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Investigation in Reuse of Decommissioned Wastewater Facility and Reclaimed Water for Culturing Paddlefish Fingerlings

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Abstract

Reclaimed water is treated wastewater that has received at least secondary treatment and basic disinfection and is reused for beneficial purposes. The goal of this study was to develop a safe and reliable sustainable aquaculture system for producing stocker fish using reclaimed water in decommissioned wastewater treatment plants (WWTP) in Kentucky. The specific objectives were (1) to monitor paddlefish, *Polyodon spathula*, growth and survival and water quality in experimental tanks with static or flow-through reclaimed water, (2) to evaluate the use of decommissioned tanks for large-scale production of phase II paddlefish, and (3) to biomonitor paddlefish grown in reclaimed water for contaminants. Phase I paddlefish (11 ± 2.6 g) were produced by feeding live *Daphnia* collected daily from the clarifier tanks with hand-pulled nets for 27 d. Phase II paddlefish were produced in four replicated 5600-L experimental tanks with static and flow-through reclaimed water. Paddlefish from the flow-through system were significantly larger (199.2 ± 61 g) and had better feed conversion ratios (2.8 ± 2.1) than those from the static system (135.5 ± 51 g; 4.1 ± 1.6). For the large-scale trial, two 1125 m^3 decommissioned digester tanks were stocked with 50,000 paddlefish larvae per tank. One tank was treated as a flow-through system with reclaimed water flowing at a rate of 280 L/min, while the other tank was treated as a static system where water was just added to replace that lost by evaporation. Survival rate (40%) and weight (194.1 ± 25.4 g) from the flow-through system were significantly different from those of the static system (31%; 147.1 ± 6.5 g). This difference could be linked to better water quality in the flow-through systems. Analyses for 38 contaminants were conducted on *Daphnia*, prepared diets, and paddlefish. All the concentration levels detected were at levels well below the FDA action limits and their permissible limits in edible food. The result from this project showed that paddlefish can be successfully produced in large-scale as stocker fish using reclaimed water in decommissioned tanks at WWTP.

Water sources for aquaculture have traditionally been groundwater, surface water, and spring water. New sources of quality water are needed as the world's water resources are impacted by increasing demand, drought, depletion, and pollution. The need for additional water supplies has been the central motivator for water reuse. In the USA, the minimum level of treatment that must be achieved for discharge from municipal wastewater treatment plants (WWTP) is a secondary treatment. There are over 15,000 WWTP in the USA providing secondary or more advanced treatment (US EPA 2008) with a combined treatment capacity representing a treated effluent flow of approximately 121 billion liters per day (NRC 2012). Only 7.4% of this reclaimed water is estimated to be reused, suggesting its additional usage in the future (Miller 2006).

There is significant interest in using reclaimed water for aquaculture (EPA Victoria 2003). The ambiguous term "reclaimed water" has been used in publications inconsistently. This has produced a negative public perception when the term reclaimed water has been used. "Water should not be judged by its history, but by its quality" (Van Vuuren cited in Haarhoff and Van der Merwe 1996). In this article, we use the same definition for reclaimed water as the Southwest Florida Water Management District (www.swfwmd.state.fl.us), which states reclaimed water is municipal water that has

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received at least secondary treatment and basic disinfection and is reused after flowing out of a WWTP for beneficial purposes such as irrigation and groundwater recharge.

In aquaculture, the term wastewater has been used to describe different qualities of water from sewage to reclaimed water (Bunting 2004). The World Health Organization guidelines for the safe wastewater use for aquaculture set a standard of $\leq 10^4$ *Escherichia coli*/100 mL and zero viable trematode eggs per liter of treated wastewater (WHO 2006). Although the United States Environmental Protection Agency (US EPA) does not have a standard set for using reclaimed water for aquaculture, a concentration of ≤200 fecal coliform/100 mL has been recommended (Levine and Asano 2004; US EPA 2012). The US EPA limit requirement for surface-water discharge of reclaimed water is 200 fecal coliform/100 mL as a monthly average (Hammer and Hammer 2012). Therefore, by WHO guidelines, the reclaimed water produced in the USA could be used for aquaculture.

With better methods for processing wastewater, many municipalities in the USA are now building new, larger facilities and decommissioning the old ones, many of which have tanks and ponds that could be converted for fish culture. Many are being needlessly demolished when recycling them as fish production facilities could save the community demolition costs, create new jobs, and generate revenue. Some new facilities are being built adjacent to the old facilities and would allow reclaimed water to be used for aquaculture. Using reclaimed water for aquaculture would generally be considered a non-consumptive use of recycled water, because the effluent water goes back to the treatment process (Mims 2009). One concern of using reclaimed water for fish production is the potential chemicals that could be present in the water, especially heavy metals and persistent organic pollutants (Sapkota et al. 2008). Monitoring fish production in reclaimed water must be conducted to ensure that it is a safe and sustainable water supply for aquaculture. Paddlefish, *Polyodon spathula*, is a native fish in the USA with high aquaculture potential. Some attributes making paddlefish a high-valued fish species are boneless white meat with a firm texture and its roe, which is processed into black caviar (Mims and Shelton 2005). The quality of their caviar has been compared to that of Sevruga, *Acipenser stellatus*, caviar (Sevrin-Reyssac 1997). In this study, we propose using paddlefish as a contaminant bioindicator in reclaimed water. Monitoring fish species should be selected based on trophic levels, mobility, longevity, sensitive to pollution, consumer safety, distribution, native to the area, and other physiological and ecological characteristics (Van der Oost et al. 2003). Because of their longevity (30 yr) and large body size, paddlefish could bioaccumulate higher contaminant levels than other fishes. In addition, paddlefish respiration (via ram ventilation) and feeding (filter feeder) requires constant swimming with its mouth open to pass relatively large water volume across their gills; this behavior made paddlefish a better indicator for contaminants in reclaimed water (Gundersen and Pearson 1992; Gundersen et al. 2000).

The goal of this study was to develop a safe and reliable sustainable aquaculture system for producing stocker fish using reclaimed water in decommissioned WWTP in Kentucky. The specific objectives were (1) to monitor paddlefish growth and survival and water quality in experimental tanks with static or flow-through reclaimed water, (2) to evaluate the use of decommissioned tanks for large-scale production of phase II paddlefish, and (3) to biomonitor paddlefish grown in reclaimed water for contaminants.

Materials and Methods

The experiment was conducted at Frankfort Water Reclamation Facility (FWRF), Kentucky, USA, with a treatment capacity of 37.5 million liters per day of primarily residential and light industrial wastes. The processing treatment includes grit removal, aeration basins (oxidation ditch), final settling (clarifier), and effluent disinfection by ozone. The applied dose of ozone ranged from 3 to 6 mg/L with a contact time of 5–10 min (Loeb et al. 2012). The reclaimed water (disinfected secondary treated wastewater) characteristics are (monthly averages) 25 mg/L biochemical oxygen demand (5-d; carbonaceous), 30 mg/L total suspended solids, and 130 *E. coli* org/100 mL. The most notable elements found in the reclaimed water were (annual average) cadmium (<2.5 μg/L), lead (<10.0 μg/L), mercury (0.069 μg/L), and selenium (11.0 μg/L).

Production of Phase I Paddlefish in Experimental Tanks

Paddlefish larvae were propagated at Kentucky State University, Aquaculture Research Center (KSU-ARC), Frankfort, Kentucky, USA, according to Mims and Shelton (2005) from wild broodstock collected in Kentucky. Exogenous feeding larvae were stocked at FWRF into six 5600-L conical-bottomed polyethylene experimental tanks. Larvae were stocked at 1500 fish/tank. Live *Daphnia* were collected daily from the 1500-m^3 clarifier tanks with hand-pulled plankton nets and were fed to satiation twice daily to fish. Settable solids were siphoned daily from tanks. Dissolved oxygen level and temperature were monitored twice a day using an YSI Model 57 Meter (Yellow Springs Instruments, Yellow Springs, OH, USA). Total ammonia, nitrite, and pH were checked twice/wk. Total ammonia (Nesslerization) and nitrite (diazotization) were measured using a HACH DR/2500 Spectrophotometer (HACH, Loveland, CO, USA; methods 8038 and 8507), and pH was measured using an Oakton Model 510 Meter (Oakton Instruments, Vernon Hills, IL, USA). Fish were harvested after 27 d and measured for weight and length. Twenty-five fish were removed randomly from each tank, placed in food-grade polyethylene bags, and kept at −84 C for later contaminant analyses. Remaining fish were feed-trained for 7 d on 1.6 mm extruded trout prepared diet (45% protein, 15% fat; EXTR450 Rangen, Buhl, ID, USA).

Experimental Tanks: Static versus Flow-Through Systems

Feed-trained phase I paddlefish produced from the previous experiment were stocked at FWRF into eight 5600-L conical-bottomed polyethylene tanks. Paddlefish were stocked at 100 fish/tank. Four experimental tanks were treated as a flow-through system, flowing 6 L/min reclaimed water with total water replacement every 16 h. The other four experimental tanks were treated as a static system replacing only water lost during tank cleaning, which was done twice/wk with 50% water exchange. All tanks were equipped with air-lift systems and supplied with liquid oxygen by oxygen diffusers.

Control tanks were set up at the KSU-ARC. Dechlorinated city water was used in six 1700-L tanks. Paddlefish were stocked at 30 fish/tank. Three tanks were treated as a flow-through system flowing 1.8 L/min dechlorinated city water with water replacement every 16 h. The other three tanks were treated as static systems with 50% water exchanged two times per week.

Paddlefish were fed twice per day at 5% body weight. Paddlefish were sampled ($N \geq 30\%$) every 2 wk to determine average weights. Fish were initially fed extruded 1.6-mm floating trout diet (EXTR 450; Rangen) for 2 wk. The next 2 wk, initial feed was gradually replaced by a 3.2-mm floating catfish diet (CAT.32; Rangen). Thereafter, fish were fed 3.2-mm floating pellet (Li'l Stike; Southern States, Richmond, VA, USA). Water quality was monitored as described previously. Fish were harvested after 120 d and measured for weight and length. Ten percent of the fish collected were randomly selected, placed in food-grade polyethylene bags, and kept at −84 C for later contaminant analyses.

Large-Scale Trial in Decommissioned Tanks: Static versus Flow-Through Systems

For testing commercial application, two 1125-m^3 decommissioned digester tanks were used. Paddlefish larvae were stocked at 50,000 fish/tank. One tank was treated as a flow-through system with reclaimed water flowing at a rate of 280 L/min; the other tank was treated as a static system where water was just added to replace that lost by evaporation. Each tank was supplied with two 0.37-Kw surface aerator (Airolator, Kansas City, MO, USA) and air diffuser to prevent stratification. Paddlefish were initially fed *Daphnia* collected from the clarified tanks for 4 wk then 1.6-mm floating trout diet (EXTR 450; Rangen) for 4 wk and gradually replaced with a 3.2-mm floating catfish diet (CAT.32; Rangen). Thereafter, fish were fed an extruded diet (Li'l Strike; Southern States) to apparent satiation twice daily. Fish were harvested after 120 d.

Contaminants Analysis

Samples were analyzed for 38 contaminants. The fish were filleted and any red muscle in the fillets was trimmed and discarded. Samples were composited to yield 100 g homogenate. Homogenates were transferred to polyethylene containers and frozen at −84 C until analysis. Analyses were done at the Department for Environmental Protection/Division of Environmental Program Support/Environmental Services Branch Laboratory in Frankfort, KY, USA. Detection limits for the contaminants analyzed were cadmium (0.18 mg/kg), lead (0.46 mg/kg), selenium (0.18 mg/kg), mercury (0.0048 mg/kg), hexachlorobenzene (1 µg/kg), alpha-BHC (3 µg/kg), beta-BHC (1 µg/kg), gamma-BHC (1 µg/kg), delta-BHC (1 µg/kg), aldrin (5 µg/kg), heptachlor (1 µg/kg), heptachlor epoxide (1 µg/kg), oxychlordane (2 µg/kg), *trans*-chlordane (2 µg/kg), *cis*-chlordane (2 µg/kg), *trans*-nonachlor (2 µg/kg), chlordane (1 µg/kg), *cis*-nonachlor (2 µg/kg), technical chlordane (5 µg/kg), 2,4′-dichlorodiphenyldichloroethylene (DDE) (1 µg/kg), 4,4′-DDE (1 µg/kg), 2,4′-dichlorodiphenyldichloroethane (DDD) (1 µg/kg), 4,4′-DDD (1 µg/kg), 2,4′-dichlorodiphenyltrichloroethane (DDT) (1 µg/kg), 4,4′-DDT (1 µg/kg), total DDT (2 µg/kg), mirex (1 µg/kg), endosulfan sulfate (1 µg/kg), aroclor 1016 (10 µg/kg), aroclor 1221 (20 µg/kg), aroclor 1232 (10 µg/kg), aroclor 1242 (10 µg/kg), aroclor 1248 (10 µg/kg), aroclor 1254 (10 µg/kg), aroclor 1260 (10 µg/kg), aroclor 1262 (10 µg/kg), aroclor 1268 (10 µg/kg), and toxaphene (10 µg/kg).

Data Analyses

Fish performance indices were calculated using the following formulae:

$$\text{Apparent feed conversion ratio (FCR)}$$
$$= \text{feed intake (dry weight)}$$
$$/\text{body weight gain (wet weight)}.$$

Fulton's $K = (W/L^3) \times 100$, where $W =$ wet weight (g) and $L =$ length (cm). Specific growth rate (SGR % increase in body weight/d) = $([\ln W_\mathrm{f} - \ln W_\mathrm{i}]/t) \times 100$, where $W_\mathrm{f} =$ final weight (g), $W_\mathrm{i} =$ initial wet weight (g), and $t =$ time (d).

Water quality was evaluated using a complete randomized design with repeated measures. Data were analyzed with the MIXED procedure in SAS Version 9.3 software (SAS Institute, Cary, NC, USA). The covariance structure, autoregressive of order 1, was used in the repeated measure model. Contaminants data were analyzed using *t*-test to determine the differences between treatments. Differences were considered statistically significant when $P < 0.05$.

Results

Production of Phase I Paddlefish in Experimental Tanks

Water quality variables for this experiment were (average ± SD) dissolved oxygen (10 ± 3 mg/L), temperature (21 ± 2.5 C), pH (7.3 ± 0.4), un-ionized ammonia (0.03 ± 0.03 mg/L), and nitrite (0.7 ± 0.7 mg/L). Mean survival of phase I paddlefish fed live food after 27 d was 57 ± 24%. Mean weight was 11 ± 2.6 g. SGR was 0.41 ± 0.05 g/d.

Experimental Tanks: Static versus Flow-Through Systems

Differences in water quality between static and flow-through systems are presented in Table 1. Afternoon dissolved oxygen and temperature and pH were not significantly different between static and flow-through systems while morning dissolved oxygen and temperature, un-ionized ammonia, and nitrite were significantly different. The only not significant difference in the interaction between treatment and time was for the afternoon temperature. Because nitrite values were as high as 5 mg/L in the static systems,

TABLE 1. *Repeated-measure analysis of water quality variables in experimental static and flow-through systems.*[a]

	Treatment		Type 3 tests of fixed effects ($P > F$)	
Variables	Static	Flow-through	Treatment	Treatment × date
D.O. am (mg/L)	9.33 ± 0.15	11.04 ± 0.14	0.0002	<0.0001
D.O. pm (mg/L)	9.32 ± 0.21	10.14 ± 0.11	0.1386	<0.0001
Temp. am (C)	22.0 ± 0.13	22.6 ± 0.09	0.0006	<0.0001
Temp. pm (C)	23.2 ± 0.12	23.5 ± 0.09	0.0993	0.0549
pH	7.02 ± 0.03	6.98 ± 0.02	0.0675	<0.0001
NH_3-N (mg/L)	0.02 ± 0.002	0.01 ± 0.001	0.0005	<0.0001
NO_2-N (mg/L)	1.84 ± 0.15	0.25 ± 0.03	<0.0001	<0.0001

D.O. = dissolved oxygen.

[a] Numbers represent mean ± SEM.

high-grade evaporated salt (Cargill, Minneapolis, MN, USA) was added to keep a chloride to nitrite–nitrogen ratio of 16:1 to prevent methemoglobinemia. At the end of the study, static systems had a survival rate of 94 ± 5.5% while survival in the flow-through was 97 ± 4.9%. Survival rates were not different ($P = 0.285$). Paddlefish in the flow-through system were larger (199.2 ± 61 g) ($P < 0.0001$) than those from the static systems (135.5 ± 51 g). Fish in flow-through systems had a significantly better FCR (2.8 ± 2.1) than fish in the static systems (4.1 ± 1.6). Fulton's condition factor was not significantly different between fish in flow-through (0.029 ± 0.00092) and static (0.029 ± 0.00077) systems.

Large-Scale Trial in Decommissioned Tanks: Static versus Flow-Through Systems

A total of 15,773 phase II paddlefish were harvested from the static digester tank. The survival rate was 31.55% and mean weight was 147.1 ± 6.5 g. A total of 20,123 paddlefish were harvested from the flow-through digester tank. The survival rate was 40.25% and mean weight was 194.1 ± 25.4 g. Paddlefish from the flow-through tank were significantly larger than those from the static system ($P < 0.05$).

Contaminants Analysis

From the 38 contaminants analyzed, only 11 contaminants were detected in *Daphnia*, nine from phase I paddlefish raised in reclaimed water, and seven from the control in city water (Table 2). Mercury was the only contaminant that was not significantly different among *Daphnia*, paddlefish from reclaimed water, and paddlefish from city water. The bioaccumulation of contaminants in phase I paddlefish that fed on wastewater-grown *Daphnia* was at low levels (Table 2).

From the 38 contaminants analyzed in phase II paddlefish culture in experimental static or flow-through systems, only nine were detected in paddlefish from static systems and eight from flow-through systems (Table 3). All the concentration levels detected were at levels well below the Food and Drug Administration (FDA) action limits and their permissible limits in edible food. Comparison of the static and flow-through systems found that residues of chlordane were significantly higher ($P < 0.05$) in the flow-through system (Table 3). Adding the means for residues of chlordane in the flow-through system, including technical, *cis*- and *trans*-chlordane, and *trans*-nonachlor, equal a "chlordane" total value of 0.11 mg/kg, which is below the FDA action level of 0.30 mg/kg for total chlordane.

The contaminants detected in the prepared diets fed to phase II paddlefish were cadmium, mercury, selenium, and technical chlordane; although cadmium was just found in the feed EXTR450. Contaminant concentrations varied across the different feed types (Table 4).

Discussion

Using treated wastewater for aquaculture is not a new idea (Edwards and Pullin 1990). With more sophisticated treatment processes for wastewater reclamation, better water quality can

TABLE 2. *Contaminants (mg/kg) analyzed from live food and phase I paddlefish that were fed* Daphnia *for 27 days and were cultured in reclaimed water or dechlorinated city water in experimental tanks.*[a]

		Phase I paddlefish	
Contaminant	*Daphnia*	Reclaimed water	City water[b]
Mercury	0.024 ± 0.02^{a}	0.018 ± 0.01^{a}	0.023 ± 0.01^{a}
Selenium	0.642 ± 0.32^{a}	Not detected^{b}	0.201 ± 0.01^{a}
Technical chlordane	0.104 ± 0.02^{a}	0.043 ± 0.00^{b}	0.035 ± 0.00^{b}
Cis-chlordane	0.016 ± 0.00^{a}	0.005 ± 0.00^{b}	0.003 ± 0.00^{b}
Trans-chlordane	0.013 ± 0.00^{a}	0.004 ± 0.00^{b}	0.004 ± 0.00^{b}
Trans-nonachlor	0.008 ± 0.00^{a}	0.004 ± 0.00^{b}	0.003 ± 0.00^{b}
Heptachlor epoxide	0.008 ± 0.00^{a}	0.003 ± 0.00^{b}	Not detected^{c}
4,4′-DDE	0.002 ± 0.00^{a}	0.001 ± 0.00^{ab}	0.002 ± 0.00^{a}
4,4′-DDT	0.001 ± 0.00^{a}	Not detected^{b}	Not detected^{b}
Hexachlorobenzene	0.002 ± 0.00^{a}	0.001 ± 0.00^{b}	Not detected^{c}
Aroclor 1254	0.034 ± 0.01^{a}	0.008 ± 0.00^{b}	Not detected^{c}
% Lipids	3.253 ± 0.51^{a}	3.355 ± 0.01^{a}	0.679 ± 0.10^{b}

[a] Values represent mean ± SD, $N = 4$. Significant differences in a line are indicated by different superscripts ($P < 0.05$).

[b] Activated charcoal was used to dechlorinate the city water.

TABLE 3. *Contaminants (mg/kg) analyzed from phase II paddlefish culture in experimental static or flow-through systems and fed prepared diets for 120 d.*[a]

Contaminant	Static	Flow-through	FDA action level
Mercury	0.021 ± 0.00^{a}	0.020 ± 0.00^{a}	1.0
Selenium	0.172 ± 0.12^{a}	0.176 ± 0.10^{a}	NE[b]
Technical chlordane[c]	0.024 ± 0.02^{a}	0.072 + 0.01^{b}	0.3
Cis-chlordane[c]	0.002 ± 0.00^{a}	0.016 ± 0.00^{b}	0.3
Trans-chlordane[c]	0.003 ± 0.00^{a}	0.016 ± 0.00^{b}	0.3
Trans-nonachlor[c]	0.001 ± 0.00^{a}	0.006 ± 0.00^{b}	0.3
Heptachlor epoxide	0.004 ± 0.00^{a}	0.016 ± 0.00^{b}	0.3
4,4′-DDE[d]	0.001 ± 0.00^{a}	Not detected^{b}	5.0
Aroclor 1254[e]	0.003 ± 0.01^{a}	0.008 ± 0.01^{a}	3.0
% Lipids	6.480 ± 1.07^{a}	7.470 ± 1.16^{a}	

[a] Values represent mean ± SD ($n = 4$). Significant differences in a line are indicated by different superscripts ($P < 0.05$).

[b] NE: Not guidelines have been established.

[c] The action level is for total chlordane.

[d] The action level for DDT, DDE, and TDE is for residues of the pesticides individually or in combination.

[e] The action level is for total polychlorinated biphenyls (PCB) aroclors. Seven aroclors were analyzed including aroclor 1016, 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268.

be achieved depending on the intended use (US EPA 2012). Reclaimed water is highly tested; in some cases, this water has been demonstrated to be safe as a source of potable water (NRC 2012). In this study, we focus on using reclaimed water (disinfected secondary treated wastewater) for aquaculture production. Although reclaimed water contains some nutrient elements, they are insufficient to meet the nutritional requirements of fish, so additional food sources (i.e., prepared diets) need to be applied.

Production of Phase I Paddlefish in Experimental Tanks

Zooplankton is a valuable source of protein, lipids, and enzymes for numerous larval fishes (Kibria et al. 1997). Abundant zooplankton population can be produced during wastewater treatment, especially during the secondary settling. Zooplankton production in tertiary lagoons also known as polishing lagoons has been calculated to be 18.6 m.t./ha/yr (Guerrin 1988), 81 kg/d (Metcalfe 1995), and as many

TABLE 4. *Contaminants (mg/kg) analyzed from prepared diets fed to paddlefish.*

	Rangen feed		Southern states
Compound	EXTR 450 1.6 mm	CAT.32 3.2 mm	Li'l Strike 3.2 mm
Diet			
% Protein	45	32	36
% Fat	16	6	6
% Fiber	<2	<5	6
Contaminants			
Cadmium	0.285	Not detected	Not detected
Mercury	0.019	0.011	0.007
Selenium	1.310	0.780	0.251
Technical chlordane	0.016	0.015	Not detected
% Lipids	8.880	8.330	4.040

as 40–84 kg/h harvested during the summer (Kibria et al. 1999). Copepods densities in these lagoons could be as high as 4356/L (Nandini 1999). Previous studies used zooplankton collected from tertiary lagoons to feed fish such as common carp, *Cyprinus carpio* (Guerrin 1988), golden shiner, *Notemigonus crysoleucas* (Metcalfe 1995), silver perch, *Bidyanus bidyanus* (Kibria et al. 1999), and Nile tilapia, *Oreochromis niloticus* (Sousa 2007). There is concern about bioaccumulation of heavy metals and persistent organic pollutants from fish fed zooplankton collected from these systems (Kibria et al. 1997; Nandini et al. 2005). In this study at the FWRF, live daphnid species grew abundantly in the nutrient-rich treated wastewater in clarifier tanks. Large daphnids are known to be the preferred primary food for paddlefish larvae (Mims et al. 1995a, 1995b). Once paddlefish reach a weight of 3–4 g, they can readily accept 1.5 mm extruded pellets (Mims et al. 2009). We demonstrated the contaminant levels in *Daphnia* collected from the clarifier tanks were at low concentrations and were not bioaccumulated into paddlefish after 27 d of feeding. In a similar study, silver perch fed for 30 d on zooplankton, *Daphnia carinata* and/or *Moina australiensis*, collected from a tertiary lagoon contained low levels of zinc, cadmium, and lead (Kibria et al. 1999). Zooplankton grown from treated wastewater could be a valuable food source for larval fish. It is important to notice, depending on the raw sewage source and the treatment process, contaminant concentration in this food source can vary.

Experimental Tanks: Static versus Flow-Through Systems

Most research on fish production using reclaimed water or partially treated wastewater has been focused on constructed wetland treatment systems. Treatment wetlands are designed to take advantage of the natural process to assist in treating wastewater. Polishing wetlands receive secondary effluents and provide tertiary or advanced treatment (Kadlec and Wallace 2009). Examples of these constructed wetlands were the Arcata marsh and sanctuary where Coho salmon, *Oncorhynchus kisutch*, were raised (US EPA 1993; Allen 1998), and the pilot scale aquaculture–wetland ecosystem that grew tilapia for food (Costa-Pierce 1998). In this study, reclaimed water was used only as a water source for aquaculture production and not as part of the treatment process. Previous studies used secondary treated wastewater without disinfection as a water source to grow Nile tilapia in Egypt (Khalil and Hussein 1997) and tilapia hybrid, *Oreochromis niloticus* × *O. aureus*, gray mullet, *Mugil cephalus*, and hybrid Chinese carp, *Hypophthalmichthys molitrix* × *Aristichthys nobilis*, in Israel (Feldlite et al. 2008). In this study, we found that paddlefish raised in flow-through system had better growth and FCR than those from the static system. This may potentially be linked to better water quality and more stable dissolved oxygen levels in the flow-through systems. While nitrite levels were significantly higher in static systems, this could have had a profound effect on growth. Due to the high nitrite concentration, it was necessary to increase the chloride level to avoid any toxic effect. Therefore, water flowing will have the advantages of greater, more stable exchange of oxygen and nitrogenous waste removal.

In addition, our results indicate that the contaminant concentrations were well below the FDA action limits and at their permissible limits in edible food in all samples. Chlordane was higher in paddlefish from the flow-through systems (0.11 mg/kg) but was still far below the maximum allowable limit (0.3 mg/kg). Although all registered uses of the pesticide chlordane were banned in USA in 1988, chlordane bioaccumulates in the environment due to slow degradation rates and high lipid solubilities (Blocksom et al. 2010). Chlordane has been detected in eggs of Ohio River paddlefish at concentrations exceeding the FDA action limit (Gundersen et al. 1998, 2000). Persistent hydrophobic chemicals may accumulate in fish through direct uptake from water by gills or skin (bioconcentration), via uptake of suspended particles (ingestion), and via consumption of contaminated food (biomagnification) (Van der Oost et al. 2003). The results of this study indicate that most chlordane came from live food. Phase I paddlefish produced in city water had the same chlordane concentration as fish produced in reclaimed water. The source of the chlordane was the *Daphnia* collected from the secondary clarifier tanks and fed to paddlefish in reclaimed water and city water. Being hydrophobic, chlordane adhered to any particle, in this case *Daphnia* fed to the paddlefish. This explains why paddlefish raised in city water had similar chlordane concentration as those raised in treated effluent water.

Large-Scale Trial in Decommissioned Tanks: Static versus Flow-Through Systems

One of the major capital investments in aquaculture is pond or tank construction. The total cost of investment will be considerably reduced if culture units already exist. Traditionally, location has been considered one of the most important factors to initiate a business in aquaculture. However, an abundant source of good-quality water is the first and foremost important need for a fish farm (Beem 1998). This study combined all these factors using reclaimed water in decommissioned wastewater treatment tanks located in rural communities. Cost-effective use of reclaimed water and decommissioned facilities for aquaculture applications necessitates producing fish relatively close to the potential consumers. As technologies are now advanced so treated wastewater is of better quality, the stigma of extensive waste treatment is becoming more obsolete. Attempts have been made to rehabilitated ponds or lagoons designed for sewage treatment for aquaculture purposes (Bunting 2004). Some examples include using stabilization ponds to produce Nile tilapia and bluespot mullet, *Moolgarda seheli*, in Egypt (Shereif et al. 1995), Nile tilapia in Peru (Nava 2001), and African catfish, *Clarias gariepinus*, in Ghana (Tenkorang et al. 2012), although these examples used partially treated wastewater without disinfection. With stricter regulations, the treatment process has to be modified or the plant be upgraded, leaving behind decommissioned tanks. The FWRF had two 1125-m^3 digester tanks that had not been in use for more than a decade. In this project, we wanted to take advantage of these unused tanks and convert them into aquaculture production systems and demonstrate their commercial application.

Paddlefish were successfully raised in large scale in decommissioned digester tanks, although survival was lower compared with experimental tanks. Paddlefish stocked in these large-scale trials were at larval stage; this stage requires a large quantity of *Daphnia.* It is estimated that paddlefish larvae requires >200 *Daphnia*/L to attain high survival and growth rates (Mims and Schmittou 1989). Lack of proper quantity of zooplankton has been cited as the major reason for reduce growth and survival of paddlefish (Mims and Schmittou 1989). Survival attained in this study (30–40%) is comparable with other studies in nursery ponds such as Michaletz et al. (1982) (8–30% survival), Mims et al. (1995a) (26–31% survival), and Mims et al. (1995b) (50–55% survival). A better method of harvest and introduction of the live food into these types of large deep tanks needs to be developed to improve survival and growth. Because of the depth (8 m) of these tanks, the tanks were kept destratified using existing diffusers installed in the digester tanks. However, the obstruction of the diffuser only allowed partial seining increasing time of labor. A crane was used to lift a tub that was filled with fish. Such equipment was already present at the WRF and easily accessible. The type of digester used in this research was an open-top aerobic digester. In other WRF or WWTP, the digesters tanks sometimes have a fixed cover that could be modified or removed to be used for aquaculture. With relatively low capital investment to repurpose these tanks, existing infrastructure could successfully be converted for aquaculture and raise fish at commercial level.

Contaminants Analysis

Some people are concerned about the safety of using reclaimed water for aquaculture. The public perception of "naturalness" where people strongly believe that "fish grown in natural bodies of water" are safer to eat than fish from reclaimed water has caused lack of acceptability of water reuse (Miller and Mosher 2005). However, the fact is in the USA: 42% of the nation's total lake acreage and 36% of the nation's total river miles have been under advisory (US EPA 2011). From this same surface water, the US aquaculture industry withdraws 78% of their freshwater used (Lovelace 2009). In this study, we were able to demonstrate the potential safety of using reclaimed water for aquaculture. All values of the tested contaminants in the muscle tissue were found below FDA action levels. Fillet is the most popular process for human consumption in the USA; other organs (i.e., liver) could have higher contaminant concentration but are not commonly eaten and were not analyzed in this study.

Contaminant levels depend on the characteristics of the sewage, degree of treatment process before use, and feed used (Bunting 2004). Bioaccumulation of contaminants from fish cultured in sewage or partially treated wastewater had yielded elevated contaminant levels not safe for human consumption (Odjadjare et al. 2011; Authman et al. 2012). Recently emerging contaminants such as pharmaceuticals, personal care products, and endocrine-disrupting chemicals have received growing attention due to potential implications for human and ecological health effects (Diamanti-Kandarakis et al. 2009). WWTP are generally not designed for removing these micropollutants (Gerrity and Snyder 2011). Ozone has demonstrated to be effective in removing micropollutants, particularly steroid hormones (Gerrity and Snyder 2011). Despite the effectiveness of ozone, using ozone at WRF in the USA has been limited with fewer than 10 WRFs currently using ozone. We had the advantage in this study to use ozone-disinfected water at Frankfort's WRF being one of them (Oneby et al. 2010). Future studies on contaminant bioaccumulation in food fish needs to be carried out using reclaimed water that has been disinfected with other types of disinfection such as ultraviolet radiation and chlorine.

Today, some states in the USA rely on reclaimed water as a source of potable water (US EPA 2012). Reclaimed water could represent a safe and sustainable alternative supply of water for aquaculture. Reuse of decommissioned wastewater plants for aquaculture could provide significant opportunities to enter aquaculture with little capital investment for initiating food fish production. The water management in this study was considered as a zero-discharge system, because the water used for aquaculture went back to the head of the plant for processing and was not released into the environment. Future studies need to be conducted with other species using this innovative reuse technology.

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