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Determining the Effects of Freshwater Inflow on Benthic Macrofauna in the Caloosahatchee Estuary, Florida

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ABSTRACT

Florida legislation requires determining and implementing an appropriate range and frequency of freshwater inflows that will sustain a fully functional estuary. Changes in inflow dynamics to the Caloosahatchee Estuary, Florida have altered salinity regimes that, in turn, have altered the ecological integrity of the estuary. The purpose of this current project is to determine how changes in freshwater inflows affect water quality, and in turn, benthic macrofauna, spatially within the Caloosahatchee Estuary and between multiyear wet and dry periods. Thirty-four benthic species were identified as being indicator species for salinity zones, and the estuary was divided into 4 zones based on differences in community structure within the estuary. Community structure had the highest correlations with water quality parameters that were common indicators of freshwater conditions resulting from inflows. A significant relationship between salinity and diversity occurs both spatially and temporally because of increased numbers of marine species as salinities increase. A salinity-based model was used to estimate inflow during wet and dry periods for each of the macrofauna community zones. The approach used here (identifying bioindicators and community zones with corresponding inflow ranges) is generic and will be useful for developing targets for managing inflow in estuaries worldwide. *Integr Environ Assess Manag* 2015;9999:XX–XX. © 2015 SETAC

Keywords: Bioindicator Ecohydrology Environmental flow Infauna Salinity

INTRODUCTION

The quality, quantity, timing, and distribution of freshwater inflows are extremely important to the health and function of any estuary (Montagna et al. 2013). Many jurisdictions have begun to make recommendations and regulations to maintain these environmental flows to estuaries. A conceptual model has emerged to identify inflow effects on estuary resources (Alber 2002; Palmer et al. 2011; Montagna et al. 2013). The relationship between biology and hydrology is complex and embedded in the food web and material flow dynamics of estuaries. Ultimately, biological resources in estuaries are affected by salinity, which in turn is affected by inflow. Because of the linkage between flow, salinity, and biology, determining the relationship between inflow and resources is a multistep approach. First, the resource to be protected is identified. Second, the salinity range or requirements of that resource are identified in both space and time. Third, the flow regime needed to support the required distribution of salinity is identified, usually using hydrodynamic and salinity transport models. These experiences led to a generic framework that inflow hydrology drives estuarine condition and estuarine condition drives biological resources. Benthic macrofauna have been identified as a resource of interest in the current, and many other investigations, because benthos are indicators of estuarine condition and indirectly of inflow hydrology.

Research and management efforts in the Caloosahatchee Estuary have focused on determining and implementing an appropriate range and frequency of freshwater inflows that will sustain a fully functional estuary (Chamberlain and Doering 1998; Doering et al. 2002; Barnes 2005; Liu et al. 2009; SFWMD et al. 2009; Balci et al. 2012). The incentive for developing minimum and optimal flow standards has largely been driven directly and indirectly by legislation from the Florida state government, for example, Northern Everglades and Estuaries Protection Program implemented in 2007 (Section 373.4595, Florida Statutes), and requirements to develop Minimum Flows and Levels (Sections 373.042 and 373.0421 of Florida Statutes). To identify targeted inflows, the South Florida Water Management District (SFWMD) has used an approach based on the responses of estuarine organisms to changes in freshwater inflow volumes and/or salinity (Chamberlain and Doering 1998). Changes in freshwater inflows and salinity have also been shown to change the distribution and dynamics of many taxa and communities in the Caloosahatchee Estuary, including submerged aquatic vegetation (Kraemer et al. 1999; Doering et al. 2002), oysters and dermo disease (La Peyre et al. 2003; Volety 2008), fauna inhabiting oyster reefs (Tolley et al. 2006), fishes (Stevens et al. 2010), and cownose rays (Collins et al. 2008).

The purpose of this current study is to create an ecohydrology method to determine how changes in freshwater inflows and salinities affect estuary function. Here, the indicators of function are benthic macrofauna abundance, community structure and abundance, and location of

All Supplemental Data may be found in the online version of this article.

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individual indicator species, and the study location is the Caloosahatchee Estuary. Benthic organisms are ideal biological indicators of changes in water quality because they are relatively immobile, have long lifespans relative to plankton, and many species are sensitive to changes in water and sediment quality (Carriker 1967; Dauer 1993). Many studies have linked benthic communities to changes in freshwater inflow and have used these indicator communities to predict freshwater inflow needs and estuarine health (for a summary see Montagna et al. 2013). These studies have identified salinities or inflow volumes that sustain particular macrofauna communities or indicator species, and also how spatial or temporal changes in salinity or inflow volumes change the composition of those same communities or indicator species. After determining what a healthy estuary-wide macrofauna community (or other target metric) is, inflow needs can then be calculated and used to manage the freshwater inflow volumes to the estuary. Ecological health is assessed by determining if ecological conditions are in an acceptable range (Montagna et al. 2013).

METHODOLOGY

Study area

The Caloosahatchee Estuary is a small (62 km^2) but significant part of the greater Charlotte Harbor estuarine complex, which is located in the southwest coast of Florida (Figure 1). Inflow to the Caloosahatchee Estuary comes mainly from the Caloosahatchee River, a highly modified tributary that is connected to Lake Okeechobee (Doering and Chamberlain 1999). Natural habitats, drainage patterns, land uses, and managed freshwater flows within the Caloosahatchee River Watershed have been altered significantly over time (Balci et al. 2012). The Caloosahatchee River provides irrigation water, drainage and potable water, and conveyance of releases to maintain water levels in Lake Okeechobee. A network of secondary and tertiary canals now overlays the Caloosahatchee River Watershed providing drainage and irrigation to accommodate citrus groves, sugar cane, cattle grazing, and urban development. Runoff and groundwater seepage from the 344 000 ha watershed and releases from Lake Okeechobee combine to deliver an annual median of 870×10^6 m^3 of freshwater to the estuary annually (Flaig and Capece 1998; Doering and Chamberlain 1999).

The Caloosahatchee Estuary has also been significantly altered (Chamberlain and Doering 1998). The Caloosahatchee River runs 70 km from Lake Okeechobee at Moore Haven (where flow is controlled by S-77) to the Franklin Lock and Dam at Olga (where flow is controlled by S-79, see Figure 1). Separating fresh and brackish water, the Franklin Lock demarcates the head of the Caloosahatchee Estuary, which extends 42 km downstream to Shell Point, where it empties into San Carlos Bay in the southern portion of the greater Charlotte Harbor system. A navigation channel has been dredged and a causeway was built across the mouth of San Carlos Bay in the 1960s. Historic oyster bars upstream of Shell Point have been mined and removed to be used in the construction of roads. Seven automobile bridges and one

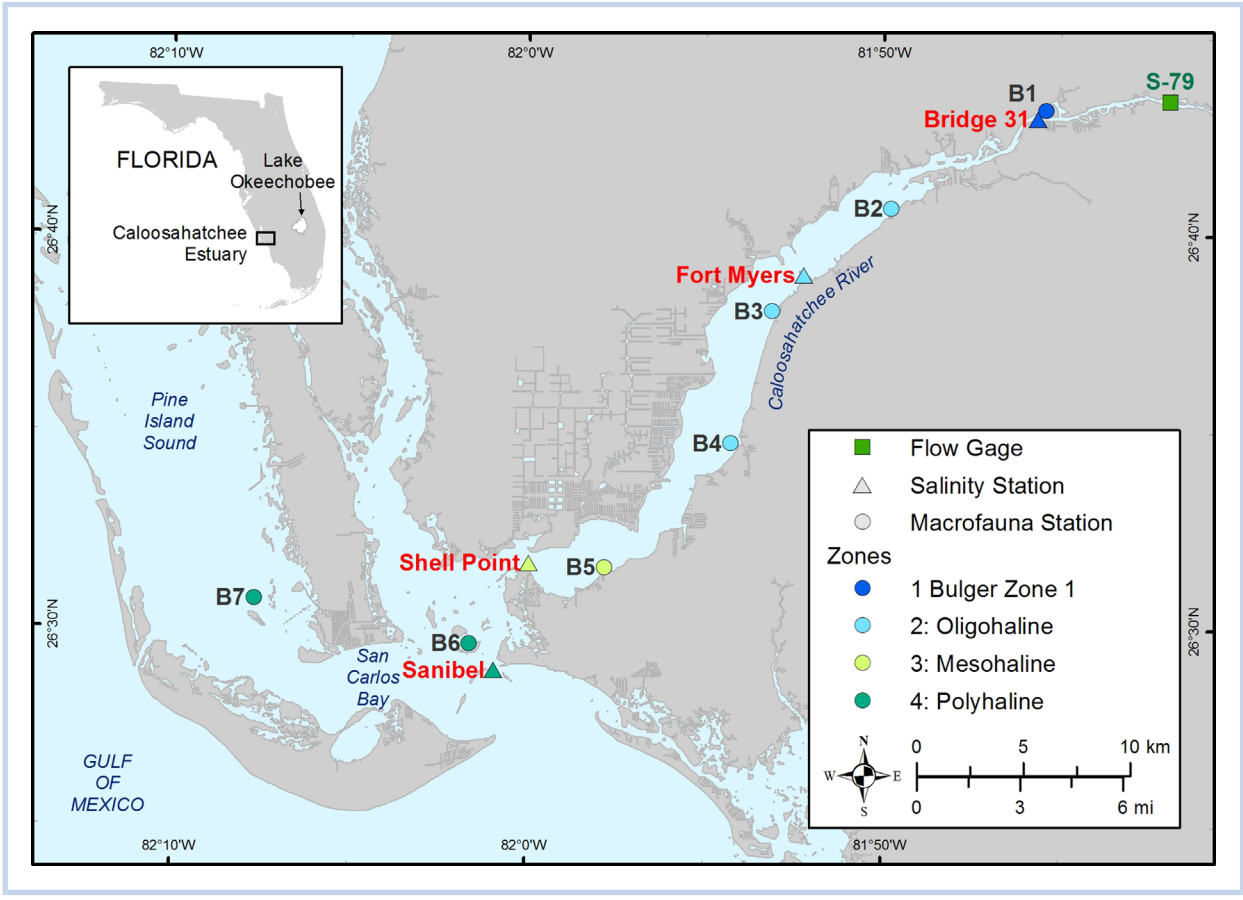

Figure 1. Map of Caloosahatchee Estuary macrofauna and salinity sampling stations showing macrofauna community zones (as in Figure 5).

railroad bridge connect the north and south shores of the estuary.

The system is highly variable on a seasonal basis (Figure 2). Large volumes of freshwater during the wet season can flush all salt from the estuary. By contrast, inflow at S-79 can stop entirely during the dry season. Saltwater intrudes to S-79, sometimes approaching 20 psu (Chamberlain and Doering 1998).

Sampling design

The study was designed to investigate benthic macrofauna distributions as related to spatio-temporal variability along the salinity gradient in the estuary. Statistical methods, including some of those used in other estuaries in Texas (Montagna et al. 2002; Palmer et al. 2011) and Florida (Montagna et al. 2008; Mattson et al. 2012) are applied to the data collected in the Caloosahatchee Estuary.

Samples were collected at 7 stations (B1–B7) (Figure 1) during 2 periods; from February 1986 to April 1989 (Period 1) and from October 1994 to December 1995 (Period 2) (Figure 2). During the first sampling period, sampling occurred 16 times at stations 1 through 6 (every 2–3 mo) and 10 times at station 7 (every 3–4 mo). Four stations (2, 4, 5, and 6) were sampled in the second period for 12 mo of the 15-mo period. The environmental conditions were different between the 2 sampling periods. Relatively low inflow rates occurred during the first period, and extremely high inflow rates occurred during the second period (Figure 2). The peak monthly inflow during the second period was the highest of any month from 1980 to 2001.

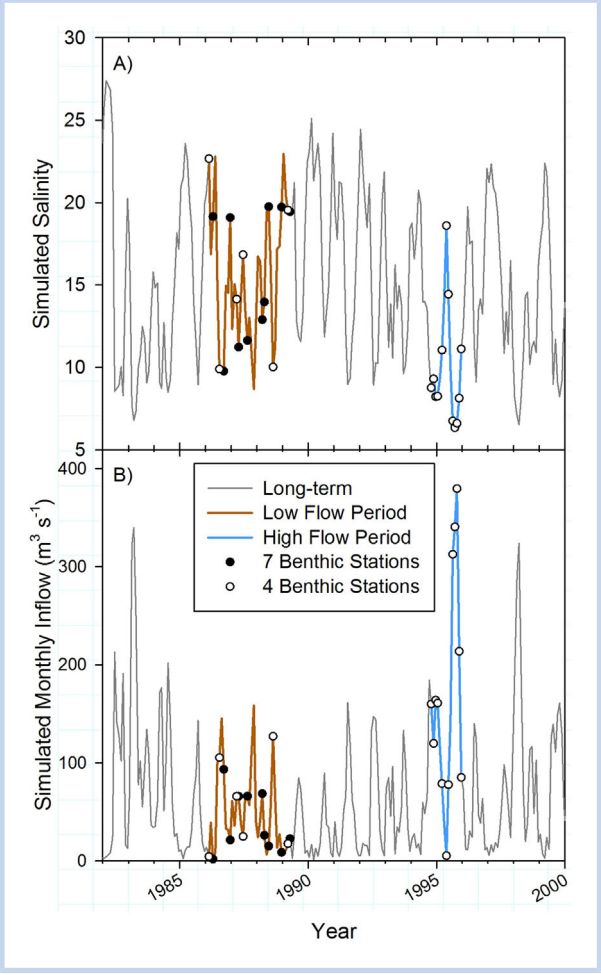

Figure 2. Mean monthly modeled salinity of benthic stations (**A**) and total estuarine inflow (**B**) over time including high and low inflow benthic sampling periods. Dots indicate when benthic samples were taken and how many stations were sampled.

To determine if seasonal variation is important to organisms, the data set was also parsed into wet seasons and dry seasons based on Liu et al. (2009). The wet season is defined as the months of June to September, and the dry season is defined as the months of October to May.

Macrofauna

Benthic samples were collected using a Wildco® petite ponar grab (0.02323 m^2). Five replicates were collected at each station within a 15 to 25 m radius. The sediment consisted of predominantly sand and shell hash. Samples were sieved on a 500-μm screen, preserved in 5% to 15% formalin buffered by Epsom salt, and stained with Rose Bengal. Invertebrates were separated from substrate by either the SFWMD (period 1) or Mote Marine Laboratory (MML) (period 2) and stored in ethanol. Staff from MML identified the dominant taxa (95% of organisms) to the species level and the remaining taxa to genera or higher taxa groups. Only 4 of the 5 replicates were processed in the second sampling period. Taxa nomenclature was verified by the authors using the Catalogue of Life internet-based checklist (Roskov et al. 2013).

Mean macrofauna abundance and diversity were calculated for each station. Macrofaunal diversity was calculated using Hill's N1 diversity index. Hill's N1 was used because it has units of number of dominant species and is more interpretable than most other diversity indices (Ludwig and Reynolds 1988). Differences in macrofauna characteristics among stations were tested on 2 subsets of the data because the sampling regime was uneven. The first subset included all 7 stations for 10 months in period 1 (dry). The second subset included 4 stations (2, 4, 5, and 6) across all months and encompasses both sampling periods.

Differences in macrofauna characteristics among stations were determined using 2-way ANOVAs with station and month-year as treatments. A linear contrast was used to test for differences among sampling periods. Post-hoc Tukey tests were run to test for differences among stations and station-period interactions. Statistical tests were performed using SAS 9.3 software on $\log_e(x+1)$-transformed data (SAS Institute 2011).

Macrofaunal community structure was analyzed using non-metric multidimensional scaling (MDS) using a Bray–Curtis similarity matrix among stations on $\log_e(x+1)$ transformed data (Clarke 1993). Relationships within each MDS were highlighted using a Cluster Analysis using the group average method. Where stations were sampled in both time periods, differences in community structure and species assemblages between periods and among zones were tested using ANOSIM, and SIMPER in Primer (Clarke and Gorley 2006).

Salinity

Mean daily salinity was calculated using a time-series modeling technique that accounts for the temporal and spatial distribution of salinity in the estuary and driving factors such as freshwater inflows, rainfall, and tide (Qiu and Wan 2013). This model has been calibrated to local salinities measured at 7 stations in the estuary (Figure 1). Verification of the relationship between salinity and freshwater inflow was undertaken using Principal Components Analysis (see Supplemental Data).

Salinity–macrofauna relationships

Relationships between previously modeled salinity (Qiu and Wan 2013) and macrofauna abundance and diversity were examined with a nonlinear model. The assumption behind the model is that there is an optimal range for salinity, and abundance and diversity values decline before and after meeting this maximum value (Montagna et al. 2002, 2008). That is, the relationship resembles a bell-shaped curve predicted with a 3-parameter, log normal model:

$$Y = ae^{\left[-0.5\left(\frac{in\frac{X}{X_c}}{b}\right)^2\right]} \quad (1)$$

This model was used to characterize the nonlinear relationship between a biological characteristic (Y) and salinity (X). The 3 parameters characterize different attributes of the curve, where a is the maximum value, b is the skewness or rate of change of the response as a function of salinity, and c the location of the peak response value on the salinity axis. For this project, samples with zero abundances were not used. The nonlinear statistical models were fit using SAS 9.3 software (SAS Institute 2011).

Verification that macrofauna communities were correlated with inflow effects was made using the BIO-ENV procedure with Primer software (see Supplemental Data).

The analyses above determine salinities where peak diversity and abundance occur, and tipping points where rapid changes in macrofauna characteristics occur with small changes in salinity. Peak abundances and diversities were determined using the nonlinear model between univariate macrofauna variables and salinity mentioned above (Montagna et al. 2002). Tipping points can be identified by using this same method but also when comparing multivariate community structure (Palmer et al. 2011). Essentially, determining salinities where peak abundance and diversity, and breaking points among community structure groups occur elucidate salinity thresholds to maintain communities in the salinity zones.

Salinity–inflow relationships

The final step was to identify inflow regimes that will yield the observed salinity thresholds. The thresholds define the boundaries among salinity zone habitats, which define the salinity gradient necessary to maintain an estuarine community. The 2 benthic sampling periods had different inflows and salinities and thus provided the basis for a low flow (period 1) and high flow (period 2) regimes (Figure 2). Based on macrofauna community structure and diversity groupings, the Caloosahatchee Estuary was divided into 4 zones. The lower quartile, median, and upper quartile of salinity were determined for each zone and sampling period combination using salinities from all dates and stations within each zone and period. The summary statistics of salinities were then converted to inflow at each salinity station within each zone (Figure 1).

The conversion from salinity (S) to flow (Q) was enabled using a 2-parameter exponential decrease model:

$$\log(Q+1) = ae^{-bS} \quad (2)$$

Flow at the S-79 gaging station was estimated for the lower, middle (median), and upper quartiles of previously modeled salinity (Qiu and Wan 2013) in each period (1 and 2) at 4 salinity stations within or adjacent to the 4 macrofauna community zones (Figure 1). The salinity stations used were Bridge 31 (zone 1), Fort Myers (zone 2), Shell Point (zone 3), and Sanibel (zone 4). Each salinity-flow conversion, along with 90% confidence intervals was calculated using SAS 9.3 software (SAS Institute 2011). These analyses allowed us to determine a minimum flow that supports the salinity zone thresholds.

RESULTS

Macrofauna

Spatial analyses of all 7 stations were compared using only the 10 months that all stations were sampled; all within the dry sampling period. Spatial and between-period comparisons of macrofauna communities that incorporated all months were enabled when only data from stations 2, 4, 5, and 6 were used.

Macrofauna abundance did not follow a linear trend moving horizontally up or down the Caloosahatchee Estuary (Figures 3A and 4A). N1 diversity followed a general pattern of low to high from upstream to downstream (Figures 3B and 4B). N1 diversity at the downstream stations 6 and 7 were significantly greater than at station 5, which in turn was significantly greater than at the upstream 4 stations.

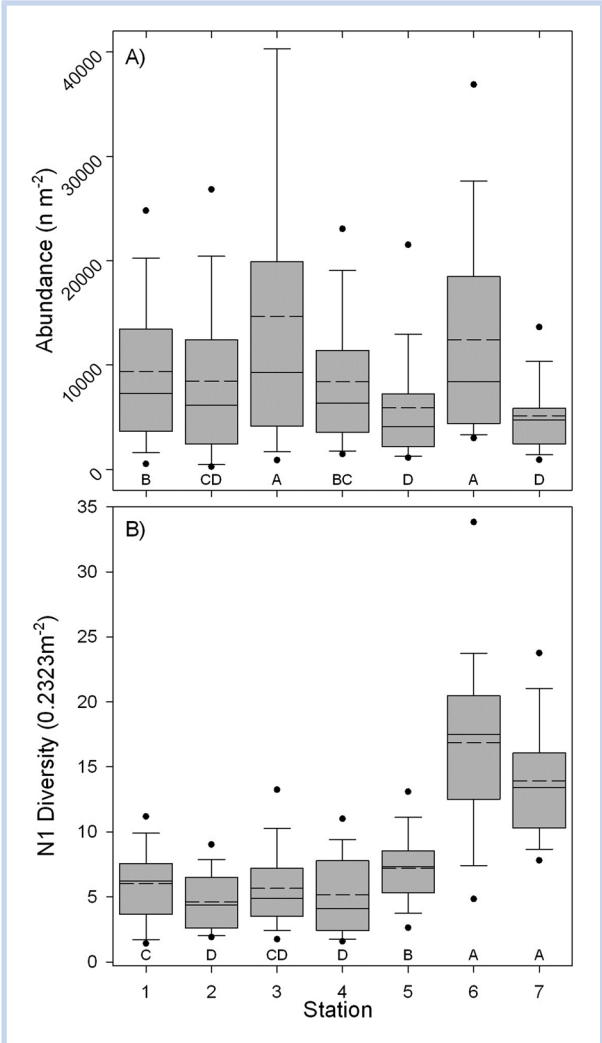

Figure 3. Boxplot of (**A**) abundance and (**B**) N1 diversity at each sampling station during simultaneous sampling in period 1 (low flow, 10 months). Dashed line indicates mean, letters represent Tukey groupings on log-transformed data.

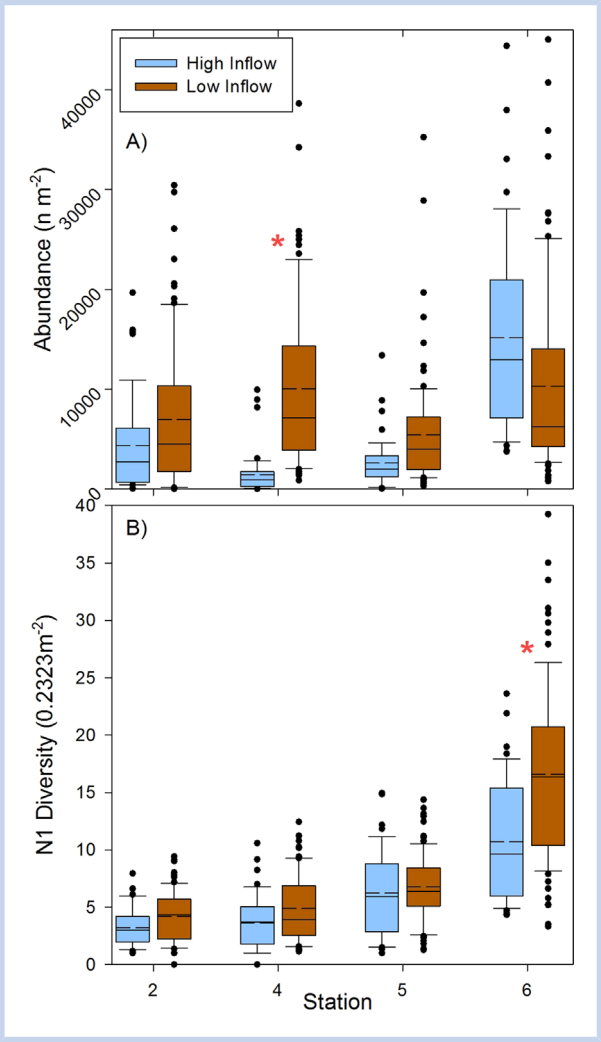

Figure 4. Boxplot of (**A**) abundance and (**B**) diversity at 4 simultaneously sampled stations in each period. Dotted line is mean. Red asterisk indicates significant difference among periods.

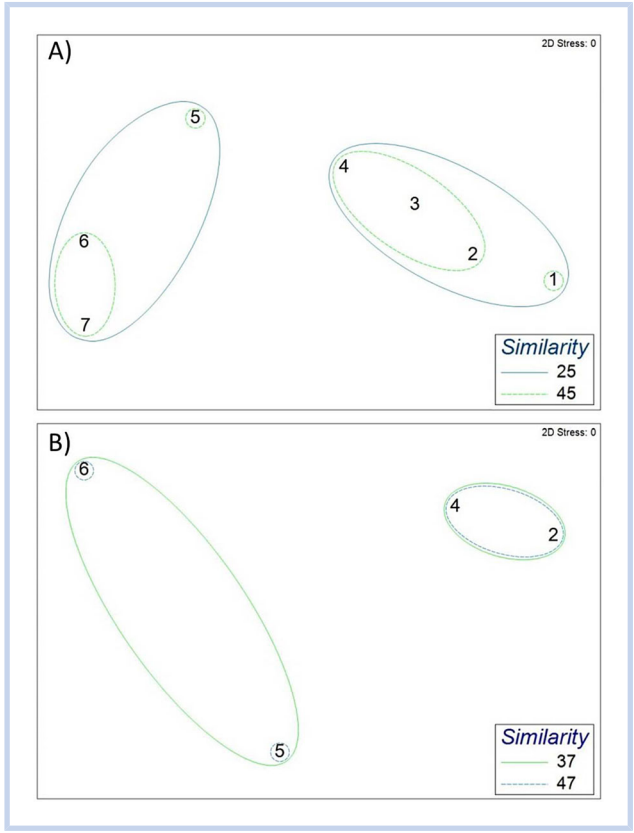

Figure 5. Multidimensional scaling plot of mean community structure at each station from February 1986 to April 1989 (**A**) and over both sampling periods (**B**).

N1 diversity, and especially abundance, were variable over the study period. Mean abundance and N1 diversity were higher in the low inflow period (mean abundance 8203 n m^{-2}, N1 8.1 $grab^{-1}$) than in the high inflow period (mean abundance 5891 n m^{-2}, N1 5.9 $grab^{-1}$). However, these differences between periods were only significant at one station for each variable. Abundance was significantly higher in the low flow period 1 than in the high flow period 2 at station 4 only (Figure 4A; Table S1). N1 diversity was significantly higher in period 1 than in period 2 at station 6 only (Figure 4B, Table S2).

Mean macrofauna community structure was grouped by location, with the upstream stations being different—from the downstream stations (Figure 5). When comparing all stations only (but only period 1), stations 1 to 4 were different from downstream station 5 to 7. These clusters were each subdivided into 2 further communities. The communities at stations 2 to 4 were significantly similar to each other (>57% similar) but not to station 1. Similarly, communities at stations 6 and 7 were significantly similar to each other (>53% similar) but not to station 5. From this clustering, the stations were divided into 4 spatial zones. Zone 1 (upstream) consists of station 1, zone 2 consists of stations 2 to 4, zone 3 consists of station 5, and zone 4 (downstream) consists of stations 6 and 7 (Figure 1).

The most abundant species within zone 1 were the amphipod *Grandidierella bonnieroides*, Conrad's false mussel *Mytilopsis leucophaeata*, and the Atlantic rangia clam *Rangia cuneata* (Table S3). In zone 2, the most abundant organisms were the cumacean *Cyclaspis varians*, the amphipod *Grandidierella bonnieroides*, and Conrad's false mussel *Mytilopsis leucophaeata*. The most abundant species in zone 3 was the amphipod *Ampelisca vadorum*, whereas the most abundant species in zone 4 were the spionid polychaete *Carazziella hobsonae*, and the myodocopid ostracod *Parasterope pollex*.

When comparing the 4 stations that were sampled in both periods, the upstream stations were divided into the same zones as determined when comparing all 7 stations in period 1 (zone 2: stations 2 and 4, zone 3: station 5, zone 4: station 6) (Figure 5B). Macrofauna communities in each of the 3 zones that were sampled in both periods were all significantly different from each other (ANOSIM: R statistic = 0.62, significance value $\leq$0.001). Polychaetes *Paraprionospio pinnata*, *Glycinde solitaria*, and *Mediomastus ambiseta* were more abundant in zones 3 and 4 than zone 2 (Table S4). The polychaetes *Aricidea philbinae*, *Tharyx dorsobranchialis*, *Prionospio perkinsi*, *Sigambra tentaculata*, and bivalve *Tellina versicolor* were all more abundant in zone 4 than in zones 2 or 3. The amphipod *Grandidierella bonnieroides* was more abundant in zone 2 than in zones 3 or 4.

The cumacean *Cyclaspis varians* decreased in abundance in period 2 (high inflow) relative to period 1 (low inflow) in zones 2 and 3 but increased in zone 4 (Tables 1 and S5). Polychaete *Streblospio benedicti* and bivalve *Mulinia lateralis* both decreased in abundance in zone 2 from period 1 to 2 but increased in abundance in zones 3 and 4. Unidentified

Table 1. Abundances of species that were discriminating between periods within all spatial zones from SIMPER analysis[a]

	Zone 2		Zone 3		Zone 4	
Species	Low inflow	High inflow	Low inflow	High inflow	Low inflow	High inflow
Cyclaspis varians	26	2	90	10	115	261
Mulinia lateralis	11	0	2	7	2	275
Streblospio benedicti	50	9	33	65	5	34
Tubificidae w/o capillary setae	1	38	1	51	6	924

[a]Abundances are detransformed to n m^{-2}.

Tubificidae without capillary setae (Oligochaeta) increased in abundance in all zones from period 1 to period 2. The isopod *Cyathura polita* and amphipod *Grandidierella bonnieroides* both decreased in abundance between dry and wet periods, however unidentified Tanypodinae (Chironomidae) increased during the same time. Many species increased in abundance from low inflow to high inflow period in zone 4, including amphipod *Cerapus tubularis*, isopod *Edotia montosa*, and bivalve *Mysella planulata*.

An MDS analysis was performed to compare community structure during wet and dry seasons (Figure S1). The wet and dry season MDS analyses used only dates in the low inflow period (period 1) because this is when all stations were simultaneously sampled. Overall, the dominant pattern where there is a large difference between stations 4 and 5 remains. There was also no difference between zones 1 and 2 in wet and dry seasons. Although stations 5, 6, and 7 were similar above the 40% level, there was a downstream shift during dry seasons, because station 6 was more similar to station 7 in dry seasons, and more similar to station 5 in wet seasons.

Salinity

Salinities were significantly higher during period 1 (i.e., the low-flow) than period 2 (i.e., the high-flow) in each zone (Table 2, Figure S2). Generally, salinity differed significantly among zones and periods. One exception is that salinities at zone 1 were not significantly different from salinities at zone 2 during the high inflow period.

Salinity–macrofauna relationships

The number of species and N1 diversity both had linear increases with salinity (Figure S3). Macrofauna abundance had no significant linear or log-normal relationship with salinity. There were significant log-normal relationships between salinity and N1 diversity within each zone, and between salinity and abundance within zone 2 (Table 3; Figures S4 and S5). Peak diversities occurred when salinities were less than 6 at the 2 upstream zones (1 and 2) and when salinities were above 28 at the 2 downstream zones (3 and 4). The relationship between abundance and salinity in zone 2 was not a good fit, even though it was significant. The abundances of 34 taxa had significant log-normal relationships with salinity (Table S6).

Salinity–inflow relationships

Modeled salinity at each of the 4 salinity stations were correlated successfully with flow at the Franklin Lock and Dam (S-79) using the exponential decrease model (Figure 6; Table 2). Flow estimates for median salinities associated with each spatial zone range from 11 to 48 m^3s^{-1} during period 1 (low inflow) to 34 to 126 m^3s^{-1} during period 2 (high inflow). The corresponding flows estimated from the lower quartile of salinity in period 1 is similar to the corresponding flows estimated from the upper quartile of salinity in period 2 (16 to 78 m^3s^{-1} and 16 to 76 m^3s^{-1}, respectively).

At low salinities (<3), the prediction equation appears to overpredict the flow as most data points were below the lower 90% limit. This is true for the gages at Bridge 31 and the Fort Meyers station.

DISCUSSION

This study assessed how the distribution, abundance, and community structure change with varying freshwater flows and used this assessment to define salinity thresholds that drive these changes. By comparing macrofauna communities along a spatial salinity gradient, useful information about the effects of temporal changes in salinity have been inferred. The linking of freshwater inflows to salinity and benthic community characteristics is simplified in the Caloosahatchee Estuary because it is a geomorphologically simple estuary. The Caloosahatchee is a classic "drowned river valley" or "coastal plains" estuary with a single large inflow source (the Caloosahatchee River) and a well-defined unidirectional salinity gradient along the narrow length of the estuary. As with other "gradient estuaries," minor changes in the salinity gradient are caused by tidal fluctuations, but much larger changes in the salinity gradient are caused by variation in river flow (Hodgkin 1994).

There is a distinct zonation of benthic communities along the salinity gradient in the Caloosahatchee Estuary. This zonation is evident when comparing N1 diversity (Figure 3B) and multivariate community structure (Figure 5) of the communities along the length of the Caloosahatchee Estuary. The positive relationship between salinity and diversity on a spatial salinity gradient is common in many estuaries (Montagna and Kalke 1992; Schlacher and Wooldridge 1996; Ysebaert et al. 1998; Sousa et al. 2006).

Diversity increased during the dry period in all 4 stations that were sampled for the duration of this study; although the increase was only significant at station 6 (Figure 4B). Similar increases in diversity during periods of decreased inflow have been observed in some—but not all—Texas estuaries (Montagna and Palmer 2012) but does not occur in one hypersaline reverse estuary (Montagna et al. 2002; Montagna and Palmer 2012). As has long been recognized elsewhere, species diversity increases with increasing salinity because of the invasion by marine species (Remane 1934). Univariate metrics of species diversity itself cannot be used to set inflow criteria because maximum diversities may indicate a marine, rather

Table 2. Salinities of each zone and corresponding flow estimates and 90% CI

Period	Salinity statistic	Zone	Salinity	Corresponding flow (m^3s^{-1})		
				Estimate	90% high CI	90% low CI
Low inflow	Lower quartile	1	0.2	77.9	66.1	91.6
		2	0.6	63.8	58.1	69.8
		3	15.1	15.7	16.4	14.9
		4	28	25.6	24.7	23.4
	Median	1	1.2	47.8	43.8	52
		2	7.1	21.7	22.1	21.2
		3	19.8	8.7	9.5	8
		4	32.5	11.3	11.9	9.6
	Upper quartile	1	4.2	15.2	16.2	14.1
		2	12.5	8.1	8.9	7.3
		3	24.9	5.1	5.8	4.5
		4	34.7	7.9	8.7	6.6
High inflow	Lower quartile	1	0.2	77.9	66.1	91.6
		2	0.2	126.4	106	150.7
		3	7.9	47.8	45.4	50.1
		4	21	152	113	170.9
	Median	1	0.2	77.9	66.1	91.6
		2	0.2	126.4	106	150.7
		3	9.9	33.7	33.1	34.1
		4	26.2	38.1	34.9	36.1
	Upper quartile	1	0.2	76.4	65	89.7
		2	3.1	55.3	51.2	59.5
		3	13.9	18.4	19.1	17.7
		4	30.5	15.8	16.2	13.8

CI = confidence interval.

than healthy estuarine environment. This is why it is important to identify the salinities associated with community zones using multivariate analyses.

In the current study, 34 taxa have been identified as being indicators of salinity (Table S6). Two taxa are calculated as being indicators of freshwater conditions (salinity <0.5), 6 as being indicators of oligohaline conditions (salinity 0.5–5), 11 as being indicators of mesohaline conditions (salinity 5–18), 10 as being indicators of polyhaline conditions (salinity 18–30), and 5 as being indicators of euhaline conditions (salinity 30–40) according to the Venice salinity classification system (Anon 1958).

Although the Venice system is widely used to divide an estuary into salinity zones, it is not biologically relevant to many estuaries, because it does not incorporate temporal variability of salinity and the objective links between the reported salinity zones and biological communities are unclear (Bulger et al. 1993). Bulger et al. (1993) divided an estuary into several overlapping zones that are based on the abundances of organisms along a salinity gradient. In the present study, we use a combination of these 2 classification schemes to name the macrofauna community zones (Table 4).

As reported in this study, Diptera (fly larvae) and the polychaete *Amphicteis floridus* (synonym *Hobsonia florida*) are good low salinity indicators (Tanypodinae in Table S5 and Ceratopogonidae and *A. floridus* in Table S6). Diptera, including those from the Ceratopogonidae and Chironomidae families are common in lower salinities or after flooding events (Kalke and Montagna 1991; Schlacher and Wooldridge 1996; Montagna et al. 2002). *Amphicteis floridus* can be abundant in habitats that have frequent low salinities (Zajac and Whitlatch 1982; Kalke and Montagna 1991; Poirrier et al. 2008).

Table 3. Log normal model relationships of N1 diversity and abundance with modeled salinity

		Parameters						
					c (salinity)			
Metric	Zone	*N*	*a* (peak)	*b* (skewness)	Estimate	90% low	90% high	*p* value
N1 diversity	1	17	7.1	2	0.7	0.2	1.2	<0.0001
	2	74	5.3	3.3	5.5	−2	12.9	<0.0001
	3	29	7.7	1.8	36.2	−99.5	171.8	<0.0001
	4	40	15	0.8	29.1	15.3	42.9	<0.0001
Abundance (n m^2)	1	17	11 726	−1.65E + 08	2 475 026			–
	2	74	11 744	−2	6.6	−0.3	13.5	<0.0001
	3	29	10	462	0			–
	4	40	10	209	0.1			–

Several polyhaline and euhaline species have been identified by this study. *Molgula* spp. (Ascidacea) is reported to have an optimal salinity range from 18 to 36 in Tampa Bay (Dragovich and Kelly 1964), which is consistent with the optimal salinity of 31 modeled in this study (Table S6). The bivalve *Mysella planulata* was modeled to have peak abundances at salinities of 22 (Table S6). The success of this model is reinforced by *M. planulata* increasing in abundance (Table S5) when mean salinities decrease from 31 in period 1 to 25 in period 2 in zone 4 (Table 2). "Unidentified Tubificidae without capillary setae" is a low salinity indicator (peak at salinity of 2) that increased in abundance when flows increased between period 1 and 2.

Despite the loss of several macrobenthic species in high-flow relative to low-flow periods, the abundance of several mobile invertebrates and fish have been documented to decrease during low-flow periods in southwest Florida estuaries (Flannery et al. 2002). These mobile species include bay anchovy and sand seatrout juveniles, mysids, and grass shrimp. A study on fish and mobile aquatic invertebrates (blue crab

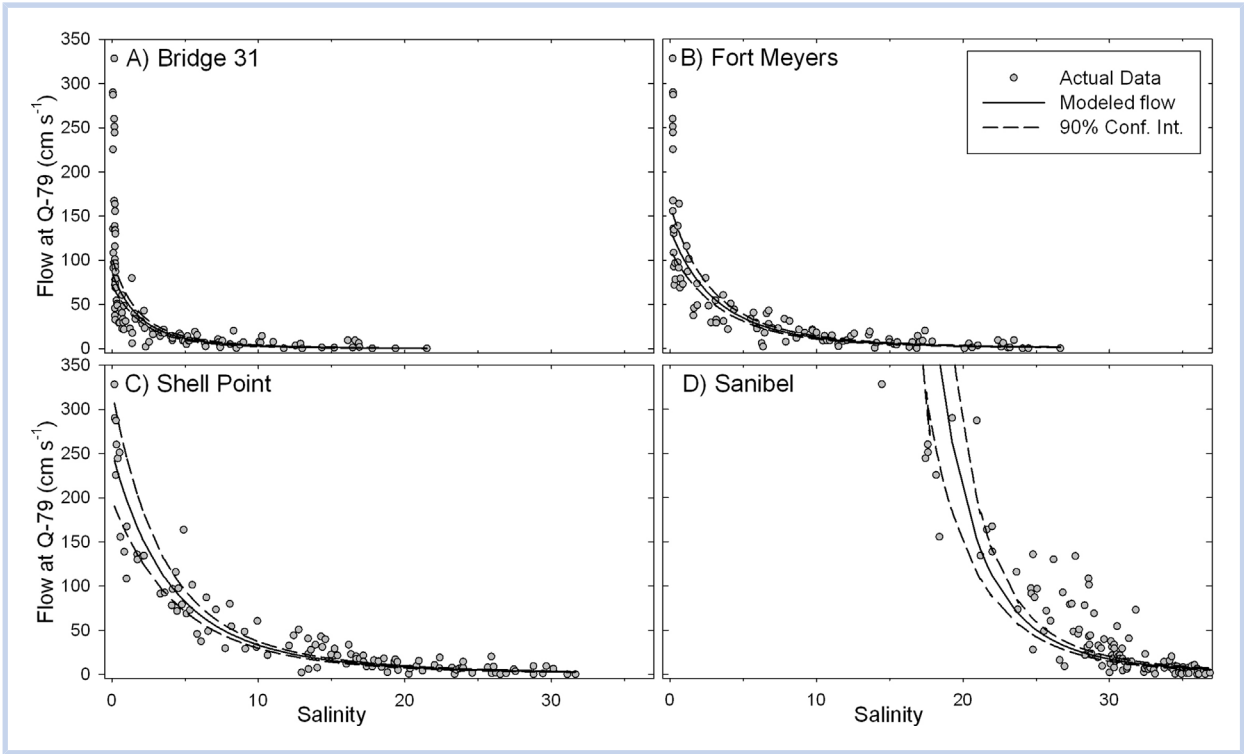

Figure 6. Actual and modeled flow-salinity relationship at Bridge 31, Fort Myers, Shell Point, and Sanibel. Upper and lower bounds are at 90% confidence interval.

Table 4. Classification of salinity zones from the Venice system[a], Bulger et al. study[b], and the current study

Venice system		Bulger study		Current study		
					Salinity quartiles	
Name	Salinities	Name	Salinities	Name	Low inflow period	High inflow period
Fresh	<0.5					
Mixohaline (brackish)	0.5–30.0	1	Fresh-4	Bulger zone 1	0.2–4.2	0.2–0.2
Oligohaline	0.5–5.0	2	14-Feb	Oligohaline	2.6–12.5	0.2–3.1
Mesohaline	5.0–18.0	3	18-Nov	Mesohaline	15.1–24.9	7.9–13.9
Polyhaline	18.0–30.0	4	16-27	Polyhaline	28.0–34.7	21.0–30.5
Euhaline	30.0–40.0	5	24-marine			
Hyperhaline	>40.0					

[a]Anon (1958).
[b]Bulger et al. (1993).

Callinectes sapidus and pink shrimp *Farfantepenaeus duorarum*) separated Caloosahatchee into 3 zones, with the lower, middle, and upper zones incorporating the reach of the benthic stations in the current study of stations 4 and 5, 2 and 3, and 1, respectively (Stevens et al. 2010). In the current study, significant differences in community structure of fish and mobile invertebrates were found among the estuarine zones, and associated species with each zone were identified. Of the 25 species with an estuarine zone preference, 9 preferred the upper zone, 6 preferred the middle zone, 5 preferred the lower zone, and 5 preferred 2 zones.

One interesting outcome when wet and dry seasons are compared is that the tipping points among the zones do not change among the freshest 3 zones in the upper reaches. However, in dry seasons, stations 6 and 7 are grouped together; whereas in wet seasons, stations 5 and 6 are grouped together. This indicates that there is a downstream shift in downstream communities during the wet seasons.

Decreases in freshwater inflows to the Caloosahatchee Estuary will result in an increase in diversity of macrobenthic fauna throughout the estuary although this may not necessarily be extrapolated to include hypersaline conditions (salinity >40). Hypersalinity was not recorded during the study period even when gaged flow (at S-79) was zero during the dry season. Future hypersalinity in the Caloosatchee Estuary is unlikely because the wet season (June–September) coincides with the hottest time of the year (summer) and tidal flushing is sufficient to prevent salinities exceeding that of the Gulf of Mexico. However, potential decreases in freshwater inflows can cause important freshwater and low-salinity species and habitats to be lost or reduced in size as these habitats are destroyed or relocated upstream (Chamberlain and Doering 1998). Maintaining low salinity habitats is integral for at least part of the life cycle of mobile species such as *Callinectes sapidus* (blue crab), *Carcharhinus leucas* (bull shark), and *Pristis pectinata* (smalltooth sawfish) (Hunt and Doering 2013) and many other species in the Caloosahatchee Estuary (Stevens et al. 2010).

CONCLUSION

Providing sufficient inflows to the Caloosahatchee Estuary allows a spatial salinity gradient, which is host to a range of organisms, including benthic communities. Decreases in freshwater inflows will allow an increase in macrobenthic diversity and changes in community composition due to an increase in marine species but will also cause a decrease of habitats that support low-salinity species. Benthic communities are not only indicators of a salinity gradient but are part of the food chain for many mobile aquatic species. Freshwater inflow rates that provide suitable conditions for these benthic communities, and indirectly other organisms, were developed in this study (Table 2). Providing such inflows during low and high inflow periods may allow the ecosystem health of the Caloosahatchee to be maintained.

The ecohydrological approach used in this study that links freshwater inflows indirectly to benthic macrofaunal communities (estuarine function) is useful for determining the effects of changes in freshwater inflows. This approach is generic and will be useful elsewhere. However, management criteria of selected indicators will need to be established alongside using this approach. Multiple indicators of inflow effects should be used to most accurately manage inflows. In the current study, diversity is not the most useful indicator of freshwater inflow because it is positively correlated with salinity and does not specifically incorporate the gains or losses of individual species that are indicative of the function of the estuary. Maintaining macrofaunal zones and the occurrence of several indicator species will be much more useful in managing inflows. The inclusion of metrics such as benthic indices of biotic integrity (Pollack et al. 2009) or β-diversity (de Juan et al. 2013) may be useful as indicators of freshwater inflow provided enough relevant information can be gathered. The quantification of functional indicators, such as the biomass of important prey items for mobile fauna, could also be useful in determining the ecosystem function of an estuary in relation to changes in freshwater inflow. However, definite food web linkages would have to be determined for this method to be successful. The accuracies of studies such as this current one will be optimized by the collection of long-term data so that a baseline is known, and replication exists to confirm effects of inflow on estuarine function.

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SUPPLEMENTAL DATA

Additional figures and tables included in the supplementary material display in more detail the spatio-temporal differences in water quality and macrofauna communities, species compositions of macrofauna communities, and relationships between salinity and individual taxa and univariate community characteristics. These additional figures and tables are useful for researchers looking to replicate the study elsewhere or looking for further details about the Caloosahatchee Estuary. Relationships between inflow, water quality, and macrofauna communities are also described. These relationships emphasize the influence of inflows on macrofaunal communities and provide justification of using salinity as a proxy for freshwater inflow.

Table S1. Post hoc Tukey groupings of macrofauna abundance at each station between periods 1 (low inflow) and 2 (high inflow).

Table S2. Post hoc Tukey groupings of macrofauna N1 diversity at each station between periods 1 (low inflow) and 2 (high inflow).

Table S3. Mean abundance of the most abundant species at all stations in Period 1.

Table S4. Discriminating species among spatial zones from SIMPER analysis.

Table S5. Discriminating species between periods within spatial zones from SIMPER analysis.

Table S6. Taxa with significant log-normal relationships between abundances and salinity.

Table S7. Pearson Correlations between the first two Principal Component (PC) factor scores from Principal Components Analysis of water quality (XXX) and monthly freshwater inflow.

Table S8. Correlations between macrobenthic communities and water quality variables resulting from the BIO-ENV procedure using data from (A) months that all seven macrofauna stations were sampled, and (B) all samples.

Figure S1. Community structure analysis by MDS for the A) dry season (October - May) and B) wet season (June - September) during Period 1. Plot is overlaid with contours from cluster analysis where similarities are 25% for dotted lines, and 44% for solid lines. Salinities are mean modeled salinities (Qiu and Wan, 2013).

Figure S2. Boxplot of daily salinities within each salinity zone during each sampling period.

Figure S3. Linear regressions of salinity with N1 diversity, number of species, Shannon diversity (H') and abundance.

Figure S4. Log-normal relationships between N1 diversity and modeled salinity within each zone.

Figure S5. Log-normal relationships between macrofauna abundance and modeled salinity within each zone.

Figure S6. Principal Components Analysis scores (A) and loading vectors (B) of water quality variables sampled in the same months as the macrobenthos during the low inflow period.

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