Hierarchical Modeling Assessment of the Influence of Watershed Stressors on Fish and Invertebrate Species in Gulf of Mexico Estuaries

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Abstract

The northern Gulf of Mexico (GoM) spans five U.S. states and encompasses estuaries that vary greatly in size, shape, upstream river input, eutrophication status, and biotic communities. Given the variability among these estuaries, assessing their biological condition relative to anthropogenic stressors is challenging, but important to regional fisheries management and habitat conservation initiatives. Here, a hierarchical generalized linear modeling approach was developed to predict species presence in bottom trawl samples, using data from 33 estuaries over a nineteen-year study period. This is the first GoM estuary assessment to leverage Gulf-wide trawl data to develop species-level indicators and a quantitative index of estuary disturbance. After controlling for sources of variability at the sampling event, estuary, state, and sampling program levels, our approach screened for statistically significant relationships between watershed-level anthropogenic stressors and fish and invertebrate species presence. Modeling results indicate species level indicators with sensitivities to landscape stressor gradients. The most influential stressors include total anthropogenic land use, crop land use, and the number of toxic release sites in upstream watersheds, as well as agriculture in the shoreline buffer, each of which was significantly related to between 21% and 39% of the 57 species studied. Averaging the effects of these influential stressors across species, we develop a quantitative estuary stress index that can be compared against benchmark conditions. In general, disturbance levels were greatest in estuaries west of the Mississippi delta and in highly developed estuaries in southwest Florida. Estuaries from the Florida panhandle to the eastern Mississippi delta had less anthropogenic stress.

Keywords: biological assessment, species indicators, anthropogenic stressors, hierarchical modeling, watershed development, Gulf of Mexico

1. Introduction

Fishing is central to the social and economic well-being of the northern Gulf of Mexico (GoM) region of the United States (U.S.), making sustainable management of fisheries a regional priority. The seafood industry in the Gulf States of Florida, Alabama, Mississippi, Louisiana, and Texas (FL, AL, MS, LA, TX) contributed $7.9 B to the 2012 U.S. Gross Domestic Product (GDP) and provided 160,000 jobs to coastal residents (NMFS 2014), while recreational fisheries provided an additional $7.8 B to the regional GDP in 2012 as a result of the activities of 3.1 M anglers (NMFS 2014). Some of the most valuable species in both the commercial and recreational fisheries, including shrimps (Family: Penaeidae), Gulf Menhaden (*Brevoortia patronus*), and Spotted Seatrout (*Cynoscion nebulosus*), have strong affiliations to estuary habitats for a portion of their life cycles. The estuaries of the GoM are subject to disturbance from a wide range of anthropogenic activities, potentially putting commercial and recreational fisheries at risk. Understanding the spatial patterns and causes of degradation to estuary fisheries and fish habitats are important science priorities.

Environmental degradation in some areas of the GoM is already advanced due to anthropogenic disturbances that include hydrologic alteration, eutrophication, toxic pollution, and overfishing (NRC, 2000; Rabalais et al., 2002; Yáñez-Arancibia and Day, 2004, Howarth and Marino, 2006). Altered patterns of freshwater inflow to GoM estuaries have resulted from upstream damming, river channelization, and water abstraction (Harwell, 1997; Flannery et al., 2002), which have led to changed salinity regimes, reduced dilution of estuary pollutants, and land subsidence (Day et al., 2000). Excessive runoff of nitrogen and phosphorus from fertilizers and urban activities in catchments has caused eutrophication, and in severe cases, low dissolved oxygen and fish kills (Rabalais et al., 2002; Diaz and Rosenberg, 2008; 2011). Toxic chemicals associated with industry, urban development, and agriculture are strongly concentrated in some areas and have been shown to negatively affect benthic organisms in the GoM (Brown et al., 2000). Some of these toxic releases originate from the petroleum industry, which is especially concentrated in coastal areas of LA and eastern TX (Adams et al., 2004). Estuarine fish and invertebrate species integrate habitat conditions differently over both time and space and can be helpful as biological indicators of larger trends in ecosystem degradation (e.g., Macauley et al. 1999).

A central challenge in biological assessment is to distinguish the responses of species to anthropogenic stress from the high levels of natural background variation common to ecological systems (Hawkins et al., 2010). This challenge is particularly acute in GoM estuaries where the daily and seasonal variability in temperature, salinity, and dissolved oxygen leads to annual variation in biological community composition and structure (Peterson and Ross, 1991; Akin et al., 2003; Baltz et al., 1993; Gelwick et al., 2001; Granados-Dieseldorff and Baltz, 2008). While high natural environmental variability can be stressful to many species, anthropogenic stress to estuaries has been shown to result in dominance by opportunistic habitat or trophic generalists, to the detriment of rare or specialized taxa (Felley, 1987; Chesney and Baltz, 2001; Lewis et al., 2011). Such findings suggest that some species may be particularly sensitive to human impacts, including even estuarine species adapted to high degrees of natural environmental variation. Many estuarine studies conducted to date have focused on responses by groups of species with similar life history or functional characteristics (i.e. community metrics; Macauley et al., 1999; Summers, 2001; Hughes et al., 2002; Meng et al., 2002; Jordan et al., 2010; Cabral et al., 2012).

However, evidence from freshwater systems suggests that community metrics may obscure biological responses to stressor gradients that can be detected in species-specific indicators (Baker and King, 2010; King and Baker, 2010). Further, individual species indicators may also allow for more direct linkages to management by focusing on populations of economically valuable taxa or taxa with high conservation value.

Multiple approaches have been used to account for natural background variation in biological assessment. One approach is to define different biological indicators within discrete salinity zones (Coates et al., 2007; Briene et al., 2010; Cabral et al., 2012) or different types of estuaries (Harrison and Whitfield, 2006). Another approach is to screen for indicators that are sensitive to anthropogenic stress, but insensitive to natural gradients (Jordan et al., 2010). Moreover, models can be developed and used to account for the effects of natural variables before testing for indicator sensitivity (Engle et al., 1994). Multivariable models (e.g. multiple linear regression) have proven useful for predicting species or community responses to both natural and anthropogenic gradients (Lewis et al., 2007; Courrat et al., 2009; Delpech et al., 2010). By controlling for natural variation with model coefficients, biological responses to one or multiple ecological stressors can be predicted at different stressor levels which can be particularly helpful for judging whether current conditions differ from a benchmark or reference condition (Hawkins et al., 2010). Without benchmarks, little context exists for interpreting the measured value of an ecological resource, which can vary substantially with natural differences among sites.

Efforts to classify the ecological status of estuaries have occurred globally over the past two decades. In the U.S., studies have focused on characterizing estuaries based on their water quality, susceptibility to pollution based on geomorphologic and flow conditions, and watershed stressors such as land cover and point sources of pollution (Bricker et al., 2008; Greene et al., 2015). Some studies have classified U.S. estuaries based on their fish populations (Gleason et al., 2011; Hughes et al., 2014), but only a few nekton species in limited regions have been modeled to connect fish presence to estuary anthropogenic stress (Toft et al., 2015). In Europe, regional and country specific multi-metric indices have been developed based on biological communities to calculate overall ecological health (Breine et al., 2007; Coates et al., 2007; Delpech et al., 2010; Cabral et al., 2012; Harrison and Kelly, 2013), but limited progress has been made toward showing strong relationships between these indices and anthropogenic stressors (Pasquad et al., 2013). Such analyses have been complicated by the additional effort required to "intercalibrate" the results from different studies in order to make intra-continental comparisons. In particular, setting regional benchmark conditions and comparing studies with different sampling protocols or metrics is an ongoing challenge not easily resolved (Poikane et al., 2014). Though much progress has been made in sampling and quantifying estuary ecological status, further efforts and techniques are needed to model species and biological communities across varying natural settings, link biological conditions to watershed stressors, and set benchmark conditions.

Hierarchical (or 'multi-level') modeling provides a potential methodology for linking watershed stressors and estuary biological condition, accounting for discrepancies among sampling programs and natural variability among estuaries. Hierarchical modeling accounts for variability among different groups of data at different spatial or organizational levels using regression coefficients (i.e., 'random effects') that vary by group as members of a common statistical hyperdistribution (Gelman and Hill, 2006). This extension of classical regression modeling accounts for intra-class correlation among data from common groups (i.e., estuaries, states, trawl programs), allowing for statistically valid hypothesis testing of group-level predictor variables (Gelman et al., 2014). Thus, for grouped data, hierarchical modeling is often an improvement over classical regression modeling in terms of both predictive performance and causal inference (Wikle, 2003a; Cressie et al., 2009; Qian et al., 2010), and these models have been used extensively to study environmental and ecological systems (Wikle et al., 1998; Wikle, 2003b; Clark and Gelfand, 2006; Bolker et al., 2009; Kashuba et al., 2010; Cuffney et al., 2011). In this study, hierarchical modeling is instrumental in controlling for the variability of species presence across different estuaries, states, and monitoring programs.

The aim of the current study is to identify key sources of watershed stress (i.e., stressors) that are related to species presence in GoM estuaries, and to aggregate these relationships to assess the relative intensity of watershed stress in each estuary using hierarchical generalized linear modeling. This new approach to assessing the biological health of estuaries allows us to: (1) combine nearly 70,000 trawl samples collected by separate research efforts; (2) control for natural environmental variation while identifying statistically significant estuary-level stressors affecting the presence of fish and invertebrate species; and (3) create an estuary stress index that quantifies the amount of anthropogenic stress affecting GoM estuaries as compared to benchmark conditions in the region.

2. Methods

2.1 Study Area

Our study spanned 33 estuaries across the five U.S. GoM states (Fig. 1). Estuary habitats were classified to include open water and wetland classes from the Coastal Change Analysis Program (C-CAP) dataset (NOAA, 2006) and the National Wetlands Inventory (NWI; USFWS, 2012). To summarize landscape influence on each estuary, it was necessary to define their spatial extents and tributary influences. The seaward extent of estuaries was limited to the 4-m depth contour based on an examination of plots of salinity-at-depth. The salinities at the defined seaward depth did not generally drop below 32 practical salinity units (psu). The landward extent of the estuaries was drawn to include open water areas as well as estuarine emergent wetlands from C-CAP. Atchafalaya Bay, LA was excluded because it is a statistical outlier in terms of its freshwater inflow and nitrogen load (from the greater Mississippi River Basin), and thus difficult to compare to other GoM estuaries. Additional coastal areas were omitted from this analysis because they did not meet the conventional definition of an estuary (i.e., a partially enclosed coastal body of brackish water with one or more rivers or streams flowing into it and with a free connection to the open sea; Pritchard, 1967) or they lacked sufficient watershed level stressor data needed for analysis. One estuary, Rookery Bay, was included in the final analysis because it had watershed level stressor data even though it did not possess any biological data (i.e., trawl data).

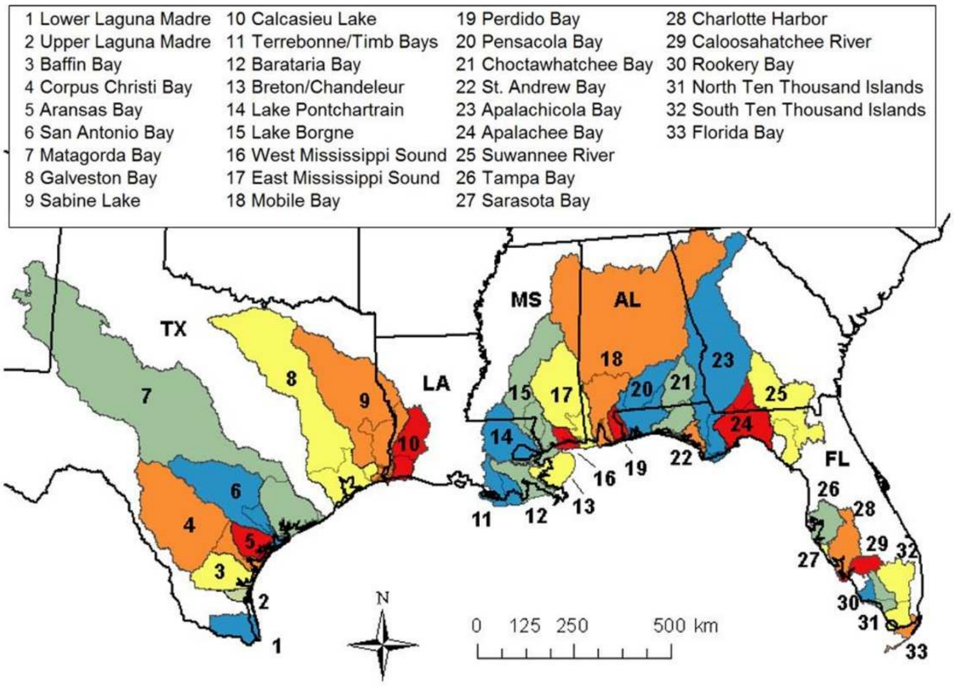


Fig. 1 Basins for the 33 estuaries and estuarine drainage areas (EDAs), representing the most downstream portion (HUC 8) of each basin, are delineated using gray lines while state lines are shown in black. For some smaller drainage basins, the total basin and EDA are equivalent (no gray line within the colored basin). The GoM includes five states: Texas (TX), Louisiana (LA), Mississippi (MS), Alabama (AL), and Florida (FL).

Data were collected at two different spatio-temporal scales. Throughout this paper, "event-level data" refer to the data recorded at the specific times and locations of individual bottom trawl samples. "Estuary-level data" refer to average conditions within estuaries or in the watersheds connecting to an estuary. Physical features include both event-level data (temperature, salinity and distance-to-shore) and estuary-level data such as estuary volume, estuary area, percent of estuary open to the sea, and average freshwater inflows. All anthropogenic stressors included in this analysis, such as toxic releases and land covers, are estuary-level data.

2.2 Trawl Events

Sampling events, performed by state and federal programs, consisted of towing otter trawls through estuary bottom waters for set distances or periods of time to determine the presence and abundance of different fish and invertebrates living in those areas. This study analyzed 69,570 bottom trawl samples from 1991 through 2009 (Table 1) gathered by the five GoM states (FL, AL, MS, LA, TX) and two federal research programs run by the U.S. Environmental Protection Agency (EPA): the Environmental Monitoring and Assessment Program (EMAP) and National Coastal Assessment (NCA). Sampling gears and protocols varied across programs (Table 1). For instance, programs used nets with varying dimensions and cod-end mesh sizes, and towed for different distances and at different speeds. Therefore, a measure of effort (hectares (ha) sampled) was derived for each sampling event, and the cod-end mesh size was recorded for each sampling program. Variation that existed in sampling protocols was accounted for by a hierarchical grouping of trawl data that allowed the model intercept to vary by sampling program (see section 2.6).

Table 1 Summary of fish and invertebrate trawl data for Florida (FL), Alabama (AL), Mississippi (MS), Louisiana (LA), Texas (TX), Environmental Monitoring and Assessment Program (EMAP), and National Coastal Assessment (NCA).

Program	# of trawls	# of species recorded	Mesh size (mm)	Trawl effort (ha)	Time period
FL	9,580	213	3.2	5.6	1991-2005
AL	2,620	102	9.5	4.2	1991-2006
MS	708	25	6.4	4.2	1991-2005
LA	23,580	209	6.4	3.2	1991-2007
TX	31,870	488	38	2.8	1991-2009
EMAP	418	157	25	5.0	1991-1994
NCA	795	244	38	5.0	2000-2004

For each bottom trawl event, fish and invertebrate species were identified and enumerated along with basic environmental data such as location, temperature, and salinity. Some trawl events yielded no data because no species were collected, while most events contained records for multiple species. Data on over 500,000 fish and invertebrate individuals collected from the trawl events were used in this study, but only species that were sampled by at least six of the seven monitoring programs and caught in a minimum of 120 trawls were used for final results (see section 2.7). This limited our study to 57 fish and invertebrate species (Table 2) whose ranges were widely distributed throughout the GoM and had sufficient data to adequately parameterize logistic models (Peduzzi et al., 1996; Allison, 2012).

2.3 Event-level Variables

Predictor variables were compiled at both the event and estuary levels. Preliminary analyses suggested that temperature, salinity and distance-to-shore were important event-level variables for the majority of species while average estuary depth was not. Temperature and salinity can determine important characteristics of fish habitat due to their effect on primary production and fish metabolism (Fry, 1971; Brett and Groves, 1979), while distance-to-shore can act as a proxy for near-shore habitats and changes in water depth. Reliable event-level depth estimates were missing for a substantial portion of the trawl data and as such this variable had to be excluded from the analysis, despite its obvious importance for fish habitat selection and trawl catch efficiency. Temperature, salinity, and distance-to-shore exhibited substantial variability as seen in Table 3. The variations in the event-level predictors reflect, to a large extent, the natural heterogeneity of estuarine conditions across the study area. While hydrologic alteration in the GoM can affect freshwater inflows and estuary salinity levels (Orlando et al., 1993; Day et al., 2000), these large-scale anthropogenic modifications were not considered directly in this study because they could not be explicitly quantified, though they are likely related to some of the available watershed stressor variables.

Table 2 Modeled fish and invertebrate species along with overall percent presence in trawl samples (T) and in estuaries (E).

Common/Scientific species name	% pres.		Common/Scientific species name	% pres.	
	T	E		T	E
Atl. Croaker/*Micropogonias undulatus*	52	85	Southern Flounder/*Paralichthys lethostigma*	5	73
Bay Anchovy/*Anchoa mitchilli*	49	85	Striped Mullet/*Mugil cephalus*	5	61
Blue Crab /*Callinectes sapidus*	42	94	Black Drum/*Pogonias cromis*	5	64
Spot/*Leiostomus xanthurus*	39	82	Striped Anchovy/*Anchoa hepsetus*	4	76
White Shrimp/*Litopenaeus setiferus*	35	70	Silver Jenny/*Eucinostomus gula*	3	82
Brown Shrimp/*Farfantepenaeus aztecus*	34	70	Lookdown/*Selene vomer*	3	73
Hardhead Catfish/*Ariopsis felis*	30	85	Star Drum/*Stellifer lanceolatus*	3	52
Sand Seatrout/*Cynoscion arenarius*	28	85	Gulf Toadfish/*Opsanus beta*	3	76
Pinfish/*Lagodon rhomboides*	27	94	Lined Sole/*Achirus lineatus*	3	73
Gulf Menhaden/*Brevoortia patronus*	20	79	Atl. Moonfish/Selene setapinnis	3	55
Silver Perch/*Bairdiella chrysoura*	20	82	Chain Pipefish/*Syngnathus lousianae*	2	76
Pink Shrimp/*Farfantepenaeus duorarum*	13	85	Crevalle Jack/*Caranx hippos*	2	52
Least Puffer/*Sphoeroides parvus*	12	70	Silver Seatrout/*Cynoscion nothus*	2	70
Bay Whiff/*Citharichthys spilopterus*	11	67	Scaled Sardine/*Harengula jaguana*	2	55
Gafftopsail Catfish/*Bagre marinus*	10	79	Blue Catfish/*Ictalurus furcatus*	2	67
Black Tonguefish/*Symphurus plagiusa*	9	76	Gizzard Shad/*Dorosoma cepedianum*	2	64
Fringed Flounder/*Etropus crossotus*	9	79	Spotfin Mojarra/*Eucinostomus argenteus*	2	52
Atl. stingray/*Dasyatis sabina*	8	73	Gulf Pipefish/*Syngnathus scovelli*	2	79
Inshore Lizardfish/*Synodus foetens*	8	97	Naked Goby/*Gobiosoma bosc*	1	73
Hog Choker/*Trinectes maculatus*	7	85	Lane Snapper/*Lutjanus synagris*	1	58
Bighead Searobin/*Prionotus tribulus*	7	85	Red Drum/*Sciaenops ocellatus*	1	64
Atl. Bumper/*Chloroscombrus chrysurus*	7	76	Atl. Threadfin Herring/*Opisthonema oglinum*	1	55
Atl. Spadefish/*Chetodipterus faber*	7	73	White Mullet/*Mugil curema*	1	48
Ground Mullet/*Menticirrhus americanus*	7	82	Bluntnose Jack/*Hemicaranx amblyrhynchus*	0.9	58
Pigfish/*Orthopristis chrysoptera*	7	82	Spanish Mackerel/*Scomberomorus maculatus*	0.8	61
Gulf Butterfish/*Peprilus burti*	6	70	Ladyfish/*Elops saurus*	0.8	64
Spotted Seatrout/*Cynoscion nebulosus*	6	79	Lined Seahorse/*Hippocampus erectus*	0.6	48
Threadfin Shad/*Dorosama petenense*	6	61	Sea Bass/*Centropristis philadelphica*	0.3	52
Atl. Cutlassfish/*Trichiurus lepturus*	5	85			

Table 3. Summary of event-level data for trawl events.

	Temperature (ºC)	Salinity (psu)	Distance-to-shore (km)
Mean	23.1	18.0	2.7
Standard deviation	6.1	11.0	3.3
2.5% quantile	11	0	0.04
97.5% quantile	31	38	14.1

All data were checked for potential outliers. Events that reported temperature values over 35º C or below 6º C were considered suspect and removed. Similarly, all non-winter records with temperatures below 10º C were excluded. In total, 285 trawl events were removed for having temperature values outside of these ranges, representing 0.4% of the original dataset. Given that salinity values above 40 psu are common in hyper-saline estuaries such as Baffin Bay, TX, no salinity values were excluded from this analysis (maximum salinity = 66 psu). Sample months were aggregated into seasons: spring (March, April, May), summer (June, July, August), fall (September, October, November), and winter (December, January, February), which were considered as a categorical variable within the models.

2.4 Estuary-level Variables

Land cover data were compiled and analyzed at three different spatial scales: basin, estuarine drainage area (EDA; Fig. 1) and shoreline buffer. Basin data covers were summarized from the entire drainage area of the estuary to the topographic divide with adjacent river basins with outlets to the sea, while EDAs were limited only to the lowest most proximate 8-digit hydrologic unit code (HUC) watershed that drains to the coast (NOAA/NOS, 1985). The shoreline buffer was defined as the 500-meter buffer inland from each estuary boundary. EDA sizes ranged from 246 km^2 to 13,690 km^2, while basin areas ranged from 330 km^2 to 121,762 km^2. For some estuaries with entirely coastal drainage areas, basin and EDA values were the same (e.g., Sarasota Bay, FL, Perdido Bay, AL, and Baffin Bay, TX; Fig 1). Land cover data from 1992, 2001 and 2006 were highly correlated (r > .98) such that all estuary-level land cover data was calculated using the 2001 National Land Cover Database (Homer et al., 2007) representing the middle of the study period and supplemented with wetland classifications from C-CAP (NOAA, 2006) and the National Wetlands Inventory (USFWS, 2012). We grouped land cover categories to reduce potential collinearity and to generate combinations of land cover types expected to have similar impacts on estuary habitat quality. The "Agriculture" class was formed by grouping crop and pasture, both of which are expected to yield excess nutrients to waterways while the "Anthropogenic" class aggregated urban, crop, and pasture into one class to represent land use predominantly affected by humans. The land cover classes adopted in the model were normalized by the total land area (A_L) of the given spatial unit (basin, EDA, or shoreline buffer), creating percent land cover values for each spatial scale. Other approaches to normalizing land cover are described below.

Watershed land cover, population, number of toxic release sites, and number of National Pollutant Discharge Elimination System (NPDES) sites (USEPA, 2015) can be interpreted as proxies for pollution, both at the basin and EDA levels. For example, crop areas may export nitrogen, phosphorous, pesticides, and various other agricultural chemicals downstream to estuaries. We would have preferred to use more direct predictors like nitrogen, phosphorus, or other pollutant loads, but reliable loading data were unavailable for many estuaries. Land-based pollution loads are often attenuated through biogeochemical processing, settling, and flushing. In the current study, it was infeasible to estimate reaction rates or settling velocities for the diverse range of potentially important pollutants. In an effort to indirectly account for settling and flushing, many estuary-level variables were normalized by estuary area (A_E) and flow (Q), in addition to A_L, creating several stressors per variable (e.g., EDA Crop/A_E, EDA Crop/Q, and EDA Crop/A_L; Table 4). Shoreline buffers are not large enough to produce significant loads, but may affect nearshore habitat conditions, and were thus normalized by only A_E. Five estuarine variables from the 2007 National Estuarine Eutrophication Assessment (Bricker et al., 2008), including estuary average salinity, estuary percent open to sea (%), hypoxic condition, and toxic algal condition (scaled from 0 = no problem to 3 = high), and eutrophication condition (scaled from 1 = low to 5 = high), were also included without normalization. In total, 47 candidate estuary-level variables were considered (Table 4). Basic estuary-level data can be found in Supplementary Material, SM 1 and SM 2.

Table 4 Estuary-level predictor variables considered in the modeling analysis using various normalization options (1 = no normalization; A_E = estuary area (km^2); Q = flow (m^3/day); A_L = total land area). Note: I(0:3) indicates an integer variable (0, 1, 2, or 3).

Variable	Unit	1	A_E	Q	A_L
Watershed					
Shoreline Urban	km^2		X		X
Shoreline Crop	km^2		X		X
Shoreline Agriculture	km^2		X		X
Shoreline Anthropogenic	km^2		X		X
Shoreline Wetlands	km^2		X		X
EDA Urban	km^2		X	X	X
EDA Crop	km^2		X	X	X
EDA Agriculture	km^2		X	X	X
EDA Anthropogenic	km^2		X	X	X
Basin Urban	km^2		X	X	X
Basin Crop	km^2		X	X	X
Basin Agriculture	km^2		X	X	X
Basin Anthropogenic	km^2		X	X	X
EDA toxic releases	#		X	X	
EDA NPDES	#		X	X	
EDA population	#		X	X	
Basin population	#		X	X	
Estuary					
Mean estuary salinity	psu	X			
Estuary percent open to sea	%	X			
Hypoxic condition	I(0:3)	X			
Toxic algal condition	I(0:3)	X			
Eutrophic condition	I(1:5)	X			

2.5 Hierarchical Generalized Linear Model

Species presence and absence in trawl samples was modeled as a binary response, where trawls that contained a given species were assigned a value of one and trawls that did not collect that species were assigned a value of zero. A common approach for modeling binary data is to use a generalized linear model that extends the framework of linear regression modeling to non-normally distributed variables by the use of a link function. These types of models are used extensively in many fields, including ecology (Guisan and Zimmermann, 2000; Guisan et al., 2002; Gelfand et al., 2005; Gelman and Hill, 2006; Latimer et al., 2006, Bolker, 2008).

$$P(y_i) = \text{logit}^{-1} (\mathbf{X_i}\,\boldsymbol{\beta}) \qquad \text{(Eq. 1)}$$

where $P(y_i)$ is the probability of fish presence in trawl i, $\mathbf{X_i}$ is a matrix of predictor variables, and $\boldsymbol{\beta}$ is a vector of corresponding model coefficients. The inverse-logit function is used to back transform the response from the continuous modeling domain ($-\infty$ to $+\infty$) to probability space values between 0 and 1: $\text{logit}^{-1}(x) = \frac{e^x}{1+e^x}$.

In addition to accounting for binary responses, our model is constructed hierarchically in order to capture different levels of organization within the data. Hierarchical models use "random effects" to account for data that are similar and can be grouped as members of a common statistical hyperdistribution (Gelman and Hill, 2006). This approach helps account for intra-class correlation among grouped data allowing for statistically valid hypothesis testing of group-level (i.e., estuary-level) predictor variables (Gelman et al., 2014). Random effects were used in this study to account for variation at the estuary, program, and state level that was not explained by other predictors in the model. Without this added model flexibility, prescreening would not have been possible without much noise from spurious correlations between predictors and watershed stressors (see Section 4.4).

2.6 Single-stressor Models

For each fish and invertebrate species, we initially screened all 47 possible estuary-level predictor variables (x_{pred}) separately while controlling for event-level variables. Logistic hierarchical models were fit using the "lme4" R package with parallel processing provided by the "doparallel" R package (R Development Core Team, 2011; Bates et al., 2013; Calaway et al., 2015). The adopted model structure is shown below.

$$P(y) = \text{logit}^{-1} (\beta_o + \beta_{season} + \alpha_{estuary} + \alpha_{state} + \alpha_{program} + \beta_{temp}*x_{temp} + \beta_{sal}*x_{sal} + \beta_{salsq}*x_{salsq} + \beta_{dist}*x_{dist} + \beta_{pred}*x_{pred}) \qquad \text{(Eq. 2)}$$

where $P(y)$ is the probability of occurrence of a given species, β_o is the overall y-intercept for the model, and β_{season} is a categorical variable used to account for seasonal differences. Random effects, $\alpha_{estuary}$, α_{state}, and $\alpha_{program}$, were assumed to follow normally distributed hyperdistributions centered around zero. For each species modeled, 32 different values of $\alpha_{estuary}$ were returned (Rookery Bay is excluded due to no trawl events), five values for α_{state} and seven values for $\alpha_{program}$. Slope coefficients β_{temp}, β_{sal}, β_{salsq}, and β_{dist} were determined for the event-level effects of temperature (x_{temp}), salinity (x_{sal}), salinity^2 (x_{salsq}), and distance-to-shore (x_{dist}), respectively. The square of salinity (x_{salsq}) was included because preliminary analysis indicated a potential non-linear (quadratic) relationship between salinity and probability of fish or invertebrate presence. β_{pred} is the slope coefficient of the estuary-level anthropogenic variable being screened. These single-stressor models were used to test the significance of each estuary-level predictor (Table 4) on the occurrence of every fish and invertebrate species (Table 2). Predictor variables were considered statistically significant when their corresponding β_{pred} coefficients were significantly different from zero at a 95% confidence level ($p<0.05$).

2.7 Multi-stressor Models

Candidate estuary-level variables for multi-stressor modeling were selected based on the single stressor modeling results. Favoring parsimonious multi-stressor models, we selected only estuary-level variables that were found to be significant for over 20% of the species. Furthermore, variables were tested for multicollinearity, and when one or more variables were found to be correlated ($|r| \geq 0.65$; Supplementary Material, SM 3), only the most significant variable was included. This process reflects two assumptions: 1) stressor variables with truly mechanistic underpinnings should affect a broad range of species across the GoM and 2) variables that represented similar mechanisms (e.g., Basin Crop/A_L is a subset of Basin Agriculture/A_L) should only enter the model once. Performing variable selection on a subset of probable stressors instead of the entire 47 dependent variables (Table 4) avoids the inclusion of spurious or "noisy" predictors and thus leads to more robust models (Cohen, 1990; Derksen and Kesselman, 1992).

For each species, all event-level variables (temperature, salinity, salinity^2, and distance to shore) and the selected candidate estuary-level variables were included together in a backward model selection procedure using the "LMERConvenienceFunctions" R package (Tremblay and Ransijn, 2015). Backward selection eliminates variables in a step-wise fashion based on statistical significance, until the final model contains only predictors significant at the 95% confidence level. Note that since multi-stressor models could have up to nine predictors (four natural variables and five anthropogenic ones; Eq. 2 and Table 5) and three levels of random effects based on preliminary modeling results, these models were only developed for species caught in at least 120 trawl events. This insures that we have at least 10 observations for each possible predictor and level of random effect (Peduzzi et al., 1996; Allison, 2012). Model selection using Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) was also explored (Sakamoto et al., 1986; Schwarz, 1978), but both of these criteria added insignificant predictors as well as eliminated significant predictors making these methods not desirable for the overall project goals.

Table 5 Summary of highly significant estuary-level stressor variables (statistically related to over 20% of species), as determined from the single-stressor models. Negative relationships indicate the percent of significant relationships that are negative. Candidate variables are those tested in the multi-stressor models.

Stressor	Species affected (%)	Negative relationships (%)	Candidate variables
Basin Anthropogenic/A_L	53	77	X
Mean estuary salinity	49	61	X
Basin Agriculture/A_L	47	70	
Basin Crop/A_L	44	72	
Basin Crop/Q	39	73	X
Basin Anthropogenic/Q	37	67	
Shoreline Agriculture/A_L	35	15	X
Basin Agriculture/Q	35	70	
EDA toxic releases/A_E	23	85	X
Basin Urban/Q	21	58	

2.8 Assessment of Estuary Level Anthropogenic Stress

Once the multi-stressor models were developed, they were used to create an estuary stress index which quantifies the amount of anthropogenic disturbance in estuaries by comparing sampled conditions to those predicted under two different benchmarks of anthropogenic disturbance. Least disturbed conditions (LDC) were defined as the minimum value of each stressor observed at the regional scale (SM 1), and minimally disturbed conditions (MDC) were predicted by setting stressor values in the models to zero. LDC and MDC are benchmarks of ecological condition frequently used to assess the degree to which the current conditions of a particular system deviate from a more desirable state (Stoddard et al., 2006; Hawkins et al., 2010). We used the region-wide stressor minima to specify LDC as an example of one benchmark to assess estuaries in the GoM, but sub-regional LDCs might also be developed and used within this same framework. To judge the amount of anthropogenic watershed stress on each estuarine system, the absolute value of each stressor coefficient was averaged across all species to determine the mean absolute rate of change in probability of species presence (on the log-odds scale) associated with each stressor. Mean absolute coefficients were then multiplied by the actual estuary-level conditions and compared to both LDC and MDC to create the estuary stress index.

3. Results

3.1 Single-stressor Models

All estuary-level stressor variables (Table 4) were screened in single-stressor models (Eq. 2) that controlled for natural variability at the event-level (e.g., temperature, salinity) as well as for intra-class correlation and unknown variability at group levels (i.e., estuary, state, program). Variables were then ranked according to the number of species with which they had significant relationships (Table 5). Basin Anthropogenic/A_L, Basin Crop/A_L, and Basin Agriculture/A_L were found to be statistically significant for over 40% of the assessed fish and invertebrate species with more than 70% of those significant relationships being negative. Of the other anthropogenic candidate stressors, Basin Crop/Q and EDA toxic releases/A_E were also negatively related to the majority of the species, while Shoreline Agriculture/A_L had a predominantly positive relationship. Mean estuary salinity was found to be a significant predictor for 49% of modeled species.

3.2 Multi-stressor Models

Multi-stressor models for individual fish and invertebrate species were developed by performing backward variable selection on event-level variables (temp, sal, salsq, and dist) and the five candidate estuary-level stressors identified in Table 5 (see Methods section for selection criteria). This resulted in a unique multi-stressor model for each fish and invertebrate species (SM 4). For example, the model for Silver Perch (*Bairdiella chrysoura*) was:

$$\begin{aligned} P(y) = \text{logit}^{-1}(&-2.92 + 0.02*[\text{temp}] + 0.04*[\text{sal}] - .001*[\text{salsq}] \\ &- 0.05*[\text{dist}] - 0.40*[\text{EDA toxic releases}/A_E] - 0.22*[\text{Basin Crop}/Q] \\ &+ 0.29*[\text{Mean estuary salinity}] + \beta_{\text{season}} + \alpha_{\text{estuary}} + \alpha_{\text{state}} + \alpha_{\text{program}}) \end{aligned} \qquad \text{(Eq. 3)}$$

The model indicates that Silver Perch presence increases with higher temperatures and in areas closer to the shore. The relationship between salinity and Silver Perch is quadratic with an optimal value at 20 psu, which is consistent with the fact that the Silver Perch prefer higher saline estuaries. Silver Perch was negatively related to EDA toxic releases/A_E and Basin Crop/Q. Note, coefficients for event-level variables are calculated in their natural units (i.e., °C) while coefficients for estuary-level variables (e.g., EDA toxic releases/A_E, estuary salinity) are based on their normalized values (with mean of zero and standard deviation of one). Therefore, a change of 1° C at the sampling spot has an effect of 0.02 on the log odds of Silver Perch presence while the change of one standard deviation of EDA toxic releases/A_E (6.3×10^{-4} releases/km^2) represents a change of 0.40 (SM 1,4). Multi-stressor models can be used to predict changes in species presence within estuarine systems due to changes in estuary-level stressors (SM 5).

The fraction of anthropogenic (agricultural plus urban) land cover in an estuarine basin (Basin Anthropogenic/A_L) was found to be the most significant anthropogenic stressor in multi-stressor models affecting 39% of species, of which 77% showed a negative relationship (Table 6). Basin Crop/Q was related to 37% of species, with 86% of those relationships being negative. Shoreline Agriculture/A_L was related to 23% of the species with most relationships being positive, and EDA toxic releases/A_E was related to 21% of the species with most relationships being negative. Average estuary salinity was related to 25% of species as well. Mean absolute values of stressor coefficients in log odds showed that Basin Anthropogenic/A_L and Basin Crop/Q exerted the largest influence on species presence throughout the GoM (Table 6).

Table 6 Percent of species significantly related to candidate estuary-level stressors in multi-stressor models and percent of those relationships that are negative. Mean absolute change is calculated by averaging the absolute value of the slope coefficients for all 57 species.

Stressor	Species affected (%)	Negative relationships (%)	Mean absolute change (log odds)
Basin Anthropogenic/A_L	39	77	.27
Basin Crop/Q	37	86	.24
Shoreline Agriculture/A_L	23	8	.15
EDA toxic releases/A_E	21	83	.14
Mean estuary salinity	25	36	.16

3.3 Assessment of Estuary Level Anthropogenic Stress

The aggregated intensity of anthropogenic stress for each GoM estuary was combined to form an estuary stress index based on the mean absolute change in log odds of species presence, comparing current to benchmark (LDC or MDC) conditions (Fig. 2). We used the mean absolute value of both positive and negative coefficients, reflecting that increases in stress-tolerant species can indicate estuary disturbance in the same way that declining sensitive species do (Felley 1987; Caddy 2000; de Leiva Moreno et al. 2000; Chesney and Baltz 2001; Lewis et al. 2011). The estuary stress index provides a concise and quantitative way to estimate the amount of anthropogenic stress affecting each estuary, and since values are additive in log odds, the contributions of individual stressors can be observed separately (Fig. 2). Basin Anthropogenic/A_L had the largest effect on species presence in the GoM, averaging nearly 70% of total deviations, and was most prevalent in the highly urbanized southwest FL estuaries (Sarasota Bay, Charlotte Harbor, Caloosahatchee River, and Tampa Bay) along with several estuaries in the western GoM (Baffin Bay, Upper and Lower Laguna Madre, and Aransas Bay, TX and Lake Borgne and Lake Pontchartrain, LA). Basin Crop/Q and EDA toxic releases/A_E both had large effects in relatively few estuaries where those stressors had elevated values (Upper Laguna Madre, Baffin Bay, Galveston Bay, and Sabine Lake, TX and Calcasieu Lake, LA) while Shoreline Agriculture/A_L was prevalent in several TX and LA estuaries.

Anthropogenic influence was also split between positive and negative effects (Fig. 3) in order to assess in which direction watershed stressors were impacting GoM estuaries. Both positive and negative components exist for the same stressor because some of the 57 species modeled are positively related to the stressor while other species are negatively related to the same stressor (Table 6; SM 4). The magnitude of negative impacts are significantly larger for Basin Anthropogenic/A_L, Basin Crop/Q, and EDA toxic releases/A_E, while the opposite is true for Shoreline Agriculture/A_L (Fig. 3).

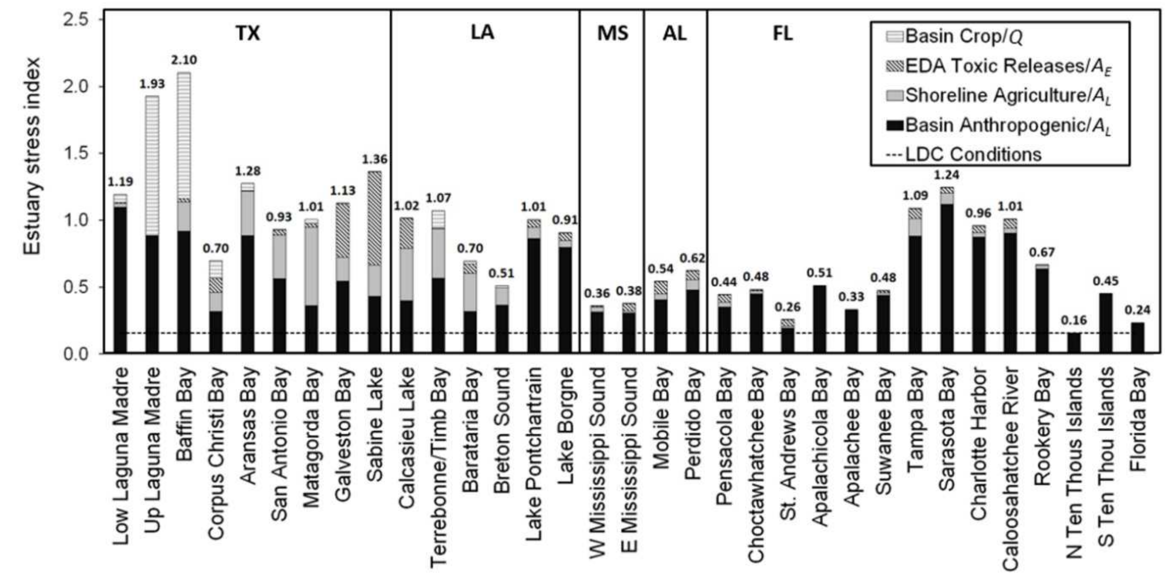


Fig. 2 Estuary stress index is the mean anthropogenic effect in logs odds based on the absolute value of model coefficients. The x-axis represents minimally disturbed conditions (MDC; all anthropogenic stressors = 0) and the dotted line represents least disturbed conditions (LDC) for the GoM (SM 1).

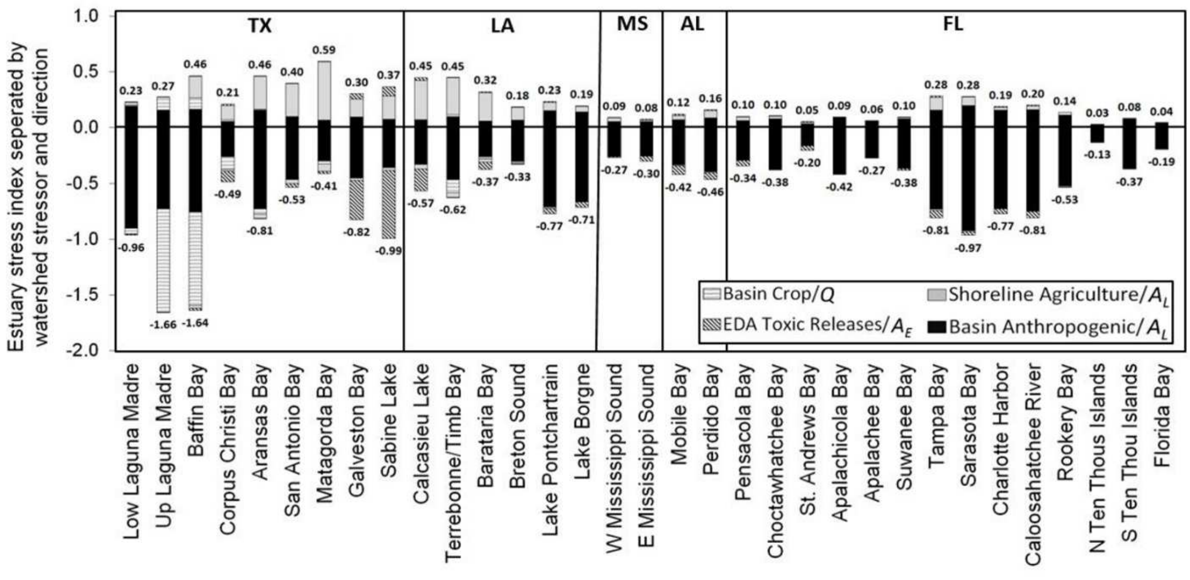


Fig. 3 Mean anthropogenic effect by estuary separated by positive and negative effects. The sum of the mean positive and mean negative effects equal the total absolute effect shown in Figure 2.

3.4 Indicator Species

Modeling individual species allowed for the identification of fish and invertebrate species whose presence had significant negative or positive relationships with anthropogenic stressors. Species with the largest negative coefficients for Basin Anthropogenic/A_L were Atlantic Moonfish (*Selene setapinnis*), Gulf Butterfish (*Peprilus burti*), Bay Whiff (*Citharichthys spilopterus*), White Shrimp (*Litopenaeus setiferus*), Blue Catfish (*Ictalurus furcatus*), Bluntnose Jack (*Hemicaranx amblyrhynchus*), Sea Bass (*Centropristis philadelphica*) and Fringed Flounder (*Etropus crossotus*) (SM 4). Species with large negative relationships with EDA toxic releases/A_E were Gulf and Chain Pipefish (*Syngnathus scovelli, Syngnathus lousianae*), Pink Shrimp (*Farfantepenaeus duorarum*), Atlantic Threadfin Herring (*Opisthonema oglinum*), Atlantic Stingray (*Dasyatis sabina*), Blackcheek Tonguefish (*Symphurus plagiusa*), Silver Perch, and Blue Crab (*Callinectes sapidus*). Several species had consistently positive responses to anthropogenic stressors including: Black Drum (*Pogonias cromis*), Gafftopsail Catfish (*Bagre marinus*), Hardhead Catfish (*Ariopsis felis*), White and Striped Mullet (*Mugil curema, Mugil cephalus*), and Lined Sole (*Achirus lineatus*).

3.5 Random Effects and Seasonality

State random effects were included to account for the potential impact of state-level fishing regulations on species presence, as well as large-scale natural variations in habitat that may occur at the state level. In addition, program random effects were included to account for variation among different trawl programs (efficiency and effort). State and program random effects were averaged across all species (Fig. 4). Mean state effects ranged from -0.3 (FL) to 0.4 (TX) and program effects vary from -0.8 (MS) to 0.6 (AL) suggesting substantial variability in the effects of different program sampling methods on the probability of species presence in trawl events. Seasonal effects were included as categorical variables. Fall had the highest mean effect while mean summer, spring, and winter offsets were 0.38, 0.36, and 0.38 lower (on log odds scale), respectively. It is important to note that event-level temperature and salinity measurements were also included as predictors in the model, so that these seasonal effects represent variability that exists in addition to seasonal patterns in temperature and salinity.

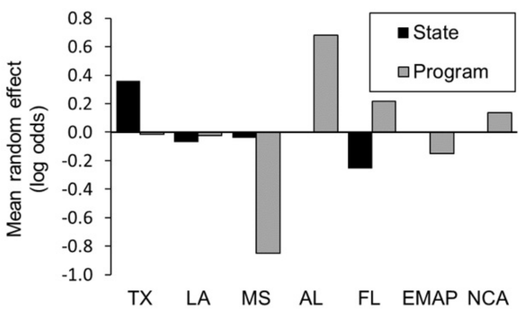


Fig. 4 Mean state and program random effects across all fish and invertebrate species.

The magnitudes of estuary-level random effects were determined by averaging across all species (Fig. 5). The relatively high random effects observed in estuaries such as Aransas Bay, San Antonio Bay, TX, Terrebonne/Timbalier Bays, LA, and Apalachicola Bay, FL point to estuaries where species presence was higher than would be expected based on the predictors and other random effects included in the models. Similarly, estuaries such as Matagorda Bay, TX and Breton Sound, LA had a lower probability of presence than would be expected. In such estuaries, other biophysical or watershed factors that were unaccounted for by this analysis may be affecting habitat quality.

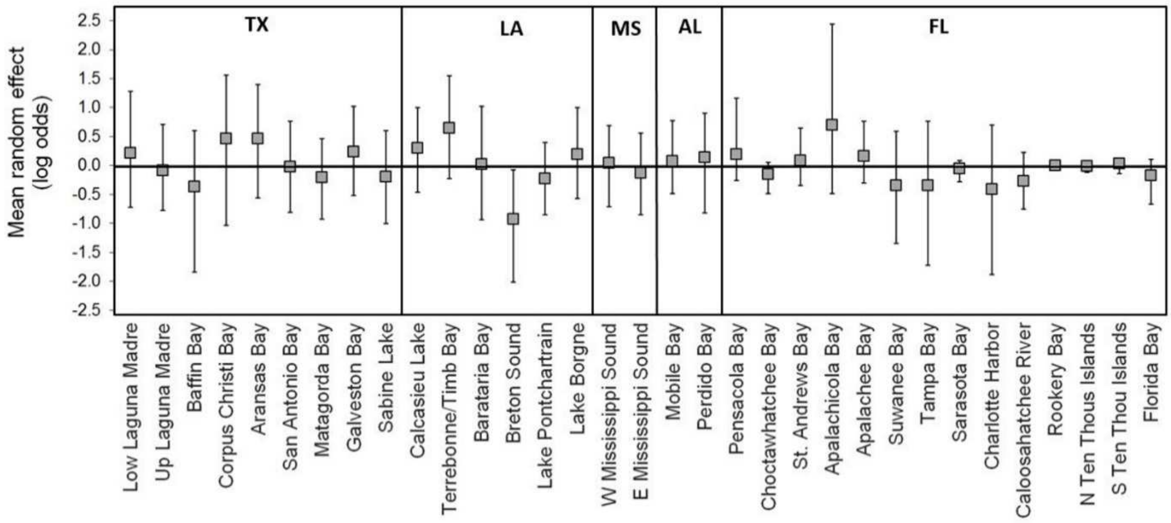


Fig. 5 Estuary random effects. Grey square indicates the mean (across species) and error bars show the 0.1 and 0.9 quantiles. Positive random effects imply species presence was under predicted by the other components of the model. Negative random effects imply the opposite. Rookery Bay does not have an estuary random effect because it had no trawl samples.

4. Discussion

4.1 Anthropogenic Stressors

Our study contrasts with previous biological assessment efforts that sought to characterize biological condition, but which were not designed to quantitatively relate that condition to watershed anthropogenic stressors (Deegan et al., 1997; Macauley et al., 1999; Summers, 2001; Hughes et al., 2002; Meng et al., 2002; Harrison and Whitfield, 2004, 2006; Breine et al., 2007; Coates et al., 2007; Uriarte and Borja, 2009; Breine et al., 2010; Delpech et al., 2010; Jordan et al., 2010; Cabral et al., 2012). Previous work in the U.S. (e.g., Greene et al., 2015) has characterized the magnitudes of anthropogenic stressors in estuarine watersheds, but there has been limited progress toward quantitatively linking these stressors to the biological condition of estuaries. In Europe, Teichart et al. (2016) used ecology quality ratios (EQR) scores from seven European countries to determine water quality stressors that affect estuarine biological condition.

Though this was a step toward linking biological condition to watershed stressors, there are still questions about how comparable EQR ratios are among countries (Poikane et al., 2014) and basin-level stressors were not explored. In this study, we demonstrate a linkage between watershed stressors at three spatial scales (basin, EDA, and shoreline) and estuarine biological condition using regression models that relate watershed stressors to the log odds of species presence while accounting for natural variation and other potentially confounding effects (e.g., systematic biases associated with different sampling programs). We also develop a quantitative index of estuary stress using mean stressor coefficients (averaged across species) and the variability in stressors across estuarine watersheds (Figs. 2, 3). We found widespread and largely negative influences of landscape stressors on both fish and invertebrate species. Our results suggest that estuaries east of the Mississippi delta to the Florida panhandle are, in general, less impacted by watershed stress than other systems in the GoM. Our indicator screening process also provides new insight into species-level responses to estuary stressors, revealing species indicators on both the tolerant and sensitive sides of the 'stress-tolerance gradient' (Whittier et al. 2007).

Our results indicate that human activities in upstream watersheds have statistically significant and largely negative relationships on a substantial portion of estuarine fish and invertebrate species. The percent of anthropogenic land cover in river basins (Basin Anthropogenic/A_L) has the strongest relationship, affecting 22 species (17 negatively) in the multi-stressor models (Table 6) including five of the ten most prevalent species in the GoM (SM 4). It accounts for nearly 70% of all absolute deviations from MDC (Fig. 3). The "Anthropogenic" land category is a composite of urban, crop, and pasture, and it generally outperformed the individual land categories in terms of predictive performance (Table 5). Nonetheless, it is interesting to consider whether one of the three land cover classes (urban, crop and pasture) plays a dominant role. The high ranking of crop and agriculture in single stressor models (Table 5) coupled with the fact that agriculture comprises 69% of anthropogenic land use within our study area, suggests that it is perhaps the dominant form of land cover leading to estuary disturbance. To further explore this, we repeated the single-stressor modeling three times, omitting estuaries in FL, TX, and the central Gulf states (LA, MS, and AL), in turn, as a simple sensitivity analysis. Basin Anthropogenic/A_L remained the top stressor when both the central state estuaries and the TX estuaries were omitted, but Basin Crop/A_L became the top stressor when FL estuaries were omitted. The increased importance of Basin Crop/A_L when Florida estuaries are excluded implies that crop production might be more influential in the western GoM. Of note, the categorical variables representing hypoxic, toxic algal, and eutrophic condition estimates (Bricker et al. 2008) were not found to be significant predictors in single-stressor models, suggesting these metrics, as calculated and used in this study, are not primary drivers of species presence in GoM estuaries.

Basin Crop/Q, reflecting crop areas normalized by average flow rate, is significantly related to 21 species (18 negatively). This stressor is likely a proxy for the concentration of crop-related pollutants entering a system. Basin Crop/Q was highest in Baffin Bay and Upper Laguna Madre, two hyper-saline southern TX estuaries, and elevated in Corpus Christi Bay, TX and

Terrebonne/Timbalier Bays, LA as well (SM 1). These results imply that as estuary flushing decreases, estuaries become more susceptible to pollution. Hydrologic alteration as well as consumptive water use by urban and agricultural sources can reduce water inflow to estuaries and the dilution of contaminants in surface water (Harwell, 1997; Day et al., 2000; Flannery et al., 2002).

The number of toxic release sites in the estuarine drainage area (EDA) normalized by the estuary area is significantly related to 12 species (10 negatively). Five of the negative relationships are with species associated with bottom environments (e.g., Atlantic Stingray, Blue Crab, Pink Shrimp, Blackcheek Tonguefish, and Hog Choker (*Trinectes maculatus*); SM 4). Galveston Bay and Sabine Lake, TX and Calcasieu Lake, LA have the highest historical levels of toxic releases, potentially due to upstream petrochemical plants (USEPA 2015; Blackburn 2015). Our findings are consistent with previous research reporting measurable negative effects of toxic contamination and releases on estuarine organisms, particularly in the benthos (Pearson and Rosenberg, 1978; Boesch and Rosenberg, 1981; Malins et al., 1985; Engle et al., 1994; Macauley et al., 1999; Brown et al., 2000).

The percent of the estuarine shoreline buffer comprised of agricultural land cover is the only anthropogenic stressor identified to be predominantly associated with an increase in species presence. It is significantly related to 13 species; 12 of these positively. Estuaries with shoreline agriculture greater than 10% are generally found in TX and LA (SM 1). One possible explanation for this finding is a positive response to incipient stress as observed in other estuary studies (Jordan and Vaas, 2000; Jordan and Smith, 2005). A possible mechanism for a positive response to stress could be greater productivity and diversity due to nutrient enrichment (i.e., a release from oligotrophy; Nixon and Buckley, 2002), or by a combination of stressors that has not reached a threshold for causing negative effects (Jordan et al., 2010). Alternatively, long-term effects of nutrient enrichment (and overfishing) have been shown to lead to the dominance of pelagic over benthic/demersal species (Caddy, 2000; de Leiva Moreno et al., 2000). Nine of the 12 species positively linked to shoreline percent agriculture are pelagic species (e.g., Gulf Menhaden, Atlantic Bumper (*Chloroscombrus chrysurus*), Ground Mullet (*Menticirrhus americanus*), and White Mullet; SM 4) and two more are catfish species which thrive in high nutrient waters (*Ariopsis felis, Bagre marinus).* More investigation into the positive relationship between shoreline agriculture and the presence of several species is warranted.

4.2 Assessment of Estuary Level Anthropogenic Stress

We adopted both MDC and LDC as possible benchmarks against which to compare estuary disturbance in order to demonstrate how our index of estuary stress could be used with both; however, each of these potential benchmarks have limitations. MDC requires a somewhat greater degree of model extrapolation, while LDC is not referenced to an absolute ecological state making it susceptible to shifting baselines as watersheds continue to develop over time (Pauly, 1995). LDC conditions, as defined in this study, do not deviate greatly from MDC (0.16 change in log odds; Fig. 2). LDC for both Shoreline Agriculture/A_L and EDA toxic releases/A_E were zero (i.e., equal to MDC) while LDC for Basin Crop/*Q* is essentially the same as MDC. The minimum LDC value for Basin Anthropogenic/A_L was 8.9% and this stressor explains the majority of the difference between MDC and LDC. The application of LDC across such a large system as the GoM might be debatable (Stoddard et al., 2006); however, comparing the MDC and LDC benchmarks in Fig. 2 suggest the overall disturbance pattern indicated by our results should be fairly robust to small changes in benchmark assumptions. Estuary rankings essentially remain the same whether they are based on deviation from LDC or MDC conditions since both benchmarks are constant across the GoM and would only change if sub-regional benchmarks are developed on the assumption that baseline conditions vary by region. Another approach that might lead to different estuary ranks would be if the estuary stress index was defined as only the negative (i.e., negative component in Fig. 3) or the net effect of watershed level stressors (i.e., the difference between the positive and negative components in Fig. 3) for specific subsets of species of interest. If system productivity (i.e., more fish for anglers) is of interest, then an estuary stress index could be created specifically for species of interest for commercial and recreational fisherman. However, both of these alternatives ignore the value of stress-tolerant species as potential indicators of anthropogenic influence (see below; Whittier et al., 2007).

4.3 Indicator Species

The estuary stress index proposed here is based on the responses of widely distributed fish and invertebrate species to different landscape anthropogenic stressors that provide insights into important regional indicator species. Forty-eight of the 57 species studied are significantly related to at least one anthropogenic stressor in the multi-stressor models (SM 4). While the overwhelming direction of response is negative, responses of species with apparent stress tolerance (i.e., those that responded positively to increasing stressor levels) are also present (see Section 3.4). Tolerant species tend to have greater behavioral and diet plasticity, greater niche breadth and may benefit from the absence of more sensitive competitors (Vázquez and Simberloff, 2002; Swihart et al., 2003; Devictor et al., 2008; Wilson et al., 2008; Segurado et al., 2011).

4.4 Hierarchical Modeling

The ability to model variability at multiple scales (i.e., estuaries, states, programs) is an important advantage of hierarchical modeling in this application. Critically, it allowed us to perform statistically valid hypothesis testing concerning which watershed anthropogenic stressors are significantly related to species presence. The hierarchical approach can be contrasted with more traditional “no pooling” and “complete pooling” approaches to regression modeling (Gelman and Hill, 2006; Qian et al., 2010; Cuffney et al., 2011). If we had developed independent models (or parameter estimates) for each estuary (no pooling), then it would be infeasible to statistically assess the significance of stressors acting across multiple estuaries. On the other hand, if all data were combined into a single model, but random effects were omitted, then we would be treating each trawl sample as statistically independent, failing to address the substantial intraclass correlation that exists among samples from the same estuary, state, and program (Gelman et al., 2014). As an example, single-stressor models (Eq. 2) were run omitting the estuary random effect ($\alpha_{estuary}$) and 40 of the 47 predictor variables listed in Table 4 were significant for over 80% of species; an implausible result related to the failure to account for intraclass correlation.

In addition, random effects allow us to characterize variability over different scales (i.e., sample, estuary, state, and programs) (Wikle, 2003a; Cressie et al., 2009). For example, program-level random effects allow us to assess the variability associated with differences in sampling protocols. The MS program only recorded 23 of the 57 modeled species, resulting in a much lower mean program random effect when compared to other state and federal programs (Fig. 4). State random effects are also of interest, with TX having the highest state random effect and FL having the lowest, though the variation across states was less than the variation across programs (Fig. 4). Exploring the causes of state-level variation could be a subject for future research, as this variation could plausibly be related to either state fishing regulations, biogeography, or other factors that vary over a large spatial scale. The ability to discern between state and program level random effects is critical, as state random effects reflect actual differences in species prevalence, whereas program random effects reflect sampling efficiency. Distinguishing between state and program random effects within the model was only possible because of the two federal interstate programs (EMAP and NCA), and this is an important reason to continue these federal trawl programs in the future.

4.5 Summary and Future Directions

This study identifies watershed anthropogenic stressors that are most strongly associated with fish and invertebrate species presence, and then used the modeled stressor relationships to develop an index of watershed stress for estuaries across the GoM. The hierarchical generalized linear model used here provides a tool to control for variability that occurs among programs, estuaries, and states, as well as among individual trawl samples. A large percentage of GoM species exhibited negative associations with anthropogenic stressors, but some positive associations were present as well. The percent anthropogenic land cover (urban, crop, and pasture) within an estuary's drainage basin was consistently ranked as a leading predictor of estuary disturbance. When determining deviations from benchmark conditions (Fig. 2), we implicitly assumed that the statistically significant predictor variables identified through hierarchical modeling are, in fact, drivers of species presence/absence. However, the empirical relationships developed in the single and multi-stressor models presented here do not directly demonstrate causal relationships, as it is well known that correlation is not necessarily causation. The results do, however, provide a data-supported line of evidence relevant to large-scale estuarine assessments and fisheries management in the GoM. Past studies have found negative effects of nitrogen loading, euthrophication, and urbanization on estuary species (Dauer et al., 2000, Diaz and Rosenberg, 2008; Coll et al., 2010), but our results are perhaps the most compelling evidence to date for widespread population-level effects on fish and invertebrate habitats in GoM estuaries by watershed stressors, given the comprehensiveness of the datasets used and the spatial extent of the study. We expect this study to motivate and focus future research efforts to understand the mechanisms by which watershed stressors affect estuary habitats. Our findings may also be useful to help guide and prioritize watershed restoration activities.

While the current study focuses on species-level responses, other studies have focused on the responses of biological assemblages (i.e., metrics of community composition, structure, and function) to regional stressor gradients (Lewis et al., 2007; Piazza and La Peyre, 2009). Acknowledging the potential advantages to species-specific indicators (see Introduction), our approach could be extended to consider the presence/absence of species functional groups that share common habitat, life history, or feeding strategies. Modeling functional groups may help corroborate the patterns documented here and provide further insights into the mechanisms by which landscape stressors act on biological communities. Further, modeling functional groups could facilitate comparison of results to previous studies that have relied on community metrics for estuarine biological assessment (Engle et al., 1994; Summers, 2001; Hughes et al., 2002; Jenkins, 2004; Jordan et al., 2010).

This hierarchical approach could be extended to consider species abundance, which is related (imperfectly) to the number of individuals of a given species collected in a trawl event. An assessment of how species abundance responds to anthropogenic stressors could also be valuable, particularly for species of social or economic importance. Many of the species found to have sensitivities to stressors in this study are also targeted by the regional commercial fishery (including shrimps, Gulf Menhaden, and Blue Crab), and species that are heavily targeted in recreational fisheries (Spotted Seatrout, Sand Seatrout (*Cynoscion arenarius*), Silver Seatrout (*Cynoscion nothus*), Ground Mullet, and Atlantic Croaker (*Micropogonias undulates*); NMFS, 2014). A natural extension of this research is to focus on the responses of economically valuable species in terms of both their probabilities of occurrence and abundances.

Finally, the hierarchical modeling approach developed in this study could be enhanced to consider additional spatial and temporal variability. Our approach does consider event-level variables like temperature and salinity that explain some intra-estuary variability, but which cannot be directly related to stressors. Like other studies before ours (Jordan et al., 2010), our assessment is not capable of estimating variation in biological condition at a sub-estuary scale. If trawl samples could be organized into different estuarine subsections (perhaps based on estuary geomorphology), then species presence could be related to more localized stressors associated with shoreline development. Shoreline hardening associated with sea walls has been shown to reduce both richness and abundance of species in near shore areas (Peterson and Lowe 2009; Gittman et al. 2016; Dethier et al. 2016), but precise estuary-level estimates of shoreline hardening were not available for this study (Gittman et al. 2015). Regarding temporal variability, future analyses could explore how species presence changes over time, with alterations in watershed development and basin flows as potential predictor variables. A better understanding of temporal trends in estuary condition could further prioritize watershed management and restoration activities.

Acknowledgements

This work would not have been possible without the contributions of Correigh Greene, Steve Brown, Harmon Brown (NOAA NMFS), Tom Minello (NOAA retired), Kirsten Larsen (NOAA NESDIS), and Moe Nelson (NOAA NOS). The Southeast Aquatic Resources Partnership Science and Data Committee provided important guidance. Hiroo Imaki, Amy Drohan, Bethany Craig, Joshua Simms, and Michelle Zapp-Sluis were instrumental in dataset assembly and creation, and data were generously provided by Florida Fish and Wildlife Conservation Commission, Alabama Department of Conservation and Natural Resources, Louisiana Department of Wildlife and Fisheries, Mississippi Department of Marine Resources, and Texas Parks & Wildlife Department. Justin Davenport and Alexey Katin of NCSU also provided important technical assistance with results processing and figures. We also thank Song Qian and Yoon Kyung Cha for their methodological input during the early phases of this work.

Funding: This work was supported by the National Oceanic and Atmospheric Administration [grant numbers NA14OAR4170299, NA15OAR4170215] and North Carolina State University.

References

Adams C.M., E. Hernandez, and J.M. Cato. 2004. The economic significance of the Gulf of Mexico related to population, income, employment, minerals, fisheries and shipping. Ocean & Coastal Management 47(11): 565-580.

Akin, S., K.O. Winemiller, and F.P. Gelwick. 2003. Seasonal and spatial variations in fish and macrocrustacean assemblage structure in Mad Island Marsh estuary, Texas. Estuarine, Coastal and Shelf Science 57(1): 269-282.

Allison, P. D. 2012. Logistic regression using SAS: Theory and application. SAS Institute.

Baltz, D., C. Rakocinski, and J. Fleeger. 1993. Microhabitat use by marsh-edge fishes in a Louisiana estuary. Environmental Biology of Fishes 36(2): 109-126.

Baker, M.E., and R.S. King. 2010. A new method for detecting and interpreting biodiversity and ecological community thresholds. Methods in Ecology and Evolution 1(1): 25-37.

Barber, M. (ed.). 1994. Environmental Monitoring and Assessment Program indicator development strategy: Project summary. US Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratory.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effect models using lme4. Journal of Statistical Software. doi:10.18637/jss.v067.i01.

Blackburn, J. B. 2015. The Book of Texas Bays (Vol. 6). Texas A&M University Press.

Bolker, B. M. 2008. Ecological models and data in R. Princeton University Press.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., and J.S.S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in ecology & evolution. 24(3): 127-135.

Boesch, D.F., and R. Rosenberg. 1981. Response to stress in marine benthic communities. In Stress effects on natural ecosystems, ed. G.W. Barrett and R. Rosenberg, 179-200. New York: John Wiley & Sons.

Breine, J.J., J. Maes, P. Quataert, E. Van den Bergh, I. Simoens, G. Van Thuyne, and C. Belpaire. 2007. A fish-based assessment tool for the ecological quality of the brackish Schelde estuary in Flanders (Belgium). Hydrobiologia 575(1):141-159.

Breine, J., P. Quataert, M. Stevens, F. Ollevier, F.A. Volckaert, E. Van den Bergh, and J. Maes. 2010. A zone-specific fish-based biotic index as a management tool for the Zeeschelde estuary (Belgium). Marine Pollution Bulletin 60(7): 1099-1112.

Brett, J.R., and T.D.D. Groves. 1979. Physiological energetics. Fish physiology 8: 279-352.

Bricker, S.B., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2008. Effects of nutrient enrichment in the nation's estuaries: A decade of change. Harmful Algae 8(1): 21-32.

Brown, S.S., G.R. Gaston, C.F. Rakocinski, and R.W. Heard. 2000. Effects of sediment contaminants and environmental gradients on microbenthic community trophic structure in Gulf of Mexico estuaries. Estuaries 23(3): 411-424.

Cabral, H.N., V.F. Fonseca, R. Gamito, C.I. Gonçalves, J.L. Costa, K. Erzini, J. Gonçalves, J. Martins, L. Leite, J.P. Andrade, and S. Ramos. 2012. Ecological quality assessment of transitional waters based on fish assemblages in Portuguese estuaries: The Estuarine Fish Assessment Index (EFAI). Ecological Indicators 19: 144-153.

Caddy, J. F. 2000. Marine catchment basin effects versus impacts of fisheries on semi-enclosed seas. *ICES Journal of marine Science*, *57*(3): 628-640.

Calaway, R., Revolution Analytics, S. Weston, and D. Tenenbaum. 2015. ‘Foreach’ Parallel Adaptor for the 'parallel' Package. CRAN. https://cran.r-project.org/web/packages/doParallel/index.html.

Chapra, S.C. 2008. Surface water-quality modeling. Waveland press.

Chesney, E.J., and D.M. Baltz. 2001. The effects of hypoxia on the northern Gulf of Mexico coastal ecosystem: A fisheries perspective. In Coastal hypoxia: Consequences for living resources and ecosystems, ed. N.N. Rabalais and R.E. Turner, 321-354. Washington: American Geophysical Union.

Clark, J. S. and A.E. Gelfand. 2006. A future for models and data in environmental science. Trends in Ecology & evolution, 21(7): 375-380.

Cressie, N., C.A. Calder, J.S. Clark, J.M.V. Hoef, and C.K. Wikle. 2009. Accounting for uncertainty in ecological analysis: The strengths and limitations of hierarchical statistical modeling. Ecological Applications 19(3): 553-570.

Coates, S., A. Waugh, A. Anwar, and M. Robson. 2007. Efficacy of a multi-metric fish index as an analysis tool for the transitional fish component of the Water Framework Directive. Marine Pollution Bulletin 55(1): 225-240.

Cohen, J. 1990. Things I have learned (so far). American psychologist, 45(12): 1304.

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Lasram, F. B. R., Aguzzi, J. and R. Danovaro. 2010. The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. *PloS one*, *5*(8), e11842.

Courrat, A., Lobry, J., Nicolas, D., Laffargue, P., Amara, R., Lepage, M., ... and O. Le Pape. 2009. Anthropogenic disturbance on nursery function of estuarine areas for marine species. *Estuarine, Coastal and Shelf Science*, *81*(2): 179-190.

Cuffney, T., R. Kashuba, S. Qian, I. Alameddine, Y. Cha, B. Lee, J. Coles, and G. McMahon. 2011. Multilevel regression models describing regional patterns of invertebrate and algal responses to urbanization across the USA. Journal of the North American Benthological Society 30(3): 797-819.

Dauer, D.M., J.A. Ranasinghe, and S.B. Weisberg. 2000. Relationships between benthic community condition, water quality, sediment quality, nutrient loads, and land use patterns in Chesapeake Bay. Estuaries 23(1): 80-96.

Day, J.W., L.D. Britsch, S.R. Hawes, G.P. Shaffer, D.J. Reed, and D. Cahoon. 2000. Pattern and process of land loss in the Mississippi Delta: A spatial and temporal analysis of wetland habitat change. Estuaries 23(4): 425-438.

de Leiva Moreno, J. I., Agostini, V. N., Caddy, J. F., and F. Carocci. 2000. Is the pelagic-demersal ratio from fishery landings a useful proxy for nutrient availability? A preliminary data exploration for the semi-enclosed seas around Europe. ICES Journal of Marine Science: Journal du Conseil, 57(4): 1091-1102.

Deegan, L.A., J.T. Finn, S.G. Ayvazian, C.A. Ryder-Kieffer, and J. Buonaccorsi. 1997. Development and validation of an estuarine biotic integrity index. Estuaries *20*(3): 601-617.

Delpech, C., A. Courrat, S. Pasquaud, J. Lobry, O. Le Pape, D. Nicolas, P. Boët, M. Giradin, and M. Lepage. 2010. Development of a fish-based index to assess the ecological quality of transitional waters: The case of French estuaries. Marine Pollution Bulletin 60(6): 908-918.

Derksen, S. and H.J. Keselman. 1992. Backward, forward and stepwise automated subset selection algorithms: Frequency of obtaining authentic and noise variables. British Journal of Mathematical and Statistical Psychology, 45(2): 265-282.

Dethier, M. N., Raymond, W. W., McBride, A. N., Toft, J. D., Cordell, J. R., Ogston, A. S., Heerhetz, S.M. and H.D. Berry. 2016. Multiscale impacts of armoring on Salish Sea shorelines:

evidence for cumulative and threshold effects. Estuarine, Coastal and Shelf Science 175: 106-117.

Devictor, V., R. Julliard, and F. Jiguet. 2008. Distribution of specialist and generalist species along spatial gradients of habitat disturbance and fragmentation. Oikos 117: 507–514.

Diaz, R.J., and R. Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. Science 321(5891): 926-929.

Díaz, R. J. and R. Rosenberg. 2011. Introduction to environmental and economic consequences of hypoxia. International journal of water resources development, 27(1): 71-82.

Engle, V.D., J.K. Summers, and G.R. Gaston. 1994. A benthic index of environmental condition of Gulf of Mexico estuaries. Estuaries 17(2): 372-384.

Felley, J.D. 1987. Nekton assemblages of three tributaries to the Calcasieu Estuary, Louisiana. Estuaries 10(4): 321-329.

Flannery, M.S., E.B. Peebles, and R.T. Montgomery. 2002. A percent-of-flow approach for managing reductions of freshwater inflows from unimpounded rivers to southwest Florida estuaries. Estuaries 25(6): 1318-1332.

Fry, F.E.J. 1971. The effect of environmental factors on the physiology of fish. Fish Physiology 6: 1-98.

Gelfand, A.E., A.M. Schmidt, S. Wu, J.A. Silander, A. Latimer, and A.G. Rebelo. 2005. Modelling species diversity through species level hierarchical modelling. Journal of the Royal Statistical Society: Series C (Applied Statistics) 54(1): 1-20.

Gelman, A., and J. Hill. 2006. Data analysis using regression and multilevel/hierarchical models. New York: Cambridge University Press.

Gelman, A., J.B. Carlin, H.S. Stern, and D.B. Rubin. 2014. Bayesian data analysis (Vol. 2). Boca Raton: Chapman & Hall/CRC.

Gelwick, F.P., S. Akin, D.A. Arrington, and K.O. Winemiller. 2001. Fish assemblage structure in relation to environmental variation in a Texas Gulf coastal wetland. Estuaries 24:285-296.

Gittman, R. K., Fodrie, F. J., Popowich, A. M., Keller, D. A., Bruno, J. F., Currin, C. A., Peterson, C.H., and M.F. Piehler. 2015. Engineering away our natural defenses: an analysis of shoreline hardening in the US. Frontiers in Ecology and the Environment, 13(6): 301-307.

Gittman, R. K., Scyphers, S. B., Smith, C. S., Neylan, I. P., and J.H. Grabowski. 2016. Ecological consequences of shoreline hardening: a meta-analysis. BioScience, biw091.

Gleason, M. G., Newkirk, S., Merrifield, M. S., Howard, J., Cox, R., Webb, M., ... and R. Fuller. 2011. *A conservation assessment of West Coast (USA) estuaries*. The Nature Conservancy.

Granados-Dieseldorff, P., and D.M. Baltz. 2008. Habitat use by nekton along a stream-order gradient in a Louisiana estuary. Estuaries and Coasts 31: 572-583.

Greene, C.M., K. Blackhart, J. Nohner, A. Candelmo, and D.M. Nelson. 2015. A national assessment of stressors to estuarine fish habitats in the contiguous USA. Estuaries and Coasts 38(3): 782-799.

Guisan, A., and N.E. Zimmermann. 2000. Predictive habitat distribution models in ecology. Ecological Modelling 135(2): 147-186.

Guisan, A., T.C. Edwards, and T. Hastie. 2002. Generalized linear and generalized additive models in studies of species distributions: Setting the scene. Ecological Modelling 157(2): 89-100.

Harrison, T.D., and A.K. Whitfield. 2004. A multi-metric fish index to assess the environmental condition of estuaries. Journal of Fish Biology 65(3): 683-710.

Harrison, T.D., and A.K. Whitfield. 2006. Application of a multimetric fish index to assess the environmental condition of South African estuaries. Estuaries and Coasts 29(6): 1108-1120.

Harrison, T. D. and F.L. Kelly. 2013. Development of an estuarine multi-metric fish index and its application to Irish transitional waters. *Ecological indicators* 34:494-506.

Harwell, M.A. 1997. Ecosystem management of south Florida. BioScience 47: 499-512.

Hawkins, C.P., J.R. Olson, and R.A. Hill. 2010. The reference condition: Predicting benchmarks for ecological and water-quality assessments. Journal of the North American Benthological Society 29(1): 312-343.

Homer, C., J. Dewitz, J. Fry, M. Coan, N. Hossain, C. Larson, N. Herold, A. McKerrow, J.N. VanDriel, and J. Wickham. 2007. Completion of the 2001 National Land Cover Database for the Conterminous United States. Photogrammetric Engineering and Remote Sensing 73(4): 337-341.

Howarth, R.W., and R. Marino. 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. Limnology and Oceanography 51(1): 364-376.

Hughes, B. B., Levey, M. D., Brown, J. A., Fountain, M. C., Carlisle, A. B., Litvin, S. Y., ... and M.G. Gleason. 2014. *Nursery functions of US West Coast estuaries: the state of knowledge for juveniles of focal invertebrate and fish species*. Arlington, Virginia: Nature Conservancy.

Hughes, J., L. Deegan, M. Weaver, and J. Costa. 2002. Regional application of an index of estuarine biotic integrity based on fish communities. Estuaries and Coasts 25: 250-263.

Jenkins, J.A. 2004. Fish bioindicators of ecosystem condition at the Calcasieu Estuary, Louisiana. USGS Open-File Report 2004-1323.

Jordan, S.J., and P.A. Vaas. 2000. An index of ecosystem integrity for Northern Chesapeake Bay. Environmental Science & Policy (3): 59-88.

Jordan, S.J., and L.M. Smith. 2005. Indicators of ecosystem integrity for estuaries. Estuarine Indicators. Boca Raton: CRC Press.

Jordan, S.J., M.A. Lewis, L.M. Harwell, and L.R. Goodman. 2010. Summer fish communities in northern Gulf of Mexico estuaries: Indices of ecological condition. Ecological Indicators 10: 504-515.

Kashuba, R., Y. Cha, I. Alameddine, B. Lee, and T.F. Cuffney. 2010. Multilevel hierarchical modeling of benthic macroinvertebrate responses to urbanization in nine metropolitan regions across the conterminous United States. USGS Scientific Investigations Report 2009-5243.

King, R.S., and M.E. Baker. 2010. Considerations for analyzing ecological community thresholds in response to anthropogenic environmental gradients. Journal of the North American Benthological Society 29(3): 998-1008.

Latimer, A.M., S. Wu, A.E. Gelfand, and J.A. Silander. 2006. Building statistical models to analyze species distributions. Ecological applications 16(1): 33-50.

Lewis, M., S. Jordan, C. Chancy, L. Harwell, L. Goodman, and R. Quarles. 2007. Summer fish community of the coastal northern Gulf of Mexico: Characterization of a large-scale trawl survey. Transactions of the American Fisheries Society 136(3): 829-845.

Lewis, M.A., L.R. Goodman, C.A. Chancy, and S.J. Jordan. 2011. Fish assemblages in three northwest Florida urbanized bayous before and after two hurricanes. Journal of Coastal Research 27(1): 35-45.

Macauley, J.M., J.K. Summers, and V.D. Engle. 1999. Estimating the ecological condition of the estuaries of the Gulf of Mexico. Environmental Monitoring and Assessment 57: 59-83.

Malins, D. C., Krahn, M. M., Myers, M. S., Rhodes, L. D., Brown, D. W., Krone, C.A., McCain, B.B. and S.L. Chan. 1985. Toxic chemicals in sediments and biota from a creosote-polluted harbor: relationships with hepatic neoplasms and other hepatic lesions in English sole (Parophrys vetulus). Carcinogenesis, 6(10): 1463-1469.

Meng, L., C.D. Orphanides, and J.C. Powell. 2002. Use of a fish index to assess habitat quality in Narragansett Bay, Rhode Island. Transactions of the American Fisheries Society 131(4): 731-742.

Nixon, S.W., and B.A. Buckley. 2002. “A strikingly rich zone”: Nutrient enrichment and secondary production in coastal marine ecosystems. Estuaries 25: 782-796.

NMFS (National Marine Fisheries Service). 2014. Fisheries Economics of the United States, 2012. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-F/SPO-137.

NRC (Natural Resource Council). 2000. Clean coastal waters: Understanding and reducing the effects of nutrient pollution. Washington: National Academies Press.

NOAA (National Oceanic and Atmospheric Administration). 2006. Costal Change Analysis Program regional land cover [GIS files]. http://coast.noaa.gov/ccapatlas/.

NOAA/NOS (National Oceanic and Atmospheric Administration/National Ocean Service). 1985. National Estuarine Inventory Data Atlas, Volume 1: Physical and Hydrologic

Characteristics. Ocean Assessments Division, National Ocean Service, National Oceanic and Atmospheric Administration. Rockville, Maryland. pp. 103.

Pasquaud, S., Courrat, A., Fonseca, V. F., Gamito, R., Gonçalves, C. I., Lobry, J., ... & H. Cabral. 2013. Strength and time lag of relationships between human pressures and fish-based metrics used to assess ecological quality of estuarine systems. *Estuarine, Coastal and Shelf Science* 134: 119-127.

Pauly, D. 1995. Anecdotes and the shifting baseline syndrome of fisheries. Trends in Ecology & Evolution 10(10): 430.

Pearson, T.H., and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology Association Review 16: 229-311.

Peduzzi, P., Concato, J., Kemper, E., Holford, T. R., and A. R. Feinstein. 1996. A simulation study of the number of events per variable in logistic regression analysis. Journal of clinical epidemiology, 49(12): 1373-1379.

Peterson, M.S. and S.T. Ross. 1991. Dynamics of littoral fishes and decapods along a coastal river-estuarine gradient. Estuarine, Coastal and Shelf Science 33: 467-483.

Peterson, M. S. and M.R. Lowe. 2009. Implications of cumulative impacts to estuarine and marine habitat quality for fish and invertebrate resources. Reviews in Fisheries Science, 17(4): 505-523.

Piazza, B.P., and M.K. La Peyre. 2009. The effect of Hurricane Katrina on nekton communities in the tidal freshwater marshes of Breton Sound, Louisiana, USA. Estuarine, Coastal and Shelf Science 83(1): 97-104.

Poikane, S., Zampoukas, N., Borja, A., Davies, S. P., van de Bund, W., & S. Birk. 2014. Intercalibration of aquatic ecological assessment methods in the European Union: Lessons learned and way forward. *Environmental Science & Policy* 44: 237-246.

Pritchard, D.W. 1967. What is an estuary: Physical viewpoint. In Estuaries, ed. G.H. Lauf, 3-5. Washington: AAAS Publications.

Qian, S.S., T.F. Cuffney, I. Alameddine, G. McMahon, and K.H. Reckhow. 2010. On the application of multilevel modeling in environmental and ecological studies. Ecology 91(2): 355-361.

R Development Core Team. 2011. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. [online] http://www.R-project.org.

Rabalais, N.N., R.E. Turner, Q. Dortch, D. Justic, V.J. Bierman Jr, and W.J. Wiseman. 2002. Nutrient-enhanced productivity in the northern Gulf of Mexico: Past, present and future. Hydrobiologia 475(1): 39-63.

Sakamoto, Y., Ishiguro, M., & Kitagawa, G. 1986. Akaike information criterion statistics.

Schwarz, G. 1978. Estimating the dimension of a model. The annals of statistics, 6(2): 461-464.

Segurado, P., J.M. Santos, D. Pont, A.H. Melcher, D.G. Jalon, R.M. Hughes, and M.T. Ferreira. 2011. Estimating species tolerance to human perturbation: Expert judgement versus empirical approaches. Ecological Indicators 11: 1623-1635.

Stoddard, J.L., D.P. Larsen, C.P. Hawkins, R.K. Johnson, and R.H. Norris. 2006. Setting expectations for the ecological condition of streams: The concept of reference condition. Ecological Applications 16(4): 1267-1276.

Summers, J.K. 2001. Ecological condition of the estuaries of the Atlantic and Gulf coasts of the United States. Environmental Toxicology and Chemistry 20: 99-106.

Swihart, R.K., T.M. Gehrig, M.B. Kolozsvary, and T.E. Nupp. 2003. Responses of 'resistant' vertebrates to habitat loss and fragmentation: The importance of niche breadth and range boundaries. Diversity and Distributions 9: 1-18

Toft, Jason D, S.H. Munsch, J. Ery, R. Cordell, K. Siitari, V.C Hare, B. Holycross, L.A. Debruyckere, and C.M. Greene. 2015. *Nursery Functions of West Coast Estuaries: Data Assessment for Juveniles of 15 Focal Fish and Crustacean Species*. Final report to the Pacific Marine and Estuarine Fish Habitat Partnership. http://www.pacificfishhabitat.org/assessment-reports/. Last Accessed January 2018.

Tremblay, A., and J. Ransijn. 2015. LMERConvenienceFunctions: Model selection and post-hoc analysis for (G) LMER models. R Package Version 2.

Uriarte, A., and A. Borja. 2009. Assessing fish quality status in transitional waters, within the European Water Framework Directive: Setting boundary classes and responding to anthropogenic pressures. Estuarine, Coastal and Shelf Science 82(2): 214-224.

USEPA (United States Environmental Protection Agency). 2015. Geospatial data download service. [GIS files] http://www2.epa.gov/enviro/geospatial-data-download-service.

USFWS (U.S. Fish and Wildlife Service). 2012. National wetlands inventory [GIS files]. http://www.fws.gov/wetlands/.

Vázquez, D.P., and D. Simberloff. 2002. Ecological specialization and susceptibility to disturbance: Conjectures and refutations. The American Naturalist 159: 606–623.

Whittier, T.R., R.M. Hughes, G.A. Lomnicky, and D.V. Peck. 2007. Fish and amphibian tolerance values and an assemblage tolerance index for streams and rivers in the western USA. Transactions of the American Fisheries Society 136(1): 254-271.

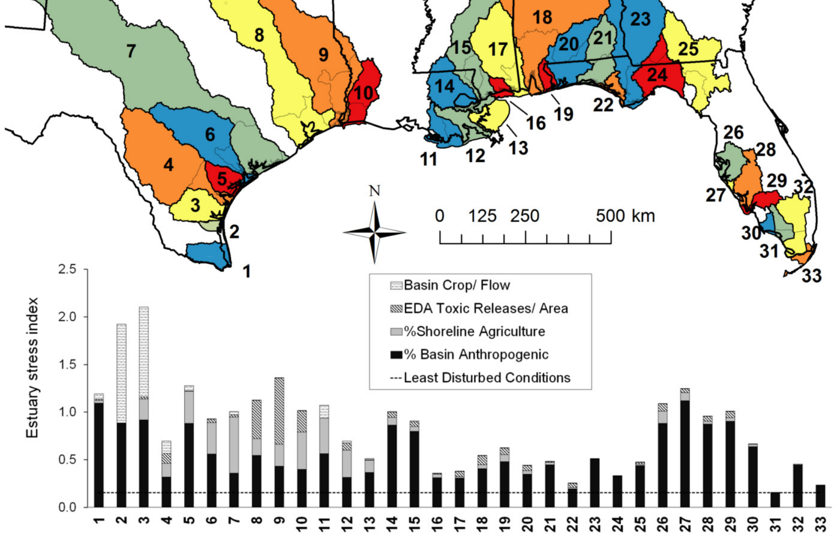
Wikle, C.K. 2003a. Hierarchical Bayesian models for predicting the spread of ecological processes. Ecology 84(6): 1382-1394.

Wikle, C.K. 2003b. Hierarchical models in environmental science. International Statistical Review 71(2): 181-199.

Wikle, C.K., L.M. Berliner, and N. Cressie. 1998. Hierarchical Bayesian space-time models. Environmental and Ecological Statistics 5(2): 117-154.

Wilson, S.K., S.C. Burgess, A.J. Cheal, M. Emslie, R. Fisher, I. Miller, N.P. Polunin, and H.P. Sweatman. 2008. Habitat utilization by coral reef fish: implications for specialists vs. generalists in a changing environment. Journal of Animal Ecology 77: 220–228.

Yáñez-Arancibia, A., and J.W. Day. 2004. The Gulf of Mexico: Towards an integration of coastal management with large marine ecosystem management. Ocean & Coastal Management 47: 537-563.



N
0 125 250 500 km
Estuary stress index
Basin Crop/ Flow
EDA Toxic Releases/ Area
%Shoreline Agriculture
% Basin Anthropogenic
Least Disturbed Conditions