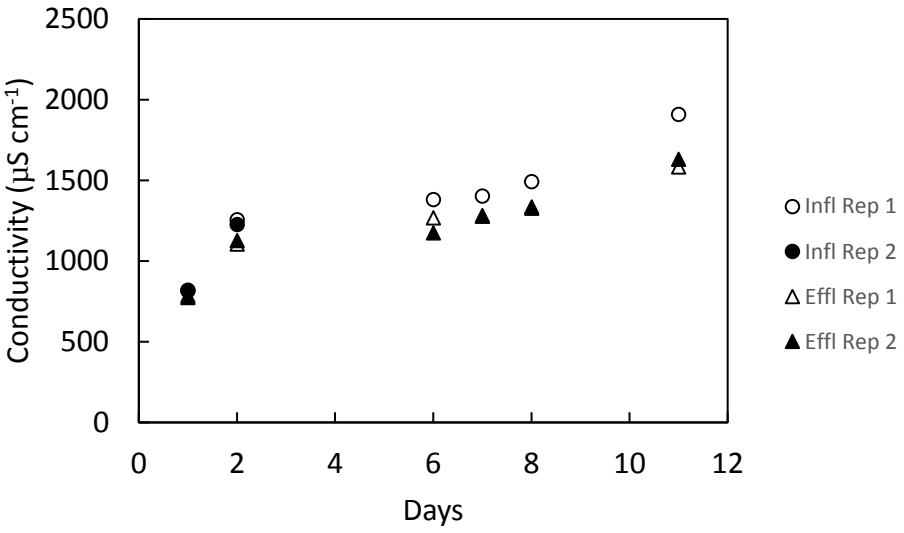

Figure 12: Conductivity in acclimatisation test with influent and effluent water

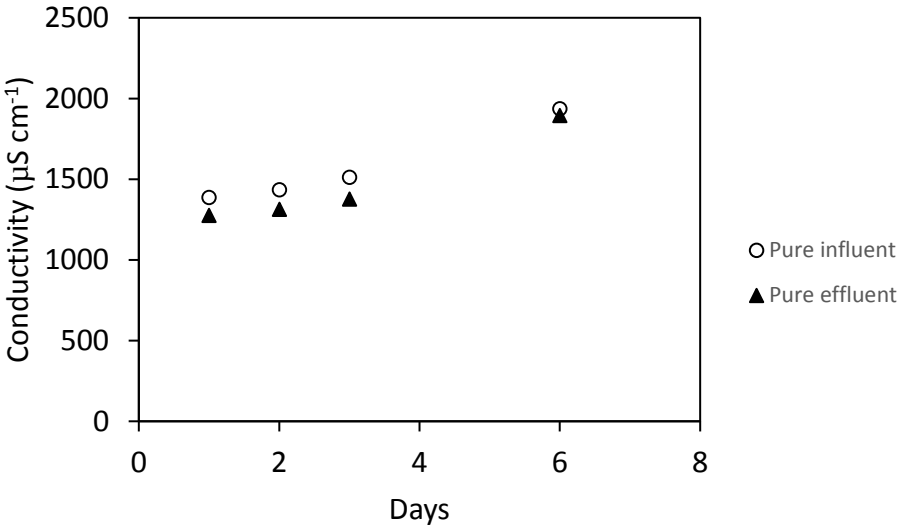

Figure 13: Conductivity in test with pure influent and effluent water

4.3 Feeding tests

4.3.1 CPOM recovery after conditioning period

Mean recovery of CPOM was 84 ± 1 % for the preliminary feeding test and 86 ± 30 % for the optimised feeding test (after one night and after 14 days of conditioning, respectively). In the optimized feeding test, mass increases were also encountered, but in general there was a decrease in mass during the conditioning period. Boxplots of fraction recovery of leaf CPOM on a DW basis are shown in Figure 14. The leftmost boxplot shows recovery from the preliminary feeding test after leaving the CPOM for one night in the filtered water, the rightmost boxplot from the optimized feeding test after a 14 days conditioning period in pond water.

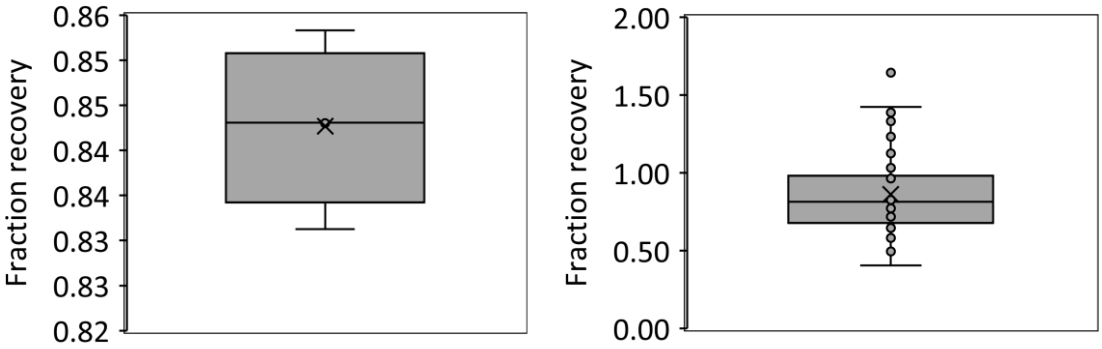

Figure 14: Recovery of leaf CPOM after 1 night in the feeding test (Left) and after 14 days conditioning in the optimized feeding test (Right). A clear distinction can be made between both conditioning periods as the longer the leaves are conditioned, the higher the variability in mass loss/gain is.

4.3.2 Preliminary feeding test

A decrease in CPOM was observed throughout the test for all time periods. CPOM after 11 days was only 66 ± 2 % of the initial CPOM when Asellidae were present, which was lower than the 79 ± 1 % of the initial CPOM for controls. Even less CPOM was still present after 22 days (57 ± 2 % and 70 ± 1 %, respectively), although this pattern was not continued towards day 33, as an increase in CPOM was observed for the C1 series (90 ± 16 %). CPOM in A1 was 48 ± 7 % after 33 days. The percentage of remaining CPOM in C1 and A1 are shown in Figure 15. Initial CPOM values are calculated as the DW of CPOM before the leaching period multiplied by the fraction recovery. Values of A1 stayed below the values of C1, yet there was no significant difference between C1 and A1 for all the periods ($p = 0.068$ for all periods). In addition, A1 was not significantly different between the periods ($p > 0.068$).

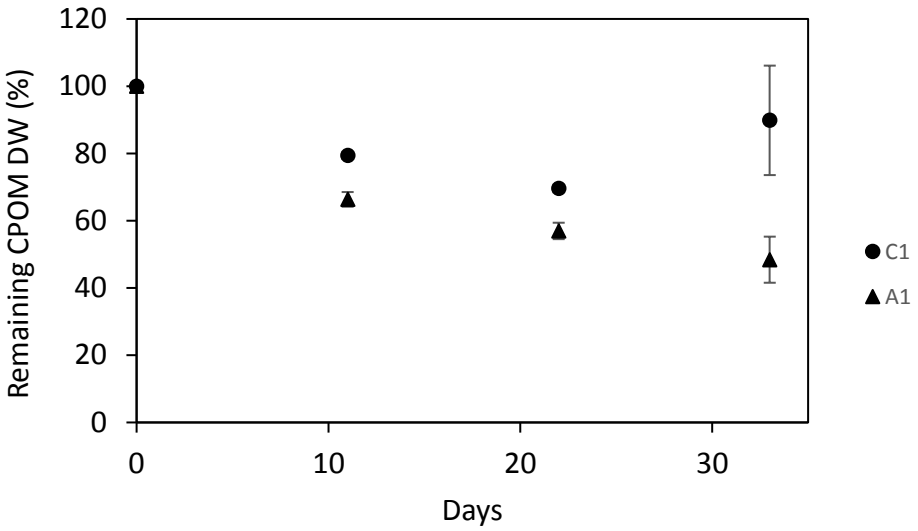

Figure 15: Percentage remaining CPOM in C1 and A1 (mean ± SD)

Results show that when consumption by Asellidae was high, microbial breakdown was low and vice versa. After 11 days, average consumption by Asellidae was 9.3 ± 1.6 mg, staying rather constant in the next 11 days, with a consumption of 9.0 ± 1.7 mg after 22 days. From then on, consumption rose steeply to a value of 29.7 ± 4.9 mg after 33 days. In Figure 16, average DW values of microbial breakdown of CPOM and consumption of CPOM by 10 Asellidae are depicted. Microbial breakdown was calculated as the difference between initial and final weights of the CPOM of the controls. Consumption of CPOM by Asellidae was then calculated as the difference between initial and final weights minus microbial breakdown. Values of consumption were not significantly different between periods ($p > 0.068$). Expressed in an overall average value, consumption rate was 0.07 ± 0.01 mg ind^{-1} d^{-1}. Microbial breakdown was 14.8 ± 0.7 mg after 11 days, rose to 21.8 ± 0.8 mg and finally decreased to 7.3 ± 11.7 mg.

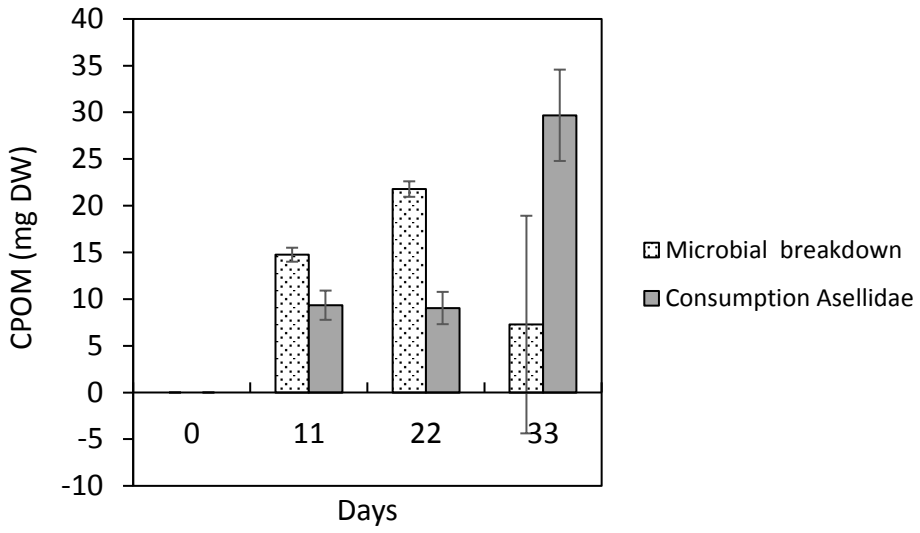

Figure 16: Microbial breakdown of CPOM and consumption of CPOM by Asellidae in the feeding test (mean ± SD). An increase in CPOM removal can be observed from day 11 till day 22 as consumption by Asellidae remains constant and the microbial breakdown increases. Similar to Figure 15, this pattern is not clearly found between day 22 and 33.

Results of FPOM will not be provided, since no discrimination could be made between initially present FPOM, feces of animals and FPOM coming from CPOM.

Only 5 % of the animals were alive at the end of the experiment. Figure 17 shows average percentage survival of the ten animals in the feeding test. In total, there were 40 animals for each period (i.e. four replicates).

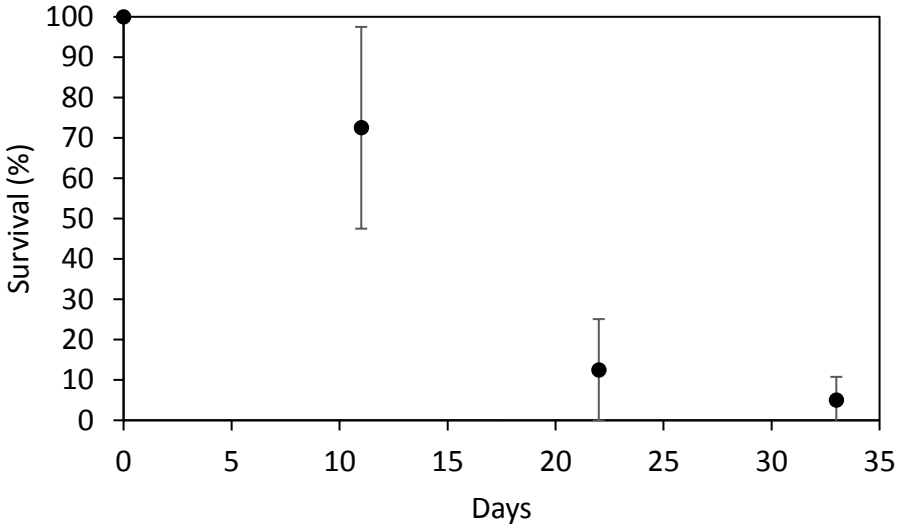

Figure 17: Percentage survival of *A. aquaticus* in the feeding test (mean ± SD). Survival drops clearly after 11 days and keeps decreasing till the end of the test.

Initial BOD concentrations of C1 and A1 were high, followed by a sharp decrease. From then on, concentrations of C1 stayed rather constant and rose slightly at the end. In A1, concentrations

rose and decreased again after 22 and 33 days, respectively. BOD in A1 was approximately the same after 11 days, higher after 22 days and lower after 33 days than in C1. An increasing trend in BOD concentration can be observed for B1 (Figure 18). COD concentrations (Appendix Figure A-2) did not follow the trends of BOD concentrations. They increased in B1, stayed rather constant in C1 and increased until 22 days in A1, yet decreased again after 33 days. BOD and COD concentrations did not differ significantly between C1 and A1 ($p > 0.068$).

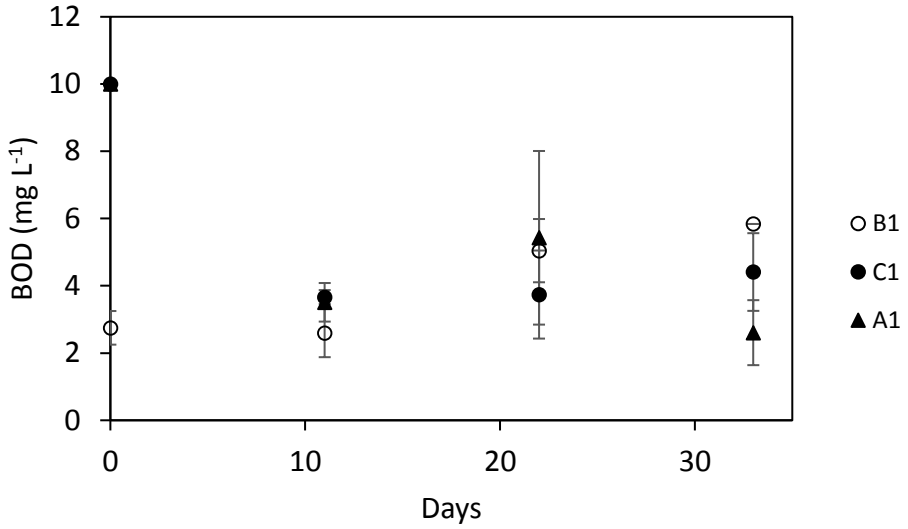

Figure 18: BOD concentrations in B1, C1 and A1 (mean ± SD). Blanks (B1) show a slight increase throughout the test, while controls (C1) seem to stabilize after 11 days. In contrast, a more sinusoidal pattern can be found when Asellidae are present, characterized by a decrease-increase-decrease.

Regarding total nitrogen (TN) concentrations, B1 and C1 show a decrease, while A1 shows a light increase after 11 days, a decrease after 22 days and again an increase after 33 days (Figure 19). Similarly, for total phosphorus (TP), a general decrease can be observed, except for A1, in which the concentration increases again after 33 days (Figure 20). C1 and A1 did not differ significantly in TN and TP concentrations ($p > 0.068$).

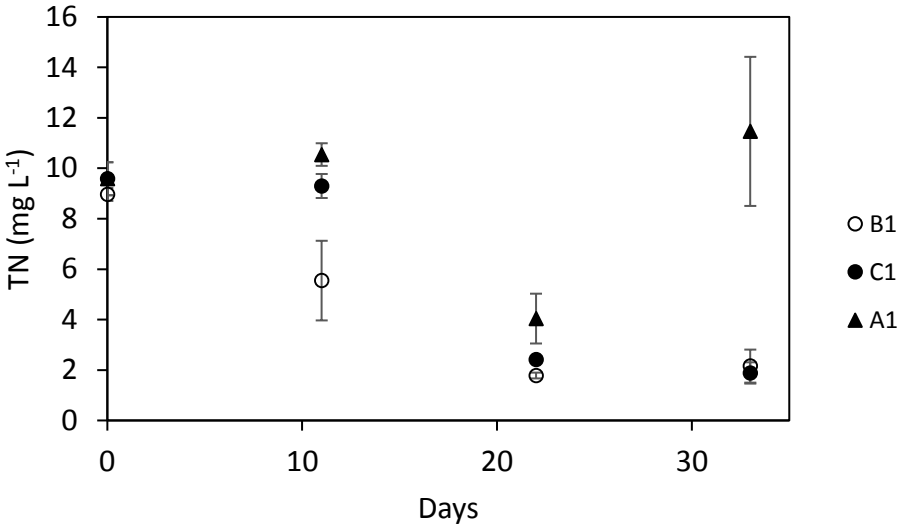

Figure 19: Total nitrogen concentrations in B1, C1 and A1 (mean ± SD). A decreasing pattern can be observed for blanks (B1) and controls (C1), though a sinusoidal pattern, different from the one in Figure 18, can be observed for the Asellidae series, consisting of increase-decrease-increase.

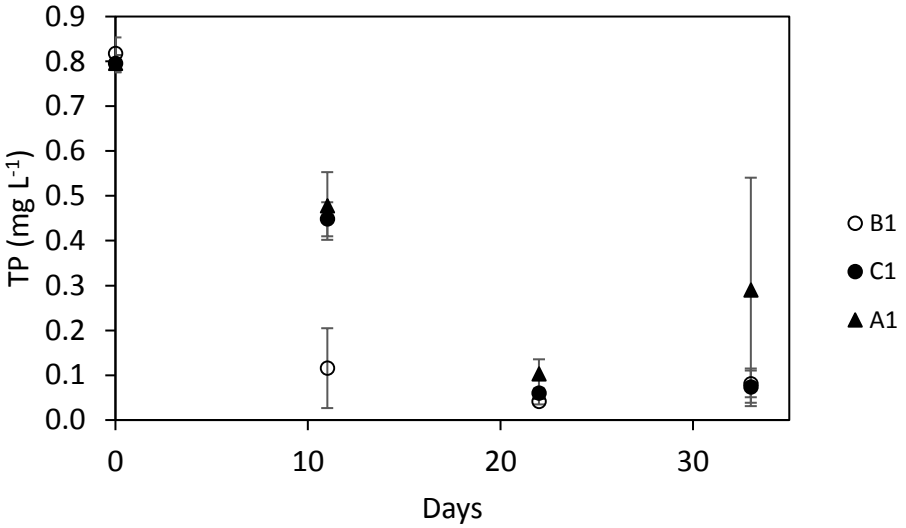

Figure 20: Total phosphorus concentrations in B1, C1 and A1 (mean ± SD). A clear decreasing pattern can be observed for all series, with only an exceptional value at day 33 for the Asellidae series, representing an increase in total phosphorus concentration.

Graphs of average temperatures, DO concentrations, conductivities and pH can be found in Appendix Figure A-3 to A-6. Weights of animals at the end of each testing period are given in Table A-1.

4.3.4 Optimized feeding test

An overall decrease in CPOM is observed for both control (C2) and Asellidae (A2) series, although less pronounced than during the preliminary feeding test (see Figure 15). CPOM lowered after 11 days for both C2 and A2 series (91 ± 5 % versus 77 ± 9 % of initial CPOM) and decreased further between day 11 and day 22 for C2 (to 73 ± 23 %), while it increased again for A2 (up to 93 ± 24 %). After 33 days CPOM was 87 ± 9 % and 73 ± 11 % for C2 and A2, respectively (Figure 21). Values were significantly different between A2 and C2 ($p < 0.01$), being lower in A2 than C2 except for the 22 days period. The remaining CPOM in A2 differed significantly between periods ($p < 0.002$), except between the 11 and 33 days period ($p = 0.221$).

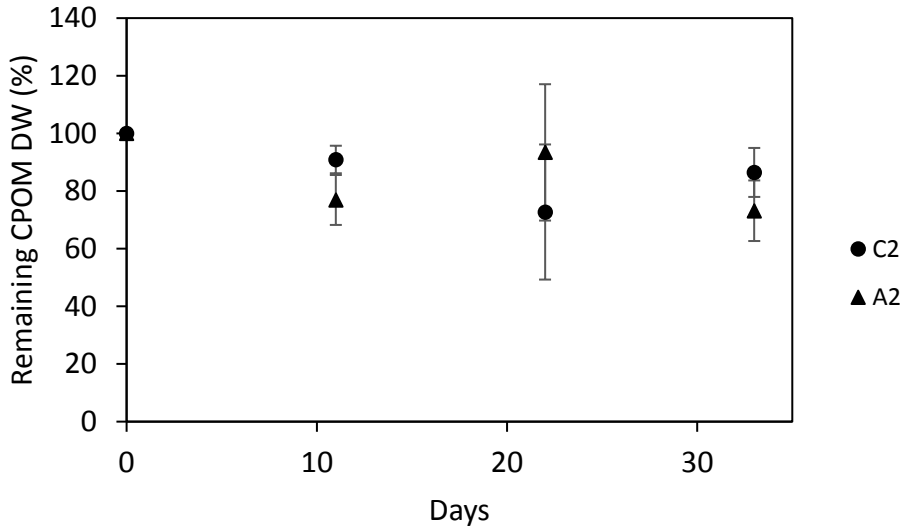

Figure 21: Percentage remaining CPOM in C2 and A2 (mean ± SD). A decrease by day 11 can be observed for both controls (C2) and Asellidae (A2), after which a rocking motion is present, consisting of decrease-increase for C2 and increase-decrease for A2.

Throughout the test, CPOM is degraded and FPOM is produced. Both factors are divided into two different subactivities, being microbial breakdown and consumption by Asellidae for CPOM degradation and microbial FPOM production and production of FPOM by Asellidae for the overall FPOM production. An overview of these activities can be found in Figure 22. The FPOM production by Asellidae consists of feces and comminuted leaves. This is calculated by subtracting the FPOM in the control series (C2) from the FPOM in the Asellidae series (A2). Due to the limited FPOM presence, FPOM after 11 days could not be measured and required the use of filters with a smaller mesh size. FPOM production was always higher in A2 than in C2, and an increasing trend can be observed for both: in C2 and A2, it was 0.5 ± 0.2 mg and 1.0 ±0.9 mg after 22 days and 1.7 ± 0.8 mg and 2.1 ± 1.9 mg after 33 days, respectively. Final FPOM was significantly higher in A2 than in C2 for both periods ($p = 0.000$). Microbial breakdown of CPOM was 0.8 ± 0.4 mg after 11 days, 2.7 ± 2.4 mg after 22 days and 1.6 ± 0.9 mg after 33 days. Consumption of CPOM by Asellidae was respectively 1.3 ± 0.8 mg, -2.0 ± 2.3 mg and 1.1 ± 1.1 mg. These values differed significantly from each other ($p = 0.000$), except for the 11 and 33 days period ($p = 0.465$). The negative value at 22

days means that the leaf squares on average increased in mass. On average, consumption rate was 0.02 ± 0.07 mg ind^{-1} d^{-1}.

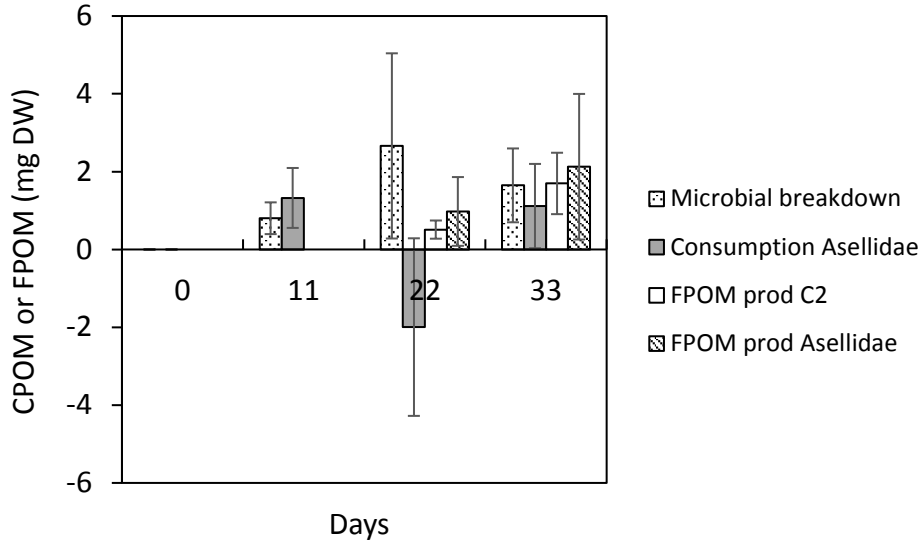

Figure 22: Microbial breakdown of CPOM, consumption of CPOM by Asellidae, FPOM production in C2 and FPOM production by Asellidae in the optimized feeding test (mean ± SD). An increase in FPOM can be observed between day 22 and day 33. The negative value of CPOM consumption by Asellidae makes it hard to infer conclusions related to the CPOM degradation.

Regarding BOD concentrations, B2 and C2 show an increase after 11 days, followed by a decrease. The same trend is observed for A2, besides a slight increase after 33 days. BOD in A2 was approximately the same or slightly higher than in C2 (Figure 23). Approximately the same trends in COD concentrations (Appendix Figure A-8) can be observed as for BOD concentrations. Both concentrations were not significantly different between C2 and A2 for all periods ($p > 0.1$).

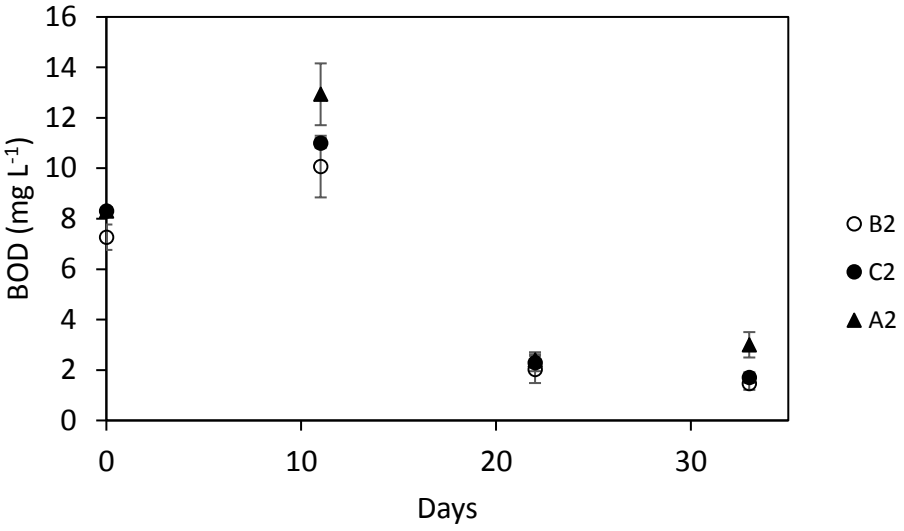

Figure 23: BOD concentrations in B2, C2 and A2 (mean ± SD). At first, after 11 days, an increase in BOD can be observed for all series. After 22 and 33 days, the BOD has already decreased to be lower than the initial BOD and remains rather stable.

Both TN and TP concentrations of A2 are always higher than those of C2. The general trend observed is an increase after 11 days, a decrease after 22 days and again an increase after 33 days. Decreasing TP concentrations can be observed for B2 and C2, while there is a decrease after 11 days, an increase after 22 days and again a decrease after 33 days for A2 (Figure 24 and Figure 25). C2 and A2 did not differ significantly in TN and TP concentrations in all periods ($p > 0.1$).

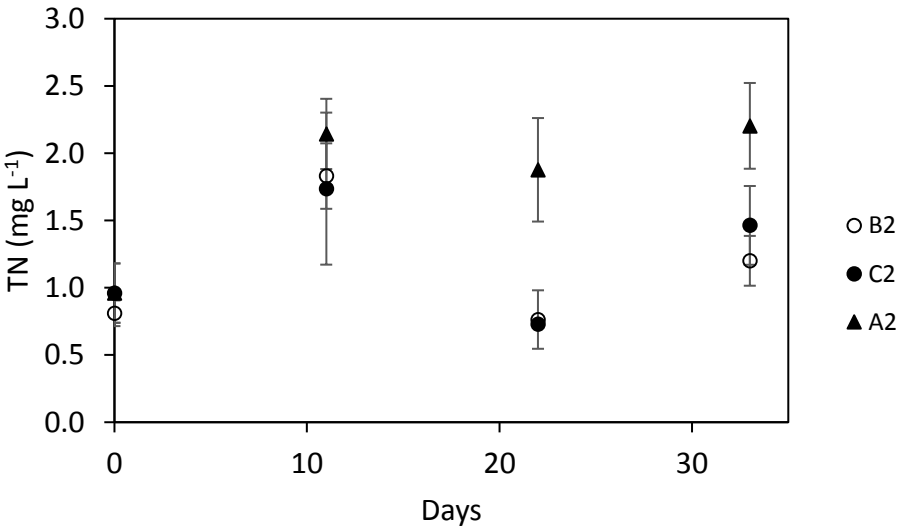

Figure 24: Total nitrogen concentrations in B2, C2 and A2 (mean ± SD). In general, an increase can be observed for all series, with a minor decrease at day 22 for both blanks (B2) and controls (C2), though at the end of the test, higher TN concentrations were obtained compared to the initial concentrations.

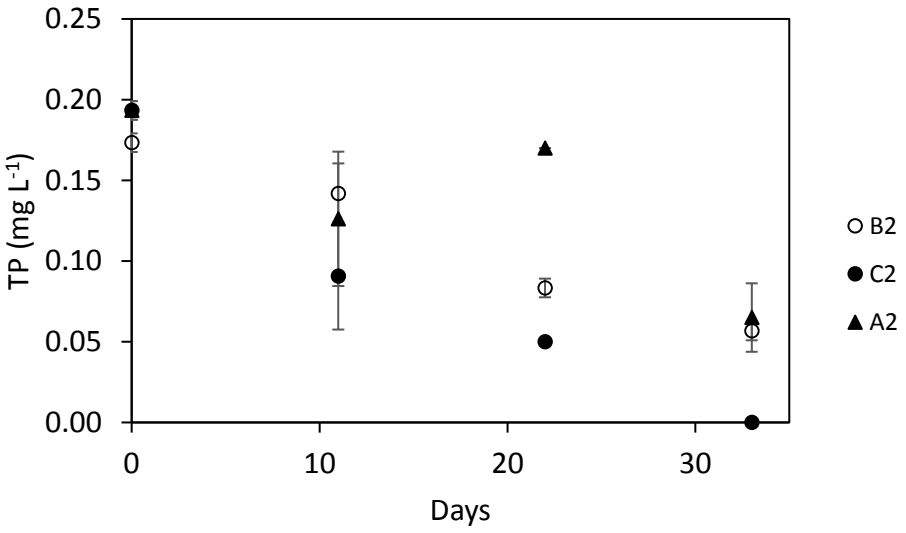

Figure 25: Total phosphorus concentrations in B2, C2 and A2 (mean ± SD). A decrease in TP concentrations is observed for all series, with a clear downward pattern for the controls (C2) and blanks (B2) series. In contrast, an increase in TP can be observed at day 22 for the Asellidae series.

Graphs of average temperatures, DO concentrations, conductivities and pH, as well as the parameters measured in the separate cups every two days can be found in Appendix (Figure A-9 to A-12 and Figure A-14 to A-17, respectively). Values of microbial breakdown, consumption by Asellidae, FPOM production, COD, TN, TP, temperature, DO, conductivity and pH of each separate cup measured after 33 days can be seen in Figure A-18 to A-28 to see the variation between the cups. Weights of animals at the end of each testing period is given in Table A-2.

4.3.6 Comparison both feeding tests

Finally, a comparison is made between consumption of CPOM by Asellidae of the preliminary feeding test and that of the optimized feeding test (Figure 26). Note that consumption in the preliminary feeding test is divided by 10, since initially 10 animals were placed in each A1 weckpot. However, biased results could be encountered in this test as the mortality of animals was not controlled. Thus care should be taken when interpreting them. Results indicate that consumption was (not significanty, p = 0.262) higher in the optimized test after 11 days, yet significantly lower after 22 and 33 days (p = 0.010 and 0.003, respectively). Overall consumption rates did not differ significantly between both tests (p = 0.066).

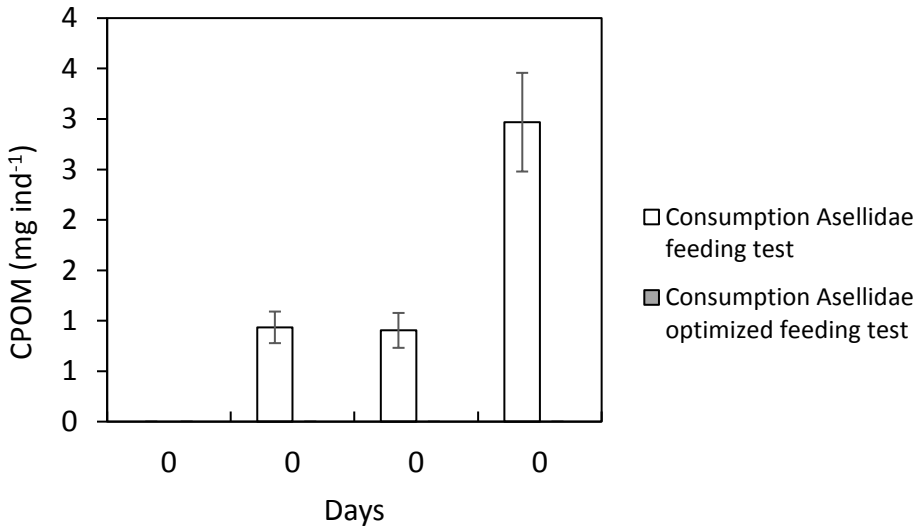

Figure 26: Comparison consumption by Asellidae between preliminary and optimized feeding test (mean ± SD). CPOM removal in the preliminary feeding test remained stable during the first three weeks, and only increased between day 22 and day 33. In contrast, a different pattern was observed for CPOM removal during the optimized feeding test, with an increase in CPOM between day 11 and day 22.

4.4 Overview

A. aquaticus consumed reed leafs (CPOM) at a rate of 0.07 ± 0.01 mg ind^{-1} d^{-1} and 0.02 ± 0.07 mg ind^{-1} d^{-1} in the preliminary and optimized feeding test, respectively. CPOM reduction was significantly higher in the Asellidae series compared to the control series in the optimized feeding test ($p < 0.01$). BOD in A1 was approximately the same after 11 days, higher after 22 days and lower after 33 days than in C1, and that in A2 was approximately the same or slightly higher than in C2. However, in both feeding tests BOD, COD, TN and TP concentrations were not significantly different between C and A ($p > 0.068$ and $p > 0.1$ for the preliminary and optimized feeding test, respectively).

5 Discussion

In this chapter, the acclimatisation test will be discussed in section 5.1, followed by the recovery of CPOM after the conditioning period for both feeding tests in section 5.2. Section 5.3 represents microbial breakdown and consumption of CPOM by Asellidae. Finally, section 5.4 discusses about COD, BOD and nutrient concentrations. Each section comprises both feeding tests.

5.1 Acclimatization test

Performing conditions of the CW at Sint-Martens-Latem are low, thus influent and effluent concentrations are relatively similar. Counterintuitive, the survival of *Asellus aquaticus* is higher in pure influent water than in pure effluent water. In the acclimatization test, no distinction can be made regarding higher survival in influent or effluent water. The low survival of animals towards the end of the acclimatization tests can be explained by increasing conductivities as more organic matter is degraded and thus more ions are produced: organic C is converted to CO_2, organic N and P are converted to NH_4^+, NO_3^- and PO_4^{3-}. In addition, evaporation of some water during the tests can cause rising conductivities. Overall, the high survival in pure influent water suggest there is a possibility for *A. aquaticus* to colonize such an ecosystem.

Mortality was mainly higher in a study of Plahuta *et al.* (2017) investigating impacts of influent and effluent water from WWTPs treating domestic and industrial wastewater on juvenile *A. aquaticus*: mortality after 96h (i.e. four days) was 95-100 % in pure influent waters and less than 20 % in pure effluent waters. This difference can be explained by the fact that the applied wastewaters of Plahuta *et al.* (2017) have a higher strength than the wastewater applied during the abovementioned acclimatization test and the potentially more efficient performance of the studied WWTPs. However, when adding sediment, mortality decreased by 42-50 % in influent waters, probably because pollutants adsorbed to the sediment and thus became less bio-available for the animals. Median lethal concentration (96h LC50) was approximately 65 vol % for influents without added sediment, and 70 vol % with added sediment. Interestingly, feeding rate and growth rate on conditioned alder leaf discs increased when animals were exposed to increasing (sublethal) concentrations of WWTP water. This can be explained by bigger amounts of micro-organisms and fungi to colonize the leaves when the water gets more concentrated, making them more nutritionally attractive to the animals.

5.2 CPOM recovery and mass losses during the tests

The variability of fraction recovery of leaf CPOM is much bigger in the optimized feeding test, probably because microbial activity had much more time to influence leaf biomass (two weeks vs. one night). Results show that mass can both be lost and gained. Mass is lost from the leaf due to loss of soluble substances and microbial breakdown. The more lignin a leaf contains, the slower will be the mass loss by microbes since this is the most recalcitrant plant constituent (Dinka *et al.*, 2004). On the other hand, mass is gained by growth of microbial biofilm. Thus an increase of the leaf mass means the microbial biofilm overcompensates the losses. But in general it can be said that there is a decrease in mass during the conditioning period.

The biggest mass loss encountered in C1 is 30.4 ± 1.1 %, 51.6 ± 6.9 % in A1 (by 10 animals), 27.3 ± 23.4 % in C2 and 26.8 ± 10.5 % in A2 (by one animal). The values of the preliminary feeding test are much bigger than the 10 % mass loss both in small- and large size litter bags found by Hietz (1992) in the first month after submersion in a lake. However, the large standard deviations in the optimized feeding test make it difficult to draw conclusions. Temperatures in the lake were highly likely to be lower than the 20°C in this study, resulting in a lower microbial and invertebrate activity. In addition, no data regarding macroinvertebrate densities were presented in that study, so possibly shredders were not abundant. Results are however more in line with the study of Alemanno *et al.* (2007), where mass loss of reed leaf litter in large-size litter bags was 56.5 ± 2.8 % after 56 days of submersion in a Mediterranean brackish lagoon.

5.3 Microbial breakdown and consumption by Asellidae

In both feeding tests, microbial breakdown was low when consumption by Asellidae was high, and vice versa. This is explained by the fact that *A. aquaticus* also feeds on the microbiological community living on the leafs. When activity of *A. aquaticus* is lower, microbial biofilm has the chance to develop. The fact that consumption of CPOM stayed rather constant after 11 and 22 days in the preliminary feeding test suggests that the activity of animals in the 22 days weckpots was low between 11 and 22 days. On the other hand, the large consumption between 22 and 33 days suggests that animals in the 33 days weckpots were more active compared to previous periods. In the optimized feeding test, results of consumption suggest that activity of Asellidae was highest in the 11 days period, very low in the 22 days period and moderate in the 33 days period. Consumption of leaf CPOM by Asellidae was expected to be higher in the optimized feeding test, since dead animals were replaced every 2 days. This was only true for the 11 days period though. In subsequent periods, consumption was higher in the preliminary feeding test, suggesting a lower activity of the animals in the optimized feeding test. The algae inhibitor and the synthetic water could play a role in this. To illustrate, 39 % of the animals were not replaced in the first 11 days, 20 % in the 22 days period and only 3 % (i.e. 1 animal) in the 33 days period. The effect of the algae inhibitor can be seen by comparing the percentage remaining CPOM in the A2 cups with and without inhibitor (Appendix Figure A-13). Values were significantly lower in the cups without inhibitor in the 22 days period ($p = 0.043$) and approximately the same in the 33 days period ($p = 0.345$). This suggests the algae inhibitor could cause *A. aquaticus* to be less active.

Values of reed leaf consumption by Asellidae in this study (0.07 and 0.02 mg ind^{-1} d^{-1} for preliminary and optimized feeding test, respectively) are below the elm leaf consumption of 0.12 mg ind^{-1} d^{-1} found by Graca *et al.* (1993b). Yet Rossi and Vitagliano-Tadini (1978) also found a *Platanus* leaf consumption of 0.07 mg ind^{-1} d^{-1}, though by *A. aquaticus* larvae. To be able to compare values expressed in mg mg $animal^{-1}$ d^{-1} in literature, values of this study were divided by average weight of animals at the end of each testing period (Appendix Table A-1 and A-2). This gave a consumption of 0.02 and 0.002 in mg mg $animal^{-1}$ d^{-1}, respectively. This is well below elm leaf consumption of 0.26 mg mg $animal^{-1}$ d^{-1} found by Graca *et al.* (1993b) and alder and oak leaf consumption of 0.038 mg mg $animal^{-1}$ d^{-1} found by Bjelke and Herrmann (2005). The lower consumption rate of reed leaves suggests that those type of leafs are less palatable for them, e.g. due to the high lignin content. To illustrate, changes in chemical composition of *P. australis* litter

during decomposition in lakes are shown in Table 9 to Table 11. In general, results vary among studies, but all found a net N mineralization in leaf litter since N content decreased during decomposition. This results in less nutritional value of the leaves for shredders. N concentrations on the other hand increased in leaves due to mass loss. Regarding fiber content, lignin content decreased slowest since this is the most recalcitrant plant constituent.

Table 9: N and P content and concentration of *P. australis* litter during decomposition in lakes

Leaves	**N**	**P**
Content	Decrease[2,3,4,5]	Increase[3]
(% of original stock)	5 to 10 % lost after first month[2]	First increase, then decrease by
	66 % lost after 628 days[4]	57 % after 628 days[4]
	87–79 % lost after 990 days[5]	40-60 % lost after first month, then increase by 100-300 % after 863 days[2]
		96–86 % lost after 990 days[5]
Concentration	Initial:	Initial:
(% of DW)	1.5 ± 0.2 %[1]	0.091 ± 0.035 %[2]
	1.03 ±0.11 %[2]	0.085 ± 0.009 %[3]
	2.17 ± 0.08 %[3]	0.117 %[5]
	1.19 %[5]	Then increase[3]
	Then increase[2,3,4,5]	Decrease to 0.035 % after 990 days[5]
	2 % after 863 days[2]	
	3.7 % after 185 days[3]	
	1.95 % after 990 days[5]	
Culms	**N**	**P**
Content	Increase[3]	Increase[3]
(% of original stock)	First decrease, then increase by	First decrease, then increase by
	19 % after 628 days[4]	67 % after 628 days[4]
	First increase by 20 %, then decrease by 40 % after 990 days[5]	60–80 % lost after 990 days[5]
Concentration	Initial:	Initial:
(% of DW)	0.5 ± 0.0 %[1]	0.041 ± 0.006 %[2]
	0.37 ± 0.06 %[2]	0.013 ± 0.003 %[3]
	0.24 ± 0.03 %[3]	0.032 %[5]
	0.24 %[5]	Then increase[3]
	Then increase[3,5]	Decrease to 0.016 % after 990 days[5]
	1.7 % after 550 days[3]	
	0.42 % after 990 days[5]	

1: Patuzzi *et al.* (2013), 2: Hietz (1992), 3: Gessner (2000), 4: Szabó and Dinka (2008), 5: Dinka *et al.* (2004)

Table 10: C:N ratio of *P. australis* litter during decomposition in lakes

Leaves	**Culms**
Decrease[1,3]	Decrease[1,3]
First increase from 37 to 45, then decrease to 20 after 628 days[2]	First increase from 204 to 355, then decrease to 57 after 628 days[2]
Decrease from 40 to 24 after 990 days[3]	Decrease from 199 to 117 after 990 days[3]

1: Gessner (2000), 2: Szabó and Dinka (2008), 3: Dinka *et al.* (2004)

Table 11: Fiber content of *P. australis* litter during decomposition in lakes

Leaves	**Lignin**	**Hemicellulose**	**Cellulose**
Content (% of original stock)	Decrease by 64 % after 628 days[1] Decrease by 70–73 % after 990 days[2]	Decrease by 90 % after 628 days[1] Decrease[2]	Decrease by 80 % after 628 days[1] Decrease[2]
Concentration (% of DW)	Initial: 10 %[2] Then increase to 26 % after 990 days[2]	Initial: 34 %[2] Then decrease to 25-33 % after 990 days[2]	Initial: 28 %[2] Then increase to 34 % or decrease to 23 % after 990 days[2]
Culms	**Lignin**	**Hemicellulose**	**Cellulose**
Content (% of original stock)	Decrease by 11 % after 628 days[1] No notable loss[2]	Decrease by 60 % after 628 days[1] Decrease[2]	Decrease by 70 % after 628 days[1] Decrease[2]
Concentration (% of DW)	Initial: 9 %[2] Then increase to 18 % after 990 days[2]	Initial: 31 %[2] Then decrease to 27-29 % after 990 days[2]	Initial: 48 %[2] Then increase to 55-56 % after 990 days[2]

1: Szabó and Dinka (2008), 2: Dinka *et al.* (2004)

The observed degradation rates allow to make an extrapolation towards field conditions. For instance, in a hypothetical CW of 200 m² treating domestic wastewater with an above ground reed biomass of 1 kg DW m^{-2} in summer (Allirand and Gosse, 1995; Graneli, 1990) and leaf to shoot dry weight ratio of 0.25 (Asaeda and Karunaratne, 2000; Graneli, 1990), the aerial leaf biomass would be 50 kg DW. Based on the results of this study, average reed consumption by *A. aquaticus* is 0.07 mg ind^{-1} d^{-1} (not taking into account the negative value in the optimized feeding test). Assuming an animal density of 1000-2000 ind m^{-2} (Mason and Bryant, 1975; Ellis, 1961), consumption of reed leaf litter by Asellidae would be 2.5-5.1 kg DW, which is 5-10 % of the total leaf litter if all leafs would shed.

However, reed consumption of Asellidae in pure wetland water should be investigated, since activity might be lower due to higher nutrient concentrations. In addition, this should be done at different, more realistic temperatures. This study was done at 20°C, but consumption will probably be lower at e.g. 5 or 10°C.

5.5 FPOM production

In this section, only FPOM production from the optimized feeding test will be discussed. In the preliminary test, FPOM was also added as food source together with CPOM, however no discrimination could be made between initially present FPOM, feces of animals and FPOM coming from CPOM. Thus those results will not be discussed. In the optimized test on the other hand, no FPOM was added as food source, so FPOM production consisting of feces and comminuted leafs could be determined to have more insight in the fate of the CPOM. Results are not easily interpreted though, since some algae growth could be observed.

Cups containing leaf squares in the optimized test still showed some algae growth, despite the algae inhibitor. However, visual comparison with cups not containing the algae inhibitor showed much more algal biomass in the latter cups. Nevertheless, results of FPOM production are biased because of this. An attempt to take algae into account was done by giving classes to C2 and A2 cups based on (subjective) visual examination. Then the FPOM in A2 was subtracted by the average of the relevant class in C2. The results are shown in Figure 27. Now, FPOM production in A2 was 0.9 ± 0.7 mg compared to 1.0 ±0.9 mg after 22 days and 1.7 ± 1.4 mg compared to 2.1 ± 1.9 mg after 33 days. The fact that FPOM production is higher than consumption by the animals suggests that the FPOM mainly consists of comminuted leaf CPOM, and to a much lesser extent of feces. Again, care should be taken to interpret the results due to algae growth.

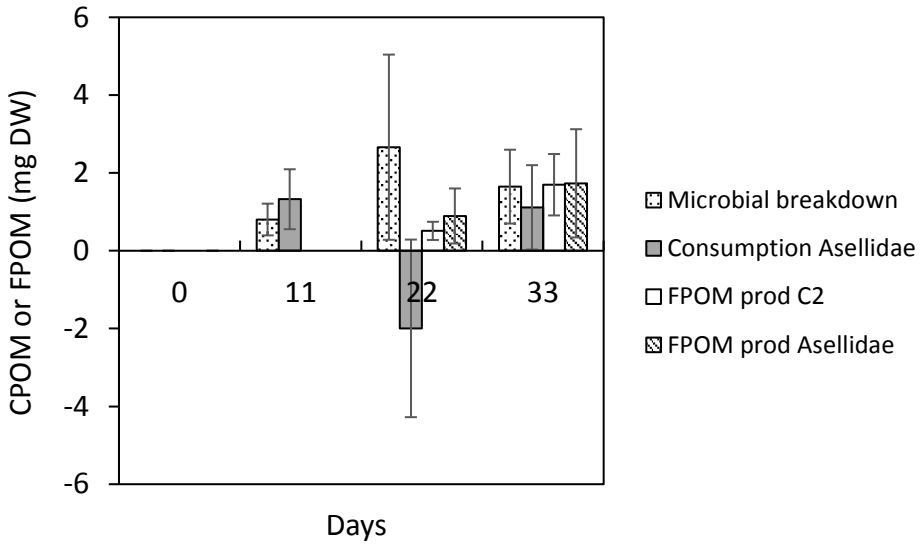

Figure 27: Microbial breakdown of CPOM, consumption of CPOM by Asellidae, FPOM production in C2 and FPOM production by Asellidae, taking algae classes into account in the optimized feeding test (mean ± SD). More FPOM is produced than CPOM is consumed.

5.7 COD, BOD and nutrient concentrations

Initial values of BOD in the preliminary feeding test were high for C1 and A1 due to hydrolysis of substances from the leaves and microbial activity during the one night leaching period. Then it fell back due to high microbial activity breaking down BOD. After that, BOD increased again in A1, explained by high activity of Asellidae, excreting carbon and producing FPOM which is broken down by microbes. At the end, BOD was lower in A1 than in C1, which suggests the high consumption causes BOD concentrations to drop; if less leaf biomass is present, less soluble substances can be released by microbial activity.
In contrast to the preliminary feeding test, BOD concentrations in the optimized feeding test increased after 11 days. This suggests initial microbial activity released soluble organic matter from the leaves. Subsequent low BOD concentrations suggest microbes have used most of the BOD in the water. BOD in A2 was always equal to or higher than C2 (although not significant), which can be explained by excretion by the animals and microbial colonization of FPOM production by shredding, releasing soluble BOD in the water.

TP and TN concentrations in Asellidae and control series were initially approximately the same as in blanks for both feeding tests, suggesting there was only leaching of organic matter, not of nutrients. The fact that nutrient concentrations in Asellidae series are always higher than in control series (although not significant) can be explained by excretion of nutrients by the animals and/or more rapid microbial degradation of organic matter: by shredding the leaf CPOM, the animals produce leaf FPOM which has a bigger specific surface area for microbial colonization. In the preliminary feeding test, the decrease in nutrient concentrations is probably due to the uptake by algae (and microbes). No further decrease in B1 and C1 at the end might be because some algae died and released nutrients back in the water. Increase of nutrients in A1 at the end can be explained by high activity of Asellidae, excreting nutrients and producing FPOM which is broken down microbially. In the optimized feeding test, there was no algae growth in B2 (this does not count for the preliminary feeding test). Thus lower nutrient values in A2 and C2 than B2 suggest algae (and microbes) took up nutrients for growth. Strangely, TP and TN values in A2 followed a reverse trend: when TN increased, TP decreased and vice versa. No clear explanation could be found for these contradicting patterns.

5.9 Observations versus simulations

Besides the performed experiments, the observed results and the extrapolations to field conditions, simplified ecosystem models can be developed to estimate the influence of *Asellus aquaticus* on the CPOM content within a wetland. To illustrate this, a simplified ecosystem model was developed of which the conceptual diagram, equations and parameter values can be found in Appendix section 12.3. Only maximum ingestion rate of leaf CPOM by Asellidae (d^{-1}) could be derived from the feeding tests, other parameters were found in literature or based on assumptions. However, some data are lacking specifically for *A. aquaticus*. In these cases, more general values for benthic macroinvertebrates from existing models such as PCLake have been used.

In the figures presented below, a comparison is made between a value for maximum ingestion rate by Asellidae from literature (0.33 d^{-1}) and from the one reported in this study (0.02 d^{-1}). Furthermore, simulations if animals are not present are shown. Conditions for the simulations were the same as in the optimized feeding test, i.e. 1 animal, an initial leaf CPOM of 9.8 mg DW and an initial microbial density of 12.5 mg m^{-2} was assumed. Figure 28 presents simulated and observed leaf CPOM degradation during a 33 days period. It can be seen that the observed A2 values after 22 and 33 days are situated in between the simulated values, and that CPOM decreases faster when animals are present. In Figure 29, the observed values represent the average BOD values from the optimized feeding test. Simulated dissolved organic matter (DOM) values when animals are present also first increase and then decrease, the latter due to rising microbial biomass (Appendix Figure A-29). DOM does not show an increase at first when animals are absent, but ends at approximately the same value as when animals are present. Simulated *A. aquaticus* biomasses decrease and detritus concentrations increase faster when animals are present due to mortality of animals as can be seen in Figure A-30 and A-31, respectively.

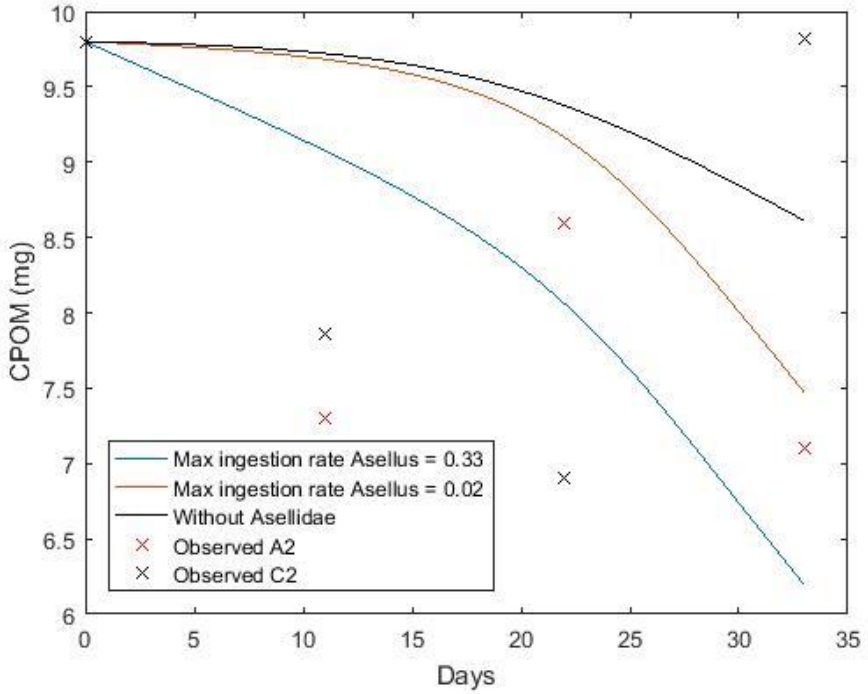

Figure 28: Simulations and observed values of leaf CPOM in a 33 days period. CPOM content decreases faster when *A. aquaticus* is present, with a faster decrease when the maximum ingestion rate equals 0.33 d^{-1}. Prediction of the observed CPOM content by the developed model is not accurate.

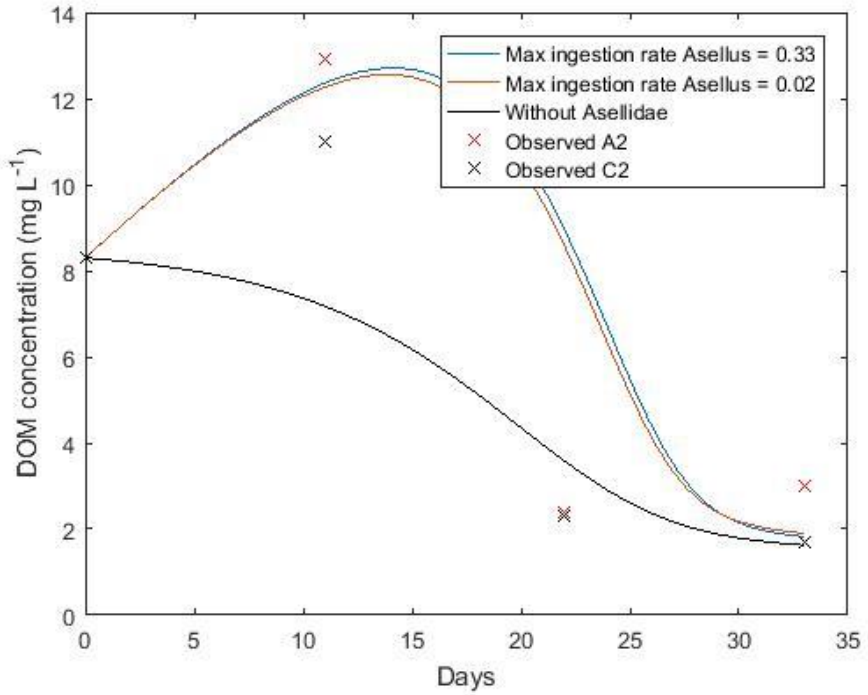

Figure 29: Simulations and observed values of dissolved organic matter (DOM) in a 33 days period. A clear increase in DOM can be observed after 11 days when *A. aquaticus* is present, followed by a decrease towards day 22 and day 33. Observed DOM content of controls (C2) and Asellidae series (A2) at day 11 and day 33 are close to the predicted concentrations.

Appendix Figures A-32 to A-36 show simulations for 1 year in more realistic conditions, i.e. initial densities of 2000 animals m^{-2} (Mason and Bryant, 1975), 0.25 kg m^{-2} leaf litter (Allirand and Gosse, 1995; Graneli, 1990) and 1.25 g m^{-2} micro-organisms (Moran *et al.*, 1988), and a temperature following a sine function with higher and lower temperatures in summer and winter, respectively. A surface area of 200 m^2 was considered. It can be seen that under these conditions, leaf CPOM falls back very rapidly to zero after approximately 65-70 days. Animals only show an added value with a maximum ingestion rate of 0.33 d^{-1}. DOM concentrations in the presence of animals first decrease and then increase again, more rapidly for the maximum ingestion rate of 0.33 d^{-1}. However, when animals are not present, values stay low after they decreased. Microbial biomasses show one large peak (highest for maximum ingestion rate of 0.02 d^{-1} and when animals are not present), causing the decrease in DOM and leaf CPOM. The *A. aquaticus* population with a maximum ingestion rate of 0.33 d^{-1} shows a peak, after which it falls back to the initial biomass. However, the population with a maximum ingestion rate of 0.02 d^{-1} declines. Thus, if the population is to be maintained, a higher rate than 0.02 d^{-1} is necessary. Detritus concentrations increase rapidly, which can be explained by the die-off of *A. aquaticus* and micro-organisms. End values do not differ considerably between the three simulated scenarios. In general, when animals are present, leaf CPOM is lower and DOM is higher. The latter does not necessarily mean that effluent BOD will be higher, since this is an increased food resource for, for instance, denitrifying bacteria.

These types of models are interesting for CW management and decision making to simulate plant litter and effluent BOD given a certain reed stand and *A. aquaticus* density. In addition, the impact of inoculating CWs with *A. aquaticus* on reed leaf organic matter, or the impact of harvesting the reed stand on organic matter in the water and microbial biomass can be simulated. Harvesting namely removes BOD from the system, yet also organic matter and nutrients to fuel microbial processes (Yang *et al.*, 2016). However, further research is needed to quantify missing parameters for *A. aquaticus*, e.g. ingestion rate of micro-organisms by Asellidae and half saturation constant for ingestion of leafs.

6 Conclusion

Available studies on macroinvertebrate presence in CWs for wastewater treatment indicate that shredders are not abundantly present in these ecosystems. Given the fact that CWs receive autochthonous plant litter every year, introducing shredder species could enhance decomposition processes of organic matter. This would be beneficial in CWs treating domestic wastewater, since most of the BOD in the effluent originates from the decay of plant litter and not from the wastewater entering the wetland. In addition, species from other functional feeding groups, for instance collectors, could thrive on the activity of these animals as well.

The 80% survival of the freshwater isopod *A. aquaticus* in pure influent water from a CW treating domestic wastewater suggests that there is potential for this animal to colonize such ecosystems. Moreover, the feeding tests on reed leaf CPOM (*Phragmites australis*, the macrophyte of choice in most CWs) indicated that *A. aquaticus* can significantly reduce the leaf biomass compared to controls ($p < 0.01$) with consumption rates of 0.07 ± 0.01 mg ind^{-1} d^{-1} and 0.02 ± 0.07 mg ind^{-1} d^{-1} in the preliminary and optimized feeding test, respectively. Although these values are generally smaller than consumption rates encountered for other leaf types, probably due to the high lignin content and low nutritional value of reed leaves, *A. aquaticus* could still reduce reed leaf litter by 5-10 % when present in a density of 1000-2000 ind m^{-2} in CWs. Additionally, FPOM production rates of 0.04-0.06 mg ind^{-1} d^{-1} were found, including comminuted plant material and feces, but biased by algae growth. In contrast to CPOM, the BOD, COD, TN and TP concentrations were not significantly different when animals were present ($p > 0.068$ and $p > 0.1$ for the preliminary and optimized feeding test, respectively). Variations can be explained by microbial activity, excretions by animals, and more rapid microbial breakdown of particulate organic matter due to FPOM production by the shredding activity of the animals. Simulations with a simplified ecosystem model indicated that leaf CPOM is lower and DOM is higher in the presence of *A. aquaticus*, compared to the absence of the animals. The latter does not necessarily mean that BOD in the effluent will be higher, since this can be considered as an increased food resource for, for instance, denitrifying bacteria.

To conclude, there is potential for *A. aquaticus* to live in CWs treating domestic wastewater and slightly improve the removal efficiency of CPOM in these natural systems. Yet, further research is needed in order to know all the impacts of *A. aquaticus* presence with respect to wetland processes and effluent quality parameters.

7 Further recommendations

Acclimatization tests with water from different CWs treating domestic wastewater and chemical characterization of the water at different points in time during the test will result in better understanding of survival of *A. aquaticus* in this type of water and what factors cause them to die. Inoculating *A. aquaticus* in real CWs treating domestic wastewater will elucidate whether or not these animals can survive in these types of ecosystems and water conditions. In fact, the survival test of *A. aquaticus* should be considered not only in CWs treating domestic wastewater but also in others. Similarly, the same can be done at different ponds comprising the wetlands where major removal or degradation of organic matter is needed.

Suggestions for improving the optimized feeding test include examining how to prevent algae growth in the cups, or determining chlorophyll a to quantify algae growth. If an algae inhibitor is used, it is advisable to also add controls without algae inhibitor to know the effect of the inhibitor on microbial breakdown. In addition, discrimination between feces and FPOM from leaves would make it possible to have insight in the whole mass balance and have better approximate model predictions. Furthermore, initial DW of *A. aquaticus* could be determined by measuring initial wet weight (WW) and a DW/WW ratio. In this way, growth of animals can be determined. Lastly, the test should be repeated with water from a CW treating domestic wastewater at cold and warm temperatures to simulate seasonality.

To further simulate real conditions, lab-scale SF CWs treating domestic wastewater with and without Asellidae could be put up to quantify the influence of *A. aquaticus* on reed litter decomposition, BOD, COD, nutrient concentrations and suspended solids in the water. The same tests could be performed with other shredder species such as some aquatic beetles, both based on feeding behavior and resilience to CW conditions. The combined effect of shredders and collectors that consume FPOM produced by the shredders would be interesting to investigate as well. The overall effect of present macroinvertebrates on reed litter decomposition in CWs could be investigated using small- and large-size litter bags as is done already in lake ecosystems (Mason and Bryant, 1975; Hietz, 1992). It can then be tested in lab-scale CWs containing these species if adding *A. aquaticus* enhances organic matter degradation.

Animals that significantly impact organic matter degradation can then be incorporated in models to simulate the fate of organic matter of plant litter in CWs. Nowadays, most models for lakes and marshes do not go deep in macroinvertebrate dynamics and just take general values for the whole macrobenthos group. In fact, different species can differ substantially in their impact. Thus modelling the effect of different animals separately and their joint impact on organic matter in CWs is not only interesting in the field of ecology, but also in CW management and decision making. For example, in early stages of a CW when colonization of animals is not that substantial yet, introducing some, such as *A. aquaticus*, could enhance cycling of organic matter and nutrients to fuel microbial processes.

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9 Appendix

9.1 Preliminary feeding test

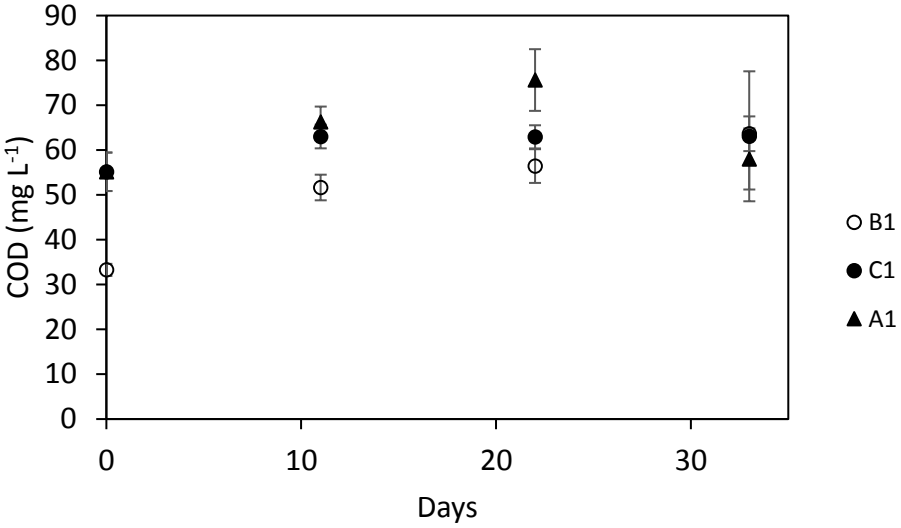


Figure A-1: COD concentrations in B1, C1 and A1 (mean ± SD)

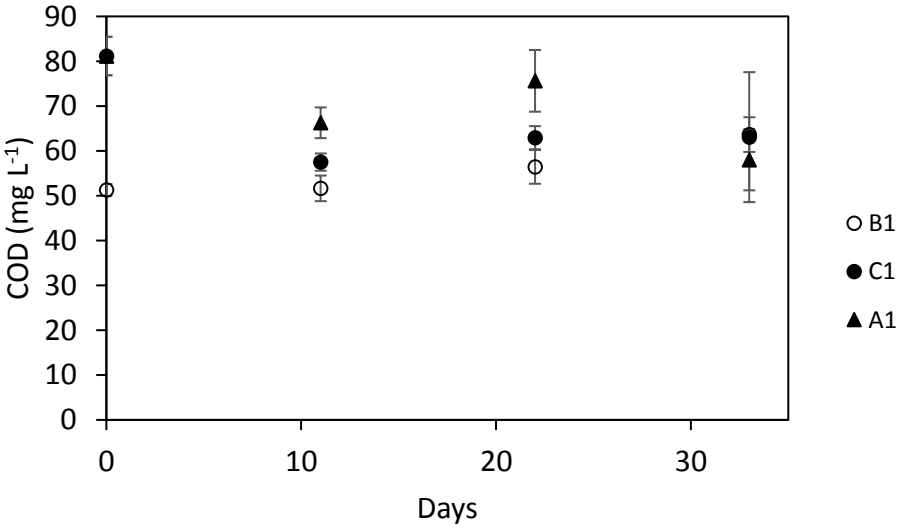


Figure A-2: Corrected COD concentrations in B1, C1 and A1 (mean ± SD)

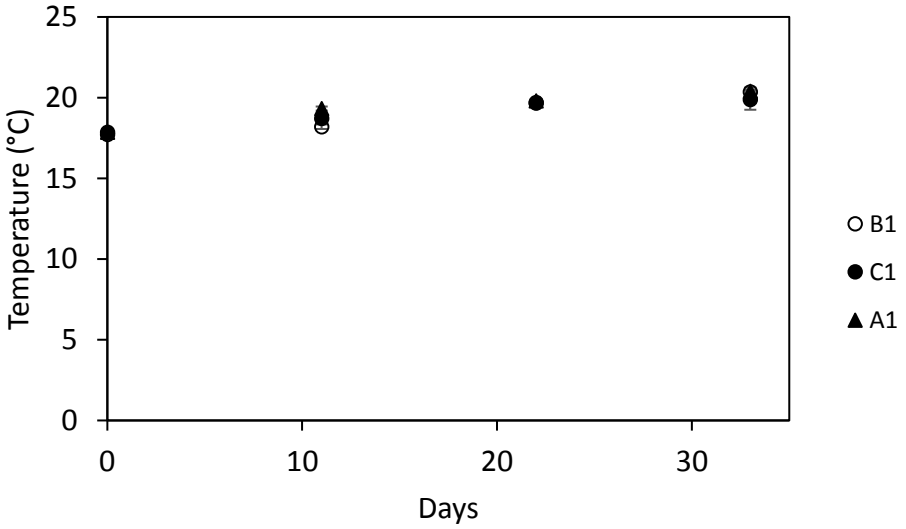


Figure A-3: Temperatures in B1, C1 and A1 (mean ± SD)

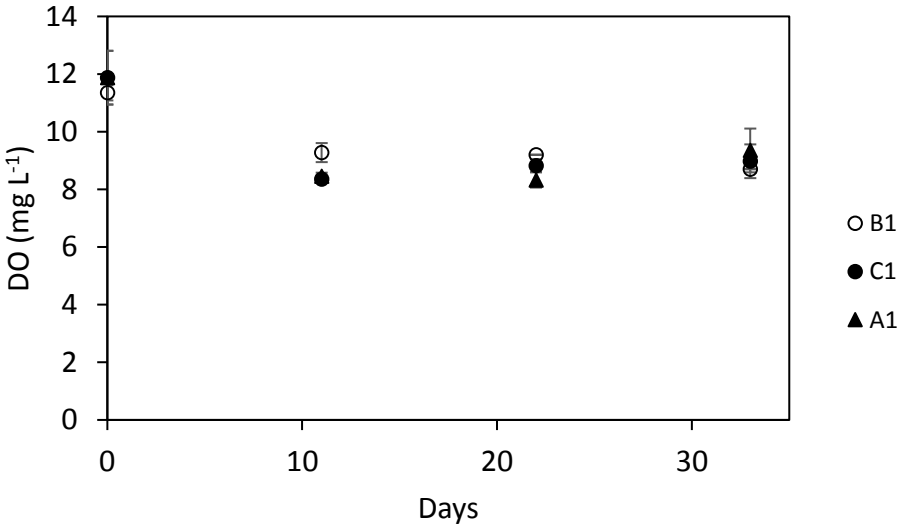


Figure A-4: Dissolved oxygen concentrations in B1, C1 and A1 (mean ± SD)

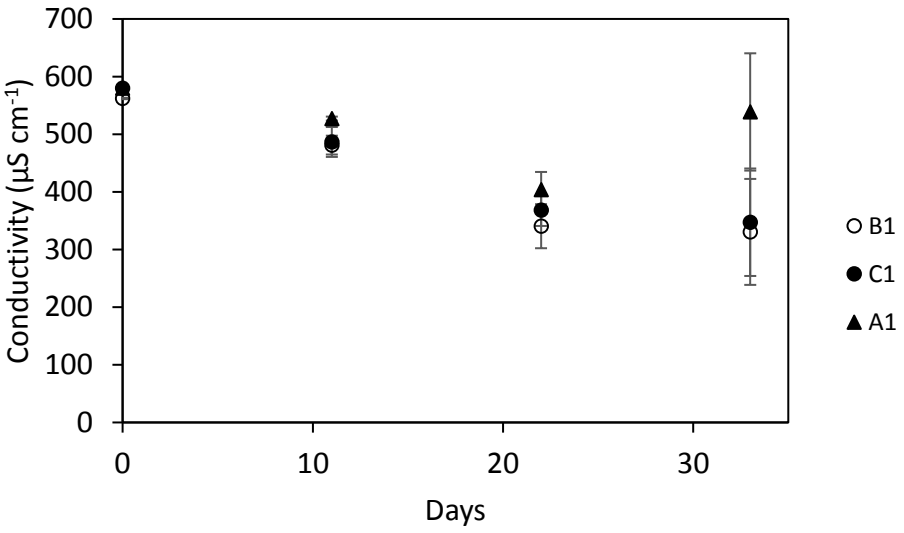


Figure A-5: Conductivities in B1, C1 and A1 (mean ± SD)

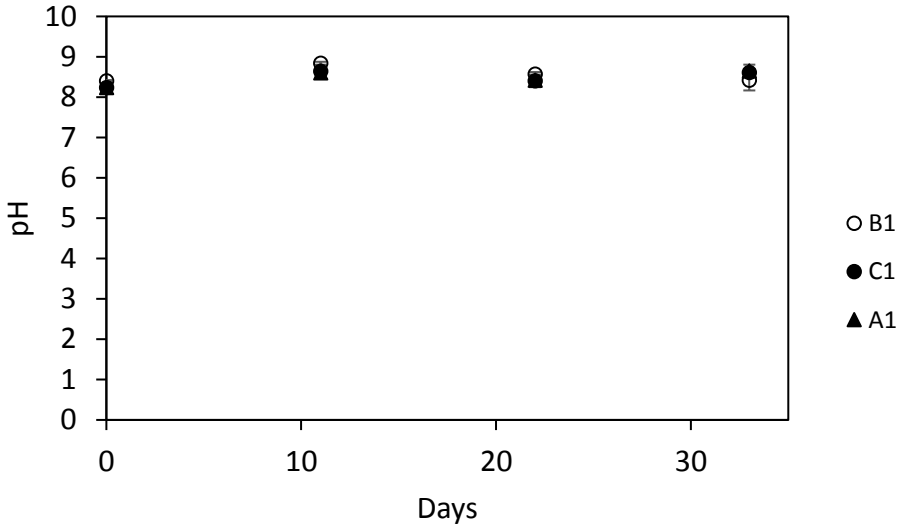


Figure A-6: pH in B1, C1 and A1 (mean ± SD)

Table A-1: Average weight of 1 *A. aquaticus* in A1 at the end of each testing period in the preliminary feeding test

Testing period	Average weight 1 *A. aquaticus* (mg DW)
After 11 days	
Replicate 1	3.1
Replicate 2	4.9
Replicate 3	3.7
Replicate 4	3.7
Average 11 days	**3.9 ± 0.8**
After 22 days	
Replicate 1	4.2
Replicate 2	5.0
Replicate 3	4.8
Replicate 4	5.7
Average 22 days	**4.9 ± 0.6**
After 33 days	
Replicate 1	No animals present anymore
Replicate 2	3.3
Replicate 3	1.9
Replicate 4	3.5
Average 33 days	**2.9 ± 0.9**

9.2 Optimized feeding test

9.2.1 Analysis of B2, C2 and A2 cups

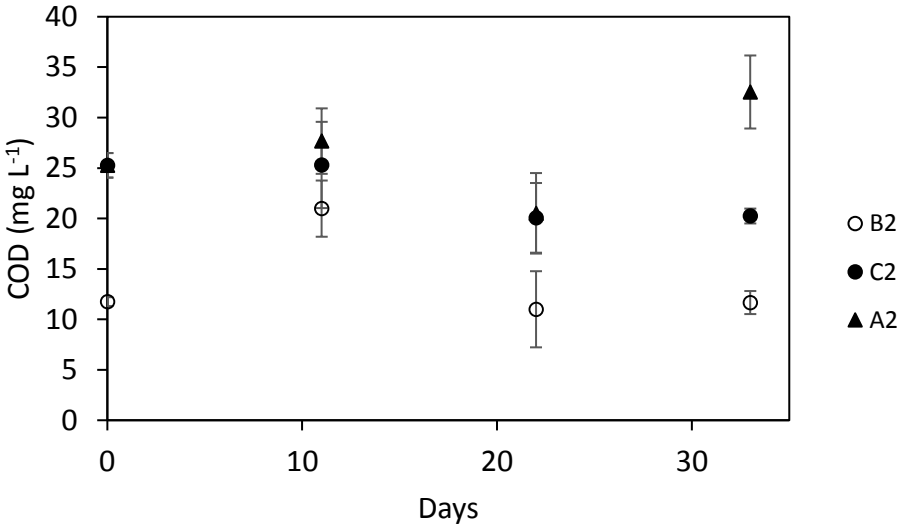


Figure A-7: COD concentrations in B2, C2 and A2 (mean ± SD)

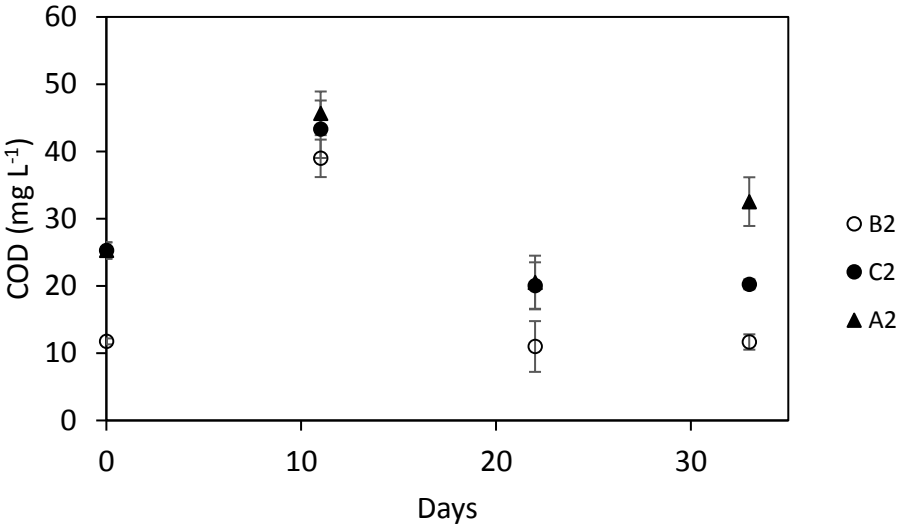


Figure A-8: Corrected COD concentrations in B2, C2 and A2 (mean ± SD)

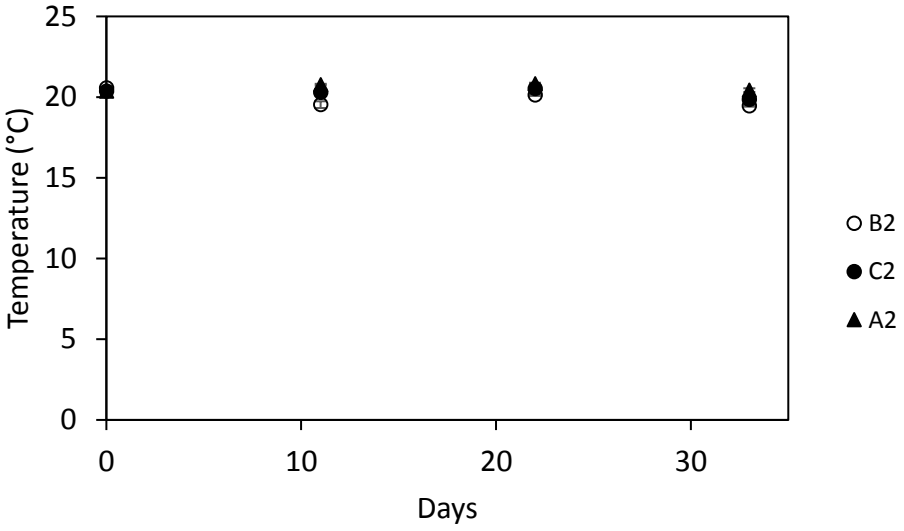


Figure A-9: Temperatures in B2, C2 and A2 (mean ± SD)

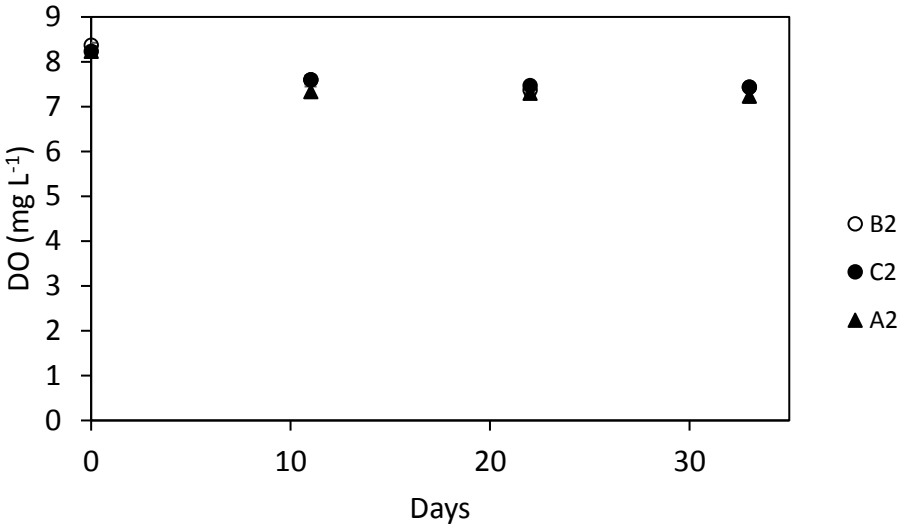


Figure A-10: Dissolved oxygen concentrations in B2, C2 and A2 (mean ± SD)

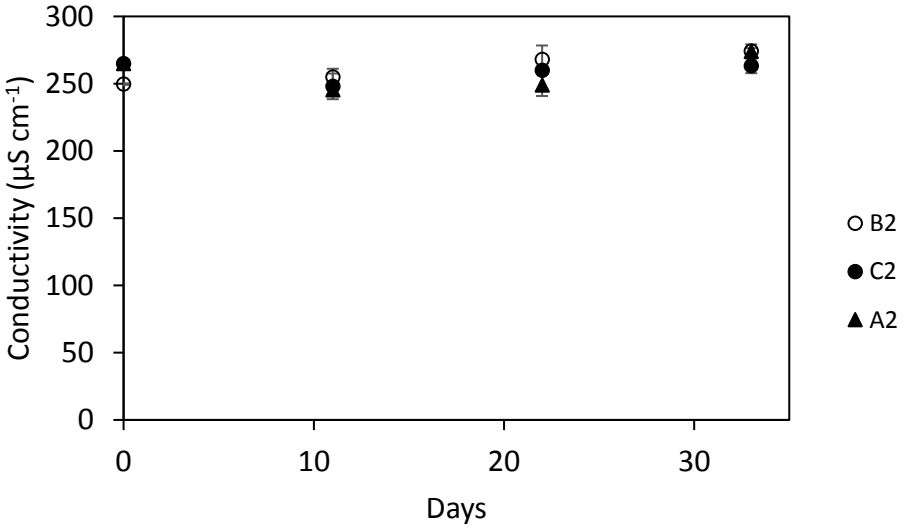


Figure A-11: Conductivities in B2, C2 and A2 (mean ± SD)

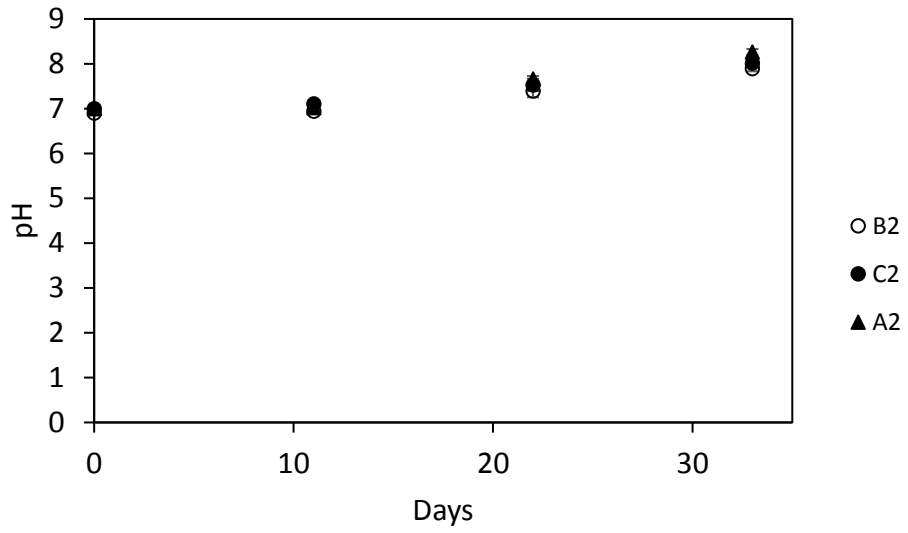


Figure A-12: pH in B2, C2 and A2 (mean ± SD)

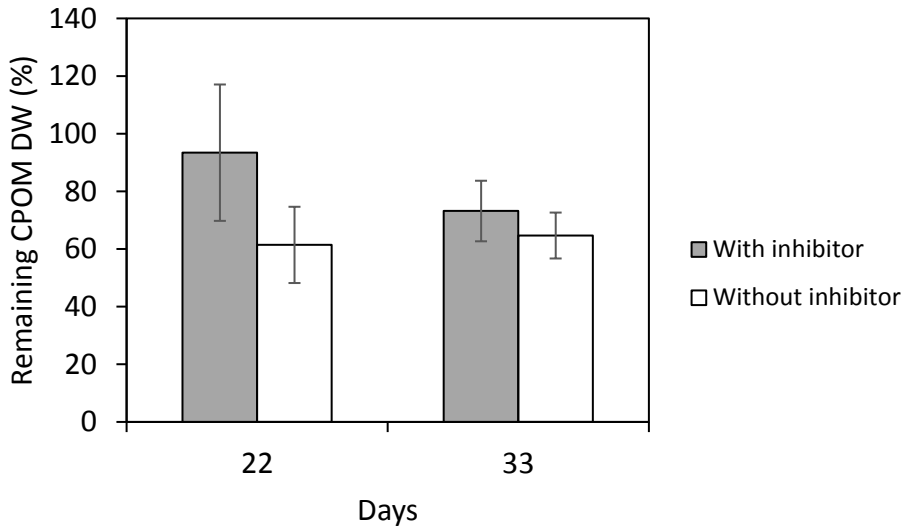


Figure A-13: Comparison percentage remaining CPOM of A2 cups with and without algae inhibitor (mean ± SD)

9.2.2 T, DO, conductivity and pH of the separate cups during the testing period

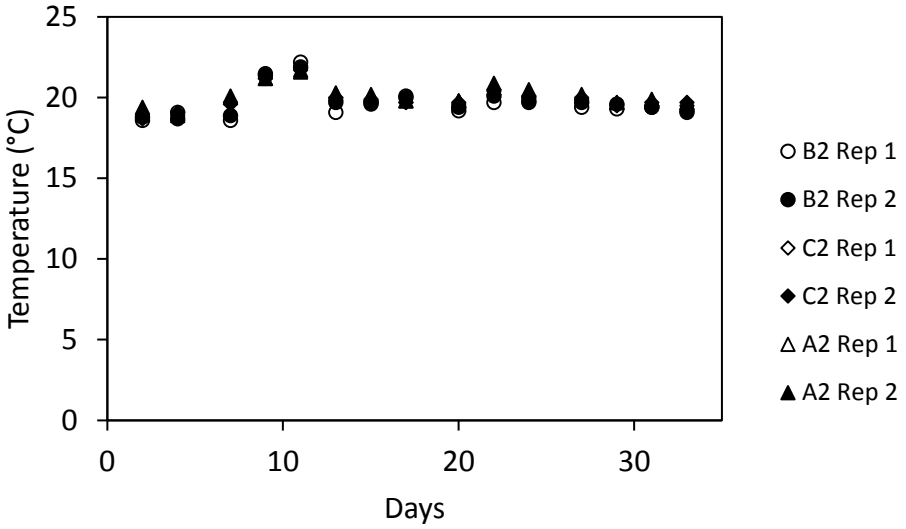


Figure A-14: Temperature during time in 2 replicates of B2, C2 and A2 from the separate cups

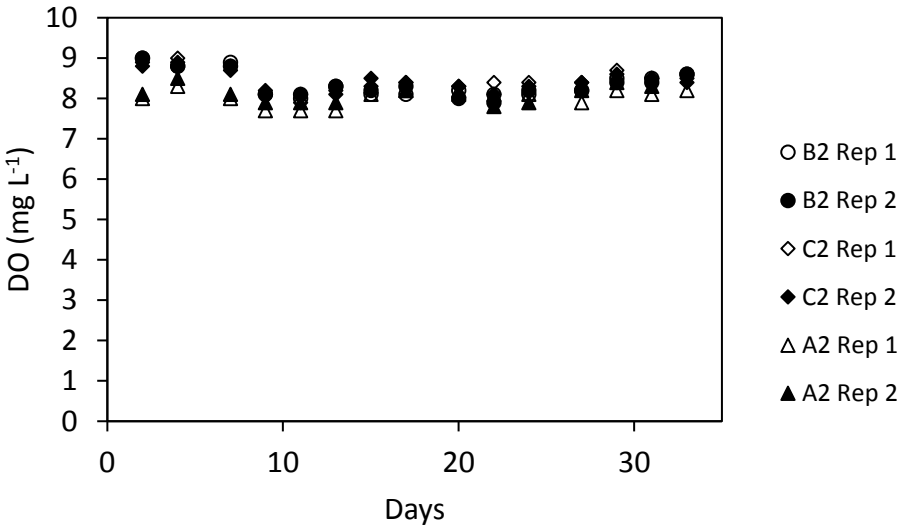


Figure A-15: Dissolved oxygen concentrations during time in 2 replicates of B2, C2 and A2 from the separate cups

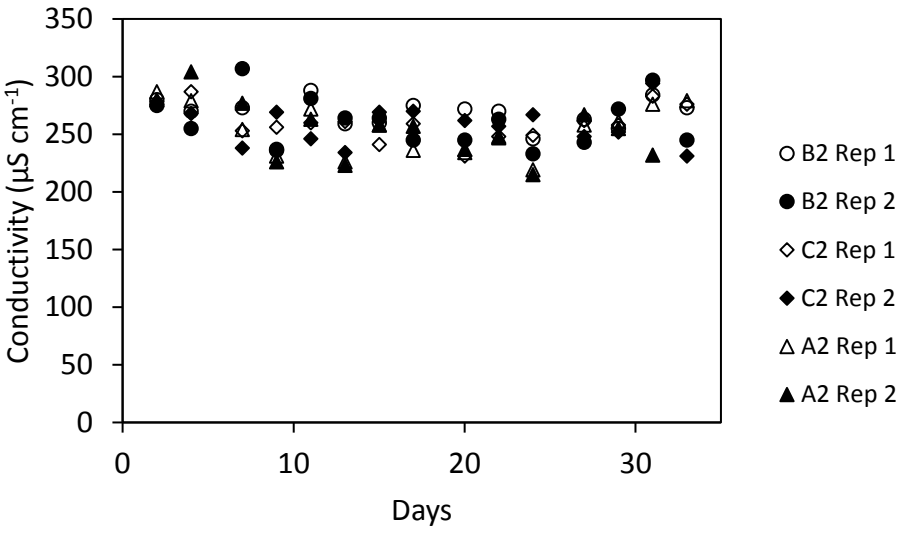


Figure A-16: Conductivity during time in 2 replicates of B2, C2 and A2 from the separate cups

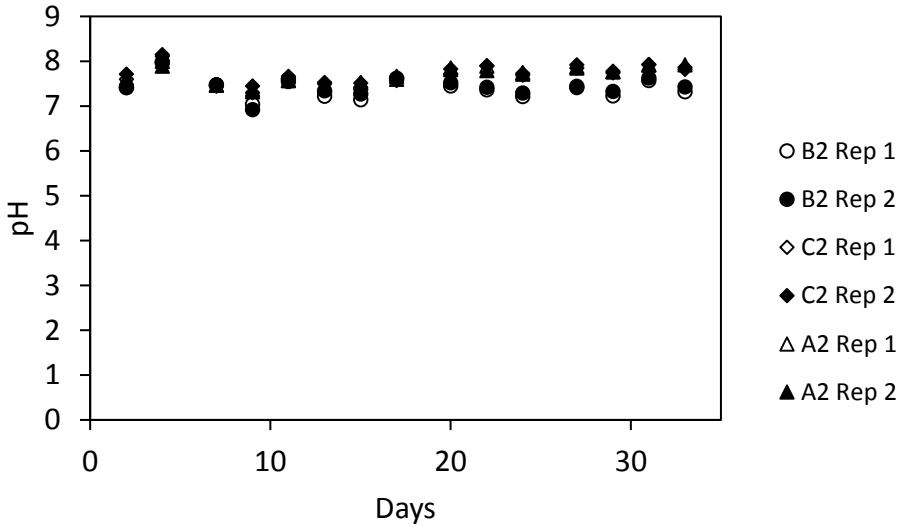


Figure A-17: pH during time in 2 replicates of B2, C2 and A2 from the separate cups

9.2.3 Analysis of the separate cups after 33 days

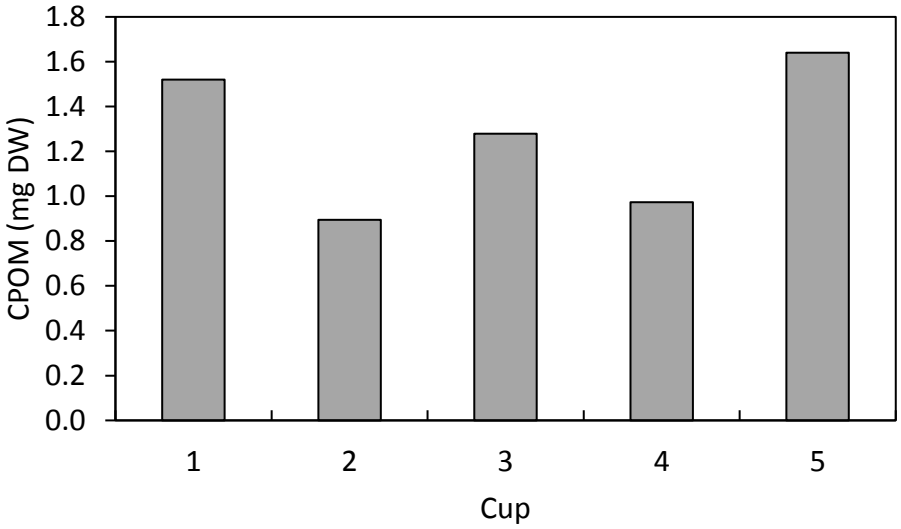


Figure A-18: Microbial breakdown in C2 of the separate cups after 33 days

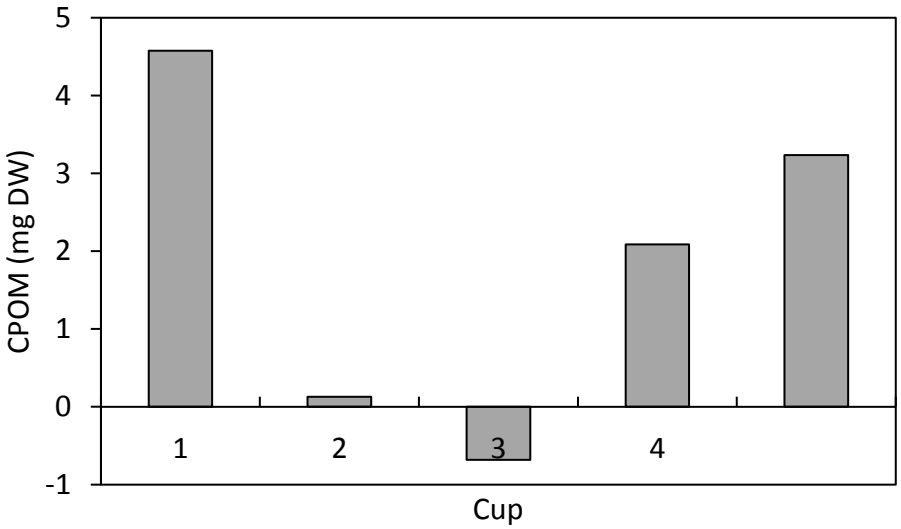


Figure A-19: Consumption of CPOM by Asellidae in A2 of the separate cups after 33 days

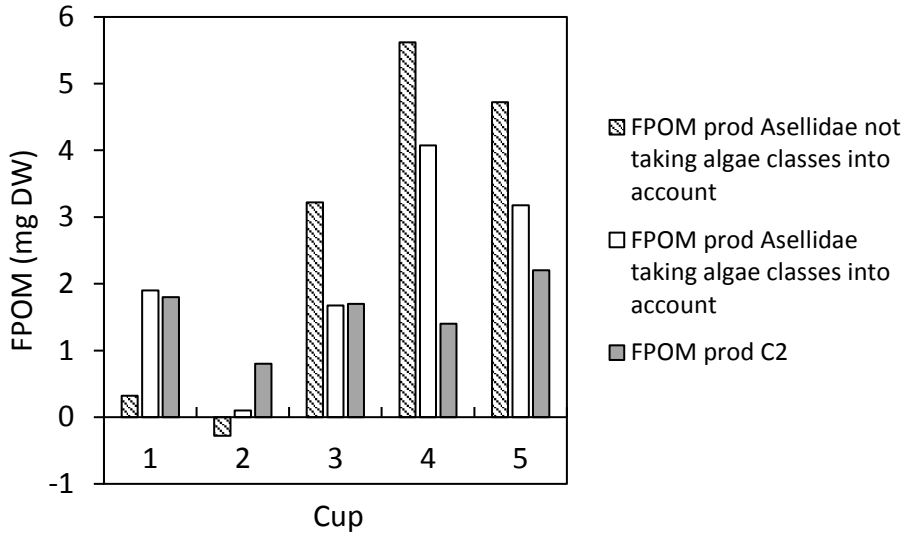


Figure A-20: FPOM production in C2 and A2 of the separate cups after 33 days

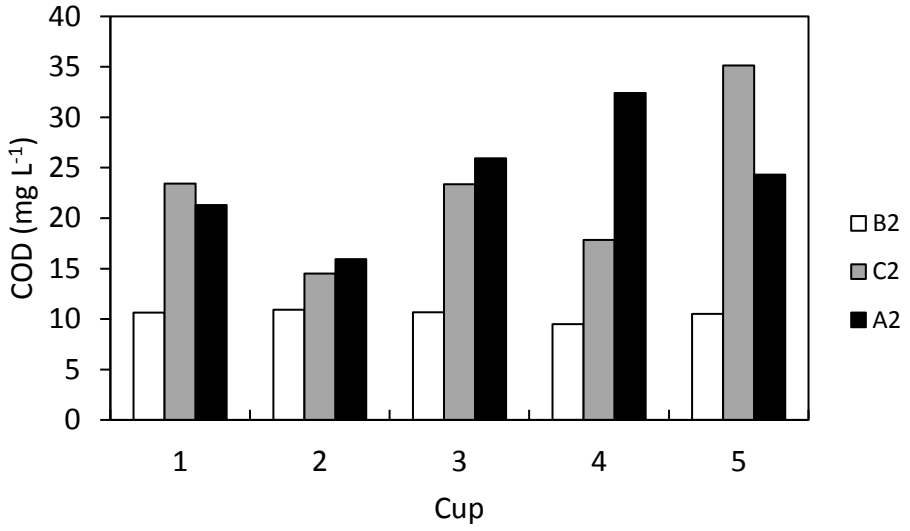


Figure A-21: COD concentrations in B2, C2 and A2 of the separate cups after 33 days

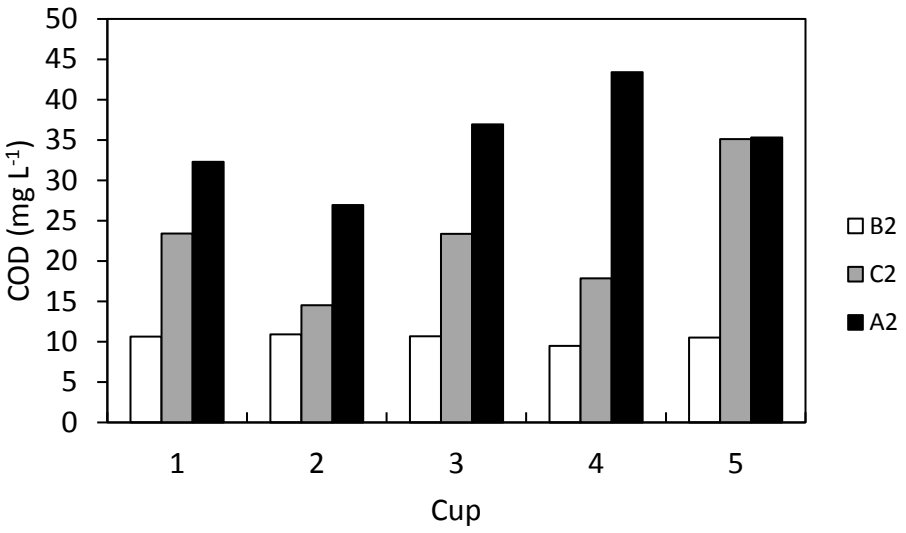


Figure A-22: Corrected COD concentrations in B2, C2 and A2 of the separate cups after 33 days

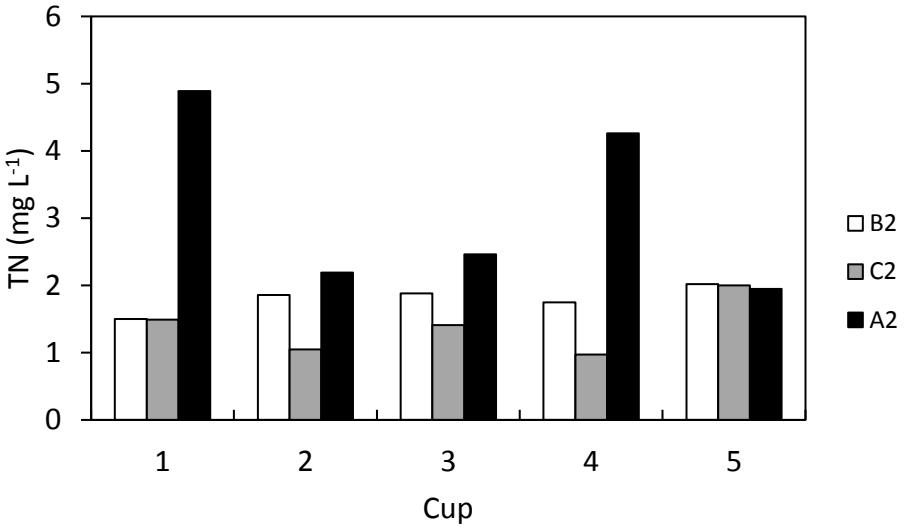


Figure A-23: TN concentrations in B2, C2 and A2 of the separate cups after 33 days

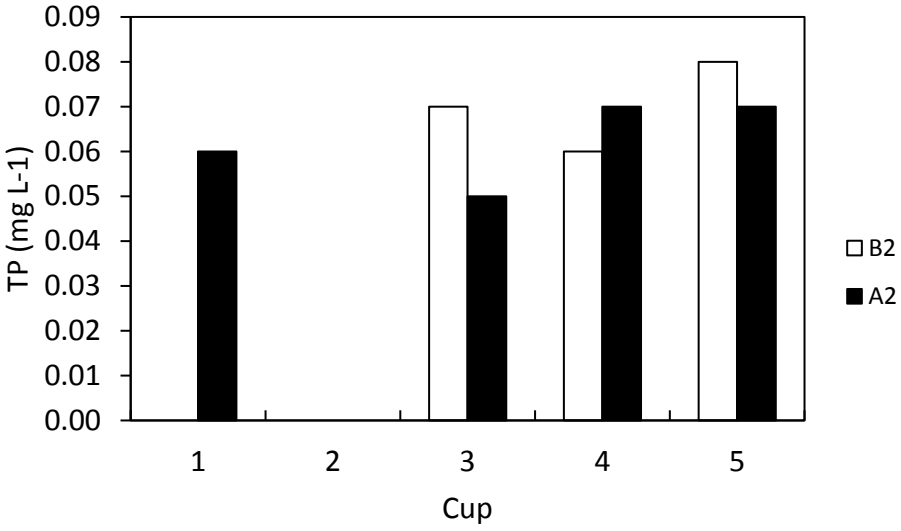


Figure A-24: TP concentrations in B2 and A2 of the separate cups after 33 days. If data are not shown, concentrations were below detection limit

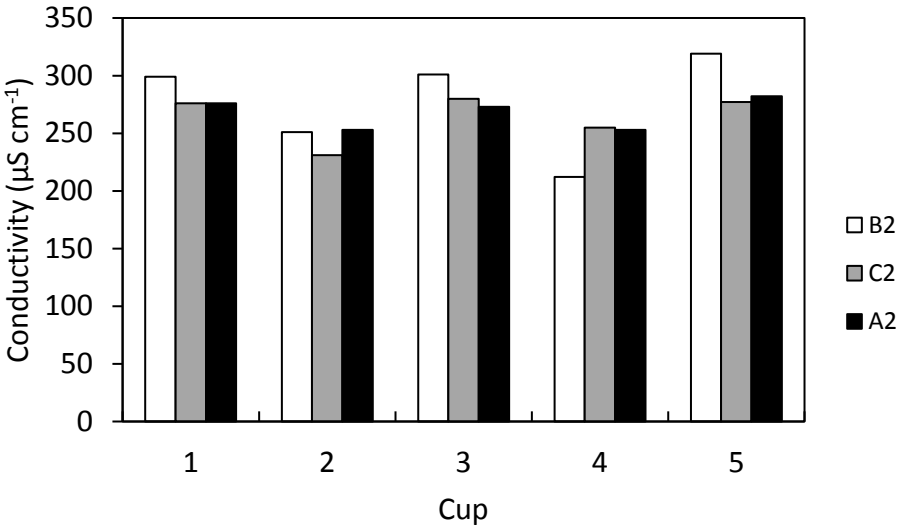


Figure A-25: Conductivities in the separate cups after 33 days

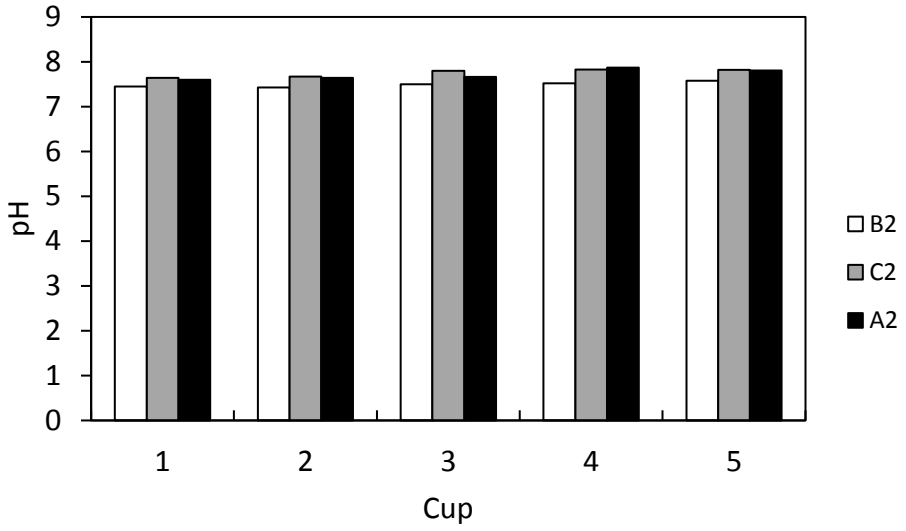


Figure A-26: pH in the separate cups after 33 days

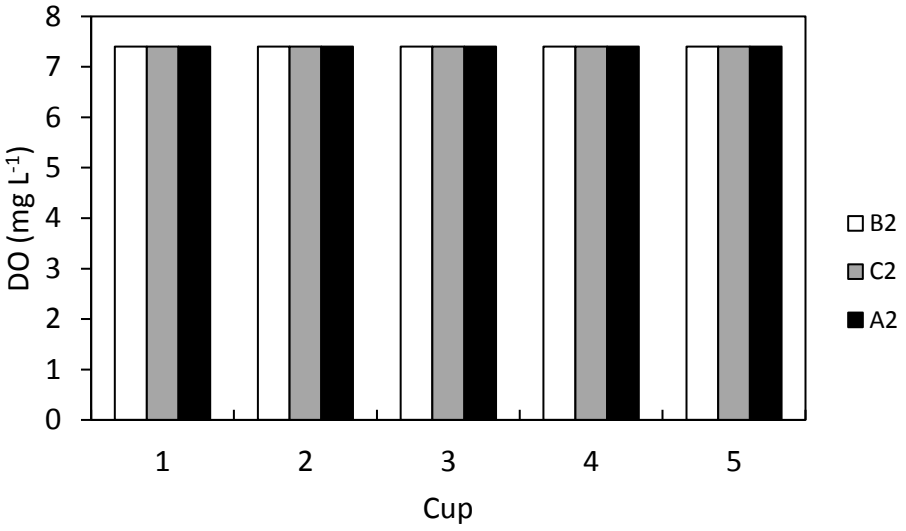


Figure A-27: Dissolved oxygen concentrations in the separate cups after 33 days

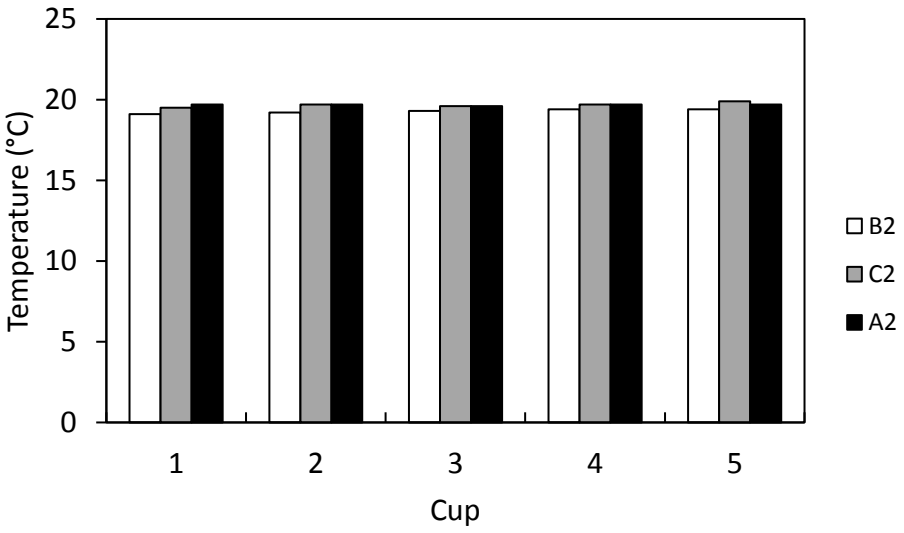


Figure A-28: Temperatures in the separate cups after 33 days

9.2.4 Weights of *A. aquaticus* individuals

Table A-2: Weight of *A. aquaticus* in A2 at the end of each testing period in the optimized feeding test

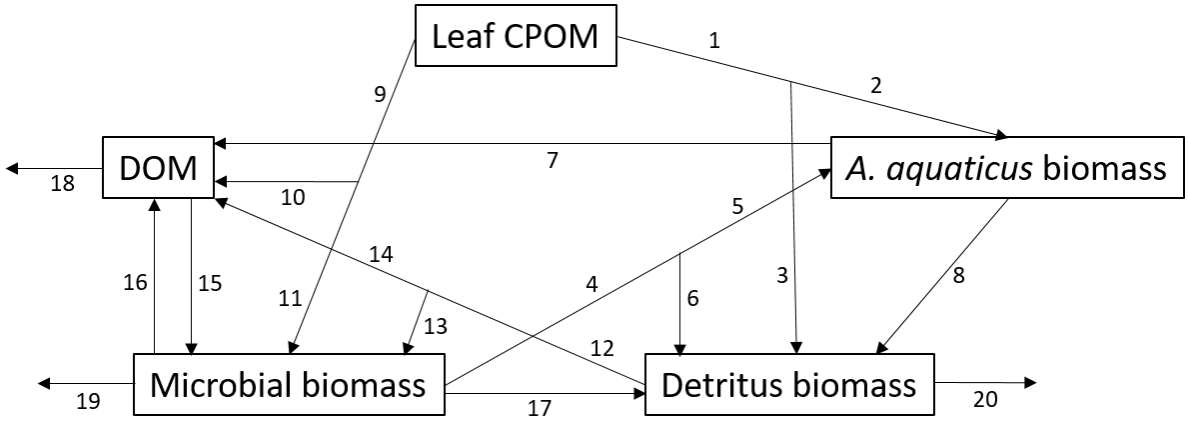
Testing period	**Weight *A. aquaticus* (mg DW)**
After 11 days	
Replicate 1	3.6
Replicate 2	3.4
Replicate 3	7.2
Replicate 4	6.2
Replicate 5	4.6
Replicate 6	4.9
Replicate 7	2.9
Replicate 8	5.7
Replicate 9	6.1
Replicate 10	5.8
Replicate 11	5.2
Replicate 12	5.0
Replicate 13	6.3
Replicate 14	4.9
Replicate 15	5.5
Replicate 16	5.4
Replicate 17	5.5
Replicate 18	6.9
Replicate 19	7.2
Replicate 20	6.5
Replicate 21	5.9
Replicate 22	3.4
Replicate 23	5.7
Replicate 24	5.4
Replicate 25	5.1
Replicate 26	6.1

Replicate 27	6.7
Replicate 28	6.0
Replicate 29	4.8
Replicate 30	10.6
Average 11 days	**5.6 ± 1.4**
After 22 days	
Replicate 1	2.9
Replicate 2	5
Replicate 3	4
Replicate 4	3.1
Replicate 5	6
Replicate 6	4.5
Replicate 7	3.1
Replicate 8	6
Replicate 9	2.3
Replicate 10	2.6
Replicate 11	8.5
Replicate 12	2.5
Replicate 13	5.8
Replicate 14	3.7
Replicate 15	2.3
Replicate 16	3
Replicate 17	2.3
Replicate 18	3
Replicate 19	5.2
Replicate 20	2
Replicate 21	3.3
Replicate 22	6.7
Replicate 23	4.8
Replicate 24	3.1
Replicate 25	7.7
Replicate 26	5.1
Replicate 27	7.5
Replicate 28	1.4
Replicate 29	2.6
Replicate 30	3.5
Average 22 days	**4.1 ± 1.9**
Cups without inhibitor after 22 days	
Replicate 1	3.7
Replicate 2	5
Replicate 3	4.7
Replicate 4	3.1
Replicate 5	1.1
Average without inhibitor 22 days	**3.5 ± 1.6**
After 33 days	
Replicate 1	7.4
Replicate 2	5.1
Replicate 3	4.2

Replicate 4	3.6
Replicate 5	6.7
Replicate 6	5.1
Replicate 7	6.7
Replicate 8	6.6
Replicate 9	5.3
Replicate 10	5
Replicate 11	7.5
Replicate 12	4.5
Replicate 13	4.8
Replicate 14	7.8
Replicate 15	6.1
Replicate 16	7.2
Replicate 17	5.6
Replicate 18	3.3
Replicate 19	3.4
Replicate 20	4.1
Replicate 21	3.9
Replicate 22	5.2
Replicate 23	7.3
Replicate 24	4.4
Replicate 25	5.4
Replicate 26	6.9
Replicate 27	3.9
Replicate 28	4.2
Replicate 29	3.1
Replicate 30	7.7
Average 33 days	**5.4 ± 1.5**
Cups without inhibitor after 33 days	
Replicate 1	6
Replicate 2	3.3
Replicate 3	3.4
Replicate 4	2.2
Replicate 5	6.2
Average without inhibitor 33 days	**4.2 ± 1.8**
Separate cups after 33 days	
Replicate 1	4.4
Replicate 2	2
Replicate 3	5.7
Replicate 4	8.1
Replicate 5	4.7
Average separate cups 33 days	**5.0 ± 2.2**

9.3 Model organic matter degradation

9.3.1 Conceptual diagram



1: Ingestion of leafs by *A. aquaticus*
2: Assimilation of leafs by *A. aquaticus*
3: Egestion (feces production) by *A. aquaticus*
4: Ingestion of micro-organisms by *A. aquaticus*
5: Assimilation of micro-organisms by *A. aquaticus*
6: Egestion (feces production) by *A. aquaticus*
7: *A. aquaticus* respiration
8: *A. aquaticus* mortality
9: Microbial breakdown of leafs
10: Conversion of leafs to dissolved organic matter (DOM) by micro-organisms
11: Assimilation of leafs by micro-organisms
12: Microbial breakdown of detritus
13: Assimilation of detritus by micro-organisms
14: Conversion of detritus to dissolved organic matter (DOM) by micro-organisms
15: Uptake of DOM by micro-organisms
16: Microbial respiration
17: Microbial mortality
18: Efflux of DOM
19: Efflux of micro-organisms
20: Efflux of detritus

Assumptions

- Respiration and mortality are first order decay processes
- Food uptake is a Michaelis-Menten process
- Microbial respiration is negligible
- Mortality is not temperature dependent, only ingestion and respiration rates
- Initial detritus biomass is zero
- Volumetric flux is assumed zero for simplicity

9.3.2 Differential equations

$$\frac{d[LeafBiomass]}{dt} = -microbial\ breakdown - ingestion\ by\ Asellidae$$

$$= -\frac{[LeafBiomass]}{[LeafBiomass] + K_{Leaf,micr}} * Breakdown_{max,leaf,micr}(20) * \theta^{T-20} * [MicrobialBiomass] - \frac{[LeafBiomass]}{[LeafBiomass] + K_{Leaf,As}} * Ingestion_{max,leaf,As}(20) * \theta^{T-20} * [AsellusBiomass]$$

$$\frac{d[AsellusBiomass]}{dt} = assimilation\ from\ leafs + assimilation\ from\ microbiology - respiration - mortality$$

$$= AE_{As} * \frac{[LeafBiomass]}{[LeafBiomass] + K_{Leaf,As}} * Ingestion_{max,leaf,As}(20) * \theta^{T-20} * [AsellusBiomass] + AE_{As} * \frac{[MicrobialBiomass]}{[MicrobialBiomass] + K_{micr,As}} * Ingestion_{max,micr,As}(20) * \theta^{T-20} * [AsellusBiomass] - k_{resp,As}(20) * \theta^{T-20} * [AsellusBiomass] - k_{mort,As}(20) * \theta^{T-20} * [AsellusBiomass]$$

$$\frac{d[DetritusBiomass]}{dt} = egestion\ Asellidae + mortality\ Asellidae + mortality\ microbiology - microbial\ breakdown\ of\ detritus - efflux$$

$$= (1 - AE_{As}) * \frac{[LeafBiomass]}{[LeafBiomass] + K_{Leaf,As}} * Ingestion_{max,leaf,As}(20) * \theta^{T-20} * [AsellusBiomass] * \frac{A}{V} + (1 - AE_{As}) * \frac{[MicrobialBiomass]}{[MicrobialBiomass] + K_{micr,As}} * Ingestion_{max,micr,As}(20) * \theta^{T-20} * [AsellusBiomass] * \frac{A}{V} + k_{mort,As}(20) * \theta^{T-20} * [AsellusBiomass] * \frac{A}{V} + k_{mort,micr}(20) * \theta^{T-20} * [MicrobialBiomass] * \frac{A}{V} - \frac{[DetritusBiomass]}{[DetritusBiomass] + K_{Detr,micr}} * Breakdown_{max,detr,micr}(20) * \theta^{T-20} * [MicrobialBiomass] * \frac{A}{V} - \frac{Q}{V} * [DetritusBiomass]$$

$$\frac{d[DOM]}{dt} = respiration\ Asellidae + respiration\ microbiology + conversion\ of\ leafs + conversion\ of\ detritus - uptake\ by\ microbiology - efflux$$

$$= k_{resp,As}(20) * \theta^{T-20} * [AsellusBiomass] * \frac{A}{V} + k_{resp,micr}(20) * \theta^{T-20} * [MicrobialBiomass] * \frac{A}{V} + (1 - AE_{micr}) * \frac{[LeafBiomass]}{[LeafBiomass] + K_{Leaf,micr}} * Breakdown_{max,leaf,micr}(20) * \theta^{T-20} * [MicrobialBiomass] * \frac{A}{V} + (1 - AE_{micr}) * \frac{[DetritusBiomass]}{[DetritusBiomass] + K_{Detr,micr}} * Breakdown_{max,detr,micr}(20) * \theta^{T-20} * [MicrobialBiomass] * \frac{A}{V} - AE_{micr} * \frac{[DOM]}{[DOM] + K_{DOM,micr}} * Uptake_{max,DOM,micr}(20) * \theta^{T-20} * [MicrobialBiomass] * \frac{A}{V} - \frac{Q}{V} * [DOM]$$

$$\frac{d[MicrobialBiomass]}{dt} = assimilation\ from\ leafs + assimilation\ from\ detritus + assimilation\ from\ DOM - ingestion\ by\ Asellidae - respiration - mortality - efflux$$

$$= AE_{micr} * \frac{[LeafBiomass]}{[LeafBiomass] + K_{Leaf,micr}} * Breakdown_{max,leaf,micr}(20) * \theta^{T-20} * [MicrobialBiomass] + AE_{micr} * \frac{[DetritusBiomass]}{[DetritusBiomass] + K_{Detr,micr}} * Breakdown_{max,detr,micr}(20) * \theta^{T-20} * [MicrobialBiomass] + AE_{micr} * \frac{[DOM]}{[DOM] + K_{DOM,micr}} * Uptake_{max,DOM,micr}(20) * \theta^{T-20} * [MicrobialBiomass] - \frac{[MicrobialBiomass]}{[MicrobialBiomass] + K_{micr,As}} * Ingestion_{max,micr,As}(20) * \theta^{T-20} * [AsellusBiomass] - k_{resp,micr}(20) * \theta^{T-20} * [MicrobialBiomass] - k_{mort,micr}(20) * \theta^{T-20} * [MicrobialBiomass] - \frac{Q}{V} * [MicrobialBiomass]$$

9.3.3 State variables and units

Table A-3: State variables of the model

State variable	Unit
LeafBiomass	mg m^{-2}
AsellusBiomass	mg m^{-2}
DetritusBiomass	mg L^{-1}
DOM	mg L^{-1}
MicrobialBiomass	mg m^{-2}

Table A-4: Constants used in the model

Constant	Explanation	Unit
A	surface area	m^2
V	volume	L
Q	volumetric flux	L d^{-1}

Table A-5: Parameters used in the model (references between brackets)

Parameter	Explanation	Value
$K_{Leaf,micr}$	half-saturation constant for microbial breakdown of leafs	10 000 mg m^{-2} (1)
$Breakdown_{max,leaf,micr}(20)$	maximum breakdown rate of leafs by micro-organisms at 20°C	0.1 d^{-1} (2)
$K_{Leaf,As}$	half-saturation constant for ingestion of leafs by Asellidae	200 g m^{-2} (3)
$Ingestion_{max,leaf,As}(20)$	maximum ingestion rate of leafs by Asellidae at 20°C	0.33 d^{-1} (3,4)
AE_{As}	Assimilation efficiency of Asellidae	0.303 (4)
$K_{micr,As}$	half-saturation constant for ingestion of micro-organisms by Asellidae	300 mg m^{-2} (2)
$Ingestion_{max,micr,As}(20)$	maximum ingestion rate of micro-organisms by Asellidae at 20°C	0.0016 d^{-1} (5)
$k_{resp,As}(20)$	first order respiration rate constant of Asellidae at 20°C	0.005 d^{-1} (6)
$k_{mort,As}(20)$	first order mortality rate constant of Asellidae at 20°C	0.001 d^{-1} (4)
$k_{resp,micr}(20)$	first order respiration rate constant of micro-organisms at 20°C	0 d^{-1} (assumption)
$k_{mort,micr}(20)$	first order mortality rate constant of micro-organisms at 20°C	0.15 d^{-1} (7)
$K_{Detr,micr}$	half-saturation constant for microbial breakdown of detritus	5000 mg m^{-2} (assumption)
AE_{micr}	Assimilation efficiency of micro-organisms	0.50 (assumption)
$Breakdown_{max,detr,micr}(20)$	maximum breakdown rate of detritus by micro-organisms at 20°C	0.5 d^{-1} (assumption)
$K_{DOM,micr}$	half-saturation constant for utilization of DOM by micro-organisms	5 mg L^{-1} (7)
$Uptake_{max,DOM,micr}(20)$	maximum uptake rate of DOM by micro-organisms at 20°C	0.7 d^{-1} (2)
θ_{As}	Arrhenius temperature correction coefficient for Asellidae	1.04 (3)
θ_{micr}	Arrhenius temperature correction coefficient for micro-organisms	1.07 (3)

1: Pepper *et al.* (2008), 2: Moran *et al.* (1988), 3: Janse (2005), 4: Jørgensen *et al.* (1991), 5: Findlay *et al.* (1984), 6: Ivleva (1980), 7: Deng *et al.* (2016)

9.3.4 Simulations over a 33 days period

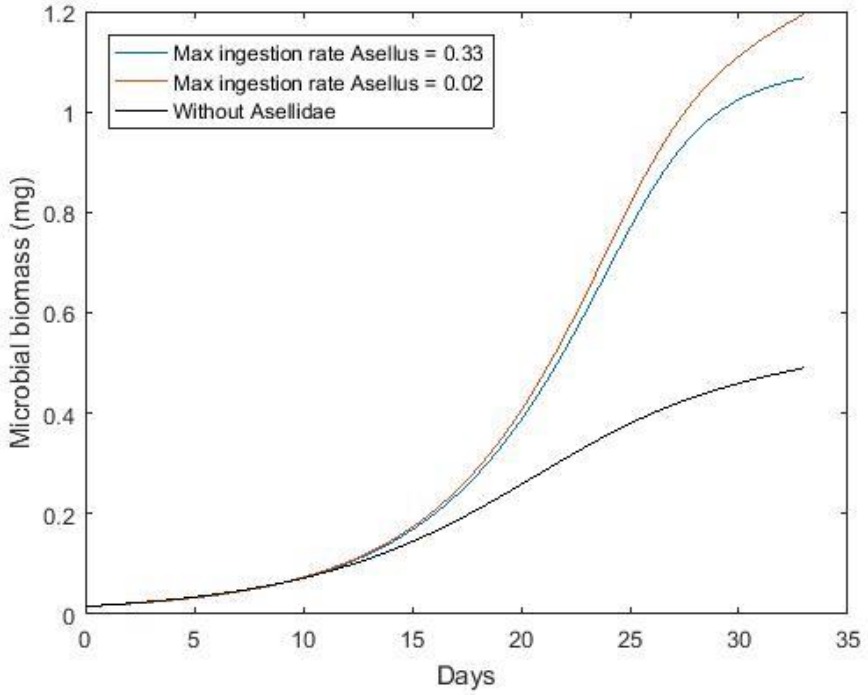


Figure A-29: Simulations of microbial biomass over a 33 days period

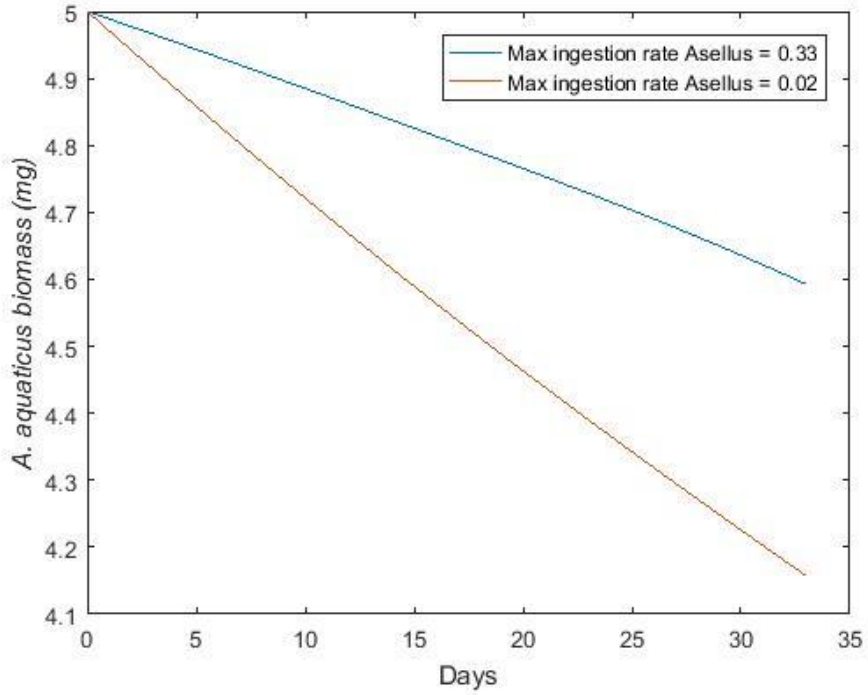


Figure A-30: Simulations of *A. aquaticus* biomass over a 33 days period

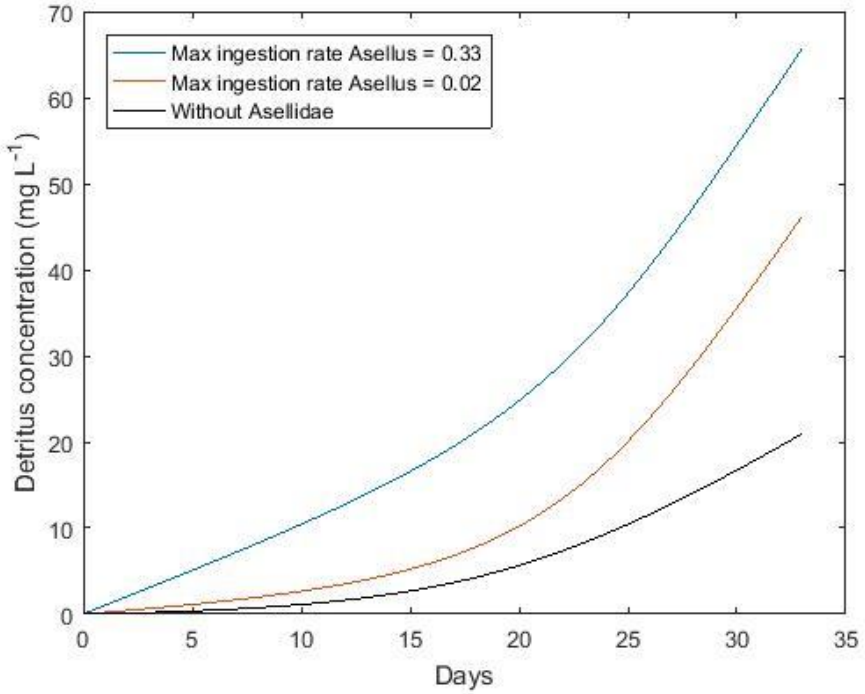


Figure A-31: Simulations of detritus concentration over a 33 days period

9.3.5 Simulations over a one year period

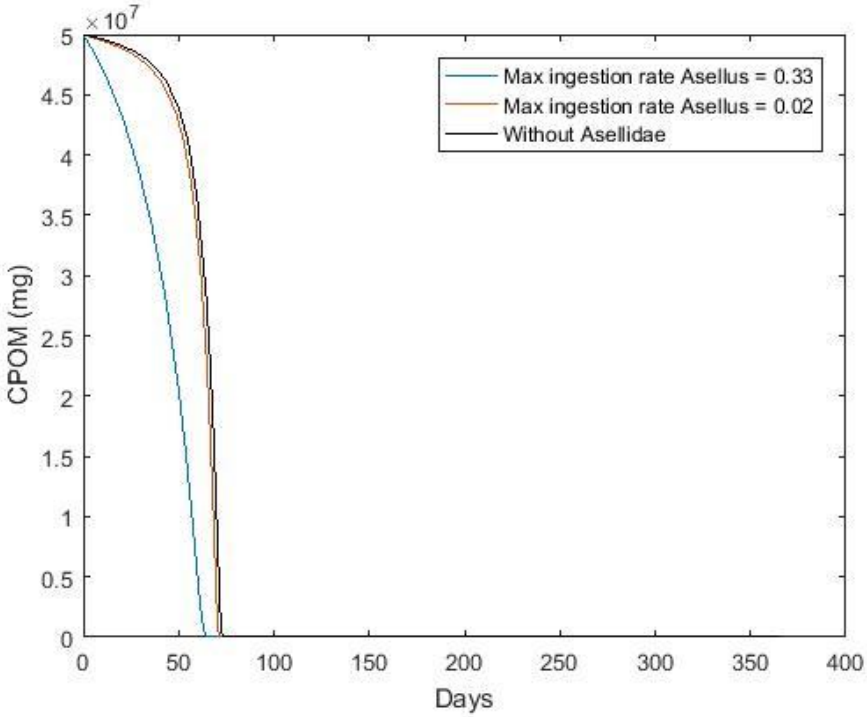


Figure A-32: Simulations of leaf CPOM over a one year period

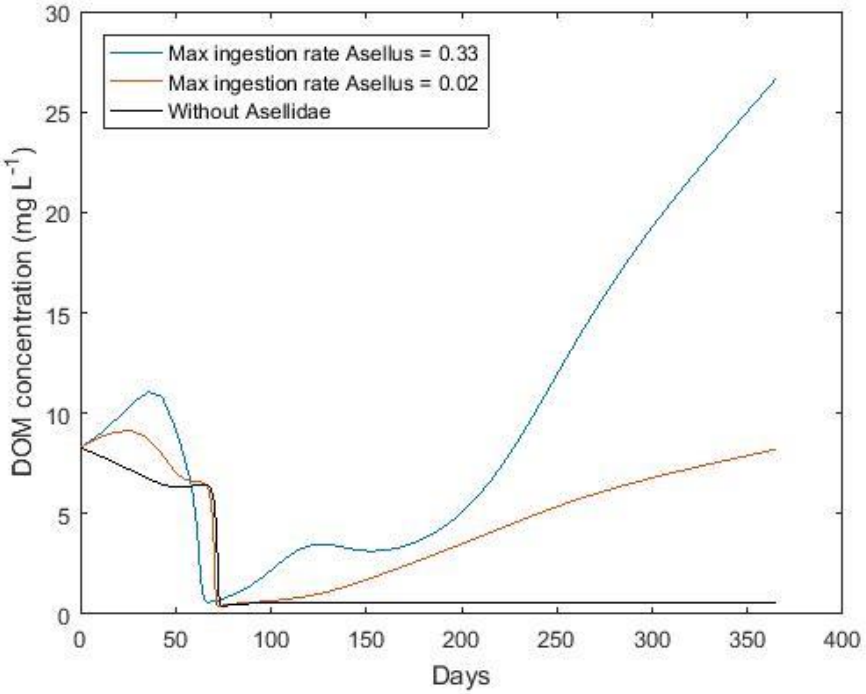


Figure A-33: Simulations of DOM concentration over a one year period

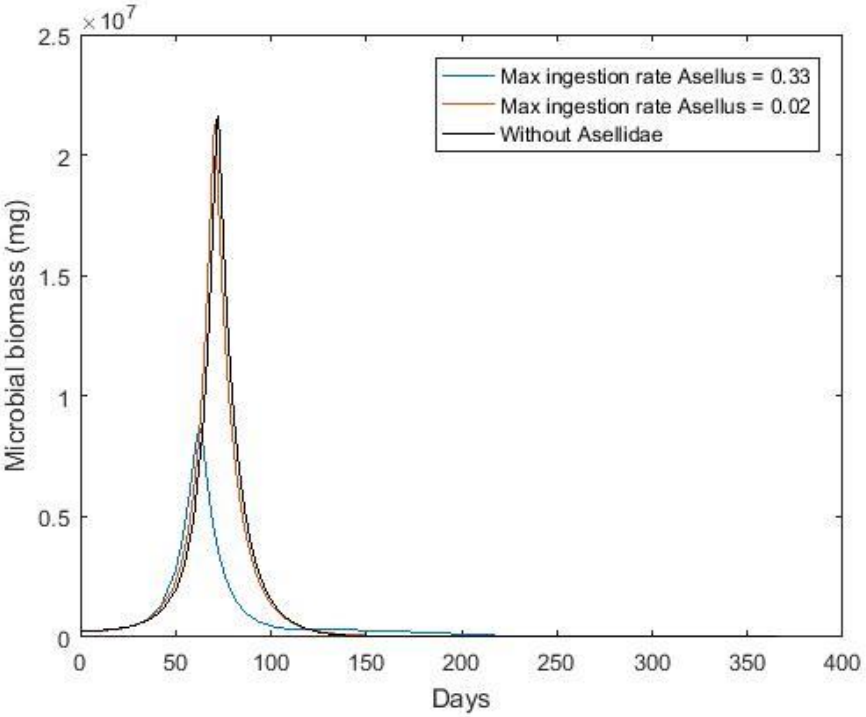


Figure A-34: Simulations of microbial biomass over a one year period

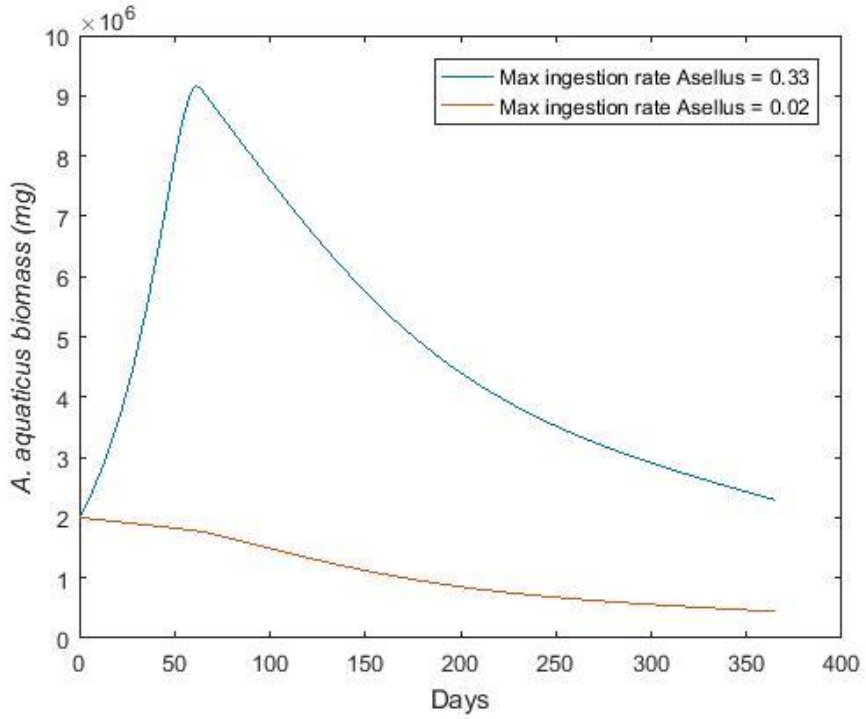


Figure A-35: Simulations of *A. aquaticus* biomass over a one year period

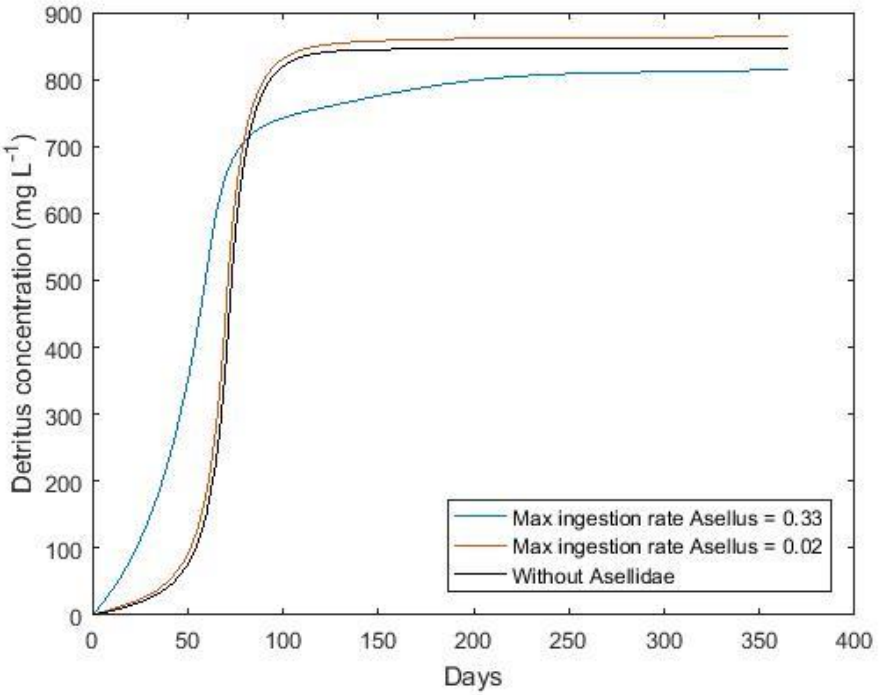


Figure A-36: Simulations of detritus concentration over a one year period