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Use of constructed wetlands with four different experimental designs to assess the potential for methyl and total Hg outputs

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Abstract

This study used 10 parallel, smallscale constructed wetlands to investigate the potential for methylmercury (MeHg) production and water quality improvement using water and sediment from a creek that is a significant source of non-point nutrient, sediment and Hg pollution to a pristine river. The 4 replicated experimental designs utilized: (1) creek or Hg-contaminated water (25–320 ng Hg L^{-1}) and creek or Hg-contaminated sediment (0.86 ± 0.52 µg Hg g^{-1}) (MW-MS), (2) Hg contaminated water and clean sediment (0.09 ± 0.03 µg Hg g^{-1}) (MW-CS), (3) clean water (effluent from a wastewater treatment plant; 4–16 ng Hg L^{-1}) and Hg contaminated sediment (CW-MS), and (4) clean water and clean sediment (CW-CS). All designs functioned as sinks for N, P, sediment, and total Hg (THg). However, designs receiving clean water as the influent exhibited the least removal.

Seasonal variations in net MeHg output were observed for designs with MW-MS and CW-MS, with concentrations peaking during warmer months. Designs with CS did not exhibit clear seasonal trends. Wetlands with CW and MS were the greatest MeHg sources. This was probably due to the fact that the treated wastewater had greater SO_4^{2-} and total organic C (TOC) concentrations, lower pH, and, in general, higher temperatures than the creek water, and to the greater pool of Hg available in the Hg contaminated sediment, all of which could lead to enhanced Hg methylation. Temperature and SO_4^{2-} correlated best with MeHg output in all designs. Although data from these small systems cannot be scaled up to predict the response in larger wetlands, results indicated that the benefits of a wetland, such as nutrient, suspended solids and THg removal, should be considered together with the risk of MeHg production.

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1. Introduction

This study used small-scale constructed wetlands to investigate their potential use to improve water quality of Steamboat Creek (SBC), Nevada, USA (Fig. 1). This creek is the largest source of pollution to the Truckee River, which ends in a terminal water body, Pyramid Lake. The pollution is derived from bank erosion, agricultural and storm water runoff, geothermal inputs, and legacy mining inputs (Reuter et al., 1992; NDEP, 1994). The creek annually contributes ~70 ton of N, ~15 ton of P, 900 ton of total suspended solids (TSS) (NDEP, 1994),

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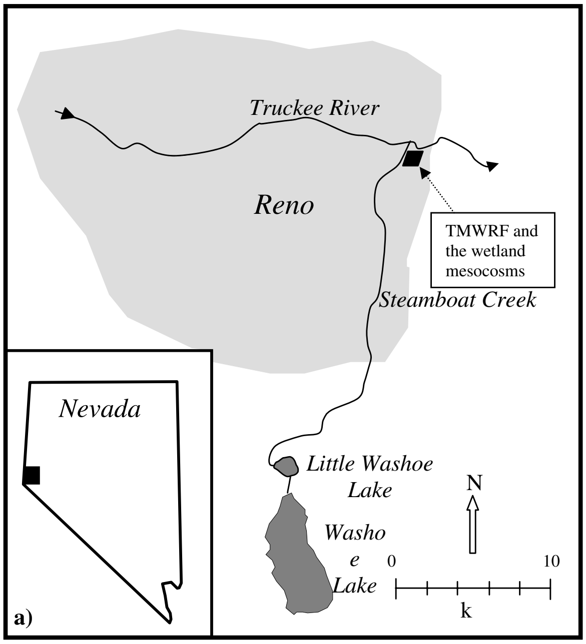

Fig. 1. Location of the study area in Nevada (a) and in the Reno area. The wetland mesocosms were situated at the confluence of Steamboat Creek and the Truckee River on the properties of the Truckee Meadows Water Reclamation Facility (TMWRF) (figure source: Scott Brown).

and about 4–10 kg of Hg to the Truckee River (Thomas, 2003).

High Hg concentrations have been reported in SBC water (24–419 ng L^{-1}; Lyons et al., 1998; Blum et al., 2001; Thomas, 2003; Gustin et al., 2005; Brown et al., 2006) and sediment (0.2–10.2 μg g^{-1}; Blum et al., 2001; Stamenkovic et al., 2004; Brown et al., 2006). Mercury in SBC water is predominantly particulate bound (75 ± 17%) (Thomas, 2003; Stamenkovic et al., 2005) and derived from mine waste produced at ore processing facilities in the creek headwaters in the late 1800s (Lyons et al., 1998; Blum et al., 2001; Thomas, 2003; Stamenkovic et al., 2005).

One option that has been considered for reduction of nutrient and suspended solid loading from SBC to the Truckee River is a large-scale wetland system at the mouth of SBC. Research has shown that constructed wetlands are a cost-effective and reliable method for implementing water quality improvements (Knowlton et al., 2002; Reed and Brown, 1992; Spieles and Mitsch, 2000; Stamenkovic et al., 2005). Wetlands have also been shown to be a sink for THg (St. Louis et al., 1994, 1996; Waldron et al., 2000; King et al., 2001; Galloway and Branfireum, 2004; Warner et al., 2005), but sites of MeHg release to downstream ecosystems (Zillioux et al., 1993; Krabbenhoft et al., 1995; Branfireun et al., 1996; Schwesig et al., 1999; Galloway and Branfireum, 2004). Since MeHg bioaccumulates in aquatic food webs and consumption of fish with MeHg is the most significant human exposure pathway, there is concern that wetland construction in the SBC watershed could increased MeHg release to the lower Truckee River. Fish collected from the Truckee River downstream from SBC have higher Hg concentrations than fish collected above the confluence (Gustin et al., 2005), demonstrating that current creek inputs are having an adverse impact.

To investigate the potential for nutrient removal and MeHg production associated with SBC water and sediment, 10 wetland mesocosms with 4 different experimental designs were constructed over ~3 a. In 2000, 3 mesocosms with SBC water as the influent were constructed, two with creek sediment and one with clean sediment (Spurkland, 2001). In July 2001, 2 additional mesocosms were constructed using Truckee Meadows Water Reclamation Facility as the wetland influent water and creek sediment as substrate (Fig. 2). The data obtained after monitoring these 5 mesocosms over a 1-a period (2002) was documented by Stamenkovic et al. (2005). In July 2002, 5 additional mesocosms with clean sediment were constructed with 3 receiving creek water and two receiving treatment plant effluent. Since the latter is discharged into SBC, these 4 replicated experimental designs mimicked scenarios for wetland water and sediment sources at the mouth of SBC.

This paper discusses the continued performance of the wetlands described in Stamenkovic et al. (2005) and of the additional wetlands added in 2002. The seasonal and temporal variations in the net MeHg and THg outputs associated with the different designs are described along with a comparison of the systems. The potential for a constructed wetland to improve the quality of the water released from SBC to the Truckee River is considered.

2. Materials and methods

2.1. Site description

Each of the 10 parallel wetland mesocosms utilized in this study had a 0.45 mil rubber liner and 0.3 m thick sediment base, and contained 3 cells of dimensions 1.8 m wide, 3 m long and 0.6 m deep,

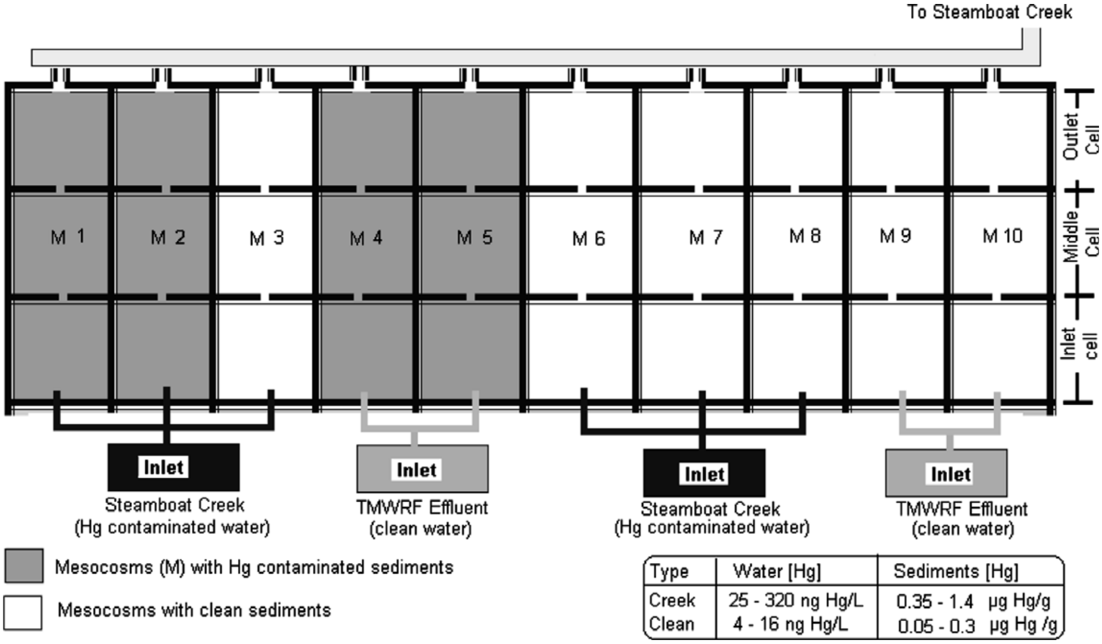

Fig. 2. Schematic for the constructed wetland mesocosms used for this study. Mesocosms 1, 2 and 3 were operational in July 2000, M4 and M5 in July 2001, and M6 through M10 in January 2003. M6 had no vegetation.

resulting in a total surface area of 16.2 m^2. The length-to-width ratio for the mesocosms was 5:1, which is within the range recommended for constructed wetland systems (Crites, 1994). Six of the mesocosms (1, 2, 3, 6, 7, and 8) utilized water from SBC (25–320 ng Hg L^{-1}) or Hg contaminated water (MW) (Fig. 2). Effluent from Truckee Meadows Water Reclamation Facility (4–16 ng Hg L^{-1}) or clean water (CW) was used as the influent water for the other 4 mesocosms (mesocosms 4, 5, 9 and 10). The water treatment facility has dual media filtration to reduce TSS and tertiary treatment for N and P removal. Water from both sources was pumped to a head tank from which a controlled flow of water was gravity fed into each wetland mesocosm. Effluent weirs at the dividers between each cell were used to maintain the desired water level. In the head tank, coarse sediment was settled out and only fine suspended particles (silt and clay) were transported into the wetlands (Brown et al., 2006). The surface flow wetlands had a discharge rate of 7 ± 4 L min^{-1} over the 2 a of the study. Flow rates were adjusted bi-weekly to ensure similar flows through all the mesocosms. The hydraulic residence time was 4–6 h. Sediments from SBC (0.86 ± 0.52 µg Hg g^{-1}), referred to as Hg contaminated sediments (MS) were used for the base of mesocosms 1, 2, 4, and 5, while substrate with lower Hg concentrations (0.09 ± 0.03 µg Hg g^{-1}), derived from the surrounding area above the creek floodplain, referred to as clean sediments (CS), was used for mesocosms 3, 6, 7, 8, 9, and 10 (Fig. 2). This provided for 4 replicated experimental designs: (1) Hg contaminated water and Hg contaminated sediment (MW-MS) (mesocosms 1 and 2); (2) Hg contaminated water and clean sediments (MW-CS) (mesocosms 3, 6, 7, and 8); (3) clean water and Hg contaminated sediments (CW-MS) (mesocosms 4 and 5); and (4) clean water and clean sediments (CW-CS) (mesocosms 9 and 10) (Fig. 2). All mesocosms were allowed at least 6 months to equilibrate before data collection was initiated. All mesocosms except 6 (no vegetation denoted by NV) were densely vegetated with approximately 70% cattails (*Typha* sp.) and the rest comprised of rushes (*Juncus* sp.), and duckweed (*Lemna* sp.). Mesocosm 3 is of similar design to 7 and 8 (all MW-CS), but it was operational in 2000, while the designs 7 and 8 were constructed in July 2002.

2.2. Sample collection and analyses

Unfiltered (total) and filtered (dissolved) water samples were collected from inflow and outflow of each mesocosm using clean sampling techniques (EPA Method 1669) and acid washed Teflon® bottles

(Keeler et al., 1995) for Hg analyses. All samples were preserved with optima HCl (0.4% by volume) and refrigerated until analysis. Samples were filtered using a peristaltic pump, acid cleaned Teflon tubing and 0.45 μm Teflon capsule filters (Swico Inc. part # FFC1011PEY). Filter blanks were obtained using the configuration above and water generated using a Millipore® Milli-Q water filtration system.

Concentrations of MeHg in water were determined following USEPA method 1630 using cold-vapor atomic fluorescence spectrophotometry (CVAFS) after distillation and aqueous phase ethylation and isothermal GC separation (Bloom, 1989). Concentrations of THg were measured after bromine monochloride oxidation, stannous chloride reduction, and purging of Hg from solution onto Au-coated quartz sand traps (Bloom and Crecelius, 1983). Mercury on traps was analyzed by dual amalgamation and CVAFS (EPA method 1631) (Bloom and Fitzgerald, 1988; Dumarey et al., 1985). Detection limits based on 3 standard deviations of reagent blanks were 5 pg L^{-1} ($n = 25$) for MeHg and 0.1 ng per 100 mL ($n = 34$) for THg. A 5-point calibration was conducted daily, before and after MeHg analysis, and verified every 10–12 samples, using certified reference material (NIST-3133). Dogfish muscle standard (DORM-2) was used as an independent quality control sample for MeHg analysis (Bloom et al., 1997; Marvin-DiPasquale et al., 2003) and measured concentrations were 115 ± 21% ($n = 91$) of the certified value. Triplicate analyses of water samples yielded an average coefficient of variation of 11 ± 10% and 18 ± 9% for MeHg and THg analyses, respectively. Extraction efficiency of MeHg ranged from 70% to 130% ($n = 62$), and 55–140% ($n = 38$) for standard additions to Millipore Ultrapure water and field samples, respectively. Bottle blanks were 0.8 ± 0.2 ng L^{-1} ($n = 42$) for THg and 0.016 ± 0.007 ng L^{-1} ($n = 51$) for MeHg, and filtered water blanks were 0.6 ± 0.5 ng L^{-1} ($n = 45$) for THg and 0.013 ± 0.006 ng L^{-1} ($n = 53$) for MeHg. Method blanks were always less then detection limit for MeHg and THg (5 pg L^{-1}, $n = 111$ and 1 ng L^{-1}, $n = 37$, respectively).

Water quality parameters were measured every other week in influent and effluent waters of each wetland mesocosm in situ and in the laboratory. Parameters measured included temperature, pH, concentration of N and P species, TSS, oxidation–reduction potential (ORP), SO_4^{2-}, electrical conductivity, and TOC (Stamenkovic et al., 2005). All field instruments were calibrated in the laboratory before each sampling period. Temperature, nutrients, electrical conductivity, pH, NO_3^-, TSS, and SO_4^{2-} were analyzed using the methods described in Standard Methods for Examination of Water and Wastewater (APHA, 1995). The ORP in water was measured using a Pt electrode and an Ag/AgCl reference electrode connected to a pH-meter (Orion Instruments, Model 290A) (Faulkner et al., 1989).

Surface sediment samples (0–5 cm) were collected 6 times from the inlet and outlet cells of each mesocosm during late spring to early fall. Samples were collected from 3 different locations within a cell, homogenized, and frozen in glass vials with Teflon lined lids until analysis, which was within 30 days of collection. The concentration of MeHg in sediment samples was determined by aqueous phase ethylation, isothermal GC separation and CVAFS (Bloom, 1989; Liang et al., 1994). Certified reference materials DORM-2 (fish muscle) (Bloom et al., 1997; Marvin-DiPasquale et al., 2003) and IAEA-405 (estuarine sediment) (Baeyens et al., 2003; Wyse et al., 2004) were used for MeHg quality assurance, and measured concentrations were 122 ± 17% ($n = 31$) and 112 ± 17% ($n = 25$) of the certified value, respectively. Analysis of THg in sediment was completed using a solid state Milestone™ Hg analyzer (USEPA method 7473) or by cold vapor hydride atomic absorption spectrometry (Lechler et al., 1997). Prior to and during sediment sample analyses for THg, calibration of instruments was verified using National Institute of Standards and Technology (NIST) reference materials: San Joaquin soil (# 2709), Peach (# 1547) and Apple leaves (# 1515), and errors were <5%. Triplicate analysis of sediment samples yielded an average coefficient of variation of 16 ± 14% and 15 ± 8% for MeHg and THg analyses, respectively.

Net system flux (μg m^{-2} day^{-1}) was calculated using ΔC, the difference between the outlet and inlet water concentrations, times the flow rate of water through the wetland, divided by surface area of the mesocosm (16.2 m^2). Flow rates were determined volumetrically.

2.3. Statistical analysis

Statistical analyses were performed using SAS (SAS Institute® Inc.) and associated macros to perform stepwise and discriminate function analysis. Correlations between ΔC MeHg, ΔC THg, and water quality parameters were evaluated using linear regression analyses. Results were considered statistically significant at $p < 0.05$. Means are reported ± standard deviation, unless otherwise noted. The area under the curve was calculated using the trapezoidal rule in Sigma Plot®.

3. Results and discussion

3.1. Water quality parameters

The two water sources for the mesocosms, MW and CW, had significantly different water quality. The MW had notably higher dissolved O_2 (DO), THg and MeHg, inorganic $N(NO_3^- + NO_2^-)$, and TSS (Table 1). In general, pH was higher in the MW relative to CW. The CW had higher SO_4^{2-} and TOC concentrations, as well as temperature (CW = 15–30 °C and MW = 5–23 °C) (Table 1). DO and pH in MW that were diverted for this study were similar to those reported by Thomas (2003) for a sampling location just above the wetland intake pumps (DO = 5.2–12.2 mg L^{-1} and pH 6.8–9.5). The TSS reported for the MW were significantly greater (280–6130 mg L^{-1}; Thomas, 2003) than that measured for the mesocosm influent (~10–90 mg L^{-1}), which was attributed to particle settling in the head tanks used to provide controlled flow of water into the wetlands (Brown et al., 2006). In general, CW chemistry was more constant throughout the year relative to MW, which exhibited variability due to changes in discharge rates and inputs from different water sources (Sierra tributaries, Truckee River via ditches, irrigation return flows, runoff and groundwater) (Thomas, 2003).

Both the DO and pH of water decreased from the inlet to the outlet of the mesocosms, as did N, P and SO_4^{2-} due to wetland biogeochemical processes (Table 1) (Mitsch and Gosselink, 2000). As the water passed from the inlet to the outlet aerobic oxidation resulted in consumption of O_2. The pH of the water decreased most likely due to microbial processes that facilitated the addition of CO_2 and the formation of H_2CO_3. M indicates mesocosm as identified in Fig. 2. The decrease in SO_4^{2-} is due to consumption by SO_4^{2-}-reducing bacteria. As a result of SO_4^{2-} reduction, sulfide is generated along with CO_2 that is produced by way of the simultaneous oxidation of organic matter. Phosphorus loss from water as it moved through the wetland is associated with sorption onto sediments and subsequent sediment settling. Nitrogen removal would result due to denitrification and uptake by plants.

Table 1
Mean ± standard deviation concentrations for water quality parameters in Steamboat Creek water (Hg contaminated water, MW) and treatment plant effluent (Clean water, CW) from December 2002 to June 2005

Parameters	Inflow	Outflow			Inflow	Outflow	
	SBC	MW-MS (M1 & 2)	MW-CS (M7 & 8)	MW-CS-NV (M6)	TMWRF	CW-MS (M4 & 5)	CW-CS (M9 & 10)
THg	71 ± 34*	32 ± 27	25 ± 17	40 ± 34	6.4 ± 3.1	7.2 ± 2.6	5.0 ± 1.1
MeHg	0.91 ± 0.61*	1.41 ± 1.12	0.99 ± 0.61	0.91 ± 0.38	0.36 ± 0.21	1.60 ± 1.22	0.81 ± 0.50
pH	7.8 ± 0.4	7.2 ± 0.3	7.3 ± 0.4	7.4 ± 0.9	7.2 ± 0.3	6.79 ± 0.58	7.3 ± 0.35
DO	8 ± 2*	5 ± 2	6 ± 2	7 ± 2	6 ± 2	4 ± 2	5 ± 3
$NO_3^- + NO_2^-$	0.34 ± 0.24*	0.08 ± 0.05	0.04 ± 0.03	0.07 ± 0.08	0.11 ± 0.06	0.02 ± 0.02	0.03 ± 0.05
TN	1.5 ± 1.6	1.0 ± 0.9	0.9 ± 0.8	1.2 ± 0.6	1.9 ± 0.4	1.3 ± 1.2	1.4 ± 1.3
TP	0.29 ± 0.23	0.15 ± 0.05	0.19 ± 0.06	0.21 ± 0.07	0.29 ± 0.07	0.23 ± 0.06	0.27 ± 0.16
OP	0.2 ± 0.07	0.16 ± 0.04	0.13 ± 0.08	0.15 ± 0.08	0.13 ± 0.07	0.14 ± 0.09	0.13 ± 0.07
TSS	25.4 ± 17.3*	4.6 ± 5.6	3.4 ± 3.6	4.6 ± 5.1	7.7 ± 5.5	5.4 ± 6.2	2.5 ± 2.4
TOC	4.9 ± 2.1*	5.2 ± 2.1	6.3 ± 3.0	5.5 ± 2.7	7.3 ± 1.5	5.8 ± 2.5	7.1 ± 3.1
SO_4^{2-}	82.2 ± 15.0*	69.9 ± 19.1	75.2 ± 15.1	73.5 ± 16.9	100.6 ± 13.1	78.8 ± 14.6	85.7 ± 13.4
ORP	206 ± 140	183 ± 95	197 ± 101	221 ± 97	225 ± 139	170 ± 104	201 ± 112

Units are for: total and methylmercury concentrations (ng L^{-1}), pH, temperature (temperature in °C) and average concentration (mg L^{-1}) of dissolved O_2 (DO), inorganic $N(NO_3^- + NO_2^-)$, total N (as TKN), total P (TP), orthophosphate (OP), total suspended solids (TSS), total organic C (TOC), SO_4^{2-} and oxidation reduction potential (ORP, mV). Detections limit: THg = 0.1 ng/100 mL, MeHg = 5 pg/L, NO_3^- = 0.01 mg/L, TN = 0.25 mg/L, TP = 0.025 mg/L, OP = 0.01 mg/L, TSS = 0.5 mg/L, TOC = 0.1 mg/L.

* Paired sample test indicated significant ($p < 0.05$) differences between MW and CW concentrations.

There was a decrease in TSS from the inlet to the outlet for only the wetlands using MW (Table 1). Mean TSS removal over the 2 a of data collection in this study for the vegetated mesocosms with MW was 73 ± 32%. The mesocosm with no vegetation and MW removed a similar quantity of TSS (70 ± 36%). TSS removal in the designs with CW were quite variable due to low and variable amounts of TSS in CW. Mesocosms with CW–MS removed 14 ± 72% and the mesocosm with CW-CS removed 43 ± 46% of the TSS. The release of sediment from the design with MS was most likely due to some scouring of fine sediment.

For 2003–2005, the percent removal of total N for mesocosms with creek water, MW-MS, MW-CS, and MW-CS-NV, averaged 38 ± 29, 30 ± 42, and 15 ± 27%, respectively. Removal was greater in the warmer months as would be expected due to promotion of bacterial activity and plant growth under these conditions. Total N removal averaged 27 ± 28% and 34 ± 45% for designs CW-MS and CW-CS, respectively. Total P removal was highest for vegetated mesocosms fed by SBC water (32 ± 28% MW-MS, 55 ± 27% MW-CS); and lower for the non-vegetated mesocosm (18 ± 23% MW-CS-NV) and those with CW (21 ± 32% CW-MS; 18 ± 59% CW-CS). Total P removal in wetlands with MW correlated with percent TSS removal ($r^2 = 0.38$, $p = 0.0003$; $r^2 = 0.25$, $p = 0.009$; $r^2 = 0.20$, $p = 0.009$, respectively). However, this relationship was not found for mesocosms with CW, which was most likely due to the low TSS in CW. Based on the data collected in MW-CS-NV, the presence of vegetation in the mesocosms enhanced nutrient removal, while TSS removal was primarily a function of change in water velocity and not vegetation.

Stamenkovic et al. (2005) reported that in 2002, removal efficiency for mesocosms with MW-MS was 43%, 30% and 70% for N, P and suspended solids, respectively. Thus, the mean annual removal efficiency of N, P and TSS in the mesocosms with MW in operation for ~5 a has remained fairly constant over time.

3.2. Mercury and methylmercury

Concentrations of THg in MW were significantly greater and more variable than in the CW mesocosms (Table 1). The MW is significantly influenced by water sources to the creek (i.e., Washoe Lake releases, slough inputs containing Truckee River and irrigation return flows, tributary inputs, and urban and agricultural runoff) and changes in flow. Thomas (2003) and Brown et al. (2006) found that TSS and THg, which is predominantly particulate bound, correlated with flow. Mesocosms with MW as influent were a sink for THg (Fig. 3), whereas mesocosms with CW-MS were most often a slight source, while those with CW-CS were a sink. The 2002 data collected by Stamenkovic et al. (2005) revealed that initially the designs with CW-MS were a strong source of THg (Fig. 3). They hypothesized that this was due to scouring of fine sediments from the wetland soil by the low TSS CW. Over time, the strength of these wetlands as a THg source has declined suggesting that the pool of fine sediments has been reduced. Total Hg removal in the mesocosm with MW-CS-NV was similar to that in the same design that was vegetated. Since most of the Hg was particulate bound, this indicates that sedimentation due to decreased velocity of water was the primary mechanism for removal. Wetlands with CW-MS were the only ones found to be a consistent source of dissolved Hg. All the other experimental designs were more often a sink for dissolved THg (Fig. 3).

Concentrations of MeHg in MW during this study (2003–2005, 0.91 ± 0.60 ng L^{-1}) were always higher than CW (0.36 ± 0.21 ng L^{-1}) with dissolved MeHg making up 65 ± 21% of the MeHg concentration in the MW and roughly the same percentage in CW (63 ± 20%). The mean difference in the MeHg concentrations between the outlet and inlet (ΔC MeHg) for this study ranged from a high of 1.2 ± 1.0 ng L^{-1} for those with CW-MS to a low of 0.12 ± 0.58 ng L^{-1} for those using MW-CS. The large range is due to seasonal variability (Fig. 4). It is noteworthy that there were differences of up to 30% in the ΔC MeHg for replicate mesocosms. This is most likely due to spatial variation in wetland processes occurring in the different systems and the individual mesocosms responses to changing environmental and water quality conditions. Although the sediment and water sources were the same for replicate mesocosms, the vegetation density varied, as would microclimates within each system.

Overall, ΔC MeHg from the experimental design CW-MS was significantly greater than other experimental designs (MANOVA test, Wilks' $\lambda < 0.0001$). These wetlands were a source of Hg all year round and exhibited clear seasonal trends with highest output in the warmer months of the year (Fig. 4). These

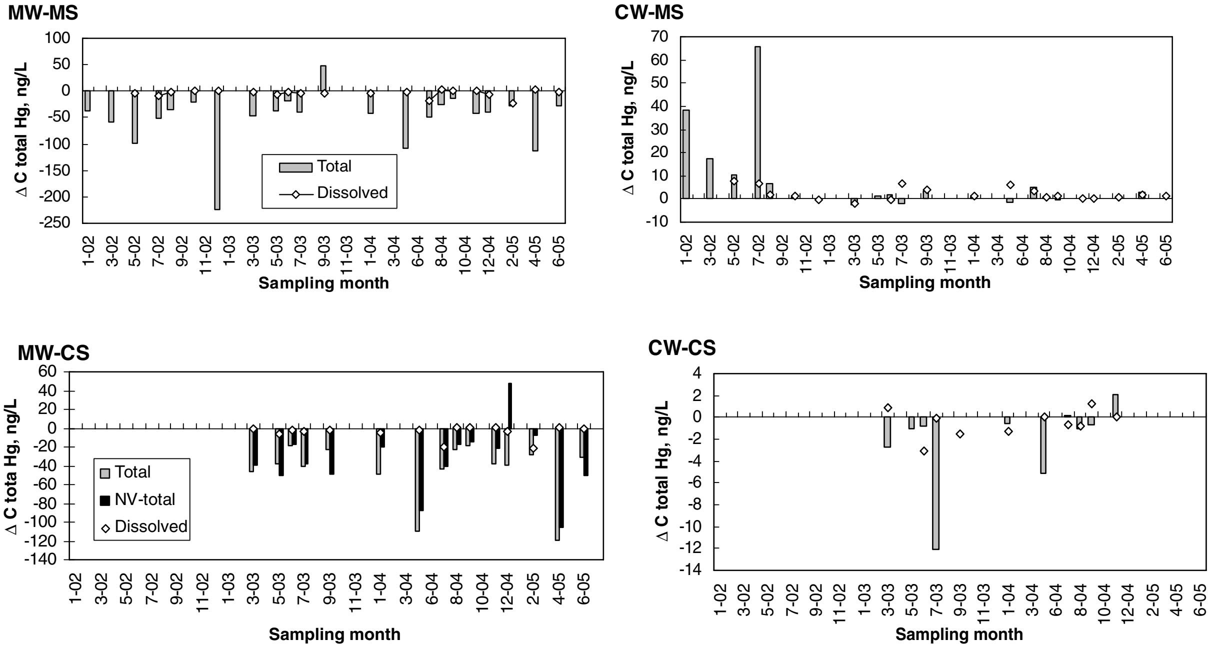

Fig. 3. Bar graphs showing ΔC total unfiltered Hg (THg) and points showing ΔC dissolved THg (THg_D) (sampling months on x-axis). Note that the scale is different for each graph. Data for 1-02 to 12-02 are from Stamenkovic et al. (2005). Negative values indicate mesocosms were a sink and positive a source. Concentrations for each experimental design are mean of replicate mesocosms. Percent difference between replicate mesocosms was ~15%.

mesocosms were monitored by Stamenkovic et al. (2005) during 2002 (Fig. 4). ΔC MeHg during their study was somewhat higher than observed in this study, as was total Hg flux for this wetland. This is thought to reflect the equilibration of the constructed ecosystem over time. The wetland with MW-MS, similar to that with CW-MS, exhibited seasonal trends in ΔC MeHg in that it was a source in the warmer months and a sink during colder months (Fig. 4). Those wetlands with CS, that became operational in July 2002, did not exhibit the clear seasonal trends that were observed for those with MS and ΔC MeHg in those wetlands were lower. Of the two designs with CW, those with MS had a statistically significant (ANOVA, $p > 0.05$) higher ΔC MeHg, which was possibly due to the larger pool of Hg in the sediment.

Stamenkovic et al. (2005) originally suggested that water chemistry was important in controlling MeHg production in the wetlands with MS. One hypothesis they suggested was that the higher N concentrations in the MW inhibited MeHg production. In recent manipulations of these wetlands, described in Gustin et al. (in press), increasing the NO_3^- concentrations in the designs with CW-MS and CW-CS by a factor similar to the difference between MW and CW (10 mg/L) did not influence ΔC MeHg. Gustin et al. (in press) suggested, based on the work of others, that the added amount was not high enough to inhibit MeHg production. An alternate hypothesis of Stamenkovic et al. (2005) was that there were higher SO_4^{2-} concentrations in CW relative to MW, since SO_4^{2-} is added in the wastewater treatment process at this facility. In this study, SO_4^{2-} was measured in the influent and effluent waters of the wetlands and concentrations in CW were greater, by 10–20 mg L^{-1}, than in MW mesocosms (Table 1).

The ΔC MeHg for MS-CS-NV was significantly less than that found for MS-CS with vegetation (ANOVA, $p < 0.05$). One hypothesis for this is that photo-demethylation was degrading the MeHg in the wetland with no vegetation. Photolytic decomposition is thought to be the primary abiotic mechanism for MeHg degradation (Ullrich et al., 2001; Sellers et al., 1996). It is also possible that biotic demethylation was occurring at a higher rate in the unvegetated mesocosm.

Using linear regression analyses, it was found that water quality parameters such as DO, pH, TOC, total N and total P that have been previously found to be correlated with MeHg production (Verta et al., 1994; Porvari and Verta, 1995; Rood,

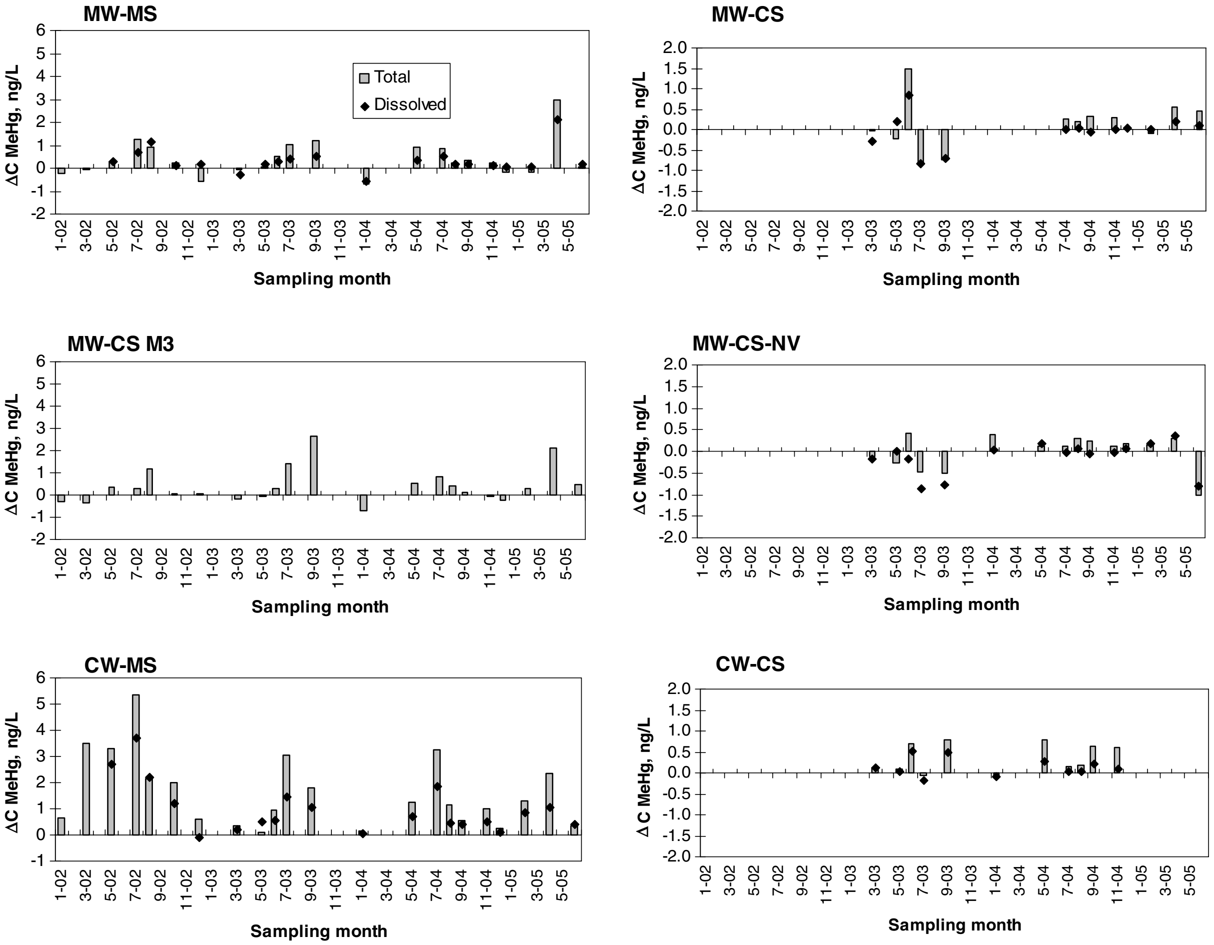

Fig. 4. ΔC MeHg (MeHg) (outlet – inlet concentration) (ng L^{-1}) (primary Y-axis) and ΔC dissolved MeHg ($MeHg_D$) (secondary Y-axis). X-axis gives sampling months. Data for 1-02 to 12-02 are from Stamenkovic et al. (2005). Negative flux indicates mesocosms were a sink and positive a source for MeHg. Concentrations for each experimental design are mean of replicate mesocosms. Percent difference between replicate mesocosms for designs was MW-MS (10 ± 7%), MW-CW (12 ± 19%), CW-MS (14 ± 10%), and CW-CS (7 ± 14%).

1996; Steffan et al., 1988; Branfireun et al., 1999; Mauro et al., 1999; Ullrich et al., 2001; King et al., 2001) were in most cases not correlated with ΔC MeHg dissolved (Table 2) and total ΔC MeHg. This lack of clear correlation of ΔC MeHg with these water quality parameters may be due to a complex interplay of environmental parameters influencing the ΔC MeHg in these constructed field systems. Two parameters that were correlated with the ΔC MeHg were the change in the SO_4^{2-} concentration between the inlet and outlet for all experimental designs and the temperature of effluent water for all designs, except MW-CW-NV (Table 2).

It is well established that SO_4^{2-} reducing bacteria are the dominant microbes that methylate inorganic Hg (Compeau and Bartha, 1985; Gilmour and Henry, 1991) and that SO_4^{2-} concentrations are important in controlling Hg methylation (Gilmour and Riedel, 1995; King et al., 1999; Branfireun et al., 1999). The concentration difference between MW and CW was ~20 mg L^{-1} and the concentration in influent waters of all mesocosms were on the order of 80–100 mg L^{-1}. A lower concentration range of 20–50 mg $L^{-1}$was suggested by Gilmour and Henry (1991) as being optimum for MeHg production. Additionally, Stamenkovic et al. (2004) investigated methylation and demethylation potential in the sediments of the wetland with MW-MS. They found low potential methylation rates in the wetland sediments, which they attributed to high pore water sulfide and dissolved organic C. This suggests that at least for the mesocosms with MW, the high SO_4^{2-} concentrations could cause sulfide formation, which will affect MeHg production. In

Table 2
Regression coefficients (r^2) and p-values for linear regressions analyses between Δ dissolved MeHg and water quality parameters

Parameters	Experimental designs				
	MW-MS (M1 & 2)	MW-CS (M7 & 8)	MW-CS-NV (M6)	CW-MS (M4 & 5)	CW-CS (M9 & 10)
Dissolved oxygen	0.003 (−) $p = 0.83$	0.40 (−) $p = 0.019$	0.32 (−) $p = 0.028$	0.33 (−) $p = 0.072$	0.10 (−) $p = 0.19$
pH	0.08 (−) $p = 0.74$	0.015 (−) $p = 0.69$	0.005 (−) $p = 0.80$	0.074 (−) $p = 0.33$	0.057 (−) $p = 0.48$
Temperate	0.35 (+) $p = 0.02$	0.41 (+) $p = 0.019$	0.06 (+) $p = 0.37$	0.39 (+) $p = 0.014$	0.49 (+) $p = 0.0016$
$NO_3^- + NO_2^-$	0.009 (−) $p = 0.74$	0.04 (−) $p = 0.52$	0.01 (−) $p = 0.72$	0.03 (−) $p = 0.53$	0.03 (−) $p = 0.30$
Total nitrogen	0.23 (−) $p = 0.10$	0.04 (−) $p = 0.59$	0.18 (−) $p = 0.13$	0.13 (−) $p = 0.17$	0.05 (−) $p = 0.42$
Total phosphorus	0.17 (−) $p = 0.14$	0.007 (−) $p = 0.73$	0.005 (−) $p = 0.79$	0.006 (−) $p = 0.15$	0.12 (−) $p = 0.15$
TSS	0.29 (−) $p = 0.091$	0.002 (−) $p = 0.97$	0.011 (−) $p = 0.71$	0.068 (−) $p = 0.36$	0.13 (−) $p = 0.19$
TOC	0.005 (−) $p = 0.80$	0.02 (−) $p = 0.66$	0.01 (−) $p = 0.67$	0.01 (−) $p = 0.71$	0.07 (−) $p = 0.33$
ORP	0.18 (−) $p = 0.10$	0.23 (−) $p = 0.071$	0.12 (−) $p = 0.21$	0.19 (−) $p = 0.11$	0.12 (−) $p = 0.24$
ΔC SO_4^{2-}	0.39 (+) $p = 0.006$	0.39 (+) $p = 0.01$	0.33 (+) $p = 0.03$	0.55 (+) $p < 0.0001$	0.34 (+) $p = 0.015$

Positive (+) sign indicates positive and negative sign (−) negative correlations.

controlled manipulations of these mesocosms, increasing the SO_4^{2-} concentrations in the wetlands with CW by ~50 mg L^{-1} did not result in an immediate or clear increase in MeHg output over ~30 days (Gustin et al., in press). Despite the correlation between SO_4^{2-} and ΔC MeHg, these observations suggest that the high SO_4^{2-} concentrations may not be the dominant reason for the higher ΔC MeHg associated with those wetlands having CW as influent. Other factors that would promote methylation in those designs with CW include higher TOC, lower pH and warmer water temperatures. Additionally, high TSS in the MW could inhibit MeHg production (cf. Ullrich et al., 2001).

Even though those mesocosms with MW-CS exhibited low ΔC MeHg, it is hypothesized that this trend could change over time since the sediment pool of Hg will increase as THg is deposited from MW to the sediments. Data from one mesocosm that was not replicated (Figs. 2 and 4, M3) and had been in operation since 2000 supports this. The ΔC MeHg from M3 was comparable to that for MW-MS, although this latter wetland originally had CS. Stamenkovic et al. (2005) also found that the M3 wetland had a Hg flux comparable with the one with MW-MS in 2002. This suggests that over time, if MW is the source of water for a larger scale wetland and clean sediments are initially used, inputs of Hg by way of the MW could result in an increase in MeHg released over time.

An annual MeHg flux for each design was calculated by integrating the area under a curve representing the MeHg flux for the different months of sampling. Flux was calculated using the ΔC MeHg for each sampling date (Fig. 4), the area of the wetland (16.2 m^2), and the flows recorded for each time of sampling. In general, the calculated annual output for the different designs exhibited the following hierarchy: CW-MS 143 μg m^{-2} a^{-1} > MW-MS 57 μg m^{-2} a^{-1} > CW-CS 29 μg m^{-2} a^{-1} > MW-CS 6 μg m^{-2} a^{-1}. The annual flux for the MW-CS with vegetation was comparable to the MW-CS-NV at10 μg m^{-2} a^{-1}, which may have been largely due to a higher flux from the latter in winter relative to summer, and vice versa for the former.

The annual flux of MeHg for all mesocosm designs was greater than that reported in the literature for natural systems, which are typically within the range of 0.1–0.5 μg m^{-2} a^{-1} (Krabbenhoft et al., 1995; St. Louis et al., 1996; Driscoll et al., 1998; Galloway and Branfireum, 2004). If one assumes the calculated annual flux from these small systems would be representative of a larger system, there are several explanations for the higher annual fluxes calculated for the mesocosms based on the data collected in this study. The first is that Hg inputs in both the CW and MW were elevated relative to pristine systems, as were the sediment Hg concentrations in both MS and CS. The SO_4^{2-} concentrations in the water of this study were also significantly elevated with respect to clean systems, which often have $\leqslant 10\ \mathrm{mg\ L^{-1}}$ or below the range thought to be optimal by Gilmour and Henry (1991). Additionally, the hydrologic characteristics (water residence time and surface flow rates) and the age of these wetland systems may have promoted Hg methylation to a greater degree than that observed in long lived natural systems.

The significantly greater flux calculated for these small-scale constructed wetlands also leads to the question – Can small scale wetlands be used to predict fluxes occurring from larger systems? Potential uncertainties in our ability to use small-scale system data for scaling include whether the MeHg produced from the small homogeneous constructed wetlands represents that found in association with a large heterogeneous wetland system. Hunt et al. (1997) showed that there was significant spatial variability in wetland biogeochemical processes. They also found substantial differences between natural and constructed systems. A larger wetland system would contain riparian and emergent wetland vegetation as well as areas with no vegetation. Differences in vegetation alone may significantly influence wetland biogeochemical cycling (Hunt et al., 1997). Additionally, the constructed wetlands were operated with a specific hydrologic experimental design, which is reflected in the data. A wetland design with different vegetation structure and hydrologic characteristics could result in significantly different nutrient processing and MeHg production capabilities. The constructed wetland system only processed a limited amount of water that was not reflective of MW from which the larger particulate material was removed. It is uncertain whether a larger wetland with different volumes of water would result in the same removal efficiencies for non-point source pollutants.

3.3. Mercury in sediments

Concentrations of THg and MeHg in sediment for inlet and outlet cells are shown in Fig. 5. Because Stamenkovic et al. (2005) sampled sediments over a year in MW-MS and CW-MS and found no seasonal trend in MeHg concentrations, sediment samples were collected 6 times during the warmer months. In general, mesocosms with MS had higher THg concentrations than those with CS. Total Hg concentrations in those mesocosms with MS were within the range reported by Stamenkovic et al. (2005) for samples collected during 2002.

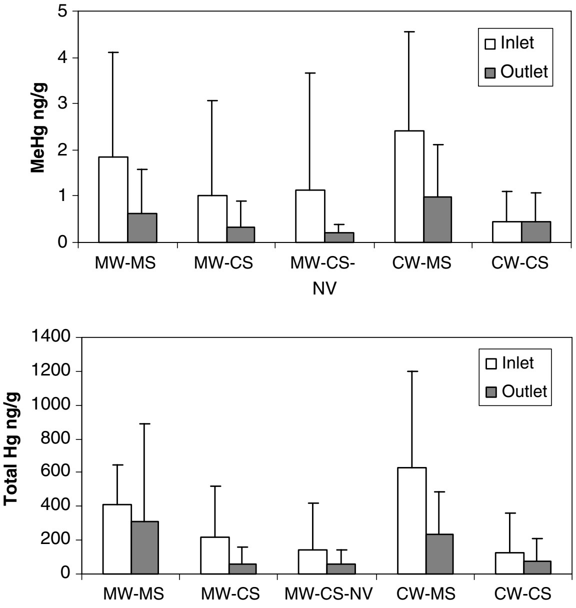

Fig. 5. Methylmercury (MeHg, top panel) and total Hg (bottom panel) concentrations in sediment of the inlet and outlet cells for each experimental design.

MeHg concentrations in MS were also similar to those reported by Stamenkovic et al. (2005). Mean MeHg concentrations in the inlet sediments of all designs, except those with CW-CS, were greater than those measured in the outlets. Higher inlet cell MeHg concentrations were also observed by Stamenkovic et al. (2005) who found this surprising given that DO and ORP decreased from the inlet to outlet. MeHg concentrations in the MW-CS and MW-CS-NV were similar. This would support the hypothesis that MeHg is produced in the latter, but photo-degradation reduced the net amount exported from the system. The concentrations of MeHg in sediment ranged from 0.03 to $7.1\ \mathrm{ng\ Hg\ g^{-1}}$ dw, and were comparable to those observed by Blum et al. (2001) and Stamenkovic et al. (2004) along SBC ($0.03–12.9\ \mathrm{ng\ g^{-1}}$).

4. Conclusions

Based on the data collected from the small-scale wetlands, the construction of large-scale wetlands at the mouth of SBC could potentially improve water quality in the Truckee River downstream of the confluence with SBC through nutrient, sediment and THg removal. Since most of the Hg is bound to fine clay-sized particulate matter (Brown et al., 2006), the hydraulic retention time and flow rates associated with a wetland system could affect the efficiency of Hg removal. Phosphorus and N removal would also be influenced by the hydrologic conditions and degree of vegetation in the wetlands. One important management consideration is that sediment-bound Hg deposited to the wetland could be remobilized and may enter the river if a flood event occurred.

Since those mesocosms with CW-MS were a greater source of MeHg than those with MW-MS, water chemistry is thought to be an important factor influencing MeHg production. Clean water had higher SO_4^{2-} and TOC concentrations, higher temperature, and lower pH and TSS concentrations than MW. These factors combined would all promote methylation of Hg in sediments interacting with this water relative to MW, suggesting that use of CW for a wetland system could exacerbate production of MeHg. This is an important observation for the water limited western USA because wastewater inputs may be used to maintain stream flows. Additionally, Hg could be remobilized if CW infiltrating the wetland scours fine-grained sediments containing Hg, which are found at the wetland soil-water interface. Data collected from the CW-MS mesocosms also indicated that if the sediment and water in a wetland have not co-existed, the wetlands could exhibit an increased flux over several years due to equilibration. This suggests that changing water sources in a wetland, such as what might occur in the western USA where water is highly managed, might result in significant changes in Hg biogeochemical cycling.

There are significant uncertainties with respect to the use of small-scale systems such as those applied here to predict how a large-scale system might behave. Spatial heterogeneity typical of large wetlands was not the case in the constructed mesocosms and the hydrologic setting applied may not be representative of a large-scale system. Management decisions for stream restoration in Hg contaminated systems, such as this one, requires weighing all the benefits and risks, and designing and operating the system to maximize the benefits.

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