Baseline aquatic contamination and endocrine status in a resident fish of Biscayne National Park

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Abstract

Surface water, sediment, and fish from Biscayne Bay, coastal wetlands adjacent to the bay, and canals discharging into the bay were sampled for determination of baseline contamination in Biscayne National Park. While the number of contaminants detected in canal waters was greater during the wet season than the dry season, no seasonal difference was evident for Biscayne Bay or coastal wetland waters. Estrogen equivalency (as 17 β -estradiol equivalents), as predicted by the Yeast Estrogen Screen, for extracts of passive water samplers deployed in canals and wetlands was elevated during the wet relative to the dry season. Generally, contamination in water, sediments, and fish was greater in the canals than in Biscayne Bay and the wetlands. Guideline levels for sediment contaminant were exceeded most frequently in canals relative to the coastal wetlands and the bay. Further investigation is necessary to better understand the impact of contaminants in Biscayne National Park.

Keywords: Biscayne Bay; Polar organic chemical integrative sampler; SPMD; yeast estrogen screen; wetland; wastewater

1 Biscayne National Park (Park) is a marine reserve encompassing the majority of Biscayne
2 Bay (Bay) in South Florida (Figure 1). Historically, freshwater entered the Bay from the coastal
3 wetlands and the Miami River. The volume and timing of that freshwater introduction was
4 critical to the health and well-being of the Bay's habitat and biota (Langevin, 2001). However,
5 dredging, channelization, and water management activities in south Florida as a result of the
6 Central & South Florida Project have altered water flow into the Bay while increasing
7 development in the region has altered water quality impacting conditions in the Bay (Bradner et
8 al., 2004; Caccia and Boyer, 2005; Lirman et al., 2008; Stalker et al., 2009; Wang et al., 2003).
9 Monitoring of surface water in the Bay and in canals entering the Bay for contaminants have
10 detected a variety of current-use pesticides (e.g., triazine herbicides, endosulfan, chlorothalonil,
11 chlorpyrifos) (Harmon-Fetcho et al., 2005; Scott et al., 2002). Another study detected legacy
12 pesticides and pollutants (DDT, dieldrin, and PCBs) within tissues of fauna within the Bay (Litz
13 et al., 2007). In addition to water contamination, sediments in the Bay are contaminated by a
14 variety of metals, particularly near Miami and in canals entering the Bay (Carnahan et al., 2008).
15 Contamination of habitats within the Park is evident, with the source(s) likely being agricultural,
16 residential, and industrial areas "upstream" of the Bay near Homestead, Florida City, and Miami.

17 A joint Federal and State effort (Comprehensive Everglades Restoration Plan,
18 <http://www.evergladesrestoration.gov/>) is underway in Florida to restore the hydrology of south
19 Florida and rehabilitate the Everglades Ecosystem. Much of the freshwater that enters the South
20 Florida Ecosystem is directed through canals to either the Gulf of Mexico or the Atlantic Ocean
21 to minimize flooding. The purpose of the Comprehensive Everglades Restoration Plan is
22 "restoration, preservation, and protection of the South Florida ecosystem while providing for
23 other water-related needs of the region, including water supply and flood protection." (CFR 33
24 Section 385.8) Rehydration of the Bay's coastal wetlands was a component of the
25 Comprehensive Everglades Restoration Plan not only to restore the adjacent wetlands but also to
26 re-establish the natural pattern of freshwater flow to the Bay (McLean and Bush, 2000).
27 However, resource managers in the Park were concerned about the proposed use of treated
28 wastewater. Contaminants associated with wastewater, from here on referred to as organic
29 wastewater contaminants (OWCs), have been implicated in endocrine disruption of fish in water
30 bodies receiving treated waste water effluents (Folmar et al., 1996; Kirby et al., 2004; Vajda et
31 al., 2008). However, the existing level of OWCs in the Bay and in canal water that enters the
32 Bay has not been reported. Canals passing through the urban and agricultural areas adjacent to
33 the Park and the surficial aquifer in the region may already transport significant amounts of
34 OWCs into the Bay regardless of any additional input from rehydration of the wetlands.
35 Therefore, the intent for the present study was collection of data that could serve as a baseline for
36 comparison with data that may be collected from the Bay and wetlands after full implementation
37 of the rehydration of the Bay's coastal wetlands.

38 Two objectives were planned for the present study. The first was to determine existing
39 contamination in the Bay by sampling surface water, sediments, and fishes from the Bay,
40 influent canals, and the coastal wetlands planned for rehydration. Water contamination would
41 indicate short term contaminant flux into the Bay, while the fish and sediment data would
42 indicate a relatively longer period of contamination at the sampled location. Also, given the
43 distinct seasonal precipitation differences in south Florida and concordant difference in
44 freshwater discharge from canals into the Bay, we sampled water during the wet and dry seasons
45 with the expectation that contaminant transport into the Bay would be affected. The second
46 objective was determination of the potential for endocrine disruption from contaminants detected

1 in waters as well as the current endocrine status of a representative resident fish species. Since
2 endocrine disruption is often reported in fish exposed to wastewater effluents (Folmar et al.
3 1996; Kirby et al., 2004; Vajda et al., 2008), we wanted to determine if existing conditions may
4 be resulting in endocrine disruption.

5 Water contamination was assessed using passive water samplers: semi-permeable
6 membrane devices (SPMD) and polar organic chemical integrative samplers (POCIS). These
7 passive samplers were deployed together at multiple locations in the Bay and canals as well as at
8 the coastal wetlands planned for rehydration (Fig. 1). Samplers were deployed in canals that
9 discharge into the Bay, at three different locations within the Bay (map ID numbers 10-12 in Fig.
10 1), three coastal wetlands (map ID numbers 13-15), and the North River (map ID number 16).
11 For each of the canals, samplers were deployed below the last structure that controls discharge
12 into the Bay (hereafter referred to as “canal downstream” locations, map ID numbers 1-3 and 5-
13 9). Samplers were also deployed upstream of the structures (“canal upstream” locations, map ID
14 numbers 5 and 17-22) in select canals near suspected OWC sources. One location in Everglades
15 National Park (North River) was selected as a reference. The deployment dates within the
16 respective seasons were selected based on rainfall data reported in the South Florida Water
17 Management District’s online database DBHYDRO
18 ([http://www.sfwmd.gov/portal/page/portal/xweb%20environmental%20monitoring/dbhydro%20](http://www.sfwmd.gov/portal/page/portal/xweb%20environmental%20monitoring/dbhydro%20application)
19 [application](http://www.sfwmd.gov/portal/page/portal/xweb%20environmental%20monitoring/dbhydro%20application)); that is, the wet season deployment dates were selected when elevated rainfall and
20 discharge into the Bay was indicated, while the dry season deployment date was selected when
21 minimal rainfall and discharge was indicated. Based on these data, the wet season samplers were
22 deployed in September and retrieved in October while the dry season samplers were deployed in
23 April and retrieved in May. Deployment and retrieval of the SPMDs and POCIS generally
24 followed the procedures described in Alvarez (2010). Performance reference compounds
25 (phenanthrene-*d*₁₀ and pyrene-*d*₁₀) were incorporated within the SPMDs at the time of sampler
26 preparation to account for factors associated with transport, deployment, and retrieval that would
27 affect contaminant accumulation (Huckins et al., 2006). A separate blank for each location for
28 both passive sampler types were used to adjust for contamination as a result of transportation,
29 deployment, and storage. The SPMDs and POCIS were anchored in place by cinder blocks and
30 suspended within the water column by Styrofoam floats such that the samplers were between 0.4
31 and 2.4 m below the water surface.

32 A solvent-rinsed stainless steel sediment corer was used to collect sediment samples.
33 Four discrete samples were collected in close proximity (within 10 m) at each of the passive
34 sampler deployment locations. The top 2 inches from each discrete sample were composited
35 within a stainless steel bowl, homogenized using a solvent rinsed stainless steel spoon,
36 subsampled (≈200 mL) into pre-cleaned glass jars, and frozen for shipment.

37 The fish taxa collected at the canal mouth and wetland locations for analyses and gonadal
38 histology were primarily those with high site fidelity (rainwater killifish [*Lucania parva*] and
39 crested goby [*Lophogobius cyprinoides*]) with the goal that the data would reflect local
40 conditions. Due to the small body size of these taxa (a few grams wet weight); fish from a
41 location were composited to provide sufficient sample size for detection of low contaminant
42 concentrations. Killifish and gobies could not be found at the Bay or North River reference
43 locations. Therefore, pinfish (*Lagodon rhomboides*) were captured from those locations for
44 residue analyses, but were not used for histology. The fish were collected either by dip net
45 (killifish) or hook and line (goby and pinfish). Fish selected for residue analyses were placed

into solvent rinsed aluminum foil and frozen, while fish selected for histology were placed into a 10 percent formaldehyde solution.

The locations and seasons at which passive water samplers were deployed, and from which sediment and fish samples were collected are listed in Table 1.

All passive samplers as well as sediment and fish samples intended for residue analysis were shipped to the Columbia Environmental Research Center of the U.S. Geological Survey. Upon receipt, a subset of POCIS samplers from each location were processed as described in Alvarez (2010) for quantification of OWCs and other relatively hydrophilic compounds while SPMDs were processed as described in Alvarez et al. (2008) for quantification of lipophilic compounds. The remaining POCIS retrieved from each location were processed as described above with the extract subjected to the Yeast Estrogen Screen (YES) (Alvarez et al., 2008; Vermeirssen et al., 2005) to evaluate estrogen equivalency (EEQ expressed as ng estradiol/L) of the chemical mixture sampled by the POCIS. Organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), polybrominated diphenyl ether (PBDE) flame retardants, and polycyclic aromatic hydrocarbons (PAHs) were found largely in SPMD extracts, while a variety of chemical classes typically associated with wastewaters (OWCs) were found in POCIS extracts. Table 2 shows a list of analytes targeted in the passive water sampler extracts.

Fish and sediment samples were homogenized and subsampled (2 g fish, 5 g dry weight sediment) for extraction and analysis. Each subsample was spiked with stable ^{13}C -labeled surrogates (^{13}C -bis-[tribromo]-phenoxyethane, ^{13}C -PBDEs, ^{13}C -PCB-155, methyl-naphthalene- d_{10} , fluoranthene- d_{10}) and subjected to heated and pressurized solvent extraction (accelerated solvent extraction – 5 percent acetone in dichloromethane, 1500 psi at 100°C), which was followed by flash S-X3 gel permeation chromatography (Peterman et al. 2015) and solid-phase extraction (basic alumina) to clean the extract. The cleaned extracts were then fractionated by basic alumina flash chromatography. Fraction 1 (30 mL of 1 percent dichloromethane in cyclopentane) contained ortho-PCBs and a few highly apolar pesticides, and Fraction 2 (15 mL 0.5 percent acetone in dichloromethane) contained most of the legacy organochlorine pesticides, PBDEs, PAHs, musk fragrances, and some OWCs (Peterman et al., 2006). Method detection limits (blank average + 3SD) ranged from 0.05 to 1 ng/g for most analytes using GC/quadrupole MS SIM for Fraction 1, and GC/High Resolution MS (GC/HRMS) for Fraction 2. Over 100 analytes were chromatographed with a short high performance capillary column (13 m x 0.15 mm i.d.) in the comprehensive GC/HRMS analyses (Peterman, 2006). In each sample set, quality control was ensured by additional quality control samples of procedure blanks, matrix blanks, and matrix spikes carried through the entire procedure of sample extraction and analysis. Also, the ^{13}C -labeled surrogates spiked into each sample and quality control sample provided additional quality control with the optimum quantitative isotope dilution GC/MS technique to accurately measure analytes at ppb levels in each sample regardless of variations in analyte recovery throughout the entire method. A list of the analytes targeted in the fish and sediment samples is shown in Table 3.

Gonadal histology was evaluated only for rainwater killifish. Histology was not evaluated for crested gobies because they are functional hermaphrodites. Killifish were collected from the canal mouths of 7 of the 9 canal downstream locations, and from all of the wetland locations. Fish were put into a 10 percent formaldehyde solution in the field and transported to the laboratory. After seven days in the formaldehyde solution, the fish were soaked in tap water for four days during which the water was changed twice per day. Then the fish were transferred to a 70 percent ethanol solution. Gonads were excised and desiccated in a solution of ethanol

1 and Safeclear II before infiltration in paraffin (Presnell and Schreiber, 1997). Tissue blocks
2 were then sectioned (4 – 10 µm thick) and mounted onto slides for staining with hematoxylin
3 and eosin. Between two and six slides were prepared for each fish. Testes were examined
4 microscopically for evidence of testis-ova as an indication of endocrine effects (Leino et al.,
5 2005; Mills and Chichester, 2005).

6 The relative level of contamination at the sampled locations was evaluated based on the
7 number of detected contaminants rather than their concentrations. Two comparisons were
8 planned for these data. The first evaluated the role of seasonality on water contamination and
9 EEQ of the POCIS extracts. For the contaminant data, the average number of detections for the
10 canal, wetland, and Bay locations were compared between the wet and dry seasons. Analysis of
11 the canal data was by the Welch's ANOVA because of the unbalanced design and
12 heteroscedasticity among the seasons (Ruxton, 2006; Welch, 1947). The Kruskal-Wallis test
13 was used for the Bay data since they were not normally distributed. The wetland data satisfied
14 the assumptions for parametric comparisons and were compared by the paired t-test. Since the
15 EEQ data satisfied assumptions for parametric analysis, the seasonal averages were compared by
16 the one-way ANOVA for the canal and Bay data while the paired t-test was used for the wetland
17 data. The second comparison evaluated relative contamination of water and sediments among
18 canal upstream, canal downstream, Bay, and wetland locations. The comparison for fish
19 contamination was among the canal downstream, Bay, and wetland locations. No fish were
20 collected from the canal upstream locations. Relative contamination was evaluated by
21 comparing the total number of detected contaminants. These comparisons were by Welch's
22 ANOVA because of the unbalanced design and heteroscedasticity, followed by the post-hoc
23 Games-Howell test of differences between locations. The level of significance for all
24 comparisons was $\alpha=0.05$. No comparisons were conducted among seasons or locations for the
25 different contaminant groups (i.e., PAHs, OCs, PBDEs, OWCs). All of the raw data are reported
26 in Supplemental Data Tables, and can also be accessed through the Department of the Interior
27 Science Base (Bargar, 2016).

28 The number of contaminants detected in the water during the wet and dry seasons
29 differed significantly for the canal locations ($p=0.04$), but not for the Bay (0.67) or wetland
30 (0.54) locations (Fig. 2). The significant difference appeared driven by PAHs and OWCs: the
31 average number of PAHs detected during the wet season (13.4 in 2009 and 14.3 in 2011) nearly
32 tripled the number detected during the dry season (5.1), while the number of OWCs detected
33 during the wet seasons (1.4 in 2009 and 3.6 in 2011) was lower relative to the dry season (5.3).
34 A greater number of PAHs were detected at the canal downstream locations of canals passing
35 predominantly through high density residential areas relative to canals passing through
36 agricultural areas (Fig. 3). The exception to this trend was canal C-111 (Map ID 9 in Fig. 1)
37 where a spike in PAH detections was found during the wet season of 2009 despite the canal not
38 passing through any nearby high density residential areas. The number of OC pesticide
39 detections during the wet season of 2011 was greater at the canal downstream locations of canals
40 that pass through agricultural areas (Map IDs 6-8) relative to canals that do not (Map IDs 1-5).
41 As is evident in Fig. 3, PAHs (32 total detections) and OC pesticides (31) were the most
42 commonly detected contaminants in water. Within the PAHs, fluoranthene and pyrene were
43 most commonly detected. Fluoranthene was detected at all locations during both seasons, while
44 pyrene was detected at all but two canal downstream locations during the dry season.
45 Hexachlorobenzene was the most commonly detected OC pesticide being found at all locations
46 during the dry season, and at all but two locations during the wet season of 2011. Concentrations

for none of the individual contaminants exceed water quality criteria recommended by the State of Florida.

The average number of contaminants detected in sediments was significantly greater ($p=0.002$) at the canal upstream locations (36.3, SE=2.72) relative to the canal downstream (17.25, SE=1.78), Bay (11.3, SE=2.33), and wetland locations (15.7, SE=1.86) (Fig. 4). The number of contaminants detected in sediments did not differ significantly ($\alpha=0.05$) among the canal downstream, Bay, and wetland locations. Concentrations for several of the contaminants exceeded published sediment quality guideline values for estimating risk to benthic invertebrates (MacDonald et al. 1996). The contaminants that exceeded the threshold effect levels (TEL) were naphthalene (TEL=34.6 ng/g), phenanthrene (86.7 ng/g), fluoranthene (113 ng/g), pyrene (153 ng/g), benzo[a]pyrene (88.8 ng/g), dieldrin (0.72 ng/g), and p,p'-DDE (2.07 ng/g). The contaminants that exceeded the probable effects level (PEL) were anthracene (PEL=245 ng/g) and benzo[a]pyrene (763 ng/g). Of the 44 total guideline exceedances, 36 were in the canals, 4 in the wetlands, 3 in the Bay (1 for each Bay location), and 1 at the North River reference location (Map ID 16). With respect to the canal locations, 29 of the 36 exceedances, including the PEL exceedances, were at the canal upstream locations.

The average number of contaminants in fish differed significantly among the canal, Bay, and wetland locations ($p=0.03$, Fig. 5). Among the sampled locations, the fewest number of contaminants were detected in fish from the wetland locations ($p<0.05$). No contaminants were found in fish at concentrations that exceeded wildlife criteria. However, the sampled fish are lower trophic level fish unlikely to have elevated contaminant concentrations.

The EEQ differed significantly among seasons for the canal ($p<0.001$) and wetland ($p=0.03$) locations (Fig. 6). The greatest EEQ (6.9 ng/L) was found at one of the wetland locations (Deering Estate – Map ID 13) during the wet season of 2011 while the lowest EEQ (0.2 ng/L) was at the North River reference location (Map ID 16) during the dry season. Generally, the EEQs were greater during the wet season than during the dry season. Interestingly, the EEQ for the North River reference location was also greater during the wet season when fewer contaminants were detected (Fig. 2), indicating that the targeted analytes may not be responsible for the elevated EEQs. Extracts of the POCIS were subsequently analyzed for the phytoestrogens daidzein, coumestrol, and genistein. A simple scatter plot of those data and the EEQ data indicated no relation.

Histological evaluation of the testes of 79 male killifish found no evidence of altered gonadal development.

The number of contaminants detected in the water during the present study reflects the variety of sources adjacent to the Bay. The landscape in the study area is a mosaic of urban development, residential neighborhoods, agriculture, and rural areas. Several of the primary canals that were sampled during the present study pass through the landscape integrating contamination from those areas. While a wide variety of contaminants were detected, there was a clear predominance of PAHs in the more northern canals that pass through high density residential areas, particularly during the wet season. The OC pesticides predominated in the southern canals that passed through agricultural areas. The northern canals pass through a landscape that is largely urban while a significant portion of the landscape through which the southern canals pass is agricultural. Urban landscapes are known to contribute significant quantities of PAHs to the aquatic environment (Heintzmann et al., 2015; Howitt et al., 2014), while agricultural landscapes contribute pesticides (Mamta and Wani, 2015; Postigo and Barcelo, 2015; Puckett et al., 2011; Vymazal and Brezinova, 2015). These contaminants may

enter the aquatic environment through surface water runoff or leaching into the groundwater, the latter of which is of particular significance in the study area. The Biscayne Aquifer is a surficial aquifer in the study area in which the groundwater level is typically less than 10 feet below land surface (Sonntag, 1987) making it relatively susceptible to contamination during significant rain events. In fact, Bradner et al. (2004) reported several different pesticides and volatile organic compounds in water drawn from the Biscayne Aquifer making it very possible that ground water directly contributes contamination to the Bay and the canals.

Greater contamination was found in the canals relative to the Bay, wetlands, and North River. The canals not only pass through and collect surface waters from the adjacent landscape, but they are also connected with other canals that pass throughout southeast Florida meaning the detected contaminants could be areas not adjacent to the Bay. Canal waters discharged into the Bay would certainly deliver contaminants to the Bay, but the concentrations in the canal water are then diluted by the Bay and ocean water. This is likely the reason many of the contaminants detected in the canals were not found in the Bay. The Bay locations could be affected by those contaminants, but dilution would decrease detectability and lower the concentrations to the point of being apparently less hazardous based on available toxicity data. While the wetlands do receive surface water runoff and its associated contaminants, they drain smaller areas immediately adjacent to the Bay, areas that do not have the same level of development as the areas through which the canals pass. An interesting phenomenon was that the number of contaminants detected in the wet season was greater only in the canals, not at the Bay, wetland, or North River locations. The number of contaminants detected in the latter locations was lower during the wet season. A similar study with greater replication in the Bay and wetland areas would clarify this apparent relationship.

Seasonal differences in canal water contamination likely reflect changes in water control operations rather than changes in natural transport processes alone (i.e., increased runoff and elevated groundwater transport during the wet season). The climate of South Florida is subtropical with a relatively cool dry season from December through May, and then a warm wet season from June through November. Water control operations in South Florida are primarily for flood control and water supply maintenance. Maintaining water supply is the primary focus during the dry season when water is withheld in water retention areas in the Everglades Ecosystem as well as in the canals. During the wet season, water is released from the water retention areas and canals passing it to the Atlantic Ocean or the Gulf of Mexico. Based on reported flow data for the water control structures in the canals sampled in the present study, the structures were not open during the dry season deployment, but were open during the wet season deployments. As a result, the samplers were largely exposed to marine waters during the dry season and a mixture of marine and canal water during the wet season. Increased water contamination at the downstream canal locations during the wet season is likely a reflection of passive sampler exposure to contaminated canal water released to the Bay. Increased contamination in aquatic systems during the wet season has been reported by others (Azevedo-Linhares and Freire, 2015; Salguero-Gonzalez et al., 2015). With regards to Biscayne Bay, increased water transport into the Bay from canals during the wet season resulted in decreased water quality (Caccia and Boyer, 2005; Lirman et al., 2008) and likely increases contaminant loading into the Bay. Based on the lack of a significant change among seasons of water contamination at the coastal wetlands and the Bay interior, those locations may be less impacted by contaminant influx from canals. This is supported by the findings of both Caccia and Boyer (2005) and Lirman et al. (2008) who report that canal water has less influence on Biscayne Bay

1 water quality at locations further from the shoreline. The lack of a seasonal influence on water
2 contamination at the wetland locations indicates they are not strongly influenced by
3 contaminants from water passing directly into the Bay from the canals.

4 Sediment contamination is likely to negatively affect benthic communities at the canal
5 upstream areas. Adverse effects to benthic invertebrates as a result of sediment contamination
6 are unlikely when concentrations are less than the TEL, but are likely if concentrations are
7 greater than the PEL (MacDonald et al., 1996). The PEL was exceeded at four canal upstream
8 locations. In addition, between 3 and 6 TELs were exceeded at all but one of the canal upstream
9 locations. Therefore, benthic invertebrates are likely affected by sediment contamination in the
10 canal upstream areas. Despite being downstream of the more contaminated canal upstream
11 areas, sediment contamination at the canal downstream locations was relatively low. No TEL
12 exceedances were found at half of the canal downstream locations. Few TELs (between 1 and 3)
13 were exceeded at the other canal downstream locations, or at the wetland and Bay locations.
14 Therefore, sediment contamination is unlikely to be negatively affecting the benthic communities
15 at the downstream areas. However, activities that disturb the benthic habitats in the canal
16 upstream areas could result in the suspension and transport of contaminated sediments to the
17 canal downstream areas and the Bay. Such activity may have resulted in a spike in the number
18 of PAHs detected at C-111 during the wet season of 2011. The water control structure upstream
19 of the deployment location was undergoing reconstruction during the deployment. Transport of
20 sediments suspended by such activities needs to be prevented in order to minimize contamination
21 of the downstream areas.

22 Adverse effects to aquatic organisms appear unlikely since none of the detected
23 contaminants exceeded any water quality criteria recommended for the State of Florida. This
24 could be interpreted as indicative of a low likelihood of adverse effects. However, there were no
25 recommended standards for most of the detected contaminants. In addition, the water quality
26 criteria do not account for the potential effect of chemical mixtures, which are recognized as
27 presenting hazards not adequately characterized by individual chemical toxicity testing (Allan et
28 al., 2012; Engraff et al., 2011; Gardner et al., 1998).

29 The YES was employed in the present study to understand the potential for endocrine
30 disruption in aquatic organisms exposed to the contaminant mixture in Bay waters. While the
31 presumption is that higher EEQs would indicate a greater potential for endocrine disruption, the
32 presence of estrogen receptor subtypes in fish with substrate affinities different from that of the
33 human estrogen receptor in the YES makes less clear a direct relation between YES EEQs and
34 estrogenic effects in fish (Yost et al., 2014). The average EEQs for the locations ranged from 0.8
35 to 4.2 ng/L depending on the location and season. Whether these results are from a single
36 contaminant or a mixture of contaminants is unknown since response in the YES is non-specific
37 with regards to the compounds. Contaminants detected during the present study that have
38 estrogenic potential include phthalates, PBDEs, DDT, dieldrin, endosulfan, bisphenol-A and
39 heptachlor as well as the natural phytoestrogens daidzein, coumestrol, and genistein (Bang et al.,
40 2012; Lyche et al., 2015; Rivero et al., 2015; Yue-Wern et al., 2007). It is also possible that the
41 contaminant(s) responsible for the elevated EEQs were not one of the targeted analytes. Several
42 guideline values have been suggested to interpret EEQs from assays like the YES. The European
43 Community document (COM(2011)876) suggested a guideline level of 0.4 ng/L (cited in
44 Johnson et al., 2013). Caldwell et al. (2012) and Wu et al. (2014) used species sensitivity
45 distributions from fish reproduction studies to derive predicted no-effect concentrations of 0.43
46 and 2 ng/L. Yet another recommended a tentative predicted no-effect concentration of 1 ng/L

(Young et al., 2002). The average EEQ measured in the present study for all seasons and locations exceeded the lowest of those predicted no-effect levels (0.4 ng/L), while over half of them exceeded the higher suggested no-effect level (2 ng/L). The EEQs were higher at the canal and wetland locations during the wet season when water discharge from the canals and surface water runoff into the wetlands is greater. Elevation of EEQs following runoff events has been reported (Alvarez et al., 2013). While the assumption would be that elevated EEQs in the canals are due to increased contamination as a result of greater discharge, the same assumption may be incorrect for the wetlands because contamination in the wetlands was not elevated during the wet season. Similar results were observed for the North River reference location. The elevated EEQs at least at the wetland and North River locations either were not the result of contaminants, or they were the result of agents not included in the list of targeted analytes. While data from the YES indicate at least a mixture of components may affect the endocrine system in fish, no evidence of endocrine disruption (testis-ova) was evident for the rainwater killifish. The lack of effect in the killifish may have been because (1) the concentration necessary to affect them is greater than the concentration necessary to result in the EEQs found during the present study, (2) they are less sensitive than the species on which the no-effect levels were based, (3) they had adapted to local conditions making them less susceptible, or (4) the causative agents are natural and do not elicit adverse effects in fish. The cause of the elevated EEQs found during the present study is not clear.

The present study found a broad array of contaminants in water and sediments of canals entering the Bay, and in lower trophic level fish living in those canals and Bay. Seasonal changes in water contamination were evident where canals entered the Bay, but not in the Bay interior or wetlands. The detected concentrations for the majority of those contaminants were less than guideline values for adverse effects in aquatic and benthic organisms. While elevated EEQs at the canal and wetland locations were consistently elevated during the wet season, the same was not true for Bay locations. Some of the EEQs indicate the potential for endocrine effects in aquatic organisms, but no disruption was evident in gonads of the sampled fish species. Several have reported the impact of canal waters upon water quality in the Bay, primarily as a result of eutrophication or salinity changes (Caccia and Boyer, 2005; Langevin, 2003; Lirman et al., 2008; Stalker et al., 2009). Relatively few papers have discussed the impact of contaminants as a result of canal water introductions to the Bay. While the use of canal water and treated wastewater to rehydrate wetlands could introduce contaminants to the Bay, data from the present study indicate existing sources. The proposed rehydration of the coastal wetlands may increase contaminant presence in those wetlands.

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FIGURES

Fig. 1. Sampling locations in Biscayne National Park and adjacent coastal area. Numbers 1-3 and 5-9 denote canal downstream sampling locations; 4 and 17-22 denote canal upstream locations; 10-12 denote Bay locations; 13-15 denote the wetland locations; and 16 denotes the reference location. See Table 1 for GPS coordinates and names for each of the locations. Stippled areas are incorporated areas of Homestead, Florida City, and Miami.

Fig. 2. Average number of contaminant detections in water at the canal, Bay, and wetland locations during the wet and dry seasons. Variance bars represent 1 SE above and below the mean. $n = 3$ at Bay and wetland locations; $n = 8$ at canal locations during the wet season 2009 and dry season 2011; and $n = 14$ during wet season 2011. Asterisk indicates significant difference among the three seasons (Welch's ANOVA, $p = 0.04$).

Fig. 3. The number of organochlorine pesticides (OC pesticides), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), and organic wastewater contaminants (OWCs) detected in the passive water samplers deployed at the canal downstream locations in relation to the deployment locations and to the predominant land-use through with the canals flow. Location 16 (North River reference) is not shown on the map, but the data are shown in the bar graphs for reference.

Fig. 4. Average number of contaminant detections in sediment samples collected from the canal upstream, canal downstream, Bay, and wetland locations. Variance bars represent 1 SE above and below the mean. Means with different letters are significantly different ($\alpha=0.05$, Games-Howell post-hoc test), n -values for the canal upstream, canal downstream, Bay, wetland, and North River locations were 7, 8, 3, 3, and 1, respectively.

Fig. 5. Average number of contaminant detections in composite fish samples collected from the canal, Bay, and wetland locations. Variance bars represent 1 SE above and below the mean. Means with different letters are significantly different ($\alpha=0.05$, Games-Howell post-hoc test). n -values for the canal, Bay, wetland, and North River locations were 8, 3, 3, and 1, respectively.

Fig. 6. Average estradiol equivalents (EEQ), as predicted by the Yeast Estrogen Screen, for chemicals present in extracts in polar organic chemical integrative for samplers deployed in the dry and wet season at locations in canals, restoration wetlands, Bay, and North River locations. Variance bars represent 1 SE above and below the mean. n for the Bay and wetland locations was 3, 8 for canals during the dry season and wet season of 2009, 14 for the wet season of 2011, and 1 for the North River. The asterisk indicates a significant difference ($\alpha=0.05$) in the mean number of detections within a season for the canals and wetlands.

Table 1. Sampling plan for all locations and each season.

Location name	Latitude	Longitude	Map ID [*]	2009 Wet Season					2010
				P	SP	F	S	P	
Canal C-3	25.705165	-80.252564	1	x	x			x	
Canal C-2	25.659893	-80.271121	2	x	x			x	
Canal C-100	25.610636	-80.307877	3	x	x			x	
Canal C-1 up	25.551905	-80.350856	4	x	x			x	
Canal C-1 down	25.541086	-80.328785	5	x	x			x	
Canal C-102	25.519336	-80.336789	6	x	x			x	
Military canal	25.489625	-80.343077	7	x	x			x	
Canal C-103	25.470178	-80.346165	8	x	x			x	
Canal C-111	25.280571	-80.440637	9	x	x			x	
North Bay	25.564088	-80.284176	10	x	x			x	
Central Bay	25.494688	-80.278338	11	x	x			x	
South Bay	25.471099	-80.284483	12	x	x			x	
Deering Estate Wetland	25.620799	-80.302368	13					x	
Cutler Wetland	25.551199	-80.315739	14					x	
Fender Point Wetland	25.506482	-80.336443	15					x	
North River	25.358423	-80.900243	16					x	
C-1 @ SW157	25.628944	-80.446889	17						
Snapper Creek Wellfield	25.698583	-80.360000	18						
C-3 @ A.D. Barnes Park	25.739856	-80.308592	19						
L31N @ SW8	25.760958	-80.498047	20						
C-103 @ HWWTP	25.463806	-80.468778	21						
C-103 @ SW147	25.474644	-80.428083	22						

P - polar organic integrative sampler; SP - semi-permeable membrane device; F - fish; S - sediment

* ID numbers used for locations shown in Figure 1.

Table 2. Analytes targeted ^a in the passive sampler extracts.

SPMD (organochlorines)	SPMD (Polycyclic aromatic hydrocarbons)
Chlorpyrifos	1,2-Dimethylnaphthalene
cis-Chlordane	1-Ethyl-naphthalene
cis-Nonachlor	1-Methylfluorene
cis-Permethrin	1-Methylnaphthalene
Dacthal	2,3,5-Trimethylnaphthalene
Dieldrin	2-Methylfluoranthene
α -Endosulfan	2-Methylnaphthalene
Endosulfan sulfate	2-Methylphenanthrene
β -Endosulfan	3,6-Dimethylphenanthrene
Endrin	3-Methylcholanthrene
Heptachlor	4-Methylbiphenyl
Heptachlor epoxide	9-Methylanthracene
Hexachlorobenzene	Acenaphthene
Lindane	Acenaphthylene
Mirex	Anthracene
o,p'-DDD	Benz[a]anthracene
o,p'-DDE	Benzo[a]pyrene
o,p'-DDT	Benzo[b]fluoranthene
Oxychlordane	Benzo[b]naphtho[2,1-d]thiophene
p,p'-DDD	Benzo[b]thiophene
p,p'-DDE	Benzo[e]pyrene
p,p'-DDT	Benzo[g,h,i]perylene
p,p'-Methoxychlor	Benzo[k]fluoranthene
PBDE-100	Biphenyl
PBDE-153	Chrysene
PBDE-28	Dibenz[a,h]anthracene
PBDE-47	Dibenzothiophene
PBDE-99	Fluoranthene
Pentachloroanisole	Fluorene
Total PCB	Indeno[1,2,3-c,d]pyrene
trans-Chlordane	Naphthalene
trans-Nonachlor	Perylene
trans-Permethrin	Phenanthrene
Trifluralin	Pyrene
α -Benzenhexachloride	
β -Benzenhexachloride	
δ -Benzenhexachloride	

Table 3. Analytes^a targeted in sediment and fish samples.

Organochlorines	Organic wastewater chemicals	PBDE congener	P
Celestolide	1-Methyl naphthalene	1	1
cis-Chlordane	2,6-Dimethyl naphthalene	2	2
cis-Nonachlor	2-Methyl naphthalene	3	3
Dacthal	Acetophenone	7	4 / 10
Dieldrin	Anthracene	15	5
Endosulfan sulfate	Anthraquinone	17	6
Endrin	Benzophenone	28	7
Galaxolide	Bromoform	49	8
Heptachlor	Caffeine	71	9
Heptachlor epoxide	Carbazole	47	12
Hexachlorobenzene	Celestolide	66	13
Methoxychlor	Chlorpyrifos	77	14
Methyl triclosan	Cotinine	100	16
Mirex	N,N-diethyltoluamide	119	17
o,p',-DDT	Diazinon	99	18
o,p'-DDD	Dichlorobenzene	85	19
o,p'-DDE	Dichlorvos	126	20 / 33
Octachlorostyrene	Diethyl phthalate	154	22
Oxychlordane	Di-n-octyl phthalate	153	24 / 27
p,p'-DDD	DL-camphor	139	26 / 25
p,p'-DDE	δ -limonene	140	29
p,p'-DDE	Fluoranthene	138	31 / 28
p,p'-DDT	Galaxolide	156/169	32
Pentachloroanisole	Indole	184	34
Pentachlorobenzene	Isophorone	183	40
Phantolide	Isopropyl benzene	191	41
Tonalide	Isoquinoline	180	42
trans-Chlordane	Menthol	171	44 / 59
trans-Nonachlor	Metalaxyl	201	45
Traseolide	Methyl salicate	204	46
α -BHC	Methyl triclosan	197	48 / 47 / 75
α -Endosulfan	Naphthalene	203	49
β -BHC	Phantolide	196	51
β -Endosulfan	Phenanthrene	205	52
γ -BHC	Phenol	208	53
δ -BHC	Pyrene	207	54
	Tonalide	206	56 / 60
	Traseolide	209	63
	Tributoxyethyl phosphate		64

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6