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Impact of treated wastewater reuse and floods on water quality and fish health within a water reservoir in an arid climate

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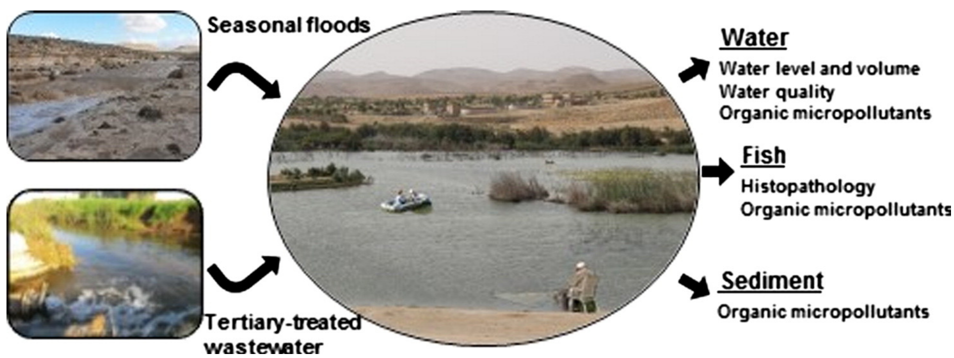
HIGHLIGHTS

- Treated wastewater (TWW) and floods are used to feed water bodies in arid regions.
- Water quality in Yeruham Reservoir (southern Israel) was mainly affected by floods.
- Organic micropollutant levels in water and sediments were low.
- Fish from the Yeruham Reservoir were healthy.
- Water reservoirs in rural arid regions may provide new economic benefits.

GRAPHICAL ABSTRACT



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ABSTRACT

Treated wastewater (TWW) reuse for agricultural irrigation is a well-established approach to coping with water shortages in semi-arid and arid environments. Recently, additional uses of TWW have emerged, including streamflow augmentation and aquatic ecosystem restoration. The purpose of the current study was to evaluate the water quality and fish health, in an artificial reservoir located in an arid climate (the Yeruham Reservoir, Israel), which regularly receives TWW and sporadic winter floods. The temporal distribution of water levels, nutrients and organic micropollutants (OMPs) were measured during the years 2013–2014. OMPs were also measured in sediment and fish tissues. Finally, the status of fish health was evaluated by histopathology. Water levels and quality were mainly influenced by seasonal processes such as floods and evaporation, and not by the discharge of TWW. Out of 16 tested OMPs, estrone, carbamazepine, diclofenac and bezafibrate were found in the reservoir water, but mostly at concentrations below the predicted no-effect concentration (PNEC) for fish. Concentrations of PCBs and dioxins in fish muscle and liver were much lower than the EU maximal permitted concentrations, and similar to concentrations that were found in food fish in Israel and Europe. In the histopathological analysis, there were no evident tissue abnormalities, and low to moderate infection levels of fish parasites were recorded. The results from the Yeruham Reservoir demonstrated a unique model for the mixture effect between TWW reuse and natural floods to support a unique stable and thriving ecosystem in a water reservoir located in an arid region. This type of reservoir can be widely used for recreation, education, and the social and economic development of a rural environment, such as has occurred in the Yeruham region.

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1. Introduction

Water quantity and quality are major factors controlling ecosystem functions and ecosystem services, which are defined as the benefits that humans can obtain from ecosystems (Boyd and Banzhaf, 2007). In water-stressed environments, supplying water for drinking and food production has always been a high priority. However, water resource management policies to control floods and to supply cultural services have also been widely applied. Many of the abovementioned uses of water require water ponding for storage. The increasing trends of water reuse and human impact on aquatic ecosystems challenge the multiple uses of water, requiring new approaches to efficiently utilize water resources (Vörösmarty et al., 2010).

One of the major approaches to efficiently utilizing water resources is through the reuse of treated wastewater (TWW), which is becoming a major strategy in coping with increasing water demand due to population growth (Levine and Asano, 2004; Tal, 2006). Accordingly, in arid and semi-arid regions, TWW reuse is rapidly expanding (Friedler, 2001; López-Serna et al., 2012). In Israel, for example, approximately 85% of TWW is reused, mostly for agricultural irrigation. Treated wastewater is also utilized for a variety of additional applications, such as irrigation of public gardens (Levine and Asano, 2004) and streamflow augmentation (Halaburka et al., 2013; Arnon et al., 2015). Regulation of TWW quality is, therefore, typically aimed at addressing the different reuse purposes and includes standards for water quality parameters, nutrients, heavy metals and other toxic elements (WHO, 2006; Israeli Ministry of Environmental Protection, 2010; USEPA, 2012).

Alongside the increasing use of TWW, there is recognition that TWW contains low concentrations (microgram-nanogram per liter) of various organic substances, which are commonly termed organic micropollutants (OMPs, Schwarzenbach et al., 2006). Organic micropollutants include, for example, pharmaceuticals and personal care products (PPCPs), polychlorinated biphenyls (PCBs), dioxins and pesticides, among others. Although wastewater treatment plants (WWTPs) reduce the levels of OMPs, their complete elimination is not achieved because these plants were not designed to deal with these types of compounds. Thus OMPs are continuously introduced into aquatic environments (Silva et al., 2012), and consequently, concerns have been raised regarding the reuse of TWW for agricultural activity (Kinney et al., 2006) and streamflow augmentation (Plumlee et al., 2012).

The development of new and sensitive analytical methods over the last several years has increased the number of chemicals that can be detected or quantified in surface waters, thus attracting the attention of researchers (Sedlak et al., 2000; Richardson, 2003; Schäfer et al., 2011; Brack et al., 2015). Consequently, every year, additional compounds are added to the list of OMPs that are of concern to aquatic biota (Luo et al., 2014; Snyder, 2014). Since TWW consists of a mixture of OMPs, rather than a single compound, the health effects that are caused by OMP exposure are related not only to the detected concentration of each chemical, but also to their integration (Gibson et al., 2005; Ankley et al., 2007; Hotchkiss et al., 2008). Additional natural stressors (e.g., reservoir water quantity and temperature) and biotic interactions (e.g., agonistic interactions and parasite burden) can influence the health-related effects. Ecotoxicological effects would, therefore, be best addressed using a multibiomarker analysis (Colin et al., 2015).

Considering the aforementioned challenges, bioindicators are becoming an attractive approach for evaluating the impact of OMPs on the aquatic ecosystem. This is particularly relevant to the subgroup of micropollutants that specifically interfere with the endocrine system, and is commonly termed endocrine disrupting chemicals (EDCs). Since the aquatic environment directly affects fish physiology (e.g., a reproductive system that is characterized as being very labile), exposure to contaminants may have profound effects on the fish (Adams et al., 1989; Scholz and Mayer, 2008).

Histopathological analysis is suitable for identifying localized changes in fish tissues following exposure to toxicants (McCarthy and Shugart, 1990; Huggett et al., 1992) the occurrence of which differed between different studies examining exposure to wastewater. Some reported no (Lozano et al., 2012) or mild and limited histopathological alterations (Escher et al., 1999), while others reported severe alterations. For example, Schmidt-Posthaus et al. (2001) reported on severe pathological alterations in internal organs and an increase in the occurrence of infectious diseases in trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) exposed to polluted river water. Bernet et al. (2004) reported on the occurrence of histopathological changes, primarily in the liver and gills of brown trout (*Salmo trutta*), following exposure to wastewater. Galus et al. (2013) reported on histopathological alterations in the kidneys and developing oocytes in zebrafish (*Danio rerio*) exposed to a pharmaceutical mixture of municipal wastewater. A comprehensive histopathological analysis was, therefore, incorporated into the present study.

Most of the studies examining the impact of OMPs on aquatic ecosystems were carried out in rivers and streams. Commonly, water samples were collected upstream and downstream from a WWTP discharge site, and then analyzed for the occurrence of OMPs (Ashton et al., 2004; Kolpin et al., 2004; Vieno et al., 2005). In several studies, fish were also collected in order to investigate the effects of TWW on fish health (Jobling et al., 2002; Schultz et al., 2010). Significantly fewer studies have inspected OMPs in water and sediments in lakes/ponds/wetlands that receive TWW (Metcalfe et al., 2003; Hoerger et al., 2014), and their effect on fish (Kavanagh et al., 2004; Kidd et al., 2007), while even fewer studies have performed multi-medium analyses of OMPs, considering together wastewater, surface water, sediment and biota, with an emphasis on estrogen bioaccumulation (Huang et al., 2013).

The recent trends and projection of increasing the reuse of TWW require the development of more efficient strategies to minimize the negative effects on the environment. It was hypothesized that under water scarcity conditions, TWW can be a reliable and sustainable water source for supporting a water reservoir for recreational activities. In order to test this hypothesis, this study evaluated the water quality and fish health in an artificial reservoir located in an arid climate, which regularly receives TWW and sporadic winter floods. The specific objectives of this study were to characterize the water quality in the Yeruham Reservoir by measuring the temporal distribution of nutrients; by measuring the occurrence of selected OMPs in water, sediment and fish tissues; and by evaluating fish health using histopathology.

2. Materials and methods

2.1. Study site description

The Yeruham Reservoir is an artificial water body located near the city of Yeruham in the Negev Desert, Israel (Fig. 1). The Negev Desert is an arid region with mean annual rainfall of <200 mm/year, mean annual temperatures of 18 °C, and an evaporation potential of over 2000 mm/year. Rainfall, temperature and evaporation data for this study were taken from the Israel Meteorological Service's website (http://www.ims.gov.il/), as recorded at the HaNegev Junction and Sede Boqer stations. Both stations are located <10 km from the Yeruham Reservoir. The reservoir was established in 1953, for the purpose of storing water from winter floods for irrigation. In order to create the reservoir, a dam of 80-m length and 15-m height was built at the intersection of the Yeruham and Revivim seasonal streams (Fig. 1). The reservoir receives flood water from the surrounding seasonal streams from sporadic rain events, which occur during the winter (November–April). When the reservoir is full, its area is 0.18 km^2, its volume is approximately 400,000 m^3, and the water level is 448.47 m above sea level.

Before the Yeruham WWTP was built in 2008, the reservoir received wastewater at different quality levels, including raw wastewater and wastewater after some stabilization in aeration ponds. Today, wastewater is treated by using conventional extended activated sludge technology and tertiary treatment by sand filtration and chlorination. Currently,

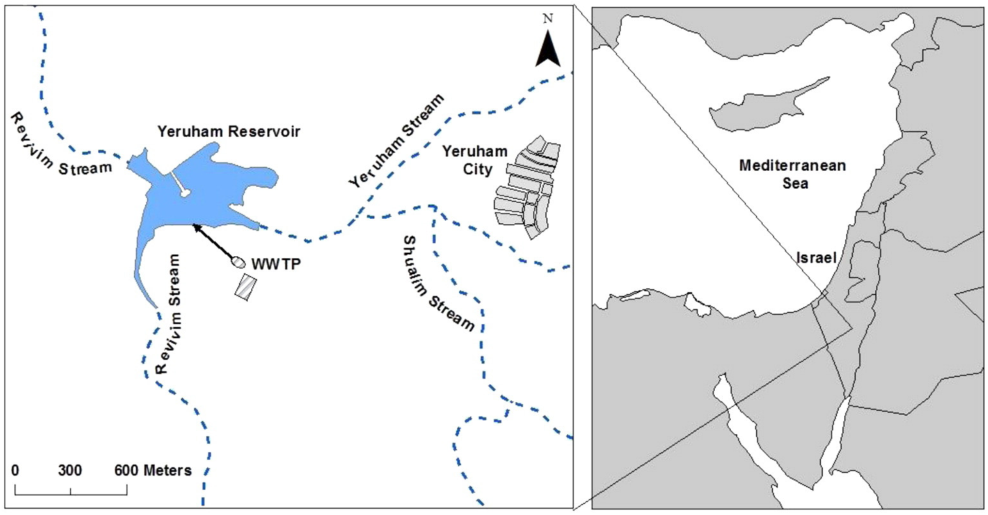

Fig. 1. Study site location and map of the Yeruham area.

the WWTP serves a population of 9700 people, and receives an average flow of approximately 2000 m^3/day of mixed domestic and industrial wastewater. The tertiary treated wastewater (TTWW) is used for irrigating trees in the local recreational park, and the excess volume is directed into the Yeruham Reservoir. The reservoir was stocked with several fish species, aiming to help maintain its ecological balance and to prevent adverse impacts, such as mosquitos, on nearby residents. The fish species stocked included tilapia (*Oreochromis* sp.), which are herbivores that feed on plants and algae, thus helping to control algal blooms and plants in the lake; and carp (*Cyprinus carpio*), which are omnivores that consume a range of natural foods and are highly resistant to different water temperatures, enabling them to survive the cold winter (unlike tilapia). Both carp and tilapia are favored by sport fishermen. Also stocked were mosquitofish (*Gambusia affinis*), in order to control mosquito larvae. All these fish species were sampled for analysis, as described below.

2.2. Water sampling, preparation and chemical analysis

Water sampling campaigns for measuring general water quality parameters were conducted monthly, along with water level measurements, from May 2013 to April 2014 (total of 12 samples). Composite samples were taken using a Masterflex peristaltic pump (Cole-Parmer, Vernon Hills, Illinois, USA) by combining 20 sub-samples distributed around the reservoir. Samples were collected from fixed locations, detected by GPS. Six sampling locations out of the 20 were located around the banks of the reservoir (and were taken using a sampling rod), and all the other 13 points were located in the reservoir and accessed by traversing it with a boat. Electrical conductivity (EC), pH, dissolved oxygen (DO) and temperature (°C) were measured in situ every month using a handheld meter (WTW Multi 3400i, Weilheim, Germany) in the early morning hours. Samples were transported to the laboratory on ice.

Immediately after arrival at the laboratory, 150 mL of the 1-L sample was filtered (MN GF-3 filter paper, Macherey-Nagel, Düren, Germany), and all samples were subsequently stored at 4 °C until analyses (<1 week). Nutrients were analyzed in triplicates, according to the standard methods (APHA, 2005) and included: nitrate (NO_3^-N), phosphate (PO_4^-P), total ammonia-nitrogen (TAN), total nitrogen (TN), total phosphorous (TP), total suspended solids (TSS), total organic carbon (TOC), dissolved organic carbon (DOC), and fecal coliforms.

Water samples for OMP analyses were collected from both the Yeruham Reservoir and the WWTP in February 2013, August 2013, March 2014, and July 2014 (a total of four samples from each location). Water samples were collected, preserved and treated before analysis according to EPA methods that are listed below. Samples were preserved by adjusting the pH to 2 using hydrochloric acid (6N). In addition, sodium sulfite was added to the treated wastewater samples from the WWTP (40 mg per 1 L of sample) in order to deactivate the free chlorine in the water, to prevent any chemical degradation of the OMPs. The samples were stored at 4 °C until extraction (<two weeks). Sixteen target compounds, including hormones, phenols and pharmaceuticals, were included in this study as follows: EDCs: estrone, 17β-estradiol, estriol, testosterone, bisphenol-A (BPA), and 4-octylphenol (4-OP); and PPCPs: carbamazepine (CBZ), triclosan (TCS), diclofenac, bezafibrate, metoprolol, propranolol, ibuprofen, ketoprofene, venlafaxin and naproxene (Masi et al., 2004). These OMPs represent several major classes, including OMPs that are commonly used to evaluate the quality of treated WW around the world.

OMPs were extracted from the water samples using the solid phase extraction (SPE) method, with Empore C_{18} extraction disks (3M, St. Paul, Minnesota, USA). The extraction of CBZ and TCS was conducted according to EPA 525.2 protocol (US EPA, 1995); all other pharmaceuticals were extracted according to EPA 1694 protocol (US EPA, 2007), and EDCs according to EPA 539 protocol (US EPA, 2010). The final extracts were stored at −20 °C until analyzed (within 28 days). The analysis of TCS and CBZ was done with a Trace GC 2000-Polaris-Ion trap mass spectrometer (Finnigan, ThermoQuest Co., Austin, Texas, USA) equipped with an Rxi®-5Sil MS column 30 m ∗ 0.25 mm ID ∗ 0.25 μm (Restek, Bellefonte, Pennsylvania, USA). EDCs and others pharmaceutical analyses were done with an ES-LC-MS/MS Xevo TQ-S Instrument (Waters, Milford, Massachusetts, USA) equipped with an Acquity UPLC BEH C_{18} 1.7 μm 2.1 ∗ 50 mm column (Waters, Milford, Massachusetts, USA) that was used for separation.

A QA/QC scheme was carried out for each batch of samples by adding the following controls: laboratory and field blanks (deionized water kept under the relevant conditions during the sampling and laboratory work) and a laboratory fortification blank and matrix (spiked blank and samples). The accuracy and precision of the methods was estimated as 70–130% (50–150% at the minimum reporting level: MRL concentration levels). Target compounds were identified by a comparison of the retention times and either full mass spectrum (EPA 525.2) or 2–3 multiple reaction monitoring (MRM) transitions (EPA 539) of the substance in the sample and its authentic standard, which were tested under the same conditions. Concentrations of the analyzed compounds were calculated using the standard internal calibration procedure. Internal standards were used as follows: Phenanthrene D_{10}, bisphenol A-D_{16}, estradiol $^{13}C_6$, estriol $^{13}C_3$, estrone $^{13}C_6$, and testosterone D_5. Analytical standards were purchased from Sigma-Aldrich. Reagents for extractions and instrumental analysis were all analytical grades and were purchased from Sigma-Aldrich and J.B. Baker.

2.3. Sediment sampling, preparation, and analysis

Two sediment grab samples were taken from the bottom of the reservoir during the winter and summer of 2014 for the measurements of the OMPs detailed in Section 2.2. Samples were collected in pre-washed glass vials (10% nitric acid and acetone), and stored at −20 °C until preparation. Preparation and analysis were modified according to Heidler and Halden (2007). Briefly, sediment samples were centrifuged under 4500 rpm for 1 h and freeze-dried for about three days. Dry samples were extracted with acetone:hexane 60:40 using an accelerated solvent extraction (ASE) device (Dionex ASE 200, Dionex; Sunnyvale, CA, USA). Extraction conditions were as follows: extraction temperature, 75 °C; extraction pressure, 1500 psi; preheating period, 1 min; static extraction period, 5 min; solvent flush, 60% of the cell volume and three extraction cycles. The volume of the extract was reduced to 3 mL using a nitrogen stream. The same sixteen OMPs that were tested in the water samples were also tested in the sediments. The samples were then cleaned with Florisil cartridges and the volume reduced again to 1 mL (TCS and CBZ analysis). For the other analytes, the extracts were completely dried, and 1 mL of MeOH was added to the dried samples. For TCS and CBZ analyses, phenanthrene D_{10} was added to the dried samples as an internal standard to evaluate the extraction efficiency, before it was inserted into the extraction cells. For the EDCs analysis, the internal standard was added just before chemical analysis. Chemical analyses were done as described in Section 2.2.

2.4. Fish sampling

In the present study, the fish inhabiting the reservoir, including all three different species mentioned above (carp, tilapia, and mosquitofish), served as bioindicators. Fish were sampled at several time points for the performance of different analyses, as described in the following. For histopathological analysis, fish were sampled in October 2013 from the main reservoir body, and 10 days later from the WWTP inlet, which was connected to the reservoir only by a small rill due to the extensive drying out of the reservoir at the end of the dry season. For OMP analysis, fish were sampled during April 2014. Fish were captured either using a net trawl that was positioned on the bank of the reservoir, with fishing rods or with hand nets. Information on the sampled fish, including species, sizes and the number sampled, are detailed in Table 1. Fish were anesthetized with clove aromatic oil (250 μL/L) and treated in compliance with the principles for biomedical research involving animals, ethics authorization number IL-78-10-2012.

2.4.1. Fish histopathology

In sampling for histopathological analysis, for small fish (1.5–2.5 cm long), the whole body was taken, and for bigger fish (7–10.5 cm long), the gills, liver, spleen, muscle, kidney and intestine were separately collected. Samples were fixed in neutral buffered formalin for 48 h and then kept in 70% alcohol until processed for histology. Decalcification was carried out in a solution containing formic acid (44%) and sodium citrate (12.5%) for 12 h prior to processing, rinsed in tap water and placed back in 70% alcohol. Processing was performed in a microwave histo-processor (RHS-1, Milstone, Italy). Samples were then embedded in paraffin blocks and sectioned at 5 μm. Finally, the sections were stained with hematoxylin and eosin (H&E).

The shapes and structures of every fish tissue sample were analyzed, and any evident abnormality or pathology was recorded. Abnormalities were observed mainly in the gills and spleen, and a ranking system was assigned to these tissues as follows: the infection levels of gill parasites were graded as high, moderate and low, corresponding to a parasite load of over 1.8, 0.9–1.8 and <0.9 parasites/filament. Spleen pathology included the occurrence of melanomacrophage centers (MMCs), and a five-grade ranking system was used for quantification. The coverage area percentage of MMCs out of the total area of the observed spleen tissue was measured using ImageJ 1.49 software, and graded as follows: grade 1: up to 0.86% coverage; grade 2: 0.86–1.37% coverage; grade 3: 1.37–3.2% coverage; grade 4: 3.2–6.06% coverage; and grade 5: above 6.06% coverage.

2.4.2. OMPs in fish tissues

The OMPs that were selected for testing in fish tissues were different from those tested in the water and the sediments. Estrogenic compounds, which were tested in the water and sediments, were also evaluated in fish due to their potential ecological impact on fish health. Other types of compounds were not tested in the tissues; instead, PCBs and dioxin compounds were tested because of the fact that fish are used for food, and these compounds are known to be a major concern in food safety (WHO-ECHS, IPCS, 1998; Marin et al., 2011). Heavy metals are also known to be of concern in food safety; however, preliminary research did not find significant accumulations of heavy metals in the sediments of the Yeruham Reservoir, and thus, they were not included in the analysis (Nishri, 2006).

For OMP analysis, liver and muscle (fillet along with the overlying skin) were taken from each fish, placed in either liquid nitrogen (liver) or dry ice (muscle) and transported to the lab, where they were moved to −80 °C until transported for analysis. Samples were sent frozen (dry ice) to BioDetection Systems BDS in Amsterdam (the Netherlands) for analysis. Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like PCBs (dl-PCBs) were analyzed in pooled samples of fish tissue using the internal modification of the DR-CALUX method (Besselink et al., 2004). The aforementioned bioassay is commonly used for evaluating food safety in Europe (EU Commission Regulation, 2011). The responses to estrogens, pseudo-estrogens and anti-estrogens were analyzed with the ER-CALUX method (Legler et al., 1999); the results are given as a sum of the estrogenic contribution of all the compounds together, in a 17β-estradiol (17β-E2) equivalence.

2.5. Data analysis

All concentrations, solute masses, and discharges are reported as mean ± standard deviation (SD). Water depth was measured from a

Table 1
Summary of fish sampling details, including fish species, numbers and sizes, sampled on different sampling dates and at different reservoir locations.

Analysis	Date	Location	Species	Number sampled	Weight (g)	Length (cm)
Histology	10-Oct-13	Main water body	Tilapia	15	N.A.	7.8 ± 1.8
			Carp	12	N.A.	7.7 ± 1.2
			Mosquitofish	12	N.A.	1.9 ± 0.2
	20-Oct-13	WWTP inlet	Tilapia	40	N.A.	3.1 ± 0.4
			Mosquitofish	40	N.A.	1.8 ± 0.3
OMPs	24-Apr-14	Main water body	Carp	46	87.8 ± 32.1	13.2 ± 1.4
			Tilapia	1	83	13

boat in 13 GPS-marked locations around the reservoir. Based on depth measurements, the bottom of the reservoir was mapped, and the temporal changes of the water volume in the reservoir were measured using Esri ArcGIS 10.2.2 software (Redlands, California, USA). All maps were projected according to the Israel Transverse Mercator grid coordinate system (ITM). Next, the water layer was created based on the water level measurements for each month, and the volume was calculated as the difference between the water layer and the topological layer using the "cut fill" tool in ArcGIS, for every water level measurement throughout the measurement period.

The values of the risk quotient (RQ) for the compounds that were found in the reservoir water are represented by the ratio between the measured environmental concentrations (MEC) and the predicted no-effect concentration (PNEC) for each compound, which were obtained from the literature (Bonvin et al., 2011; Caldwell et al., 2012).

A two-tailed Pearson correlation analysis was carried out to determine the dominant water source (TWW vs. floods) affecting the water quality in the reservoir by using SigmaPlot 12.5 software. The analysis was done on the mass of water quality variables (TAN, NO_3^-, TN, TP, TSS, and fecal coliforms) and the rainfall (cumulative volume of water). The mass was calculated by multiplying the measured concentrations by the relevant volume at each period of time (either the volume of the reservoir or the discharge from the WWTP).

The percent of occurrence of different histopathological alterations between sampling sites for a specific fish species, and for different fish species within the same site was compared by a z-test using Sigmaplot 13 software. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Seasonal changes of water level and volume in the Yeruham Reservoir

The water level in the Yeruham Reservoir was strongly affected by the seasonally related processes of water gain and loss. When first taking the measurements (end of winter 2013), the reservoir was filled almost to its maximal capacity (Fig. 2). As the dry season progressed, the water level dropped, reaching a minimum of 217 cm below the crest of the dam, and only the deeper section of the reservoir remained accessible by boat (water depth > 50 cm, which represents 14% of the maximal reservoir area). The first rain event during November 2013 resulted in a flood, and the water level increased by 113 cm. The water level continued to increase during the winter with additional rain events, and after four days of intensive rain during March 2014, the reservoir reached its maximum capacity with water spilling over the dam.

The GIS-based volume of the reservoir highly correlated with the changes in the water level ($R^2 = 0.997$, data not shown). The maximum water volume that could be stored in the reservoir was 422,000 m^3, and the minimum volume of 76,000 m^3 was recorded during October 2013 (Fig. 3). The contribution of effluents from the WWTP to the reservoir during the year was continuous, but relatively small in comparison to the contribution from floods. The mean discharge of TTWW was 26,000 ± 9000 m^3/month. On the other hand, water input from floods and rain events occurred as discrete events during the winter months (Fig. 2). The contribution from a single flood can be an order of magnitude higher than the monthly discharge, as calculated, for example, during the Nov. 2013 flood, which added to the reservoir 164,000 m^3.

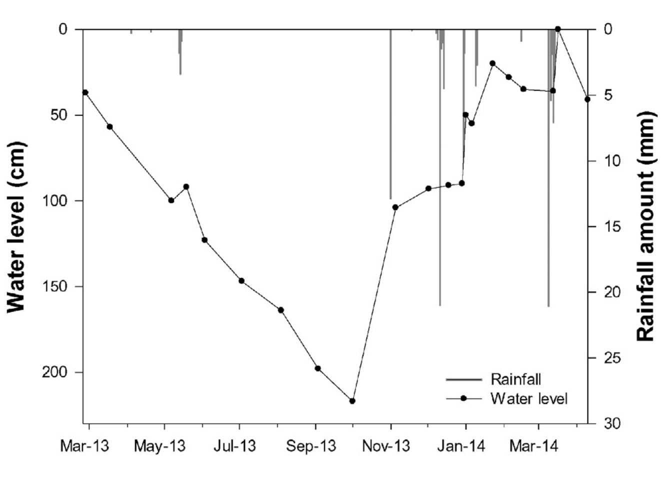

Fig. 2. Precipitation and temporal changes in the water level in the Yeruham Reservoir. Zero level marks the crest of the dam.

The evaporation rate records from the Israel Meteorological Service indicate that the evaporated volume from the reservoir ranges between 13,000 m^3/month during the winter and 57,000 m^3/month during the summer (Appendix A). Thus, the decline in the water level during the summer implies that the daily contribution of the WWTP was smaller than the water loss processes (the sum of evaporation and percolation). Water balance calculations, based on evaporation, WWTP discharge and total volume in the reservoir, revealed that percolation is a significant pathway for water loss ($p < 0.01$, Appendix A).

3.2. Water quality in the Yeruham Reservoir

The water quality parameters that were measured in the composite samples in the Yeruham Reservoir and the TTWW are displayed in Table 2 and Fig. 4, while a detailed description of the spatial distribution of the sampling and chemistry appears in Appendix B. The nutrient concentrations were higher in the TTWW than in the reservoir, showing the capacity for nutrient uptake and dilution. Pathogen concentrations, on the other hand, were much lower in the TTWW than in the reservoir. All of the abovementioned parameters were strongly affected by floods and the runoff processes from the surrounding soils and the potential re-suspension of the reservoir sediments. For example, concentrations of the dissolved inorganic nitrogen compounds, TAN and NO_3^-N, were quite stable during most of the year, and slightly fluctuated around 0.034 ± 0.024 mg/L and 0.51 ± 0.35 mg/L, respectively (Fig. 4), with the exception of high values during Nov. 2013. The Nov. 2013 sample was measured four days after the first rain event of the winter, and TAN and NO_3^-N levels reached 0.25 mg/L and 2.06 mg/L, respectively. Unequivocal increases after the first rain event in Nov. 2013 were also measured in fecal coliforms and TSS concentration, and another significant increase in fecal coliforms was observed after the floods in March 2014 (Table 2).

The correlation coefficient between the mass of water quality parameters in the reservoir and the rainfall was positive, except for TP (Table 3). On the other hand, the correlation between the mass of water quality parameters in the reservoir and the mass of nutrients that entered from the WWTP was negative in four out of six parameters. In the cases where a positive correlation was found for both sources of water, the correlation with rainfall was always stronger (Table 3). This type of analysis suggests that flood events were more influential on the water quality of the reservoir.

A disturbance effect caused by the floods (Grimm and Fisher, 1989) resulted in increased concentrations and a decline in the water quality of the reservoir. However, the reservoir resilience was high, which led to a quick recovery as illustrated by the return to the low concentrations in the next month (Fig. 4 and Table 2). Sánchez-Carrillo and Álvarez-Cobelas (2001) described similar fluctuations in nitrogen concentration in a semi-arid shallow wetland, where maximum nitrogen concentrations were highly correlated with rainfall and floods, especially after a long drought, but dropped shortly after the rain event. Fecal coliform concentration is another parameter supporting the observation that floods constitute the main factor influencing the water quality in the reservoir. Due to the strict regulation of TWW quality in Israel, the fecal coliform concentrations in the TTWW were 6 ± 8 CFU/100 mL, while the concentrations in the reservoir were 266 ± 388 CFU/100 mL (Table 2). These results indicated that the source of fecal coliforms

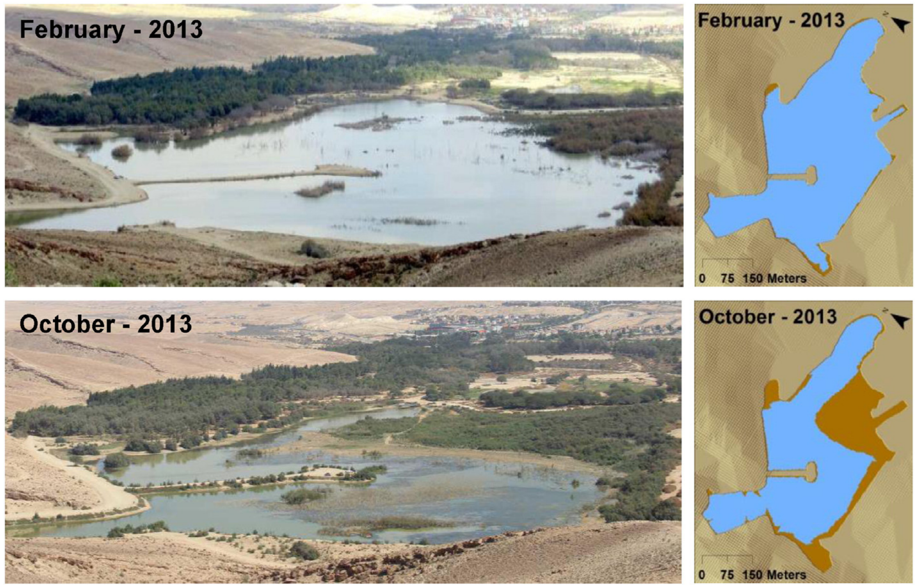

Fig. 3. A photograph showing the Yeruham Reservoir in February 2013 (almost at maximum capacity) and October 2013 (minimum capacity). ArcGIS model images of the lake surface area are illustrated on the right panel next to each photograph.

was not the TTWW, but the feces of animals found in the surrounding area of the reservoir that were washed into the reservoir with the floods.

3.3. OMPs in water and sediment samples

Estrone, carbamazepine, diclofenac and bezafibrate were the only OMPs that were found in the Yeruham Reservoir out of 16 tested compounds (Table 4 and Appendix C). These compounds were detected in all water sampling campaigns. In only two samples did the estrone concentration exceed the predicted no-effect concentration (PNEC) for fish (Table 4). The concentration of estrone in the TTWW was, in some cases, lower than the concentrations in the reservoir (Appendix C). This may be explained by leaching from nearby animal farming, which further suggests the strong influence of human activity in the catchment. Despite the rural nature of the catchment, the combination of floods and the relatively low level of human activity seems to have a strong effect on water quality in the reservoir.

The concentrations of the other OMPs were lower than the PNEC. For example, the PNEC for CBZ was 1.66 times higher than the highest concentration found in the reservoir (Table 4). In laboratory exposure of Japanese medaka fish (*Oryzias latipes*), the CBZ median lethal concentration (LC_{50}) was 35.4 mg/L (Kim et al., 2007), four orders of magnitude higher than the highest concentration found in the TTWW samples.

OMPs are characterized by various physicochemical characteristics. For many of the OMPs, adsorption to sediments is a major pathway in the environment. Estrone was the only compound that was measured in the water (Table 4) and adsorbed to the sediments (0.02 ± 0.0 μg/kg). In addition, BPA and 4-OP were found only in the sediments, and their concentrations were 2.08 ± 0.68 μg/kg and 2.05 ± 0.5 μg/kg, respectively. The concentration of OMPs in the sediment is important, since contaminant accumulation can affect bottom dwelling fish, like carp (Lozano et al., 2012). Nevertheless, the concentrations of BPA in the reservoir, for example, were very low compared to reported concentrations in aquatic sediments (Huang et al., 2012).

3.4. Bioaccumulation of OMPs in fish tissues

Bioaccumulation of OMPs in fish is significant in terms of both fish health and human health-related aspects, since sport fishing is a popular activity at the reservoir and the caught fish are used for human consumption. Forty six carp and one tilapia were collected from the

Table 2
The annual mean ± SD of measured concentrations of nutrients and other water quality parameters in the Yeruham Reservoir. Bold text marks the results that exceed the Israeli Standards for water reuse[a] (stream discharge category).

Parameter	Israeli standards for water reuse	Annual mean ± SD (TTWW)	Annual mean ± SD (reservoir)	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
TN (mg/L)	10	7.37 ± 1.77	2.87 ± 1.47	1.44	2.01	1.44	1.67	6.22	3.02	3.52	2.70	1.98	2.34	5.04	3.06
NO_3^--N (mg/L)	–	4.36 ± 1.14	0.64 ± 0.54	0.54	0.27	0.42	0.86	0.82	1.15	2.06	0.53	0.76	0.05	0.09	0.16
TAN (mg/L)	1.5	1.92 ± 0.55	0.05 ± 0.06	0.01	0.04	0.00	0.04	0.03	0.06	0.25	0.01	0.08	0.01	0.04	0.04
TP (mg/L)	1	3.82 ± 2.12	1.69 ± 1.32	1.04	N.A.	0.67	**4.51**	**1.77**	**1.99**	**3.73**	**1.05**	0.44	0.36	0.60	**2.47**
PO_4^--P (mg/L)	–	N.A.	0.26 ± 0.23	0.59	0.22	0.20	0.69	0.26	0.62	0.27	0.06	0.16	0.03	0.03	0.02
Fecal coliforms (CFU)	200	6 ± 8	266 ± 388	12	31	31	47	**397**	59	**976**	**280**	90	27	**1203**	40
TOC (mg/L)	–	N.A.	28.5 ± 22.2	12.4	17.6	31.3	16.3	25.4	22.3	26.1	29.5	16.3	16.3	95.8	33.1
TSS (mg/L)	10	5.65 ± 3.39	31.3 ± 21.6	7.7	**19.7**	**26.7**	**27.0**	**45.3**	N.A.	**86.0**	**48.0**	**11.5**	9.7	**25.7**	**36.7**
Temp (°C)	–	N.A.	20.75 ± 4.5	25.5	23.4	28.5	26.2	23.4	19.9	18.9	12.4	13.5	14.8	19.7	19.7
EC (mS/cm)	–	1.29 ± 0.28	1.39 ± 0.53	0.88	1.09	N.A.	1.48	1.53	1.59	0.95	1.01	0.84	1.18	2.45	2.33
DO (mg/L)	>3	N.A.	6.6 ± 1.2	7.3	6.4	5.6	6.0	7.6	7.1	7.9	N.A.	7.7	7.5	6.9	3.8
pH	7–8.5	N.A.	7.82 ± 0.97	8.51	8.55	8.37	8.32	7.98	**8.60**	7.34	7.42	7.65	N.A.	**8.35**	N.A.

N.A. = not available.
[a] Israeli Ministry of Environmental Protection (2010).

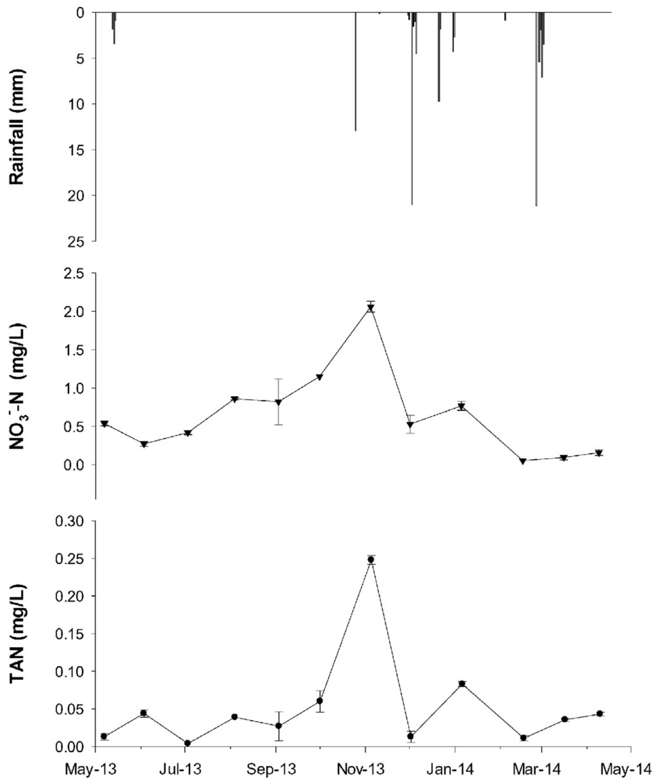

Fig. 4. Precipitation and temporal changes in NO_3^--N and TAN concentrations in the Yeruham Reservoir.

Table 4
PNECs, occurrence and concentrations of selected OMPs in water from the Yeruham Reservoir and their RQs.

Compound	Number of samples	Range (ng/L)	Mean + SD (ng/L)	PNEC (ng/L)	# of occurrences of RQ > 1
Estrone	4[1]	1–15	6.93 ± 5.38	6[a]	2
CBZ	4[1]	278–1500	744.5 ± 461	2500[b]	0
Diclofenac	2[2]	4–39	22 ± 17.6	100[b]	0
Bezafibrate	2[2]	8	8	1191[b]	0

[1] Analyzed in samples collected in the summer and winter of 2013 and 2014.
[2] Analyzed in samples collected in the summer and winter of 2014 only.
[a] Caldwell et al. (2012).
[b] (Bonvin et al. (2011).

Yeruham Reservoir. Liver and muscle (including the muscle tissue and overlying skin) were sampled and pooled together for analysis. It was found that equivalent concentrations of PCDD/PCDF and dl-PCBs were much lower than the EU standards for human consumption in the muscle (and overlying skin) and liver, constituting ~4.8% and ~6.5% of the maximal permitted values, respectively (Table 5). The measured equivalent estrogen concentrations in the fish muscle (and overlying skin) and liver were 0.51 and 0.07 ng 17β-estradiol eq./g wet weight, respectively.

In a study carried out by Huang et al. (2013), the bioaccumulation of estrogens (estrone, 17α-ethynylestradiol, 17β-estradiol and estriol) was measured in muscle, gill and liver extractions of Crucian carp sp. and Carp sp. by GC–MS. Only 17β-estradiol was found in the fish groups raised in the laboratory control and in a slightly contaminated field control site. Concentrations in the field-control-raised fish (n = 24) were 1.5–1.6 ng/g dry weight (DW) in the muscle and 2.3–2.5 ng/g DW in the liver (0.35–0.38 ng/g wet weight and 0.54–0.59 ng/g wet weight, respectively, by assuming 76.31% moisture content in carp, according to information on the USDA website: http://ndb.nal.usda.gov). Water from the fish culture site contained 1.2 ± 0.4 ng 17β-estradiol/L. Laboratory control fish (n = 24) contained lower 17β-estradiol levels: 0.9–1.1 ng/g DW and 1.7–1.8 ng/g DW in the muscle and liver, respectively (0.21–0.26 ng/g wet weight and 0.40–0.43 ng/g wet weight, respectively). Since the water and feed in the laboratory conditions were found to be hormone free, it was concluded by Huang et al. (2013) that 17β-estradiol naturally exists in fish. Another piece of supporting evidence for natural hormone levels was published by Houtman et al. (2007). In their study, 14 male breams (*Abramis brama*) were collected from two lakes with minimal exposure to anthropogenic influences as a control group. Equivalent estrogen concentrations in liver samples were determined by the ER-CALUX bioassay, revealing levels of 11.8 ng 17β-E2 eq./g lipid (equal to 2.24 ng 17β-E2 eq./g liver, assuming that lipids constituted 19% of the liver weight in bream) (Sakamoto and Yone, 1978; Kalegeropoulos et al., 1992). The aforementioned data suggests that the estrogen levels in fish from the Yeruham Reservoir are similar to those from fish grown in uncontaminated water.

3.5. Histopathological analysis

Thirty-nine fish (12 mosquitofish, 15 tilapia and 12 carp) were sampled from the reservoir, and 10 days later, 40 small tilapia and 40 mosquitofish were sampled near the TTWW inlet into the reservoir. Protozoan parasites, including *Trichodina* sp., *Ichthyobodo* sp. and *Apiosoma* sp., were found in 33% of the fish (total of 40 infected fish), on the gills, at varying infection levels: low infection levels in 17.5% of the fish; moderate infection levels in 12.5% of the fish; and high infection levels in 3% of the sampled fish. Apiosoma sp. was the most prevalent and occurred in 93% of the infected fish (Fig. 5a), while Ichthyobodo sp. and Trichodina sp. were found only in 7.5% of the infected fish. Infection rates were the highest in tilapia, with 68% infected tilapia from the TTWW inlet and 53% infected tilapia from the main water body. The infection rate in carp was 41.66%, while no parasites were found on the gills of the mosquitofish.

Epitheliocystis was present at low levels on the gills (1–3 cysts per fish) in 11% of the examined fish; of the affected fish, 54% were tilapia from the inlet, 38% were tilapia from the main water body, and 8% were mosquitofish from the TTWW inlet. Focally occurring hyperplasia was present on the gills of 12% of the examined fish, at low levels (1–2 hyperplastic foci per fish). Of the affected fish, 50% were tilapia from the main water body, 36% were carp, 7% were mosquitofish, and 7% were tilapia from the inlet.

Melanomacrophage centers (MMCs) were found in the spleens of 20% of the examined fish: 62% of them at very low-low levels (graded 1–2 out of 5), 30% at moderate levels (grade 3), and 8% of the fish at high levels (graded 4–5, Fig. 5b). The occurrence of MMCs was highest in tilapia from the main water body (60%), followed by tilapia from

Table 3
Pearson correlation coefficient of the nutrient mass from the reservoir compared with the nutrient mass from the WWTP, and with the rainfall amount. Bold numbers represent a significant correlation ($p < 0.05$).

Variables	WWTP vs. reservoir	Rainfall vs. reservoir
TAN	−0.112	0.438
NO_3^-	−0.259	0.22
TN	0.235	**0.632**
TP	−0.263	−0.161
TSS	0.0268	0.159
Fecal coliforms	−0.108	**0.637**

Table 5
Concentrations of PCDD/PCDF and dl-PCBs (concentration of PCDD/PCDF only appears in brackets) in the muscle and liver of carp from the Yeruham Reservoir, presented as pg CALUX TEQ g^{-1} wet tissue weight, and concentrations reported in fish from natural water bodies affected by TWW, presented as pg WHO-TEQ g^{-1a} or pg CALUX TEQ g^{-1b}.

Organ	Yeruham Reservoir fish	Reported in the literature				EU maximal permitted levels (pg WHO-TEQ g^{-1} product)[c]
		Mean	Min.	Max.	Reference	
Muscle & overlying skin	0.31 (0.14)	1.55 (0.28)	0.08 (0.06)	5.48 (0.78)	Marin et al. (2011)[a]	6.5 (3.5)
		N.A.	2.69 (1.27)	5.14 (2.48)	Karl et al. (2010)[a]	
		N.A.	0.84 (0.22)	14.11 (5.67)	Piskorska-Pliszczynska et al. (2012)[a]	
		N.A.	0.05 (0.03)	8.00 (2.48)	Zacs et al. (2013)[a]	
		0.076 ± 0.032 (0.045 ± 0.026)	0.048 (0.026)	0.14 (0.13)	Julshamn et al. (2013)[a]	
		0.15 (0.119)	0.025	0.27	Israeli Ministry of Health (2013)[b]	
Liver	1.3 (0.78)	14.2 ± 11.2 (2.5 ± 1.4)	1.0 (0.3)	151 (9.2)	Julshamn et al. (2013)[a]	20
		27.45 ± 19.14 (5.74 ± 4.25)	9.51 (1.64)	70.61(13.85)	Struciński et al. (2013)[a]	

N.A. = not available.
[a] pg WHO-TEQ g^{-1}.
[b] pg CALUX TEQ g^{-1}.
[c] Commission Regulation (EU) 2011.

the inlet (23%), mosquitofish from the main water body (17%), and mosquitofish from the inlet (10%), and no MMCs were observed in carp spleen. The difference in MMCs levels in tilapia collected at the main water body was significantly higher than in the inlet. MMCs were also found in the kidneys of 7.6% of tilapia from the inlet, at low to moderate levels (graded 1–3). In one of the sampled mosquitofish, diffuse necrosis was seen in the liver, affecting hepatocytes.

In the muscle, focal dystrophy of the muscle fibers was evident in three fish (two carp and one tilapia from the inlet), and a lesion caused by *Larnea* sp. was observed in the muscle of one mosquitofish from the inlet. Normal structural development of gonads appeared in tilapia and in mosquitofish from both sites, with no evident intersex occurrence. No gonads were seen in carp. The data of this histopathological analysis is summarized in Appendix 0.

In general, the highest rates of gill parasites, epitheliocystis, hyperplasia and MMCs were found in the tilapia, with significantly higher infection rates compared to mosquitofish. Tilapia are known to be sensitive to low temperatures, and in most tilapia species, growth and feeding stop below 15 °C and reproduction below 20 °C (Wohlfarth and Hulata, 1983). Low temperature stress affects the functioning of the immune system (Ndong et al., 2007; Sharon et al., 2015), which is commonly manifested in increased ectoprotozoan infection rates in cichlid fish (Paperna, 1996). At the time of fish sampling (Oct. 2013), the water temperature in the early morning hours was 19.9 °C; therefore, this factor is suggested to be the cause for the high ectoparasitic infection rates in tilapia. Epitheliocystis, caused by chlamydial organisms, is common in all cichloid species and in common carp, both cultured and wild fish (Paperna, 1996). The occurrence of epitheliocystis only in the tilapia further supports the assumption that the tilapia were more sensitive at the time of sampling due to environmental conditions (low temperature). However, the occurrence of parasites reflects a healthy and functioning ecosystem (Hudson et al., 2006), and at low to moderate infection prevalence and intensity levels, a fish population can be considered healthy.

Fish's spleen stroma contains haemopoietic tissue, and the occurrence of MMCs is common in the spleen. No MMCs were evident in the liver, although other researchers have reported their occurrence in this location (Agius and Roberts, 2003). Enlarged MMC aggregates normally occur after active phagocytosis, the morphology and size of which are affected by physiological changes such as age, starvation, and pathological, inflammatory, and immunological processes. An increase in the size and frequency of MMCs can be related to exposure to environmental pollutants, but also to infectious diseases and natural processes (Agius and Roberts, 2003). The difference in MMCs levels between tilapia samples in the main water body and the inlet probably arises from difference in fish age, as fish from the main water body were older, based on their larger size than those from the inlet (7.8 as compared to 3.1 cm), thus were exposed to various factors that induce MMCs development for a longer time period. Since the occurrence of MMCs in the fish from the reservoir was fairly low, along with the finding of parasite presence in fish with evident MMCs, we assume that the parasitic infection was the stimulus for their elevated occurrence.

3.6. Field implications

The Yeruham Reservoir was created in the 1950s with the aim of collecting flood water for irrigation. Since then, it has evolved from a water storage reservoir into a recreation lake and a nearby park. Due to the inadequate environmental and social conditions in the Yeruham

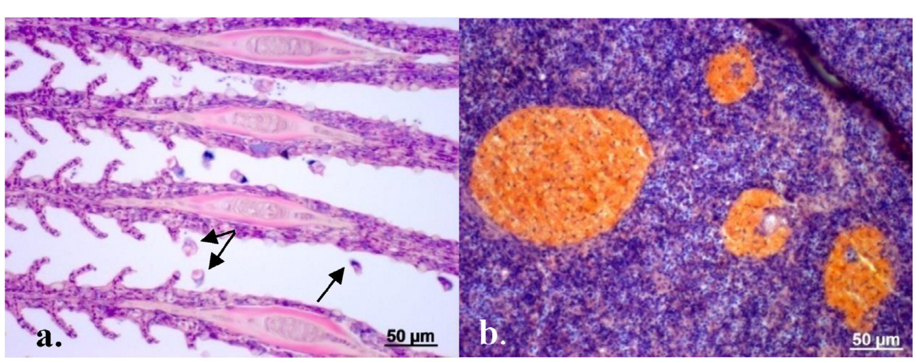

Fig. 5. Histopathology of fish from the Yeruham Reservoir: (a) Tilapia gills infected with a high level of *Apiosoma* sp. (arrows point at three parasites as an example). (b) Tilapia spleen with MMC (orange colored spots) ranked as grade 5 ("high level").

area, agriculture was never really developed, and thus the reservoir was left ignored for several decades, which led to wastewater discharge and water quality deterioration. Water collection in reservoirs was, and still is, viewed as a way to increase the available water resources. However, the construction of dams, reservoirs and other engineered water systems can negatively impact the environment. The stunning and unique example provided by the Yeruham Reservoir can serve as a model for other human-made ecosystems in arid regions around the world.

A key aspect of evaluating the environmental status of reservoirs is evaluating their physical, chemical and biological characteristics. The changes in the water depth, volume and area of the reservoir are key parameters for maintaining this ecosystem because they affect the circulation of the water, the biogeochemical processes, and the fish population and ecology due to habitat changes (Tockner and Stanford, 2002; Rayner et al., 2015). Maintaining a reservoir in an arid zone requires an understanding of the water balance to ensure that the reservoir will not become dry too often. Since floods are a major contributor to the water budget, a relatively simple analysis of rainfall distribution and flood frequency are an essential first step. The data shown here on water balance and quality (Figs. 2–4, Tables 2 and 4, and Appendix A) illustrates that the advances in wastewater treatment make WWTPs an ideal continuous water source for recreational reservoirs that can ensure the maintenance of wet conditions during drought years. The contribution of TTWW should be aimed, at least, at compensating for evaporation and percolation. In our study site, the relatively good water quality and the abundance of water throughout the year provided the basic conditions for a flourishing ecosystem. In a biological survey (BioBlitz) held in the Yeruham Reservoir in 2015, 537 species of plants and animals were recorded, including mammals (14), birds (179), reptiles (21), insects, arthropods, amphibians, fish and more. The identified plant species (146) included protected species, endemic species and also wetland species (Yoram Zvik, personal communication). This relatively high number of species, similar to recorded data from British Columbia, Canada (497 species in Whistler in 2013 (http://www.whistlerbioblitz.ca) and 488 species in Burnaby Lake in 2010 (http://bioblitz.burnabylakepark.ca)), shows that the reservoir created an ecological niche that evolved into a unique wetland ecosystem in an arid environment, with high biodiversity. We assume that these species were able to evolve due to the elimination of wetlands in more developed urbanized regions (e.g. Levin et al., 2009).

The uniqueness of the Yeruham Reservoir lies in the fact that it is located in a rural region with an arid climate, whereas most published studies examining the effect of treated wastewater addition to aquatic ecosystems are from semi-arid areas (e.g., Mediterranean climate) or from temperate climates and urbanized environments (Plumlee et al., 2012; Bischel et al., 2013). In fact, most human-made aquatic systems, including wetlands, ponds and streams, are reported to be in heavily populated areas (Brooks et al., 2006; Bischel et al., 2013; Terrado et al., 2014). Aquatic systems and "greening" environments are presented as positive developments in urban regions (Greenway, 2005; Lundy and Wade, 2011), while most human-made structures in rural regions are considered to constitute interferences with the natural system (Havel et al., 2005). However, we claim that even in rural settings, significant benefits can also accrue from the reuse of treated wastewater in aquatic ecosystems as occurs in the Yeruham Reservoir. After the WWTP in Yeruham switched to tertiary treatment, the odor and the water quality in the reservoir improved, and people began to visit it since social behavior in semi-arid and arid climates has always been affected by water (García-Llorente et al., 2012).

The Yeruham Reservoir began to attract visitors and very quickly developed into a recreational park. Although unintentional, the particular setting of Yeruham as a rural settlement in an arid zone shifted the use of the reservoir from water supply and flood control (e.g., Bangash et al., 2013; Terrado et al., 2014) to recreation and tourism. Although we did not run an ecosystem service analysis, it is clear that after several decades of the reservoir's surroundings being ignored, a seven million dollar investment over the next three years in landscape development and tourism infrastructure is expected to advance the use of the reservoir for tourism and education (Yeruham Economic Development Company, personal communication). It is also expected that this investment will have vast social, economic and ecological implications for the region. Today, recreation and tourism activities include picnicking, sport fishing, hiking and biking, which are all strongly dependent on a rich and variable biological ecosystem.

The lack of data from rural area case studies is an obstacle to developing robust methods for evaluating the potential use of TTWW for ecosystem manipulation. In the developed world, the adoption of advanced technologies for water supply can be turned into an opportunity. The water shortage in the southeastern Mediterranean has led Israel to develop desalination programs to provide drinking water, while maximizing the reuse of treated wastewater for agricultural irrigation. This strategy may lead to occasional and often seasonal surpluses of TWW, which can be used to support aquatic ecosystems for recreation. These surpluses are now used in Israel for streamflow augmentation (Arnon et al., 2015), a method that is becoming more popular in other regions with semi-arid climates (e.g., Eckhardt, 2004; Bischel et al., 2013; Halaburka et al., 2013).

Augmentation of recreational ponds with TWW offers enormous opportunities. Even the total dependence of an aquatic system on TWW is not unimaginable, as demonstrated, for example, by the San Antonio River Walk (Eckhardt, 2004). With proper water treatment and with the use of new developments in continuous online water quality monitoring (Henderson et al., 2009; Boënne et al., 2014), there should not be any hesitation in using TWW to restore wetland environments that were drastically damaged by humans in the last century, both locally (e.g., Levin et al., 2009) and globally (Dahl, 1990; Davidson, 2014).

4. Conclusions

The comprehensive measurements of water levels, water quality, OMPs in water and sediments, and fish health in the Yeruham Reservoir show the relative influence of seasonal floods and continuous TTWW discharge on the reservoir dynamics. The Yeruham Reservoir provides an example of successfully integrating the nontraditional use of TWW for supporting ecosystems in water-stressed environments. We believe that due to the increasing pressure on freshwater resources, on one hand, and the increase in TWW quantity and quality, on the other hand, more TWW will be allocated in the future for ecosystems and recreation.

It is likely that the combined use of flood waters and TWW will play a key role in the development and maintenance of reservoirs, which will support the economy, tourism, education and recreation, especially in semi-arid and arid regions. However, further investigation and monitoring are required in order to assess the risks related to public health, for example due to fish consumption. It should be emphasized that in the current example of the Yeruham Reservoir, floods were the dominant factor that affected the water quantity and quality, while the WWTP provided effluent with a more predictable volume and composition. Thus, the combination of flood water and TWW reuse increases the confidence in the water supply to the reservoir throughout the year without jeopardizing the water quality or fish health.

Acknowledgments

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Appendix A

The water balance calculation was performed by adding or subtracting volumes of water from the reservoir volume (based on the GIS calculations). During the dry periods, evaporation and discharge from the WWTP to the reservoir were considered. During the wet periods, input from floods was considered by calculating the volume of the reservoir based on the increase in measured water level (GIS calculations). A comparison between the water balance volumes (V_{WB}) and the GIS volumes (V_{GIS}), which was performed with a paired *t*-test, revealed significant differences between the V_{WB} and V_{GIS} ($p = 0.009$). These differences were attributed to water percolation (P). A positive linear correlation between the calculated percolation and the measured water levels was observed and further support that the differences in mass balance are due to percolation (Fig. A1).

Table A1
Water level, volume and water balance parameters during dry periods.

n	T	ΔT (d)	h (m)	V_{GIS} (m^3)	A_{GIS} (m^2)	E (m)	E_{GIS} (m^3)	E_{day} (m^3)	Eff (m^3)	V_{WB} (m^3)	P (m^3)	P_{day} (m^3)
1	19.5.13		447.55	259,152	169,256							
2	3.6.13	15	447.24	209,754	155,045	0.1663	28,147	1876	13,825	244,829	35,075	2338
3	3.7.13	30	447.00	173,161	149,300	0.31	48,064	1602	31,690	193,380	20,219	674
4	4.8.13	32	446.83	148,167	144,486	0.3171	47,343	1479	15,851	141,669	−6498[a]	−203
5	3.9.13	30	446.49	100,758	134,387	0.2851	41,193	1373	21,978	128,952	28,195	940
6	1.10.13	28	446.30	75,749	128,807	0.223	29,968	1070	17,783	88,572	12,824	458
7	18.12.13		447.56	260,847	169,437							
8	29.12.13	11	447.57	262,544	169,604	0.0316	5354	487	14,659	270,152	7608	692
9	1.1.14		447.97	331,711	176,028							
10	6.1.14	5	447.92	322,930	175,333	0.0093	1637	327	8445	338,519	15,589	3118
11	23.1.14		448.27	385,265	180,546							
12	5.2.14	13	448.19	370,861	179,619	0.048	8666	667	15,591	392,190	21,329	1641
13	17.3.14		448.47	421,591	182,569							
14	10.4.10	24	448.06	347,634	177,709	0.1541	28,134	1172	16,214	409,671	62,036	2585

T – date of water level measurements.
ΔT (d) = $T_n - T_{n-1}$. Time intervals between measurements in days.
h (m) – measured water level, in meters, above sea level.
V_{GIS} (m^3) – reservoir volume accepted from the GIS model, based on h.
A_{GIS} (m^2) – reservoir area, as wet area, from the GIS model.
E (m) – cumulative evaporation between ΔT, based on data from the Israel Meteorological Service website, Sede Boqer station.
E_{GIS} (m^3) = $E_{(n)} \times A_{GIS(n-1)}$. Evaporated volume between ΔT.
E_{day} (m^3) = E_{GIS} / ΔT. Evaporated volume per day between ΔT.
Eff (m^3) – cumulative volume of effluent discharged from the WWTP between ΔT.
V_{WB} (m^3) = $V_{GIS(n-1)} - E_{GIS(n)} + Eff_{(n)}$. Theoretical volume of the reservoir based on water balance.
P (m^3) = $V_{WB} - V_{GIS}$.
P_{day} (m^3/day) = P / ΔT. Percolated volume per day between ΔT.
[a] A negative P value due to a negative difference between V_{WB} and V_{GIS}, can be considered a negligible percolation.

Appendix B

B.1. Spatial distribution of nutrients in the Yeruham Reservoir

Fig. 4 and Table 1 contain information on the temporal distributions of various chemical concentrations in the Yeruham Reservoir. In Fig. 4 and Table 1, each sampling event is represented by a single analysis of a composite sample, composed of 20 water samples from 13 locations that

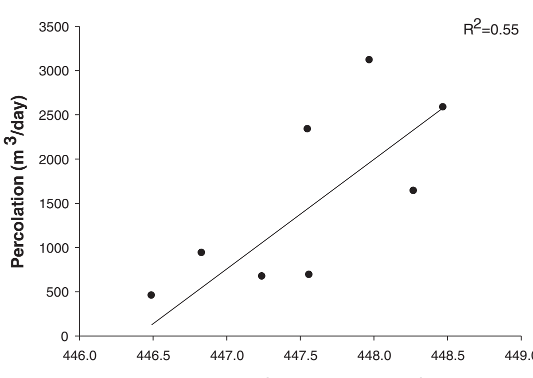

Fig. A1. Linear regression describing the link between water level in the reservoir and the calculated percolation.

were taken from the reservoir. The detailed sampling and analysis processes were carried out twice. The first sampling campaign was in March 2013 (end of the wet season). Water samples were taken from 13 sampling locations that are shown in Fig. B1. Locations 1–6 were sampled from the reservoir banks using a sampling rod, while locations 7–13 were taken from the reservoir using a boat. Samples from two different depths, 10 cm below the water surface (odd sample numbers) and 50 cm above the sediment (even sample numbers), were taken in locations 7–13 (Fig. B1). A similarly detailed sampling scheme was also performed during August 2013 (dry season). However, only 13 samples were taken during this sampling campaign, since in most of the wet areas around the reservoir, the depth was much <1 m, and it made no sense to sample from different depths.

B.2. Depth differences

The nutrient concentrations from the two different depths of the Yeruham Reservoir are shown in Table B1. No significant differences were found between the two depths during winter 2013 (total shallow vs. total deep, Wilcoxon signed rank-test, $p = 0.397$). No significant differences were found for any of the nutrients (Table B1).

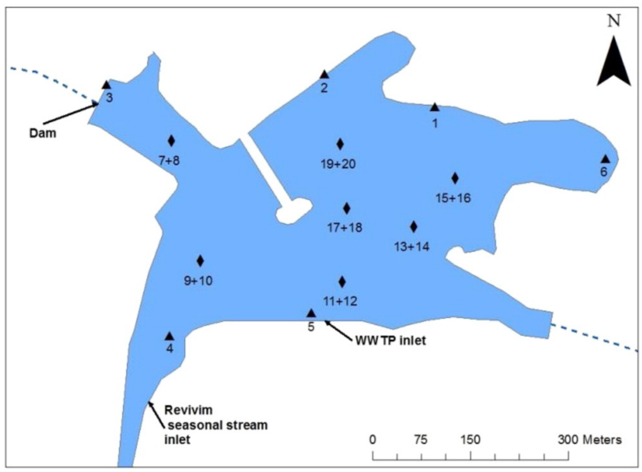

Fig. B1. Distribution of sampling points from which discrete and composite samples were taken in the Yeruham Reservoir. Samples 1–6 (triangles) were taken from the banks using a sampling rod, while samples 7–20 (diamonds) were taken from the middle of the reservoir using a boat.

B.3. Spatial distribution

In order to assess the spatial distribution of nutrients in the different sampling locations around the reservoir, water samples from all 20 sampling locations were analyzed. The results are shown in Fig. B2. In general, the composite sample represents well the water quality in Yeruham Reservoir, while only in one case, the composite concentration of TAN during summer 2013 was significantly lower than the range of individual samples as shown by the Box-Whisker presentation.

Table B1
Nutrient concentrations (mg/L) in shallow (10 cm below water surface, odd sample number) and deep (50 cm above the bottom, even sample number) sections of the Yeruham Reservoir.

Sampling locations	TAN		NO_3^-		TN		PO_4^-		TP		TSS	
	Shallow	Deep	Shallow	Deep	Shallow	Deep	Shallow	Deep	Shallow	Deep	Shallow	Deep
7–8	0.520	0.586	0.77	0.72	2.94	2.09	0.18	0.19	0.29	1.08	18	7
9–10	0.555	0.521	0.77	0.77	2.44	5.95	0.20	0.18	0.57	8.08	19	10
11–12	0.505	0.570	0.88	0.83	1.62	2.46	0.20	0.20	3.47	6.16	8	9
13–14	0.409	0.439	0.72	0.83	2.41	1.75	0.16	0.16	1.01	1.08	19	16
15–16	0.454	0.437	0.77	0.72	1.86	2.31	0.16	0.15	0.70	2.24	8	10
17–18	0.459	0.468	0.77	0.88	2.08	3.18	0.17	0.18	0.78	0.72	12	11
19–20	0.467	0.414	0.77	0.77	1.94	1.59	0.18	0.16	0.68	7.24	12	12
Statistical analysis	Two-tailed paired *t*-test ($p = 0.605$)		Two-tailed paired *t*-test ($p = 0.313$)		Wilcoxon signed rank-test ($p = 0.813$)		Two-tailed paired *t*-test ($p = 0.058$)		Two-tailed paired *t*-test ($p = 0.345$)		Two-tailed paired *t*-test ($p = 0.168$)	

Appendix C

Table C1
Concentrations and minimum quantification limit (MQL) of analyzed OMPs in water from the Yeruham Reservoir and TTWW from the Yeruham WWTP.

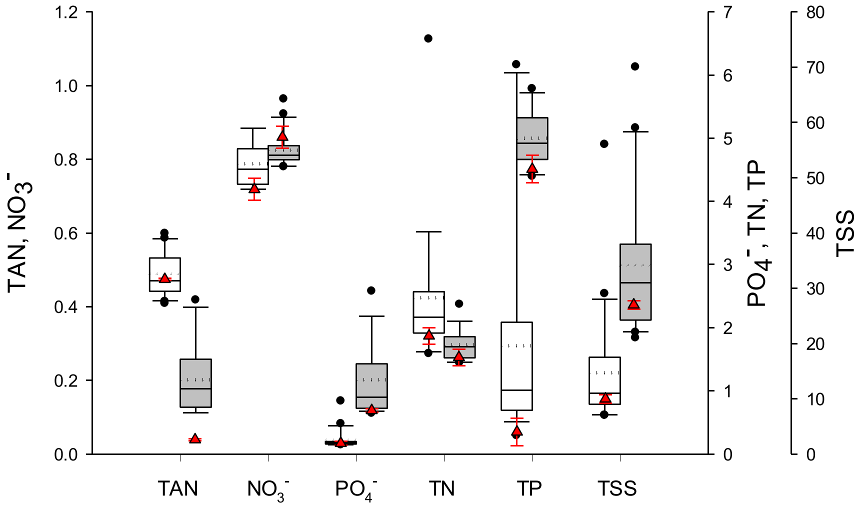

Fig. B2. Spatial analysis of nutrient concentrations (mg/L) in winter (white bars) and summer (gray bars) of 2013 is displayed using a Box-Whisker presentation; the solid horizontal line indicates the median value, the dashed horizontal line indicates the mean value, the upper and lower boundaries of the box indicate the 25th and 75th percentile values, and error bars indicate the 10th and 90th percentile values. Outrange values are marked with black circles, and composite sample concentrations are marked with red triangles. The composite sample error bar represents the analytical error.

	Compound	Units	MQL	Winter 2013		Summer 2013		Winter 2014		Summer 2014	
				Reservoir	TTWW	Reservoir	TTWW	Reservoir	TTWW	Reservoir	TTWW
EDCs	Estrone	ng/L	0.5	15	N.D.	3.3	2.6	8.4	1.6	1.0	8.4
	17β-Estradiol	ng/L	0.5	N.D.	N.D.	N.D.	N.D.	–	–	–	–
	Estriol	ng/L	0.5	N.D.	N.D.	N.D.	N.D.	N.D.	9.4	N.D.	8.1
	Testosterone	ng/L	0.5	–	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Bisphenol-A (BPA)	μg/L	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	4-Octylphenol (4-OP)	μg/L	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
PPCPs	Carbamazepine (CBZ)	μg/L	0.05	0.278	1.38	1.5	2.6	0.5	1.1	0.7	1.9
	Triclosan (TCS)	μg/L	0.05	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.3
	Caffeine	μg/L	0.05	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Diclofenac	ng/L	5	–	–	–	–	4	105	39.2	146
	Bezafibrate	ng/L	5	–	–	–	–	8	22.2	8	66.1
	Metoprolol	ng/L	5	–	–	–	–	N.D.	10.3	N.D.	55.5
	Propranolol	ng/L	5	–	–	–	–	N.D.	–	N.D.	–
	Ibuprofen	μg/L	0.1	–	–	–	–	N.D.	–	N.D.	–
	Ketoprofene	ng/L	250	–	–	–	–	N.D.	–	N.D.	–
	Venlafaxin	μg/L	0.1	–	–	–	–	N.D.	–	N.D.	–
	Naproxene	ng/L	250	–	–	–	–	N.D.	–	N.D.	–

N.D. = not detected.
– = not measured.

Appendix D

Table D1
Summary of the histopathological analysis.

Collection site	Species	Number of fish	Average length ± SD (cm)	Sex (no.)	Organ	Histopathological findings	% affected
Main water body	Mosquitofish	12	1.9 ± 0.2	♀ (4)	Gills	Hyperplasia: low level (1 fish; 1 focus).	8
				♂ (5)		Total affected	8[†]
				N.D. (3)	Spleen	MMCs: level 2 (1 fish) and level 4 (1 fish).	16
					Liver	Necrosis: low level of focal diffuse necrosis (1 fish).	8
	Tilapia	15	7.8 ± 1.8	♀ (3)	Gills	Parasite infection: low level (4 fish), moderate level (3 fish), high level (1 fish).	53
				♂ (4)		Epitheliocystis: low infection level (5 fish, 1–2 cysts in each).	33
				N.D. (8)		Hyperplasia: low level (7 fish, 1–2).	46
						Total affected	80
					Spleen	MMCs: levels 1–2 (6 fish), level 3 (2 fish) and level 4 (1 fish).	60[a]
	Carp	12	7.7 ± 1.2	N.D.	Gills	Parasite infection: low level (4 fish) and moderate level (1 fish).	42
						Hyperplasia: low level (5 fish, 2 foci in each).	42
						Total affected	58
					Muscle	Focal dystrophy of muscle fibers (2 fish).	17
TTWW inlet	Mosquitofish	40	1.8 ± 0.3	♀ (15)	Gills	Epitheliocystis: low infection level (1 fish with 1 cyst).	2.5
				♂ (15)		Total affected	2.5[†]
				N.D. (10)	Spleen	MMCs: level 1 (3 fish) and level 3 (1 fish).	10

(continued on next page)

Table D1 (*continued*)

Collection site	Species	Number of fish	Average length ± SD (cm)	Sex (no.)	Organ	Histopathological findings	% affected
					Muscle	Lesion in muscle fibers caused by Larnea (1 fish).	2.5
	Tilapia	40	3.1 ± 0.4	♀ (27)	Gills	Parasite infection: low level (13 fish), moderate level (11 fish), high level (3 fish).	67.5
				♂ (12)		Epitheliocystis: low infection level (7 fish, 1–3 cysts in each).	17.5
				N.D. (1)		Hyperplasia: low level (1 fish, 2 foci).	2.5
						Total affected	72.5*
					Spleen	MMCs: levels 1–2 (5 fish), level 3 (4 fish).	22.5b
					Kidney	MMCs: levels 1–3 (6 fish).	15
					Muscle	Focal dystrophy of muscle fibers (1 fish).	2.5

N.D. = sex could not be determined as the gonads were not seen; a, b, different letters denote statistical differences in % affected fish between collection sites for the designated fish species and pathological manifestation; †, *, different symbol denote significant differences between sampled species from a certain location. $p < 0.05$.

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