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Multivariate approach for identifying environmental indicator species in estuarine systems

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ABSTRACT: Over time, several indices have been developed for assessing estuarine environmental conditions. In many cases, these indicators were chosen at least partly based on human value judgements that may or may not be justified or defensible. We present a new, 2-step multivariate approach that impartially identifies a suite of ecological indicators and then determines their relationship to environmental conditions in San Antonio Bay, Texas. A total of 12 fisheries indicator species were identified by the PRIMER BEST analysis procedure, including *Leiostomus xanthurus*, *Bairdiella chrysoura*, *Pogonias cromis, Portunus gibbesii*, *Callinectes similis*, *Ictalurus furcatus*, *Harengula jaguana*, *Polydactylus octonemus*, *Lolliguncula brevis*, *Macrobrachium ohione*, *Opisthonema oglinum*, and *Libinia dubia*. This suite of indicators showed the highest rank correlation with variation in the environmental variables measured. Subsequent redundancy analysis of the relationship between these indicators and environmental variables revealed salinity as the most influential variable shaping the indicator assemblage, with turbidity the second most influential. When freshwater inflow increased or salinity was low, *I. furcatus*, *M. ohione*, and *O. oglinum* were at their highest relative numbers in the assemblage; conversely, when salinity was high, *L. brevis*, *L. dubia*, and *C. similis* were at their highest relative abundances. Additionally, the euryhaline species *L. xanthurus* and *B. chrysoura* were negatively related to water turbidity. The 2-step analysis presented provides a statistically robust way to impartially identify biological indicators most responsive to the environmental variables of interest, and thus provides managers with a robust method for monitoring the effects of their management actions.

KEY WORDS: Indicator assemblages · Estuarine condition · Salinity · Turbidity · Multivariate analysis · Texas

1. INTRODUCTION

Estuaries are some of the most productive ecosystems in the world (McLusky & Elliott 2004). However, physical processes in estuaries result in severe environmental gradients, particularly for salinity. Depending on factors such as the volume of freshwater inflow, tidal range, wind velocity, and geomorphology, environmental conditions can vary not only among different bay systems but also within a single estuary (McLusky & Elliott 2004). In a ‘typical’ estuary, salinities range from near 0.5 psu at the head to 35 psu at the lower reaches and flux diurnally depending on factors such as tidal phase or bay circulation. In estuaries with high levels of evaporation and low inflows, such as the Laguna Madre of Texas, USA, salinities may range even higher (e.g. 40–50+ psu). In response to this constantly changing environment, organisms living in the estuary must either move to avoid these extreme conditions or adapt in order to survive, grow, and reproduce. Species and community responses to these stressors are reflected in changes to the biotic assemblage (community composition or structure as mediated through species diversity, spatial distribution, or relative abundance).

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Various indices or metrics have been developed over the years to help monitor ecological conditions in estuaries. Some of these include using the phytoplankton community (Paerl et al. 2003, 2010, Lacouture et al. 2006), benthic assemblages (Weisberg et al. 1997, Pollack et al. 2009), and fish guilds (Hughes et al. 2002, Sheaves et al. 2012). Other estuarine condition indices have involved using habitat indicators, community indicators, economic indicators, protected species, and/or sensitive species (Warwick 1993, Thompson & Gunther 2004, Gilliers et al. 2006, Stachelek & Dunton 2013).

In Texas, there have been several studies using indicator species to investigate estuarine ecological conditions. Most indices were developed in relation to freshwater inflows or the salinity of the system. Through the mid-1990s to mid-2000s, Texas Parks and Wildlife Department (TPWD) and the Texas Water Development Board jointly published freshwater inflow recommendations for all of Texas' 7 major bays, using fishery species that were judged to be economically or ecologically important indicators for each bay system (e.g. Longley 1994, Lee et al. 2001, Kuhn & Chen 2005). In more recent years, at the direction of the Texas Legislature, a number of expert science teams and basin and bay stakeholder committees were established to make recommendations to the state of Texas on the amount of freshwater inflow needed for Texas' 7 major bays. Nearly all these studies also chose various indicator species as part of their analyses (e.g. Nueces River and Corpus Christi and Baffin Bays Basin and Bay Expert Science Team 2011). Other studies such as Pollack et al. (2009) focused on benthic species to develop a freshwater inflow indicator of biotic integrity (FIBI), which reflected the ecological condition in the Lavaca-Colorado estuary (Matagorda Bay). Additionally, at the Nueces Delta preserve of Texas, Stachelek & Dunton (2013) chose 3 emergent plants for study and determined that *Spartina alterniflora* could serve as a good indicator of ecosystem condition, because its abundance closely tracked variations in freshwater inflow in this highly saline system. In most, if not all of these examples from Texas estuaries, indicators were derived from species subjectively chosen by the investigators using their own reasoning, interests, or value judgements.

Unlike these previous studies, we utilized statistical methods to objectively choose, from an entire fisheries community, the indicator species most correlated with and sensitive to the environmental conditions measured in the estuary. San Antonio Bay, TX, was chosen as the test case for this new methodology, with the null hypothesis being that there was no correlation between the biotic assemblage (fish community) in the bay and the environmental variables measured (water salinity, temperature, turbidity, dissolved oxygen, and depth). This 2-step multivariate method first provides managers with a way to impartially identify indicators particularly sensitive to the environmental variable of interest (using PRIMER). The second step involves relating these indicators to the environmental variables such that the variable(s) that is the main driver of the species response is identified and the nature of the relationship revealed (using Canoco). Knowing this relationship, managers may then monitor the response of these indicators to verify the effect and effectiveness (i.e. success) of their management actions for the ecological community.

2. MATERIALS AND METHODS

2.1. Study area

Along the nearly 600 km coastline of Texas, there are 7 major bays and estuaries. San Antonio Bay is located in the mid-region between Matagorda Bay and Aransas Bay and is the home and feeding area to a wide range of ecologically and economically important fish and wildlife. The San Antonio Bay system encompasses the bay itself and several extensions, including Hynes Bay, Guadalupe Bay, and Espiritu Santo Bay (Fig. 1). At the bay's southwest edge lies the Aransas National Wildlife Refuge, which is the primary wintering ground (along with the surrounding bays and marshes) for one of the rarest birds in North America, the endangered whooping crane *Grus americana*. The bays making up the San Antonio Bay system are generally shallow, averaging 2 m in depth, and cover an area of approximately 530 km^2 (Diener 1975). Tides for this area are mixed with a mean tidal range of 0.06–0.09 m, and bay salinity from river to sea typically ranges from 0.5–25 psu; the major source of freshwater inflow comes from the San Antonio and Guadalupe Rivers, which converge only a few miles upstream of their mouth. Seawater exchange with the Gulf is relatively small and mainly via the Matagorda Jetties to the north with some exchange also possible through Pass Cavallo and Aransas Pass.

2.2. Trawl community data

The trawl data employed in this study come from the TPWD resource monitoring program which

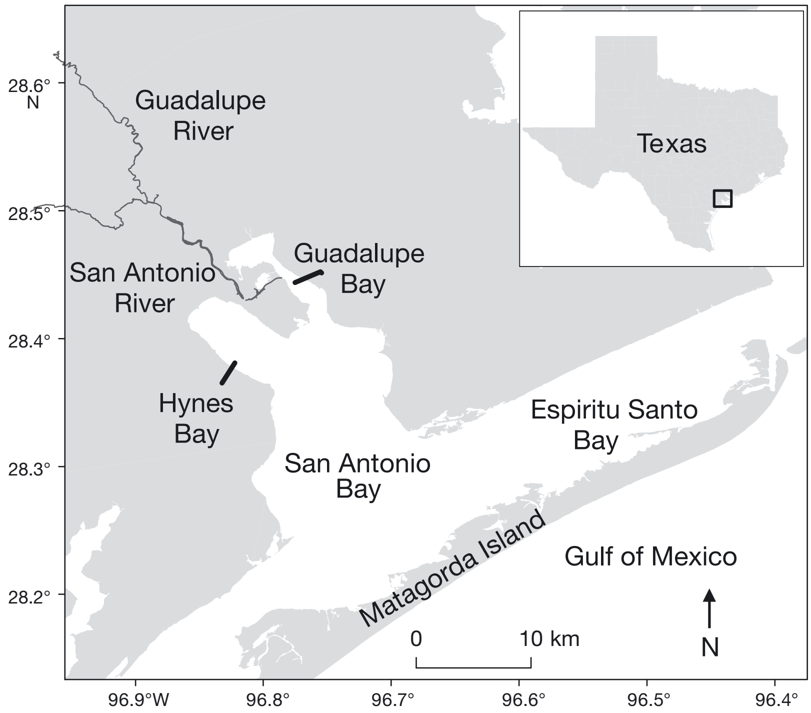

Fig. 1. San Antonio Bay study system

started in 1982 and continues to the present time. A total of 20 trawl samples are collected monthly in each major bay system in Texas. These trawls are taken in a stratified random fashion based on a grid system defined by 1 min latitude by 1 min longitude lines, such that each month 10 are taken from the upper and lower portions of the bay. These samples are also split evenly across the first and second half of the month. In cases where a trawl station is too shallow (<1 m at mean low tide) or has obstructions and cannot be sampled, an adjacent grid is chosen to collect the sample. At each station, the trawl is towed behind the boat at 3 mph (~5 km h^{-1}) for 10 min in a circular manner. The otter trawl net measures 5.7 m wide along the headrope at the mouth, 7.0 m wide along the footrope, and is made of 38 mm nylon multifilament mesh throughout. Each 10 min tow covers an area of roughly 0.46 ha. These TPWD bay trawls sample juveniles and sub-adults of fishery species utilizing Texas estuaries. All specimens (>5 mm) collected in each sample are identified, measured, counted, and then standardized to catch per unit effort (CPUE), i.e. number of animals caught per hour. At each station, water sample variables are also measured ~0.3 m off the bay bottom prior to trawl collection. Environmental variables recorded include temperature (°C), salinity (psu), dissolved oxygen (ppm), turbidity (NTU), and depth (m) (TPWD 2002). These data are publicly available by making a public information request to TPWD (https://tpwd.texas.gov/).

2.3. Data analysis

Biological (i.e. CPUE) and environmental data collected by the TPWD resource monitoring program from 1 January 1987 to 31 December 2015 were used for this study (n = 6960 sampling events). Species caught in less than 15% of trawl samples over this entire study period were excluded from the study, with 115 (of 240) species remaining for further analysis. CPUE values for each monthly sampling event were averaged by year to provide a single, annual mean CPUE for each of these remaining species. Environmental variables were processed in a similar manner and expressed as annual mean values for each individual variable. In addition, a categorical variable was added to the environmental data table to indicate the annual freshwater inflow condition, as proposed by Tolan (2013). 'Dry' years were those where the mean annual salinity was ≥85th percentile of all annual salinity values over the 29 yr study period, years with values ≤15th percentile were classified as 'wet', and values in between these 2 extremes were classified as 'normal' years.

Two software packages, PRIMER (v.6.0) and Canoco (v.5.0), were employed in this study. PRIMER's 'BEST' routine was used to carry out a full search of all possible subsets of species/taxa which exhibited the best match in their multivariate among-sample patterns with that of the associated environmental variables (Clarke & Warwick 2001). This subset of species will be referred to as 'indicators', 'indicator species', or 'indicator assemblages' of environmental conditions. It is generally assumed that the degree to which these 2 patterns match reflects the extent to which environmental conditions impact or explain the indicators' biotic patterns (Clarke & Warwick 2001). In the BEST procedure, the biotic data were ($\log_{10}$ +1) transformed to down-weight the more abundant taxa and then converted into a Bray-Curtis dissimilarity matrix. The environmental variables were normalized (mean = 0; SD = 1) and the Euclidean distances among samples were calculated. The measure of correlation between these 2 matrices, Bray-Curtis dissimilarity and Euclidean distance, was evaluated by a Spearman correlation coefficient, which ranges from −1 to +1.

The BEST routine identifies the indicators most closely correlated with the pattern in the environmental variables, but it does not identify which environmental variables from the suite considered may be the most influential. Therefore, after the indicator species were identified using BEST, the 2 matrices were then used to perform a redundancy analysis (RDA) in Canoco to explore the relative importance/influence of the environmental variables used and identify which, if any, were the main drivers of the relationship with the indicator assemblage identified. The indicator species assemblage served as the response variables for the RDA organized as year-by-species (case-by-species). The matrix of environmental data (salinity, temperature, depth, dissolved oxygen, and turbidity) served as explanatory variables and were arranged in the format of year-by-variable (case-by-variable). RDA is a linear model and often considered an extension of principal component analysis (PCA). RDA was chosen instead of a unimodal canonical correspondence analysis (CCA) because the ordination diagnostics from a pilot CCA run indicated that the relationship between the response and explanatory variables was nearly linear. These diagnostic tests also showed a short environmental gradient of <3 SD, hence the linear context was deemed most appropriate.

RDA output consisted of (1) ordination diagrams and a summary of total variation explained, (2) identification of the relative importance of the explanatory variables, and (3) a parsimonious RDA model explaining the variation in response variables (Ter Braak & Smilauer 2012). The ordination diagrams (biplot or triplot) graphically provide information on the main structure of the indicator assemblages, and their links to each environmental variable and the constrained ordination axes. The significance value calculated from the overall RDA analysis was not of interest, since the BEST analysis already identified the indicator species with the most significant overall relationship to the environmental variables. However, since BEST does not determine the relative significance of individual environmental variables as mentioned earlier, the RDA was used to elucidate their relative importance.

Additionally, the significance of salinity (a proxy for freshwater inflow) as an environmental driver was further investigated using a partial RDA for variation partitioning (VP). VP allows for further investigation of the relative contribution of subject environmental variables to the total variation seen in the biotic (indicator species) data. This method allows for an analysis of the unique explanatory power of each independent (environmental) variable separately (Borcard et al. 1992, Peres-Neto el al. 2006, Bienhold et al. 2012). For this purpose, the environmental data were divided into 2 groups: group 'a', which included only salinity, and group 'b', which consisted of turbidity and temperature (see explanation below). Group 'c' was also included to display the shared effects of these 3 variables. Two partial RDA models were run to calculate the variance uniquely contributed by each group (a and b) and their joint effect (a + b). The variance explained by salinity alone can be retrieved from a partial model controlling for the group b variables. A similar process controlling for salinity can be repeated to find the variance exclusively contributed by group b variables. The joint effect in which salinity and group b variables cannot be separated, possibly due to collinearity, can then be calculated by subtracting the unique effects from the total explained variance available from the full model run.

3. RESULTS

3.1. Environmental conditions of the estuary

Over the 29 yr of trawl samples studied, 240 unique taxa were identified. Variations in the annual values of the 5 environmental variables including salinity, temperature, turbidity, sampling depth, and dissolved oxygen are shown in Fig. 2. Of those variables, values for turbidity and salinity varied the most, followed by temperature, dissolved oxygen, and depth. Annual mean salinities fluctuated from 7.42–27.91 psu (mean ± SD: 17.81 ± 6.52 psu), while turbidities ranged from 8.55–38.37 NTU (22.54 ± 8.24 NTU). Annual mean values for the remaining 3 environmental variables (temperature, dissolved oxygen, and depth) were 22.5 ± 0.65°C, 7.91 ± 0.40 ppm, and 1.87 ± 0.08 m, respectively. Based on the 29 yr of salinity data, annual salinities ≥26.58 psu were categorized as 'dry' (≥85th percentile) and occurred in 2009, 2011, 2013, and 2014, and salinities ≤10.66 psu were categorized as 'wet' (≤15th percentile) and occurred in 1987, 1992, 2003, and 2004. The remaining 21 yr between these 2 extremes were classified as 'normal' years.

3.2. Indicator assemblage

Based on the environmental characterization of the estuary using PRIMER, the 'best' minimal set of fish-

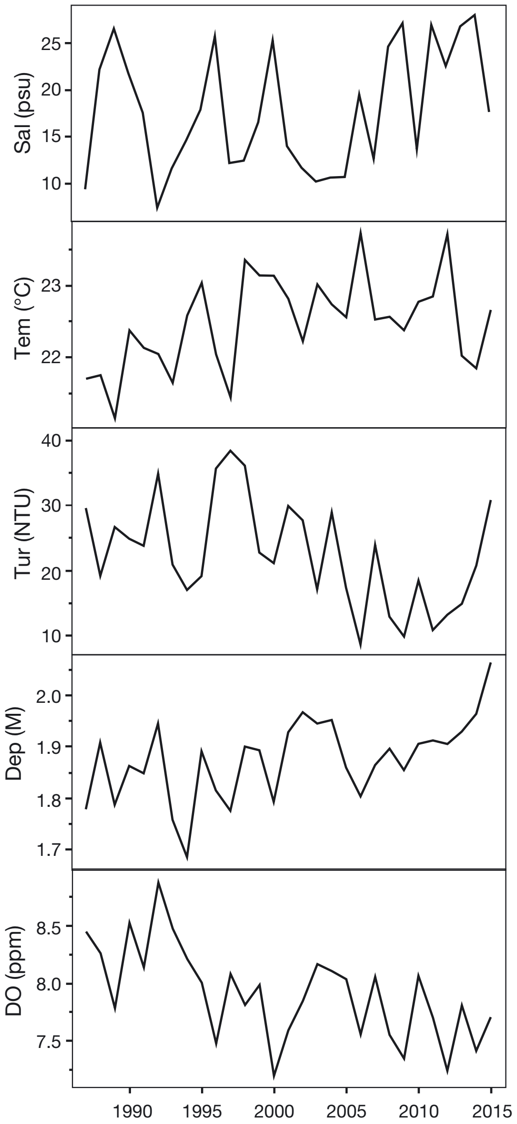

Fig. 2. Mean annual variation in environmental variables (1987–2015) for San Antonio Bay (Sal: salinity; Tem: temperature; Tur: turbidity; Dep: depth; DO: dissolved oxygen)

ery species that closely tracked changes in environmental conditions over the 29 yr study period was composed of spot *Leiostomus xanthurus*, silver perch *Bairdiella chrysoura*, black drum *Pogonias cromis*, iridescent swimming crab *Portunus gibbesii*, lesser blue crab *Callinectes similis*, blue catfish *Ictalurus furcatus*, scaled sardine *Harengula jaguana*, Atlantic threadfin *Polydactylus octonemus*, Atlantic brief squid *Lolliguncula brevis*, Ohio shrimp *Macrobrachium ohione*, Atlantic thread herring *Opisthonema oglinum*, and longnose spider crab *Libinia dubia*. Furthermore, since TPWD bay trawls typically sample mostly juveniles or sub-adults, this indicator assemblage can be further understood to be composed of younger age classes of these species. This suite of species had a rank matrix correlation coefficient of Rho = 0.563 ($p < 0.001$). Of these 12 species, 7 (Atlantic brief squid, Atlantic threadfin, Atlantic thread herring, lesser blue crab, longnose spider crab, iridescent swimming crab, and scaled sardine) are considered to prefer higher salinities. They are typically found in the open Gulf or in high salinity areas of lower bays. In contrast, blue catfish and Ohio shrimp are more oligohaline, mainly found in the upper or more freshwater portions of the estuary. The remaining 3 (black drum, silver perch, and spot) are euryhaline organisms common or endemic over a wide range of salinities in estuaries.

3.3. Indicator species–environment relationship

The significant relationship between the indicator species and environmental variables established above using the BEST procedure was then followed up with the use of a RDA model to clarify which, if any, particular environmental variables were driving the relationship. Based on the full RDA model, total variation in indicators was 103.33 and the environmental variables accounted for 55.2% of this variation. The first 2 RDA axes explained almost 75% of this fitted (explained) variation.

Graphic outputs for the RDA were displayed as ordination biplots. They summarize not only the species–environment relationships, but also the correlations among variables including those between the indicator species and those between explanatory variables. The ordination diagram for the analysis (Fig. 3) includes 3 major components: (1) the explanatory variables (red vector lines), (2) the indicator species (blue vector lines), and (3) the hydrological conditions (wet, dry, normal; red, solid triangles). The projection of environmental variables shown in Fig. 3 reveals that the first RDA axis was mainly related to salinity, and affected both stenohaline species (e.g. Atlantic brief squid, Atlantic threadfin, longnose spider crab, and lesser blue crab) and oligohaline species (e.g. blue catfish and Ohio shrimp). The second RDA axis was most strongly related to turbidity and was seen to negatively impact euryhaline species such as spot and silver perch. Since the cosine of the angle between vector lines (for the species variables or environmental variables), approximates the correlation between the corresponding variables, the close to right angle relationship between salinity and turbidity vectors suggests that

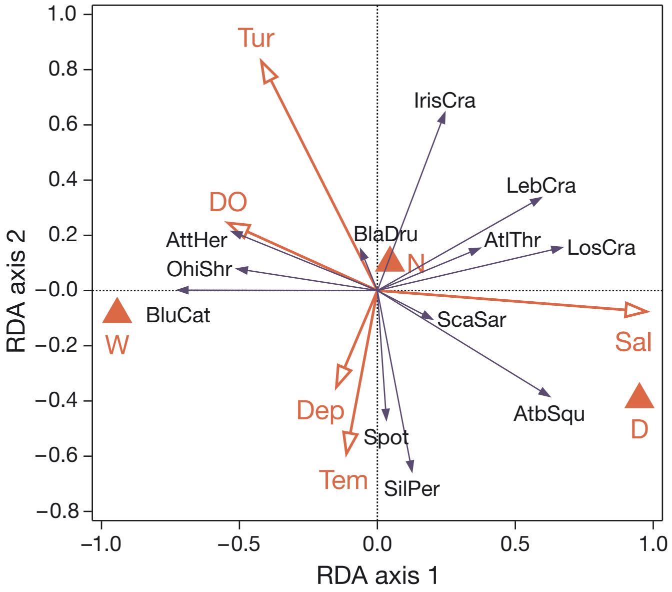

Fig. 3. Redundancy analysis (RDA) biplot showing the first 2 constrained axes, 12 indicator species (blue vectors), 5 quantitative environmental variables (red vectors), and 3 hydrological levels (solid red triangles, with D = dry, N = normal, and W = wet years). Dry, wet, and normal denote the individual years where annual mean salinities were ≥85th percentile, ≤15th percentile or in between these values for the 29 yr study period, respectively. Sal: salinity; Tem: temperature; Tur: turbidity; Dep: depth; and DO: dissolved oxygen. AtbSqu: Atlantic brief squid; AtlThr: Atlantic threadfin; AttHer: Atlantic thread herring; BlaDru: black drum; BluCat: blue catfish; IrisCra: iridescent swimming crab; LebCra: lesser blue crab; LosCra: longnose spider crab; OhiShr: Ohio shrimp; ScaSar: scaled sardine; SilPer: silver perch; and Spot: spot

these 2 environmental variables have independent effects on the structure of the indicator assemblage. Similarly, the positions of indicator species in the ordination diagram supported the known relationships for how these species cluster and relate to environmental gradients for the age classes sampled. For example, the diagram clearly displays that Atlantic brief squid, longnose spider crab, and lesser blue crab were positively correlated with each other and increased in relative abundance (i.e. CPUE) with increasing salinity.

3.4. Building a parsimonious RDA model

In addition to establishing the overall relationship between environmental variables and the indicator assemblage, 2 additional questions were of interest: (1) which environmental variable in the model was the most important, and (2) were there simpler models (with fewer explanatory variables) that still sufficiently explained the biotic pattern? Table 1 shows the effects of the various simple and conditional explanatory (environmental) variables arranged in decreasing order of importance. Simple term effects denote the amount of variation each environmental variable individually explains in the indicator species matrix if all other variables are excluded from the model. Conditional term effects denote the amount of variation explained when the particular environmental variable listed is added to the variable (or variables) above it in the model equation. Conditional terms are added in such a way that the environmental variable that explains the most additional variance is added in next. Both simple and conditional effects suggest that salinity and turbidity were the most dominant factors, respectively, driving the indicator response, and only those 2 variables (salinity and turbidity) qualified for the final, more parsimonious model with an α ≤ 0.05 threshold based on the conditional effects analysis. Note that the percent of variation explained for turbidity's conditional ef-

Table 1. Relative importance of environmental variables to indicator species based on results of redundancy analysis. Simple term effects denote the amount of variation each environmental variable individually explains in the indicator species matrix if all other variables are excluded from the model. Conditional term effects denote the amount of variation explained when the particular environmental variable listed is added to the variable (or variables) above it in the model equation. Sal: salinity, Tur: turbidity; DO: dissolved oxygen; Tem: temperature; Dep: depth. Dry, wet, and normal denote the individual years where annual mean salinities were ≥85th percentile, ≤15th percentile or in between these values for the 29 yr study period, respectively

	% Variation explained	pseudo-*F*	p-value
Simple term effects			
Sal	25.3	9.1	0.002
Tur	15.1	4.8	0.006
DO	11.1	3.4	0.008
Dry	9.8	2.9	0.018
Wet	9.0	2.7	0.032
Tem	6.6	1.9	0.072
Dep	3.5	1.0	0.448
Normal	3.2	0.9	0.478
Conditional term effects			
Sal	25.3	9.1	0.002
Tur	12.0	5.0	0.002
DO	5.0	2.2	0.074
Dry	4.9	2.2	0.066
Dep	4.1	1.9	0.104
Wet	3.0	1.5	0.212
Tem	0.9	0.4	0.846

fect does not decrease much compared to its simple term effect. This is because of the roughly orthogonal (independent) relationship between salinity and turbidity noted for Fig. 3 above. Temperature played a minor role in explaining variation in the indicator species in this study; however, Tolan (2013) observed that community structure was significantly different across seasons in Texas estuaries. Therefore, temperature was also included in the final, parsimonious model. Compared with the full model, the final one had 4 fewer variables, explained 40.3% of the total variation in indicator species (versus 55.2% for full model), and was significant (p = 0.002).

3.5. Variation partitioning

Results from the VP are displayed in Table 2. The shared portion, c, is not an interaction term, so it cannot be tested for significance. Salinity alone contributed roughly 19% of the total variation in indicator species, which was nearly twice what group b explained (10.6%). Furthermore, on a per-variable basis (compare the mean square values in the last column), salinity was a much stronger predictor (0.200 vs. 0.075). Mean square values estimate the relative strength of the unique effects (groups a and b) and are obtained via dividing the variation by the corresponding number of predictors.

4. DISCUSSION

The main purposes of this study were to present a unique, 2-step analysis method for impartially selecting biological indicators that provides an accurate reflection of the environmental conditions of the ecosystem, and to show how to identify which of these environmental variables is the main driver for the indicator assemblage overall as well as for each individual indicator species. Indicators best reflecting the response to the environmental variable of interest can then be used by managers as a check to verify whether management actions are having the expected or desired effect on abiotic conditions. In order to demonstrate the usefulness of this methodology, we presented a relatively simplified version of the analysis using fisheries and environmental data from a long-term data set for San Antonio Bay, Texas.

Many indicators have been developed to assess the condition of estuarine habitats (Gilliers et al. 2006), ranging from those based on biological resources to those based on human use and governance (Thompson & Gunther 2004). Investigators must clearly establish what the candidate indicator being considered will actually be measuring. In many cases, the chosen indicator may be subject to human bias and may not truly be an effective measure of the condition(s) being monitored. However, in his review of analytical methods for best estimating freshwater inflow needs, Estevez (2002) noted the importance of clearly identifying how these indicators respond to the environmental variables of interest. The National Research Council (NRC 2000) has provided suggestions to help in choosing appropriate indicators and determining whether the chosen candidate satisfies their suggested criteria.

Investigators must also consider whether a single indicator or a suite of indicators would best represent the desired conditions to be monitored. In recent years, there has been a trend of moving away from a simple index towards more complex, multivariate approaches (Harrison & Whitfield 2004, Love & May 2007, Sheaves et al. 2012). One example of this is the index of biotic integrity, which has been applied to the assessment of benthic conditions or the ecological condition of estuaries (Weisberg et al. 1997, Engle & Summers 1999, Pollack et al. 2009).

Regardless of which index is used, the chosen indicator should respond to changes in the condition of interest in the estuary, preferably contemporaneously, rather than lagging behind ecosystem changes (Gunther & Jacobson 2002). Fish assemblage structure has proven to be a useful tool both for monitoring (Whitfield & Elliott 2002) and evaluating the condition of estuarine systems (Ley & Halliday 2003, Sheaves 2006). Working on fish community-level responses to freshwater inflows in Texas estuaries, Tolan (2013) suggested that either lower trophic level fish, such as Gulf menhaden *Brevoortia pa-*

Table 2. Variation partitioning results for 2 subgroups of environmental variables (group a: variation explained by salinity; b: variation explained by turbidity + temperature; c: shared variation). –: no value calculated

	Group	Variation	% of explained variation	% of all variation	df	Mean square
	a	0.189	57.1	18.9	1	0.200
	b	0.106	32.1	10.6	2	0.075
	c	0.036	10.8	3.6	–	–
Total explained		0.331	100.0	33.1	4	0.134
All variation		1	–	100.0	28	–

tronus, bay anchovy *Anchoa mitchilli*, and Gulf killifish *Fundulus grandis*, or fish showing a definite salinity response (e.g. Atlantic croaker *Micropogonias undulatus*, pinfish *Lagodon rhomboides*, and white mullet *Mugil curema*) should be utilized in freshwater needs studies. Using fish assemblages, Gelwick et al. (2001) identified (1) indicators for different wetland habitats in a Texas estuary, and (2) the associated indicator species optima for salinity, oxygen, and sampling depth. For example, the indicators selected for the lower brackish zone of Matagorda Bay were Gulf menhaden, bay anchovy, silver perch *Bairdiella chrysoura*, and spotted seatrout *Cynoscion nebulosus* at salinities >15 psu, dissolved oxygen of 7–10 ppm, and depth <0.5 m (Gelwick et al. 2001).

The present study shows how the PRIMER BEST routine can be used to identify an unbiased subset of indicator species from the community sampled. This methodology, by design, picks indicators that are the most sensitive to change in the environmental variables measured, thus directly addressing the concern mentioned above about choosing indicators that are most likely to respond to changes in ecosystem condition. The use of RDA thereafter allows for identification of the main environmental drivers of the biological response, and of the species from the indicator assemblage that are most responsive to those drivers.

A total of 12 indicator fishery species were identified through the multivariate analysis presented here, and they strongly responded to variation in environmental gradients as represented by the 2 canonical axes (Fig. 3), which are linear combinations of the 5 environmental variables measured. Furthermore, the sampling method targeted fairly mobile juvenile or sub-adult age classes, so the indicators identified in this analysis had the added benefit of being capable of responding near instantaneously to changes in environmental conditions and avoiding the issues with time lags mentioned above. Additionally, since data for this study came from a long-term trawl sampling program (1987–2015) collected in San Antonio Bay, changes—such as those driven by dry and wet year conditions or other disturbances—should be captured by this study effort.

Using annual means for the CPUE and environmental variable data simplified analysis, making the data set more manageable and also had the benefit of making the results more intuitive and understandable (i.e. a single annual value is simpler to present and understand than a multitude of year × season combinations, etc.). If an investigator desires a more detailed analysis, a shorter time period (e.g. monthly) can be employed for finer scale review of environmental patterns. For the purposes of the present study, a simplified data set was used in order to focus on presenting the methodology; however, it is acknowledged that using mean annual values necessarily muted variation in the data which may be of interest, such as any within-year variation in CPUE or environmental variables. The authors caution other individuals using the techniques outlined here to weigh the relative costs and benefits of using the many different options available to them in structuring their data sets and to realize the limitations of any structure chosen.

Additionally, individuals using this methodology should be aware that the indicator species identified could include those that are relatively less common and therefore judged to not be as ecologically important. The indicators identified by the present analysis ranged from some of the most abundant and most commonly caught species in this bay system to some that were fairly common but relatively less abundant and less frequently caught. For example, spot *Leiostomus xanthurus* was the most common of the indicator species identified. Sampling showed it to have the 3rd highest annual CPUE overall compared to all other species in this bay system (240 total species caught), and it was caught in all 29 yr. Iridescent swimming crab *Portunus gibbesii* was the least commonly caught of the indicator species identified. It had the 71st highest annual CPUE overall compared to all other species in this bay system and was caught in 23 of 29 study years. Six of the 12 indicator species identified were among the top 27 most common species captured in this bay system (spot 3rd, Atlantic brief squid *Lolliguncula brevis* 7th, black drum *Pogonias cromis* 19th, silver perch 20th, lesser blue crab *Callinectes similis* 21st, and blue catfish *Ictalurus furcatus* 27th). Interestingly, 2 other species typically considered to be among the most ecologically or economically important fishery species in Texas bays, spotted seatrout and red drum *Sciaenops ocellatus*, were 39th and 77th highest (respectively) in terms of their annual CPUEs and were caught in 27 and 17 of the 29 sampling years (respectively). In order to avoid major concerns about the ecological importance of the indicators chosen when using this methodology, managers would be wise to place some constraints (as we did) on the range of species that can be considered for the analysis in order to ensure that extremely rare (and thus hard to regularly monitor) species are not considered as possible indicators. We would argue that as long as steps are taken to remove rare species from consideration as possible indicators using our methodology, the species that are identified by the procedure should be considered ecologically important, because they have been shown to be highly sensitive to, and indicative of, the environmental conditions in the ecosystem.

It is also worth pointing out that the indicator species determined for this system are definitely not flexible and are likely not applicable to other even nearby estuaries. Sheaves & Johnston (2009), working on fish data collected from 21 tropical Australian estuaries (spanning over 600 km), observed that fish assemblages from adjacent estuaries may not be the most similar when compared with those 50 or 100 km apart. As a result, the chance for the same set of indicator species to be selected in 2 neighboring estuaries or in nearby biogeographical regions seems to be very low. In addition, estuarine fish are believed to be site-specific; they are adapted locally to the demanding environmental conditions (Elliott & Quintino 2007). Therefore, even if the same set of indicator species were identified in 2 adjacent estuaries, they might still differ in their response to a similar environmental gradient or set of stressors.

Even so, the graphical output of the RDA analysis summarized well both the patterns and the indicator species–environment relationships in San Antonio Bay. The biplots (Figs. 3 & 4) clearly answer several underlying questions, such as what was (1) the relationships between indicator species and environmental variables, (2) the correlations among indicator species, (3) the correlations among explanatory variables, and (4) the relationships among the 29 yr of samples (Zuur et al. 2007, Borcard et al. 2011).

It is worth mentioning again that the individual RDA axes are a linear combination of environmental variables and represent different levels of environmental conditions in the bay system. For example, when salinity increases (from left to right in Fig. 3), the abundance of higher salinity species, such as the lesser blue crab and Atlantic brief squid, should also increase. On the contrary, when salinity declines, species that prefer lower salinities, such as blue catfish and Ohio shrimp *Macrobrachium ohione*, should increase in relative abundance. Interestingly, it was noted that higher salinities appeared to negatively affect Atlantic thread herring *Opisthonema oglinum*, which are typically thought to prefer high salinities as adults. However, this result is explained by the fact that TPWD bay trawls typically sample juveniles and sub-adults, and younger age classes of Atlantic thread herring are known to have more of an affinity for fresher estuarine conditions (Finucane & Vaught 1986, Vega-Cendejas et al. 1997).

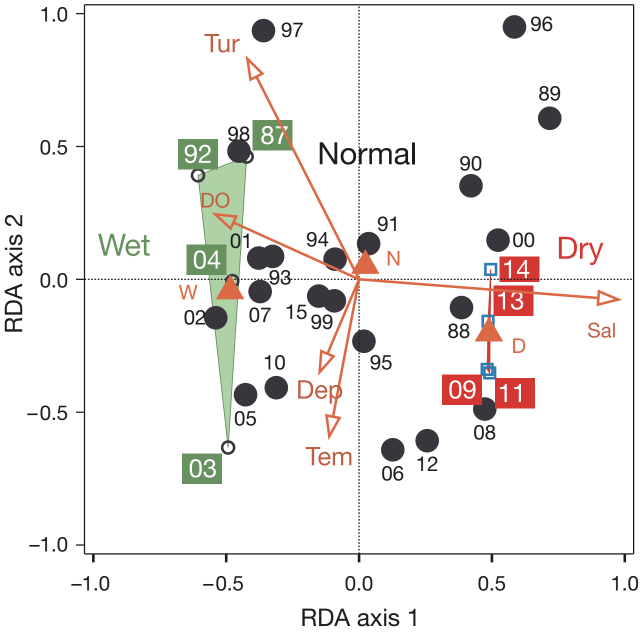

Fig. 4. Ordination diagram displaying separation of years (1987–2015 denoted as 87–15) belonging to the 3 hydrological conditions: dry, normal, and wet years, when annual mean salinities were ≥85th percentile, ≤15th percentile or in between these values for the 29 year study period, respectively. Dry conditions are represented by the 2 digit years in red boxes on the right side of the figure; wet conditions are represented by the 2 digit years in green boxes and green polygon at the left side of the figure; normal conditions are represented by the 2 digit years not in colored boxes. Sal: salinity; Tem: temperature; Tur: turbidity; Dep: depth; and DO: dissolved oxygen

The relationship for the euryhaline species, spot and silver perch, to RDA axis 2 can be understood in a similar manner (Smilauer & Leps 2014). These 2 species increase in abundance under low turbidities. Fig. 4 illustrates how the annual samples collected under different hydrological conditions are positioned in the ordination space. Dry years with low inflows/high salinity are located on the right side of RDA axis 1; wet years with high inflows/low salinity aggregate at the left side of axis 1. The remainder of the normal years generally lie between the dry and wet groups.

Another benefit of the 2-step method presented here is that once sensitive indicator species and their main environmental drivers have been identified, they can be used for other analyses as well. For example, if there is a desire to develop a predictive model for a given indicator species (Porter & Scanes 2015), a Poisson or negative binominal model can be used to display how an identified indicator species'

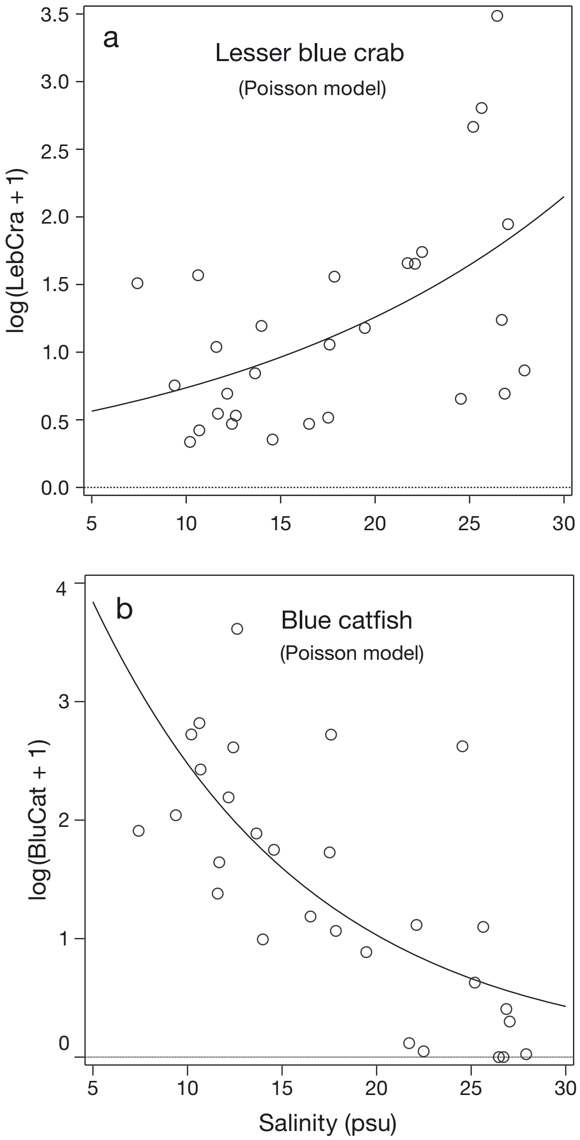

Fig. 5. Relationship between species abundance and salinity for (a) lesser blue crab (LebCra) and (b) blue catfish (BluCat) in San Antonio Bay, TX, from 1987–2015

abundance fluctuates with the environmental driver. An example of this secondary analysis from this study is presented in Fig. 5, which illustrates the lesser blue crab–salinity relationship, and the blue catfish–salinity relationship over the study period of 1987–2015. Note that these 2 examples agree well with what the biplot displays regarding how the 2 species respond to the first RDA axis—a strong salinity gradient.

In conclusion, the 2-step, multivariate approach presented here efficiently related an indicator species assemblage to the environmental conditions measured in its ecological system. Step 1 impartially identified the subset of species which were most sensitive to changes in the environmental variables measured, and step 2 identified how these indicators responded to the environmental variables as a group and individually. The value of this method for managers is that it helps them to impartially identify indicator species that have been shown to truly respond to their measured environmental variable of interest, and thus also allows the manager to use these species to determine whether management actions are having the expected or desired effect. The present analysis identified a unique indicator species assemblage for the San Antonio Bay system that showed measurable variations in abundance based on changes in environmental conditions in the bay system, thus refuting our null hypothesis. For the present study, this would mean that if managers increased freshwater inflows into the San Antonio Bay system, they should see a corresponding increase in the relative abundance of blue catfish and Ohio shrimp in bay trawl samples and a decrease in the relative abundance of Atlantic brief squid, longnose spider crab *Libinia dubia*, and lesser blue crab. Verifying these patterns in the indicators species' responses would thus provide validation of the success and effectiveness of management actions on the biological community.

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LITERATURE CITED

Bienhold C, Boetius A, Ramette A (2012) The energy-diversity relationship of complex bacterial communities in Arctic deep-sea sediment. ISME J 6:724–732

Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. Ecology 73: 1045–1055

Borcard D, Gillet F, Legendre P (2011) Numerical ecology with R. Springer, New York, NY

Clarke K, Warwick R (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. PRIMER-E, Plymouth

Diener RA (1975) Cooperative Gulf of Mexico estuarine inventory and study—Texas: area description. NOAA Tech Rep NMFS CIRC-393

Elliott M, Quintino V (2007) The estuarine quality paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed area. Mar Pollut Bull 54:640–645

Engle VD, Summers JK (1999) Refinement, validation, and application of a benthic condition index for northern Gulf of Mexico estuaries. Estuaries 22:624–635

Estevez ED (2002) Review and assessment of biotic variables and analytical methods used in estuarine inflow studies. Estuaries 25:1291–1303

Finucane JH, Vaught RN (1986) Species profile of Atlantic thread herring *Opisthonema oglinum* (Lesueur 1818). NOAA Tech Memo NMFS-SEFC-182

Gelwick FP, Akin S, Arrington DA, Winemiller KO (2001) Fish assemblage structure in relation to environmental variation in a Texas Gulf coastal wetland. Estuaries 24: 285–296

Gilliers C, Le Pape O, Désaunay Y, Bergeron JP, Schreiber N, Guerault D, Amara R (2006) Growth and condition of juvenile sole (*Solea solea* L.) as indicator of habitat quality in coastal and estuarine nurseries in the Bay of Biscay with a focus on sites exposed to the Erika oil spill. Sci Mar 70:183–192

Gunther A, Jacobson L (2002) Evaluating the ecological condition of the South Bay: a potential assessment approach. Center for Ecosystem Management and Restoration report to Department of Environmental Services San Jose, CA

Harrison TD, Whitfield AK (2004) A multi-metric fish index to assess the environmental condition of estuaries. J Fish Biol 65:683–710

Hughes JE, Deegan LA, Weaver MJ, Costa JE (2002) Regional application of an index of estuarine biotic integrity based on fish communities. Estuaries 25:250–263

Kuhn NL, Chen G (2005) Freshwater Inflow recommendation for the Sabine Lake estuary of Texas and Louisiana. CS RP V3400-825A (3/05). Texas Parks and Wildlife Department, Austin, TX

Lacouture RV, Johnson JM, Buchanan C, Marshall HG (2006) Phytoplankton index of biotic integrity for Chesapeake Bay and its tidal tributaries. Estuar Coasts 29: 598–616

Lee WY, Buzan D, Eldridge P, Pulich WM (2001) Freshwater inflow recommendation for the Trinity-San Jacinto Estuary of Texas. Texas Parks and Wildlife Department, Austin, TX

Ley J, Halliday I (2003) Role of fishing closures and habitat in conserving regional estuarine biodiversity: a case study in Northern Queensland, Australia. In: Beumer JP, Grant A, Smith DC (eds) Aquatic protected areas: What works best and how do we know? Western Australian Marine Research Laboratory, North Beach, p 562–577

Longley WL (ed) (1994) Freshwater inflows to Texas bays and estuaries: ecological relationships and methods for determination of needs. Texas Water Development Board and Texas Parks and Wildlife Department, Austin, TX

Love JW, May EB (2007) Relationships between fish assemblage structure and selected environmental factors in Maryland's coastal bays. Northeast Nat (Steuben) 14: 251–268

McLusky DS, Elliott M (2004) The estuarine ecosystem: ecology, threats, and management. Oxford University Press, Oxford

NRC (National Research Council) (2000) Ecological indicators for the nation. National Research Council, Washington, DC

Nueces River and Corpus Christi and Baffin Bays Basin and Bay Expert Science Team (2011) Environmental flows recommendations report: final submission to the Environmental Flows Advisory Group, Nueces River and Corpus Christi and Baffin Bays Basin and Bay Area Stakeholders Committee, and Texas Commission on Environmental Quality, Austin, TX. www.tceq.texas.gov/assets/public/permitting/watersupply/water_rights/eflows/20111028nuecesbbest_recommendations.pdf

Paerl HW, Valdes LM, Pinckney JL, Piehler MF, Dyble J, Moisander PH (2003) Phytoplankton photopigments as indicators of estuarine and coastal eutrophication. Bioscience 53:953–964

Paerl HW, Rossignol KL, Hall SN, Peierls BL, Wetz MS (2010) Phytoplankton community indicators of short- and long-term ecological change in the anthropogenically and climatically impacted Neuse River estuary, North Carolina, USA. Estuar Coasts 33:485–497

Peres-Neto PR, Legendre P, Dray S, Borcard D (2006) Variation partitioning of species data matrices: estimation and comparison of fractions. Ecology 87:2614–2625

Pollack JB, Kinsey JW, Montagna PA (2009) Freshwater inflow biotic index (FIBI) for the Lavaca-Colorado estuary, Texas. Environ Bioindic 4:153–169

Porter AG, Scanes PR (2015) Scavenging rate ecoassay: a potential indicator of estuarine condition. PLOS ONE 10:e127046

Sheaves MJ (2006) Scale dependent variation in composition of fish fauna among tropical estuarine sandy embayments. Mar Ecol Prog Ser 310:173–184

Sheaves MJ, Johnston R (2009) Ecological drivers of spatial variability among fish fauna of 21 tropical Australian estuaries. Mar Ecol Prog Ser 385:245–260

Sheaves MJ, Johnston R, Connolly RM (2012) Fish assemblages as indicators of estuary ecosystem health. Wetlands Ecol Manage 20:477–490

Smilauer P, Leps J (2014) Multivariate analysis of ecological data using Canoco 5. Cambridge University Press, Cambridge

Stachelek J, Dunton KH (2013) Freshwater inflow requirements for the Nueces delta, Texas: *Spartina alterniflora* as an indicator of ecosystem condition. Tex Water J 4: 62–73

Ter Braak C, Smilauer P (2012) Canoco reference manual and user's guide: software for ordination (ver 5.0). Microcomputer Power, New York, NY

TPWD (Texas Parks and Wildlife Department) (2002) Marine resource monitoring operations manual. Texas Parks and Wildlife Department, Austin, TX

Thompson B, Gunther A (2004) Development of environmental indicators of the condition of San Francisco estuary. San Francisco Estuary Institute, Oakland, CA

Tolan JM (2013) Estuarine fisheries community-level response to freshwater inflows. In: Wurbs R (ed) Water resources planning, development and management. InTech, London, p 73–94

Vega-Cendejas ME, Mexicano-Cíntora G, Arce AM (1997) Biology of the thread herring, *Opisthonema oglinum* (Pisces: Clupeidae) from a beach seine fishery of Campeche Bank, Mexico. Fish Res 30:117–126

Warwick RM (1993) Environmental impact studies on marine communities: pragmatical considerations. Aust J Ecol 18:63–80

Weisberg SB, Ranasinghe JA, Dauer DM, Schaffner LC, Diaz RJ, Frithsen JB (1997) An estuarine benthic index of biotic integrity (B-IBI) for Chesapeake Bay. Estuaries 20:149–158

Whitfield AK, Elliott M (2002) Fishes as indicators of environmental and ecological changes within estuaries: a review of progress and some suggestions for the future. J Fish Biol 61:229–250

Zuur AF, Ieno EN, Smith M (2007) Analyzing ecological data. Springer, New York, NY

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