

Lessons from the “Living-Filter”: an in-reservoir floating treatment wetland for phytoplankton reduction prior to a water treatment works intake

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Abstract - The Living-Filter is a novel floating emergent treatment wetland design that combines a hydroponic plant-bed with curtains and baffles. Thames Water Utilities and AquaticEngineering designed and installed the Living-Filter in Farmoor II reservoir, Oxfordshire, UK, in July 2012. The aim is to reduce the phytoplankton biomass prior to the water treatment works intake. The plant-bed (1 m H x 21m W x 10 m L) supports plants of *Phragmites australis*, *Phalaris arundinacea* and *Carex acutiformis*, with a combined system of curtains and baffles that aid the water to flow through the Living-Filter. All the water treated at the water treatment works has passed through the Living-Filter. The roots and a plastic fabric increase the surface area for biofiltration processes and the rhizosphere provides refuge for zooplankton. The research attempts to answer the question: Can the Living-Filter be used as an in-reservoir pre-treatment filtration process for reducing the phytoplankton biomass onto the water treatment works? Weekly surveys were carried out from July to October 2013 at sixteen sampling sites to measure physico-chemical and biological variables. The results for the first year of study show: 1. Soluble manganese, dissolved inorganic nitrogen and nitrites were significantly lower after treatment through the Living-Filter.

2. Chlorophyll-*a* removal efficiency was up to 45% during the first seven weeks. 3. Zooplankton abundance was more stable within the rhizosphere than in the water column. 4. The aerial shoots of the plants developed well, but based on root development only *Phalaris* and *Carex* should be considered for future designs. This work highlights the potential of the Living-Filter for phytoplankton reduction, but it also addresses questions that will further improve the system.

Keywords: *Living-Filter, floating treatment wetland, phytoplankton removal, zooplankton, reed-beds, in-reservoir pre-treatment filtration*

1. INTRODUCTION

Eutrophic drinking water reservoirs are seasonally affected by nuisance phytoplankton blooms (micro-algae and cyanobacteria) (Jöhnk *et al.*, 2008; Reynolds *et al.*, 2012; O’Neil *et al.*, 2012). The presence of phytoplankton blooms is associated with operational challenges in water supply treatment works (WTWs) (Henderson *et al.*, 2008; Jarvis *et al.*, 2008) and these can be due to the increased number of suspended cells in the water or the production of secondary metabolites (Jarvis *et al.*, 2008). Both the cells and polysaccharide exudates are associated with disruption of the coagulation process by inhibiting the adhesion of colloidal particles to form stable floc blankets, and therefore increasing the chemical dose demand (Sun *et al.*, 2013). Filter blocking leads to a decrease in filtration running times which reduces water production rates and as a result the increase in frequency of filter backwashing shortens their lifespan (Bauer *et.al.*, 1998; Ribau and Joao, 2006). The water industry has traditionally solved the problem by increasing the number of processing steps at WTWs for the treatment of reservoir water using new chemicals or innovative technology (Parsons and Jefferson, 2006). Over the past five decades attempts to manage blooms and reduce the

phytoplankton biomass in reservoirs have been made using chemical, physical and bio-ecological techniques (Purcell *et al.*, 2013).

Thames Water Utilities Ltd., UK (TW) identified a seasonal (July - November) increase in the coagulant (poly-aluminium chloride 10%) dose demand in a water treatment works (WTW) associated with an increase in chlorophyll-a levels (as a measure of phytoplankton biomass) in Farmoor II reservoir, Oxfordshire, UK. To reduce the chlorophyll-a prior to the WTW intake, TW and AquaticEngineering designed and installed the Living-Filter, an innovative ecological pre-treatment process. The Living-Filter is a hydroponic, floating plant-bed structure consisting of *Phragmites australis*, *Phalaris arundinacea* and *Carex acutiformis* (thereafter *Phragmites*, *Phalaris* and *Carex*). The roots are interspaced with flexible sheets of two layers of monofilament fabric (thread diameter 0.5 mm and mesh size (0.8 x 0.5 cm) of non-enriched and non-coated plastic material (the fabric), and therefore the roots develop hydroponically without a nutrient enriched substrate. The Living-Filter can be classified as a horizontal subsurface flow, floating emergent treatment wetland (HSSF-FTW) (Headley and Tanner, 2012). A key feature of the Living-Filter shared with other types of HSSF-FTW is that the flow and treatment occur primarily within the root-zone of the plants in a hydroponic context, but the main difference that there is no gravel, soil or other substrate (Headley and Tanner, 2012). Novel to the Living-Filter is the combination of a floating emergent treatment wetland (FTW) (the plant-bed) with submersed curtains and baffles, which aid the water flow through the Living-Filter, so that all the water abstracted at the WTW intake has passed through the roots. Moreover, applications of FTW in water supply reservoirs are scarce (Garbett, 2005; Li *et al.*, 2010; Wang, 2012) and this is the first attempt to reduce phytoplankton biomass at a reservoir WTW intake.

The HSSF-FTWs are ecological engineering (EE) designs, which are based on ecological science; they introduce an artificial design with elements copied from nature (Mitsch, 2012). These systems have been mainly used to remove nutrients and metals from waste waters (Tanner and Headley, 2011; Vymazal, 2013; Fonder and Headley, 2013) and most are installed in shallow (<6 m depth) water bodies. The roots of the hydroponic plants are fundamental in FTW, as physical, chemical and biological processes take place within the root system. These processes include the filtration of particulates (entrapment and deposition) (De Stefani *et al.*, 2011), the direct uptake of nutrients and metals from the water column (Marchand *et al.*, 2014) and the provision of surface area for the attachment of biofilm for biochemical and degradation processes (Kyambadde *et al.*, 2005; Osem *et al.*, 2007). Furthermore, the microorganisms living within the rhizosphere are also important in biodegradation processes (Song *et al.*, 2009; Headley and Tanner, 2012).

Promoting vegetation coverage to increase zooplankton with the assumption that the increase in zooplankton will intensify grazing on phytoplankton is one of the objectives of biomanipulation (Muylaert *et al.*, 2010). Another objective is to increase zooplankton by reducing zooplanktivory (fish fry), known as top-down mechanisms in biomanipulation (Ger *et al.*, 2014). Biomanipulation attempts to restore a water body from a turbid to a clear state, by using top-down, bottom up or both mechanisms. Bottom-up aims to prevent the availability of nutrient enrichment and release for phytoplankton production (Carpenter *et al.*, 1987, 1995; Moss, 2007). Farmoor II is a man-made freshwater reservoir that lacks natural vegetation and therefore it is expected that the roots of the plant-bed of the Living-Filter will provide some refuge to the existing zooplankton community, consequently contributing towards the reduction of phytoplankton biomass at the WTW intake. The roots and fabric of the plant-bed could

play a role in reducing phytoplankton biomass by the physical filtration processes of entrapment and deposition of suspended particles (i.e. phytoplankton cells), as well as chemical processes.

This paper aims to answer an overarching question: Can the Living-Filter be used as an in-reservoir pre-treatment filtration system for the reduction of phytoplankton biomass onto a water treatment works? The following research questions are posed 1) Are there physico-chemical and biological changes upstream and downstream the plant-bed of the Living-Filter? 2) Are there physico-chemical and biological changes within the plant-bed? 3) Will the Living-Filter provide refuge to zooplankton? 4) What lessons can be learned from this field-scale floating treatment wetland?

2. MATERIALS AND METHODS

2.1. Study site: Farmoor II reservoir and the Living Filter

Farmoor I and II reservoirs are bankside pumped storage reservoirs from the river Thames. Farmoor II maximum and mean depths are 11.8 m and 6.0 m, with a 9.3×10^6 m³ volume capacity and an area of 1 km². The distribution of the three plant species that covers 178 m² of the Living-Filter's floating plant-bed is shown in Figure 1-A. The plant-bed is a modular galvanized caged structure, with a platform area of 210 m² (10 m W x 21 m L), of which 32 m² is wooden decking to access the sampling sites. The structure is 20 m away from the WTW intake (Figure 1-B). Two impermeable curtains run from the dam and alongside the plant-bed. The curtains run downwards from the surface water level to the sediment bed (10 m H x 65 m W) (Figure 1-A and 1-B). There are two baffles between the plant-bed and the WTWs intake: the first runs two metres downwards from the surface; the second runs from two metres below the water surface to the sediment bed (Figure 1-B). The water flows into the Living-Filter at the plant-

bed, passes through the roots and the fabric, over the second baffle and under the first baffle to the WTW intake (Figure 1-A and 1-B).

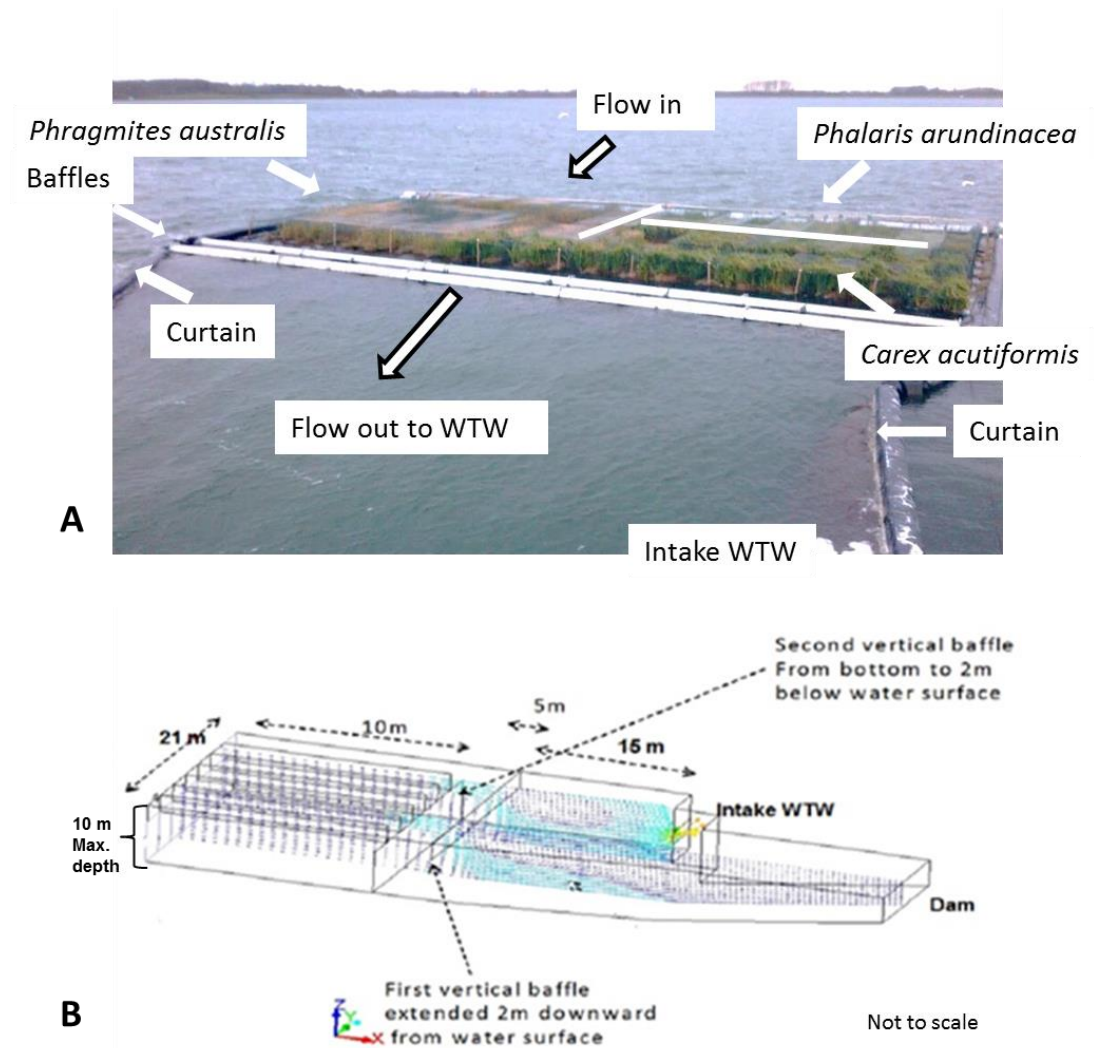


Figure 1. A- Living-Filter six months after installation in winter 2012-2013 showing: the plant-bed and plant distribution, direction of flow, and the position of the curtains and baffles. B- Schematic side view of the Living-Filter with dimensions of the plant-bed (10 m W x 21 m L), the distance between the two baffles (5 m) and the distance between the 1st baffle to the WTW intake (15 m). (Not to scale).

To facilitate the surveys and monitoring of the Living-Filter, three floating walkways were built. The plant-bed structure consists of seven modules made with a

galvanized steel mesh sheet (labelled from A to G, Figure 2-A) with dimensions 1.2 m Height x 21 m Width x 10 m Length. The diameter of the mesh in the sheets is 7.6 cm L x L. Each module (3 m W) has three cages and is separated from other modules by a baffle that runs downwards 2 m from the water surface. Each cage is 1.2 m Height x 1 m Width x 3.3 m Length and contains 10 m² unfolded area of plastic fabric (Figure 2-B).

The plants were less than a year old when first placed on the plant bed in July 2012. They were grown in 10 cm thick coconut coir mats, and although the mats were lost soon after the plants were positioned, the plants had rooted. (Figure 2-B). The plant-covered area was estimated for each cage and used to calculate the plant coverage of the plant-bed structure. The initial plant coverage was 85% (179 m² from a total of 210m²), with the remaining 15% (31 m²) taken up with wooden decking on the plant-bed.

The Living-Filter sampling sites can be seen in Figure 2-A. Stage 1 (S1) is the area outside the boundaries of the Living-Filter and stage 2 (S2) is within the plant-bed structure. Inside the Living-Filter enclosure is stage 3 (S3) which is immediately downstream of the plant-bed structure and stage 4 (S4) which is 10 m away from the WTW intake. Stages 1, 3 and 4 are depth sampling sites (in the water column) where samples are taken at three depths each 1, 4 or 5 (depending on the thermocline) and 8 m (Figure 2-C). In S2, from left to right, modules A to D have primarily *Phragmites*. Modules E to G have *Phalaris*. Hence, by species coverage *Phragmites* covers 50% of the total plant-bed and *Phalaris* (23%) and *Carex* (27%) (Figure 1-A). There is only one sampling depth (1m) for each sampling site A to G in S2 (Figure 2-A).

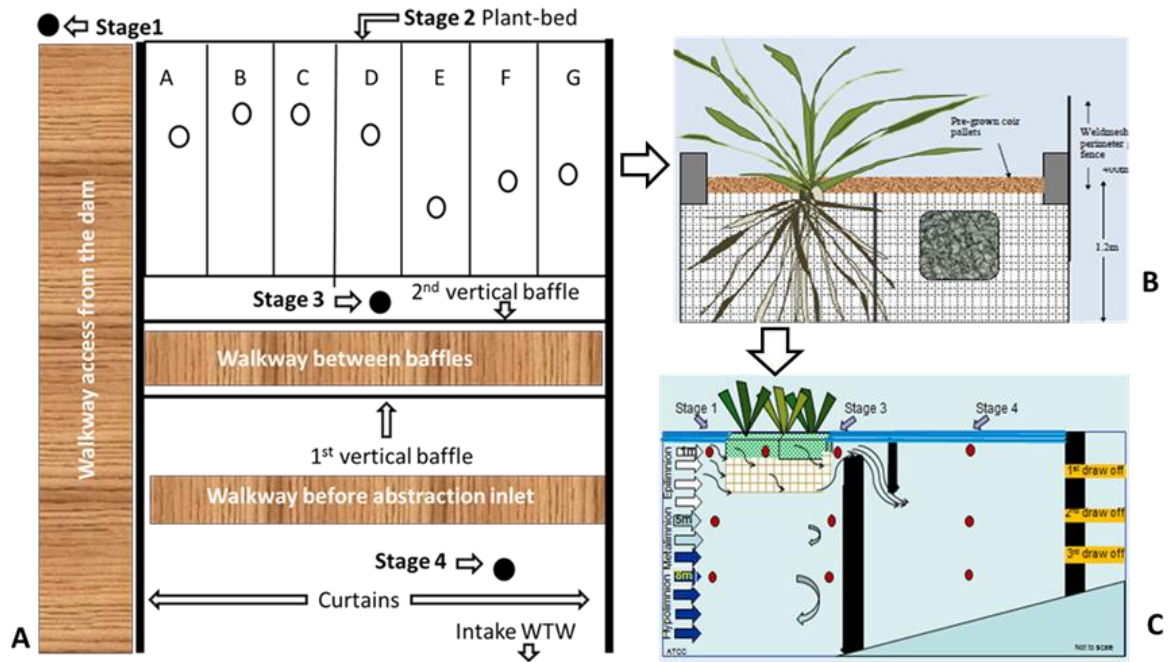


Figure 2. A- A schematic aerial view of the Living-Filter showing the walkways, plant-bed structure, curtains, baffles and stages 1-4. Stages 1, 3 and 4 sampling sites are shown by dark circles. Stage 2 comprises the labelled modules (A to G) and S2 sampling sites are shown by clear circles. **B-** Schematic cross-section showing plant and roots positions in the galvanized cage structure (1.2 m H) (AquaticEngineering, UK consultants unpublished) **C-** Schematic cross-section of the floating plant-bed in relation to the baffles and depth sampling sites for stages 1, 3 and 4. (Not to scale).

2.2. Sampling design and collection

To investigate changes in the physico-chemical and biological variables, 17 weekly surveys (July to October 2013) were carried out at 16 sampling sites (located in stages 1 to 4). The biological variables monitored were zooplankton (identification and biomass) and chlorophyll-a (a surrogate measure of phytoplankton biomass). The scope of sampling at S1, S3 and S4 was to allow investigation of the changes in the water quality upstream and downstream the plant-bed of the Living-Filter. The scope of

sampling within the plant-bed (S2) was to explore differences in water quality between the sampling sites (A to G). Differences in physico-chemical and biological variables between all stages 1, 2, 3, and 4 were investigated via samples taken at 1 m depth, where the average value of the variable for the sites A to G in S2 was taken for the comparisons with S1, S3 and S4 at 1 m depth.

Depth samples were taken at positions based on historical stratification data from TW that were confirmed with data taken during the current survey. Hence, 1 m corresponds to the epilimnion (0-3 m), 4 or 5 m corresponds to the metalimnion and 8-11 m corresponds to the hypolimnion. The thermocline was generally weak with temperature differences of 1°C -1.5°C in the water column from the top (0 m) to the bottom (11 m) of the reservoir.

Dissolved oxygen (DO) and temperature (Ta) (°C) depth profiles were taken with a Hach Intellical LDO rugged sensor from 23rd August to 14th October 2013. Samples for physico-chemical and biological analyses were collected with a Van Dorn Water Sampler (~ 2 L capacity), between 09:00 and 13:00 hours. Samples were pooled and transferred to a plastic container, of approximately 5.5 L. The Van Dorn sampler was deployed at least three times per each depth measurement at stages 1, 3 and 4.

Samples from Stage 2 (sites A, B, C, D, E, F and G) were taken with a Phil Water Sampler. The bottle was refilled 11 times for each sampling site to fill the 5.5 L containers. To facilitate sampling within the roots and fabric-filled cages (Figure 2-B), a square of 20 cm L was cut off from the top face of the galvanized cage and a piece of pipe (15 cm diameter x 50 – 70 cm length) was inserted to create a permanent sampling point. The samples were taken once the bottle passed through the pipe and reached the bottom (1 m depth) of the galvanized stainless steel structure. All samples were kept in

shade conditions until taken for chemical and chlorophyll-a analysis to Spencer House, a United Kingdom Accredited Service (UKAS) laboratory.

2.3 Physico-chemical and chlorophyll-a variables measured

The physico-chemical variables measured and analysed were DO, Ta, pH, chloride (Cl), suspended solids (SS), alkalinity (Alk): iron (Fe), manganese (Mn), aluminium (Al), soluble Fe (sFe), soluble Mn (sMn) and soluble Al (sAl), total oxidised nitrogen (TON), ammonium (NH₄), ammonia (NH₃), nitrite (NO₂), silica (Si), total phosphorus (TP) and soluble reactive phosphorus (SRP), biological (BOD) and chemical (COD) oxygen demand, particulate organic carbon (POC) total organic carbon (TOC), dissolved organic carbon (DOC) and chlorophyll-*a* (Chla). In water and wastewater TON is the sum of nitrate and nitrite. The variable dissolved inorganic nitrogen (DIN) is the sum of the concentrations of nitrate and ammonia.

2.4. Chlorophyll *a* loading and removal efficiency

To investigate whether filtration physical processes for the removal of Chla take place with the Living-Filter, the Chla loading and removal efficiency was calculated. Thames Water provided the daily hydrological flow data used to estimate the weekly Chla loadings. The hydrological flow range was 45 x10³ m³- 90 x10³ m³ (average of 65 x10³ m³).

2.4.1 Chlorophyll *a* loading

Calculations for Chla loading on S2 were made by using Eq. 1 and Eq. 2:

$$\text{Chla loading} = r * V \quad (1)$$

$$r = g \text{ m}^{-3} * t \quad (2)$$

where r is grams of Chla per cubic metre per unit time, $t = (t_2 - t_1)$ in this case week one (w) is subtracted from week two ($w_2 - w_1$), so that time refers to Chla $\text{g m}^{-3} w$ and V is the volume of the plant-bed cage ($V=210 \text{ m}^3$):

The loading difference between S1-S3 and S1-S4, was calculated by using Eq. 1 and Eq. 2, but $V=\text{m}^3$ is the average inflow to the works of each sampled week.

2.4.2 Chlorophyll a removal efficiency

The removal efficiency (RE %) was calculated based on Chla concentration and the volume of the inflow water to the works using Eq. (3), where C_i and C_f (mg L^{-1}) is the initial and final concentration; C_i is upstream of the plant-bed and C_f is Stage 3 or Stage 4

$$\text{RE}\% = \frac{(C_i - C_f)}{C_i} \times 100 \quad (3)$$

2.5 Biological organisms in the Living-Filter system

2.5.1 Plants and root development

To assess the aerial and root plant development of *Phragmites*, *Phalaris* and *Carex*, three individuals of each plant species were randomly chosen 18 months after installation. The length of the aerial shoot from the base to the highest point and the root length from the longest distance from the root apex to the base of the aerial shoot were measured.

2.5.2 Zooplankton identification, quantification and biomass

To identify and estimate zooplankton abundance and biomass, a subsample of 1L from the 5.5 L containers was filtered through a 47 mm Millipore nitrate cellulose membrane of 5 μm pore size, to enable the collection of small rotifers. A vacuum pump was applied at a low pressure differential of 0.3 atm, but stopped when the water had drained completely to avoid damaging their morphology. The membrane was removed

and placed inside a 50 ml Falcon tube. The retained filtrate was rinsed with 1-2 ml (depending on the loading on the membrane) of ultrapure water (UPW) and 0.5 – 1ml of carbonated water added drop by drop as a buffer. Ethanol 74% was then added in a 1:3 ratio (1 part UPW + carbonated water and 3 parts of ethanol). A subsample of 1 ml was obtained with a calibrated automatic volumetric pipette with a wide mouth tip and placed onto the Sedgwick-Rafter chamber (20 mm W x 1 mm D x 50 mm L). All organisms were counted under a Leica Wild 3M stereoscope microscope using x16 ocular for larger organisms. The x40 ocular is used for counting small zooplankton and to take body measurements for biomass estimation. Zooplankton abundance was calculated as the number of organisms per m³. Initially estimated in organisms per L using Eq. 4, where N = number of organisms counted per ml; V_s = Volume of the sample in ml (after re-suspension with UPW and alcohol); and V_f = Volume of reservoir water filtered.

$$n = NV_s/V_f \quad (4)$$

n is multiplied by 1000 to obtain the number of organisms per m³ (Wetzel and Likens, 2000). The zooplankton biomass (*Daphnia* spp., *Cyclops* spp. and *Diaptomus* spp.) was obtained by weighing at least 35-50 individuals of each taxon as described by McCauley (1984).

2.6 Statistical analyses

Data management and exploratory statistical analysis were undertaken using Excel (Microsoft © 2010), Minitab v. 17 and SPSS v. 22 software. Further inferential statistical analyses were used for testing the research hypothesis. Parametric tests were used if data met the required assumptions, otherwise non-parametric tests were used. Statistical comparison of the physico-chemical and biological variables between the

sampling sites at stages S1, S2, S3 and S4 were carried out by using a Paired t-test for the variables that met the parametric assumptions. Non-parametric data were analysed with Friedman's test for repeated measures, where the sampling week was taken as the repeat level factor, and significant findings ($\alpha=0.05$) were followed up with the Wilcoxon sign-rank test applying a Bonferroni correction. The effect size was calculated to estimate the magnitude of the significant effect of the statistic test that was studied. The criterion of interpretation of the effect size is: small effect (<0.3), medium effect (>0.3 and <0.5), large effect (>0.5) (Nakagawa and Cuthill 2007; Field 2009).

3. RESULTS

3.1. Exploring physico-chemical and chlorophyll-a variables in the Living-Filter system

Physico-chemical and Chla variables were measured and analysed upstream and downstream of the plant-bed and within the plant-bed of the Living-Filter to investigate possible changes of these variables induced by the Living-Filter (downstream) when compared to the variables measured upstream. Also to observe changes between the sites within the plant-bed as they were dominated by different species of plants.

The results of DO and Ta measurements taken at each depth (mean \pm SE) for S1, S3 and S4 from 23rd August to 14th October are shown in Figure 3. The results showed the DO decreased with depth at all stages, although there was a considerable decrease ($> 0.5 \text{ mg L}^{-1}$) at specific depths, at five metres in S1, S3 and in S4 at five and seven metres.

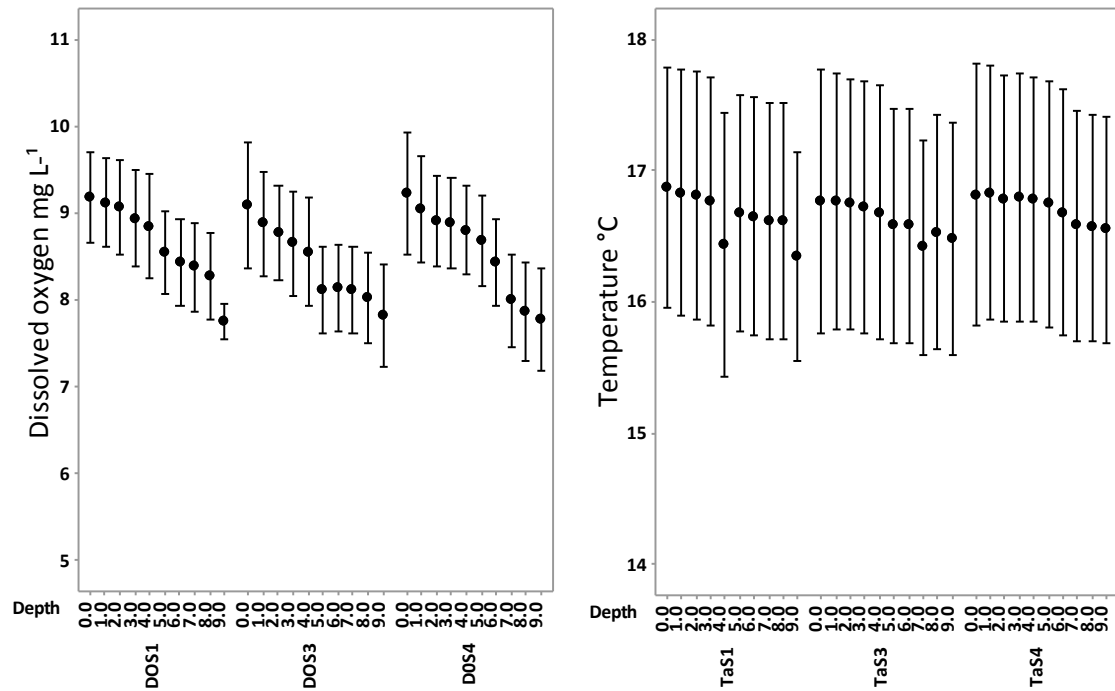


Figure 3. Interval bars (mean \pm SE) for dissolved oxygen (DO) (mg L^{-1}) and temperature (Ta) ($^{\circ}\text{C}$) depth profiles (Depth) of the water column at stages S1, S3 and S4 from 23rd August to 14th October ($n=60$)).

The results (mean \pm SE) for S1, S2, S3 and S4 for selected variables (Fe, Mn, sFes, Mn, SS, pH, COD and BOD) showing significant variation in their mean, are shown in Figure 4. The mean of Mn and Fe for S1, S3 and S4 is smaller than most sites in S2, whilst the mean for sMn in S1 is higher than the means of the other stages. Suspended solids, BOD and COD means are highest at site E.

Figure 5 shows the interval plots of the mean \pm SE for DIN, NO₂, NH₄, TP, TOC, DOC, POC and Chla. This figure shows higher means for TP, POC and Chla in S2 compared to S1, S3 and S4 and low NH₄ mean concentration in S2E and S2F.

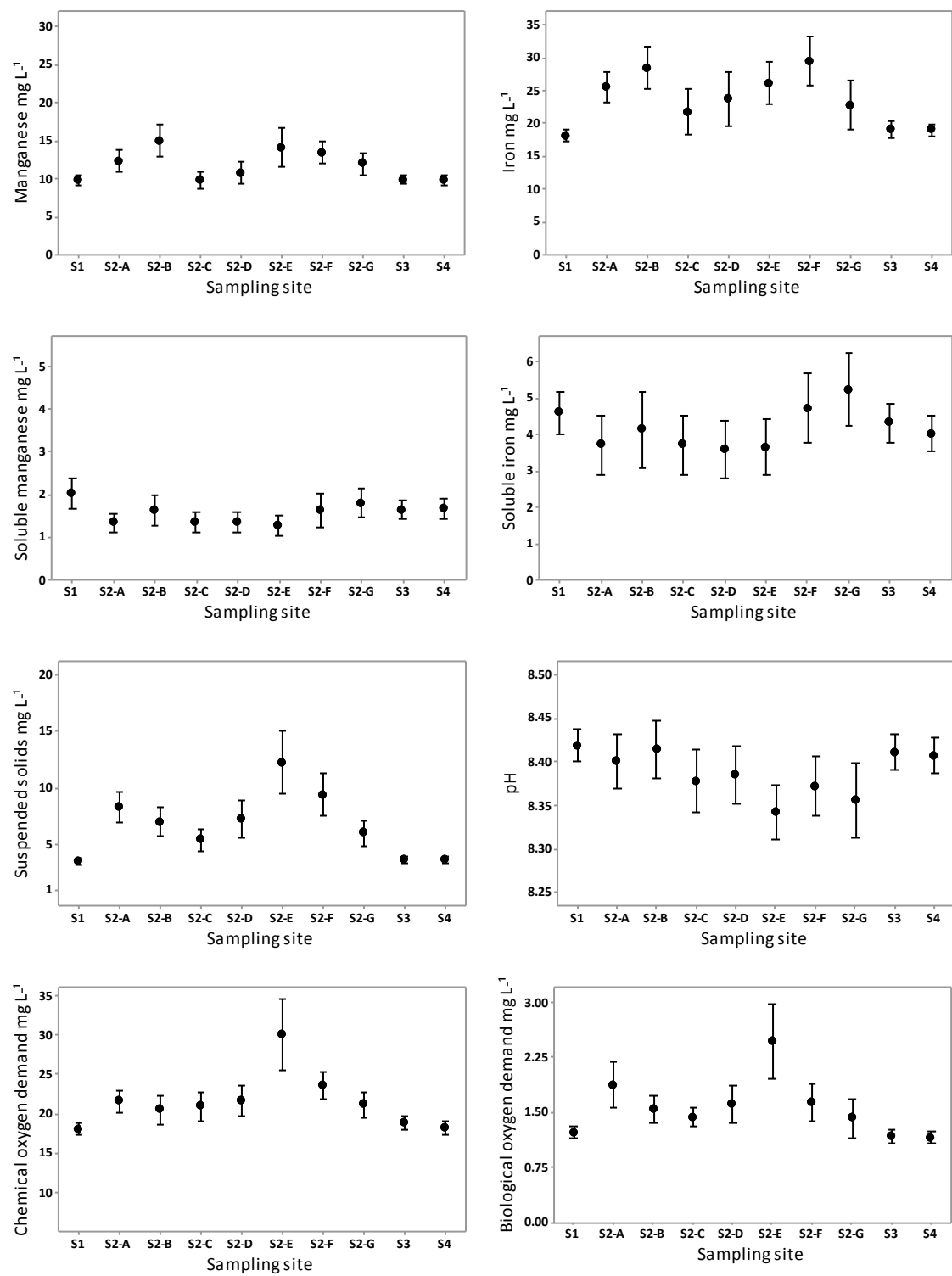


Figure 4. Interval bars (mean \pm SE) for manganese, iron, soluble manganese, soluble iron, suspended solids, pH, chemical and biological oxygen demand. Stages S1, S3 and S4 include all sampling sites (min. $n=46$; max. $n=51$). The interval bars are for each site at S2 (A, B, C, D, E, F, G) (min. $n=15$; max. $n=17$).

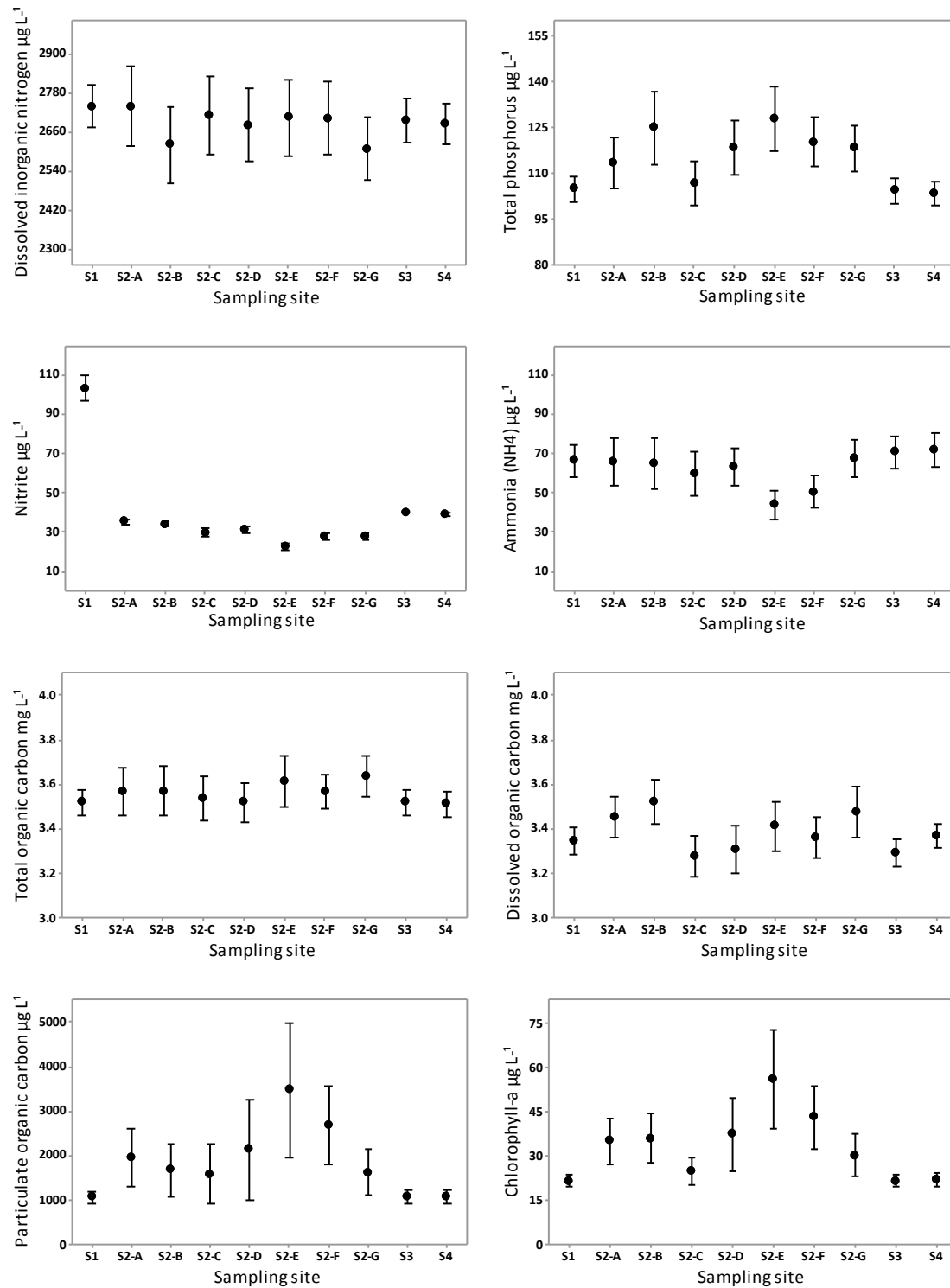


Figure 5. Interval bars (mean \pm SE) for dissolved inorganic nitrogen, total phosphorus, nitrite, ammonium, total, dissolved and particulate organic carbon and chlorophyll-a. Sampling sites S1, S3 and S4 include all depths (min. $n=46$; max. $n=51$). The interval bars are for each site at S2 (min. $n=15$; max. $n=17$).

Table 1 summarizes the significant statistical results for the tests of paired differences and repeated measures. Dissolved oxygen was significantly different between S1, S3 and S4 (data from all depths), with $S1 > S3$ and $S1 > S4$. At 1 m depth, significant differences in DO occurred between most sites, showing a reduction in DO levels in S2 compared to S1 and S4, and with both results having a large effect size. Significant temperature differences between S1, S3 and S4 showed that temperature at S3 was reduced compared to S1, with a small effect; and it was also reduced at S3 compared to S4 (medium effect). Significant differences upstream and downstream of the plant-bed were found between S1-S3 for sMn, where S1 scores were higher than S3.

Also significant were the differences between S1, S3 and S4 for the variables DIN and NO_2 where S1 was higher than S3 and S4. Ammonium was higher at S4 than S1. The t-test and Wilcoxon sign-rank test revealed a significant increase in Fe, Mn, SS, BOD, COD, TP, POC and Chla in S2 compared to S1, S3 and S4; whilst pH was significantly lower in S2, both with a large effect.

Table1. Comparison of physico-chemical and biotic variables between sites sampled at the Living-Filter: upstream and downstream of the plant-bed and within the plant-bed (average of sites A to G). The results from the test used (Matched-pair *t*-test, Friedman's and Wilcoxon sign-rank test), level of significance, degrees of freedom, number of samples and effect size are included.

Living-Filter System	Site / Paired differences	Measured Variable	Paired t <i>t</i>	Friedman's χ	Wilcoxon's sign-rank <i>T</i> <i>z</i>		Significance <i>p</i> <i>df</i>			Effect size <i>n</i> <i>r</i>	
Up & down stream Depths (0-9m)	Stages S1-S3-S4	DO		49.45			0.000	<0.05	2	60	
	S1-S3				3	-5.75	0.000	<0.017		60	0.52
	S1-S4				15	-2.53	0.005	<0.017		60	0.23
	Stages S1-S3-S4	Temp.		21.98			0.000	<0.05	2	60	
	S1-S3				11	-2.69	0.003	<0.017		60	0.25
	*S3-S4				4	-4.24	0.000	<0.017		60	0.39
Depth (1m)	All stages										
	S1-S2	DO	3.96				0.011	<0.05	5	6	0.87
	S2-S4		-3.38				0.020	<0.05	5	6	0.83
	S1-S2	Fe	-4.28				0.001	<0.05	16	17	0.73
	S2-S3		4.13				0.001	<0.05	16	17	0.71
	S2-S4		5.14				0.000	<0.05	16	17	0.79
	S1-S2	Mn	-4.15				0.001	<0.05	16	17	0.72
	S2-S3		4.6				0.000	<0.05	16	17	0.65
	S3-S4		4.87				0.000	<0.05	16	17	0.58
	S1-S3	sMn	2.05				0.046	<0.05	47	48	0.29
	S1-S3	DIN	3.13				0.002	<0.05	49	50	0.41
	S1-S4		4.66				0.000	<0.05	49	50	0.55
	S1-S3	NO ₂	9.74				0.000	<0.05	48	49	0.81
	S1-S4		9.66				0.000	<0.05	48	49	0.81
	S1-S4	NH ₄	-2.74				0.008	<0.05	49	50	0.36

(Continues)

Living-Filter System	Site / Paired differences	Measured Variable	Paired t <i>t</i>	Friedman's χ	Wilcoxon's sign-rank		Significance			Effect size	
					<i>T</i>	<i>z</i>	p-value	<i>p</i>	<i>df</i>	<i>n</i>	<i>r</i>
	S1-S2	SS	-3.57				0.003	<0.05	16	17	0.67
	S2-S3		4.07				0.001	<0.05	16	17	0.71
	S2-S4		4.01				0.001	<0.05	16	17	0.70
	S1-S2	pH	2.72				0.015	<0.05	16	17	0.56
	S2-S3		-2.69				0.016	<0.05	16	17	0.56
	S2-S4		-2.54				0.023	<0.05	15	16	0.55
	S1-S2	BOD	-2.83				0.013	<0.05	15	16	0.59
	S2-S3		3.22				0.006	<0.05	15	16	0.64
	S2-S4		4.32				0.001	<0.05	15	16	0.74
	*S1-S2	COD			2	-3.48	0.000	<0.007	16	17	0.60
	S2-S3				4	-2.82	0.002	<0.007	16	17	0.48
	*S1-S2				3	-3.34	0.000	<0.007	16	17	0.57
	S2-S3	TP			3	-3.34	0.000	<0.007	16	17	0.57
	S2-S4				2	-3.48	0.000	<0.007		17	0.60
	*S1-S2				0	-3.62	0.000	<0.007		17	0.62
	S2-S3	POC			1	-3.57	0.000	<0.007		17	0.61
	S2-S4				1	-3.64	0.000	<0.007		17	0.62
	*S1-S2				4	-2.64	0.003	<0.007		17	0.45
	S2-S3	Chla			3	-2.91	0.001	<0.007		17	0.50
	S2-S4				4	-2.68	0.003	<0.007		17	0.46

Significant differences between the sites A, B, C, D, E, F and G on the plant-bed were found for D.O (Table 2). Site E showed the lowest DO with a large effect size. Manganese was higher at site C than A, B and F, all with a medium effect size. Site E was lower in pH than sites A, C and F, with medium to large effect size. Suspended solids at Site C was lower when compared to A, B and F with medium to high effect. Nitrite was higher at sites A, B and C than D, E, F and G; site E was the lowest for NO₂. Site E had higher significant POC than the other sites, with a large effect size for most of these results.

Table 2. Comparison of physico-chemical variables between sites sampled at the plant-bed of the Living-Filter. The results from the test used (Matched-pair *t*-test, Friedman's and Wilcoxon sign-rank test), level of significance, degrees of freedom, number of samples and effect size are included.

Site Paired differences	Measured Variable	Paired t t	Friedman's χ	Wilcoxon's sign-rank T	z	p-value	Significance p	df	n	Effect size r
Stage 2	DO									
A-E		3.4				0.019	<0.05	5	6	0.84
B-E		3.51				0.017	<0.05	5	6	0.84
C-E		2.95				0.032	<0.05	5	6	0.80
D-E		3.69				0.014	<0.05	5	6	0.47
E-F		-5.32				0.003	<0.05	5	6	0.61
E-G		-3.45				0.018	<0.05	5	6	0.44
Stage 2	Mn		15.26			0.018	<0.05	6	16	
A-C				2	-2.53	0.005	<0.007		16	0.45
B-C				2	-2.99	0.001	<0.007		16	0.53
*C-F				2	-2.52	0.004	<0.007		16	0.45
Stage 2	pH		14.22				<0.05	6	16	0.00
A-E				4	-2.96	0.001	<0.007		16	0.52
C-E				5	-2.64	0.003	<0.007		16	0.47
C-G				3	-2.69	0.003	<0.007		16	0.48
*E-F				3	-2.53	0.005	<0.007		16	0.45
Stage 2	SS		22.54				<0.05	6	14	
A-C				3	-2.64	0.003	<0.007		16	0.47
B-C				3	-2.74	0.005	<0.007		15	0.50
*C-E				3	-2.89	0.001	<0.007		16	0.51

(Continues)

Site	Measured	Paired t	Friedman's	Wilcoxon's sign-rank	Significance				Effect size				
Paired differences	Variable	t	χ	T	z	p-value	p	df	n	r			
*C-F	NO ₂		34.31	2	-2.61	0.003	<0.007		16	0.43			
*D-E				3	-2.84	0.001	<0.007		16	0.50			
E-G				4	-2.59	0.004	<0.007		17	0.44			
Stage 2							<0.05	6	15				
A-D				2	-2.7	0.002	<0.007		17	0.46			
A-E				0	-3.52	0	<0.007		16	0.52			
A-F				2	-3.39	0	<0.007		17	0.58			
A-G				0	-3.4	0	<0.007		16	0.60			
B-E				2	-3.01	0.001	<0.007		15	0.55			
B-F				4	-2.51	0.005	<0.007		16	0.44			
B-G				3	-2.76	0.002	<0.007		16	0.49			
C-E				3	-2.5	0.005	<0.007		15	0.46			
D-E				2	-3.34	0	<0.007		17	0.57			
E-F				4	-2.68	0.005	<0.007						
Stage 2				POC		29.89				<0.05	6	16	
*A-E	3	-2.96	0.001				<0.007		17	0.51			
*B-E	4	-2.38	0.008				<0.007		16	0.42			
*C-E	2	-3.15	0				<0.007		16	0.56			
*C-F	2	-3.1	0				<0.007		16	0.55			
*C-G	4	-2.48	0.005				<0.007		16	0.44			
*D-E	2	-3.01	0.001				<0.007		17	0.52			
E-F	3	-2.72	0.002				<0.007		17	0.47			
E-G	2	-2.89	0.001				<0.007		16	0.51			
Stage 2	SRP		16.51						0.011	<0.05	6	15	
B-D							4	-2.39	0.007	<0.007		15	0.44
B-E							4	-2.79	0.002	<0.007		15	0.51

<0.007 and <0.017 are one-tailed level values of significance after the Bonferroni correction. *the test was based on the negative ranks

3.2 Chlorophyll loading and removal efficiency

3.2.1 Chlorophyll loading

The loading of Chla followed a seasonal trend for Chla productivity. High Chla loads were seen from 19th August (week 8) until 23rd September (week 13), with a maximum load in week nine (Figure 6).

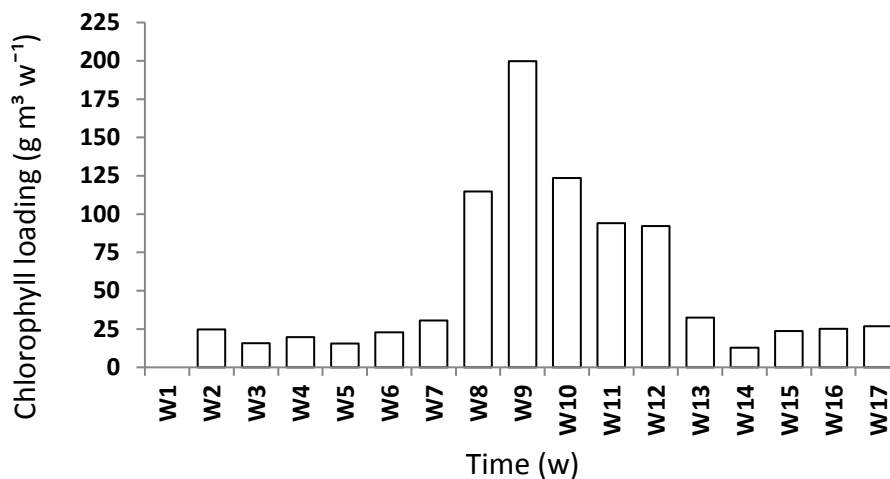


Figure 6. Weekly (w1-w17) chlorophyll-a loading onto the plant-bed of the Living-Filter from 2nd July (w1) to 31st October (w17).

3.2.2 Chlorophyll Removal Efficiency

Results for the removal efficiency (RE%) of chlorophyll-*a* was up to 45% between S1-S3 and up to 40% between S1-S4, during the first seven weeks of the survey. After this period, some RE% was observed (on only five occasions). There were three large negative removal efficiencies or releases ($\geq 90\%$) of Chla biomass, when Chla was higher downstream than upstream. The first large release corresponded to the start of September, which was the period when the first cyanobacteria bloom was observed. The other two releases were observed six weeks later at the end of summer. In the context of a filtration process, similar events take place when there is a breakthrough the filter resulting in a) no retention or b) the retained particles are

resuspended (one event after week seven and two between S1-S4 and also S1-S3 in week 13 (Figure 7).

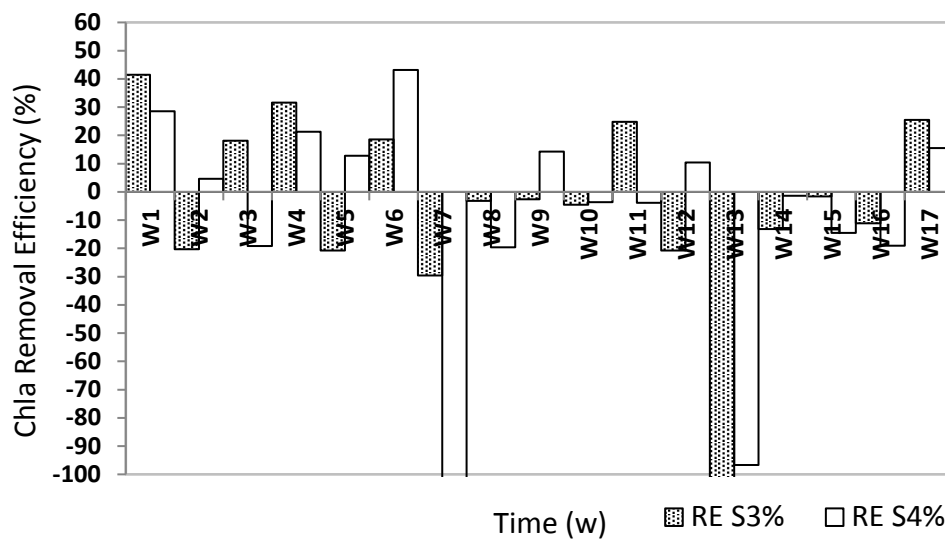


Figure 7. Weekly (w1-w17) chlorophyll-a removal efficiency (%) at Stage 3 (RE S3%) and Stage 4 (RE S4%) from 2nd July to 31st October.

3.3. Biological variables

3.3.1 Assessment of the plants and root development

Three individual plants from each of the three species were sampled. The size of the plant's aerial shoots was 0.50-0.70 m when first positioned on the cages. *Phragmites* showed the tallest aerial shoots with shortest roots. *Carex* roots were the longest, and *Phalaris* roots showed greater density and abundant fine roots (Table 3).

Table 3. Aerial shoot and root length (mean±SE) showing dimensions of the plant species sampled (n=3) from the Living-Filter plant-bed in 2013 and 2014.

Plant species	Aerial plant high (m)		Root (m)
	2013	2014	2014
	mean±SE	mean±SE	mean±SE
<i>Phragmites</i>	1.66 ±0.24	2.08±0.26	0.15±0.08
<i>Phalaris</i>	1.53±0.12	1.1±0.36	1.5±0.15
<i>Carex</i>	1.1±0.23	1.4±0.18	1.74±0.12

The plant species coverage (m²) on the plant-bed is shown for years 2012 and 2013 in Table 4, with most of the lost plants in 2013 being from the side of the structure facing the open water.

Table 4. Plant species coverage (m²) on the plant-bed structure for years 2012 and 2013.

	Year 2012	Year 2013
	(m ²)	(m ²)
Plant coverage	179	115
<i>Phragmites</i>	90	45
<i>Phalaris</i>	41	26
<i>Carex</i>	48	44

3.2.2 Zooplankton composition, abundance and biomass

A total of 22 taxa of zooplankton and aquatic organisms were identified. The juvenile phases (Nauplii) of *Cyclops* spp. and *Diaptomus* spp. were included in the analysis of the zooplankton composition (Table 5).

Table 5. Relation of the zooplankton taxa (genera, order or phyla) or aquatic organism ranked by abundance from the water column (Stage 1, 3 and 4) and the plant-bed (S2).

S1, S3 and S4	Rank.	Plant-bed (S2)
<i>Daphnia spp.</i>	1	<i>Cyclops spp.</i>
<i>Keratella spp.</i>	2	<i>Daphnia spp.</i>
<i>Nauplii small</i>	3	<i>Keratella spp.</i>
<i>Cyclops spp.</i>	4	<i>Chydorus spp.</i>
<i>Diaptomus spp.</i>	5	<i>Nauplii small</i>
<i>Asplanchna spp.</i>	6	<i>Diaptomus spp.</i>
<i>Nauplii large</i>	7	<i>Nauplii large</i>
<i>Polyarthra spp.</i>	8	<i>Nais</i>
Oligochaeta	9	<i>Asplanchna spp.</i>
<i>Brachionus spp.</i>	10	<i>Canthocamptus</i>
Mite	11	Ostracoda
<i>Canthocamptus spp.</i>	12	<i>Polyarthra spp.</i>
Mosquito larvae	13	Mite
<i>Bosmina spp.</i>	14	Mosquito larvae
<i>Chydorus spp.</i>	15	<i>Gammarus spp.</i>
Bryozoa	16	<i>Bryozoa</i>
Ostracoda	17	<i>Bosmina spp.</i>
Nematode	18	<i>Brachionus spp.</i>
Chrionomid	19	Chrionomid
	20	Mollusca
	21	<i>Limnae spp.</i>

The relative abundance of the identified taxa was compared between stages S1, S3, S4 and S2. *Daphnia* spp. was the most abundant species at S1, S3 and S4 and *Cyclops* spp. was most abundant at S2 (Figure 8), the organisms that correspond to the numbered taxa in Figure 8 can be found in Table 5.

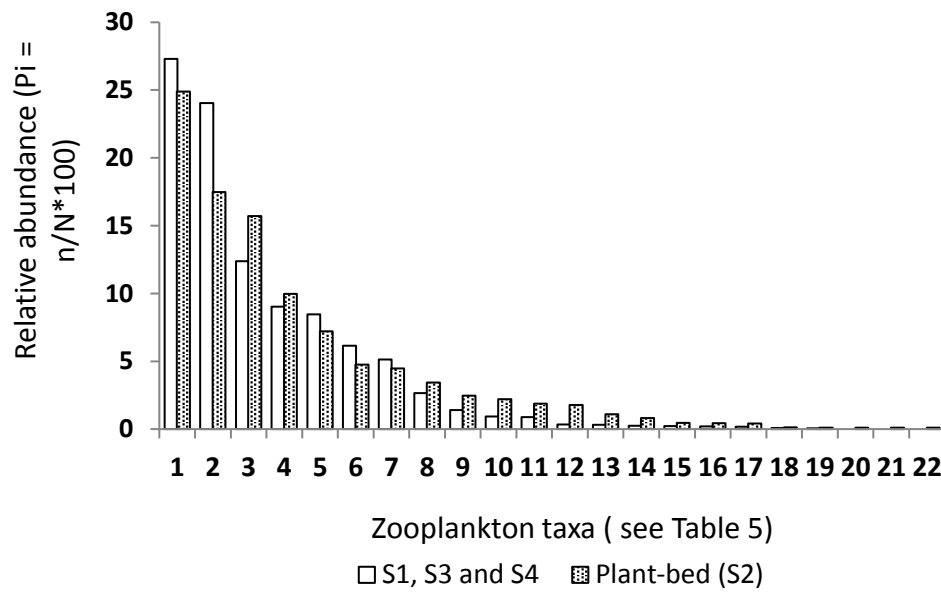


Figure 8 Relative abundance (%) of zooplankton and aquatic organisms, $P_i = n/N \cdot 100$, zooplankton taxa (see Table 5).

Dispersion of the zooplankton taxa (Relative abundance %) at 1, 5 and 8 m depths from stages S1, S3 and S4 (Figures 9, 10 and 11) is shown according to Table 5.

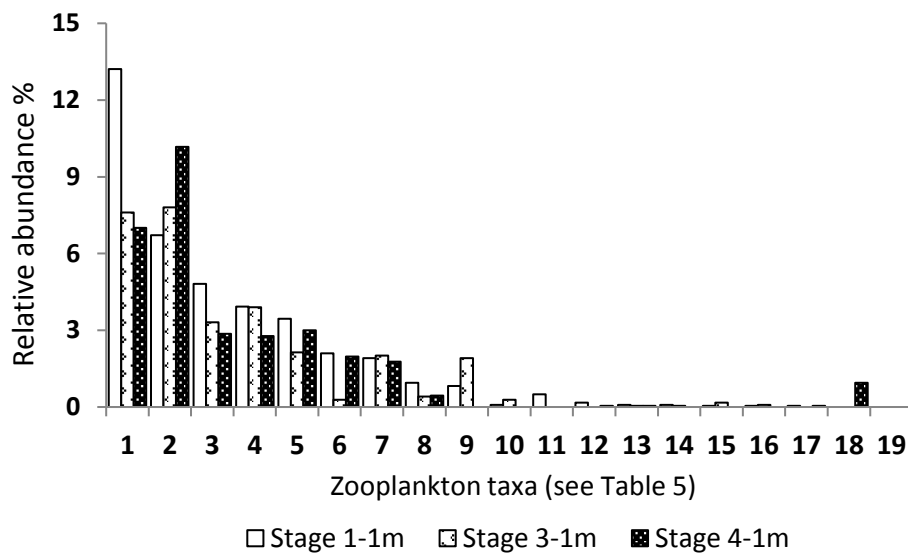


Figure 9. Dispersion of the zooplankton taxa (%) at 1m depth at S1, S3 and S4.

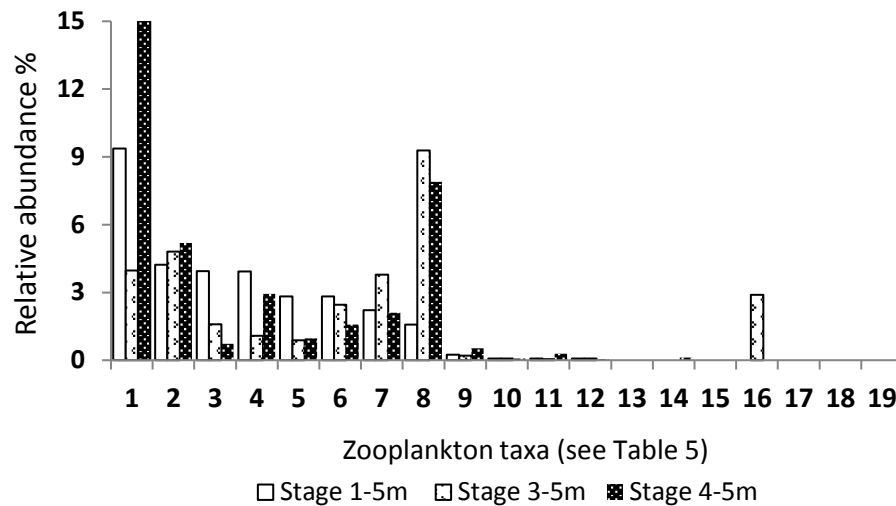


Figure 10. Dispersion of the zooplankton taxa (%) at 5m depth at S1, S3 and S4.

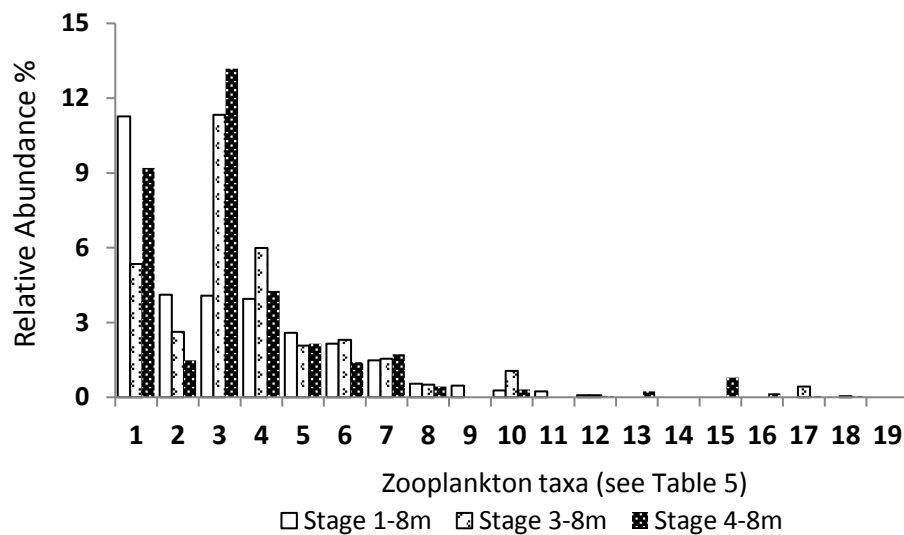


Figure 11. Dispersion of the zooplankton taxa (%) at 8m depth at S1, S3 and S4

Daphnia spp. and *Cyclops* spp. were significantly more abundant at S1, with a medium effect size for the differences S1-S3 (0.36) and a small effect size for S1-S4 (0.25) (Table 5). Statistically significant differences were found with the Friedman's test between the sampling sites of S2 for *Daphnia* spp., *Keratella* spp. and *Canthocamptus* spp. The paired differences of sites E-F were significant with a large effect size. No significant differences were found between other pairs with the Wilcoxon sign-test. (Table 6)

Table 6. Comparison of zooplankton abundance between sites of the Living-Filter, upstream and downstream of the plant-bed and within the plant-bed. Results for Friedman's and Wilcoxon sign-rank test, level of significance, degrees of freedom, number of samples and effect size are included.

Living-Filter	Site / Pair differences	Measured Variable	Friedman's χ	Wilcoxon's sign-rank			Significance			Effect size r
				T	z	p -value	p	df	n	
Upstream & Downstream Depths (0-9m)	Stages S1-S3-S4	<i>Daphnia</i> spp.	26.16			0.000	<0.05	2	45	
	S1-S3			5	-3.37	0.000	<0.017		45	0.36
	S1-S4			9	-2.33	0.009	<0.017		45	0.25
	Stages S1-S3-S4	<i>Cyclops</i> spp.	19.22			0.000	<0.05	44	45	
	S1-S3			7	-3.56	0.000	<0.017		45	0.37
	S1-S4			9	-2.96	0.001	<0.017		45	0.31
Plant-bed	Stage 2	<i>Daphnia</i> spp.	12.99			0.043	<0.05	6	14	
	E-F			0	-2.81	0.001	<0.007	6	14	0.54
	Stage 2	<i>Keratella</i> spp.	15.12			0.019	<0.05	6	14	
	Stage 2	<i>Canthocamptus</i> spp.	15.66			0.016	<0.05	6	14	

A decline in zooplankton numbers can be seen following a seasonal trend. Drastic fluctuations are observed at S1, S3 and S4 (w6) and (w13-14) (Figure 12).

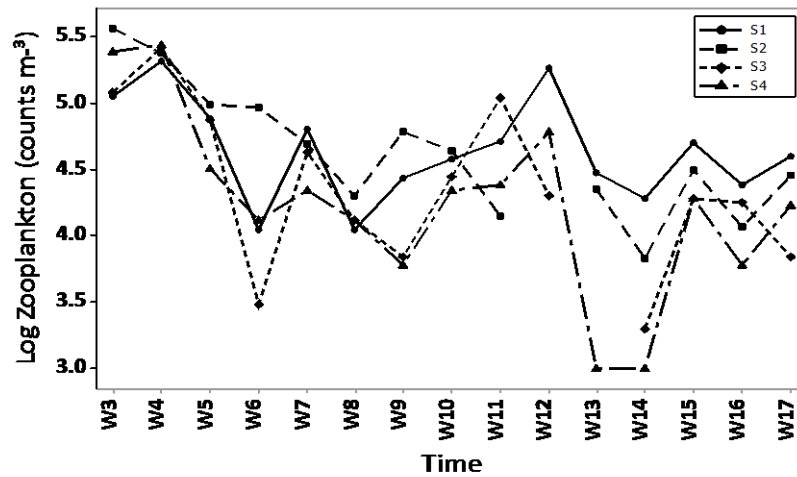


Figure 12. Time series of logarithm counts of zooplankton per cubic metre (m^{-3}) for 15 weeks at 1 m depth from 22nd July to 31st October 2013. S1=continuous line, S2=dashed line, S3=dotted line and S4=dashed-dotted line.

No significant relationships were found between the zooplankton biomass (*Daphnia* spp, *Cyclops* spp. and *Diaptomus* spp.) and Chla biomass Figure 13.

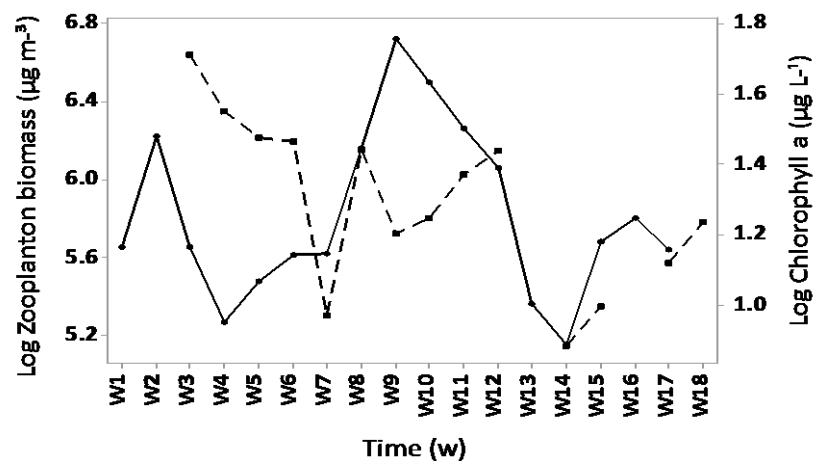


Figure 13. Time series of the logarithm for average zooplankton biomass (dashed line) ($\mu\text{g m}^{-3}$) and of chlorophyll-a (continuous line) ($\mu\text{g L}^{-1}$) at S3.

4. DISCUSSION

Thames Water and Aquatic Engineering designed and installed the Living-Filter, a novel hydroponic, floating emergent treatment wetland, with the aim of reducing the phytoplankton biomass prior to the water treatment works intake, mainly by physical filtration processes and by providing refuge for zooplankton to increase the grazing pressure on phytoplankton.

4.1 Were there physico-chemical and chlorophyll-*a* differences upstream and downstream the water entered the plant-bed of the Living-Filter?

Surveys of the water column were undertaken to identify physico-chemical changes induced by the Living-Filter upstream (S1) and downstream (S3 and S4) of the plant-bed (S2).

Significant differences for DO, temperature, pH, sMn, DIN, NO₂ and NH₄ were found indicating the Living-Filter may have induced these physico-chemical differences between the stages. The differences in DO, higher in S1 (all depths) may be related to the demand of oxygen for biochemical processes in S2. The lower temperatures in S3 may be a consequence of the shading effect on the water column by the plant-bed (Table 1). Furthermore, at 1m depth it was found that S2 differed from the other stages by a significant decrease in DO and increased COD and BOD (Table 1). This provides further evidence that biochemical processes are taking place: either biochemical activity originating from the roots themselves, or the biofilm attached to the roots and fabric, or the organisms within the rhizosphere, or a combination of all these. As found by Kyambadde *et al.* (2005) and Osem *et al.* (2007), these activities could be related to the uptake of nitrogen and denitrification/nitrification processes. In this study DIN and NO₂ are significantly higher upstream of the plant-bed, as well as sMn. The increase in NH₄ in S4

might be related to biochemical processes enhanced by the increased temperature in S4. This increase in temperature should be monitored further and addressed in future designs to prevent promoting conditions for phytoplankton growth. The quality of the water supplied to the WTW was not affected by the Living-Filter installation during the period of study.

The significant increase in SS, TP, POC and Chla in S2 (Table 1), with particularly high means at site E and F for Chla and POC (Figure 5), strongly suggests that the plant-bed has a role at retaining particulates. These results indicate that the increase in particulates may be phytoplankton cells, as Chla is also increased. Furthermore, the increase in TP could be related to the retention of cyanobacteria cells, which have a role in the cycling of nitrogen and phosphorus in water bodies and store polyphosphate granules in their cells (Cottingham *et al.*, 2015).

4. 2 Were there physico-chemical and chlorophyll-a differences within the plant-bed?

Surveys within the plant-bed (S2) were undertaken to investigate whether physico-chemical differences could relate to the coverage of the plant species.

Physico-chemical differences were found between sites in S2. Site E is markedly different from other sites (Figures 4 & 5), with statistically significant low DO, pH, NO₂, POC and SS (Table 2). The site is the furthest sampling point away from where the inflowing water first enters the plant-bed, and is surrounded by *Carex acutiformis* plants (Figure 2-A). *Carex acutiformis* roots are the longest with well-developed plants above the water (Table 3). Site F, close to *Phalaris arundinacea* and *Carex acutiformis*, showed similar means to site E for SS, POC and Chla. *Carex acutiformis* and *Phalaris arundinacea* covered about 50% of the plant-bed in 2012, however 8.3% of *Carex acutiformis* and 36.5% of *Phalaris arundinacea* was lost in 2013 (Table 4). Site C showed higher Mn, pH,

SS and POC and sites A and B showed higher significant differences for NO₂ and SRP. These sites are surrounded by *Phragmites*, which showed a well-developed aerial shoot and rhizome, but root length of less than 20 cm. *Phragmites* plants lose about 70% in root biomass over winter, which are regrown during Spring/Summer, providing little density/surface area for particle trapping or biofilm attachment. Initially *Phragmites australis* covered 50% of plant-bed, but 50% of the plants were lost in 2013 (Table 4). Nevertheless, the growth of the plants on the Living-Filter indicates that the influx of nutrients to the reservoir water suffices their nutritional requirements. The annual TP in eutrophic Farmoor II reservoir is $\geq 80 \mu\text{g L}^{-1}$. These results indicate that future designs should include protection of the plants on the windward periphery exposed to erosion effect caused by the waves.

4.3 Filtration and removal of chlorophyll-*a* by the Living-Filter

To investigate whether physical filtration processes for the removal of Chla (as a surrogate of phytoplankton biomass) can take place within the Living-Filter, Chla loading and removal efficiency were estimated.

Results for the Chla removal efficiency (<45%) were very promising in the first seven weeks of the survey when the loadings were low (Figure 6). The Chla loading doubled between weeks 8 and 12, which could have overloaded the plant-bed system. As the phytoplankton cells accumulate, the removal efficiency is initially enhanced as the size of the macro-pores between the roots/fibres of the fabric is reduced causing the flow to short-circuit along the less resistant root systems (Knowles *et al.*, 2011). However, the strength of attachment of the trapped and adhered particles will also depend on the shear forces of the inflowing water, hence high flow velocities are likely to cause greater shear stress and the trapped and adhered particles become detached. There appears to be a six-

week cycle of Chla releases (Figure 7), however, two cycles are insufficient to confirm if these observations are related to physical filtration processes.

The Living-Filter partially fulfilled its aim by showing removal figures for almost half of the period of study, but results lead to other questions: 1) Is the Living-Filter the right size for this reservoir based on Chla load and the inflow of water to the WTW? 2) Is the flow velocity too high? The Living-Filter flow velocity of 10-20 m h⁻¹ compared to a slow sand filter flow velocity 0.2-0.6 m h⁻¹, a known and effective pre-treatment process also based on ecological trophic processes. 3) Is the Living-Filter appropriate for the reservoir's depth? Similar floating emergent treatment wetlands are generally operated in water bodies less than 2-3 m depth. As the system matures, it is expected that the roots will grow deeper into the water column increasing the likelihood of physical filtration processes.

4.4 Has the Living-Filter provided refuge for zooplankton?

Zooplankton samples from the water column and the plant-bed were analysed in an attempt to answer this question.

Daphnia spp., a generalist cladoceran organism, is the most abundant taxon in the water column, whilst *Cyclops spp.*, a specialist copepod (Ger *et al.*, 2014), is the most abundant taxon within the plant-bed (Figure 8). Although statistically *Daphnia spp.* was more abundant in site E than F on the plant-bed, results showed that *Daphnia spp.* and *Cyclops spp.* were significantly more abundant upstream or away from the plant-bed (S1), where there is no root coverage for refuge. Although these were unexpected results, other authors have found a similar pattern which is known as the “shore-avoidance” hypothesis (Hulsmann *et al.*, 1999). Zooplankton avoid diurnal planktivory by migrating horizontally away from the rich macrophyte shore, however, other researchers have also found the

opposite pattern (Moss *et al.*, 1991). Moreover, Schou *et al.* (2009) using floating plastic plants in cages demonstrated differences in the diurnal horizontal migratory pattern for *Daphnia* spp. and *Cyclops* spp. in a eutrophic lake. They found higher abundances of *Daphnia* spp. in the open water whilst *Cyclops* spp. was in the pelagic zone, but *Cyclops* spp. aggregated at night within the cages. The Living-Filter is installed in a eutrophic reservoir and our results might have been influenced by the time of sampling (9:00-13:00). However, the abundance trend of the zooplankton community from each stage (S1, S2, S3 and S4) showed the most stable community abundance was within the plant-bed (S2), despite the downward temporal trend observed for each stage as the surveys approached autumn (Figure 12). Zooplankton communities able to graze on phytoplankton and cyanobacteria include four major groups: rhizopods, ciliates, rotifers and crustacean (Gerphagnon *et al.*, 2015), and of these, rotifers and crustaceans were identified in this study (Table 3) and their vertical distributions are presented in Figures 9, 10 and 11. These initial results suggest that the Living-Filter might be providing refuge for the zooplankton. However, further analysis on their spatial distribution with surveys observing their migratory patterns will be necessary to further support these observations.

4.5 Conclusions

The Living-Filter, as a novel floating emergent treatment wetland used as a pre-treatment process for the reduction of phytoplankton biomass at a WTW intake, is a promising technology. Results showed that up to 45% of chlorophyll-a (as surrogate of phytoplankton biomass) was removed during the first seven weeks of the study indicating that the Living-Filter partially fulfilled expectations as a pre-treatment biofilter. The Living-Filter appeared to behave as a saturated filter with some releases after this period.

The zooplankton community appeared to benefit by the refuge provided by the rhizosphere of the Living-Filter with stable abundances within the plant-bed. Although *Daphnia* spp. and *Cyclops* spp. abundances were significantly higher before the Living-Filter plant bed these results might have been influenced by behavioural patterns in eutrophic waters which need further investigation.

The plant selection for future Living-Filter designs should include species with the potential to develop dense and lengthy roots, and therefore, *Carex acutiformis* and *Phalaris arundinacea* are preferred to *Phragmites australis*.

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