Combined, the conditional inference tree analyses suggest that water quality responses in southwest Florida tidal creeks are subject to both natural and anthropogenic influences including nutrient inputs, hydrologic conditions and instream processes related to the influence of the buffer area of the creeks. Agricultural land use types were found to be associated with increased TN and TP concentrations (e.g., Frog and Sugarhouse). Few creeks had any agricultural activity in the watershed and in creeks with agricultural activity there could be other factors in these watersheds that may also contribute to elevated TN and TP concentrations. This should be further investigated. The number of outfalls observed in the surveyed portion of the creeks was also influential segregating the nutrient response data indicating non-point source pollution also affects the distribution of nutrient response in the creeks. Other factors such and the landscape development intensity index were not deterministic in segregating either the nutrient, chlorophyll or DO responses in these creeks though the natural buffer proportion consistently appeared as an influential parameter affecting these distributions. Creeks with a high proportion of natural vegetation in the buffer were associated with lower bottom DO % saturation values which is an important finding supporting the contention that mangrove dominated creeks should be considered wetland environments and evaluated as such in the regulatory context. Chlorophyll concentrations were primarily related to nitrogen inputs, though organic phosphorus was also a correlative factor. Landscape level factors were less influential in identifying thresholds in the water column chlorophyll distributions and the overall percent explained by the model was lower than for the nutrient endpoints. Benthic chlorophyll was only weakly related to the landscape metrics and overall there was a large degree of unexplained variability in the benthic chlorophyll data. Overall, these results suggest that, based on the scale at which we evaluated them, near field effects including instream water quality and riparian buffer attributes were more closely related to water quality responses than landscape level effects. Evaluation of water quality responses to landscape level attributes are more likely to express themselves over longer time scales such as annual geometric averages but this evaluation would require additional data in these tidal creeks.

4.3 Dissolved Oxygen as an Indicator of Adverse Effects

The FDEP did an excellent job of summarizing the science and management issues related to DO effects on biological integrity and the issues associated with using the measure as an indicator of adverse effects in their revised DO criteria document (FDEP 2013a). However, as stated earlier, the data collected as part of this study suggested that the DO criteria as specified in Florida Administrative Code (F.A.C.) were not a reliable indicator of adverse effects in southwest Florida tidal creeks. That is, the conceptual model used by FDEP suggests that increased nutrient loadings result in lower DO concentrations, which in turn result in adverse effects to fish and wildlife was not supported. In FDEP's technical support document, the authors go to great lengths to describe the role of wetlands in depressing DO concentrations and recommending biological confirmation prior to establishing total maximum daily loads based on DO exceedence analysis. The following excerpts from FDEP 2013a summarize that sentiment.

> *Natural estuaries especially subject to low DO include those receiving significant drainage from wetlands or marshes, those in areas surrounded by mangrove forests or tidal marshes, or those estuaries where salinity stratification occurs (Hendrickson et al. 2003)……*
>
> *….When estuaries are minimally disturbed by humans and are characterized by a healthy, well balanced aquatic community, it is critical that natural low DO not be misinterpreted as a response solely to anthropogenically derived nutrients or oxygen demanding substances.*

The revised FDEP DO standards for estuaries were based on experimental laboratory (LC50) experiments (FDEP 2013a). The LC50 denotes the DO concentration where 50% of test organisms expire within a certain period of time (e.g., 1 hour). Recruitment curves were developed to translate the LC50 information based on acute and chronic toxicity values for the four most sensitive Florida species. The four most sensitive species in this case were: *Morone saxatilis*, (striped bass), *Chasmodes bosquianus* (striped blenny), *Dyspanopeus sayi* (Say mud crab), and *Octopus burryi* (Burry's octopus). The final recruitment curve based on these species is provided in Figure 24 copied from FDEP 2013a.

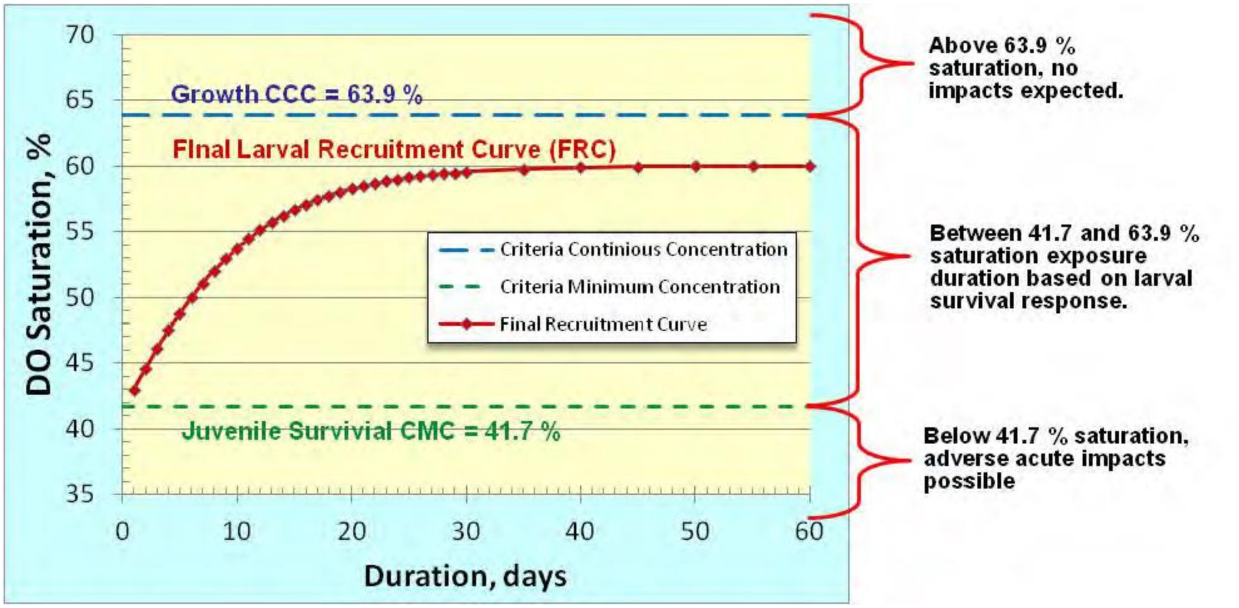

Figure 24. FDEP final recruitment curve used to establish revised marine DO standards (FDEP 2013a).

The final proposed criteria for Class II and III marine waters are expressed as:

- The daily average shall not be below 42% saturation more than 10% of the time
- The weekly and monthly averages of 51% and 56% respectively.

The state accounts for areas that transition between salt and freshwaters, e.g. tidal creeks, depending on the conductivity/salinity at the time the DO was measured. For example, if the DO of waterbody measured within a transitional zone as defined by FDEP, and that water's conductivity is below 4,580 umhos/cm or 2.7 PSU, than the applicable freshwater is applied. Conversely, the marine standard is applied if the conductivity exceeds that threshold. However, none of the four most sensitive species used to develop the final recruitment curve for the marine standard were found in the tidal creeks we sampled and these species are not typically found in any of the southwest Florida open estuary systems sampled by the FIM program. In addition, the fisheries data we collected in the sampled tidal creeks suggest that, despite DO conditions not meeting the current standards, many of these creeks were healthy and productive systems providing nursery and refugia for important fish species such as Snook. Therefore, alternative analyses were pursued to identify potential alternative DO criteria for southwest Florida creeks.

Data on acute DO toxicity (i.e., LC50) provided in Appendix E of FDEP 2013a were subset for species observed in the sampled tidal creeks and used to evaluate potential alternative

DO criteria for tidal creeks under the assumption that the acute threshold values are indicative of conditions that these species would avoid. These species include: *Anchoa mitchilli; Farfantepenaeus duorarum; Gobiosoma bosc; Lagodon rhomboides; Leiostomus xanthurus; Palaemonetes pugio; Sciaenops ocellatus* and *Palaemonetes vulgaris.* Because estuarine dependent species have temporally specific utilization patterns in tidal creek, the concentration based LC50 acute values were converted to percent saturation values based on the average temperature and salinity of each creek for the month sampled and a weighted average of all species was then calculated based on the probability of occurrence for each species each month. The results are presented in Figure 25 with spline smoothing curves used to display the creek specific response. Because the LC50 experiments are concentration based, the required percent saturation necessary to maintain a specific percent saturation changes as a function of temperature and salinity. Using the highest percent saturation of any month (i.e., July) two potential alternative criteria would be ca. 23% using Forked Creek which was the most sensitive of all creek curves, or ca. 20% if the average of all creeks for the most sensitive month were used. If only considering the most sensitive species, (*Anchoa mitchilli*), using the LC50 value of 2.1 mg/l, the results are displayed in Figure 26 and indicate that potential values would be ca. 31% if using the most sensitive creek, and ca. 27% using the average of all creeks. Using the 31% value and the 10% criterion would resolve the exceedence rate for 6 additional creeks; however, even the most lenient of these criteria (i.e. 20%) would result in exceedences for the three creeks with the most undeveloped watersheds in our study (Table 5). The combined results of these analyses to derive potential alternative site-specific DO criteria suggest that while it

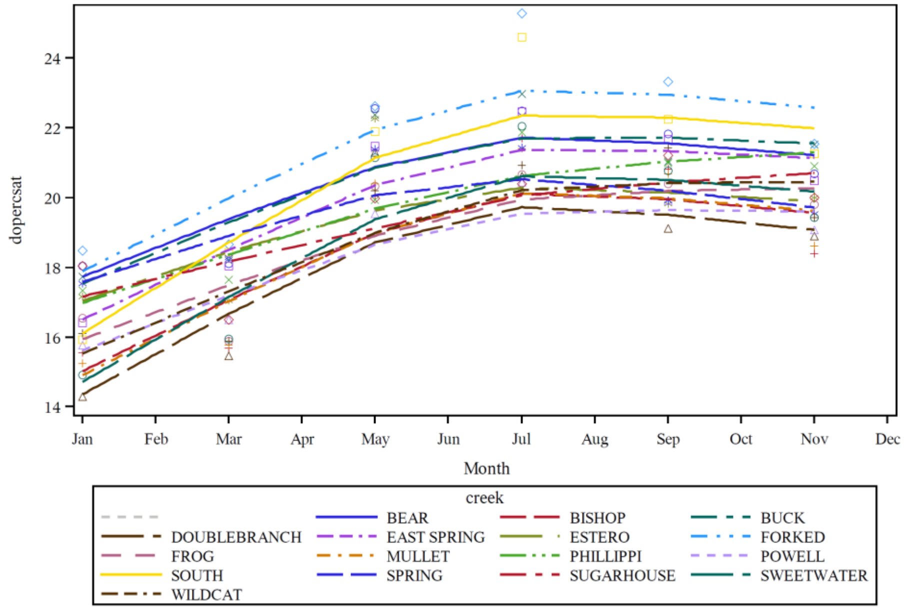

Figure 25. Results of alternative analysis to derive a site-specific DO standard for southwest Florida tidal creeks using LC50 values for several species observed during the study that were listed in FDEP 2013a Appendix E.

is possible to derive locally specific alternative standards for tidal creeks, if compliance with those criteria is forced to rely on the 10% exceedence rate, alternative standards would not ameliorate the issues associated with compliance with the current standards. Therefore, it is recommended that alternative distributional cutpoints for identifying exceedence rate thresholds be considered that are not associated with the tails of the DO distribution.

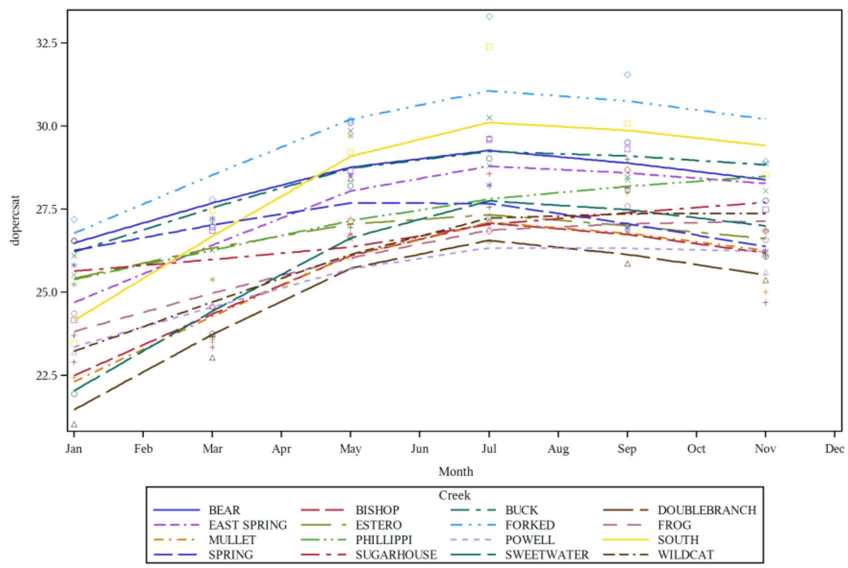

Figure 26. Results of alternative analysis to derive a site specific DO standard for southwest Florida tidal creeks using the LC50 value of 2.1 mg/l for *Anchoa Mitchilli*.

Table 5. Distributional statistics for water column average DO percent saturation with the 10th percentile highlighted indicating the values relative to that used to evaluate compliance with current DO standards in estuaries. * indicates creeks with most undeveloped watersheds.

Creek	p10	p25	p90	p100
Bear *	14.0	25.4	115.0	119.0
Bishop	28.5	52.0	92.0	155.0
Buck *	16.3	30.0	104.0	153.0
Doublebranch	57.5	59.8	95.0	97.7
East Spring	57.0	67.8	93.8	104.1
Estero	34.2	40.9	97.0	142.0
Forked	42.0	55.0	126.3	162.8
Frog	40.3	42.5	78.0	93.0
Mullet	24.0	32.3	64.3	80.0
Phillippi	41.6	49.2	89.0	111.9
Powell	24.8	28.0	52.5	84.5
South *	17.5	48.4	87.3	100.0
Spring *	21.5	27.5	84.0	93.0
Sugarhouse	40.0	46.0	79.5	83.0
Sweetwater	36.1	49.0	101.0	120.0
Wildcat	39.0	53.4	91.8	93.8

As an additional line of evidence in relating DO to fish communities we evaluated long term fisheries monitoring data collected by the Florida Fish and Wildlife Conservation Commission's Fisheries Independent Monitoring (FIM) program which has been collecting fish and *in situ* field chemistry data (including DO) in larger tidal rivers in Tampa Bay for over 15 years. For example, in Figure 27 Margalef's species richness index was plotted as a function of binned DO percent saturation midpoints for boat set seines collected in the larger Tampa Bay tidal rivers. The boat set seine is a similar gear type to the raft seines used to sample tidal creek shorelines. A horizontal line is provided in this panel of plots at a value 2.5 as a reference point. While data collected using the trawl gear used to sample bottom waters in the channel of these systems was more responsive to DO, there was little evidence to suggest that any of the diversity metrics calculated for the boat set seine gear in this long term database were adversely impacted by low DO conditions for data collected. Plots for both gear types and all diversity metrics are provided in Appendix D. While no nutrient data were associated with these fish collections, the data were also sufficient to evaluate the probability of occurrence of several individual fish species collected in this study as a

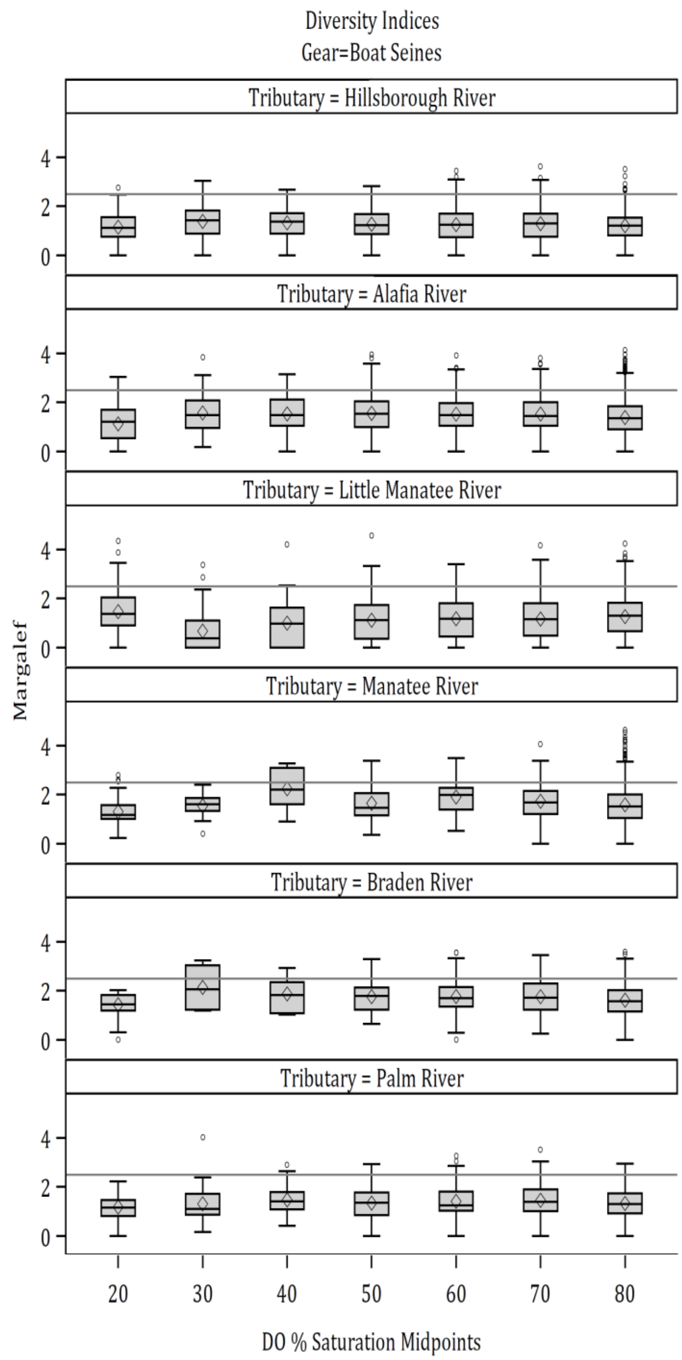

Figure 27. Distribution of Margalef's species richness by DO percent saturation category in larger Tampa Bay tidal rivers. Data were collected independent of this study and were provided upon request.

function of DO concentrations (and DO % saturation). To identify a potential threshold indicative of an adverse effect, we investigated the relationship between DO and fish probability of occurrence in this long term database.

Individual species response to DO in the long term FIM database was evaluated using logistic regression (Proc Logistic: SAS Institute, 2014) for the species used in the acute toxicity experiments described above as well as for Snook. Logistic regression estimates the probability of occurrence of a specific binary response (e.g., the presence of a particular fish taxon) as a function of either categorical or continuous predictors. We evaluated these relationships using DO as both a continuous and categorical explanatory variable in separate analysis. For the categorical analysis, DO categories were derived by binning DO concentrations in 1 mg/l increments between 2 and 8 mg/l rounded to the nearest integer value. Similarly, we binned DO % saturation values in 10 % increments between 20 and 80% saturation. Any values above the maximum category were included in the maximum category and any values below the minimum (i.e. 25%) were included in the minimum category. The lowest category was used as the reference group for each analysis such that the odds of occurrence were evaluated relative to the lowest category (i.e. the odds ratio). For each fish taxa, months were eliminated from the analysis if no fish were ever captured in that month and river. Additionally, a seasonal term was added to the model in an attempt to account for seasonal differences in the probability of occurrence. The seasonal levels were defined as Summer (April – September) and Winter (October – March) with summer used as the reference group for the comparison.

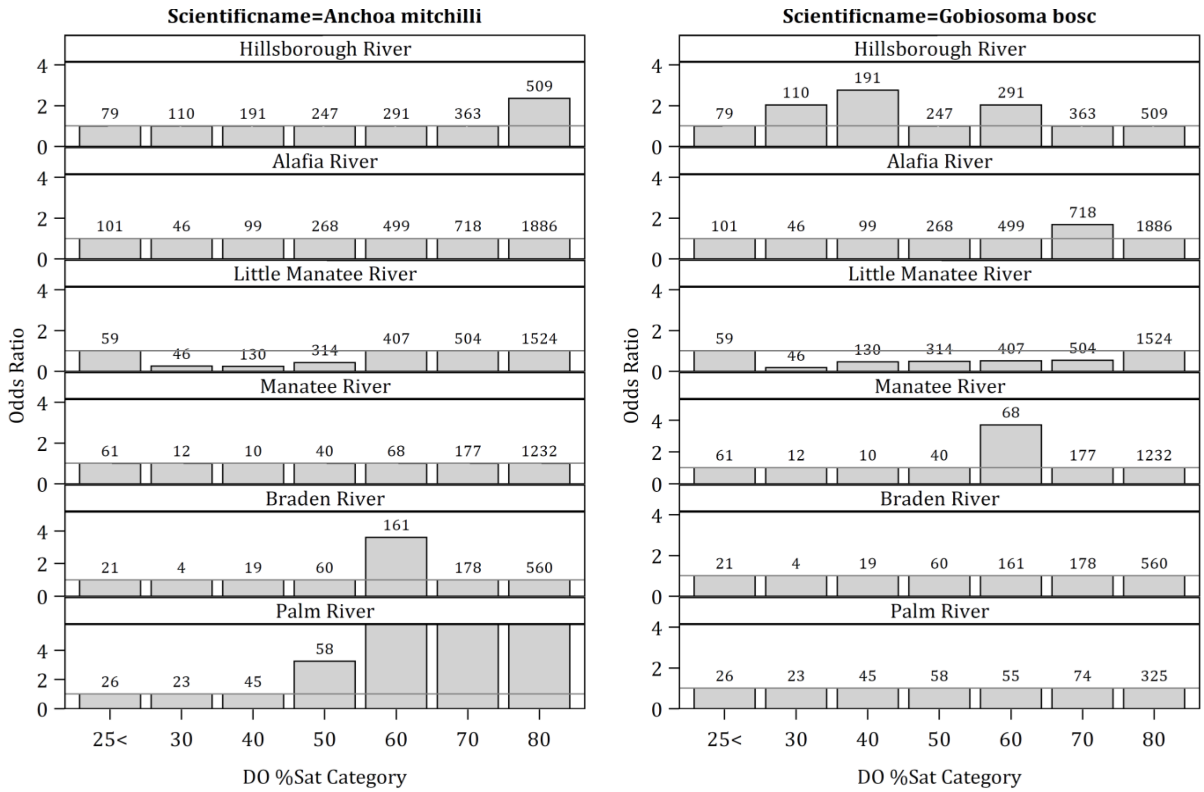

Figure 28. Results of logistic regression predicting the probability of occurrence of Bay Anchovy (left) and Naked Goby (right) based on DO percent saturation categories in Tampa Bay tidal rivers.

The results of the logistic regression analysis are summarized using bar graphs of the odds ratio estimates relative to the reference group (i.e. samples collected when DO %sat was less than 25%).In the bar graphs, values of 1 represent equal (non-significant) likelihood relative to the reference group. Values less than one indicate that the likelihood of capture significantly decreased relative to the reference group, while values greater than one indicate that the likelihood of occurrence increase relative to the reference group. It is important to note that the odds ratio is asymmetric around the value of 1 so bars below 1 appear to be smaller differences but are not necessarily. The numbers plotted above each bar represent the number of observations for each group. Figure 28 displays the results for *Anchoa Mitchilli* and *Gobiosoma Bosc*, two commonly collected species that occur year round in Tampa Bay Rivers. The results were highly system dependent for these two species (and for all other species tested) with no effect in some rivers, protective effects in others and antagonistic effects of low DO in other systems. For example, in the Little Manatee River which is the most natural river system of all of the rivers evaluated, both Bay Anchovy and the Naked Goby responded negatively to increased DO category relative to the reference group, while in other systems there were no significant effects. In the case of Bay Anchovy in the Palm River (a highly physically altered system), a significant increase in probability of occurrence as a function of DO above 45% relative to the reference category was observed. For Snook, there were indications that increased DO values would increase the probability of capture relative to a DO saturation value of 25% in some rivers (e.g., Alafia River results in Figure 29a) but in other rivers there was not a statistically significant effect. For Red Drum in the Alafia River, the likelihood of capture was significantly reduced at DO values above 25% (Figure 29b) while for most other rivers there was not a significant relationship. Plots for other species evaluated are provided in Appendix E. Together, these results suggest that while DO was significantly related to the probability of occurrence in some cases, there was no unified response to DO in these systems. Rather, the response was conditional and likely mediated by other factors such as the relationship between static habitat factors and dynamic water quality attributes as described by Browder and Moore 1981.

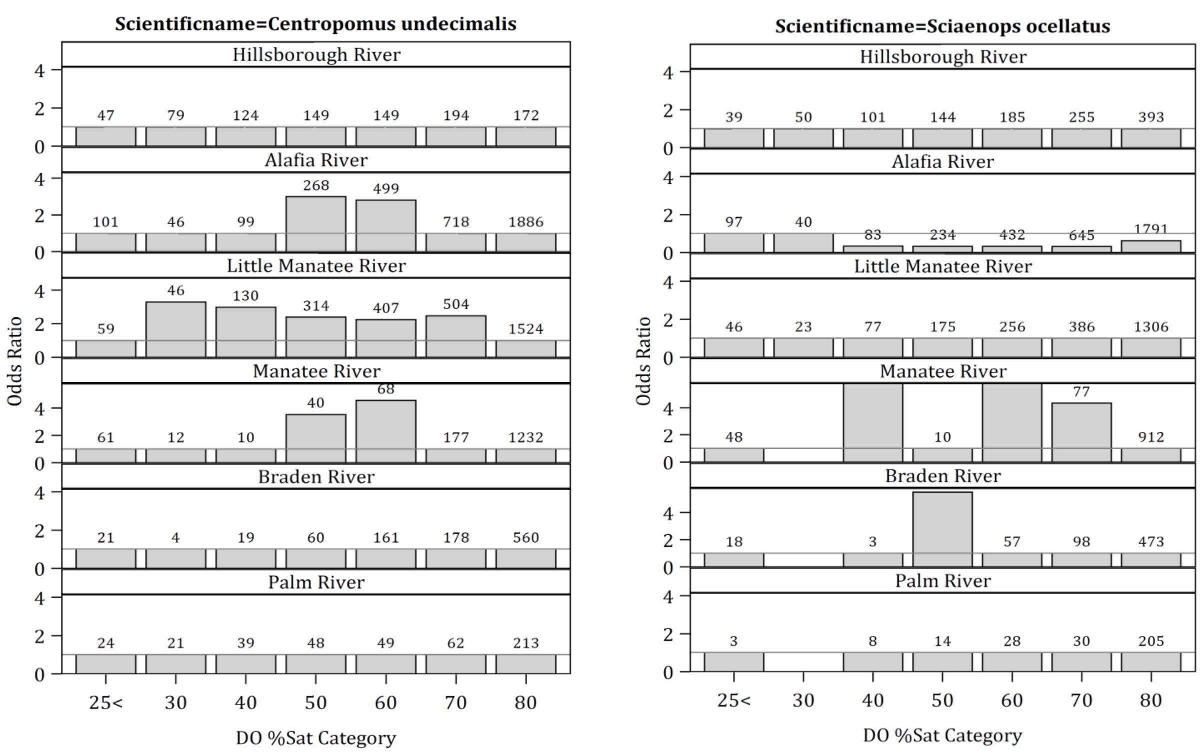

Figure 29. Results of logistic regression predicting the probability of occurrence of Common Snook (left and Red Drum (right) based on DO percent saturation categories in Tampa Bay Tidal Rivers.

In summary, we suggest that the experimental evidence used to develop Florida's marine DO standards should be observable in the empirical data in order to be appropriate for the systems to which they are applied. The combined results of the analysis of DO and fish response suggest limited and inconsistent evidence that, in evaluating observational data, DO is an reliable indicator of adverse effects for fish communities in southwest Florida tidal creeks. While the seining method may not capture the deepest portion of the creek, it does sample the entire water column (unlike trawl gear) and is the most effective method to sample fish communities in tidal creeks. Therefore, while the DO standards may be satisfactory for open bay estuarine environments, the estuarine criterion is not recommended as a mechanism for evaluating adverse effects in southwest Florida tidal creeks without biological confirmation. We recommend that alternative formulations of the DO standard be considered if DO is to be used as a water quality standard for southwest Florida tidal creeks. The alternative formulations should in particular include a statistic representative of something other than the tail of the DO distribution (i.e., the 10^{th} percentile). Additional studies are likely required to derive alternative standards for DO in southwest Florida tidal creeks but the results presented within should provide additional insights useful towards that effort.

4.4 Nutrients as a Conservative Substance

One of the most significant findings of this study was that the nutrient concentrations in the tidal portions of these creeks were generally higher than expected based on dilution of the source water by mixing with estuarine waters and in some cases could be in excess of the source water concentrations. This finding is counter to the EPA/FDEP considered method for establishing downstream protection values for tidal creeks based on the dilution curve method (EPA 2012) and provides insights into the potential instream processes that may contribute to this result. The sampling design for this study was constructed, in part, to specifically test whether nutrients acted as conservative substances in tidal creeks. By collecting water quality samples at the upstream source water station and in the downstream tidal portions of the creek on the same date (generally within a four hour window) the observed source water concentration was assumed to be representative of the expected influence of the source water on tidal creek concentrations. Using a modification of the freshwater fraction equation (Sheldon and Alber, 2006), we calculated the expected concentration of nutrients in the tidal portion of the creek as a function of the source water