Technical Support Document:

Derivation of Dissolved Oxygen Criteria to Protect Aquatic Life in Florida's Fresh and Marine Waters

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DEP-SAS-001/13

Prepared by:
Florida Department of Environmental Protection
Division of Environmental Assessment and Restoration

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Executive Summary

Dissolved oxygen in our waterways is necessary to support respiration of aquatic biology that live in Florida waters. As with humans, aquatic species also require oxygen. The Department sets dissolved oxygen levels to ensure that there is enough oxygen in our waterways to allow growth and reproduction of Florida aquatic species. Once set, regulatory programs are implemented to ensure that oxygen levels are met and aquatic species are protected.

Florida's current dissolved oxygen (DO) criteria were adopted more than 30 years ago and were based on limited information regarding the response of southern warm water species to DO conditions. Due to natural phenomena, Florida's DO concentrations do not relate well to the existing DO criteria in many of Florida's healthy fresh and marine water systems. Our temperatures and geology introduce variables that the out of date DO criteria does not consider. Consequently, the Department of Environmental Protection (FDEP) collected a comprehensive array of scientific information to serve as the basis for accurately revising the existing DO criteria, and consulted a peer review committee to receive expert feedback on the revised criteria.

The FDEP conducted an extensive statewide freshwater DO study during 2005 to 2006 in lakes and streams to collect data required to fully assess the accuracy of the current criteria and to revise the State's DO criteria. The study confirmed that DO concentrations in approximately 70 percent of the minimally disturbed streams and 52 percent of the minimally disturbed lakes sampled during the study do not relate well to the existing criteria of 5 mg/L (with 10 percent or more of the measurements falling below the criteria naturally).

After evaluating data from the DO study, FDEP determined the minimum DO levels that fully protect healthy, well balanced aquatic communities using information from unimpacted waterways in different regions of the State. FDEP derived the revised freshwater DO criteria using the relationship between the daily average DO condition (percent saturation of DO) and a measure of stream aquatic life health, the Stream Condition Index. FDEP determined the DO saturation required to achieve healthy biological conditions, an average SCI score of 40 (healthy), at the 90th percentile confidence interval.

FDEP selected DO percent saturation rather than concentration because a) the daily average DO saturation provided the best correlation with SCI scores, and b) saturation automatically accounts for the inherent relationship between temperature and DO. FDEP developed different regional criteria to account for the observed regional differences in measured DO levels and biological expectations, and used the confidence interval to add a protective safety factor accounting for the uncertainty in the relationships and the naturally expected diel fluctuations in the DO levels.

Based on the results of the regional relationships (using regression models) between aquatic biology health and DO condition (average SCI scores and the daily average DO saturations), daily average DO levels of 67, 38, and 34 percent saturation for the Panhandle West, Peninsula, and Big Bend + Northeast bioregions, respectively, were determined to support healthy, well balanced biological communities.

To derive revised DO criteria for Florida's marine waters, FDEP used the USEPA Virginian Province approach using fish and invertebrate species known to inhabit Florida's waters. The

Virginian Province method utilizes observed laboratory responses of species sensitive to DO levels to calculate DO concentrations and durations that will protect against adverse (acute and chronic) effects to aquatic life.

The application of the Virginian Province method calculated a minimum allowable DO condition criterion (percent saturation of 42 percent). To ensure additional protection against chronic effects, FDEP also added minimum weekly and monthly average DO concentrations of 51 percent saturation and 56 percent saturation, respectively. Maintaining weekly and monthly average DO concentrations at or above these levels will protect against the adverse effects of low DO on the reproduction (larval recruitment) of sensitive species.

Based on the analyses conducted, the revised DO criteria for Florida's Class I and III freshwaters are expressed as:

> *No more than ten percent of the daily average percent DO saturation values shall be below 67 percent in the Panhandle West bioregion, or 38 percent in the Peninsula and Everglades bioregions, or 34 percent in the Big Bend and Northeast bioregions.*

The revised DO criteria for Florida's Class II and III marine waters developed from the application of the USEPA Virginian Province approach to Florida specific fish and invertebrates is expressed as:

> *The daily average percent DO saturation shall not be below 42 percent in more than ten percent of the values.*
>
> *AND*
>
> *The weekly- and monthly average percent DO saturations shall not be below 51 and 56 percent, respectively.*

For a summary of the applicable percent saturation DO criteria over a range of expected Florida temperatures, see **Figure 35** on page 80.

Dissolved Oxygen conditions change throughout a given day based on temperature and photosynthesis. Comparing data and information with the daily average freshwater DO criteria is best assessed using daily average values calculated from data collected throughout the day by a recording meter (diel monitoring data). If diel monitoring data are not available, a single sample generally collected by field staff may be used to assess the DO criterion by making a "time-of-day" translation of the daily average criterion as described in **Section 4.5.4**. By taking into account the natural daily fluctuation in DO levels, use of the "time-of-day" translation of the daily average criterion will help minimize assessment errors resulting from the use of a single sample collected at a given time of day.

FDEP also evaluated whether the revised criteria are expected to impact threatened and endangered aquatic species. The majority of threatened or endangered species with high DO requirements are located in the western Panhandle, where the proposed DO criteria would increase. In portions of the Suwannee, New, and Santa Fe Rivers inhabited by the Gulf Sturgeon and Oval Pigtoe mussel, the proposed DO criteria were modified to assure that the sturgeon and mussel were fully protected. An additional modification was made to assure protection of any potential spawning of Shortnose or Atlantic Sturgeon in portions of the St. Johns River. A description of the modified criteria, developed in conjunction with the US Fish and Wildlife Service and NOAA, is provided in **Appendix I**. The new DO criteria are fully protective of threatened and endangered species throughout the state of Florida.

To avoid incorrectly listing a natural waterbody, such as an aquatic preserve, as impaired, FDEP plans to use an EPA-sanctioned provision that takes into account the natural DO regime. If the natural background DO condition of a waterbody does not attain the criteria, the DO condition associated with the natural condition must be maintained by not allowing more than a 0.1 mg/L deviation below the DO concentration associated with the natural background DO levels. For marine waters, no more than a 10% deviation from the natural background DO can be allowed if it is demonstrated that sensitive resident aquatic species will not be adversely affected, using the procedure described in **Appendix H** of this Technical Support Document.

FDEP is also including a standard that would protect waterways that have DO conditions naturally better than the protective concentration. The clause would require that those ambient DO levels be maintained, except as provided under Rule 62-302.300 and 62-4.242, F.A.C. (anti-degradation provisions). Those waterbodies that have conditions better than the percent saturation standards being established will be considered impacted if there has been an adverse change in DO conditions (a statistically significant decreasing trend in DO levels, or an increasing trend in the range of daily DO fluctuations, at the 95 percent confidence level) and a causative pollutant is identified. This trend will be determined using modern statistical procedures (a one-sided Seasonal Kendall test for trend), after controlling for or removing the effects of confounding variables, such as climatic and hydrologic cycles, quality assurance issues, and changes in analytical methods.

1 Introduction

1.1 *Purpose of Report*

The purpose of this report is to provide the technical basis for the development of revised water quality criteria for dissolved oxygen (DO) for aquatic life protection in Florida's fresh and marine waters. The DO criteria for freshwaters presented in this report were developed by the Florida Department of Environmental Protection (FDEP) based on two lines of evidence:

1. Analysis of data collected during an extensive statewide DO/nutrient study conducted during 2005 and 2006 and supplemented with additional data collected during 2010. These analyses revealed a significant relationship between Florida's stream macroinvertebrate index [the Stream Condition Index (SCI)] and DO and temperature; and,
2. An analysis of empirical DO data from several minimally disturbed systems that represent natural background DO distributions expected in Florida freshwaters.

The DO criteria for Florida's marine waters described in this report were developed by FDEP based on an application of the methodology developed by the U.S. Environmental Protection Agency (USEPA) for the Virginian Province (USEPA, 2000). EPA's Virginian Province approach uses knowledge regarding the biological response of sensitive aquatic organisms to hypoxic stress to derive DO criteria that provide sufficient protection from acute and chronic effects of exposure to low DO levels in marine waters.

1.2 *Background Information*

Dissolved oxygen (DO) is the amount of oxygen gas contained in water, and aquatic biota depend on it for respiration. Depending on duration and magnitude, when DO is reduced below an organism's physiological requirements, harm may occur, potentially resulting in mortality or detrimental sublethal effects. The amount of dissolved oxygen expected in aquatic environments (*i.e.,* DO saturation concentration) is depended on water temperature and salinity. While decreases in atmospheric pressure (*e.g.*, as experienced in high, mountainous areas) can also affect DO, Florida's low elevations make this factor insignificant. As described by Henry's Law and the Ideal Gas Law, the DO concentration at saturation decreases as the water temperature and salinity increases. The empirical DO saturation versus temperature relationship for water has been studied extensively and is well understood (Benson and Krause 1984).

1.2.1 Freshwater DO Criterion

Florida's current DO criteria for Class I and III freshwaters states that DO concentrations "*Shall not be less than 5.0 mg/L*" (Rule 62-302.530 (30), F.A.C.). Additionally, the criteria indicate that normal daily and seasonal fluctuations above these levels shall be maintained. It is important to note that the current 5.0 mg/L criterion applies to all places and at all times in Class I and III waters.

The last revision to Florida's DO criteria for freshwaters occurred in 1979, largely based on early water quality criteria recommendations from the Federal Water Pollution Control Administration (FWPCA 1968) and EPA (EPA 1972). This initial 1970s guidance concerning the establishment of appropriate DO criteria was based on very limited scientific information. Specifically, there was inadequate data regarding the response of freshwater organisms to low DO concentrations, therefore the criteria were largely driven by the responses of sensitive freshwater game fish (largely coldwater species) to depressed DO levels. The Florida DO criteria were subsequently modified slightly during the late 1970's based on additional EPA guidance (EPA 1976).

In the approximately 40 years since the EPA recommendations on DO criteria were adopted into Florida's water quality standards, considerable knowledge has been gained concerning the biological response to low DO levels, as well as the nature of Florida's aquatic systems. This information has greatly improved the knowledge base needed to develop more appropriate revised criteria for both Florida's fresh and marine waters.

1.2.2 Marine DO Criteria

Florida's current DO criteria for Class II and III marine waters specify that DO concentrations "*Shall not average less than 5.0 mg/L in a 24-hour period and shall never be less than 4.0 mg/L."*) (FAC 62-302.530). Additionally, the criteria indicate that normal daily and seasonal fluctuations above these levels shall be maintained. Florida adopted the existing DO criteria for marine waters in the early 1970's, again largely based on early water quality criteria recommendations from EPA.

EPA guidance regarding DO criteria for marine waters recognizes that there are a number of natural conditions that can result in DO levels below the recommended criteria and acknowledged that in these cases, the default criteria would not be appropriate. Additionally, the recommended DO criterion was qualified with the following statement; *"The committee would like to stress that, due to a lack of fundamental information on the DO requirements of marine and estuarine organisms, these requirements are tentative and should be changed when additional data indicate that they are inadequate"* (FWPCA 1968).

1.2.3 Naturally Low DO Conditions

When FDEP's predecessor agency (Department of Environmental Regulation) adopted its current DO criteria in 1979, it did not explicitly include language regarding an acceptable departure from natural conditions as it did for other natural stressors (*e.g.,* conductivity, pH, and temperature). The repercussions of this oversight did not become readily apparent until the implementation of the Total Maximum Daily Load (TMDL) Program. Without a specific natural background clause for DO, numerous natural waters were identified as impaired for DO and placed on the Clean Water Act (CWA) 303(d) list.

The most recent EPA guidance (EPA, 1986) recognized that waterbodies can exhibit low DO concentrations under some natural conditions stating that:

> "*Naturally-occurring dissolved oxygen concentrations may occasionally fall below target criteria levels due to a combination of low flow, high temperature, and natural oxygen demand. These naturally-occurring conditions represent a normal situation in which the productivity of fish or other aquatic organisms may not be the maximum possible under ideal circumstances, but which represent the maximum productivity under the particular set of natural conditions. Under these circumstances the numerical criteria should be considered unattainable, but naturally-occurring conditions which fail to meet criteria should not be interpreted as violations of criteria. Although further reductions in dissolved*

> *oxygen may be inadvisable, effects of any reductions should be compared to natural ambient conditions and not to ideal conditions. "*

The 1986 EPA guidance further states that:

> *"Where natural conditions alone create dissolved oxygen concentrations less than 110 percent of the applicable criteria means or minima or both, the minimum acceptable concentration is 90 percent of the natural concentration. These values are similar to those presented graphically by Doudoroff and Shumway (1970) and those calculated from Water Quality Criteria (NAS/NAE, 1973). Absolutely no anthropogenic dissolved oxygen depression in the potentially lethal area below the 1-day minima should be allowed unless special care is taken to ascertain the tolerance of resident species to low dissolved oxygen."*

With regard to DO criteria, an "exceedance" is considered to occur when the DO concentration is less than the criterion. Even though Florida did not make an allowance for DO concentrations below the DO criteria resulting from natural conditions, it is clear from the early guidance that EPA did not intend for naturally low DO waters to be considered in violation of the DO criteria. It follows then that these waters would not be identified as being impaired and placed on the CWA 303(d) list. Given the variety of physical, biological, chemical, and climatological factors that are capable of producing waters with naturally low DO conditions, FDEP's current DO criteria are overly simplistic and do not accurately reflect natural variability in DO or thresholds necessary to protect aquatic life.

1.3 *Florida Ecosystems with Naturally Low DO*

Persistent, naturally low DO concentrations below the existing DO criteria have been documented in many of Florida's minimally disturbed and healthy fresh and marine water systems. Natural freshwater systems subject to low DO include those receiving significant drainage from wetlands or marshes, waterbodies downstream of springs or other groundwater sources, and many streams during low or no flow periods.

1.3.1 Freshwater Systems with Naturally Low DO Levels

Florida's swamp/wetland drainages are characterized by low velocity flows and high color levels resulting from large inputs of Colored Dissolved Organic Matter (CDOM) from humic substances. The highly colored water and heavy shading typical of many Florida streams limits light penetration, which reduces photosynthetic DO production. In addition to increasing color levels, the high level of dissolved organic matter results in elevated natural biological oxygen demand that depletes the oxygen in the water. The limited photosynthetic activity and high oxygen demand results in naturally low DO values, with values typically ranging down to 3.0 mg/L or less.

Many Florida springs also have naturally low DO levels. The Florida Geological Survey Bulletin No. 66, *Springs of Florida*, includes DO measurements from the 1970's and early 2000's that indicate many Florida springs vents exhibit DO values well below the current 5.0 mg/L DO criterion (FGS 2004). For example, Orange, Rock, Alexander, Silver, and Ponce De Leon Springs were measured to have DO values of 0.4 mg/L, 1.0 mg/L, 1.1 mg/L, 2.4 mg/L, and 3.4 mg/L, respectively (FGS 2004). FDEP organized a Springs Monitoring Program (SMP) that collected water quality, habitat, and biological community data in 18 major springs in Florida from 2000 to 2007 (FDEP 2008). The sampled springs included Wekiwa, Ichetucknee, Volusia Blue, Rainbow, Fanning, Manatee, Wakulla, Ponce de Leon, Alexander, Rock, De Leon, Silver Glen, Juniper, Troy, Orange Grove, Peacock, Silver, and Blue Hole (FDEP, 2008). Natural factors that limited biological health (benthic invertebrates) at many of the springs included low DO with values below 3 mg/L noted at Troy, Volusia Blue, Manatee, Wekiva, and Alexander Springs. In Rock, Orange Grove, Peacock, and Silver Springs, fluctuating low DO were occasionally recorded.

Figure 1 shows the typical DO regime in Blue Springs in Volusia County over a four month period in 2010 where the diel DO range varies between 0.5 and 2.0 mg/L. It should also be noted that this gauging station is approximately 500 meters downstream of the headspring, and the DO is still lower than current Class III standards or the proposed revised criteria. **Figures 2 and 3** show typical sections of Blue Spring run.

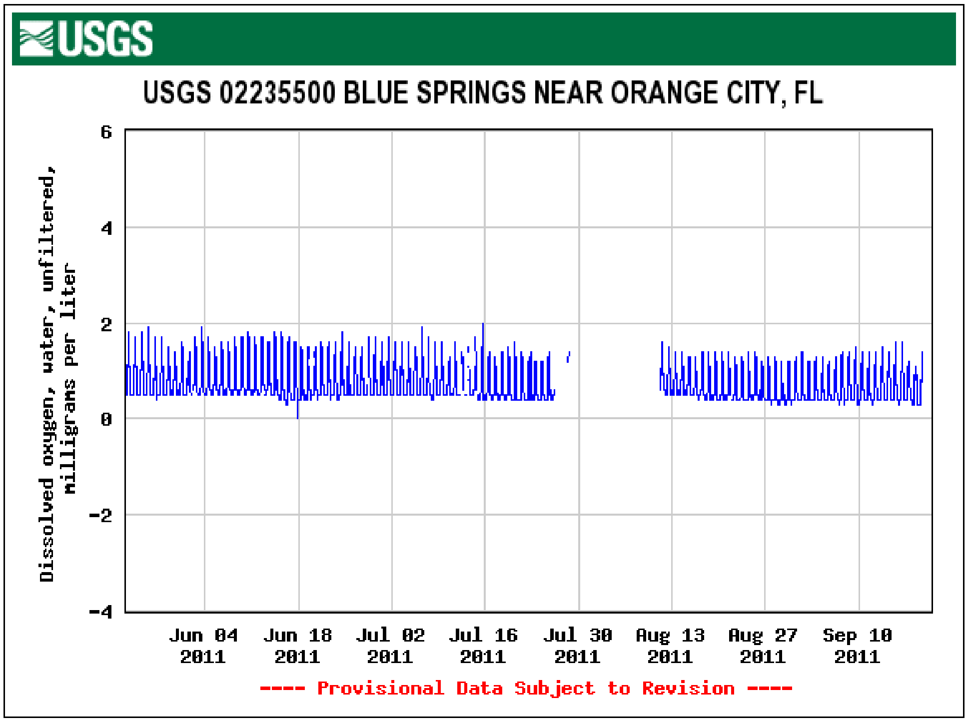


Figure 1. Typical summer DO regime in Blue Springs in Volusia County over a four month in 2010 where the diel DO range varies between 0.5 and 2.0 mg/L. The monitoring station is located approximately 500 meters downstream of the headspring.

Figure 2. Blue Spring run approximately 50 meters downstream of the spring vent.

Figure 3. Blue Spring run approximately 550 meters downstream of the spring vent.

In 2007 and 2008, the DO concentration in Blue Spring based on monthly surface grab samples averaged 0.02 mg/L (0.3 percent saturation) at the headspring and increased to 4.4 mg/L (56.6 percent saturation) approximately 500 meters downstream (SJRWMD 2010). Results from sondes deployed during this same period are shown in **Table 1**. These low DO levels are caused by the depletion of DO during long residence times of ground waters prior to being discharged from the spring vents. Residence times for the groundwater discharged by Florida's springs can range from several weeks to thousands of years (Hanshaw *et al.* 1965). Additionally, many streams receive low DO groundwater seepage, which influences the DO regime, even in area without obvious spring boils.

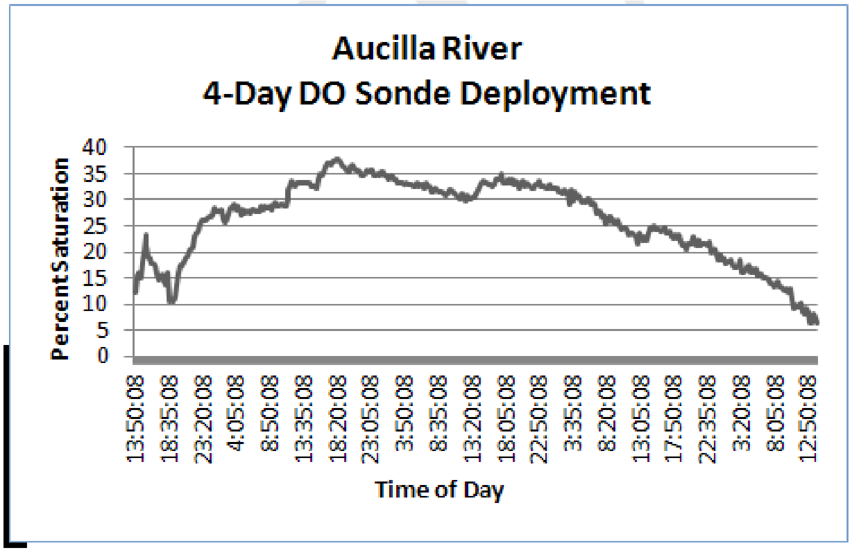
Table 1. Blue Springs DO statistics for 2007/2008 based on recording data Sondes deployed at three locations in the spring run. Stations are based on their distances downstream of the spring vent.

Parameter	Station	Average	Median	Maximum	Minimum	Std Dev	N
DO (mg/L)	35 meter	0.04	0.03	0.14	0.00	0.03	127
	355 meter	0.44	0.46	0.80	0.04	0.15	190
	570 meter	0.83	0.78	3.75	0.11	0.40	284
DO (% saturation)	35 meter	0.22	0.23	1.69	0.00	0.3	127
	355 meter	4.35	4.85	9.39	0.43	1.8	190
	570 meter	9.34	8.48	49.4	1.31	5.17	284

Florida also has many streams that exhibit naturally low DO values during low or no flow conditions, which in turn is dependent upon the amount of rainfall in any given year. Under prolonged dry periods, some streams may change into a series of disconnected pools or go completely dry, exhibiting low DO levels during these low-flow or no-flow conditions due to the lack of reaeration and naturally suppressed photosynthetic oxygen production. Furthermore, the low DO conditions often occur simultaneously with or are exacerbated by high summertime temperatures.

Many larger system and their headwaters also experience low DO levels associated with low flow conditions. Organisms, to some extent, have adapted to these hydrological conditions. For example, the Aucilla River at Highway 90 in Jefferson County (**Figure 4**) is a minimally disturbed site with a Landscape Development Intensity Index (LDI) of 1.11 that has been shown to support a healthy macroinvertebrate community even though it exhibits low DO levels. For example, during August 2010, the site passed the SCI (41 points) during a period when the average DO concentration during a four-day instrument deployment (4 days of DO measurements every 15 minutes) was 2.10 mg/L and the average percent saturation was 26.35 % (**Figure 5**). The *in situ* DO reading taken during the SCI sampling was 1.38 mg/L.

Figure 4. Aucilla River at Highway 90 in August 2010 during a period of low flow.



Statistic	**DO (%)**	**DO (mg/L)**
Average	**26.35**	**2.10**
Median	**28.25**	**2.28**
Maximum	**37.90**	**3.00**
Minimum	**6.40**	**0.51**
StDev	**7.64**	**0.61**

Figure 5. Summary of DO measurements made in the Aucilla River during four days of continuous (every 15 minutes) measurements collected in August 2010 in conjunction with a passing SCI score indicating a healthy biological community.

The results of the 2005-2006 statewide DO study, as described in greater detail in Section 2 of this document, contain many additional examples of minimally disturbed freshwater systems (*i.e.,* lakes and streams) that commonly exhibit low DO levels below the current DO criteria due to natural conditions such as high shading, receiving significant drainage from wetlands or marshes, large spring or groundwater inputs, high temperatures, and low or no flow conditions. Most of these waters with naturally low DO levels have also been shown to support healthy biological communities. This information indicates that the existing DO criteria are inaccurate for many Florida waters and need to be revised to provide the appropriate level of protection to the State's freshwaters.

1.3.2 Marine Systems with Naturally Low DO Levels

Low DO conditions also commonly occur in many of Florida's marine (estuarine and coastal) waters. In a review of the DO conditions in Florida's estuarine and coastal systems, Windsor (1985) indicated that many of the state's marine waters commonly exhibited low DO levels that did not meet the 4.0 mg/L state water quality criteria. Windsor (1985) also acknowledged that in many cases the low DO conditions observed were due, at least in part, to natural phenomena. More recently, FDEP (2011) has compiled and reviewed available diel DO monitoring data from various estuaries and marine systems around the state. This data review also confirms that many of the state's marine waters have naturally low DO conditions during at least a portion of the year.

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). Additionally, many Florida estuaries are valued for their dense seagrass beds, which provide critical habitat for many fish and other organisms. The combination of photosynthetic DO production during the day and respiration at night can result in dramatic diel swings in DO concentration in the grassbeds, with the DO levels ranging from substantially below the current criteria at night (*e.g.,* < 2 mg/L) to well above the criteria during the day (*e.g.,* > 8 mg/L). As predicted by Henry's Law, low DO conditions commonly occur simultaneously with high summertime temperatures. Salinity also plays a role in the amount of DO in marine waters, in that higher salinity waters are proportionately lower in DO saturation.

DO concentrations in Fakahatchee Bay, located in a minimally disturbed, predominantly natural estuarine area (the Ten Thousand Island Aquatic Preserve) are frequently below the existing criteria. Fakahatchee Bay is surrounded by extensive mangrove forests and is located downstream of a predominantly undeveloped Everglades watershed, with more than 80% of the watershed consisting of conservation lands (*e.g.,* Fakahatchee Strand State Park, Cape Romano Aquatic Preserve, Big Cypress National Preserve).

The DO concentrations have been monitored continuously (*i.e.,* measured at 15 minute intervals) by the Rookery Bay National Estuarine Research Reserve since January 2002. The DO data collected in Fakahatchee Bay show the expected seasonal and daily fluctuations, with the DO concentrations being inversely related to water temperature (**Figure 6**). Note that during the summer months (mid-June through mid-September) none of the daily average DO concentrations met the current 5.0 mg/L criterion. For the period from January 2002 through May 2010, 37 % of the measured daily average DO concentrations were below the current 5.0 mg/L daily average criterion.

The bay DO data indicate that DO concentrations are also typically below the existing 4.0 mg/L instantaneous limit for nearly half of the day during the summer months (**Figure 7**). Based on the January 2002 through June 2010 period of record, approximately 21 percent of the measured DO concentrations were below the 4.0 mg/L criterion.

Despite the periodic low DO conditions, Fakahatchee Bay supports a very productive fishery as well as other biological (shellfish, sea grass, etc.) communities (FDEP 2000). However, as the result DO concentrations frequently being below both the existing 4.0 mg/L instantaneous limit and the 5.0 mg/L daily average DO concentration criterion, Fakahatchee Bay could erroneously be determined to be impaired for DO even though the observed DO levels represent natural DO conditions from a system with minimal anthropogenic input.

A similarly low DO regime was observed in the East Bay portion of the Apalachicola estuary system in northern Florida. East Bay receives an abundance of organic matter inputs from the Tate's Hell State Forest, a watershed predominantly consisting of swamp forest and wet pine flatwoods. East Bay is the epicenter of secondary productivity for the Apalachicola system, a bay renowned its beneficial yield of oysters, crab, shrimp, and finfish (Livingston 2010). In fact, 90% of Florida's oysters and 10% of the Nation's oysters are produced in Apalachicola Bay.

The DO concentrations in East Bay have been continuously monitored as part of the NERR Program since January 2002. **Figure 8** illustrates the daily average DO concentrations in East Bay over the period of record. As in Fakahatchee Bay in South Florida, the DO concentrations in East Bay are inversely related to temperature, with the lowest DO levels occurring during the late summer. The low DO concentrations in the surface waters during the summer are the result of both the higher water temperatures and increased rainfall, which transports larger amounts of organic matter from the natural forested area upstream of the bay, resulting in greater natural DO demand.

Over the eight year period of record, approximately 29 percent of the daily average DO concentrations in East Bay were below the current 5.0 mg/L criterion, with 18 percent of the individual DO measurements below the 4.0 mg/L instantaneous limit. Despite having minimal anthropogenic inputs (similar to Fakahatchee Bay), East Bay could erroneously be determined to be impaired based on the frequent DO levels below both the existing 4.0 mg/L instantaneous limit and the 5.0 mg/L daily average DO concentration criterion.

Additionally, the low DO concentrations in the bottom waters in East Bay are further exacerbated by the stratification that frequently occurs in the deeper water during the summer. During these periods, the water is not mixed vertically and the denser seawater settles to the bottom while the incoming freshwater remains near the surface. Most of the oxygen sources (*e.g.,* photosynthesis, and re-aeration, etc.) occur predominately in the surface waters, while most of the oxygen sinks occur in the bottom waters (the oxygen demand resulting from the respiration of bottom organisms and the decomposition of the organic material in the sediment). Due to the density gradient formed during stratification (which acts as a physical barrier), bottom waters become isolated from the oxygenated freshwater near the surface and DO concentrations in the bottom water can quickly become depleted.

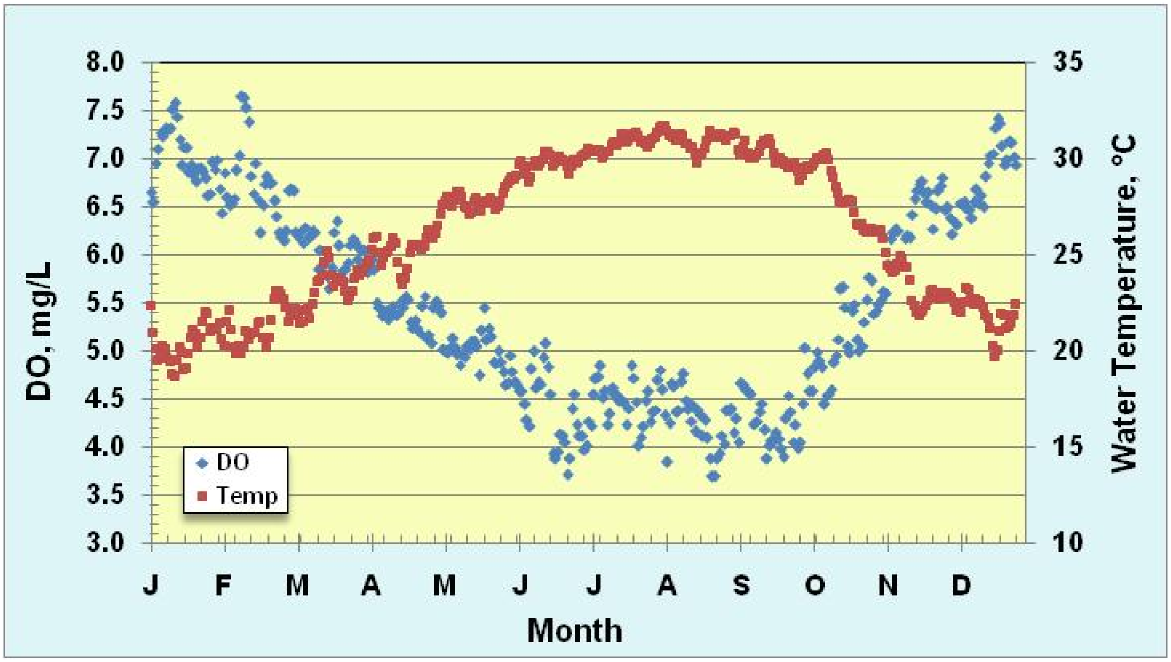


Figure 6. Average daily mean DO concentrations and water temperatures for Fakahatchee Bay calculated using data collected from January 2002 through May 2010.

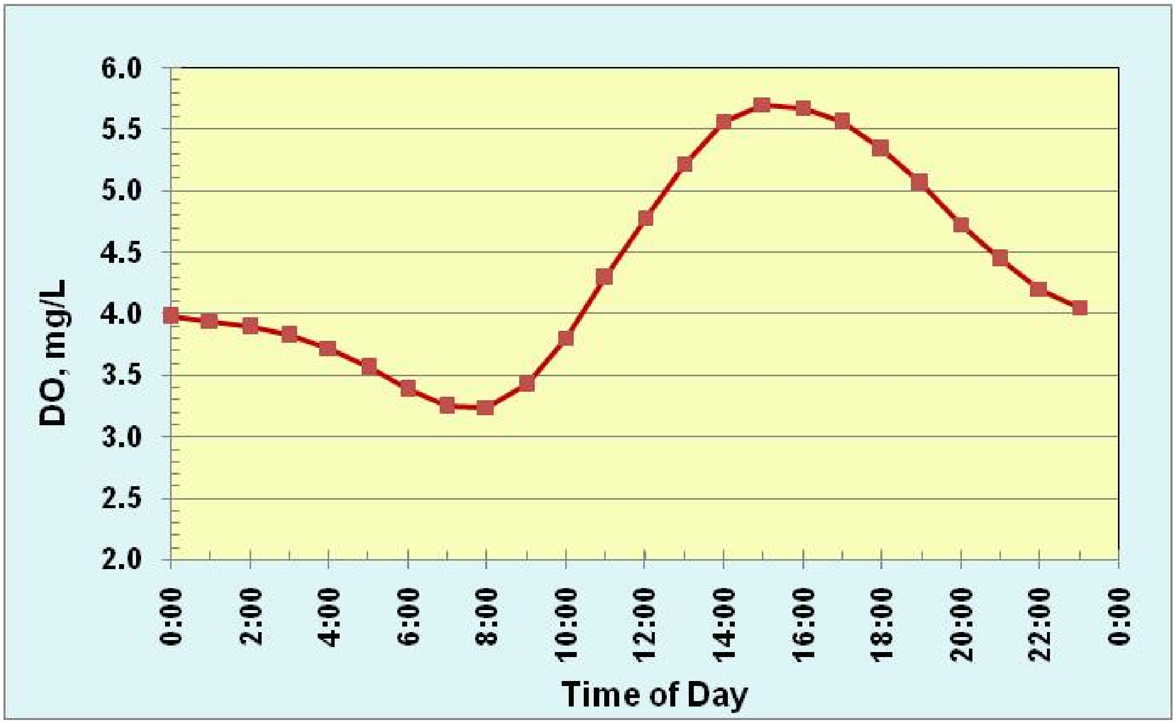


Figure 7. Average diel fluctuation in DO concentrations for Fakahatchee Bay during the summer months (June through September) calculated using data collected from January 2002 through May 2010.

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. For example, because drainage from natural wetlands, swamps, marshes, and mangrove forests typically contain high Colored Dissolved Organic Matter (CDOM) and elevated natural nutrient levels (especially, nitrogen), it is important to not erroneously identify nutrients as the pollutant responsible for low DO levels when the low DO is actually the result of natural conditions. Listing these waters as impaired may result in valuable resources to be erroneously expended in an attempt to rectify a natural condition. Conversely, when natural conditions limit available assimilative capacity (*e.g.,* for oxygen demanding substances), it is important to limit anthropogenic inputs (by means of permits or TMDLs) into the systems to prevent impairment.

Seagrass beds are a vital component of Florida's coastal ecology and economy that provide nutrition and shelter to animals that are important to marine fisheries, provide critical habitat for many other animals (*e.g.,* wading birds, manatees, and sea turtles), and improve water quality (Thayer *et al.* 1997, 1999; Livingston 1990; Kenworthy *et al.* 1988; McMichael and Peters 1989; Stedman and Hanson 1997; Valentine *et al.* 1997). A link has been established between seagrass abundance and the abundance of juvenile finfish and shellfish that is related to habitat structure (Heck *et al.* 2003). In systems where seagrasses occur, nearly all of the commercially and recreationally valuable estuarine and marine animals depend on seagrass beds as refuge or habitat for parts or all of their life cycles (Kikuchi and Peres 1977; Thayer *et al.* 1978, 1984; Kikuchi 1980; Ogden 1980; Thayer and Ustach 1981; and Wingrove (1999).

Short *et al.* (2000) list 13 important ecological services provided by seagrasses:

- Primary production (food for animals and support for fisheries and wildlife)
- Canopy structure (habitat, refuge, nursery, settlement and support of fisheries)
- Epibenthic and benthic production (support of food webs)
- Nutrient and contaminant filtration (improved water quality)
- Sediment filtration and trapping (improved water quality)
- Epiphytic substratum (support of secondary production, production of carbonate sediment)
- Oxygen production (improved water quality, support of fisheries)
- Organic-matter production and export (support of estuarine and offshore food webs)
- Nutrient regeneration and recycling (support of primary production)
- Organic-matter accumulation (support of food webs)
- Dampening of waves and currents (prevention of erosion/resuspension)
- Seed production/vegetative expansion (self maintenance of habitat)
- Self-sustaining ecosystem (recreation, landscape-level biodiversity)

Despite their critical beneficial roles in supporting various marine/estuarine communities, seagrass beds are characterized by wide diel fluctuations in DO concentrations due to extensive photosynthetic production during the day and respiration at night. The oxygen consumption through respiration commonly results in the DO concentration falling below the current 4.0 mg/L criteria for several hours per day. For example, within a seagrass bed in Sarasota Bay, which has experienced approximately a 50 percent increase in seagrass coverage in recent years, DO concentrations range from near 1 mg/L around sunrise to approximately 8 mg/L in mid-afternoon, with concentrations being below the current 4.0 mg/L criteria for 10 to 12 hours per day (**Figure 8**).

This observed low DO phenomenon (*i.e.*, natural waterbodies exhibiting DO levels below the existing DO criteria) is a common occurrence around Florida, as well as in other southeastern states. It is important to note that these naturally low DO waterbodies are able to maintain healthy biological communities and in many cases are highly productive critical habitat areas despite the low DO concentrations found during at least a portion of the year. Since Florida's existing DO criteria would erroneously indicate that these systems are impaired, despite supporting healthy biological communities and designated uses, it can logically be concluded that the existing criteria are subject to high Type I Error (erroneously concluding that a healthy site is degraded) and that more appropriate DO criteria should be developed.

1.4 *Peer Review*

The scientific information and approaches for revising the DO criteria presented in this document were reviewed by an expert panel (the DO Peer Review Committee), consisting of:

Dr. Jim Heffernan- Florida International University;
Dr. Kyeong Park- University of Alabama;
Dr. Tom Frazer- University of Florida;
Dr. Matt Cohen- University of Florida;
Dr. Douglas McLaughlin- National Council Air and Stream Improvement;
Dr. Robert Diaz- Virginia Institute of Marine Science;
Dr. Rich Batiuk- Environmental Protection Agency; and
Dr. Michael Kaller- Louisiana State University.

Responses to verbal peer review comments received during the first DO Peer Review Committee workshop are presented in **Appendix G**.

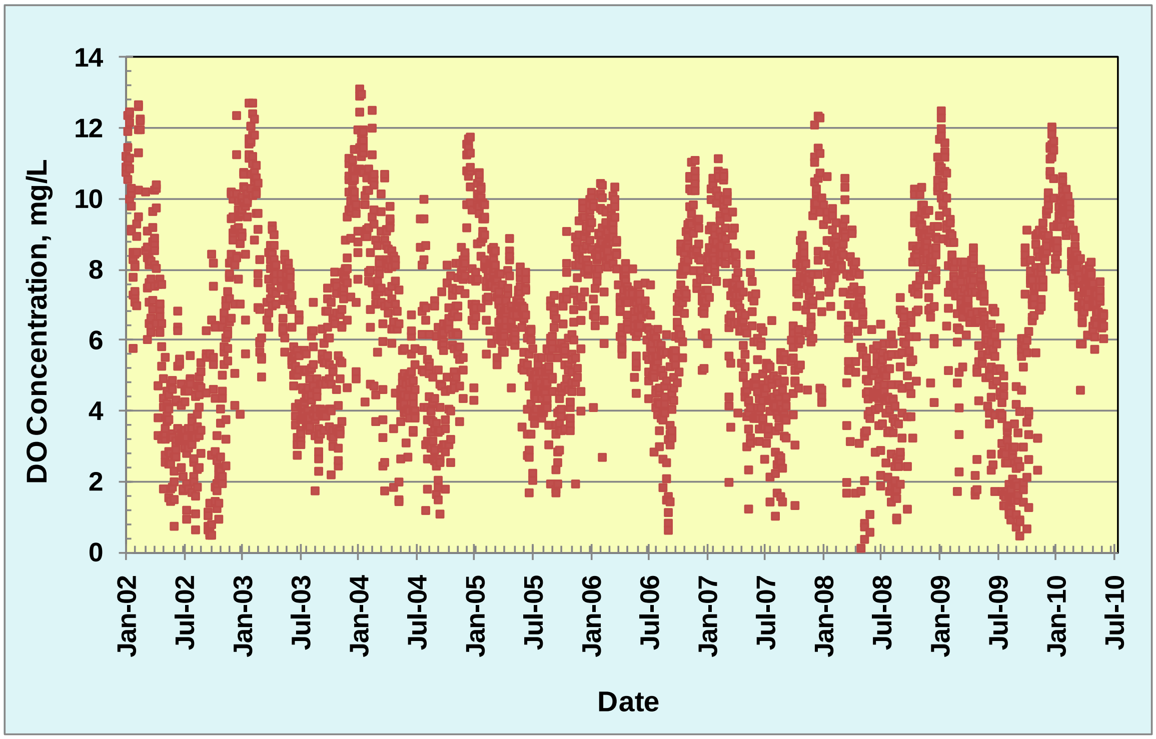


Figure 8. Daily average DO concentrations for East Bay (Apalachicola) during the January 2002 through June 2010 period of record.

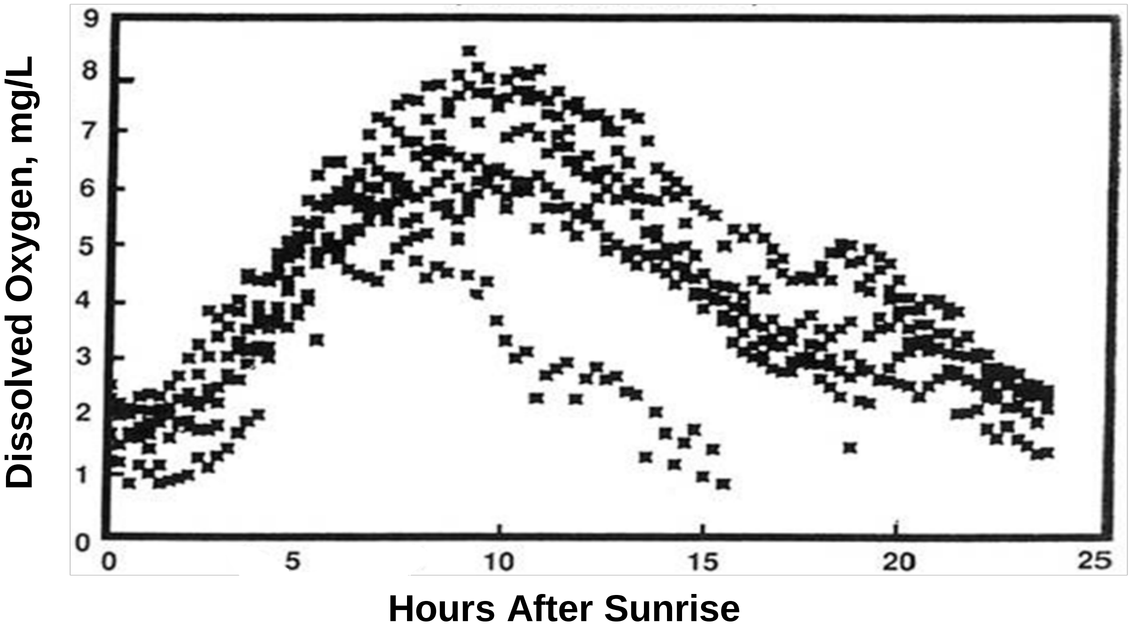


Figure 9. Dissolved Oxygen measurements collected over a seven-day period from a seagrass bed in Sarasota Bay (Tomasko *et al.* 1992).

2 Results of FDEP's Statewide Freshwater Nutrient and DO Study

To collect the data necessary to fully assess the accuracy of the existing DO criteria and to develop revised criteria for freshwater systems, FDEP conducted an extensive statewide nutrient and DO study during 2005 and 2006. The study consisted of quarterly sampling for one year at approximately 160 stream sites and 150 lake sites across Florida. The monitoring sites for the study were selected to represent the range of biogeochemical types and sizes of lakes and streams found in Florida. Additionally, sampling sites were chosen to provide a relatively even spatial distribution to assure regional differences were captured in the data collected. The study sites included both reference sites (those sites determined to be minimally affected by human disturbance, as evidenced by a landscape development intensity index (LDI) ≤ 2), and non-reference sites with a range human influence (*i.e.,* LDI > 2). The locations of the lake and stream sites monitored during the DO study are provided by bioregion in **Figure 10**.

The LDI used in the selection of study sites is a land use based index of potential human disturbance calculated using coefficients corresponding to specific land use categories within a drainage basin that could affect the water quality, hydrology, and habitat of a given site (Brown and Vivas 2003). Land uses are assigned an LDI coefficient based on the intensity of human activity determined from the level of non-renewable energy inputs. The coefficients were normalized on a scale from 1 to 10 with a coefficient of 1.0 representing natural lands and a coefficient of 10.0 being associated with the highest intensity landuses (*e.g.,* central business district or power plant).

The LDI is calculated as the area-weighted value of the land uses within an area of influence. Using the land use coefficients and the percent area occupied by each land use as determined by GIS landuse coverages, the LDI is calculated as follows:

$$LDI_{Total} = \Sigma\ (LDC_i * \%LU_i)$$

Where,

LDI_{Total} = Landscape Development Intensity Index (LDI) for the area of influence

$\%LU_i$ = percent of total area of influence in landuse i

LDC_i = landscape development intensity coefficient for land use i

Brown and Reiss (2006) identified an LDI break point of less than or equal to 2.0 to identify minimally disturbed reference sites with limited anthropogenic inputs and an LDI of greater than 2.0 to designate areas with increasing levels of human disturbance based on an evaluation of diatom, macrophyte and macroinvertebrate assemblages [*i.e.,* Florida Wetland Condition Index (FWCI)] in 193 depressional wetlands in Florida. Based on their analysis of the biological communities from areas grouped based on the LDI, they concluded that an LDI of 2.0 represented a very conservative break point between potentially disturbed sites and reference conditions. A more detailed discussion concerning the LDI and its use can be found in Section 7.2.1 of FDEP's Technical Support Document for the Development of Numeric Nutrient Criteria for Florida Lakes, Spring Vents and Streams which can be found at: http://www.dep.state.fl.us/water/wqssp/nutrients/docs/tsd-nnc-lakes-springs-streams.pdf.

Monitoring during the study included a) 4-day, (comprised of partial deployment day, three full days of monitoring, and partial retrieval day) multi-sensor (DO, temperature, pH, and conductivity) sonde deployments (with measurements at 15 minute intervals), b) water quality sampling for nutrients, color, chlorophyll, TOC, and turbidity, and c) conducting SCI, habitat assessment, and qualitative periphyton surveys. SCI, habitat assessment, and periphyton were only collected at stream sites, and phytoplankton samples were collected from lake sites. The biological sampling was conducted semiannually during two of the four quarterly monitoring events (*i.e.,* one dry season and one wet season).

To supplement the data gathered during the 2005-2006 study, 25 stream sites were monitored during 2010 using methods similar to those used during the 2005-2006 study. The 25 sites monitored during 2010 were selected to focus on streams that often exhibit DO conditions below the 5.0 mg/L current criteria, but support healthy macroinvertebrate community as evidenced by passing SCI scores. The locations of sites monitored during the 2005-2006 and 2010 DO studies are provided by bioregion in **Figure 10**.

Results of preliminary analyses of data collected during the 2005-2006 DO study indicate that many minimally disturbed sites exhibited a significant portion of the diel DO measurements (collected every 15 minutes) below the existing 5.0 mg/L criterion. **Table 2** indicates that approximately 70 percent of stream sites and 52 percent of the lake sites with an LDI score of 2 or less (indicating minimal anthropogenic inputs) exhibited more than 10 percent of the measurements below the 5.0 mg/L criterion. These minimally disturbed sites could erroneously be considered to be impaired and potentially listed for TMDL development, despite the fact that no anthropogenic pollutant sources were responsible for the low DO.

An interesting observation is that lake sites with minimal anthropogenic disturbance (*i.e.,* LDI ≤ 2) exhibited significantly lower average DO concentrations, and a greater percent of measurements below the 5.0 mg/L existing criteria, compared to sites with more human disturbance (**Table 1**). Stream sites did not exhibit this trend to the same extent, but there was a tendency for minimally disturbed sites to have more frequent DO concentrations below the current criteria than sites with greater anthropogenic inputs. It should be noted that the LDI used to distinguish reference and non-reference sites is based on land-use in a 100 meter buffer surrounding the waterbody and does not necessarily account for physical or hydrologic alterations to the systems. While the LDI is highly correlated with biological condition, it is not a direct measure of the biological health of the system.

The higher DO levels observed at sites with increased human disturbance likely results from greater productivity within the non-reference waterbodies. This conclusion is supported by the higher chlorophyll-a concentrations and larger average daily DO ranges found at the non-reference sites (**Table 2**) however, the cause of the increased productivity is less clear. Reference sites consistently exhibited higher color and TOC (total organic carbon) levels that may inhibit photosynthetic oxygen production and result in greater oxygen demand for the reference sites compared to non-reference systems. The differences in color and TOC levels between reference and non-reference systems may reflect less development around systems dominated by wetlands, which naturally produce and release high levels of color and TOC that often contribute to naturally low DO conditions. Differences in nutrient loading between reference and non-reference sites may also contribute to the observed differences in production and DO levels, even though no consistent trend in nutrient concentrations was observed.

To refine the above analysis, the DO regime at stream sites that have been demonstrated to support healthy biological communities (*i.e.,* sites with passing SCIs ≥ 40) in addition to being characterized by minimal anthropogenic influence (*i.e.*, LDI ≤ 2) was examined. Healthy stream sites with minimal human inputs also exhibited a significant portion of the diel DO measurements below the existing 5.0 mg/L criterion. More specifically, approximately 25 percent of the sites that pass the SCI and have an LDI index of 2 or less exhibited daily average DO concentrations (average daily average for the three full days during the deployment calculated using measurements collected every 15 minutes) below 5.0 mg/L (**Table 3**). For these same sites, approximately 31% of the daily minimum DO concentrations (average daily minimum for the three full days during the deployment calculated using measurements collected every 15 minutes) were below the 5.0 mg/L criterion.

The data summary (**Table 3**) also indicates that there are significant regional differences in DO levels. In general, the minimally disturbed sites exhibiting healthy biological conditions within the Panhandle bioregion (**Figure 10**) had the highest natural DO concentrations and the lowest number of measurements below the 5.0 mg/L current criteria, while the lowest DO concentrations and the highest number of sites not achieving the current criteria are observed in the Peninsula bioregion. However, there is considerable overlap in the natural DO ranges recorded across bioregions.

In addition to regional differences, there is significant seasonal variation in DO concentrations at minimally disturbed sites with healthy SCI scores (**Figure 11**). The observed seasonal variations are primarily in response to changes in water temperature. As illustrated in **Figure 11**, there is an inverse relationship between DO concentrations and water temperature. Since low DO conditions are exacerbated by increased temperatures, DO concentrations below the current 5.0 mg/L DO criteria most commonly occur between May and September in conjunction with high water temperatures, but can occur anytime of the year.

The summary of the results from the Statewide DO study confirm that the current DO criteria are not appropriate and do not accurately reflect the natural DO regime of many Florida streams.

Many of the Site Specific Alternative Criteria (SSACs) that have been established for Florida waters to acknowledge naturally low DO conditions have been based on the 10th percentile of measurements collected at minimally disturbed sites supporting healthy biological communities. The preliminary assessment of the data collected during the 2005-2006 DO study (**Table 3**) suggests that a revised statewide DO criterion established at the 10th percentile of the reference site DO measurements would be approximately 3.8 mg/L (or 44 percent saturation). However, this type of "reference site" method for developing criteria is not the preferred approach because a direct relationship between DO and the biological health of the system, which is necessary to derive an impairment threshold, is not defined. While criteria developed using the reference site approach are considered inherently protective of the reference condition, the exact level of protection afforded by the criteria cannot generally be determined since an accurate impairment threshold is not established. Due to these limitations, the reference site approach was not used to establish the revised DO criteria proposed in this document. Instead, the proposed criteria were based on further analyses that established direct relationships between observed DO levels and biological responses. The methods used to derive the revised DO criteria for Florida's fresh and marine waters are discussed in detail in Sections 4 and 5 of this document, respectively.

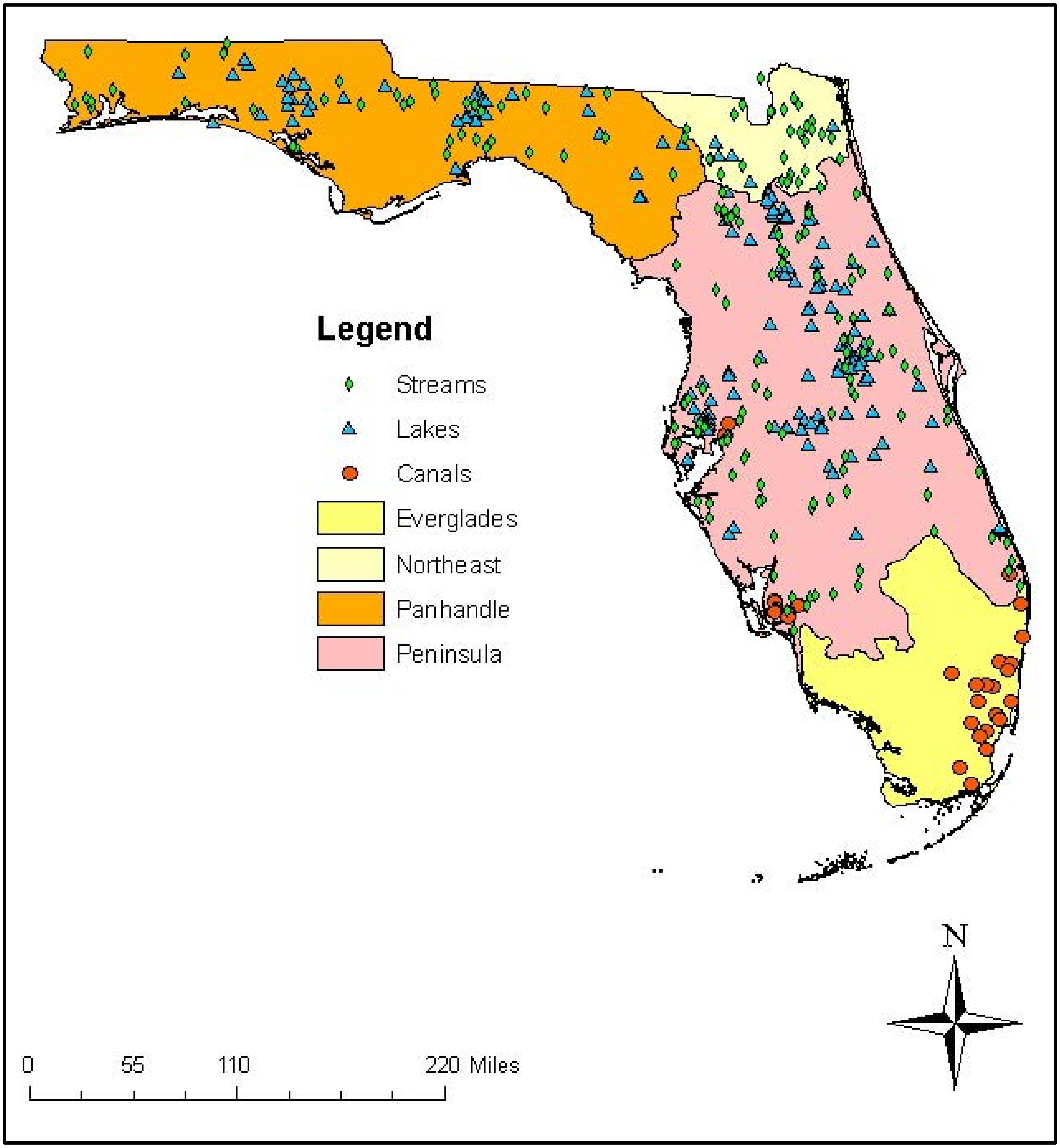


Figure 10. Location of sites monitored during either the 2005-2006 and/or 2010 statewide DO studies and the bioregions of Florida. *(Note: data collected from canal sites and sites within the Everglades bioregion were not used in the derivation of the proposed revised DO criteria.)*

Table 2. Results of preliminary analysis of DO data collected at lake and stream sites during the 2005-2006 Statewide DO Study.

Region	Waterbody Type	Reference*	Count	Average Deployment Minimum DO Concentration, mg/L	Deployment Average DO Concentration, mg/L	Deployment Average Maximum DO Concentration, mg/L	Average Daily Range, mg/L	Deployment Average DO Saturation, %	Average % Excursions from Current DO Criteria	% of sites with >10% Excursions	Average Total Nitrogen, mg/L	Average Total Phosphorus, mg/L	Average Chlorophyll-a, µg/L	Average Color, PCU	Average TOC, mg/L	Average SCI Score
Statewide	Lake	Non-reference	69	6.29	7.72	9.34	1.89	92.17	6.5	17.4	0.75	0.03	6.16	42.56	10.84	
Statewide	Lake	Reference	82	5.21	6.55	7.88	1.52	76.03	20.9	52.4	0.90	0.06	4.77	171.90	19.80	
Statewide	Stream	Non-reference	84	4.26	5.53	7.51	2.17	63.04	39.1	67.9	1.06	0.19	3.60	120.42	18.49	31.2
Statewide	Stream	Reference	80	4.80	5.58	6.72	0.93	61.10	39.0	70.0	0.97	0.08	1.46	208.19	24.86	46.4
Northeast	Lake	Non-reference	1	6.23	8.64	12.16	4.00	102.08	0.87	0.0	1.01	0.08	11.68	73.75	17.73	
Northeast	Lake	Reference	4	5.58	6.98	8.03	1.27	83.20	6.46	50.0	0.99	0.04	4.09	264.69	26.23	
Northeast	Stream	Non-reference	8	3.62	5.24	7.63	2.54	57.35	45.21	75.0	1.15	0.13	4.89	137.02	22.62	25.9
Northeast	Stream	Reference	18	4.99	5.74	6.79	0.69	61.52	35.99	72.2	1.10	0.09	1.01	323.37	38.34	55.7
Panhandle	Lake	Non-reference	14	5.64	7.41	9.33	2.38	85.61	13.41	35.7	0.66	0.03	7.78	59.06	9.88	
Panhandle	Lake	Reference	28	5.08	6.20	7.32	1.36	70.58	27.08	50.0	0.63	0.02	4.02	87.73	12.70	
Panhandle	Stream	Non-reference	11	5.16	6.35	8.07	1.93	69.03	25.70	54.5	0.65	0.07	5.89	82.14	9.64	29.1
Panhandle	Stream	Reference	27	5.45	6.32	7.43	1.06	67.87	28.69	51.9	0.67	0.06	1.15	121.49	13.26	45.7
Peninsula	Lake	Non-reference	54	6.47	7.79	9.29	1.72	93.75	4.78	13.0	0.76	0.03	5.62	37.56	10.96	
Peninsula	Lake	Reference	50	5.25	6.71	8.19	1.62	78.57	18.49	54.0	1.05	0.08	5.25	211.99	23.31	
Peninsula	Stream	Non-reference	65	4.19	5.40	7.32	2.10	62.30	40.99	69.2	1.11	0.22	2.60	125.93	19.51	32.3
Peninsula	Stream	Reference	35	4.17	4.90	6.10	0.94	55.36	49.10	82.9	1.13	0.09	1.94	219.53	27.46	42.4

* Reference classification based solely on LDI which estimates anthropogenic inputs based on surrounding land-use. The LDI does not account for physical or hydrologic alterations to the system and does not address the biological health of the system. Reference = sites with LDI ≤ 2, Non-reference = sites with LDI > 2

Table 3. Summary statistics for diel DO data collected during 2005-2006 statewide DO study at stream sites with LDI ≤ 2 and healthy biological community (SCI ≥ 40). Samples were collected quarterly over one year, under the temperature regime described in Figure 6.

Region	Statistic	Individual DO Measurements, Concentration,mg/L (% saturation)	Daily Average DO Concentration, mg/L (% Saturation)	Daily Minimum DO Concentration, mg/L (% Saturation)	Daily 10th Percentile DO Concentration, mg/L (% Saturation)	Daily Maximum DO Concentration, mg/L (% Saturation)	Daily DO Range Concentration, mg/L (% Saturation)	Average Total Nitrogen, mg/L	Average Total Phosphorus, µg/L	Average Color, PCU	Average Chlorophyll-a, µg/L
Statewide	Count	53280 (52992)	185 (184)	185 (184)	185 (184)	185 (184)	185 (184)	185	185	184	185
Statewide	Average	6.33 (68.57)	6.33 (68.57)	5.98 (65.38)	6.04 (65.96)	6.69 (72.56)	0.71 (7.18)	0.88	71.63	224.8	0.93
Statewide	Std Dev	1.94 (18.1)	1.9 (17.64)	1.89 (18.23)	1.89 (18.17)	1.91 (17.47)	0.6 (7.26)	0.59	94.30	202.4	2.04
Statewide	Minimum	0.87 (9.9)	1.15 (14.4)	0.96 (10.87)	1.03 (12.62)	1.71 (21.53)	0.06 (0.77)	0.07	4.00	5.0	0.10
Statewide	10th Percentile	3.81 (44)	3.9 (45.43)	3.62 (41.87)	3.68 (42.29)	4.06 (47.23)	0.2 (2)	0.08	4.00	5.00	0.10
Statewide	25th Percentile	4.92 (56.8)	5 (56.61)	4.5 (52.3)	4.59 (52.68)	5.23 (61.67)	0.34 (2.99)	0.43	19.00	60.00	0.10
Statewide	Median	6.5 (71.1)	6.59 (72.02)	6.16 (67.33)	6.23 (68.01)	6.95 (75.9)	0.5 (4.47)	0.80	41.00	150.0	0.50
Statewide	75th Percentile	7.72 (82.2)	7.73 (81.48)	7.34 (79.38)	7.45 (79.6)	8.07 (85.23)	0.92 (8.21)	1.17	93.00	350.00	1.10
Statewide	90th Percentile	8.83 (90.5)	8.79 (89.92)	8.47 (88.02)	8.55 (88.3)	9.05 (93.25)	1.42 (16.34)	1.56	154.40	517.50	1.80
Statewide	Maximum	13.84 (149.8)	10.91 (97.4)	10.48 (95.83)	10.57 (95.87)	11.17 (104.5)	3.81 (46.33)	3.80	794.00	1250.0	22.40
Statewide	Percent below 5 mg/L	25.82%	25.41%	30.81%	29.73%	21.08%					
Statewide	% Sites with >10% below 5 mg/L	30.81%									
Northeast	Count	15264 (15264)	53 (53)	53 (53)	53 (53)	53 (53)	53 (53)	53	53	53	53
Northeast	Average	6.03 (64.65)	6.03 (64.65)	5.75 (62.22)	5.8 (62.67)	6.29 (67.51)	0.54 (5.29)	1.09	92.46	352.8	0.62
Northeast	Std Dev	1.85 (16.97)	1.83 (16.81)	1.86 (17.67)	1.86 (17.47)	1.78 (16.12)	0.36 (3.76)	0.72	89.25	211.8	0.69
Northeast	Minimum	0.87 (9.9)	1.81 (20.25)	0.96 (10.87)	1.12 (12.62)	2.59 (30.4)	0.06 (0.77)	0.13	5.00	25.0	0.10
Northeast	10th Percentile	3.53 (40.2)	3.63 (40.87)	3.2 (37.85)	3.23 (38.42)	3.97 (43.86)	0.2 (1.9)	0.20	7.08	69.20	0.10
Northeast	25th Percentile	4.56 (53.2)	4.57 (53.61)	4.31 (51.93)	4.37 (52.68)	4.89 (58.2)	0.31 (2.7)	0.58	22.00	200.00	0.10
Northeast	Median	6.33 (67)	6.46 (66.6)	6.12 (63.27)	6.15 (63.47)	6.77 (72.07)	0.45 (4.27)	0.90	65.00	333.3	0.50
Northeast	75th Percentile	7.35 (78.6)	7.35 (79.46)	7.08 (76.67)	7.14 (77)	7.51 (81.03)	0.63 (6.1)	1.35	127.00	450.00	1.00
Northeast	90th Percentile	8.33 (83)	8 (82.64)	7.79 (81.52)	7.83 (81.67)	8.21 (84.13)	1.06 (11.08)	2.26	223.00	600.00	1.10
Northeast	Maximum	9.92 (95.4)	9.15 (92.66)	8.91 (91.23)	8.98 (91.33)	9.43 (94.3)	1.82 (19.53)	3.80	360.00	1250.0	3.60
Northeast	Percent below 5 mg/L	29.80%	30.19%	33.96%	33.96%	28.30%					
Northeast	% Sites with >10% below 5 mg/L	33.96%									

Table 3. Continued.

Region	Statistic	Individual DO Measurements, Concentration, mg/L (% saturation)	Daily Average DO Concentration, mg/L (% Saturation)	Daily Minimum DO Concentration, mg/L (% Saturation)	Daily 10th Percentile DO Concentration, mg/L (% Saturation)	Daily Maximum DO Concentration, mg/L (% Saturation)	Daily DO Range Concentration, mg/L (% Saturation)	Average Total Nitrogen, mg/L	Average Total Phosphorus, mg/L	Average Color, PCU	Average Chlorophyll-a, µg/L
Panhandle	Count	20736 (20448)	72 (71)	72 (71)	72 (71)	72 (71)	72 (71)	72	72	71	72
Panhandle	Average	7.43 (79.56)	7.43 (79.56)	7.04 (76.3)	7.12 (77)	7.83 (83.63)	0.79 (7.33)	0.64	59.98	105.6	1.04
Panhandle	Std Dev	1.68 (14.04)	1.62 (13.45)	1.6 (14.49)	1.61 (14.5)	1.62 (12.55)	0.63 (7.4)	0.45	118.43	122.4	1.88
Panhandle	Minimum	3.31 (38.6)	3.97 (47.39)	3.39 (39.63)	3.41 (39.87)	4.34 (50.73)	0.08 (1.23)	0.07	4.00	10.0	0.10
Panhandle	10th Percentile	4.95 (57.87)	5.03 (58.39)	4.81 (52.3)	4.84 (52.63)	5.55 (61.73)	0.2 (2.33)	0.09	4.00	10.00	0.10
Panhandle	25th Percentile	6.53 (73.2)	6.61 (73.29)	6.09 (66.85)	6.32 (69.22)	7.03 (78.13)	0.36 (2.95)	0.30	6.00	40.00	0.10
Panhandle	Median	7.48 (83.05)	7.49 (83.17)	7.18 (80)	7.23 (80.37)	7.78 (85.33)	0.64 (4.87)	0.52	24.50	60.0	0.50
Panhandle	75th Percentile	8.63 (90.3)	8.56 (89.87)	8.22 (88.02)	8.32 (88.28)	9.01 (93.08)	0.97 (8.17)	0.83	74.25	112.50	1.10
Panhandle	90th Percentile	9.51 (93.5)	9.42 (92.99)	8.88 (91.67)	8.95 (91.97)	9.84 (97.3)	1.42 (15.73)	1.44	118.90	250.00	1.80
Panhandle	Maximum	13.84 (149.8)	10.91 (97.4)	10.48 (95.83)	10.57 (95.87)	11.17 (104.5)	3.31 (40.3)	2.00	794.00	600.0	10.70
Panhandle	Percent below 5 mg/L	10.44%	9.72%	12.50%	11.11%	5.56%					
Panhandle	% Sites with >10% below 5 mg/L	12.50%									
Peninsula	Count	17280 (17280)	60 (60)	60 (60)	60 (60)	60 (60)	60 (60)	60	60	60	60
Peninsula	Average	5.27 (59.03)	5.27 (59.03)	4.91 (55.25)	4.97 (55.8)	5.68 (63.93)	0.77 (8.67)	0.98	67.23	252.6	1.06
Peninsula	Std Dev	1.61 (16.41)	1.56 (15.79)	1.55 (15.8)	1.54 (15.65)	1.62 (16.89)	0.72 (8.96)	0.49	57.09	192.8	2.86
Peninsula	Minimum	0.99 (12.5)	1.15 (14.4)	1.02 (12.8)	1.03 (12.92)	1.71 (21.53)	0.11 (1.1)	0.07	12.00	5.0	0.10
Peninsula	10th Percentile	3.13 (34.1)	3.15 (34.34)	2.96 (31.43)	3.07 (31.67)	3.66 (37.91)	0.19 (2.25)	0.08	14.36	5.00	0.10
Peninsula	25th Percentile	4.14 (48.1)	4.17 (49.83)	3.97 (46.28)	4 (47.32)	4.41 (53.03)	0.34 (3.26)	0.71	31.75	80.00	0.10
Peninsula	Median	5.38 (63)	5.38 (63.36)	5.02 (60.73)	5.03 (60.92)	5.67 (66.13)	0.48 (5.05)	0.98	48.00	200.0	0.50
Peninsula	75th Percentile	6.41 (71.1)	6.34 (72.1)	6.01 (67.19)	6.05 (67.34)	6.95 (75.28)	1.01 (11.23)	1.27	68.88	350.00	1.10
Peninsula	90th Percentile	7.26 (76.8)	7 (75.03)	6.7 (72.48)	6.73 (72.79)	7.65 (82.58)	1.67 (19.97)	1.49	140.15	532.50	1.62
Peninsula	Maximum	9.25 (109.9)	8.31 (79.22)	8.01 (77.4)	8.1 (77.83)	8.5 (94.47)	3.81 (46.33)	2.30	292.00	900.0	22.40
Peninsula	Percent below 5 mg/L	40.77%	40.00%	50.00%	48.33%	33.33%					
Peninsula	% Sites with >10% below 5 mg/L	50.00%									

Daily DO statistics based on three full-days of measurements collected every 15 minutes during each quarterly deployment.

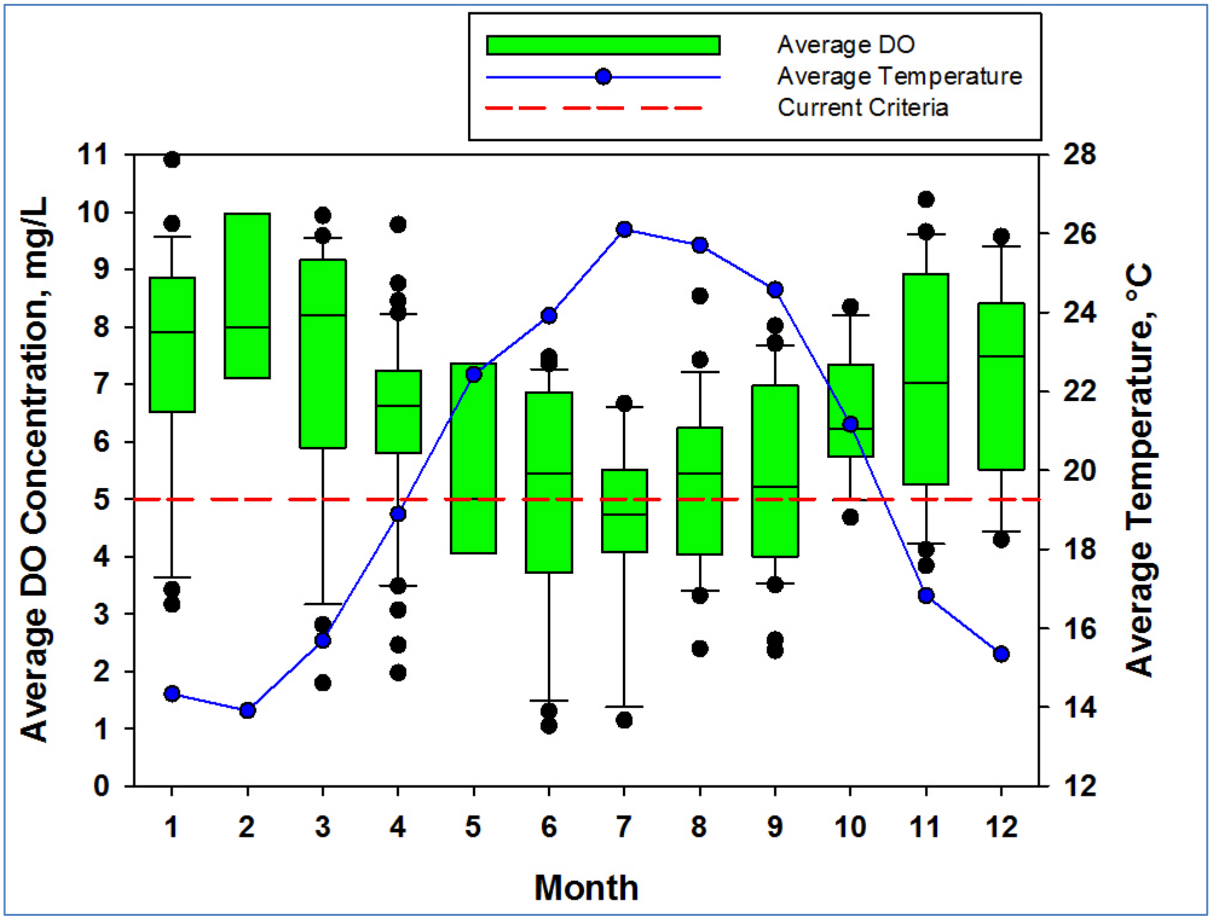


Figure 11. Average DO concentration and water temperature by month for minimally disturbed sites (*i.e.*, LDI ≤ 2) that also exhibit a healthy macroinvertebrate community (*i.e.*, SCI ≥ 40).

3 Development of SSACs

In recognition of the fact that the currently applicable water quality criteria may not be appropriate for some of Florida's waterbodies, Florida's Water Quality Standards allow for the development of Site Specific Alternative Criteria (SSAC) that more accurately reflect the levels required to maintain healthy biological communities under natural conditions (Rule 62-302.800, Florida Administrative Code). To be approved for a Type I SSAC, a petition must demonstrate that an alternative criterion is more appropriate for a specified portion of waters of the state and:

- Document that the proposed alternative concentrations that are different from the otherwise applicable Class III criteria exist because of natural background conditions;
- Establish the levels and duration of the naturally-occurring concentrations, and other parameters or conditions that may affect it;
- Describe the historical and existing biology, including variations that may be affected by the parameters in question;
- Show that normal fluctuations of an analyte are being maintained; and
- Show that the designated use is being attained and not adversely affecting adjoining waters.

Alternative DO criteria derived using the reference site approach (establishing the expected DO regime based on data collected at minimally disturbed waterbodies) are considered to be inherently protective. However, as no cause and effect relationship is identified, the exact level of protection provided by the reference site approach is not easily assessed and can vary based on a number of factors, including the appropriateness of the reference waterbody, the method used to derive the new criterion, and the sufficiency and robustness of data available.

To date, there are 13 State-approved DO SSACs for Florida waters that have been derived using various methods. Most of the currently approved DO SSACs have been Type I SSACs and have been derived using a reference site approach. Reference sites, also known as benchmark sites, are waterbody segments affected by only very minimal human influence, as described by an LDI of 2 or lower, optimal habitat (*e.g.,* >120 in streams), support healthy biological communities (*i.e.,* SCI ≥ 40), and little human modification of the system's hydrology.

The provisions for a Type II SSAC are more flexible since they allow for SSACs to be based on other "generally accepted scientific method or procedure to demonstrate with equal assurance that the alternative criterion will protect the aquatic life designated use of the water body". One Type II DO SSAC has been developed for the Lower St. Johns River estuary using the measured responses of multiple sensitive organisms exposed to low DO (a modification of the USEPA Virginian Province approach).

Examples of the DO SSACs developed for Florida's waters using various techniques are provided in **Appendix A**. The development of SSACs for individual waterbodies to address a global problem with the existing inaccurate DO criteria is both time consuming and costly. Details concerning the development of revised DO criteria that more accurately capture the natural conditions and biological DO requirements in Florida's fresh and marine waters are provided in Sections 4 and 5 of this document, respectively.

4 Development of Revised Freshwater DO Criteria

Extensive statewide DO monitoring has demonstrated that the current 5.0 mg/L DO criteria is not appropriate for a substantial number of Florida waterbodies due to natural conditions that result in DO levels below the existing criteria. To provide a comprehensive solution that provides an adequate and consistent level of protection, the statewide DO criteria should be revised using scientifically defensible methods. Additionally, the development of more appropriate statewide DO criteria would avoid the time and expense involved with the development of site specific criteria for all of the waterbodies for which the current 5.0 mg/L criteria is not appropriate. However, it should be noted that even after the revisions proposed in this document are adopted, site specific criteria would still be needed to address unique conditions in some locations.

4.1 *Use of Freshwater Stream Invertebrates*

The FDEP decided to rely on freshwater stream macroinvertebrates to determine protective freshwater DO criteria for both streams and lakes because research has shown that the DO requirements of species in flowing waters are higher than those of species in lentic environments, and that invertebrates are generally more sensitive to low DO than are fish. In a study of 35 lowland streams in southwestern Louisiana, Justus *et al.* (2012) found that biological thresholds based on taxa richness, species diversity, and total abundance were higher for invertebrates than for fish. This suggests that as DO levels decrease, biologically significant effects are observed in the invertebrate population prior to being observed in fish.

Fox *et al.* (1937) demonstrated that stream dwelling larval mayfly species (*Baetis*) were able to successfully tolerate low DO down to 2.8 mg/L, where as larval mayfly species such as *Leptophlebia* and *Cloeon* , which are found in lakes, could withstand 2.1 and 1.3 mg/L DO, respectively. The authors noted the effect of habitat on the oxygen sensitivity and oxygen uptake rates of many freshwater organisms, and concluded that lentic taxa were better adapted to low DO than their lotic counterparts. Based on field data, Hobbs and Hall (1974) concluded that crayfish found in lentic environments could tolerate lower DO than crayfish found in flowing streams, and based on experimental evidence, that pond and lake dwelling crayfish had better survival and lower mortalities in low DO than did lotic crayfish.

Stream macroinvertebrates as a whole, typically require higher DO for their growth and survival, and are generally more susceptible to decreases in DO concentrations, than are Florida fishes (Jim Estes, Fish and Wildlife Conservation Commission, personal communication, 2010). This is consistent with the work of Davis (1975), who demonstrated that macroinvertebrates required a higher DO concentration than fish. Davis (1975) provided comparisons of DO requirements for a variety of fish and invertebrates, and concluded that a DO regime designed to protect macroinvertebrates would also protect the fish that share their habitat as well. Although the Davis work was conducted in Canada, Florida warmwater fish species (*e.g.*, black bass) are generally less sensitive to low DO than coldwater species found in northern latitudes. It was shown in 1933 by Fox and Simmonds that certain species of mayfly nymphs (*Baetis rhodam*) and caddis larvae (*Hydropsyche sp.*) from a swift stream have a considerably higher oxygen consumption than nearly related and equal sized ephemerid mayflies (*Chloeon dipterum*) and trichopterids (*Limnopktlus vittatus*) from a pond, and that, within the confines of a single species of isopod crustacean (*Asellus aquaticus*), individuals from a swift stream consume more oxygen than animals from sluggishly flowing water (Fox and Simmonds 1933). The oxygen uptake was in each case measured under standard and similar conditions. Further evidence was obtained for the same phenomenon with other species of ephemerids and confirmed that species from rapidly flowing water have a higher rate of metabolism than those from stagnant water (Fox et al 1934).

Game fish such as largemouth bass and bluegill have been shown to be able to survive at DO levels as low as 0.92 and 1.5 mg/L, respectively (Moss and Scott 1961). Dudley *et al.* (1975) discovered high mortality of largemouth bass hatchlings at a DO concentration of 1.0 mg/L and complete mortality when levels were lower. Coutant (1985) and Krouse (1968) suggested striped bass could survive at DO levels as low as 3.0 mg/L. As the DO concentrations fall below 3.0 mg/L, the fish become increasingly stressed Coutant (1985) with mortality occurring when DO concentrations reached 1.0 mg/L (Krouse, 1968).

Campbell and Goodman (2004) and Jenkins *et al.* (1995) studied juvenile shortnose sturgeon, an endangered species, under controlled laboratory conditions. Their tests concluded that high mortality occurred in the species when DO concentrations were 2.7 mg/L or less for fish less than 3 months old. Three to five month old fish did not survive when levels were 2.2 mg/L. Moss and Scott (1961) found that the critical DO level for survival for channel catfish was 0.95 mg/L. Similarly, Carlson *et al.* (1980) found significant reductions in growth of channel catfish at 1.0 mg/L. However, Andrews *et al.* (1973) found that channel catfish experienced significant reductions in growth when DO levels were below 3.0 mg/L.

While the DO levels associated with significant mortality in warm water fish species is usually quite low, generally in the range of 2 mg/L or less, there are numerous reports of laboratory studies that indicate some fish species can have sub-lethal effects such as changes in behavior, reduced growth rates, increased energy expenditure, and changes in reproductive behavior at higher DO levels (Brown-Peterson *et al*. 2005). Based on a review of available literature on the DO levels associated with sub-lethal effects, EPA (1986) identified a response threshold between 5 and 6 mg/L for non-salmonoid, warm water, freshwater fish. However, it is important to acknowledge, as EPA (1986) does in their guidance, that in many locations and times (such as Florida blackwater stream systems, groundwater dominated systems, or naturally stratified marine waters) natural conditions do not attain the sub-lethal effects DO response threshold. In these cases, the DO levels may not be optimal, but the organisms may have adapted to the naturally lower DO levels, or the organisms simply would not be expected to occur under the natural DO regime.

More recent studies (Brown-Peterson, et al., 2011) have provided evidence that highly controlled small-scale laboratory studies do not adequately mimic field conditions or responses. Many of the laboratory experiments conducted have not considered all of the factors that influence the biological response under field conditions such as flow, diel DO cycles, or adaptive mechanisms. Additionally, these studies indicate that organisms from the same species but from different locations can have dramatically different response to exposure to low DO levels. This finding suggests that organisms may possess a mechanism by which they can adapt to naturally low DO levels and do not exhibit sub-lethal effects until lower DO concentrations. Kaller *et al.* (2008) also demonstrate how the fish communities of Louisiana streams (similar to those in Florida) have become adapted to living in low DO, low flow, warm water environments. These results help explain how many of Florida waterbodies having low DO levels (naturally below the specified 5 – 6 mg/L sub-lethal response threshold) continue to support healthy biological communities.

In aquatic systems where the natural DO levels are above the sub-lethal effects level, lowering the DO levels below the threshold may induce adverse effects on some sensitive species within the community, which may in turn affect biological community structure and function. To protect against this from occurring, the revised DO criteria includes a provision that requires the continued maintenance of the existing DO regime in aquatic systems having DO levels naturally higher than the minimum criteria. A description of the provision that would require that naturally high DO levels be preserved is provided in Section 8 of this report. This inclusion of this provision will not allow the DO concentrations in waterbodies with naturally high DO levels to decrease to levels where additional sub-acute effects to sensitive populations may occur (*i.e.*, beyond those that may occur under the natural DO regime).

Because Florida invertebrates, as a group, are more sensitive to low DO than are Florida fishes, FDEP explored using the multi-metric Stream Condition Index (SCI) macroinvertebrate tool to determine the DO level that is sufficient to support a healthy, well-balanced community. Although there may be examples of extremely tolerant species of stream invertebrates (*e.g.,* "blood worms") that can tolerate DO concentrations below those of many fish species (Landman *et al.* 2005; Surber and Bessey 1974; Connolly *et al.* 2004), it should be noted that the SCI metrics recognize the differences in individual species sensitivity and tolerance, and are scored accordingly (see below), to assure that a balanced community, including reproducing populations of representative sensitive taxa, are present in streams with scores greater than 40. The sensitive taxa assessed during the SCI scoring process include a wide variety of plecopterans, trichopterans, empheropterans, odonates, etc., as listed in LT 7000, found at: http://www.dep.state.fl.us/water/sas/sop/sops.htm. This sensitive taxa list was produced by evaluating the responses of 1,195 Florida taxa to a human disturbance gradient (Fore 2007), and is the most comprehensive list of sensitive taxa for Florida.

Several authors have suggested that freshwater stream invertebrate communities in low gradient Gulf Coast streams tend to be comprised largely of generalists (Adams *et al.* 2004; Williams *et al.* 2005; Johnson and Kennedy 2003; Kaller and Kelso 2007). Invertebrate communities with high proportions of generalists are able to respond with more plasticity to highly variable environmental conditions. Whereas most fish species will migrate in and out of an area depending upon the current conditions, benthic invertebrates typically remain sessile until conditions approach lethal limits, at which point those species with the capacity to do so will attempt to drift out of the affected area (Connolly *et al.* 2004). Based on field observations by DEP staff, it is not uncommon for fishes to be absent or rare in many of the natural, small and shallow stream segments sampled by FDEP even though these streams support healthy, well-balanced benthic macroinvertebrate communities. Because the invertebrates are more representative of the resident community than are the transient fish populations, it is logical to develop water quality criteria to protect the macroinvertebrate community. Additionally, since the number of sensitive macroinvertebrate taxa is an important metric within the SCI, and because stream segments with passing SCI scores generally contain multiple sensitive species, it is reasonable to utilize the SCI to develop water quality criteria to protect the benthic invertebrate community in streams, and subsequently, apply the criteria to lakes.

Greater detail concerning the development and application of the SCI can be found in **Appendix B** of this document and in the DEP guidance document "*Sampling and Use of the Stream Condition Index (SCI) for Assessing Flowing Waters: A Primer*" (FDEP 2007), which can be found at: http://www.dep.state.fl.us/water/sas/qa/docs/62-160/sci-primer-102411.pdf

4.1.1 Relationship between SCI metrics and DO Saturation

Since the response of the SCI to DO levels is the basis of the derivation of the proposed freshwater DO criteria presented in this Technical Support document, it is important to establish that there are significant predictable relationships between at least some of the component metrics and DO levels that result in the overall response of the SCI to DO used to derive the proposed criteria.

The relationships between the 10 individual macroinvertebrate metric that comprise the SCI and DO levels were examined and are presented in **Appendix C**. Because of the known regional differences in biological expectations that are incorporated into the SCI as well as observed spatial differences in DO levels, the relationships between the SCI metrics and DO were examined separately for the Panhandle West, Panhandle East + Big Bend, and Peninsula bioregions.

Predictably, the observed relationships varied across regions and by metric. Generally, the strongest responses to DO levels were for metrics that were measures of the pollution sensitive portion of the macroinvertebrate community such as; number of sensitive taxa, number of clinger taxa, and number of Ephemeroptera (mayfly) taxa. All of these metrics exhibited a positive response to DO, as expected, with the number of sensitive taxa increasing with increasing DO levels. Spatially, the strongest relationships between the metrics indicative of the pollution sensitive taxa and DO levels were generally found in the Panhandle West bioregion where the biological expectation is higher and a greater number of sensitive organisms are typically found in conjunction with higher DO levels. In contrast, metrics that describe portions of the community that are more pollution tolerant such as percent very tolerant individuals, and percent dominant taxa exhibited less significant responses to DO and tended to decrease with increasing DO levels (**Appendix C**).

The results of the evaluation of the individual SCI metrics followed expected patterns and confirms that the macroinvertebrate community is responding to DO levels and that the relationships between the SCI scores and DO levels are not the random result of a combination of the individual metrics. This finding supports the use of the SCI versus DO relationships in the derivation of the proposed freshwater DO criteria described in this document. Even though some of the individual metrics exhibit stronger relationships with DO than does the composite SCI, they were not directly used in the derivation of the criteria since no impairment threshold has been indentified for the metrics.

4.2 *Derivation of Revised DO Criteria for Florida's Freshwaters*

4.2.1 Initial Analyses of State-Wide DO/Nutrient Study Data

Initial regression analyses of the daily average DO data collected during the 2005-2006 and 2010 statewide DO/nutrient studies revealed that the invertebrate SCI score responds positively to increasing DO concentrations (**Figure 12**), as expected, since macroinvertebrates require some level of DO for survival and can become stressed at low DO levels above those needed for survival. To avoid biasing the results, the daily average DO concentrations were calculated using only the three full days of measurements within the four-day deployment (initial partial deployment day + three full days + partial retrieval day). The average DO concentrations were paired with the SCI score collected during that deployment for the regression analysis. Because the invertebrate community in natural systems is influenced by many other factors in addition to DO (*e.g.,* conductivity, habitat availability, flow regime, other pollutants), the ordinary least squares regression relationship between all of the SCI scores and DO concentration is statistically significant, but only explains a small portion of the SCI variability among streams, as indicated by a relatively low coefficient of determination ($r^2 = 0.218$) (**Figure 12**).

To allow a clearer assessment of the SCI response to DO levels, a number of steps were taken to reduce the variability in the data and improve the relationship. First the data from the 2005-2006 and 2010 DO studies were screened to minimize the influence of other anthropogenic and natural confounding factors known to affect the invertebrate community and the SCI score. Sites used to further explore the relationship between the SCI score and DO concentration were limited to sites having Habitat Assessment (HA) scores greater than 110 points (on a scale of 8 to 160, with 8 being the worst possible habitat and 160 being the best possible habitat), Landscape Development Intensity (*i.e.,* LDI) Index scores of two or less (on a scale of 1-10 with one being totally undisturbed and 10 being a highly urbanized stream), conductivities less than 300 µmhos/cm, and nitrate-nitrite concentrations less than 0.35 mg/L.

The nitrate-nitrite screening level was based on the 0.35 mg/L criteria for springs developed by FDEP and adopted by the USEPA. The LDI threshold of two or less was previously developed and used by FDEP in the development numeric nutrient criteria and has been accepted by the USEPA as generally indicative of minimally disturbed sites with limited anthropogenic inputs. The screening thresholds for conductivity and habitat assessment scores were established at levels that were found to no longer adversely affect the SCI score.

To minimize errors caused by variability in the SCI scores, sites with two SCIs performed during the same year that varied by more than 20 points were assessed to determine if sampling errors (*e.g.,* samples collected during rapid fluctuations in water level, or during periods of no flow, etc) likely resulted in abnormally high variation between the samples. The 20 point threshold was selected based on the known variability of the SCI measurements. Any SCI data associated with samples found not to be collected in accordance with the FDEP SOPs and SCI primer (FDEP, 2007) were omitted from further analyses.

The results of the regression analysis using the screened data indicates a slightly improved relationship that was statistically highly significant ($r^2 = 0.252$, $p = 0.001$) as shown in **Figure 13.** This relationship provides further support that sites with daily average DO concentrations well below the current criterion of 5.0 mg/L can still support healthy macroinvertebrate communities, as evidenced by acceptable SCI scores.

Since the preliminary analysis of the data from the statewide study indicated significant regional differences in the DO concentrations observed, the regression analysis was repeated using regional datasets to further assess any spatial differences in the SCI versus DO relationship. The regional datasets were created by separating the screened data by the same three bioregions (*i.e.,* northeast, panhandle, and peninsula) utilized in the calculation of the SCI scores, as illustrated in **Figure 13**.

The relationships between the SCI scores and daily average (*i.e.,* average of three full days of deployment) DO concentrations for the individual bioregions are provided in **Figure 14**. The results of the regional regression analysis suggest that there are regional differences in the SCI versus DO relationships with the Panhandle bioregion requiring slightly higher DO levels to support healthy macroinvertebrate communities (*i.e.,* SCI scores ≥ 40) compared to the Peninsula and Northeast bioregions. This finding is consistent with the regionalization of the SCI that requires more sensitive organisms in the panhandle to achieve a passing SCI score.

While the data screening and regionalization improved the relationship between the SCI scores and DO concentrations, the coefficients of determination (r^2 values of 0.24 - 0.50) explained only a portion of the variation. However, temporally matched short-term DO and SCI data do not adequately account for the variability in DO concentrations, which change in response to a number of factors (*e.g.,* natural diel cycle, temperature, flow) more rapidly than changes in either the invertebrate community or SCI score. In other words, the invertebrate community does not respond instantaneously to changes in DO concentration (or other non-lethal parameters). Instead, the invertebrate community present in a waterbody represents an integration of the environmental conditions (including DO concentrations) over an extended period.

Attempting to improve the SCI versus DO relationships by minimizing the variability caused by the natural short-term fluctuations in DO concentrations, the relationships were re-examined using data averaged over longer periods. **Figure 15** provides the relationship between the averages of the SCI scores versus the daily average DO concentrations by individual bioregions. The results indicate that averaging the data over longer periods improves the relationship considerably for the Panhandle and Peninsula bioregions. The relationship for the Northeast bioregion decreased slightly; however, given the limited range in SCI scores, especially below 40, the reliability of the relationship using the averaged data for that region is questionable.

The stronger relationships found using the average SCI and average DO data confirm that DO is a significant factor determining the biological health of the system. The annual average SCI versus DO relationships indicate that annual average DO concentrations between 3.0 and 5.5 mg/L are generally required to support healthy macroinvertebrate populations depending on the region. Revised DO criteria could be developed using these stronger relationships, however, establishing DO criteria as annual averages is not consistent with the planned application of the criteria and would probably not provide adequate protection to the sensitive biological communities. Since there can be substantial seasonal variations in DO levels, annual average DO criteria would not protect against shorter term low DO conditions that could adversely impact biological communities. Due to this limitation, the averaged SCI and DO data were not utilized to derive the proposed revised DO criteria. Additional efforts to minimize variability in the SCI versus DO relationships using other techniques are described below.

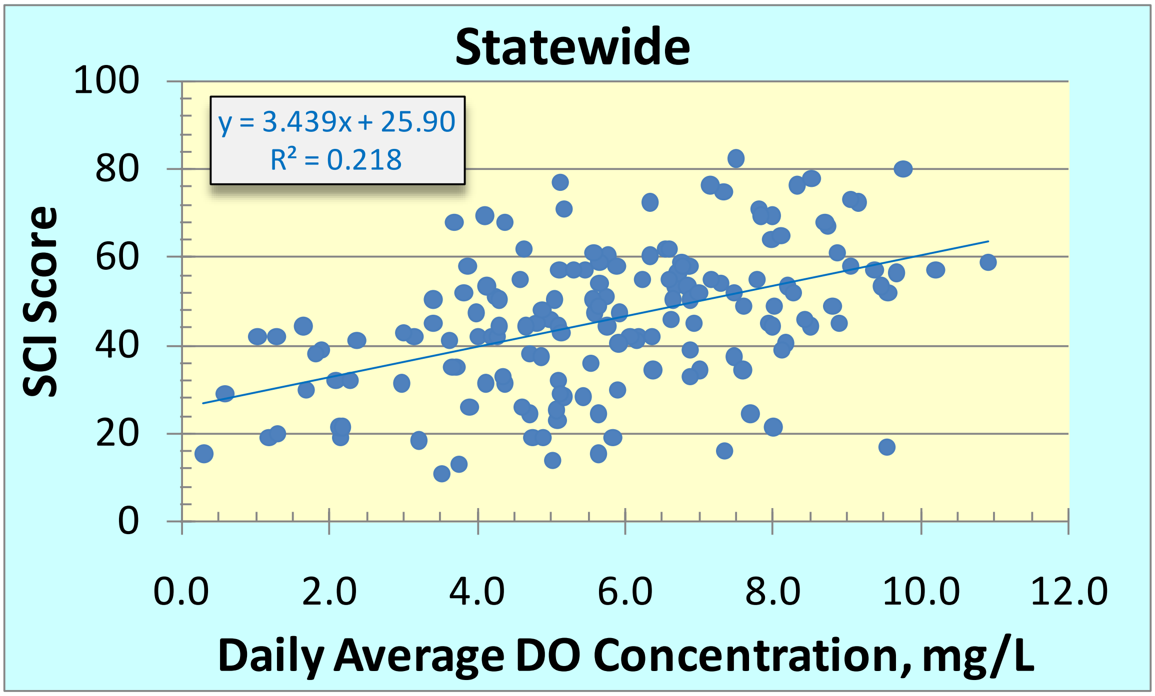


Figure 12. Relationships between SCI and average daily DO concentrations at statewide sites with LDI ≤ 2. Data collected during 2005-2006 and 2010 DO studies.

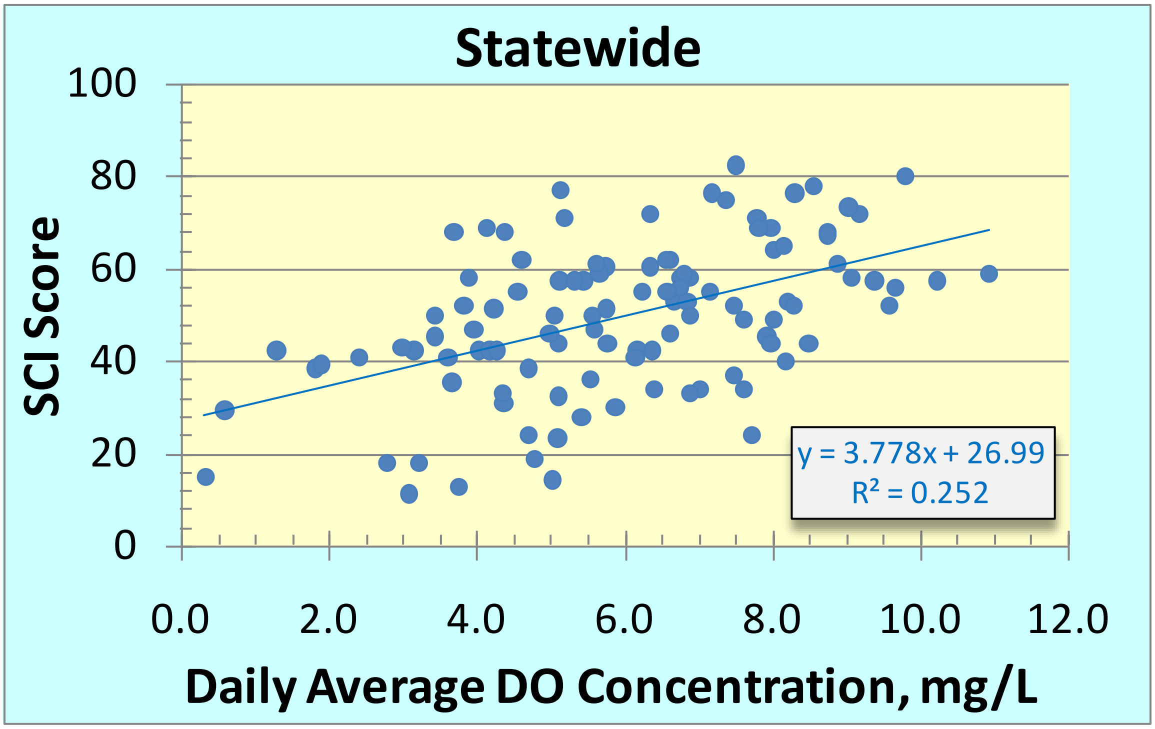


Figure 13. Relationships between SCI and average daily DO concentrations for statewide sites. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment ≥ 110, NOx ≤ 0.35 mg/L).

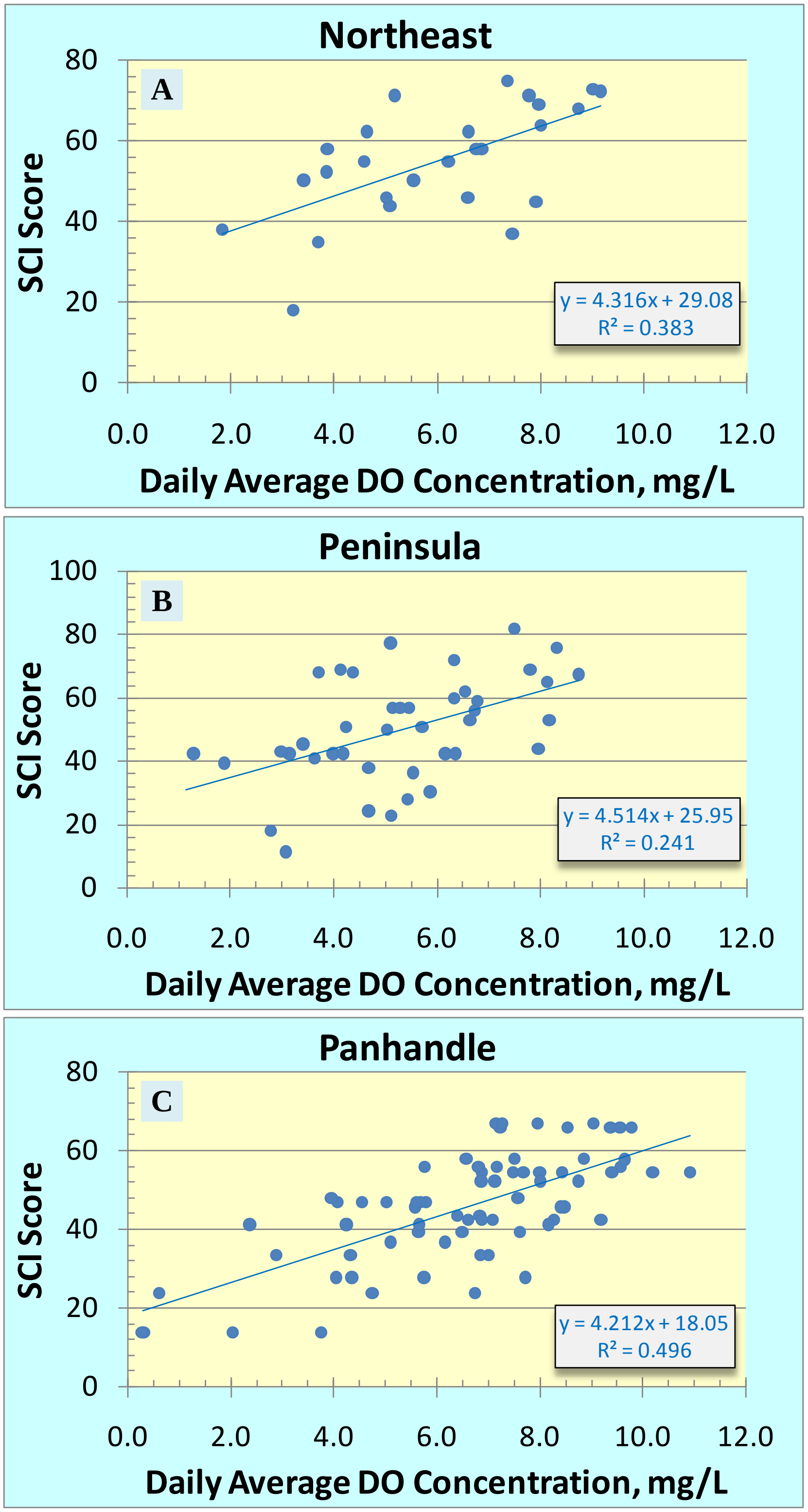


Figure 14. Regional relationships between SCI and average daily DO concentrations for A) Northeast bioregion, B) Peninsula, and C) Panhandle sites. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment ≥ 110, NOx ≤ 0.35 mg/L).

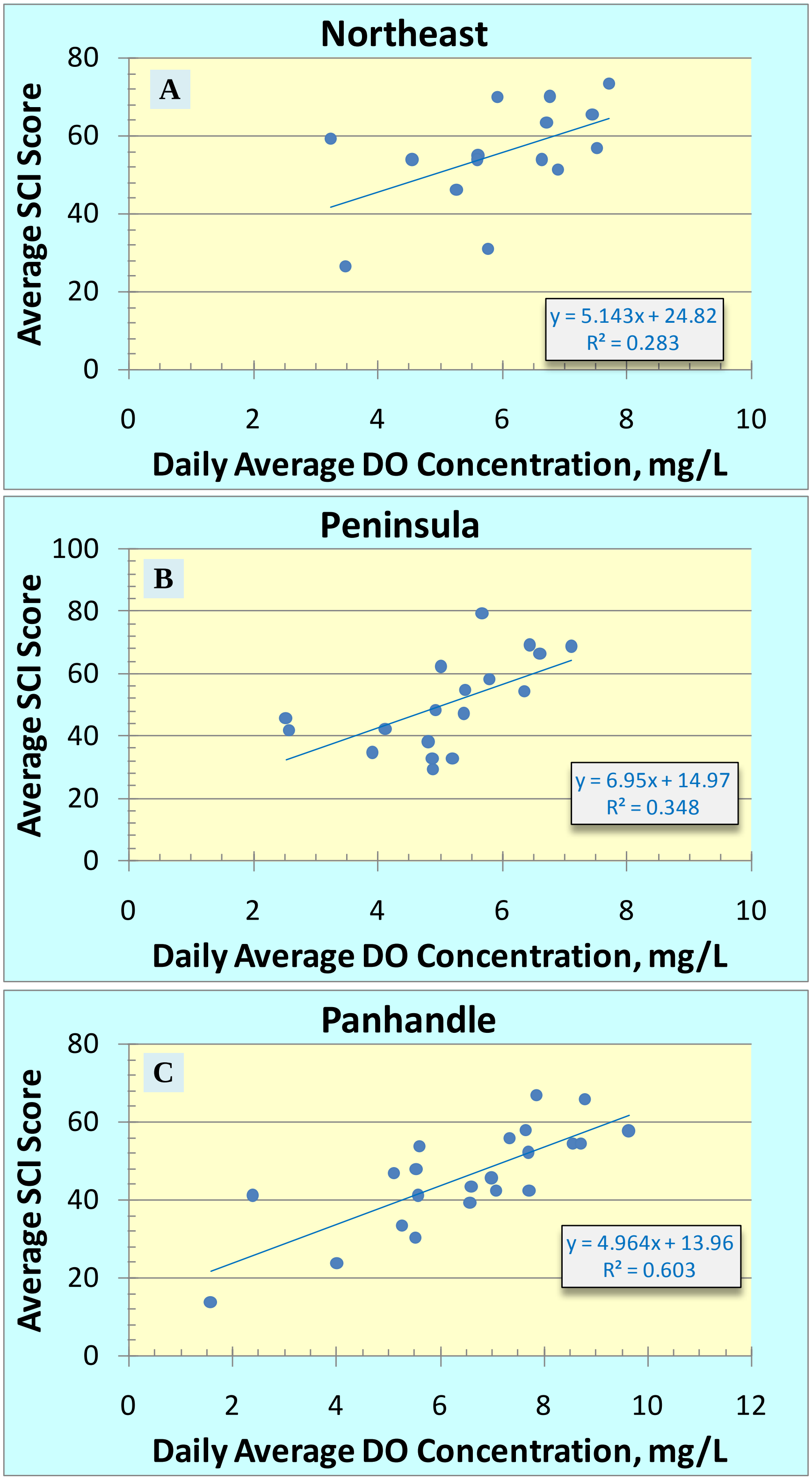


Figure 15. Regional relationships between annual average SCI scores and average DO concentrations for A) Northeast bioregion, B) Peninsula, and C) Panhandle sites. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment ≥ 110, NOx ≤ 0.35 mg/L).

4.2.2 DO Versus Temperature Relationship

To further refine the SCI versus DO relationship, factors that influence the short-term changes in DO concentrations can be taken into account or included in the analyses. From Henry's Law and the Ideal Gas Law, it is known that the expected concentration of DO in water is dependent on temperature, with the DO saturation concentration decreasing with increasing water temperature. The empirical DO saturation versus temperature relationship for water has been studied extensively and is well understood (Benson and Krause 1984).

Data from minimally disturbed (*i.e.,* LDI ≤ 2) sites that support healthy biological communities (*i.e.,* SCI ≥ 40) were used to determine the expected DO versus temperature relationship for natural streams in Florida. **Figure 16** provides the DO versus temperature relationships for minimally disturbed Florida streams that support healthy invertebrate communities using three full day deployment average DO and temperature levels. This figure also provides the empirical DO saturation versus temperature relationship for comparison. Since streams in the southeastern US rarely approach DO saturation naturally (see Kaller and Kelso 2007), the DO versus temperature relationship observed in "reference" streams is not expected to match the empirical relationship. Instead, the observed stream relationship is expected to be offset downward to some extent, but follow the same general trend with DO concentrations decreasing as stream temperature increases. The lines of best fit through the center of the data are offset from the empirical relationship, but have similar slopes as the empirical line, indicating that the observed DO concentrations are responding, as expected, to changes in water temperature. The noise in the relationships results from the differences in other factors that influence the DO concentrations besides temperature (*e.g.,* re-aeration, photosynthetic activity, respiration, and natural oxygen demand) across sites.

While the DO versus temperature relationship derived on reference site conditions could be used to develop DO criteria, DEP prefers criteria with a direct linkage to biological response. Deriving criteria by using the reference approach alone (*i.e*., without biological confirmation) may result in a high Type I error rate when the criteria are applied. Alternate analyses were conducted to link the DO versus temperature relationship to healthy biology (SCI >40) are described below.

4.2.3 Multiple-Linear Regression Analysis

To directly relate the observed DO versus temperature relationship to biological health of the system, a multiple-regression was conducted between SCI (dependent variable) and DO and water temperature. As described for the previous analyses, the data used in the multiple-linear regression were screened to minimize the effects of natural and anthropogenic factors on the SCI score and DO concentrations. The SCI scores in the screened dataset were paired with the average daily (*i.e.,* average of three full days) DO concentrations for the deployment in which the macroinvertebrate samples were collected.

The results of the analyses indicated a statistically highly significant ($p < 0.001$) relationship. The equation resulting from the regression analysis can be expressed as:

$$\mathbf{SCI = (4.60 * DO) + (0.58 * Temperature) + 10.71}.$$

Since the healthy biological community threshold for the SCI is a score of 40, the equation above was solved for DO after inserting a SCI score of 40. Solving the equation provides a DO versus temperature relationship that is directly tied to a supported healthy biological community. The solved equation is:

$$\mathbf{DO = (-0.126 * Temperature) + 6.35}$$

The solved equation is plotted in **Figure 17** along with the empirical DO saturation versus temperature curve and the data from the original DO-temperature relationship that passed the SCI for comparison. The equation has a slope similar to the empirical DO versus temperature relationship, indicating that the predicted DO concentrations are responding to temperature, as expected. At a given temperature, sites with DO concentrations higher than the level calculated by the equation would be considered to support healthy biological conditions, while sites with a DO concentration below the level predicted by the equation would be less likely to support healthy conditions. **Figure 17** also indicates that a DO criterion based on this equation would have a low Type I error rate, with approximately eight percent of the sites exhibiting healthy macroinvertebrate communities falling below the line predicted by the equation.

The multiple regression approach assumes that DO and temperature are both independent variables. However, this may not be a valid assumption, since DO concentration is inherently related to temperature as described by Henry's Law and the Ideal gas law. Therefore, the validity of the results can be drawn into question. Due to this issue, the results of the multiple-linear regression were not used to derive the final proposed DO criteria. Further analyses using alternative methods of incorporating the effect of temperature on DO levels into the SCI response are provided below.

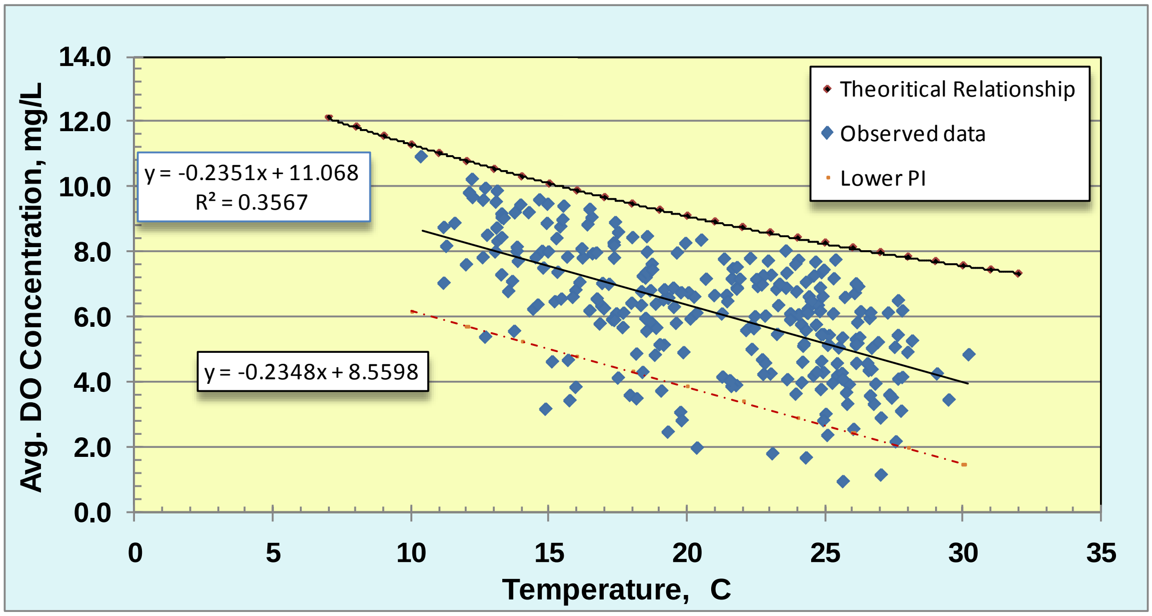


Figure 16. Daily average water temperature versus daily average DO concentration relationship for sites with passing SCI from 2005-2006 DO study with lower 90% prediction interval. Empirical DO concentration versus water temperature relationship also provided for comparison.

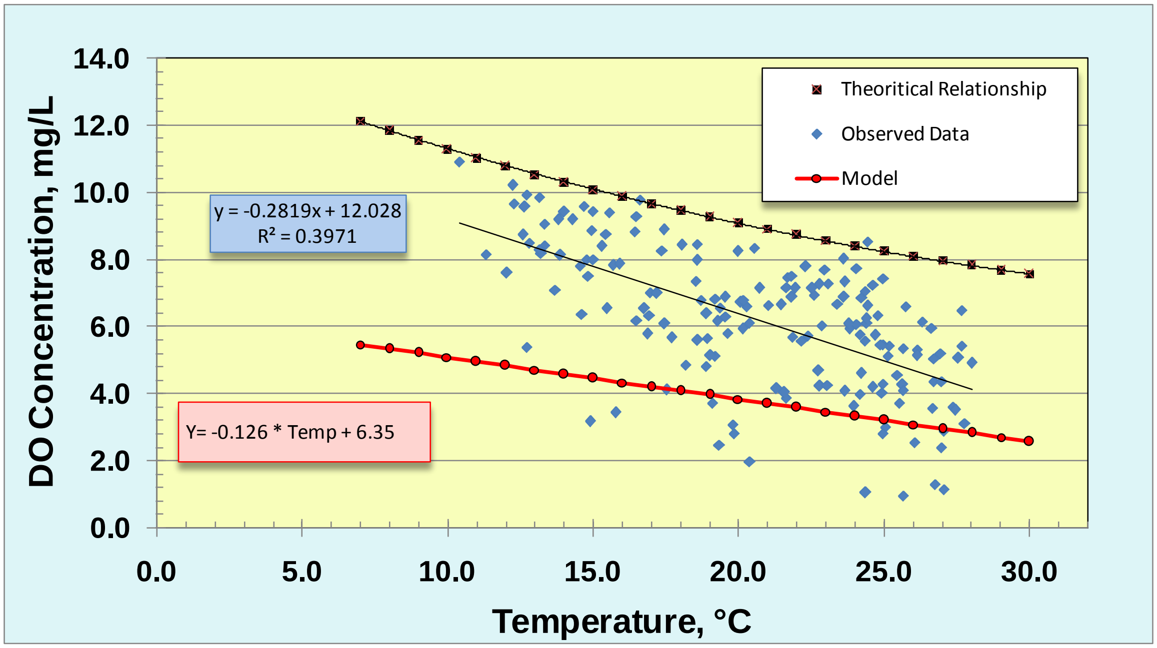


Figure 17. DO versus water temperature relationship derived by solving the equation resulting from the multiple regression equation of SCI versus DO and water temperature using an SCI score of 40, as shown by the red line, and referred to as "model". Also provided are: 1) the empirical DO versus temperature relationship; and 2) the observed relationship based on data from the 2005-2006 and 2010 DO studies passing the SCI and screened based on LDI, conductivity, HA, and nitrate-nitrite.

4.2.4 Linear Regression between SCI and DO Saturation

Given concerns about the statistical assumption by the multiple-linear regression analysis that DO and temperature are independent variables, an alternative approach was investigated to incorporate the effect of temperature on the response of SCI to DO levels. The alternative approach explicitly modeled DO concentration as a function of temperature; that is, percent saturation was used as the independent variable in the regression analysis.

Additionally, during the derivation of revised DO criteria it was recognized that the bioregions utilized by the SCI needed to be re-evaluated to better define the macroinvertebrate expectations across the State. When the SCI bioregions were initially developed, there were relatively little data available for some of the bioregions, particularly the eastern portion of the Panhandle region. However, FDEP has subsequently collected thousands of new SCIs throughout the state. In addition, better Geographic Information System (GIS) tools are now available allowing more accurate spatial resolution.

The re-analysis of the SCI regionalization indicated that the Panhandle bioregion should be divided into the Panhandle West and Big Bend bioregions because they have significantly different taxa at reference sites. This subdivision is consistent with the BioRecon regional divisions, which were previously split into a Panhandle east and west. During the SCI re-evaluation, the bioregion boundaries were also redrawn to be consistent with watershed boundaries. The original bioregion boundaries bisected watersheds which meant that the biological expectation in some streams changed upstream or downstream of an imaginary line across the stream. Greater detail concerning the re-analysis of the SCI bioregions and the results of that evaluation are provided in **Appendix B**. As part of the re-assessment, the SCI equations were also updated and SCI scores were recalculated. Revised SCI scores based on the results of the re-regionalization effort are identified as SCI-2012 below to differentiate them from the original SCI.

Based on the previous findings that suggested regional differences in DO levels and the SCI response to DO, the regression analyses were initially conducted on the regional datasets to determine if there were apparent regional differences in the SCI versus DO relationships. The results of the initial analysis (**Figure 18**) indicate very similar SCI/DO relationships for the Northeast and Big Bend bioregions. The analysis also suggested that the relationship for the Panhandle West was distinctly different from those observed for the other bioregions. The relationship for the Peninsula bioregion was more similar to those for the Northeast and Big Bend bioregions; however, it was not clear if the differences observed were statistically significant.

To further investigate the regional differences in the SCI/DO relationships, a forward selection stepwise regression analysis was conducted to statistically evaluate the effect of bioregion on SCI-2012 response to DO saturation. Because the SCI pass/fail threshold of 40 points is based on the average of two samples, the SCI-2012 scores for the sites were averaged and used as the response variable in the regression analysis to be consistent with the development and application of the SCI. The averaged SCI-2012 scores in the screened dataset were paired with the daily average percent DO saturation based on the three full days of measurements during each deployment. Each of the three bioregions was entered into the analysis as dummy variables. The minimum Bayesian Information Criterion (BIC) was used to choose the best model.

The analysis indicated that the Panhandle West bioregion was a significant effect; that is, the SCI response to DO saturation was clearly different in the Panhandle West than that found in any of the other bioregions (**Table 4**). The results of the stepwise regression also indicated that the SCI-2012 versus DO saturation relationships for the Big Bend and Northeast bioregions were not significantly different statistically and that these two bioregions could be combined for further analyses. The analyses also indicated that the SCI-2012 response to DO level in the Peninsula bioregion was similar to the response observed in the Big Bend and Northeast bioregions with the differences not being statistically different at the 95 percent confidence level, but were significant different at the 90 percent confidence level. A backward elimination stepwise regression yielded the same results.

Based on the results of the stepwise regression, a regional linear regression analysis was conducted to determine the percent DO saturation necessary to support a healthy macroinvertebrate community, as evidenced by a passing SCI-2012 score of ≥ 40. As previously described, the data were screened to minimize the effects of natural and anthropogenic factors on the SCI score and DO level. The averaged SCI scores (to be consistent with the development and application of the SCI) in the screened dataset were paired with the daily average percent DO saturation based on the three full days of measurements during each deployment. The resulting dataset was spatially divided into the Panhandle West, Peninsula, and the Big Bend + Northeast regions consistent with the results of the stepwise regression.

The results of the regional linear regression analysis of the SCI-2012 versus daily average DO saturations conducted on the data from the Panhandle West, Peninsula, and Big Bend + Northeast bioregions are provided in **Figure 19**. In addition to the linear regression, a number of non-linear fits to the data were attempted; however, none of the other fits consistently provided better results. Therefore, the linear regressions (**Figure 19**) provided the best regionalized models of the SCI versus DO saturation relationships for Florida streams that also incorporates the effect of temperature on the expected DO levels.

These regional regression models were used to determine the daily average DO concentrations necessary to support a healthy macroinvertebrate community (*i.e.,* pass the SCI). Solving the regression equations for an SCI score of 40, indicates that daily average percent DO saturation of 62, 32, and 26 percent are protective of the designated use of aquatic life us support for the Panhandle West, Peninsula, and Northeast + Big Bend bioregions, respectively, as indicated by acceptable SCI scores. However, the proposed criteria also need to consider the uncertainty in the analyses and natural diel fluctuations of DO levels in surface waters. The natural diel variation in DO levels are discussed in greater detail below.

A revised DO criterion derived in this manner could be expressed as either a percent saturation values (*e.g.,* minimal percent DO saturations of 62, 32, and 26 %) or as a concentration calculated as a function of temperature. Statement of the criteria as a saturation provides a simpler and more straightforward expression, while the criteria expressed as a temperature dependent concentration is more consistent with the expression of the current criteria.

Table 4. Summary of the step regression history for average SCI versus daily average DO saturation. The best model includes the Panhandle and daily average DO saturation as model parameters.

Step	Model Parameter(s)	Sig Prob	Seq SS	RSquare	AICc	BIC
1	Daily Average DO Sat	0	6598.833	0.1525	1637.129	1646.948
2	Average DO SAT, Panhandle West	0	5806.747	0.2867	1604.219	1617.27
3	Average DO SAT, Panhandle West, Peninsula	0.084	460.9297	0.2973	1603.268	1619.529
4	Average DO SAT, Panhandle West, Big Bend	0.5446	56.45439	0.2986	1605.015	1624.465
5	Average DO SAT, Panhandle West, NE	0.1383	339.7787	0.2945	1604.075	1620.337

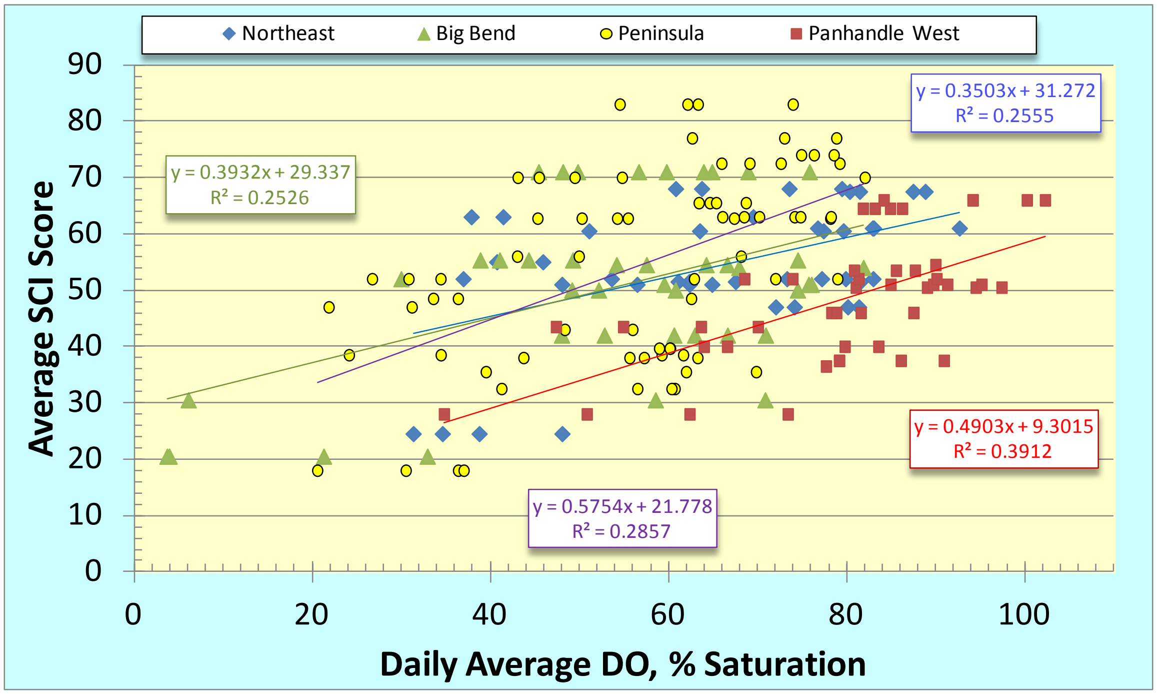


Figure 18. Regional relationships between average SCI score and average daily DO percent saturation for the Panhandle West, Big Bend, Northeast, and Peninsula bioregions. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment ≥ 110, NOx ≤ 0.35 mg/L).

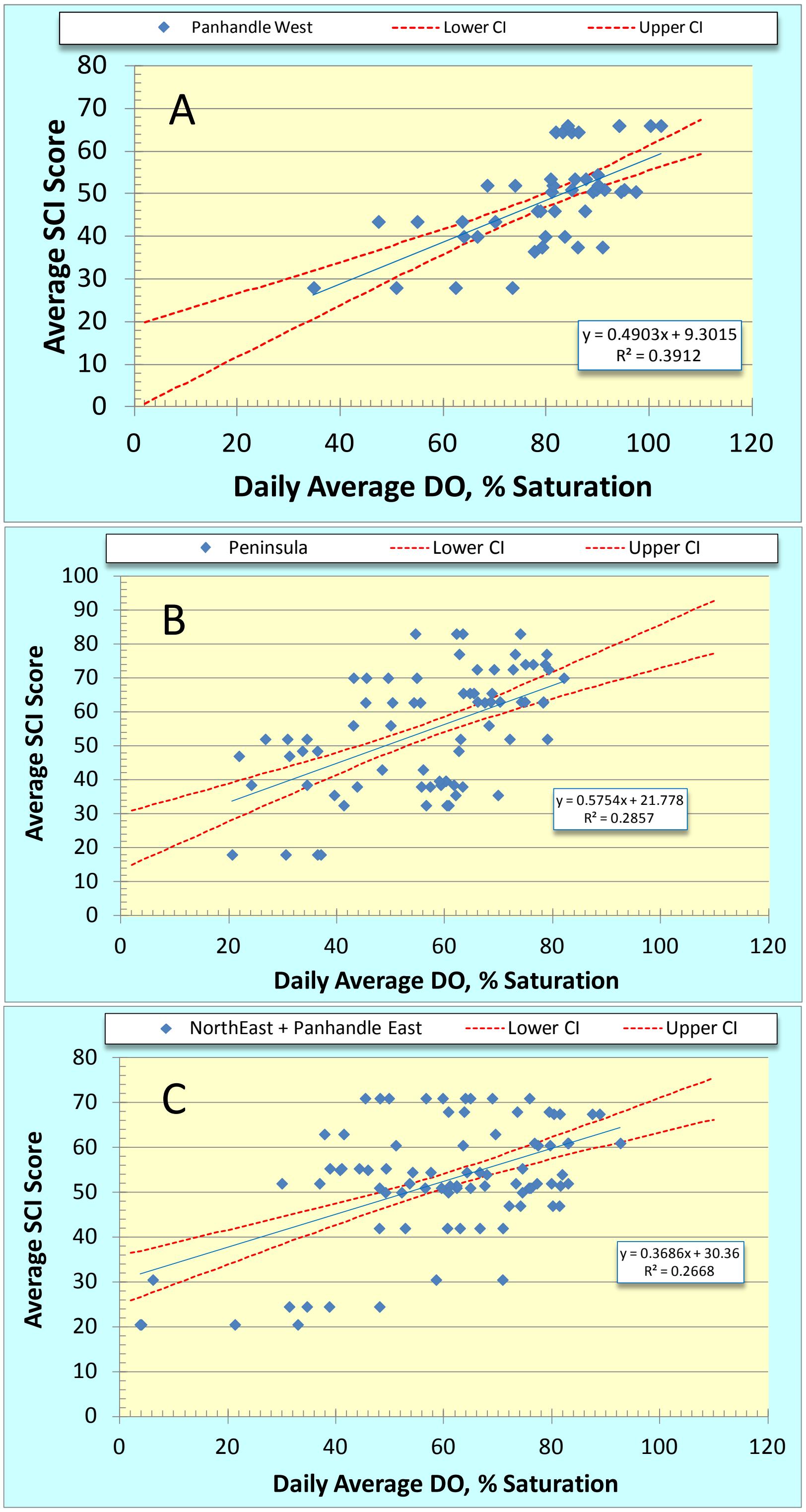


Figure 19. Regional regression relationships between average SCI score and average daily DO percent saturation for A) Panhandle West, B) Big Bend + Northeast, and C) Peninsula bioregions including 90 percent confidence intervals. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment ≥ 110, NOx ≤ 0.35 mg/L).

4.2.5 Natural Diel DO Fluctuation

In natural waterbodies, DO exhibits a diel cycle depending on the level of production and respiration in the waterbody. The proposed freshwater DO criteria were derived as daily average percent DO saturation. Ideally, data from continuous (*i.e.*, every 15 to 30 minutes) DO measurements recorded by deployed data sondes would be used to accurately calculate the daily average DO levels that could then be compared to the criteria to assess compliance. However, most water quality sampling programs do not deploy recording data Sondes, and instead, collect instantaneous grab samples. Due to the natural diel DO fluctuations, the time of day for the instantaneous DO measurements could be an important consideration.

Figure 20 illustrates the average diel fluctuations for percent DO saturation in streams with LDI ≤ 2 and SCI ≥ 40 and in lakes with LDI ≤ 2 with the typical time of day for the average daily percent DO saturation. As shown in the figure, the typical diel DO range for streams is much less than for lakes due to the heavy shading common for most Florida streams, which limits photosynthetic DO production. The average diel DO range for minimally impacted stream sites supporting healthy macroinvertebrate communities is 0.75 mg/L (or 7.6 % saturation). In other words, a grab sample collected anytime during the day would on average be within plus or minus 0.38 mg/L of the daily mean DO concentration. Since the expected accuracy of the instruments normally used to measure DO is plus or minus 0.3 mg/L, the average diel range in streams is nearly within the measurement accuracy.

An analysis of the diel DO data collected in streams as part of the 2005 – 2006 and 2010 DO studies indicates that DO measurements collected anytime during the normal 8:00 am to 5:00 pm work day would be expected to be within approximately 7 percent of the daily mean. Therefore, if continuous data are not available, instantaneous grab samples collected during the workday could be substituted as an estimate of the 24-hour average and used to assess compliance with the proposed criteria with minimal error.

The natural diel fluctuations in minimally impacted Florida lakes are considerably greater than for streams, averaging 1.5 mg/L (or 20 percent saturation). As shown in **Figure 20**, the daily average DO concentration in lakes typically occurs just after noon (*i.e.*, 12:15 pm). An analysis of the diel DO data collected in lakes as part of the 2005 – 2006 statewide DO study indicates that DO measurements collected anytime during the normal 8:00 am to 5:00 pm work day would, on average, be expected to be within approximately 12 percent of the daily mean.

Methods used to consider the natural diel fluctuations in DO levels in the derivation and application of the criteria are discussed in subsequent sections of this document.

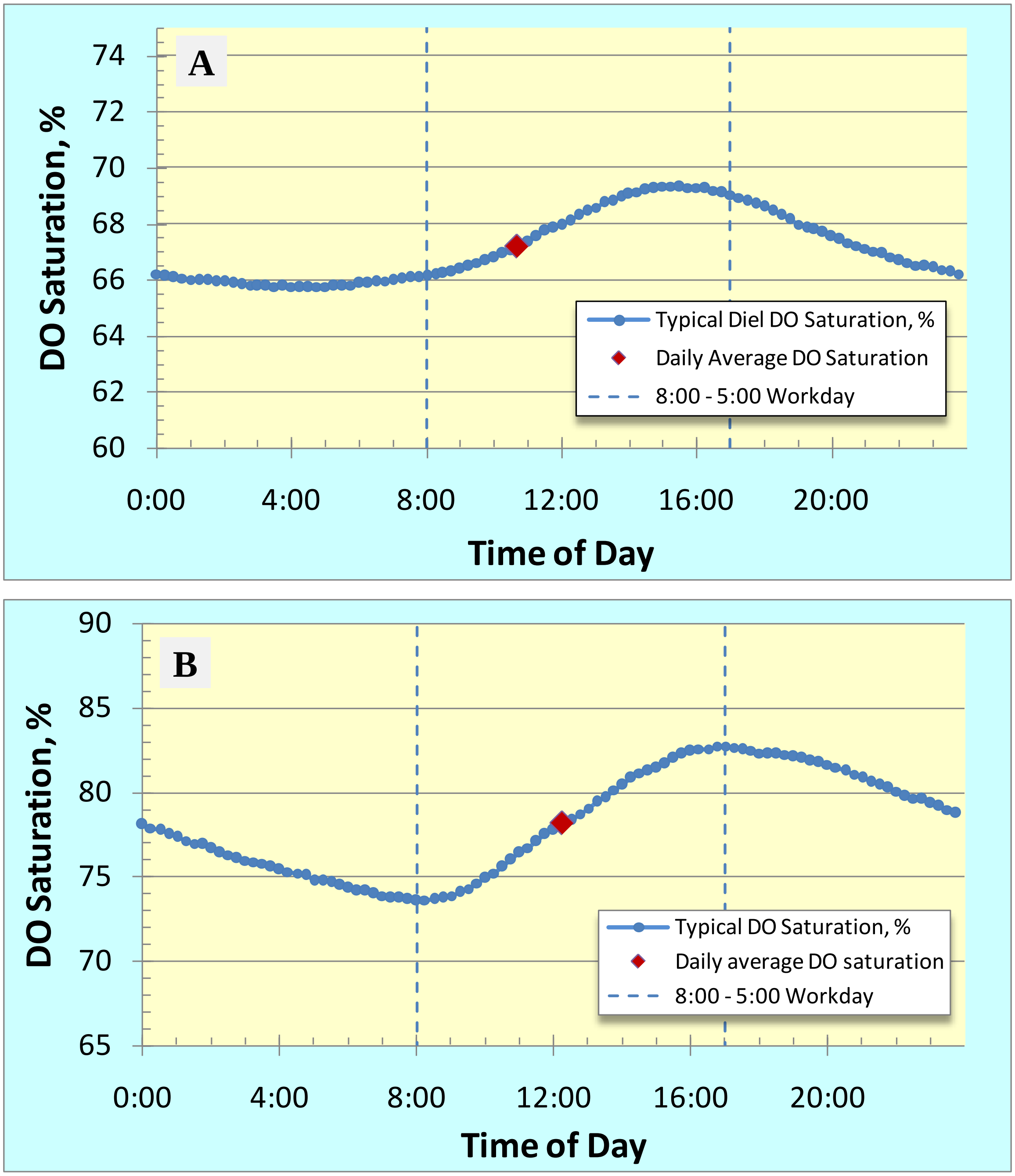


Figure 20. Typical diel fluctuations for percent DO saturation in A) streams with LDI ≤ 2 and SCI ≥ 40 and B) lakes with LDI ≤ 2 (surface readings). Also provided are the typical time of day for mean percent DO saturations and normal 8:00 – 5:00 workday.

4.3.1 Derivation of Proposed Criteria

Even though the relationships between the average SCI-2012 scores and the daily average DO saturation levels are highly significant statistically, there is a level of uncertainty in the relationships. To define the uncertainty in the relationships, a 90 percent confidence interval was calculated around the regression lines as shown in **Figure 19**. There is 90 percent confidence that the true SCI-2012 versus daily average DO saturation relationship is between those confidence bounds. Therefore, using the lower 90 percent confidence bound, there is 90 percent confidence that the true SCI-2012 versus DO saturation relationship is at or to the left of that line. Using the point at which the SCI = 40 line crosses the lower confidence bound, instead of the regression line, as the criteria increases the confidence that the criteria is protective of the sensitive macroinvertebrate community. The potential DO criteria based on the lower confidence bound of the SCI-2012 versus daily average DO saturation relationship are 67, 38, and 34 percent saturation, for the Panhandle West, Peninsula, and Big Bend + Northeast bioregions, respectively.

Another method for deriving water quality criteria recommended in USEPA guidance is using an upper percentile of the distribution from a set of minimally impacted reference sites. FDEP utilized this approach for developing numeric nutrient criteria for Florida streams and to establish numerous DO SSACs as described in **Appendix A**. Typically, FDEP has used the 10th (or 90th in the case of nutrients) percentile of the reference distribution as the threshold for water quality criteria. During the derivation of the proposed revised DO criteria for Florida's freshwaters, the distribution of DO levels for reference sites was also examined to support the results of the SCI-2012 versus daily average DO saturations regressions. **Table 5** compares the results of the regression analyses to the reference DO saturation distribution.

In the case of both the Panhandle West and Big Bend + Northeast + Peninsula bioregions, the proposed criteria agree well with the 10th percentile of the reference distribution. The 10th percentile for the Panhandle West reference sites is 68% compared to the proposed criteria of 67% saturation based on the regression analysis. Similarly, the 10th percentile for the Big Bend + Northeast bioregion reference distribution is 39% compared to the 34% resulting from the regression analyses. The 10th percentile for the Peninsula bioregion reference distribution is 34% compared to the 38% resulting from the regression analyses. Therefore, the reference distribution is another line of evidence that supports the proposed criteria based on the results of the regression analyses.

The proposed DO criteria are also supported by a study of 35 lowland streams in southwestern Louisiana. Justus et al., (2012) found statistically significant biological thresholds of 2.6 and 2.3 mg/L for invertebrates and fish, respectively. The DO thresholds were based on analyses of taxa richness, species diversity, and total abundance. Because the lowland streams included in this study are comparable to those in many portions of Florida and most of the invertebrate and fish species found in the Louisiana study also occur in Florida, the results can be expected to be transferable. In addition, the criteria being proposed for Florida waters are above the biological thresholds for Louisiana streams, the proposed criteria for the Big Bend, Northeast, and Peninsula bioregions can are be expected to be protective of the expected invertebrate and fish populations.

Because the potential criteria derived based on the lower confidence bound are 8 to 31 percent higher than the thresholds based on the actual regression line, they would help minimize the influence of the sampling time of day on the Type II error rate (*i.e.,* identifying impaired sites as unimpaired) for future compliance assessments. Additional steps could be taken in the application of the proposed criteria to further minimize the error associated with using instantaneous measurements to estimate the daily average DO level to assess compliance. These could include requiring that the data used for compliance assessment be collected within a narrower time range that more closely approximates the daily average DO level. However, most of the information DEP uses for surface water assessments consists of "found data", and placing a time restriction on the use of this found data could greatly reduce the data available for assessment purposes. Alternatively, the temporal distribution of the data available for a waterbody could be evaluated during the assessment process and additional data could be collected if needed to provide a relatively uniform temporal distribution of data (*e.g.*, all samples not collected in afternoon) and an accurate assessment of the DO conditions.

Table 5. Comparison of the results of the regional SCI-2012 versus average daily DO saturation regression analyses with the distribution of DO saturation levels at reference sites with LDI ≤ 2 and SCI scores ≥ 40.

Parameter	**Panhandle West**	**Peninsula**	**Northeast + Big Bend**
SCI-2012 versus Daily Average DO Saturation Results			
Regression SCI=40	**62**	**32**	**26**
Lower CI SCI=40	**67**	**38**	**34**
Reference Site (LDI ≤ 2, SCI ≥ 40) DO Distribution			
Count	50	91	89
Avg	83.5	58.7	60.2
Median	85.9	62.9	62.3
5th percentile	63.8	25.9	27.4
10th percentile	68.3	33.6	38.7
25th percentile	79.9	77.6	48.0

4.4 *Freshwater DO Criteria Summary and Conclusions*

The existing freshwater DO criteria of 5.0 mg/L at all times has been shown to be inaccurate for many natural Florida streams and lakes that typically exhibit DO concentrations below 5.0 mg/L even though they support healthy biological communities. Several approaches were evaluated to derive a protective but more accurate DO criteria for Florida freshwaters including:

1. Using a reference site distributional approach to derive a criteria that is inherently protective, but without identifying a threshold, below which impairment is likely;
2. Using a regression analysis that included measures of biological health (*i.e.,* SCI) and DO concentrations to determine the DO concentration protective of a healthy, well balanced community;
3. Conducting a multiple-linear regression analysis of SCI scores versus DO concentration and temperature to account for the influence of temperature on the DO levels required to support healthy biological communities; and
4. Derivation of regional SCI versus DO saturation relationships to determine the DO saturation (which may be converted to temperature dependent DO concentration) protective of a healthy, well balanced community.

After considering the strengths and weaknesses associated with the various approaches described above, the FDEP concluded that the most robust method for deriving the revised DO criteria was to use the lower confidence limit for the regional regression relationship between the average SCI-2012 and the daily average DO saturation to determine the DO level required to achieve a minimum SCI score of 40, because:

- The regional DO saturation provided the best correlation with SCI scores indicative of healthy biological communities;
- The inherent relationship between temperature and DO is automatically incorporated into the criteria;
- The regional criteria account for the observed regional differences in measured DO levels and biological expectations.
- The use of the lower confidence interval for the average SCI-2012 versus daily average DO saturation takes into account the uncertainty in the relationships and the naturally expected diel fluctuations in the DO levels. This allows the criteria to be applied to continuous diel measurements or grab samples collected during the typical sampling day;
- DO saturation is fully protective of a healthy, well balanced aquatic community, both in lentic and lotic waters;
- The proposed criteria based on the SCI-2012 versus DO saturation regression analyses are supported by the reference distribution of DO saturation levels; and
- The resulting criteria are subject to an acceptable Type I error rate.

As shown in **Figure 19**, the daily average DO saturations of 67, 38, and 34 percent, shown to be protective of healthy aquatic communities in the Panhandle West, Peninsula, and Big Bend + Northeast bioregions, respectively, based on the 90 percent confidence bounds around the regional SCI-2012 versus DO saturation regression analysis are easily converted into a temperature dependent DO concentration relationship (**Figure 21**). The DO concentrations resulting from the percent DO saturation criteria over the range of water temperatures expected in Florida Panhandle and Northeast and Peninsula streams are provided in **Table 6**. The average statewide summer water temperature for the summer months (May – October) is approximately 25 °C, while the average temperature during the winter months (January – April and November – December) is 16 °C. The monthly statewide average water temperature for minimally disturbed Florida streams is provided in **Figure 6**.

Based on the analyses conducted, the recommended revised DO criteria for Florida's Class I and III freshwaters could be expressed as:

> *No more than ten percent of the daily average percent DO saturation values shall be below 67 percent in the Panhandle West bioregion or 38 percent in the Peninsula bioregion or 34 percent in the Northeast and Big Bend bioregions*[1].

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[1] As described in Section 7, the Department also plans to include a natural conditions clause.

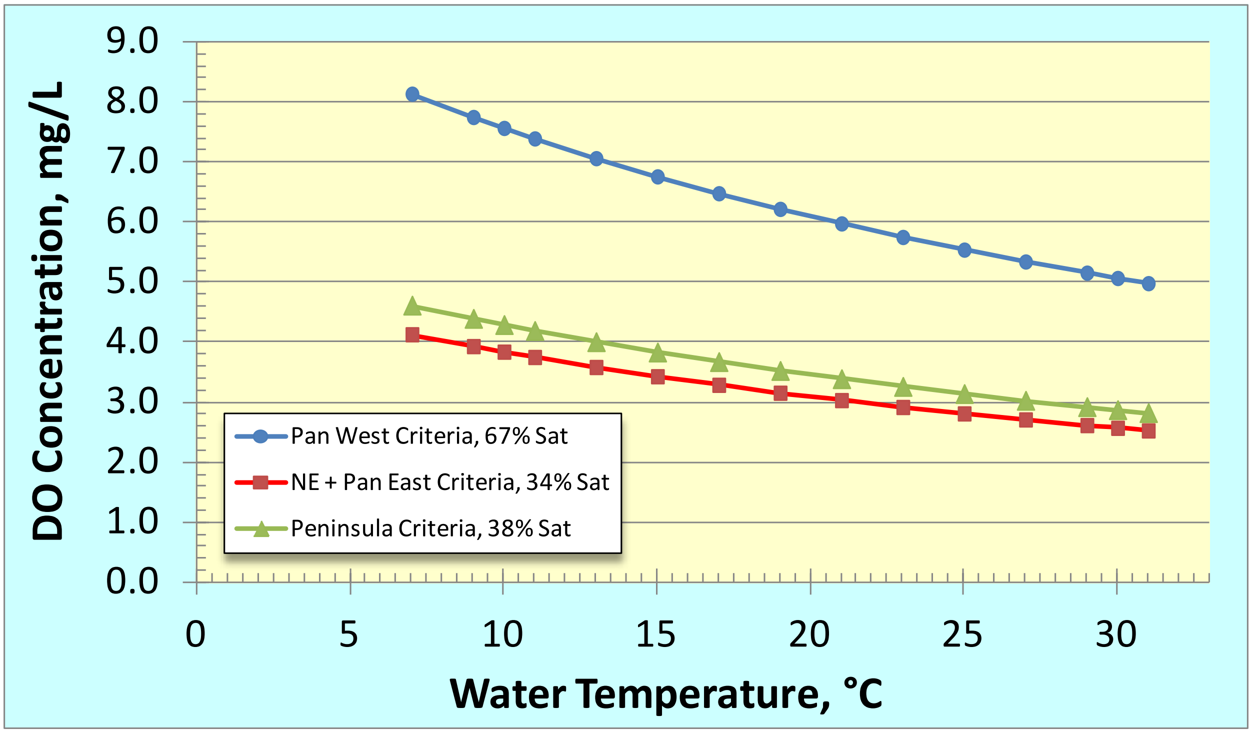


Figure 21. Potential temperature dependent DO criteria derived by solving the linear regression equations for the average SCI versus daily average percent DO saturation (provided in Figure 19) for a SCI score of 40.

Table 6. DO concentrations resulting from proposed percent DO saturation criteria for a range of water temperatures typically expected in Florida Panhandle West, Peninsula, and Northeast + Big Bend bioregion streams.

Temp, °C	NE + Pan East Criteria, 34% Sat	Peninsula Criteria, 38% Sat	Pan West Criteria, 67% Sat
8	4.0	4.5	7.9
10	3.8	4.3	7.6
12	3.7	4.1	7.2
14	3.5	3.9	6.9
16	3.4	3.8	6.6
18	3.2	3.6	6.3
20	3.1	3.5	6.1
22	3.0	3.3	5.9
24	2.9	3.2	5.6
26	2.8	3.1	5.4
28	2.7	3.0	5.2
30	2.6	2.9	5.1

4.5 Application of the Proposed Freshwater DO Criteria

The FDEP plans to evaluate the proposed criteria, for purposes of ambient 303(d) assessments, using the provisions in Florida's Impaired Waters Rule (Chapter 62-303, F.A.C.) using the binomial hypothesis test which allows no more than 10 percent of the values collected during an assessment period to be below the DO criteria. Under the current Impaired Waters Rule, samples collected within 4 days are averaged, but FDEP plans to revise the IWR so that individual grab sample DO data would not be averaged (would be assessed independently), while a daily average value would be used to represent continuous DO records from Sondes. Considerations related to the time of day sampled are discussed in Section 4.2.5. Several additional issues regarding the application of the proposed DO criteria to Florida's freshwaters are discussed below.

4.5.1 Application of Peninsula Criteria to Everglades Bioregion

Because there are few natural streams in the Everglades bioregion in south Florida and the SCI has not been calibrated for this region, the SCI versus DO relationship could not be used to independently derive DO criteria for this area. Additionally, a SSAC for the wetlands within the Everglades Protection Area (EPA) has been developed and adopted and will remain in effect. Because the proposed DO criteria for the Peninsula bioregion is believed to be fully protective of the biological communities within the remaining natural waterbodies as well as the limited communities inhabiting the man-made or altered waterbodies that predominate this area, the proposed Peninsula criteria will also apply to freshwaters within the Everglades bioregion where SSACs have not been adopted.

4.5.2 Application of the Criteria to Lakes

Although DEP attempted to develop an invertebrate Lake Condition Index, the data indicated that lake invertebrates responded more strongly to natural differences in water clarity, nutrient levels , and color than to human disturbance or DO (Fore 2007), so it would be difficult to use lake invertebrates to establish DO criteria. While DEP was able to develop a Lake Vegetation Index that was correlated to human disturbance, plants are not generally viewed as susceptible to low DO as are animals. Since Florida does not have a lake bioassessment tool for fish, an alternate, but fully protective method, is needed to develop lakes DO criteria.

Based upon the information presented in Section 3.1, it has been established that the DO requirements of sensitive species in flowing waters (streams) are generally higher than those of species in lentic environments (lakes), and that sensitive invertebrates are generally more sensitive to low DO than are fish and other lentic species. Therefore, based on the available information, the recommended stream DO criteria derived using the SCI response to DO is considered to be fully protective of lake communities.

Because there are generally fewer DO sensitive species typically found in the lakes and because the DO requirements of lentic organisms are generally lower, application of the stream criteria to lakes may result in a higher Type I error rate (incorrectly concluding that a healthy lake is impaired) than in streams. However, because the analyses presented in this report are much more scientifically defensible than DO criteria adopted by Florida in the 1970s that were predominantly based on responses of northern salmonid fish, FDEP concluded that application of the recommended stream criteria to lakes is the best available scientific approach.

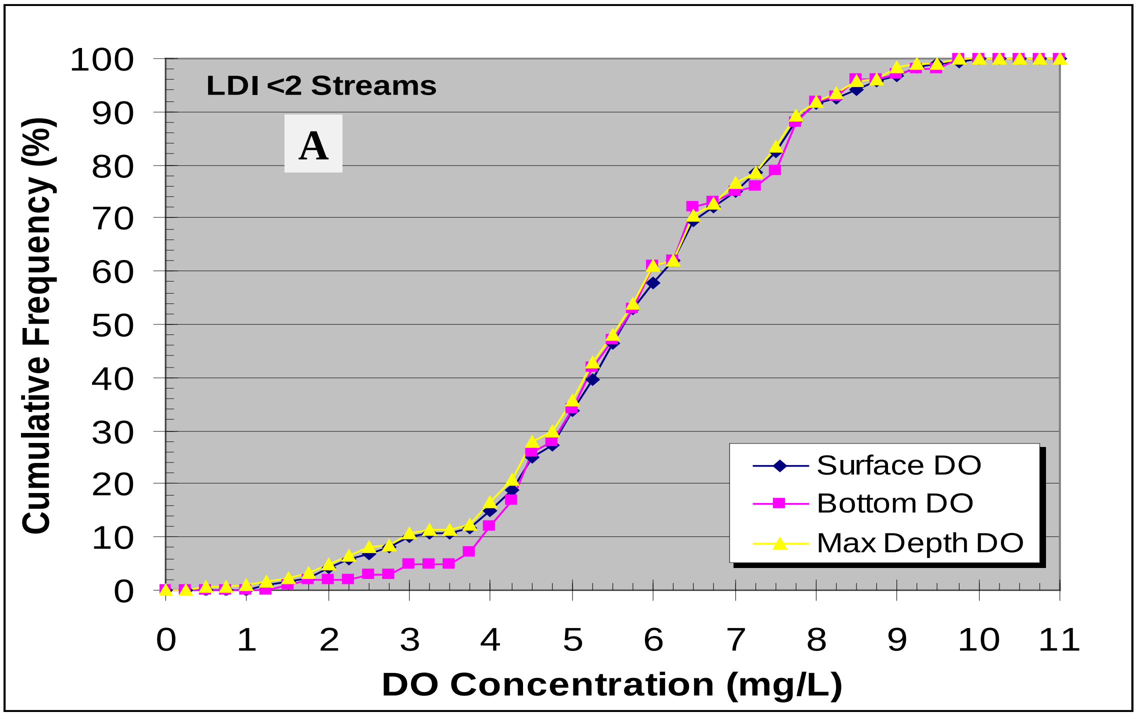
4.5.3 Sampling Depth

Most Florida streams are shallow and well mixed and significant vertical differences in DO are not generally found in minimally impacted (LDI ≤ 2) streams (**Figure 22A**) based on the data collected during the statewide DO study. This suggests that, in streams, the recommended DO criteria can applied to data collected throughout the water column. However, vertical differences are more pronounced in minimally disturbed lakes (**Figure 22B**), where the median difference in DO concentrations between the surface and bottom was approximately 1.3 mg/L, with 20 percent of the lakes having 2.2 mg/L or more difference in DO concentrations between the surface and bottom. As a result, the sampling depth for the collection of data to assess compliance with the DO criteria in lakes is a more important consideration than in streams.

Additionally, it is difficult to accurately measure bottom water column DO because of interference or interactions with (generally low DO) sediments. To ensure that an accurate and consistent measurement of the water bodies' health is obtained, Kaller *et al.* (2010) recommended that DO monitoring should take place in the upper two meters of the water column (or upper half of the water column depending on depth) due to the influence of water temperature, flooding levels, water movement, and depth on DO levels and stratification.

This sampling strategy is protective of lake fish and invertebrates because they most often utilize the littoral zone (higher areas) for habitat and nursery areas. Assuring that the upper portion of the water column complies with the criteria provides sensitive organisms adequate passage zones and refuge from naturally low DO conditions found near the bottom. Due to the naturally low DO levels, non-mobile organism inhabiting the lake bottoms are generally not sensitive to low DO conditions. Additionally, because low DO levels are typically expected to occur in the bottom waters of lakes due to natural phenomena (stratification, sediment oxygen demand, respiration, etc.), sampling the upper portion of the water column will limit the number of healthy lakes erroneously listed as impaired due to natural conditions.

Based on this available information, the proposed freshwater DO criteria can be applied to data collected throughout the water column in streams. For lakes, it is recommended that the application of the proposed DO criteria be limited to data collected in upper two meters of the water column. This recommendation is consistent EPA-approved DO criteria in other states that specify that DO measurements for assessment of ambient waters be taken from the top half of the water column.



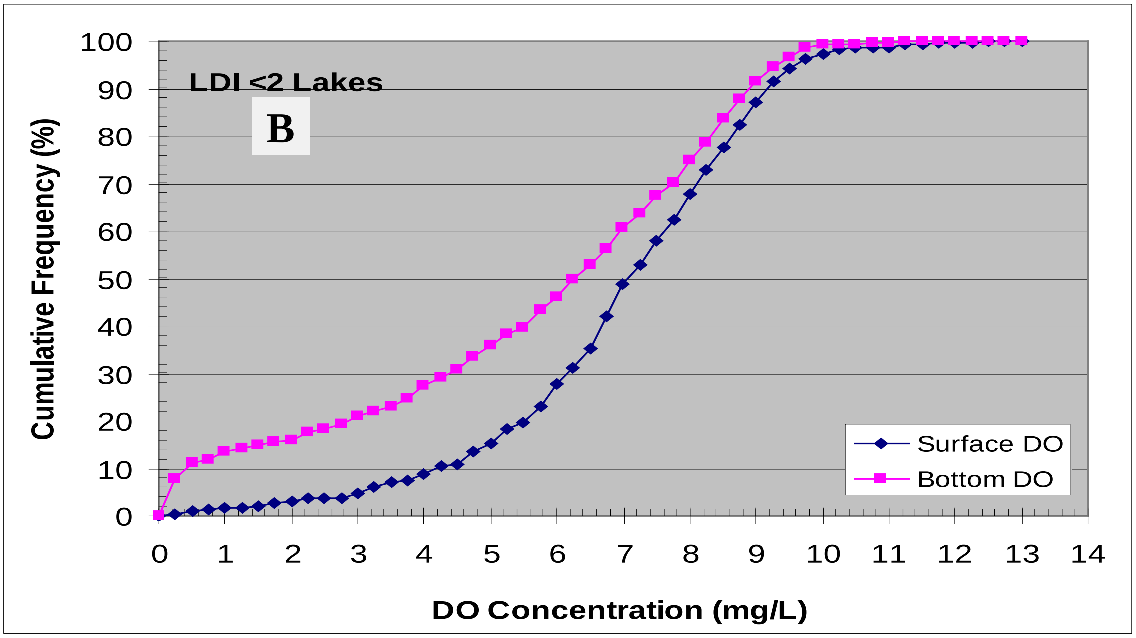


Figure 22. Comparison of surface (0.5 m below the surface) and bottom (0.5 m above the bottom) DO concentrations in minimally impacted (*i.e.*, LDI ≤ 2) Florida streams and lakes sampled as part of the 2005 – 2006 statewide DO study.

4.5.4 Sampling Time of Day

The proposed freshwater DO criteria were derived as daily average percent DO saturations. Ideally, compliance with the criteria would be assessed using daily average DO levels calculated from diel monitoring data, however, most water quality sampling programs only collect instantaneous grab samples. Therefore, due to the natural diel DO fluctuations described above, the time of day for the instantaneous DO measurements could be an important consideration in future assessments of the criteria.

Basing the proposed criteria on the confidence bounds of the SCI-2012 versus Daily average DO saturation relationship helps to assure that the criteria are protective regardless of the distribution of sample collection times in the dataset for the waterbody. However, as an additional protective measure, FDEP has developed typical diel DO curves for lakes and streams in each bioregion based on the data collected during the Statewide DO study described in Section 2. These diel curves can then be used to derive a time-of-day translation of the applicable daily average DO criterion based on the time-of-day at which the sample is collected. The typical diel curves for streams and lakes are provided in **Figures 23 to 25** for each DO bioregion.

To derive the time-of-day specific translations of the daily average criteria, the typical DO diel curves for each region and waterbody type (*i.e.*, red curves shown in **Figures 23 to 25**) were shifted so that the daily average was equal to the proposed daily average criteria applicable to that region (*i.e.*, blue curves in the figures). A polynomial equation was then fitted to each of the shifted curves with the resulting equations being provided in **Figures 23** to **25** and in **Table 7**. The fitted curves depicted by the polynomial equations represent the daily DO regime at a site exactly meeting the daily average DO criteria with a typical diel fluctuation. Therefore, to achieve the daily average DO criterion, the measured DO level at any specific time of day would be expected to be at or above the level predicted by the curve. This method would require higher DO levels during the afternoon when DO levels are typically higher due to increased photosynthetic activity and allow lower DO levels in the morning when DO levels are naturally lower due to less photosynthesis and more respiration.

Therefore, to assess the proposed criteria using grab samples, the equations in **Table 7** can be used to provide a time of day specific translation of the daily average criteria that could be compared to the measured DO levels. Alternatively, the equations could be used to derive a time of day adjustment that would be applied to the measured DO level. The adjusted DO value would then be compared to the proposed daily average criteria. In either case the result would be the same. If diel data were available, a true daily average would still be calculated and directly compared to the daily average criteria to assess compliance.

Before making a final assessment of compliance with the DO criteria using grab samples, the data should be reviewed to evaluate the potential influence that the distribution of sampling times had on the results of the assessment. The assessment will be most accurate when there is an even distribution of samples during the day. In cases where the available data is not evenly distributed over the day, especially when there is a lack of samples collected early in the day, additional data may need to be collected before a reliable assessment can be made. The additional data collection would likely include continuous diel monitoring so that an accurate compliance assessment could be made.

Table 7. Fitted polynomial equations describing the expected DO levels for a site with a daily average DO level equal to the criterion and with a typical diel DO fluctuation.

Region	**Equations for the Time of Day Interpretation of Criteria** [*]
Streams	
Northeast + Big Bend	$1.1844 \times 10^{-13} \bullet T^5 – 4.1432 \times 10^{-10} \bullet T^4 + 4.7729 \times 10^{-7} \bullet T^3 – 1.9692 \times 10^{-4} \bullet T^2 + 0.02314 \bullet T + 31.24$
Peninsula + Everglades	$1.9888 \times 10^{-13} \bullet T^5 – 6.8941 \times 10^{-10} \bullet T^4 + 7.8373 \times 10^{-7} \bullet T^3 – 3.1598 \times 10^{-4} \bullet T^2 + 0.03551 \bullet T + 33.43$
Panhandle West	$9.0851 \times 10^{-14} \bullet T^5 – 2.9941 \times 10^{-10} \bullet T^4 + 3.1560 \times 10^{-7} \bullet T^3 – 1.0851 \times 10^{-4} \bullet T^2 + 0.006285 \bullet T + 65.61$
Lakes	
Northeast + Big Bend	$1.4578 \times 10^{-13} \bullet T^5 – 5.5607 \times 10^{-10} \bullet T^4 + 7.0683 \times 10^{-7} \bullet T^3 – 3.1879 \times 10^{-4} \bullet T^2 + 0.02817 \bullet T + 34.19$
Peninsula + Everglades	$1.3709 \times 10^{-13} \bullet T^5 – 5.0496 \times 10^{-10} \bullet T^4 + 6.1352 \times 10^{-7} \bullet T^3 - 2.5817 \times 10^{-4} \bullet T^2 + 0.01960 \bullet T + 37.14$
Panhandle West	$7.1190 \times 10^{-14} \bullet T^5 – 2.6420 \times 10^{-10} \bullet T^4 + 3.2247 \times 10^{-7} \bullet T^3 – 1.3607 \times 10^{-4} \bullet T^2 + 0.01071 \bullet T + 66.35$

* *T in the equations is the time of day in minutes past midnight.*

Additionally, because DO is not a direct pollutant, but instead is a response to other pollutants, waterbodies for which the available data indicate that the DO criteria are not met will undergo a more detailed assessment to determine the causative pollutant(s) responsible for the low DO levels prior to the waterbody being listed as impaired. This assessment can include: 1) the collection of additional data if needed, 2) a review of the biological health of the system to determine if the low DO conditions are having an adverse effect on the biological communities, 3) an assessment of nutrient, chlorophyll, and BOD data to determine if excessive levels of these pollutants can account for the DO levels observed, and 4) an evaluation of the diel DO range that could help determine if the observed low DO condition is natural or could suggest a cause for the low DO levels.

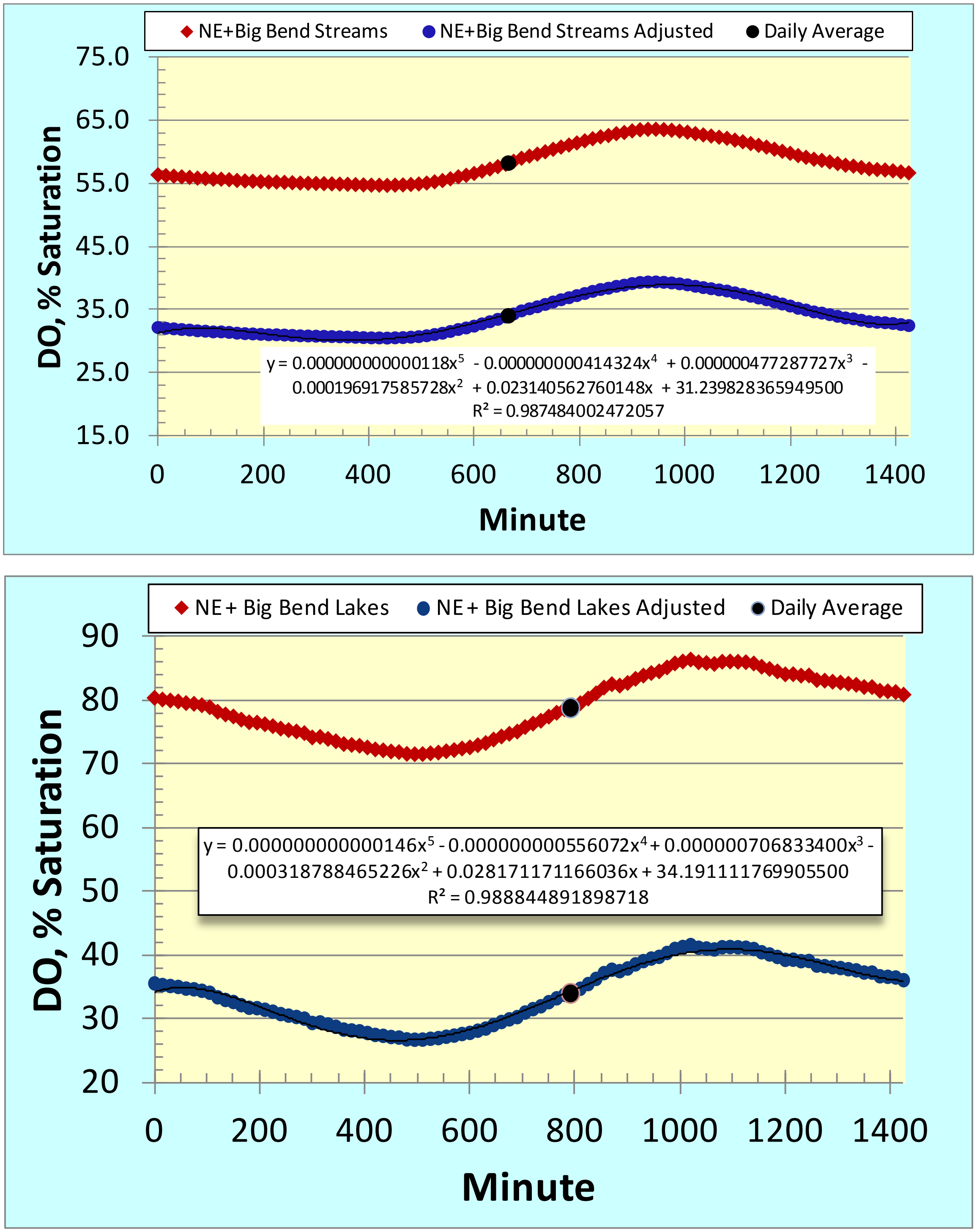


Figure 23. Typical diel DO fluctuations and adjusted criteria curves for streams (top graph) and lakes (bottom graph) in the Northeast and Big Bend bioregions.

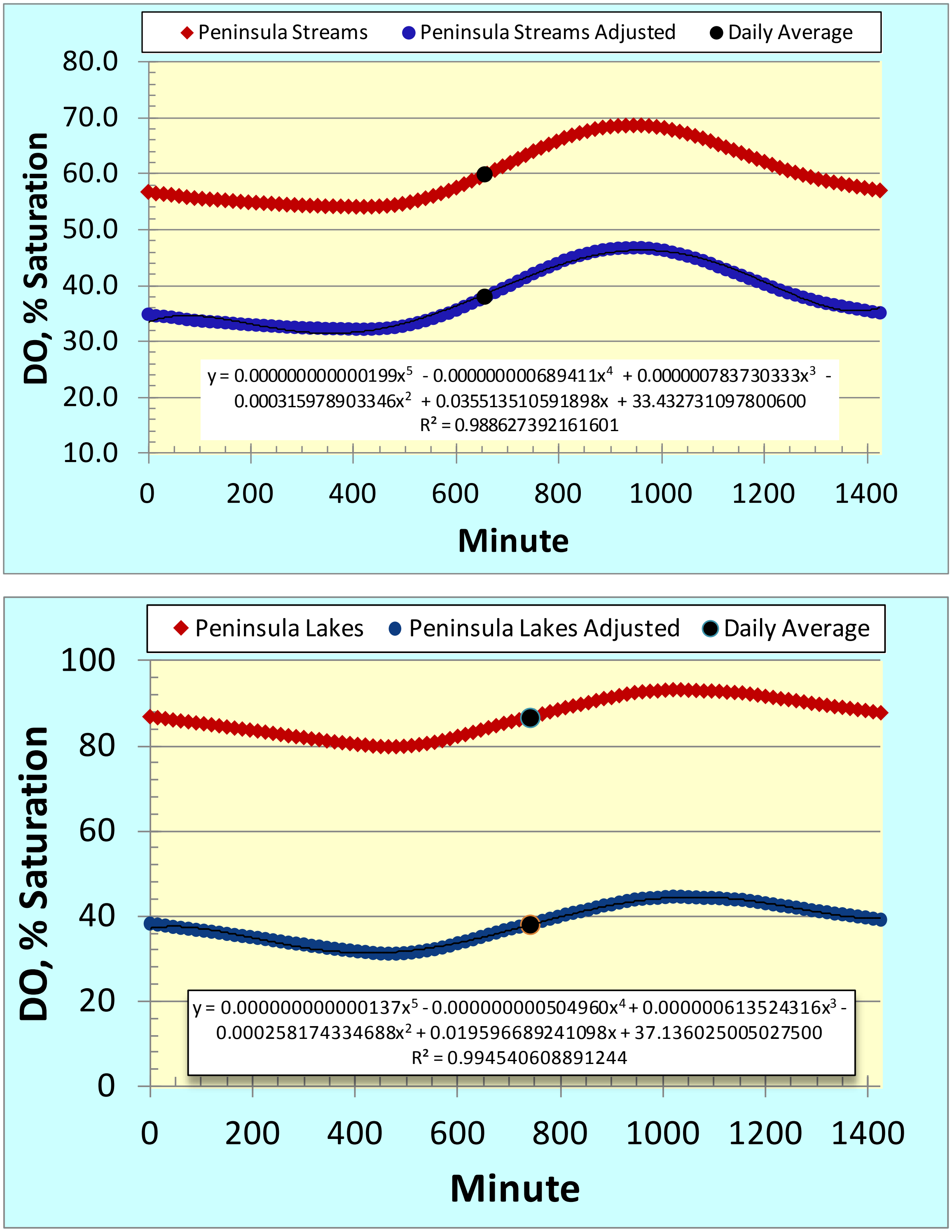


Figure 24. Typical diel DO fluctuations and adjusted criteria curves for streams (top graph) and lakes (bottom graph) in the Peninsula bioregion.

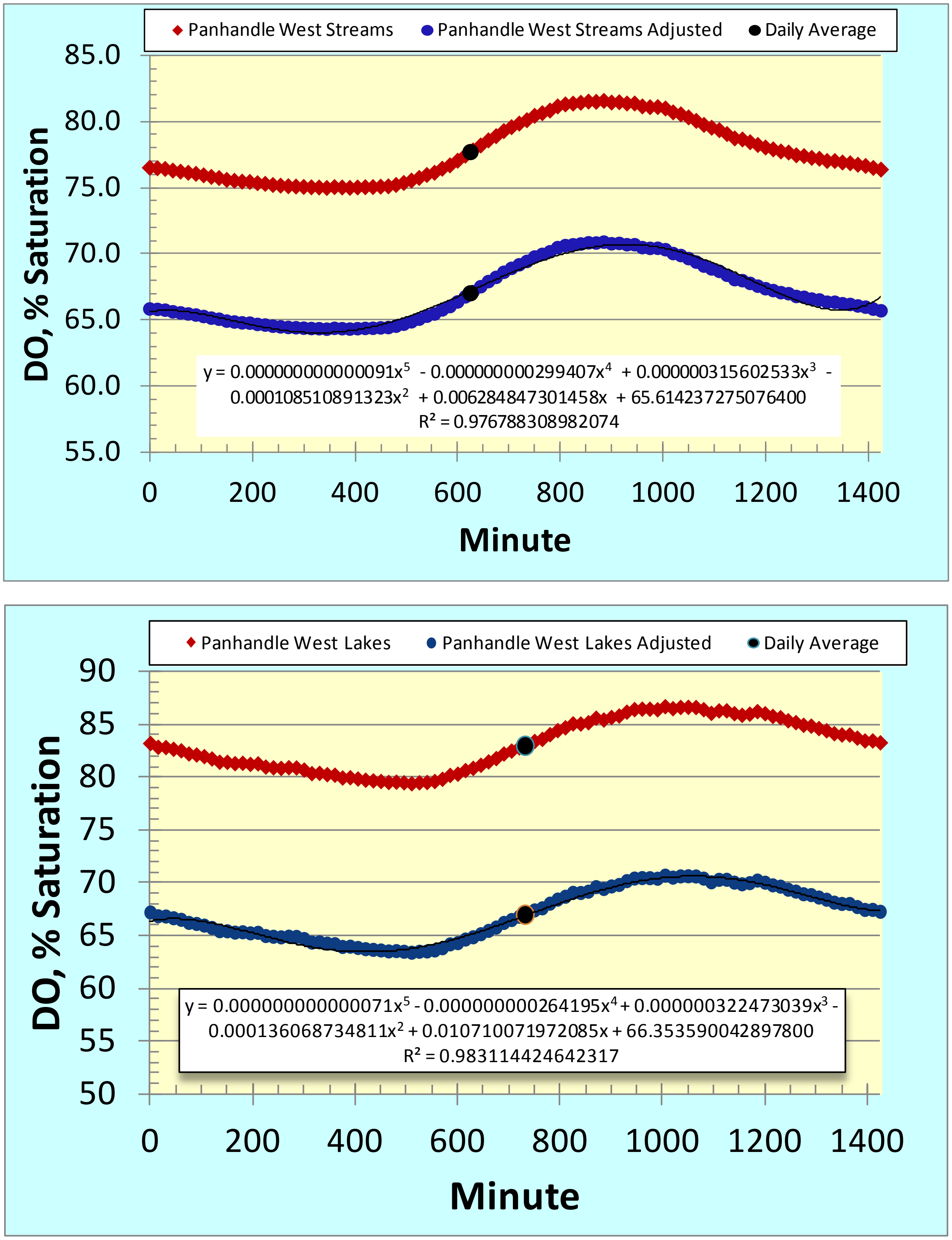


Figure 25. Typical diel DO fluctuations and adjusted criteria curves for streams (top graph) and lakes (bottom graph) in the Panhandle West bioregion.

5 Development of Revised Marine Water DO Criteria

The FDEP evaluated the USEPA Virginian Province approach as a scientifically defensible method for deriving more accurate DO criteria for Florida's marine waters. Application of the Virginian Province method would be a comprehensive solution that provides an adequate and consistent level of protection, while avoiding the time and expense involved with the development of site specific criteria for all of the waterbodies for which the current criteria are not appropriate. However, it should be noted that site specific criteria will likely still be needed in some situations.

5.1 *Summary of the USEPA Virginian Province DO Criteria Approach*

The USEPA Virginian Province document (EPA 2000) recommends an approach for deriving DO levels necessary to protect coastal and estuarine organisms in the Virginian Province (Cape Cod, Massachusetts to Cape Hatteras, North Carolina) based on laboratory dose-response data similar to the approach routinely used to set criteria for toxics (Stephan et al, 1985). The method is accepted by the United States Environmental Protection Agency (USEPA or EPA) Region 4 and has been used by several coastal States (including Florida) to develop DO criteria.

The EPA Virginian Province methodology represents a synthesis of current knowledge regarding biological responses to hypoxic stressors in aquatic ecosystems. This approach considers the response to both continuous and cyclic exposures to low DO levels to derive criteria that are protective of aquatic life. The aquatic life based approach utilized for the Virginian Province (EPA 2000) identifies three important components of the resulting criteria, as follows:

- The Criterion Continuous Concentration (CCC), which is defined as a DO concentration above which continuous exposure is not expected to result in unacceptable chronic effects to sensitive biological communities.
- The Criterion Minimum Concentration (CMC), which is defined as a daily mean DO concentration below which any exposure for a 24-hour period or longer would result in unacceptable acute effects (mortality) to sensitive organisms.
- The Final Recruitment Curve (FRC), which is a function that defines the maximum allowable exposure duration at DO concentrations between the CMC and CCC necessary to prevent unacceptable reductions in seasonal larval recruitment for sensitive species. Since the effects of low DO depend on both the duration and intensity of exposure, the FRC allows shorter exposure durations as the DO level decreases.

Aquatic life and its uses are assumed to be fully supported as long as DO concentrations remain at or above the (CCC) chronic criterion for growth. Conversely, if DO concentrations fall below the juvenile/adult survival criterion (CMC), low DO would be expected to result in unacceptable mortality to some of the most sensitive species. When DO conditions are between these two values, further evaluation of the duration and intensity of low DO is needed to determine whether the level of oxygen can support the most sensitive members of a healthy aquatic community (EPA 2000). This evaluation is conducted by comparing the monitored data in a given waterbody to the FRC developed for the species expected to be present. An overview of the USEPA Virginian Province approach is provided in **Appendix D**. A more detailed discussion of the Virginian Province method and the derivation of the various components of the criteria can be found in *Ambient Aquatic Life Water Quality Criteria for Dissolved Oxygen (Saltwater): Cape Code to Cape Hatteras* (referred to as the Virginian Province) (USEPA 2000) and *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan *et al.* 1985). Details concerning the application of the Virginian Province Approach to derive revised DO criteria for Florida's marine waters are provided below.

5.2 *Application of Virginian Province Approach to Florida Waters*

The EPA recommended approach for the Virginian Province is based on conservative assumptions using species found in cooler northern waters, and therefore, it is understandable that the DO criteria generated for the Virginian Province may potentially be overprotective if applied directly to warmer Florida waters. EPA (2000) and Thursby (2003) suggested that the FRC developed for the Virginian Province may be overprotective for areas to the south, because recruitment seasons lengthen and larval development times decrease with increased distance south from the Virginian Province. Both factors would act to decrease the sensitivity of the FRC, suggesting that the curve shown in **Figure 26** should logically be shifted down on the lower left quadrant to more accurately reflect Florida conditions.

Additionally, while many of the species used to derive the Virginian Province criteria (USEPA, 2000) also occur in Florida, there are several that are not found in Florida. The species not found in Florida include some of the most sensitive species (Maine lobster, for example) used to derive the various components of the Virginian Province criteria. However, the species present in Florida waters appear to bracket the general range of DO sensitivities seen in the entire complement of species used by USEPA (2000). To derive the most accurate DO criteria for Florida's marine waters, the general Virginian Province method developed by the USEPA was applied using data for Florida specific species. A description of the data used and the derivation of the proposed revised DO criteria for Florida marine waters are provided below.

5.2.1 Data Sources and Selection

Data were gathered from primary literature and published government reports and their supporting material. Acute toxicity data were compiled from laboratory toxicity data describing acute dose response to low DO, and calculations were based on the DO value lethal to 50 percent of test organisms (LC_{50}). Data were gathered from studies exposing organisms to experimental DO concentrations for 24 to 96 hours. Both time points produce relatively similar LC_{50} values (USEPA 2000). Acute DO data were gathered for a total of 49 species of salt water organisms. The available data were evaluated for suitability of use on the basis of (1) the quality of experimental methods used, (2) sufficiency of documentation of resulting toxicity values, and (3) suitability of experimental organisms to represent species relevant to Florida's estuarine/coastal waters. Studies conducted at water temperatures outside the range typical for Florida waters were not considered appropriate for further use. Experiments using water temperatures less than 15 degrees Celsius (°C) were excluded. Documentation for a study needed to be rigorous enough to allow for repetition of the methods. Organisms were suitable for use in developing criteria if the species, genus, or a closely related species occurred in Florida's estuarine/coastal waters. Of the 49 species available, 26 were considered appropriate for use in developing revised DO criteria for Florida. A complete list of all acute and chronic DO data gathered, experimental conditions, and notes regarding the species occurrence in Florida waters and the appropriateness of the data for use in deriving revised DO criteria is provided in **Appendices E and F**, respectively.

5.2.2 Derivation of Criterion Minimum Concentration (CMC) for DO in Florida Waters

Acute DO data for 26 species of salt water organisms in 25 genera were considered appropriate for use in derivation of revised DO criteria for Florida's marine waters (**Table 8**). A complete tabulation of the data regarding the acute response of organisms to DO is provided in **Appendix E**. The 26 species included in the calculation of the CMC for Florida waters exhibited LC_{50}s that ranged from 0.43 to 2.17 mg/L. The four most sensitive species are: *Menidia beryllina* (inland silverside), *Trachinotus carolinus* (pompano), *Cynoscion nebulosus* (spotted seatrout), and *Harengula jaguana* (scaled sardines) with LC_{50}s ranging from 1.63 to 2.17 mg/L. The final acute value (FAV) is calculated using the following series of equations modified from Stephen *et al.* (1985) (USEPA, 2000):

$$S^2 = \frac{\sum((\ln GMAV)^2) - ((\sum(\ln GMAV))^2/4)}{\sum(P) - ((\sum\sqrt{P}))^2)/4)}$$

$$S = \sqrt{S^2} \qquad P = R/(n+1)$$

$$L = (\sum(\ln\ GMAV) - S(\sum(\sqrt{P})))/4$$

$$A = S(\sqrt{0.95}) + L$$

$$FAV = e^A$$

Where:
R = rank of sensitivity
GMAV = Genus mean acute value
FAV = Final Acute Value

The FAV calculated consistent with USEPA's Virginian Province Approach (USEPA, 2000) using the four most sensitive species is 2.07 mg/L. Because the FAV is calculated using the LC_{50}s (*i.e.*, the concentration that is lethal to 50 percent of the organisms) a correction is needed to result in a criterion that would allow a maximum of five percent of the organisms to be affected. Therefore, the 2.07 mg/L FAV was multiplied by the average LC_5/LC_{50} ratio (*i.e.,* 1.35) for the species used, resulting in a CMC of 2.8 mg/L. Exposure to daily average DO concentrations below 2.8 mg/L should be avoided to prevent unacceptable acute effects (*i.e.,* mortality) to sensitive organisms.

5.2.3 Derivation of Criterion Continuous Concentration (CCC) for DO in Florida Waters

Acceptable data for the chronic (*i.e.,* growth) effects of exposure to low DO concentrations were available for 19 species of salt water organisms found in Florida's marine waters, representing 18 genera (**Table 9**). A complete list of the data regarding the chronic effects of low DO to marine organisms is provided in **Appendix F**. The 19 species included in the calculation of the CCC for Florida waters exhibited species mean chronic values (SMCV) ranging from 1.34 to 4.67 mg/L. The four most sensitive species are: *Litopenaeus setiferus* (northern white shrimp), *Paralichthys lethostigma* and *Paralichthys dentatus* (same genus, summer flounder and southern flounder, respectively), *Libinia dubia* (longnose spider crab), and *Dyspanopeus sayi* (Say mud crab), with genus mean chronic values (GMCV) ranging from 3.46 to 4.67 mg/L.

In accordance with the USEPA's Virginian Province Approach (USEPA, 2000), the CCC is calculated using the same equations specified for the CMC. Based on the four sensitive genera, a CCC of 4.9 mg/L was determined. Therefore, long-term continuous exposure to DO concentrations above 4.9 mg/L should not result in unacceptable chronic effects to sensitive organisms.

5.2.4 Derivation of Final Recruitment Curve (FRC) for DO in Florida Waters

Sufficient dose-response and developmental data for inclusion in the Virginian Province Larval Recruitment Model developed by USEPA were available for 10 species of salt water organisms known to inhabit Florida's marine waters (**Table 10**). The 10 species included in the calculation of the FRC for Florida waters exhibited genus mean acute values (GMAV) ranging from 0.86 to 2.54 mg/L. The four most sensitive species are: *Morone saxatilis*, (striped bass), *Chasmodes bosquianus* (striped blenny), *Dyspanopeus sayi* (Say mud crab), and *Octopus burryi* (Burry's octopus) with GMCVs ranging from 2.15 to 2.54 mg/L. The FRC developed by applying the USEPA's Virginian Province Approach (USEPA, 2000) to Florida specific species is provided in **Figure 27**. Exposures to daily average DO concentrations between the CMC of 2.9 mg/L and the CCC of 4.8 mg/L for durations less than predicted by the FRC should not result in unacceptable recruitment effects (*i.e.,* 5% recruitment loss) to the early developmental stages of sensitive organisms.

Table 8. Data for acute response to low DO conditions for species used to derive CMC for Florida marine waters. Species ranked in order of sensitivity from least sensitive to most sensitive.

Rank	Genus	Species	Common Name	SMAV (LC_{50})	GMAV (LC_{50})	LC_5/LC_{50}
1	Spisula	Spisula solidissima	Atlantic surfclam	0.43	0.43	1.63
2	Rithropanopeus	Rithropanopeus harrisii	Harris mud crab	0.51	0.51	
3	Prionotus	Prionotus carolinus	northern sea robin	0.55	0.55	1.45
4	Eurypanopeus	Eurypanopeus depressus	flat mud crab	0.57	0.57	
5	Leiostomus	Leiostomus xanthurus	spot	0.75	0.75	1.16
6	Scophthalmus	Scophthalmus aquosus	windowpane flounder	0.81	0.81	1.48
7	Palaemonetes	Palaemonetes pugio	daggerblade grass shrimp	0.72	0.86	1.53
7b	Palaemonetes	Palaemonetes vulgaris	marsh grass shrimp	1.02	0.86	1.37
8	Ampelisca	Ampelisca abdita	amphipod	0.90	0.90	
9	Brevoortia	Brevoortia tyrannus	Atlantic menhaden	1.04	1.04	1.53
10	Crassostrea	Crassostrea virginica	eastern oyster	1.15	1.15	
11	Stenotomus	Stenotomus chrysops	scup	1.25	1.25	
12	Mugil	Mugil cephalus	striped mullet	1.38	1.38	
13	Americamysis	Americamysis bahia	mysid shrimp	1.40	1.40	1.16
14	Callinectes	Callinectes sapidus	blue crab	1.40	1.40	
15	Paralichthys	Paralichthys dentatus	summer flounder	1.41	1.41	1.19
16	Farfantepenaeus	Farfantepenaeus duorarum	pink shrimp	1.41	1.41	
17	Sciaenops	Sciaenops ocellatus	red drum	1.45	1.45	
18	Litopenaeus	Litopenaeus setiferus	northern white shrimp	1.47	1.47	
19	Morone	Morone saxatilis	striped bass	1.58	1.58	1.24
20	Lagodon	Lagodon rhomboides	pinfish	1.61	1.61	
21	Syngnathus	Syngnathus fuscus	pipe fish	1.63	1.63	1.17
22	Menidia	Menidia beryllina	inland silversides	1.63	1.63	
23	Trachinotus	Trachinotus carolinus	pompano	1.74	1.74	
24	Cynoscion	Cynoscion nebulosus	spotted seatrout	1.88	1.88	
25	Harengula	Harengula jaguana	scaled sardines	2.17	2.17	

Table 9. Data for chronic response to low DO conditions for species used to derive CCC for Florida marine waters. Species ranked in order of sensitivity from least sensitive to most sensitive.

Rank	Genus	Species	Common Name	NOEC, mg/L	HOEC, mg/L	SMCV, mg/L	GMCV, mg/L
1	Fundulus	Fundulus grandis	Gulf killifish	1.34	1.34	1.34	1.34
2	Crassostrea	Crassostrea virginica	Eastern Oyster	1.50	1.50	1.50	1.50
3	Leiostomus	Leiostomus xanthurus	Spot	2.00	1.50	1.73	1.73
4	Cyprinodon	Cyprinodon variegatus	Sheepshead Minnow	2.25	1.75	1.97	1.97
5	Cynoscion	Cynoscion regalis	Weakfish	2.00	2.00	2.00	2.00
6	Acartia	Acartia tonsa	copepod	2.14	2.14	2.14	2.14
7	Palaemonetes	Palaemonetes pugio	Daggerblade Grass Shrimp	1.50	1.50	1.50	2.18
7b	Palaemonetes	Palaemonetes vulgaris	Marsh Grass Shrimp	4.41	2.60	3.15	2.18
8	Americamysis	Americamysis bahia	Mysid Shrimp	3.29	2.39	2.67	2.67
9	Micropogonias	Micropogonias undulatus	Atlantic croaker	2.70	2.70	2.70	2.70
10	Brevoortia	Brevoortia tyrannus	Atlantic Menhaden	4.00	2.00	2.83	2.83
11	Mercenaria	Mercenaria mercenaria	Northern quahog	4.20	2.40	3.17	3.17
12	Jordanella	Jordanella floridae	Florida flagfish	5.38	2.00	3.28	3.28
13	Menidia	Menidia menidia	Atlantic silverside	3.90	2.80	3.30	3.30
14	Morone	Morone saxatilis	Striped Bass	3.40	3.40	3.35	3.35
15	Litopenaeus	Litopenaeus setiferus	Northern White Shrimp	4.00	3.00	3.46	3.46
16	Paralichthys	Paralichthys dentatus	Summer Flounder	5.14	3.30	3.97	4.09
16b	Paralichthys	Paralichthys lethostigma	Southern Flounder	5.37	3.40	4.22	4.09
17	Libinia	Libinia dubia	Longnose Spider Crab	5.30	4.11	4.67	4.67
18	Dyspanopeus	Dyspanopeus sayi	Say Mud Crab	5.86	3.92	4.67	4.67

Table 10. Species used to derive Final Recruitment Curve for Florida marine waters.

Species	Genus	Common Name	GMAV (LC50), mg/L	Length of Recruitment Season (days)	Duration of Larval Development (days)
Palaemonetes pugio	Palaemonetes	grass shrimp	0.86	100	12
Sciaenops ocellatus	Scianops	red drum	1.76	90	21
Dyspanopeus sayi	Dyspanopeus	mud crab	1.80	66	21
Menidia beryllina	Menidia	silverside	1.94	42	14
Libinia dubia	Libinia	spider crab	2.05	66	21
Eurypanopeus depressus	Eurypanopeus	mud crab	2.11	66	21
Cancer irroratus	Cancer	rock crab	2.15	65	35
Morone saxatilis	Morone	striped bass	2.41	49	28
Chasmodes bosquianus	Chasmodes	stripped blenny	2.50	210	21
Octopus burryi	Octopus	Burry's octopus	2.54	300	28

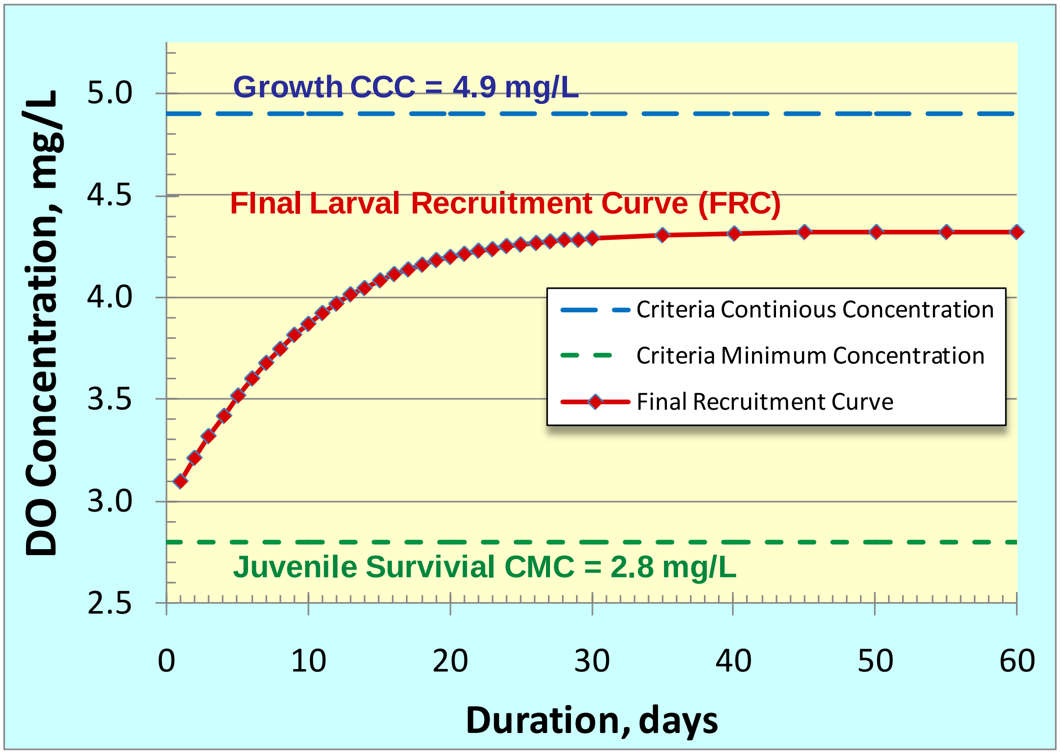


Figure 26. Results of the application of USEPA's Virginian Province Approach to data for Florida specific species to develop revised DO concentration criteria for Florida's marine waters. The three components (*i.e.*, CMC, CCC, and FRC) of the criteria are provided.

5.3 *Developing Saturation Based DO Criteria Using the Virginian Province Approach*

In the freshwater analysis, described in Section 4, DO saturation provided a slightly better correlation with the biological response (*i.e.*, SCI) than did DO concentration. This suggests that DO saturation is a better predictor of the biological health of the system. Additionally, the use of a saturation-based DO criteria accounts for the known seasonal (*i.e.*, temperature) effects on the expected DO levels in natural waterbodies. In contrast, a criterion expressed as a concentration requires that a fixed minimal DO level be maintained throughout the year regardless of the water temperature.

To be consistent with the proposed expression of the proposed DO criteria for freshwaters, the U.S. EPA Virginian Province Approach was used to develop saturation based DO criteria for Florida's marine waters. The percent saturation criteria were derived by converting the DO concentrations used in the development of the concentration based criteria to percent saturations using the experimental water temperature and salinity for the individual tests. The same Virginian Province methodology (USEPA, 2000) described above for the concentration based criteria was then applied to the percent saturation data to derive the three components (*i.e.*, CMC, CCC, FRC) of the criteria.

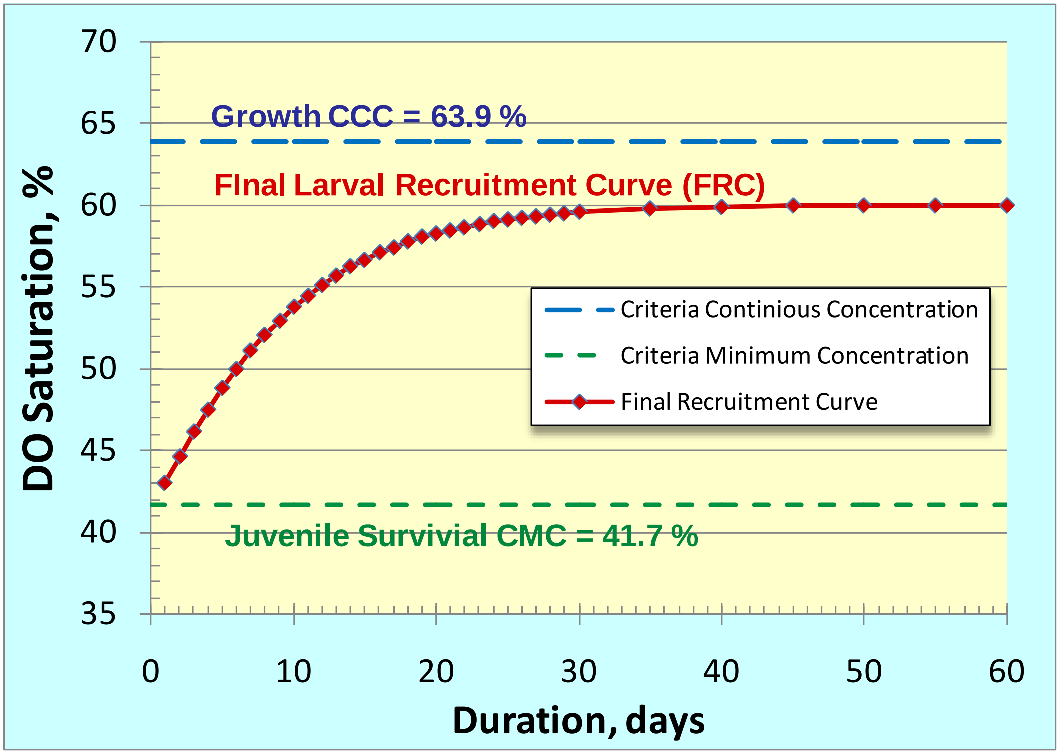


Figure 27. Results of the application of USEPA's Virginian Province Approach to data for Florida specific species to develop revised DO percent saturation criteria for Florida's marine waters. The three components (*i.e.*, CMC, CCC, and FRC) of the criteria are provided.

The 26 species used in the calculation of the concentration based CMC for Florida waters exhibited LC_{50}s that ranged from 5.99 to 32.53 percent saturation. The four most sensitive species are; *Menidia beryllina* (inland silverside), *Trachinotus carolinus* (pompano), *Cynoscion nebulosus* (spotted seatrout), and *Harengula jaguana* (scaled sardines), with LC_{50}s ranging from 24.77 to 32.53 percent saturation.

The final acute value (FAV) calculated in accordance with the Virginian Province methodology using the four most sensitive species is 30.9 percent saturation. A CMC of 42 percent saturation was then calculated by multiplying the FAV by the average LC_5/LC_{50} ratio of 1.35 to adjust the result to allow a maximum of five percent of the organisms to be affected. Exposure to daily average DO levels below 42 percent saturation should be avoided to prevent unacceptable acute effects (*i.e.*, mortality) to sensitive organisms.

Similarly, the data for the 20 species in 18 genera used to calculate the concentration based CCC were converted to percent saturations with the species mean chronic values (SMCV) ranging from 19.35 to 62.61 percent saturation. Based on the percent saturation data, the four most sensitive species are: *Litopenaeus setiferus* (northern white shrimp), *Paralichthys lethostigma* and *Paralichthys dentatus* (same genus, summer flounder and southern flounder, respectively), *Libinia dubia* (longnose spider crab), and *Dyspanopeus sayi* (Say mud crab). The genus mean chronic values (GMCV) for the four most sensitive genera ranged from 50.86 to 62.61 percent saturation. The CCC calculated using the same equations specified for the CMC is 64 percent saturation, indicating that long-term continuous exposure to DO concentrations above 64 percent saturation should not result in unacceptable chronic effects (*e.g.,* growth) to sensitive organisms.

The 10 species included in the calculation of the FRC for Florida waters exhibited genus mean acute values (GMAV) ranging from 0.86 to 2.54 mg/L. The four most sensitive species are; *Cancer irroratus* (rock crab), *Morone saxatilis*, (striped bass), *Chasmodes bosquianus* (striped blenny), and *Octopus burryi* (Burry's octopus), with GMCVs ranging from 2.15 to 2.54 mg/L. The FRC determined by converting the concentration based data from the application of the USEPA's Virginian Province Approach to percent saturation is provided in **Figure 27**. Exposures to daily average DO concentrations between the CMC of 42 percent saturation and the CCC of 64 percent saturation for durations less than predicted by the FRC should not result in unacceptable recruitment effects (*i.e.,* greater than 5% recruitment loss) to the early developmental stages of sensitive organisms.

5.4 Summary of Proposed DO Criteria for Florida's Marine Waters

In accordance with guidance from the USEPA, the Virginian Province Method was applied to data available for Florida specific species. The results of the analyses are provided in **Figure 26** for concentration based data and **Figure 27** for percent saturation based data. A CMC value of 42 percent saturation and a CCC value of 64 percent saturation were calculated using the recommended EPA approach. To avoid unacceptable acute effects (*i.e.,* mortality) to sensitive organisms, the daily average DO level shall not be allowed to fall below the 42 percent saturation CMC value (unless the low DO is determined to be a natural condition). Note that when a daily average is calculated, half of the values during the day tend to be above the average and the other half of the values tend to be below the average.

Concentrations above the 64 percent saturation CCC are protective of all adverse chronic effects resulting from exposure to low DO concentrations, although many healthy Florida marine systems exhibit periods that naturally fall below this level. At DO concentrations between the CMC and CCC, the allowable duration of exposure is provided by the FRC. Durations of exposure at or above those described by the FRC are protective against adverse larval recruitment effects for sensitive species (no more than a 5% reduction in sensitive taxa recruitment due to low DO).

There are several options for incorporating the durations of exposure provided by the FRC into DO criteria. For example, in the DO SSAC for the lower St. Johns River, continuous monitoring is required to develop an annual cumulative frequency distribution for DO concentrations between the CMC and CCC. The frequency of low DO levels within specified ranges are then compared to those predicted by the FRC to evaluate compliance with the SSAC. Because requiring continuously deployed DO Sondes is not feasible for statewide criteria, DEP considered other ways to incorporate the FRC information. In a simpler application, several states have derived minimum 7- and/or 30-day average DO concentrations from the FRC and apply those without the need for continuous monitoring. The minimum 7- and 30-day average concentrations, which DEP expresses as weekly and monthly averages, taken from the FRC developed for Florida's marine waters are 51 percent saturation and 56 percent saturation, respectively.

Due to the better relationship found between the biological response (*i.e.*, SCI) and DO saturation compared to concentration in the freshwater analysis, as well as the inherent adjustment of the DO levels to account for the natural seasonal (*i.e.*, temperature) effects, the DO Peer Review Committee recommended that the saturation based DO criteria be adopted for marine waters. **Figure 28** provides the DO concentrations over the range of water temperatures expected in Florida resulting from the three components of the percent DO saturation criteria for predominately marine waters at a salinity of 15 ppt. DO data collected as concentrations can be converted to percent saturation using temperature and salinity data collected in conjunction with the DO measurements using the equations as provided in APHA, 1989:

$$DO_{sat} = (Exp((-139.34411 + (157570.1/Temp) - (66423080/Temp^2) + (12438000000/Temp^3) - (862194900000/Temp^4)) - (Sal * (0.017674-(10.754/Temp)+(2140.7/Temp^2)))))$$

$$\% DO = (DO_{measure/} DO_{sat})*100$$

Where:

DO_{sat} = DO concentration in mg/L at 100 % saturation,
Temp = water temperature in °K (°C + 273.15 = °K)
$DO_{measure}$ = Measured DO concentration in mg/L.
Sal = Salinity in part per thousand (ppt)

Therefore, the proposed DO criteria for Florida's Class II and III marine waters could be expressed as:

> *The daily average DO concentration shall not be below 42 percent saturation in more than 10 percent of the values.*
>
> *AND*
>
> *The weekly and monthly average DO percent saturations shall not be below 51 and 56 percent, respectively.*

It is recognized that while EPA's Virginian Province approach provides a method for criteria development that would be protective of sensitive species that inhabit Florida's estuarine/coastal waters, some Florida waters exhibit natural DO levels below those derived using this approach. Although these naturally low DO systems are healthy and often highly productive, the early life stages of the sensitive species used in the development of the revised DO criteria may naturally not occur in these waters during the periods of naturally low DO levels (although older life stages may be unaffected). These waters will still require additional efforts to develop Site Specific Alternative Criteria (SSAC) that consider the species naturally found there during low DO conditions, or be assessed by the "deviation from background" approach, discussed below.

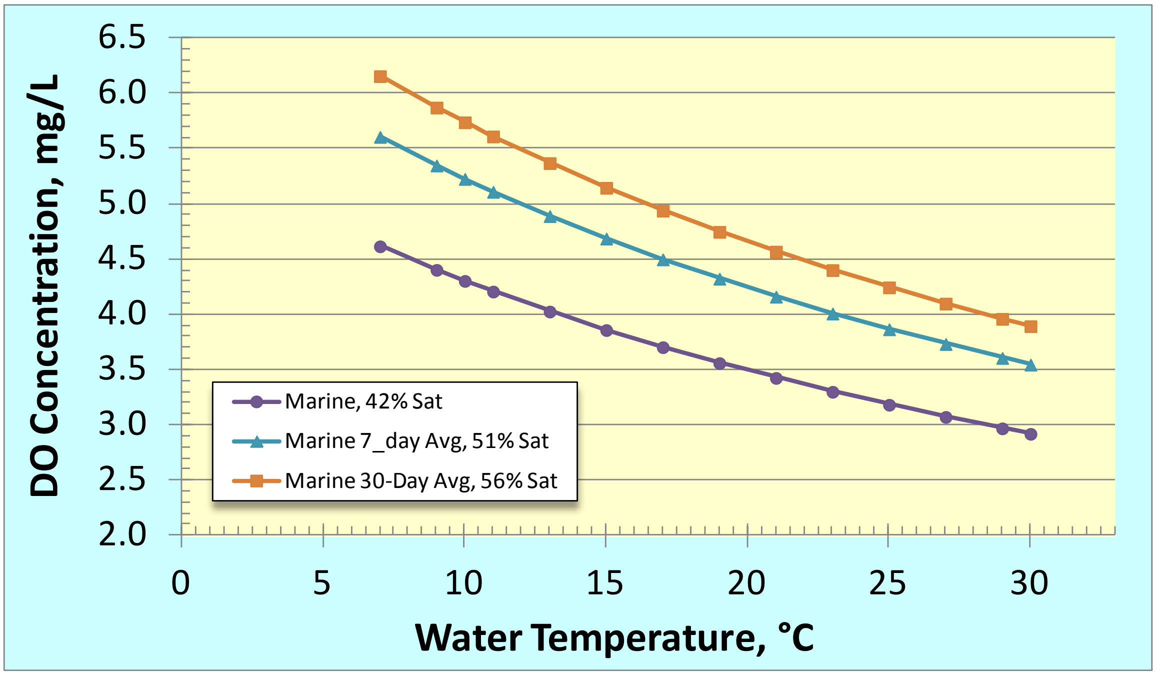


Figure 28. Temperature dependent DO concentrations at 15 ppt salinity resulting from proposed marine DO criteria developed using the Virginian Province Approach.

5.5 *Application of the Proposed Marine DO Criteria*

The proposed criteria will be evaluated, for purposes of ambient 303(d) assessments, using the provisions in Florida's Impaired Waters Rule (Chapter 62-303, F.A.C.) using the binomial hypothesis test which allows no more than 10 percent of the measurements collected during an assessment period to be below the criteria. Additional issues regarding the application of the proposed DO criteria to Florida's marine waters are discussed below.

5.5.1 Sampling Time of Day for Marine Criteria

In natural waterbodies, DO exhibits a diel cycle depending on the level of production and respiration in the waterbody. The proposed DO criteria for Florida's marine waters were derived as daily, weekly, and monthly average percent DO saturations. Ideally, data from continuous (*i.e.*, every 15 to 30 minutes) DO measurements recorded by deployed data Sondes would be used accurately calculate the average DO levels that could then be compared to the criteria to assess compliance. However, most water quality sampling programs throughout Florida do not deploy recording data Sondes, and instead, collect instantaneous grab samples. Due to the natural diel DO fluctuations, if the instantaneous measurements are used to compare to the criteria developed using the daily average percent DO saturation, the time of day for the instantaneous DO measurements could be an important consideration.

The typical range in natural diel fluctuations in minimally impacted Florida estuaries and marine waters are greater than observed for Florida lakes and streams, averaging 2.4 mg/L. As shown in **Figure 29**, the daily average DO concentration in estuaries and marine waters typically occurs just after noon (*i.e.,* 12:10 pm), with the minimum occurring at approximately 8:00 am and the maximum occurring near 5:00 pm. An analysis of diel DO data collected in estuaries and marine waters in different parts of the State as part of the National Estuary Research Reserve (NERR) Program indicates that DO measurements collected anytime during the normal 8:00 am to 5:00 pm work day would, on average, be expected to be within approximately 20 percent of the daily mean. The level of diel fluctuation in marine waters vary considerably with presence of seagrass, level of freshwater input, tidal flushing, amount of organic input, and sediment type.

Steps could be taken in the application of the proposed criteria to further minimize the error associated with using instantaneous measurements to estimate the daily average DO level to assess compliance. These could include requiring that the data used for compliance assessment be collected within a narrower time range that more closely approximates the daily average DO level. However, most of the information DEP uses for surface water assessments consists of "found data" and placing a time restriction on the use of this found data could greatly reduce the data available for assessment purposes.

As an alternative solution, FDEP plans to evaluate the temporal distribution of data available for sites that are identified as potentially impaired (these waters are placed on the "planning list" for further assessment). Before making a final assessment, FDEP will conduct a more in-depth review of the data to evaluate the potential influence that the sampling time had on the results.

In some cases, conclusions can still be made using data not temporally distributed throughout the day. For example, if an assessment conducted using mostly data collected early in the morning (when the lowest DO levels typically occur) indicates that the site achieves the criteria, it can be confidently concluded that the sites does achieve the criteria. Likewise, if an assessment conducted using mostly data collected late in the afternoon (when the highest DO levels typically occur) indicates that the site does not achieve the criteria, it can be confidently concluded that the sites fails to achieve the criteria. In other cases where the available data is not evenly distributed over the day, additional data would be required to be collected before a reliable assessment could be made. The additional data collection would likely include continuous diel monitoring so that an accurate compliance assessment could be made.

In addition, because DO is not a direct pollutant, but instead is a response to other pollutants, waterbodies for which the available data indicate that the DO criteria are not met will undergo a more detailed assessment to determine the causative pollutant(s) responsible for the low DO levels prior to the waterbody being listed as impaired. This assessment can include; a) the collection of additional data if needed, b) a review of the biological health of the system to determine if the low DO conditions are having an adverse effect on the biological communities; c) an assessment of nutrient, chlorophyll, and BOD data to determine if excessive levels of these pollutants can account for the DO levels observed, and d) an evaluation of the diurnal DO range that could help determine if the observed low DO condition is natural or could suggest a cause for the low DO levels.

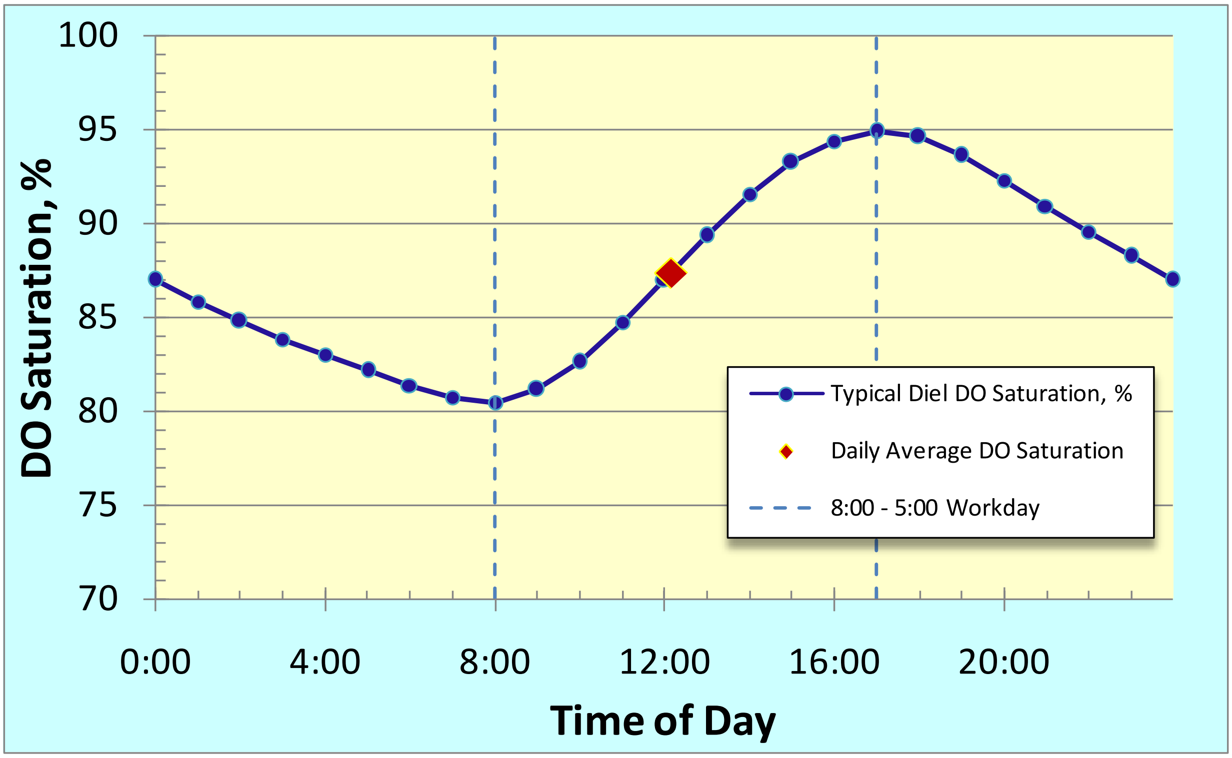


Figure 29. Composite diel DO curve based on hourly records from Apalachicola NEER sites Cat Point and Dry Bar sites, GTM NEER Pine Island, San Sebastian, and Fort Matanzas sites, and Rookery Bay NEER Fakahatchee Bay site.

5.5.2 Data Requirements to Calculate Weekly and Monthly Average DO Levels

The FDEP conducted a statistical boot-strapping analysis to determine the minimum number of grab samples needed to accurately estimate the weekly and monthly average DO saturation level. Because DO measurements can consist of either instantaneous grab samples collected at random times throughout the day or continuous (every 15 to 60 minutes) measurements collected by a deployed sonde, the analysis was conducted using both instantaneous grab samples and daily average data calculated from diel measurements collected every 15 minutes. The analysis consisted of randomly selecting from 1 to 30 instantaneous grab samples or daily average DO saturation levels calculated from diel data. The data used in the analysis were collected as part of the National Estuary Research Reserve's monitoring program in Florida estuaries. The random sampling was performed 10,000 times, with the results being used to calculate a weekly or monthly average DO concentration. The average calculated from the randomly selected samples was then compared to the true weekly or monthly average of the dataset. The variance across the random data pulls was also examined. **Figures 30 and 31** provide the results of the analyses for the monthly average using daily average DO saturation calculated from diel data and instantaneous grab samples, respectively. Similarly, **Figures 32 and 33** illustrate the results of the analysis for the weekly average.

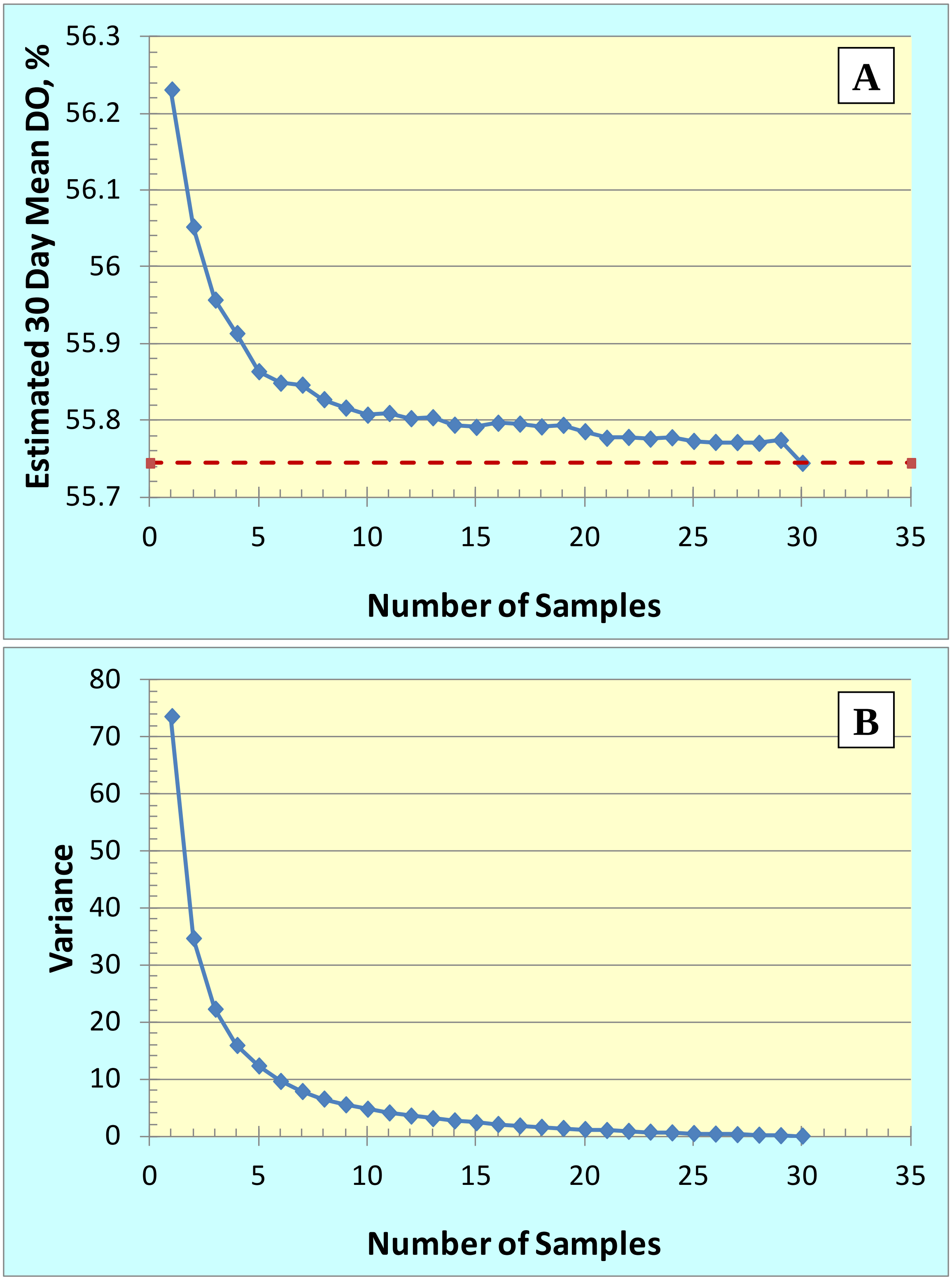


Figure 30. Results of statistical bootstrapping analysis to determine minimum data requirements to accurately calculate the 30-day mean percent DO saturation using daily average data calculated using diel measurements. Graph A shows the estimated 30-day average basd on number of random samples with red dashed line depicting true 30-day average. Graph B provides the variance across 10,000 random iterations.

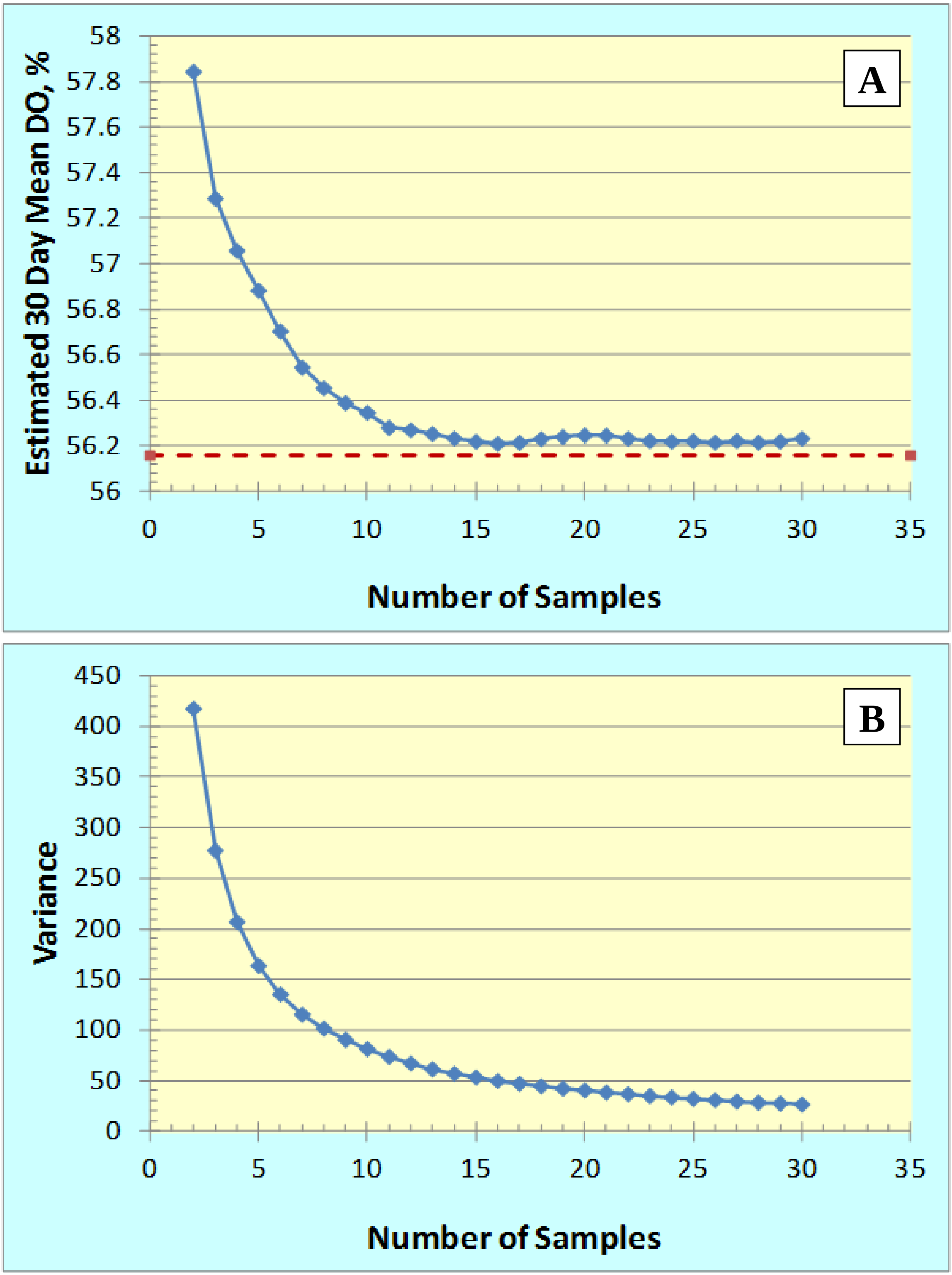


Figure 31. Results of statistical bootstrapping analysis to determine minimum data requirements to accurately calculate the 30-day mean percent DO saturation using grab samples. Graph A shows the estimated 30-day mean basd on number of random samples with red dashed line depicting true 30-day average. Graph B provides the variance across 10,000 random iterations.

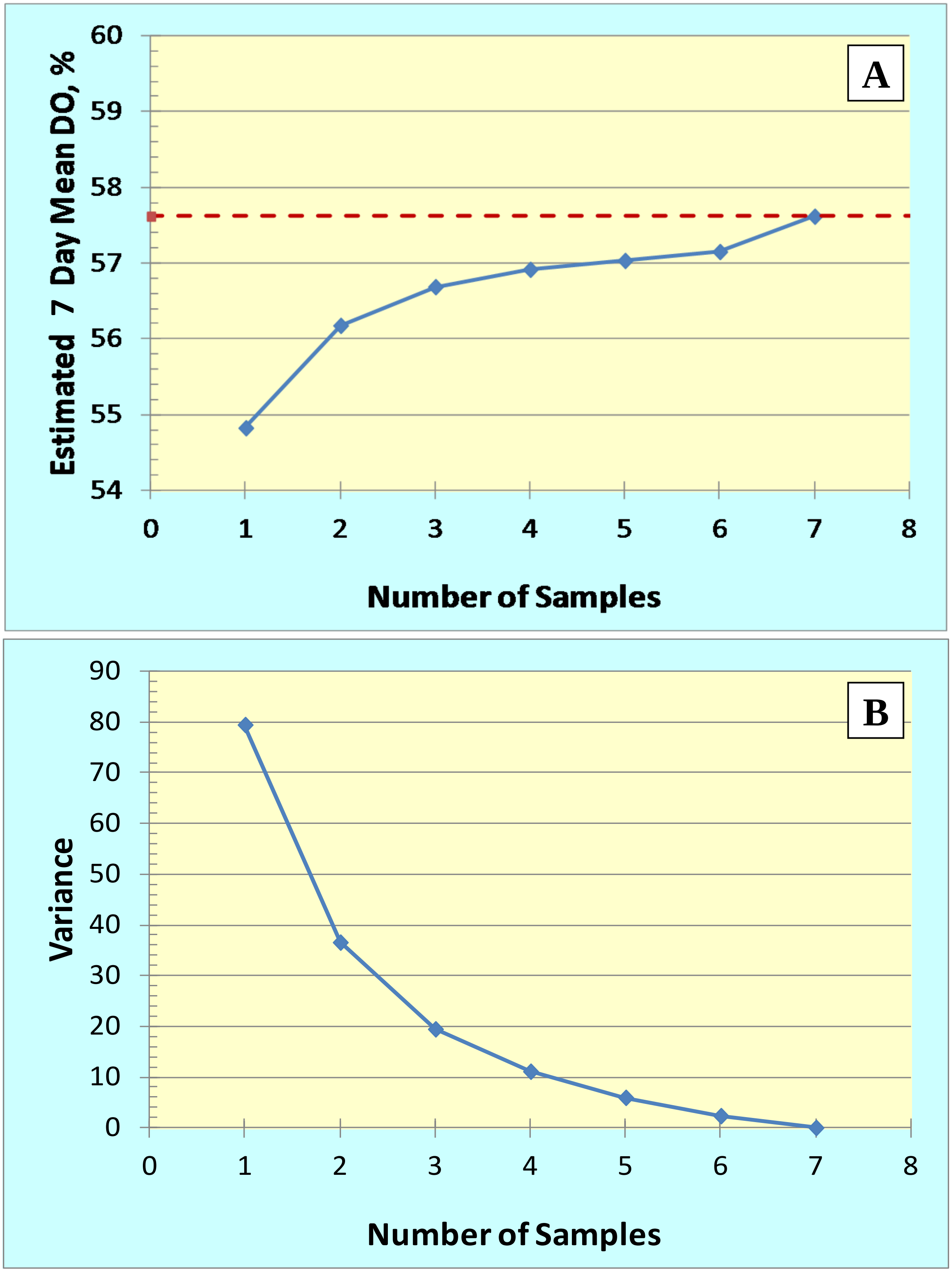


Figure 32. Results of statistical bootstrapping analysis to determine minimum data requirements to accurately calculate the 7-day mean percent DO saturation using daily average data calculated using diel measurements. Graph A shows the estimated 7-day mean basd on number of random samples with red dashed line depicting true 7-day average. Graph B provides the variance across 10,000 random iterations.

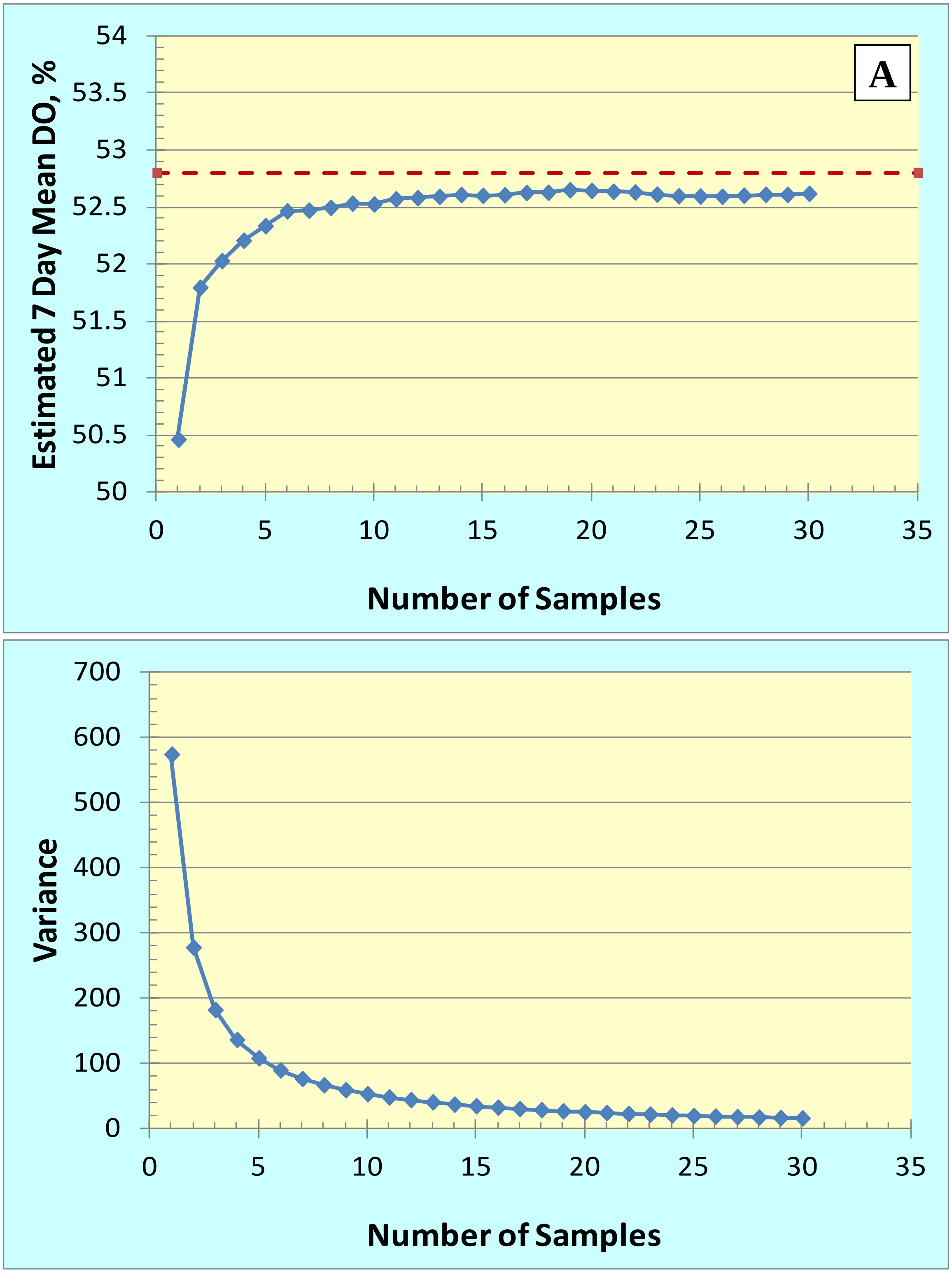


Figure 33. Results of statistical bootstrapping analysis to determine minimum data requirements to accurately calculate the 7-day mean percent DO saturation using grab samples. Graph A shows the estimated 7-day mean basd on number of random samples with red dashed line depicting true 7-day average. Graph B provides the variance across 10,000 random iterations.

The results of the analyses show that, as expected, the calculated weekly and monthly averages approach the true average for the dataset as the number of samples increase. Additionally, the variance across the calculated averages also decreases with increasing sample size. The goal of this analysis is to determine the number of samples required to provide an accurate estimate of the true mean and minimize the variance across samples in order to minimize potential error rates while not requiring an unreasonable number of samples. For the monthly average, the analyses indicate that either a minimum of three daily averages calculated from diel monitoring or at least ten instantaneous grab samples are required to accurately estimate the monthly average percent DO saturation. Either the three daily averages or the ten instantaneous grab samples provided average estimates of the true 30-day average within 0.2% saturation with relatively low levels of variance (**Figures 30 and 31**).

The result of the analysis for the weekly average indicate that either a minimum of three daily averages calculated from diel monitoring or at least ten instantaneous grab samples are required to accurately estimate the weekly average percent DO saturation (**Figures 32 and 33**). Because the natural major factors that cause short-term (≤30-days) fluctuations in DO levels (*e.g.*, variations in biological activity resulting from changes in light levels, water velocity, water temperature, tides, etc.) can have similar effects over both weekly and monthly periods, it is not surprising that the number of samples needed for the weekly average are very similar to those for the monthly average.

Based on these results, the FDEP has determined that the weekly average DO levels may be estimated using a minimum of:

1. Three daily average DO concentrations calculated from three full-days of diel measurements during the week. Diel measurements should be collected at least once every hour, with a minimum of 24 measurements per day (this is the preferred method), or
2. Ten instantaneous DO grab samples, collected during at least three days of the week and at least four hours apart. Grab samples should be distributed evenly between the morning and afternoon.

Additionally, the FDEP has determined that the monthly average DO levels may be estimated using a minimum of:

1. Three daily average DO concentrations calculated from three full-days of diel measurements collected during different weeks of the month. Diel measurements should be collected at least once every hour, with a minimum of 24 measurements per day (this is the preferred method), or
2. Ten instantaneous DO grab samples, collected from a minimum of ten different days of the month. Grab samples should be distributed evenly between the morning and afternoon.

6 Consideration of Threatened and Endangered Species

Four fish species and seven freshwater mussel species that occur in Florida have been listed as threatened or endangered under the Endangered Species Act (ESA) passed by Congress in 1973. Seven additional mussel species found in Florida have been proposed for listing as either threatened or endangered under the ESA. A list of the threatened and endangered aquatic species that occur in Florida that could be potentially affected by the proposed DO criteria is provided in **Table 11**.

The purpose of the ESA is to protect and promote recovery of imperiled species and the ecosystems upon which they depend. To accomplish this objective, the ESA affords additional protection to threatened and endangered species to prevent: 1) damage to, or destruction of, a species' habitat; 2) overutilization of the species for commercial, recreational, scientific, or educational purposes; 3) disease or predation; 4) inadequacy of existing protection; and 5) other natural or manmade factors that affect the continued existence of the species. When one or more of these factors imperils the survival of a species, the United States Fish and Wildlife Service (FWS) takes action to protect it.

The ESA protects endangered and threatened species and their habitats by prohibiting the "take" of listed animals. "Take" is defined as "to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect or attempt to engage in any such conduct." Through regulations, the term "harm" is defined as "an act which actually kills or injures wildlife. Such an act may include significant habitat modification or degradation where it actually kills or injures wildlife by significantly impairing essential behavioral patterns, including breeding, feeding, or sheltering."

A brief discussion of the threatened and endangered species found in Florida waters as well as any potential effects of the proposed DO criteria is provided below.

6.1 *Sturgeon*

All modern sturgeon species spawn in fresh water, and some species spend their entire lives there. A number of sturgeon species, however, spend their adulthood in oceanic environments, while some adult sturgeon live in brackish water (McEnroe and Cech 1987). Two sturgeon species occurring in Florida (*i.e.,* Gulf sturgeon, *Acipenser oxyrinchus desotoi* and shortnose sturgeon, *Acipenser brevirostrum*) have been shown to be particularly sensitive to low oxygen levels (Wakeford 2001) and are provided special consideration due to their listing as threatened and endangered, respectively, under the ESA. Information concerning these two sturgeon species and their DO requirements are provided below.

6.1.1 Shortnose Sturgeon

The shortnose sturgeon (*Acipenser brevirostrum*) is an endangered fish species that occurs in large coastal rivers and estuaries of eastern North America. It is an anadromous fish living mainly in the slower moving riverine waters or nearshore marine waters along the east coast of North America, and migrating periodically into faster moving freshwater areas to spawn. Shortnose sturgeon, unlike other anadromous species, do not appear to make long distance offshore migrations. They are benthic feeders. Juveniles are believed to feed on benthic insects and crustaceans. Mollusks and large crustaceans are the primary food of adult shortnose sturgeon.

Nineteen distinct population segments inhabit rivers ranging from the Saint John River in New Brunswick, Canada, to the St. Johns River in Florida. In the southern portion of the range, they are found in the St. Johns River in Florida; the Altamaha, Ogeechee, and Savannah Rivers in Georgia; and, in South Carolina, the river systems that empty into Winyah Bay and the Santee/Cooper River complex that forms Lake Marion.

Shortnose sturgeon was originally listed as an endangered species by FWS in March 1967, under the Endangered Species Preservation Act. Pollution and overfishing, including bycatch in the shad fishery, were listed as principal reasons for the species' decline. Shortnose sturgeon remained on the endangered species list when Congress passed the ESA in 1973. A recovery plan for the shortnose sturgeon was finalized in December 1998. No critical habitat areas have been identified for the shortnose sturgeon.

Table 11. Florida fish and invertebrate species listed as either threatened or endangered under the Endangered Species Act. (E = endangered, T = threatened, CH = critical habitat)

Common Name	Genus species	Status
Fish		
Shortnose sturgeon	*Acipenser brevirostrum*	Endangered
Gulf sturgeon	*Acipenser oxyrinchus desotoi*	Threatened/CH
Smalltooth sawfish	*Pristis pectinata*	Endangered/CH
Okaloosa darter	*Etheostoma okaloosae*	Threatened
Mussels		
Fat three ridge	*Amblema neislerii*	Endangered/CH
Chipola slab shell	*Elliptio chipolaensis*	Threatened/CH
Purple bank climber	*Elliptoideus sloatianus*	Threatened/CH
Shiny rayed pocketbook	*Hamiota (=Lampsilis) subangulata*	Endangered/CH
Gulf moccasin shell	*Medionidus penicillatus*	Endangered/CH
Ochlockonee moccasin shell	*Medionidus simpsonianus*	Endangered/CH
Oval pigtoe	*Pleurobema pyriforme*	Endangered/CH
Tapered pigtoe	*Fusconaia burkei*	Proposed (T)
Narrow pigtoe	*Fusconaia escambia*	Proposed (T)
Round ebony shell	*Fusconaia rotulata*	Proposed (E)
Southern sand shell	*Hamiota australis*	Proposed (E)
Fuzzy pigtoe	*Pleurobema strodeanum*	Proposed (T)
Southern kidney shell	*Ptychobranchus jonesi*	Proposed (E)
Choctaw bean	*Villosa choctawensis*	Proposed (E)

6.1.2 Gulf Sturgeon

Gulf sturgeon are anadromous, spending cooler months (October or November through March or April) in estuarine or marine habitats, where they feed on benthic organisms such as isopods, amphipods, lancets, molluscs, crabs, grass shrimp, and marine worms (Wakeford, 2001). In the spring, gulf sturgeon return to their natal river, where the sexually mature sturgeon spawn, and the population spends the next 6–8 months there before returning to estuarine or marine waters for the winter (Fox *et al.* 2000; Wakeford 2001).

Historically, Gulf sturgeon occurred from the Mississippi River east to Tampa Bay. Sporadic occurrences were recorded as far west as the Rio Grande River in Texas and Mexico, and as far east and south as Florida Bay (Wooley and Creteau 1985). The sub-species' present range extends from Lake Pontchartrain and the Pearl River system in Louisiana and Mississippi respectively, east to the Suwannee River in Florida. Gulf sturgeon require specific ecosystem conditions to survive. In their riverine habitat, sturgeon require waters that have large areas of diverse habitat; natural variations in flow, velocity, temperature, and turbidity; free-flowing sections to provide suitable spawning sites; and uninhibited access to upriver spawning sites. During their early-life-history stages, sturgeon require bedrock and clean gravel or cobble substrate for eggs to adhere to and for shelter for developing larvae (Wakeford 2001). Young-of-the-year sturgeon appear to disperse widely, using extensive portions of the river as nursery habitat. Larvae and juveniles in their sensitive life stages generally utilize waters where temperature is approximately 20^{o} C or less during the summer. In Florida, these year-round cool temperature waters are restricted to Floridan Aquifer springs, some of which (*e.g.,* Anderson Spring in Suwannee county) are well known sturgeon breeding areas.

The Gulf Sturgeon was listed as a threatened species under the ESA on September 30, 1991 (Federal Register, Vol. 56, No. 189, September 30, 1991), with the critical habitat areas being finalized in March 2003. The critical habit areas for the Gulf Sturgeon are tabulated in **Tables 12 and 13** for freshwater and estuarine/marine habitats, respectively. Also, the critical habitat areas located within Florida are illustrated in **Figure 34**.

Table 12. Approximate linear distance of the riverine critical habitat units for the gulf sturgeon. Main stems are listed first with tributaries being indented. (Reproduced from Federal Register Vol 68, No. 53. 3/19/2003).

Critical Habitat Unit River Systems	State	River Kilometers	River Miles
1. Pearl (East, West, and all tributaries)	Louisiana/Mississippi	632	393
Bogue Chitto		163	101
2. Pascagoula	Mississippi	203	126
Leaf		164	102
Bouie		10	6
Chickasawhay		232	144
Big Black Creek		8	5
3. Escambia	Florida/ Alabama	117	73
Conecuh		127	79
Sepulga		11	7
4. Yellow	Florida/ Alabama	154	96
Blackwater		18	11
Shoal		13	8
5. Choctawhatchee	Florida/ Alabama	249	155
Pea		92	57
6. Apalachicola	Florida	254	158
Brothers		24	15
7. Suwannee	Florida	293	182
Withlacoochee		19	12
Total Freshwater		**2,783**	**1,730**

Table 13. Approximate area of the estuarine and marine critical habitat units for the gulf sturgeon. Main stems are listed first with tributaries indented. (Reproduced from Federal Register Vol 68, No. 53. 3/19/2003).

Critical Habitat Unit Estuarine and Marine Systems	State	Kilometers^2	Miles^2
8. Lake Borgne	Louisiana/ Mississippi/ Alabama	718	277
Little Lake		8	3
Lake Pontchartrain		763	295
Lake St. Catherine		26	10
The Rigolets		13	5
Mississippi Sound		1,879	725
MS near shore Gulf		160	62
9. Pensacola Bay	Florida	381	147
10. Santa Rosa Sound	Florida	102	39
11. Near shore Gulf of Mexico	Florida	442	171
12. Choctawhatchee Bay	Florida	321	124
13. Apalachicola Bay	Florida	683	264
14. Suwannee Sound	Florida	546	211
Total		**2,783**	**1,730**

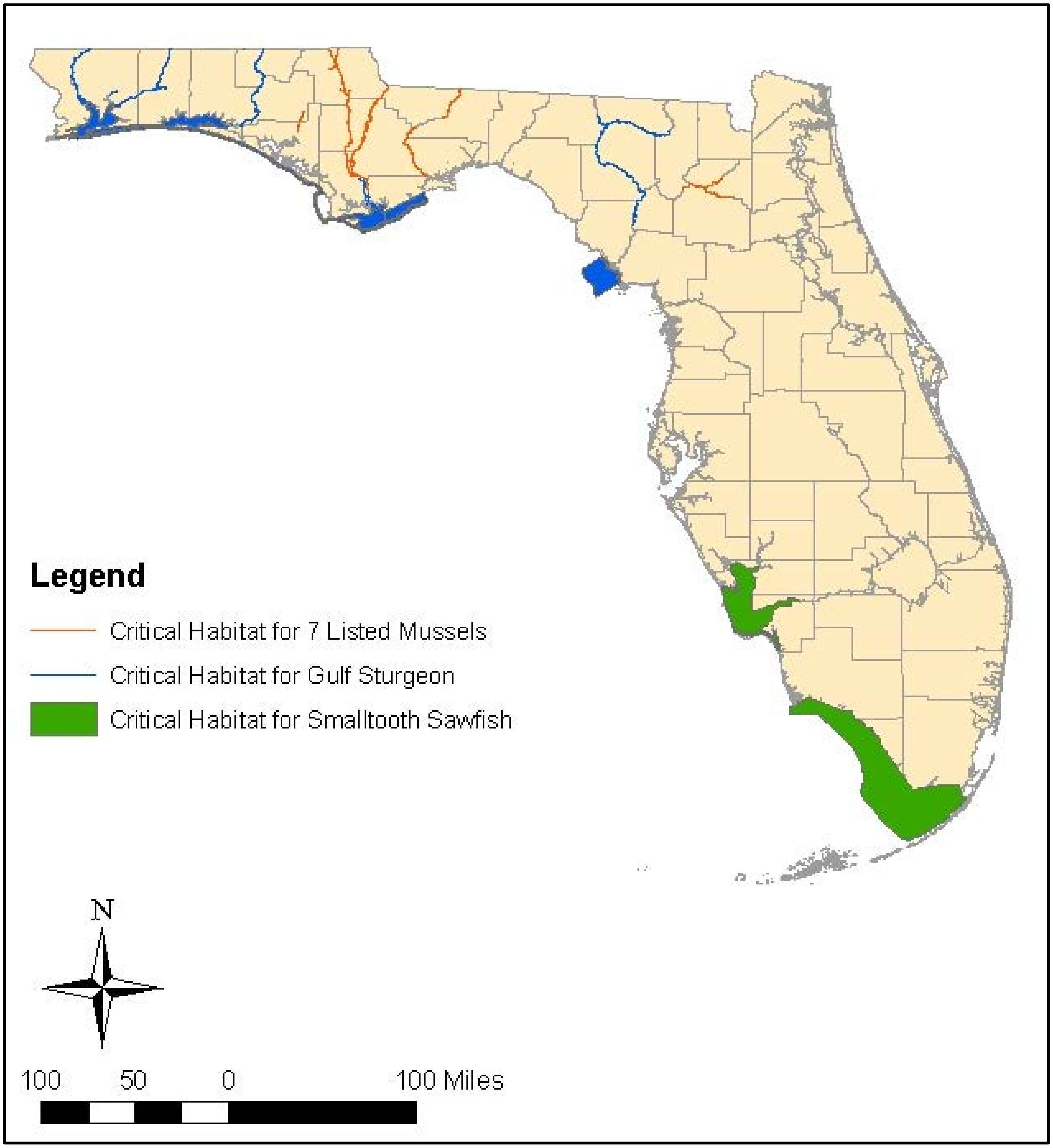


Figure 34. Areas identified as critical habitat areas in Florida for fish and aquatic invertebrates listed as either threatened or endangered under the Endangered Species Act.

6.1.3 Dissolved Oxygen requirements of Sturgeon

Many sturgeon species are oxyphilic, requiring high levels of oxygen. The sensitivity of sturgeon to low DO conditions appears to decrease as the fish matures. Jenkins *et al.* (1993) found that juvenile (< 25 days old) shortnose sturgeon experienced complete mortality (100 %) when exposed to DO concentrations of 2.5 mg/L for six hours. However, the tolerance to this low DO level increased with age, with 96 percent mortality for fish 32 days old, 86 percent mortality for fish 64 days old, and only 12 percent mortality for fish 104 to 310 days old.

Additionally, the research indicated that young sturgeon were also very sensitive to exposure to high salinity (> 7 ppt) with the sensitivity also decreasing with age of the fish. This finding confirms the need of the young sturgeon, which exhibit a higher sensitivity to low DO conditions, to remain in freshwater for several months prior to returning to marine waters for the winter. Kynard's (1997) study of the shortnose sturgeon also indicates that the young-of-the-year sturgeon remain in freshwater for approximately a year and that the most sensitive larval and juvenile life stages of the sturgeon were not utilizing marine waters.

Research has also shown that sturgeon's sensitivity to low DO levels may be exacerbated by high ambient water temperatures (> 28 °C). At high temperatures, concomitant low levels of DO may be lethal. Secor and Gunderson (1997) found that exposure of young-of-the-year Atlantic sturgeon to DO levels of approximately 3 mg/L (*i.e.*, 2-3 mg/L) in unsealed tanks for 10 days resulted in a mean survival of 75 percent at low temperature (approximately 19 °C) compared to 0 to 50 percent (average of 12.5 percent) survival at high temperature (approximately 26 °C). When exposed to low DO levels at high temperature in sealed tanks that prevented access to the air-water interface, all of the sturgeon died and mortality occurred much more rapidly (*i.e.*, within the first 30 hours). This suggests that sturgeon can utilize the relatively oxygen-rich water near the air-water interface to survive short-term exposures to low DO levels.

Because only two DO (*i.e.,* 2-3 and 6-7 mg/L) and temperature (*i.e.,* ~19 and ~26 °C) treatments were utilized in their studies, more precise estimates of the DO and temperature levels that became stressful to the sturgeon are not possible. Additionally, the authors (Secor and Gunderson, 1997) suggest that the response to low DO and high temperature of the specimens from the Hudson River population used in their study could potentially be different from the response of more southern populations. The Hudson River rarely becomes hypoxic compared to more southern waters (*e.g.*, Chesapeake Bay and south) that naturally exhibit higher temperatures and lower DO levels. Therefore, it is feasible that the more southern populations of sturgeon could exhibit a different (*i.e.*, less sensitive) response to low DO and high temperature than observed in the study.

Because the sensitive early life stages of sturgeon do not utilize marine waters and the less sensitive older sturgeon move to the marine waters in the cooler months when DO levels are normally higher, the proposed marine DO criteria of 42 percent minimum daily average with weekly and monthly averages of 51 and 56 percent saturation, respectively, are expected to be protective of the Shortnose or Gulf sturgeon. At temperatures of 20 °C or less, which are typical in the fall and winter when the sturgeon are present, minimum DO concentrations of 3.8 mg/L or higher and monthly average DO levels of 5.1 mg/L will be required to achieve the proposed criteria described above (**Figure 35**).

The critical freshwater habitat that has been identified for the Gulf Sturgeon within Florida is located in the Big Bend and Northeast bioregions, respectively (**Figure 34**). The most sensitive early life stages of the gulf sturgeon can occur in the Suwannee, Santa Fe, and Withlacoochee Rivers in the spring and fall while young Atlantic and shortnose sturgeon may occupy portions of the St. Johns River in the spring in February and March. Research has suggested that these early life stages of the sturgeon can require DO levels slightly above the 34 percent saturation criteria proposed for the Big Bend and Northeast bioregions. Research has also indicated that the DO requirements of the sturgeon decreases as they age. Therefore, to assure that the early life stages of the sturgeon are fully protected, the proposed DO criteria was modified for portions of the Suwannee, Santa Fe, Withlacoochee, and St. Johns Rivers where young sturgeon may be found.

A description of the modified criteria developed in conjunction with the U.S. Fish and Wildlife Service (USFWS) and NOAA's National Marine Fisheries Service (NMFS) is provided in **Appendix I** along with a description of the areas in the Suwannee, Santa Fe, Withlacoochee, and St. Johns Rivers that may be inhabited by young sturgeon. With this modification, the proposed DO criteria is expected to be fully protective of all life stages of the Gulf, Atlantic, and Shortnose Sturgeons in both their fresh and marine water habitats within Florida based on the best available information.

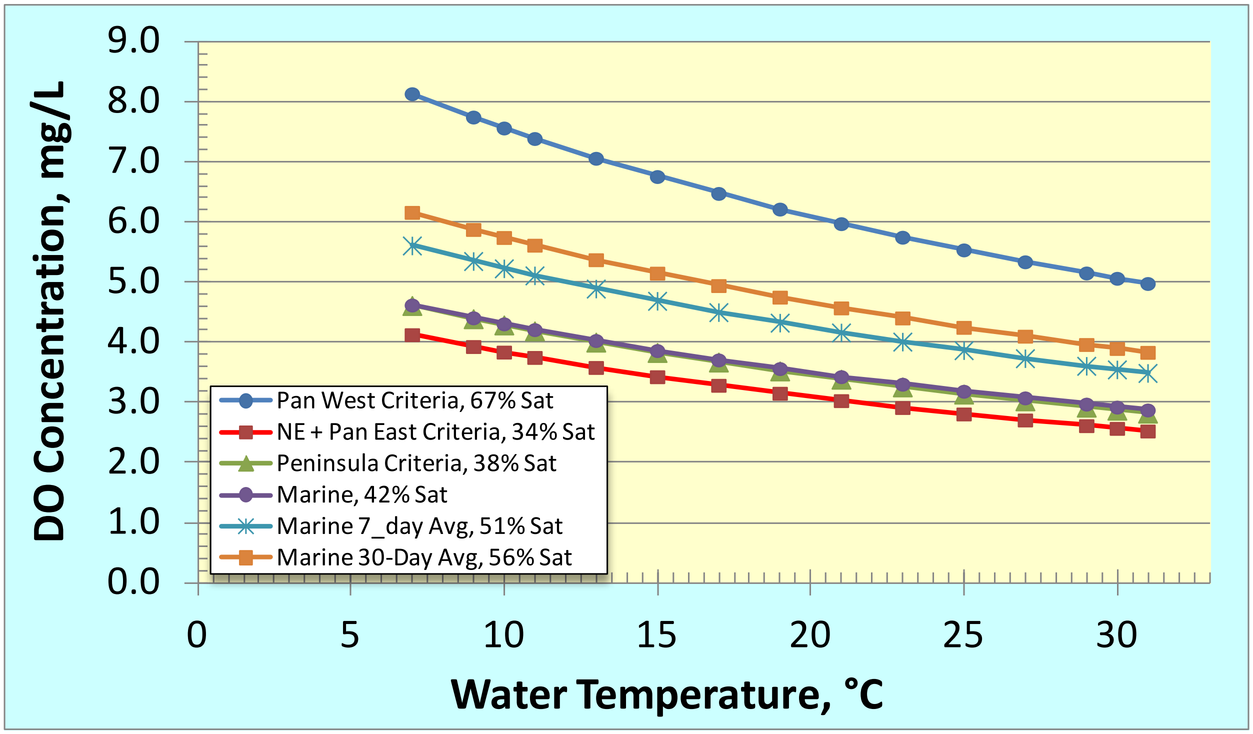


Figure 35. Dissolved oxygen concentrations resulting from the proposed DO saturation criteria for Florida's fresh and marine waters over the range of expected water temperatures. DO concentrations for marine criteria calculated using a salinity of 15 ppt.

6.2 *Okaloosa Darter*

The Okaloosa darter, *Etheostoma okaloosae*, is a small, perch-like fish (maximum size of 49 millimeters Standard Length) that is listed as threatened under the ESA.

The endemic Okaloosa darter is known to occur in only six clear stream systems that drain into two Choctawhatchee Bay bayous in Walton and Okaloosa Counties in the northwest Florida panhandle. Okaloosa darters are currently found in the tributaries and the main channels of the following six streams: Toms, Turkey, Mill, Swift, East Turkey, and Rocky Creeks. Approximately 90 percent of the 457 square kilometer (176 square mile) watershed drainage area that historically supported the Okaloosa darter is under the management of Eglin Air Force Base (AFB), with approximately 99 percent of the stream length in the darter's current range is within the boundaries of Eglin AFB. Eglin AFB encompasses the headwaters of all six of these drainages, and the remainder of the these streams flow out of Eglin AFB into the urban complex of the Cities of Niceville and Valparaiso (Federal Register, Vol. 76 (63), Friday, April 1, 2011 18087- 18103).

The Okaloosa darter was first listed as endangered under the ESA on June 4, 1973 (38 FR 14678) due to its extremely limited range, habitat degradation, and apparent competition from a possibly introduced related species, the brown darter. A recovery plan for the darter was completed on October 23, 1981, and revised on October 26, 1998. As a result of the habitat restoration activities completed as identified in the recovery plan and the subsequent stabilization/ growth of the population, the Okaloosa darter was reclassified from endangered to threatened on April 1, 2011 (Federal Register, Vol. 76 (63), Friday, April 1, 2011 18087- 18103).

Sensitivity to low DO conditions has not been identified as a factor contributing to the decline of the Okaloosa darter, and there is no scientific literature that suggests that the Okaloosa darter requires DO levels higher than those required by the sensitive macroinvertebrates (required to pass the SCI) used in the derivation of the proposed DO criteria. Additionally, the proposed DO criteria for the Florida Panhandle West bioregion, which encompasses the known habitat of the Okaloosa darter, is higher than the State's current 5.0 mg/L criteria for much of the year (**Figure 35**) and only drops slightly below 5.0 mg/L during the highest water temperatures. More specifically, during the 2005 – 2006 year long DO study discussed in Section 2, the median water temperature for minimally impaired sites used in the derivation of the criteria was 20.5 °C with a 90th percentile of 24.9 °C. The corresponding DO criteria for the median and 90th percentile water temperatures would be 5.3 and 4.8 mg/L, respectively. Therefore, the evidence indicates that the proposed DO criteria are fully protective of the Okaloosa darter.

6.3 *Freshwater Mussels*

Seven freshwater mussel species were listed as either threatened or endangered (five as endangered and two as threatened) on March 16, 1998 (63 FR 12664). An additional seven species have been proposed to be listed as either threatened or endangered. A complete tabulation of the mussel species currently listed as threatened or endangered as well as those proposed for listing is provided in **Table 11**. The seven listed mussels are restricted to a few river basins (Apalachicola-Chattahoochee-Flint, Ochlockonee, Suwannee, and Econfina Creek) in Alabama, Florida, and Georgia. Historically, they were distributed across hundreds of stream miles in these basins and now survive in a few relatively small, isolated populations scattered throughout their former range. The Florida critical habitat areas for the listed mussel species are shown in **Figure 34**. In Florida, the habitat range for these mussels is limited to the panhandle bioregion, except for the Oval Pigtoe (*Pleurobema pyriforme*) which also occurs in the Big Bend bioregion.

According to the FWS Recovery Plan for the listed mussels, habitat alteration, including impoundments, channelization, gravel mining, contaminants, sedimentation, and stream-flow depletion, is likely the principal cause of these species' decline in range and abundance. Genetic factors associated with increasingly small and isolated populations and the introduction of invasive alien species may present additional obstacles to their recovery. Brim-Box and Williams (2000) also identify deforestation, intensive upland agricultural development, river impoundments, and declines in native host fish species as the major factors adversely affecting mussel diversity and abundance. Infrequent natural disturbances such as floods and droughts may further affect mussels by causing physiological stress or death to individuals or populations already stressed by habitat alteration.

In a study of the effects of drought conditions on mussel populations, Johnson *et al.* (2001) found that low DO levels during periods of very low flow (and low water velocity) were highly correlated to mussel mortality in the Flint River Basin. Based on weekly field observations, they found that mussel morality was significantly higher when DO levels were below 5.0 mg/L compared to above 5.0 mg/L, especially when flow velocity at the substrate surface fell below 0.01 m/s. The 5.0 mg/L threshold used in the analysis was selected somewhat arbitrarily based on visual examination of a scatter plot of the data collected. Due to the variability in confounding factors during the field observations, a more refined threshold could not be determined. Additionally, Johnson *et al.* (2001) found significant species differences in the response to low DO conditions with *Elliptio crassidens*, *Lampsilis subangulata*, *Medionidus pencilatus* and *Pleurobema pyriforme* experiencing the highest mortality under hypoxic conditions (*i.e.,* DO <5 mg/L). *Elliptio complanata* and *Villosa vibex* had lowest mortality under hypoxia and withstood long-term exposures to DO levels below 5.0 mg/L.

The proposed DO criteria for the Florida Panhandle West bioregion, which encompasses the known habitat of the threatened or endangered mussel species, except the Oval Pigtoe, is higher than the State's current 5.0 mg/L criteria for much of the year (**Figure 35**) and only drops slightly below 5.0 mg/L during the highest water temperatures. More specifically, during the 2005 – 2006 year long DO study discussed in Section 2, the median water temperature for minimally disturbed sites used in the derivation of the criteria was 20.5 °C with a 90th percentile of 24.9 °C. The corresponding DO criteria for the median and 90th percentile water temperatures would be 5.3 and 4.8 mg/L, respectively. Therefore, the proposed DO criteria are expected to fully protect the threatened or endangered mussel species in the Panhandle West bioregion.

In the Big Bend bioregion, the proposed DO criteria was modified in portions of the New and Santa Fe Rivers inhabited by the Oval Pigtoe mussel to assure the mussel was fully protected. A description of the modified criteria developed in conjunction with the USFWS is provided in **Appendix I** along with a description of the areas in the New and Santa Fe Rivers inhabited by the Oval Pigtoe mussel. With this modification, the proposed DO criteria is expected to be fully protective of the Oval Pigtoe mussel habitats within Florida based on the best available information.

6.4 Smalltooth Sawfish

Smalltooth sawfish are another endangered tropical marine and estuarine fish species found in Florida (NMFS, 2009). Prior to around 1960, smalltooth sawfish occurred commonly in shallow waters of the Gulf of Mexico and eastern seaboard up to North Carolina, and more rarely as far north as New York. Subsequently, their distribution has contracted to peninsular Florida and, within that area, can only be found with any regularity off the extreme southern portion of the state. The current distribution is centered in the Everglades National Park (including Florida Bay). Smalltooth sawfish in the United States generally inhabit shallow waters of inshore bars, mangrove edges, and seagrass beds, but are occasionally found in deeper coastal waters.

Although time-series abundance data are lacking, publication and museum records, negative scientific survey results, anecdotal fisher observations, and limited landings per unit effort data (from Louisiana) indicate that smalltooth sawfish have declined dramatically in U.S. waters over the last century (NMFS 2009). The smalltooth sawfish was listed as endangered under the ESA on April 16, 2001 (Federal Register Vol. 66, No. 73, 19414) with a recovery plan finalized in January 2009 (NMFS 2009). The critical habitat for the sawfish within Florida is shown in **Figure 34** (Norton *et al.* 2012).

Loss and/or degradation of habitat and excessive overexploitation and collection as bycatch in fishing nets have been identified as the potential factors most directly leading to the decline in abundance and distribution of the smalltooth sawfish. Because sawfish are relatively slow growing, take several years to reach maturity, and have low fecundity, their population numbers are slow to recover from excessive loss.

Little is known about smalltooth sawfish life history or environmental requirements, but some inferences can be drawn from the locations where they are found. Based on public and research observations, gravid females enter estuaries briefly for parturition with the juveniles occupying the lower reaches of rivers, estuaries, and coastal bays for approximately the first 3 years of their life (up to approximately 2.5m total length) (Simpfendorfer *et al.* 2008 and 2010, Scharer *et al.* 2012). Based on research encounters, sawfish have been found at depths from less than 1 meter to over 150 meters, salinities from 1.98 to 38.60 ppt, DO concentrations from 3.5 to 9.1 mg/L, and water temperatures from 20.9 to 33.2°C (Waters *et al.* 2011). Because most of these observations came from small to very small juvenile fish, when most species are most sensitive to low DO conditions, this limited information suggests that the smalltooth sawfish is not more sensitive to low DO levels than the sensitive species used to derive the proposed criteria. This conclusion is supported by data collected by the Rookery Bay National Estuarine Research Reserve (NERR) in the Ten Thousand Islands area of the Southwest Florida coast. The Ten Thousand Islands area is located adjacent to Everglades National Park and is one on the most pristine estuarine areas in Florida with little anthropogenic input. The sawfish encounter data collected in Ten Thousand Islands area show that sawfish were most often captured in Faka Union and Fakahatchee Bays, with encounters recorded during all months of the year except January and December (Unpublished data provided by Patrick O'Donnell, Rookery Bay NERR). The DO conditions associated with the locations of the sawfish captures ranged from 3.2 to 7.6 mg/L with temperatures from 18.6 to 33.8°C and salinities from 5.5 to 38.6 ppt. Approximately 38 percent of the sawfish captures occurred at DO concentrations below 5.0 mg/L with 15 percent of the captures occurring at DO levels below 4.0 mg/L. Additionally, the Rookery Bay NERR maintains continuous diel data recorders that have measured DO levels in both Faka Union and Fakahatchee Bays since 2002. **Figures 6 and 7** summarize the data that has been collected in Fakahatchee Bay and **Figure 36** provided the data collected in Faka Union Bay. Data from both bays indicate daily average DO levels below 5.0 mg/L occur commonly, especially in the summer months. In Faka Union Bay, where the majority of the sawfish captures occurred, 44% of the daily average DO levels were below 5.0 mg/L.

Smalltooth sawfish also have been documented to commonly occur further north along the southwest Florida coast in the Charlotte Harbor and Caloosahatchee River estuaries located in more urbanized areas and are much more highly influenced by anthropogenic inputs, including nutrients. In fact, the Caloosahatchee River and Estuary have been determined to be impaired by excessive nutrient inputs and is currently undergoing TMDL development. The higher nutrient inputs to these systems results in greater productivity which can in turn influence of the factors such as the DO levels. Poulakis *et al.* (2010 and 2011) studied the abiotic affinities of the smalltooth sawfish in the Charlotte Harbor and Caloosahatchee River estuaries. Based on their captures of sawfish, they reported that sawfish had an affinity for high DO levels above 6.0 mg/L. Additionally, the authors indicate increasing electivity index values for DO levels up to 12 mg/L. It should be noted that at temperature and salinity levels typically found in this area, DO concentrations above 8 mg/L represent supersaturated DO conditions characteristic of nutrient enriched areas, but not often found in more pristine areas.

While this study provides much very useful information concerning the sawfish, extreme care should be exercised in the interpretation and extrapolation of the results to other waterbodies for use in establishing regulatory water quality limits for parameters such as DO for a number of reasons including: 1) in addition to being a biotic requirement, DO is a response variable that is influenced by inputs of other parameters such as nutrients and organic material and can be correlated to other waterbody characteristics such as water velocity, amount of shading, color, and depth; 2) as described previously, the Charlotte Harbor and Caloosahatchee River estuaries are more nutrient enriched than other waterbodies in the sawfish range, resulting in higher levels of primary production which can artificially raise DO levels; 3) the abiotic affinities reported do not represent biotic requirements, they only characterize the conditions at the time and location where the sawfish were captured; and 4) based on discussions with the authors (Gregg Poulakis and Philip Stevens personal communication) the DO measurements reported were often taken later in the day when the highest DO levels typically occur. Additionally, in nutrient enriched waters such as the Caloosahatchee River estuary, the diel DO fluctuation is commonly more than 3 mg/L. Therefore, the results of this study, in conjunction with the data from more pristine areas, may suggest that sawfish have an affinity for areas with greater primary production resulting from anthropogenic nutrient inputs as is common for other fish species.

In additional studies in the Caloosahatchee River estuary between 2005 and 2007, Simpfendorfer *et al.* (2011) found that salinity and temperature were important environmental factors influencing the movement and location of smalltooth sawfish. However, the study did not include DO level as a variable.

At 20° C and 20 ppt salinity, the proposed marine DO criterion CMC of 42 percent saturation will result in a required minimum daily average DO of 3.4 mg/L, which is a level empirically observed to support juvenile sawfish in areas with limited anthropogenic inputs. Additionally, to satisfy the proposed marine DO criteria, both the weekly and monthly average must be met, meaning average DO conditions must be maintained well above 3.4 mg/L (*e.g.*, 4.2 mg/L at 25° C and 20 ppt salinity). Because juvenile smalltooth sawfish have been shown to inhabit natural areas where daily low DOs of < 4 mg/L commonly occur, the proposed marine DO criteria are expected to fully protect the smalltooth sawfish.

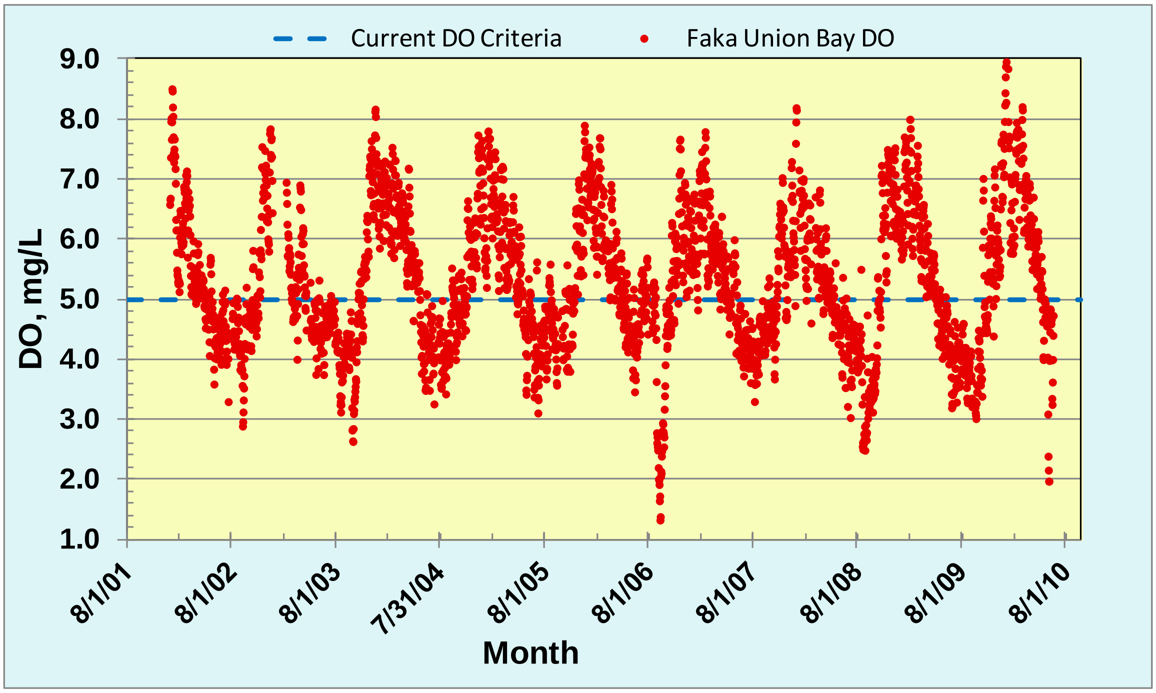


Figure 36. Daily average DO concentrations for Faka Union Bay determined from diel monitoring data (collected every 15 minutes) by the Rookery Bay National Estuarine Research Reserve from January 2002 through June 2010. Dashed line indicates Florida's currently daily average marine DO criteria of 5.0 mg/L

7 Deviation from Background

The USEPA (1986) guidance on freshwater DO criteria recognized that under some circumstances, natural conditions can result in DO concentrations below the generally applicable criteria stating:

> *Where natural conditions alone create dissolved oxygen concentrations less than 110 percent of the applicable criteria means or minima or both, the minimum acceptable concentration is 90 percent of the natural concentration. These values are similar to those presented graphically by Doudoroff and Shumway (1970) and those calculated from Water Quality Criteria 1972 (NAS/NAE, 1973). Absolutely no anthropogenic dissolved oxygen depression in the potentially lethal area below the 1-day minima should be allowed unless special care is taken to ascertain the tolerance or resident species to low dissolved oxygen.*

Similarly, in their guidance on marine DO criteria, USEPA states that:

> *"A daily average of 5.0 mg/L and no less than 4.0 mg/L at all times. If it is determined that the natural condition in the waterbody is less than the values stated above, then the criteria will revert to the natural condition and the water quality standard will allow for a 0.1 mg/L deficit from the natural dissolved oxygen value. Up to 10 percent deficit will be allowed if it is demonstrated that resident aquatic species shall not be adversely affected" (USEPA, 1980).*

To recognize that some Florida waters will naturally exhibit DO levels below the proposed criteria, language similar to that provided in EPA guidance will be included in the proposed criteria to allow an alternative criteria to be established at natural background levels or at a minimal deviation from natural background conditions if it is demonstrated that the biological communities present are not adversely affected. For example, FDEP would require that DO data be coupled with a site-specific evaluation of marine taxa, compared to those taxa expected in similar, minimally disturbed conditions, to demonstrate that the deviation from natural background is protective. For predominately marine waters, FDEP has developed an approach for determining the allowable deviation from natural background that would result in a less than 5% loss in larval recruitment in marine waters using a spreadsheet model developed by the USEPA. The spreadsheet model is based on the same concepts incorporated into the Virginian Province Method used to derive the proposed criteria. Required inputs to the model include available species/genus level spawning, developmental, and DO sensitivity data as well as an estimate of the natural background DO levels. The approach will require that the model be run for a number of resident organism covering different taxonomic groups that are sensitive to low DO levels to assure minimal biological effects. This approach is detailed in **Appendix H** along with acceptable methods that can be used to accurately estimate natural background DO levels in both fresh and marine waters.

Inclusion of such language in Florida standards would reduce the number of minimally disturbed waterbodies that are incorrectly placed on the 303(d) list and provide a more accurate, implementable TMDL target as well as reducing the need for SSACs.

8 Maintaining Existing DO Conditions

To protect aquatic systems that have DO levels naturally higher than the minimum protective concentrations, FDEP plans to include a clause in the DO criteria that would require that these higher ambient DO levels be maintained, except as allowed under Rule 62-302.300, and 62-4.242, F.A.C. (anti-degradation provisions). During the National Pollutant Discharge Elimination System (NPDES) permitting process, a discharger must show that any lowering of DO below existing ambient levels is clearly in the public interest, or such lowering will not be allowed (lowering of DO to below the criteria is presumed not to be in the public interest). During this process, FDEP will take into account the variability associated with hydrologic and climatic cycles, as well as variability associated with analytical measurements.

During the Impaired Waters Rule assessment process, FDEP will place waterbody segments on the Verified List of impaired waters if there has been a statistically significant decreasing trend in DO levels, or an increasing trend in the range of daily DO fluctuations, at the 95 percent confidence level. When evaluating changes over time, confounding, or exogenous variables, such as natural random phenomena (*e.g.*, rainfall, flow, and temperature) often have considerable influence on the response variable in question (*e.g.*, DO level). By statistically accounting for confounding influences, the background variability is reduced so that any trend present can be better observed. This trend will be determined using a one-sided Seasonal Kendall test for trend, after controlling for or removing the effects of confounding variables, such as climatic and hydrologic cycles, quality assurance issues, and changes in analytical methods. Additionally, the anthropogenic pollutant *(e.g.*, BOD, ammonia, etc.) causing the lowered DO must be identified to place a waterbody segment on the verified impaired waters list.

9 Implementation of the Proposed DO Criteria for Compliance Assessment Purposes

This section provides a brief description of the intended methodology for use in assessing compliance with the proposed DO criteria. This methodology will assure that the proposed DO criteria are appropriately applied statewide in a manner that is consistent with the derivation of the criteria.

9.1 *Freshwater Criteria*

The regional daily average DO criteria for freshwaters will preferentially be assessed using diel monitoring data when available. For the purpose of assessing the proposed DO criteria a full day of diel data shall consist of 24 hours of measurements collected at a regular time interval of no longer than one hour. If diel monitoring data are not available, compliance with the criteria can be assessed using discrete measurements (*i.e.*, results from grab samples) or a combination of diel data and discrete measurements. If diel monitoring data are available, an arithmetic average will be calculated for each full day of monitoring data. That daily average will then be compared to the applicable daily average criterion to evaluate compliance with the DO criteria. Only diel monitoring covering a full 24 hour period will be assessed in this manner. Diel monitoring data covering less than a full 24-hour period will be assessed as discrete measurements as described below.

Time of Day Considerations

If diel monitoring data are not available, discrete measurements will be used to evaluate compliance with the criteria by taking into account the time of day in which the samples were collected. The equations provided in **Table 7** in **Section 4.5.4** will be used to provide a time of day specific translation of the daily average criteria. To assess compliance with the daily average criterion, each measured DO level will be compared to the time of day specific translation calculated based on the time at which the sample was collected.

If multiple instantaneous DO samples are available in a day, the time of day specific translation of the daily average criterion will be calculated for each individual sample. Achievement of the daily average DO criteria for that day will be assessed by comparing the average of the actual DO measurements collected during the day against the average of the calculated time of day specific translations for each sampling time. If the average of the instantaneous DO values is greater than or equal to the average of the calculated values, the daily average DO criterion is achieved. The evaluation of multiple samples collected during a day in this manner will be considered as a single sample for assessment purposes.

For waterbody assessment purposes, the daily average for each spatially independent sampling site will be considered as a separate sample for evaluation of the daily average criterion even if collected on the same day. For example, daily average DO concentrations calculated for five spatially independent sites within a waterbody segment will be considered as five separate samples. Using the binomial assessment methodology described in the Impaired Waters Rule (Chapter 62-303, F.A.C.) the daily average criterion will be achieved if no more than the 10 percent of the separate samples collected during the assessment period have DO values below the applicable criterion.

9.2 *Marine Water Criteria*

As described for freshwaters, the proposed DO criteria for marine waters will preferentially be assessed using diel monitoring data when available. If diel monitoring data are not available, compliance with the criteria can be assessed using discrete measurements or a combination of diel data and discrete values. If discrete measurements are utilized in the assessment of compliance, the effect of time of day at which the samples were collected should be considered prior to making a final decision. As described for the freshwater criteria, compliance with the marine daily average criterion will be assessed using the binomial methodology described in the Impaired Waters Rule (Chapter 62-303, F.A.C.) which uses a 10 percent exceedance frequency. The assessment will be based on the daily average DO levels from spatially independent sampling sites as outlined for the freshwater criteria.

The data sufficiency requirements for calculating the 7- and 30-day average DO concentrations for marine waters are described in **Section 5.5.2** of this report. Additionally, the proposed criteria indicates that the 7- and 30-day components of the marine DO criteria are not achieved if more than one exceedance of the DO criteria occurs in a 12-week or one-year period, respectively.

To assure that the assessment is representative of the entire waterbody, the 7- and 30-day average DO concentrations will be calculated as waterbody averages. To calculate a 7- or 30-day average, the daily average DO concentrations for all spatially independent sampling sites within the waterbody (as calculated for the daily average criteria) will be averaged to provide a daily waterbody average. Each of the daily waterbody averages occurring during the 7- or 30-day period will be averaged to provide a waterbody average that will be used to assess compliance with the criteria.

Additionally, in areas that fluctuate between freshwater and marine conditions, only DO data with associated specific conductivity levels at or above 4,580 µmhos/cm (or 1,500 mg/L chloride, or 2.7 PSU salinity) will be used to calculate 7- and 30-day average DO concentrations for assessment of the marine criteria as described in Section 9.3 below.

9.3 Application of DO Criteria in Tidal Areas that Fluctuate between Freshwater and Marine Conditions

In tidal areas that fluctuate between saline and freshwater conditions, the applicable DO criteria will be based on the specific conductivity (if specific conductivity data is not available corresponding chloride or salinity levels may be used as specified below) level at the time of sampling. For the purpose of assessing compliance with the proposed DO criteria, the area from 5.1 km (3.2 miles) upstream to 5.1 km downstream of a FDEP Geographic Information System coverage that depicts the landward extent of saltwater vegetation (the "line of tide") will be considered as a transitional zone that may potentially fluctuate between marine and freshwater conditions. For assessment purposes, DO data collected in this area should be matched with salinity/specific conductance measurements (*i.e.*, salinity/specific conductance measured within 15 minutes of the DO measurement) to determine the applicable criteria.

For data collected within the transitional zone, if the specific conductivity level collected in conjunction with a DO measurement (*i.e.*, within 15 minutes of the discrete DO measurement) is less than 4,580 µmhos/cm (or 1,500 mg/L chloride, or 2.7 PSU salinity), the applicable freshwater DO criterion will apply to that DO measurement. If the specific conductivity level collected in conjunction with a DO measurement is at or above 4,580 µmhos/cm (or 1,500 mg/L chloride, or 2.7 PSU salinity), then the marine DO criteria will apply. Additionally, within the transitional zone, only DO measurements with corresponding specific conductivity levels at or above 4,580 µmhos/cm will be used to calculate 7- and 30-day average DO concentrations for assessment of the marine criteria, as indicated above.

In areas upstream of the transitional zone, the freshwater DO criteria as described in Section 9.1 can confidently applied without the need to pair DO and salinity/specific conductance measurements. Similarly, DO data collected in the area downstream of transitional zone can be assessed using the marine DO criteria without needing to evaluate salinity data (note however, that DO percent saturation measurements are adjusted based on salinity, and that this function is normally carried out internally by the instrument).

Additionally, if historical salinity/specific conductance data indicates that an area is consistently either fresh or marine water with little fluctuation above and below the threshold values, the DO data from that area can be assessed using the appropriate criteria without the need to pair the DO data with salinity/specific conductance measurements.

9.4 Determination of Causative Pollutant for DO Impairment

Low dissolved oxygen is not a direct pollutant, but rather, a response to either anthropogenic pollutants (*e.g*., BOD, ammonia) or to specific natural conditions (*e.g.,* natural decomposition of leaf litter, stagnant flow, etc.). Therefore, waterbodies for which the available data indicate that the DO criteria are not being achieved will undergo a more detailed assessment to determine the causative pollutant(s) responsible for the low DO levels prior to the waterbody being listed as impaired. This assessment can include: 1) the collection of additional physical data (*e.g*., flow, rainfall, channel morphology, etc.), including an assessment of potential human inputs (*e.g*., Landscape Development Intensity Index, evaluation of NPDES discharges, etc.), 2) a review of the biological health of the system to determine if the low DO conditions are having an adverse effect on the biological communities, 3) an assessment of water quality data, including nutrient, chlorophyll, and BOD data to determine if excessive levels of pollutants can account for the DO levels observed, 4) an evaluation of the natural DO regime for the waterbody, and 5) an evaluation of the diel DO range. Based on the site-specific data, the Department will determine if the observed low DO condition is in response to a pollutant or is a natural condition. Additional information on determining background conditions is found in Appendix H.

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Appendix A:

Examples of DO SSACs for Florida Waters

Examples of DO SSACs for Florida Waters

In recognition of the fact that the generally applicable criteria are not always appropriate for some of Florida waterbodies, Florida's Water Quality Standards allow for the development of Site Specific Alternative Criteria (SSAC) that more accurately reflect the levels required to maintain healthy biological communities under natural conditions (Rule 62-302.800, Florida Administrative Code). To be approved for a Type I SSAC, a petition must demonstrate that an alternative criterion is more appropriate for a specified portion of waters of the state and:

- Document that the proposed alternative concentrations that are different from the otherwise applicable Class III criteria exist because of natural background conditions;
- Establish the levels and duration of the naturally-occurring concentrations, and other parameters or conditions that may affect it;
- Describe the historical and existing biology, including variations that may be affected by the parameters in question;
- Show that normal fluctuations of an analyte are being maintained; and
- Show that the designated use is being attained and not adversely affecting adjoining waters.

Most DO SSACs adopted to date have been Type I SSACs and have been derived using a reference site approach. Reference sites, also known as benchmark sites, are waterbody segments affected by only very minimal human influence, as described by an LDI of 2 or lower, optimal habitat (*e.g.,* >120 in streams), and little human modification of the system's hydrology. Alternative DO criteria derived using the reference site approach (establishing the expected DO regime based on data collected at minimally disturbed waterbodies) are considered to be inherently protective. However, as no cause and effect relationship is identified, the exact level of protection provided by the reference site approach is not easily assessed and can vary based on a number of factors, including the appropriateness of the reference waterbody, the method used to derive the new criterion, and the sufficiency and robustness of data available.

The provisions for a Type II SSAC are more flexible as they allow for SSACs to be based on other "generally accepted scientific method or procedure to demonstrate with equal assurance that the alternative criterion will protect the aquatic life designated use of the water body". One Type II DO SSAC has been developed for the Lower St. Johns River estuary using the measured responses of multiple sensitive organisms exposed to low DO (a modification of the USEPA Virginian Province approach).

EPA has previously approved 13 State-approved DO SSACs for Florida waters. However, the development of SSACs for individual waterbodies to address a global problem with the existing DO criteria is both time consuming and costly.

1. Examples DO SSACs Based on Reference Conditions

1.1. Amelia River DO SSAC

In the Amelia River estuary, DO levels observed during the summer are below 4.0 mg/L approximately 10% of the time due to natural conditions. Extensive marsh and swamp systems adjacent to the system contribute vast amounts of leaf litter, which in turn causes the waters to contain high amounts of organic tannins, lignin, and other humic acid substances. These naturally occurring water quality conditions contribute to periodic low DO in the estuary.

A DO SSAC was developed for the Amelia River using the reference site approach. Due to its minimal level of human disturbance, the nearby Nassau River, which is part of the Nassau River-St. Johns River Marshes State Aquatic Preserve, was selected as the reference system for the derivation of the Amelia River DO SSAC. The DO regime in the Nassau River exhibited similar seasonal DO patterns as observed in the Amelia River. The documented seasonal variations were found to be strongly linked to seasonal temperature fluctuations. All recorded values below the existing criterion were associated with temperatures above 20°C, which occurred during the warmer summer months. Based on the reference conditions observed in the Nassau River estuary, the Amelia River estuary SSAC specifies that during the summer months of July through September the instantaneous DO concentration shall not fall below 3.2 mg/L and that a daily average DO concentration of 5.0 mg/L shall be maintained. During the other months, the existing marine criterion remains in effect.

1.2. Econfina/Fenholloway River SSAC

The DO concentrations in the Fenholloway River and adjacent coastal area exhibited expected seasonal variations; however, concentrations lower than the existing DO criteria occurred throughout the year and were much lower than those measured in the Nassau River. A DO SSAC for the Fenholloway River was derived based on the DO regime in the Econfina River, which was selected as a minimally disturbed reference system based on its surrounding benign land-use, lack of anthropogenic inputs, the presence of healthy biological communities, and its similarity to the Fenholloway. In streams where the method applies, healthy biological communities are those with Stream Condition Index (SCI) average scores of 40 or greater (see discussion in section 4.2).

Due to the natural seasonal fluctuations in the expected DO levels, the SSAC petition requested that the SSAC vary on a seasonal basis. **Figure A1** provides a monthly summary of the DO data from the Lower Econfina River and estuary. Based on similarity in DO levels among the months, the DO data were grouped into three seasonal periods for the purpose of derivation and application of the SSAC. The three periods are 1) April 1 through September 30, 2) February 1 through March 31, and October 1 through November 30, and 3) December 1 through January 31.

The DO SSAC for the Fenholloway River consisted of two components for each seasonal period, which were derived as:

- the 10th percentile of the daily average DO levels (*e.g.,* 2.1 mg/L during the summer); and
- the 10th percentile of the daily DO minimum concentrations (*e.g.,* 1.2 mg/L during the summer).

The results of the analyses are summarized in **Table A1**.

1.3. Everglades SSAC

In the Everglades open-water slough communities, where light penetration is high, photosynthetic activity by periphyton and submerged aquatic vegetation (P/SAV) result in increasing oxygen concentrations during daylight hours. At night, respiration and sediment oxygen demand (SOD) draw oxygen concentrations down. The combination of high DO production during the day and the respiration and oxygen demand from the organic rich sediment results in strong natural diel DO fluctuations.

Recognizing that a single value criterion does not adequately account for the wide natural daily (diel) and seasonal fluctuations in DO concentrations observed in the marsh, FDEP developed an algorithm to account for the major factors (*e.g.,* time of day and temperature) influencing natural background DO variation in the Everglades. The algorithm uses sample collection time and water temperature to model the observed natural sinusoidal diel cycle and seasonal variability. This model provides a lower DO limit (DOL) for an individual monitoring station and is described by the equation:

$$\mathrm{DOL}_i = [-3.70 - [1.50 \text{ sine } (2\pi/1440\ t_i) - (0.30 \text{ sine } (4\pi/1440\ t_i))] + 1/(0.0683 + 0.00198\ C_i + 5.24 \cdot 10^{-6} \cdot C_i^2)] - 1.1$$

Where:

DOL_i is the lower limit for the ith annual DO measurement in mg/L

t_i is the sample collection time of the ith annual DO measurement

C_i is the water temperature associated with the ith annual DO measurement in °C

To fully account for seasonal and annual variability in marsh DO concentrations, the SSAC is assessed based on a comparison between the annual average of multiple (*e.g.,* monthly) DO measurements made throughout the year and the average of the corresponding DO limits specified by the above equation for that year. In other words, annual average observed DO at a monitoring station is to be compared to the annual average of all DOL_i determinations for that year.

2. Example of DO SSAC Derived Using Virginian Province Method

To develop the DO SSAC for the marine portions of the lower St. Johns River (LSJR), FDEP used a modification of the USEPA Virginian Province method (USEPA, 2000). This method utilizes the measured response of multiple sensitive organisms exposed to low DO conditions. These data are then used to determine the DO level necessary to protect sensitive taxa from significant decreases in recruitment or growth. The resulting growth and larval recruitment response curves were used to derive the DO SSAC for the Lower St. Johns River.

In accordance with EPA recommendations for the Virginian Province (USEPA, 2000), the DO range was divided into intervals. For the proposed LSJR SSAC, intervals were established from 4.0 to 4.2 mg/L; 4.2 to 4.4 mg/L; 4.4 to 4.6 mg/L; 4.6 to 4.8 mg/L; and 4.8 to 5.0 mg/L based on the applicable portions of the larval population recruitment/ survival function and the larval growth function. The applicable larval population recruitment/survival curve or larval growth curve can then be used to derive the acceptable exposure durations for each interval.

Since the biological effect of low DO exposure is cumulative across the DO intervals, the fractional exposures within each range would be summed as proposed by the USEPA (2000). The final DO SSAC for the LSJR was expressed as the sum of the fractional exposures between 4.0 and 5.0 mg/L which can be expressed as:

$$\left(\begin{array}{c}\textbf{Total Fractional}\\ \textbf{Exposure}\end{array}\right)=\frac{\begin{array}{c}\textbf{Days between}\\ \textbf{4.0 - < 4.2 mg/L}\end{array}}{\textbf{16 day Max}}+\frac{\begin{array}{c}\textbf{Days between}\\ \textbf{4.2 - < 4.4 mg/L}\end{array}}{\textbf{21 day Max}}+\frac{\begin{array}{c}\textbf{Days between}\\ \textbf{4.4 - < 4.6 mg/L}\end{array}}{\textbf{30 day Max}}+\frac{\begin{array}{c}\textbf{Days between}\\ \textbf{4.6 - < 4.8 mg/L}\end{array}}{\textbf{47 day Max}}+\frac{\begin{array}{c}\textbf{Days between}\\ \textbf{4.8 - < 5.0 mg/L}\end{array}}{\textbf{55 day Max}}$$

Where the number of days within each interval is based on the **daily average** DO concentration. To achieve the SSAC, the annual sum of the fractional exposures must be less than 1.

Given the ubiquitous nature of low DO conditions in estuarine waters throughout the state, the Virginian Province method could also be used to develop more appropriate regional or statewide criteria. Not only would the application of this method on a broader spatial scale be more efficient and cost effective than developing criteria for individual waterbodies, it would provide an equal and known level of protections to all state waters. For example, the application of this method to the Amelia River may yield satisfactory results. However, it should be noted that some waterbodies exhibit DO levels that may naturally fall below the levels derived using this method and may continue to require SSAC development.

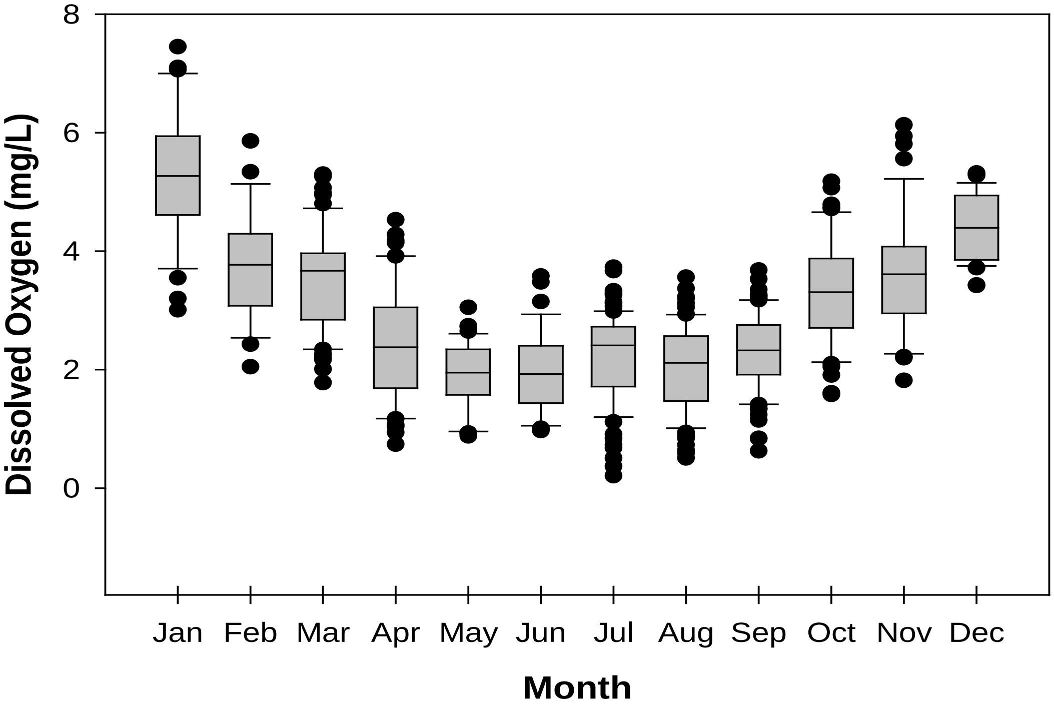


Figure A1. Monthly distribution of daily minimum dissolved oxygen concentrations at Econfina River and estuary monitoring sites.

Table A1. Seasonal dissolved oxygen SSAC for estuary and coastal areas based on the 10th percentiles of dissolved oxygen concentrations at

Annual Period	Daily Average DO Criteria (mg/L)[1]	Daily Minimum DO Criterion (mg/L)[2]
River/Estuarine Areas		
April – September	2.1	1.2
February - March and October – November	3.4	2.4
December – January	5.0	3.6
Adjacent Coastal Areas		
April – September	3.2	1.7
February - March and October – November	5.0	3.5
December – January	5.0	4.0

[1] The 24-hour average DO at any location within the described area shall not be less than the level specified for that seasonal period.

[2] No more than 10 percent of the measurements made during a 24-hour period at any location within the described area shall be less than the minimum level specified for that seasonal period.

Appendix B:

Detailed Description of the Stream Condition Index (SCI)

The Stream Condition Index (SCI)

1 General Information

Florida's Stream Condition Index was built upon EPA's "rapid bioassessment" concept proposed in the early 1990s. The Human Disturbance Gradient (HDG) approach was used to select a series of 10 metrics that respond predictably to anthropogenic disturbance. To identify effective biological metrics, the HDG was composed of four factors known to affect aquatic communities: 1) The Landscape Development Intensity Index (Brown and Vivas 2006), 2) Habitat Assessment scores (DEP SOP), 3) Hydrologic Modification Score (DEP SOP), and 4) Water column ammonia concentration (Fore *et al.* 2007).

The 10 selected attributes metrics were chosen to:

- Represent a comprehensive array of indicators of biological integrity (necessary to demonstrate whether species composition, diversity, and functional organization is comparable to that of natural habitats within a region);
- Provide meaningful and predictable assessment of human effects; and
- Avoid redundancy by excluding less robust metrics that were similar to those with a demonstrated ability to predict human disturbance.

The SCI is comprised of the following metrics:

- Number of Total Taxa
- Number of Clinger Taxa
- Number of Long Lived Taxa
- Percent Suspension Feeder and Filterer Individuals
- Number of Sensitive Taxa
- Percent Tanytarsini Individuals
- Percent Very Tolerant Individuals
- Number of Ephemeroptera Taxa
- Number of Trichoptera Taxa
- Percent of Individuals in the Dominant Taxon

These metrics were combined into a dimensionless index (scoring from 0 to 100) that provides a synthesis of the health of the invertebrate community.

The current Florida Stream Condition Index (SCI) was developed in 2004, and adjustments were made in lab counting procedures to reduce variability of results in 2007. The DEP expends great efforts to ensure that data are produced with the highest quality, both in the field and in the lab. Samplers and lab technicians follow detailed Standard Operating Procedures (SOPs), and additional guidance for sampling and data use is provided through a DEP document entitled, *Sampling and Use of the Stream Condition Index (SCI) for Assessing Flowing Waters: A Primer* (DEP-SAS-001/11).

Samplers are only approved to conduct the SCI after passing a rigorous audit with the DEP, and laboratory taxonomists are regularly tested and must maintain >95% identification accuracy. To be scientifically defensible, stream systems being evaluated against the SCI should be morphologically identifiable as streams, so that potential human influences can be discerned (the reference streams should be compared to streams, reference streams should not be compared to a system with lake-like or wetland-like conditions).

1.1 *2012 Adjustments to SCI Regionalization*

Designed to serve as a spatial framework for the assessment and monitoring of ecosystems, ecoregions denote areas within which ecosystems (and the type, quality, and quantity of environmental resources) are generally similar (Omernik 1987). By identifying homogeneous units that reflect the inherent capacities and potentials of ecosystems, ecoregions stratify the environment by its probable response to disturbance (Bryce *et al.* 1999).

Griffith *et al.* (1994) identified twenty ecological sub-regions in Florida through a statewide analysis of patterns in geology, physiography, vegetation, climate, soils, land use, wildlife, and hydrology (**Figure B1**). Compilation of this map, performed at the larger 1:250,000-scale, was part of a collaborative project between EPA and FDEP during 1991-1993. Explanation of the methods used to define the ecoregions is given in Omernik (1995), Gallant *et al.* (1989), and Griffith *et al.* (1994). Based on a Non-metric Multidimensional Scaling Analysis (NMDS) of reference site community similarity, three stream Bioregions were established in 1996 (Barbour *et al.* 1996). These original bioregions, within which different expectations were established for the SCI metrics based on natural patterns, included the Panhandle, the Northeast, and the Peninsula (note that the SCI is not calibrated for the Everglades region, where few natural streams exist) (Griffith *et al.* 1994; **Figure B2**).

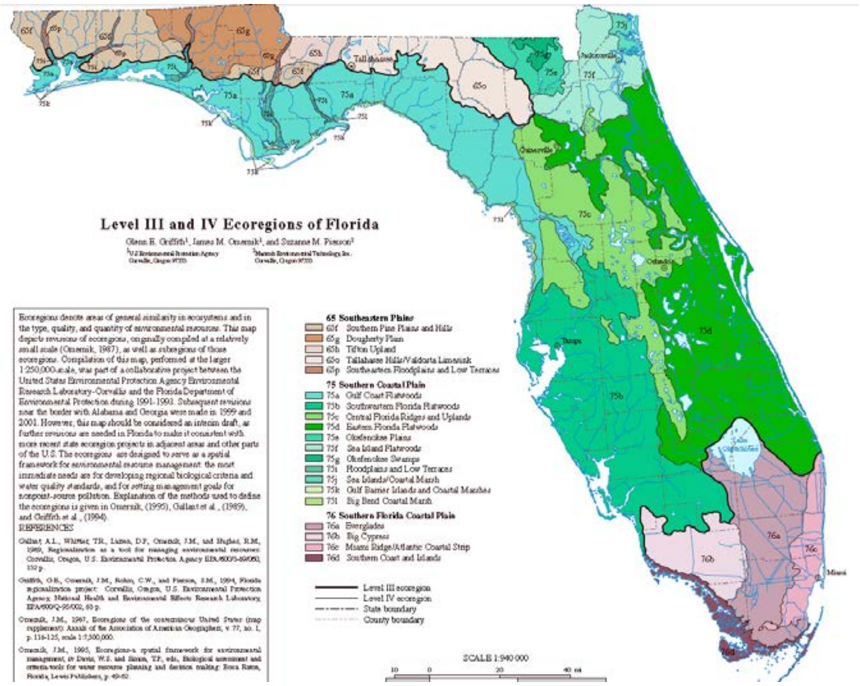


Figure B1. Ecological sub-regions of Florida (after Griffith *et al.* 1994).

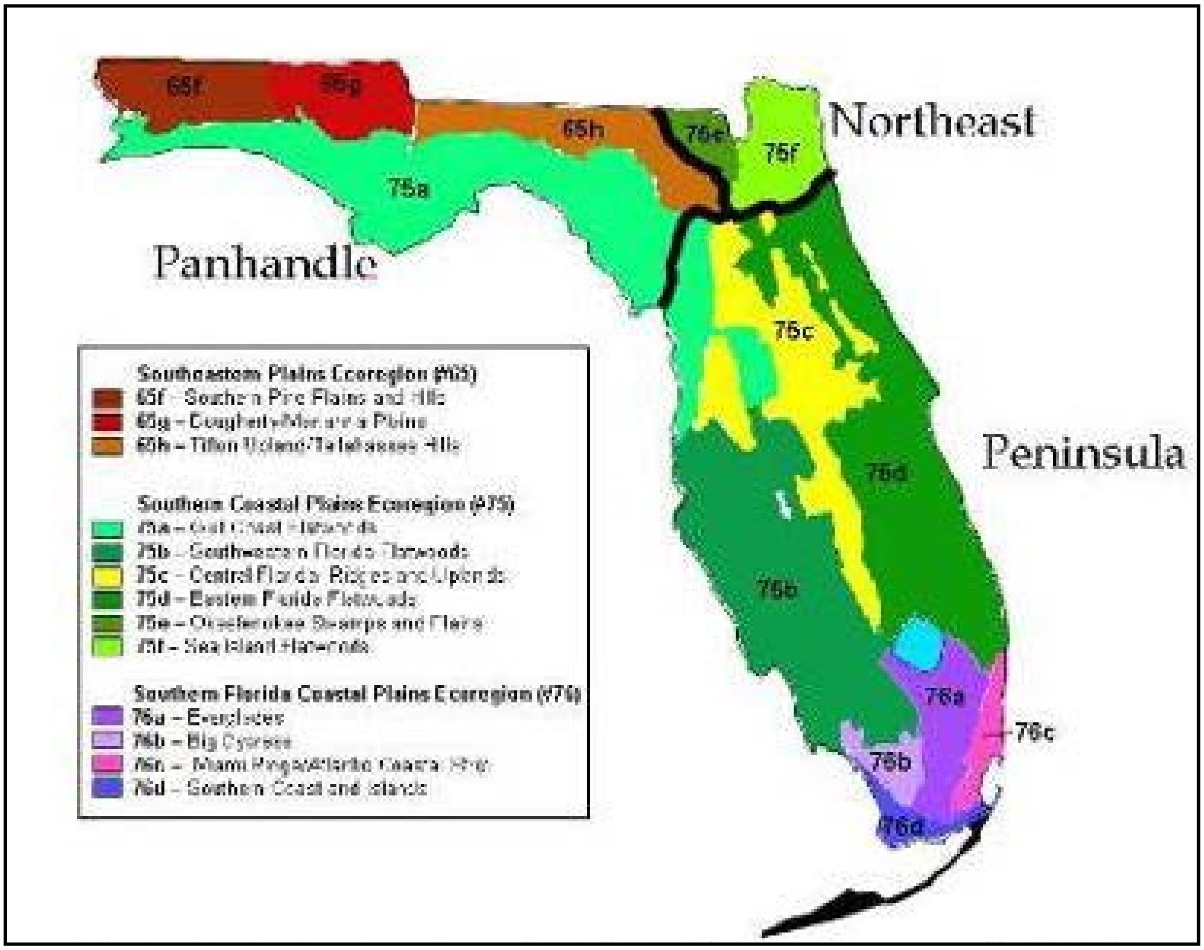
Through the use of these original bioregions over time, FDEP noted the following technical issues that prompted a reanalysis of the bioregions:

- The original regionalization was conducted before current Geographic Information System tools were available, meaning new technological improvements could produce better spatial resolution;
- FDEP has collected thousands of new SCIs throughout the state after the original bioregions were established, and these data had not been spatially analyzed to determine if the original bioregions continued to best explain variability in the data;
- Original bioregion boundaries bisected watersheds, meaning many streams were subjected to different biological expectations, when simply moving upstream and downstream of an imaginary line across the stream (see example in **Figure B3**);
- Using the BioRecon tool, different biological expectations had been established east and west of the Ochlockonee watershed, however, the Panhandle was lumped into a single region for the SCI.

In 2012, FDEP performed the following steps to reassess the regionalization component of the SCI:

- Using high resolution GIS (National Hydrography Database) and site specific knowledge of streams, FDEP adjusted the bioregional boundaries to be consistent with watersheds (consisting of the Hydrologic Unit Code 10 coverages, to prevent bioregions from bisecting any streams);

- FDEP conducted Non-metric Multi-Dimensional Scaling analysis (NMDS)on the Bray-Curtis similarity of taxa communities in reference site samples (habitat assessment score ≥ 110 and LDI ≤ 2) to determine if any regional boundaries merited adjustment, especially with regard to the Panhandle East / West regions, because the BioRecon method previously identified separate regions in this area; and
- FDEP performed Principal Components Analysis (PCA) on existing SCI metrics (# clinger taxa, % filter feeders, sensitive taxa, etc.) to determine which regions separated when data were summarized as SCI metrics.



FigureB2. Original 2007 bioregions.

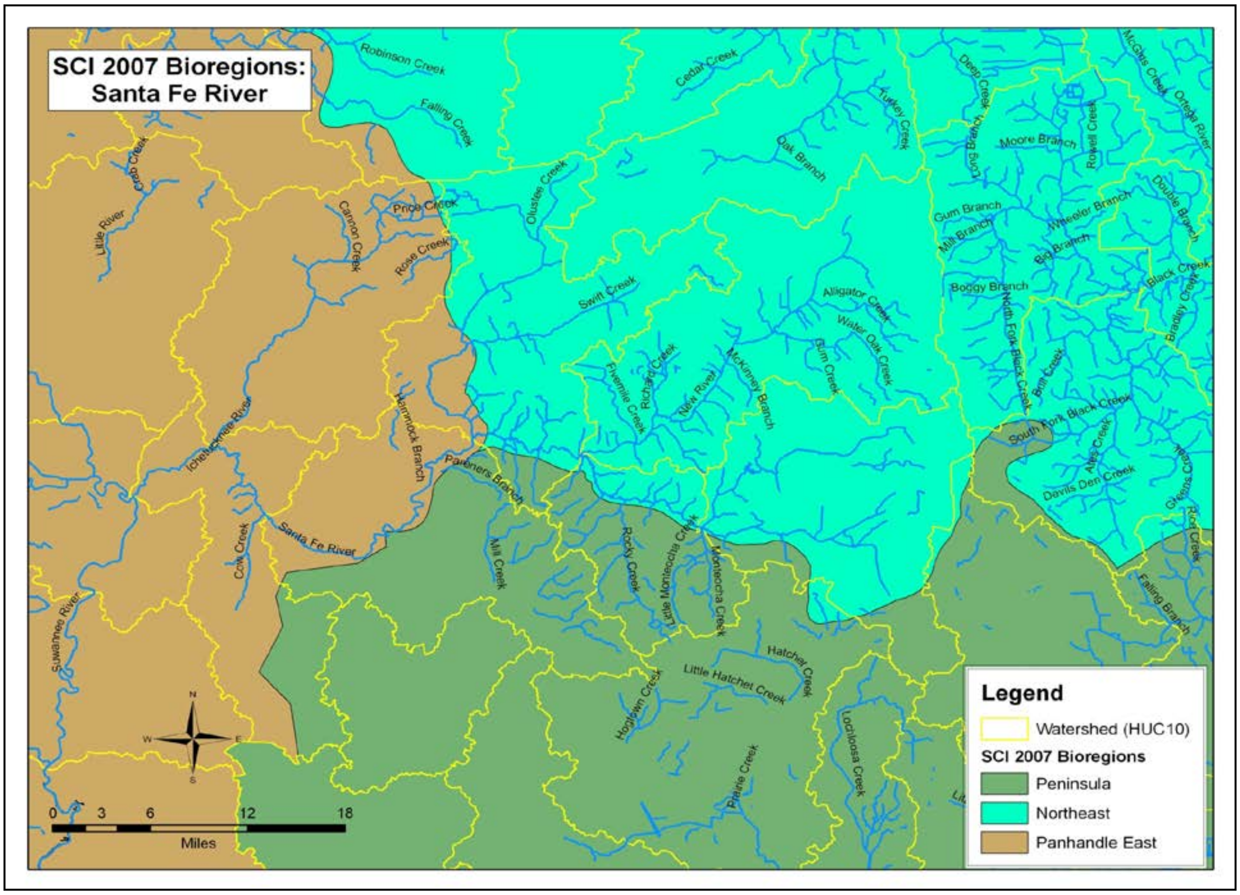


Figure B3. As seen in this example of the Santa Fe and Black Creek watersheds, original Bioregions cross watershed boundaries.

Results of NMDS analysis of the similarity of reference site taxa showed that the Panhandle East was different from the Panhandle West, with very little overlap (**Figure B4**). This supports the establishment of a new Panhandle East, or Big Bend Bioregion, with biological expectations that are different from the Panhandle West. Results of NMDS analysis of reference site taxa data showed that the Big Bend exhibited sufficient differences with the Northeast Region to maintain these as separate regions (**Figure B5**). Finally, NMDS analysis demonstrated that there was sufficient overlap in reference sites in the north and south Peninsula to maintain this area as a single region (**Figure B6**). Based on these analyses, the Panhandle bioregion is separated into two distinct bioregions, referred to as the "Panhandle West" and the "Big Bend" (or Panhandle East). The analysis also showed that the Northeast and Peninsula bioregions should be maintained as originally described, with minor adjustments at the borders to prevent bisecting watersheds. All bioregion borders were changed from the Griffith *et al.* (1994) subecoregions to Hydrologic Unit Code 10 boundaries so that entire watersheds would be in the same bioregion. For example, in the Santa Fe and Black Creek watersheds, the old bioregion boundaries bisected the watershed as shown in **Figure B3**, however, the new 2012 bioregions were modified to be consistent with watershed boundaries (*i.e.*, Bioregions do not bisect watersheds) as depicted in **Figure B7**. **Figure B8** provides a general overview of the changes to the SCI regionalization with **Figures B9 – B12** providing greater detail regarding the location of the boundaries between SCI regions.

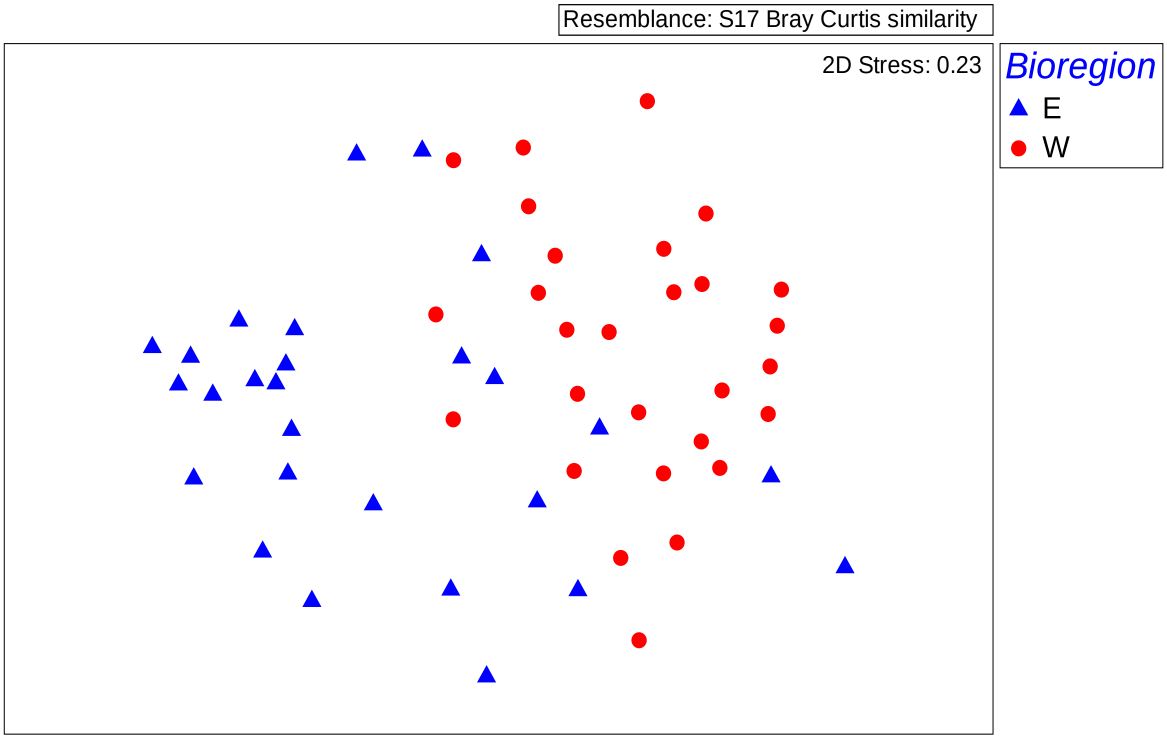


Figure B4. Results of Non-metric Multi-Dimensional Scaling (NMDS) analysis of reference site taxa data from sites east and west of the Ochlockonee watershed.

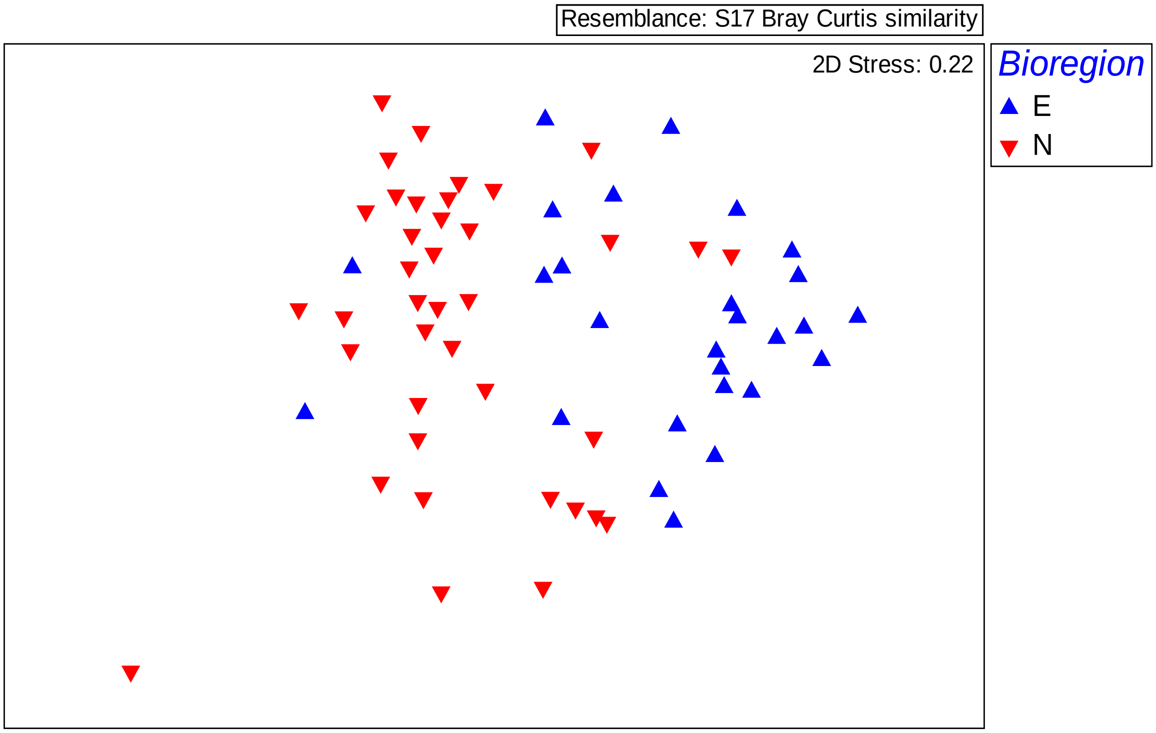


Figure B5. Results of Non-metric Multi-Dimensional Scaling (NMDS) analysis of vetted reference site taxa data from sites in the Panhandle East and existing Northeast Bioregion.

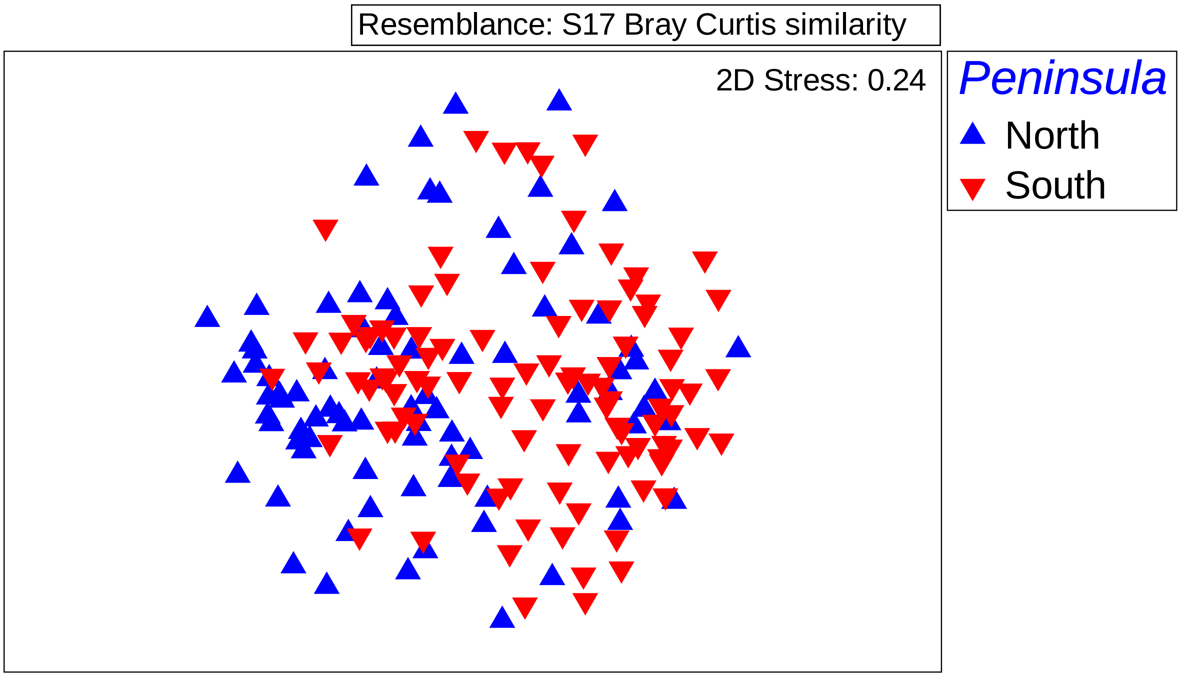


Figure B6. Results of Non-metric Multidimensional Scaling (NMDS) analysis of reference site taxa data from sites north and south in the Peninsula Bioregion.

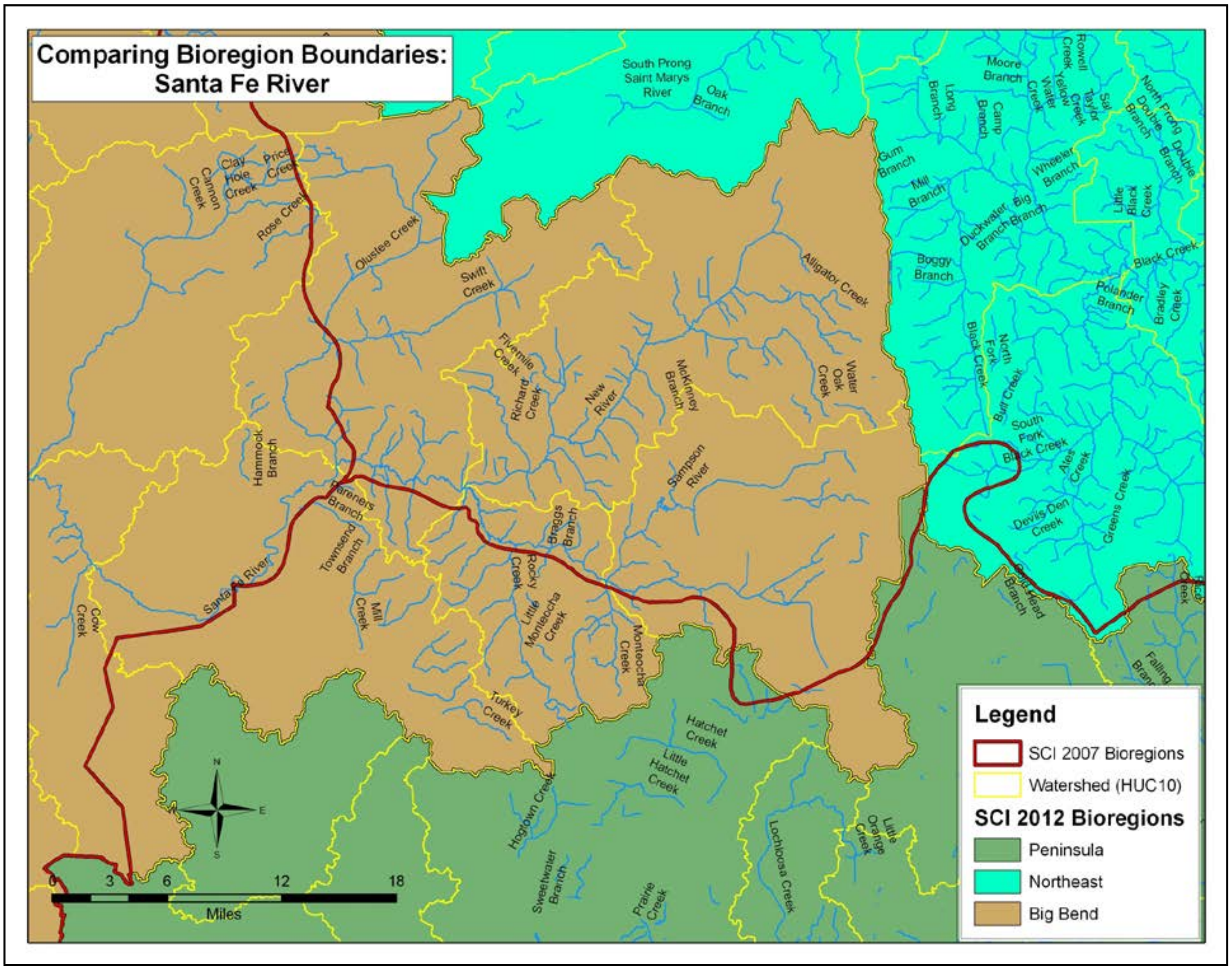


Figure B7. As seen in the example of the Santa Fe and Black Creek watersheds, the new 2012 Bioregions were modified to be consistent with watershed boundaries (*i.e.*, Bioregions do not bisect watersheds). Old Bioregion boundaries for this watershed are depicted in Figure B3.

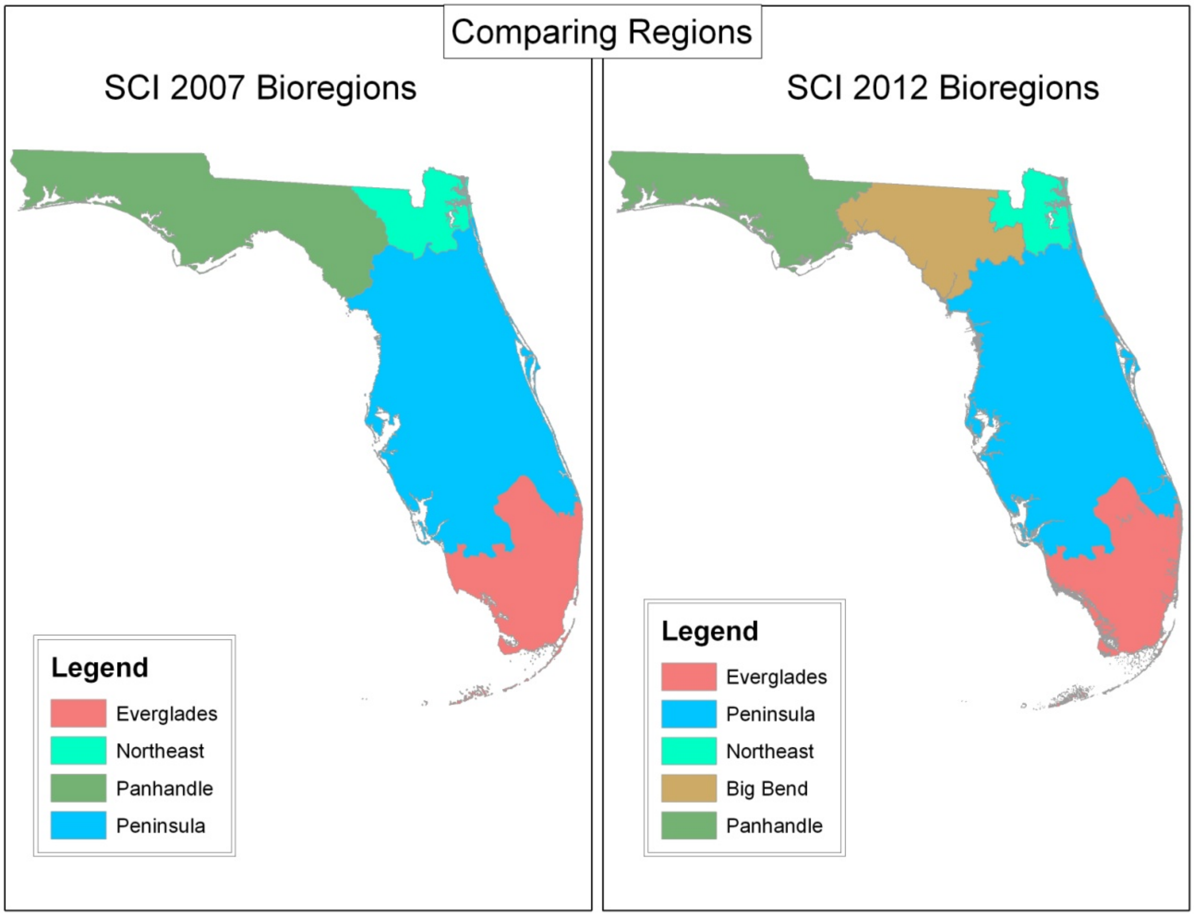


Figure B8. A comparison of the current SCI 2007 bioregions (on left) with the new 2012 bioregions for BioRecon and SCI (on right).

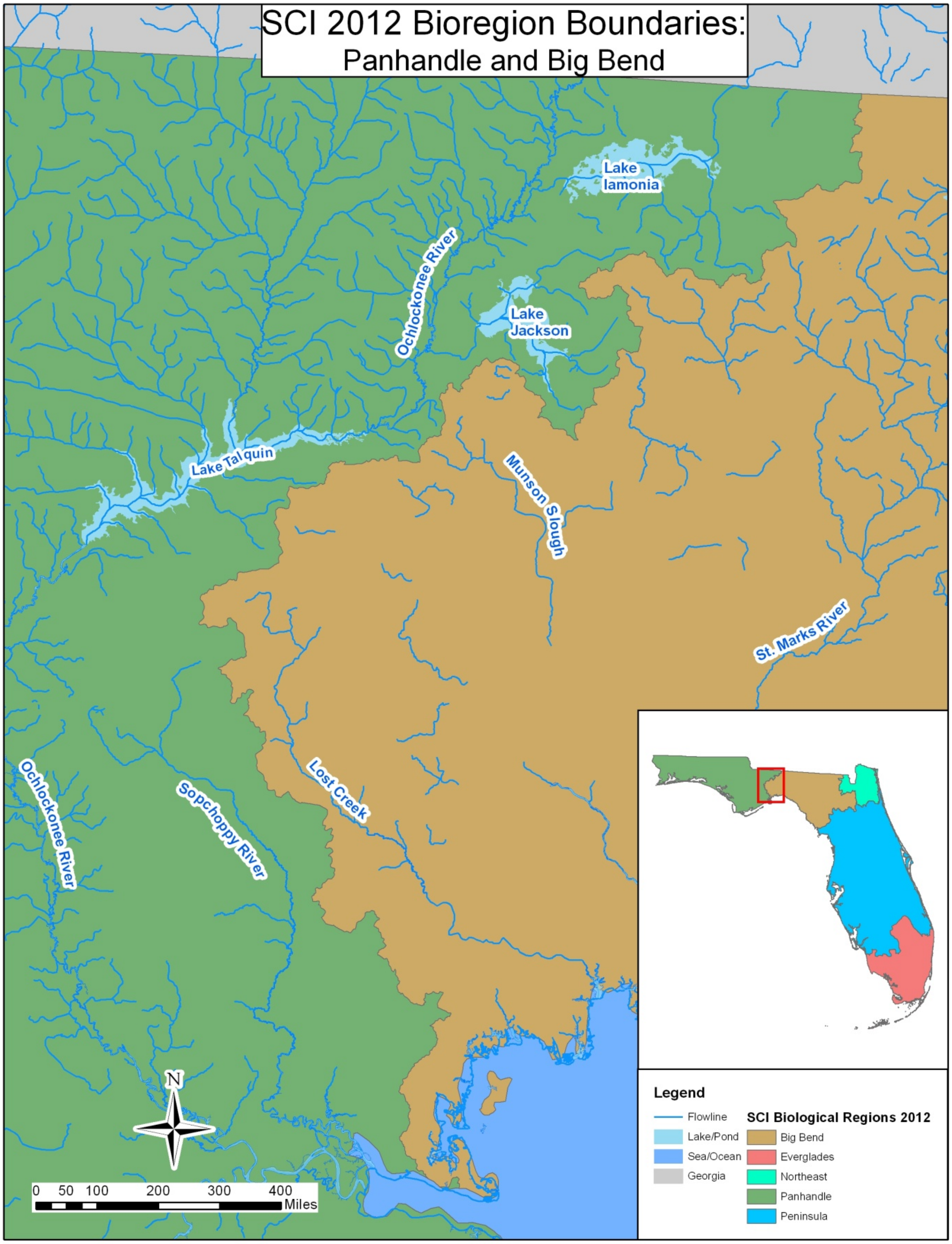


Figure B9. Detail view of boundary between Panhandle West and Big Bend SCI 2012 bioegions.

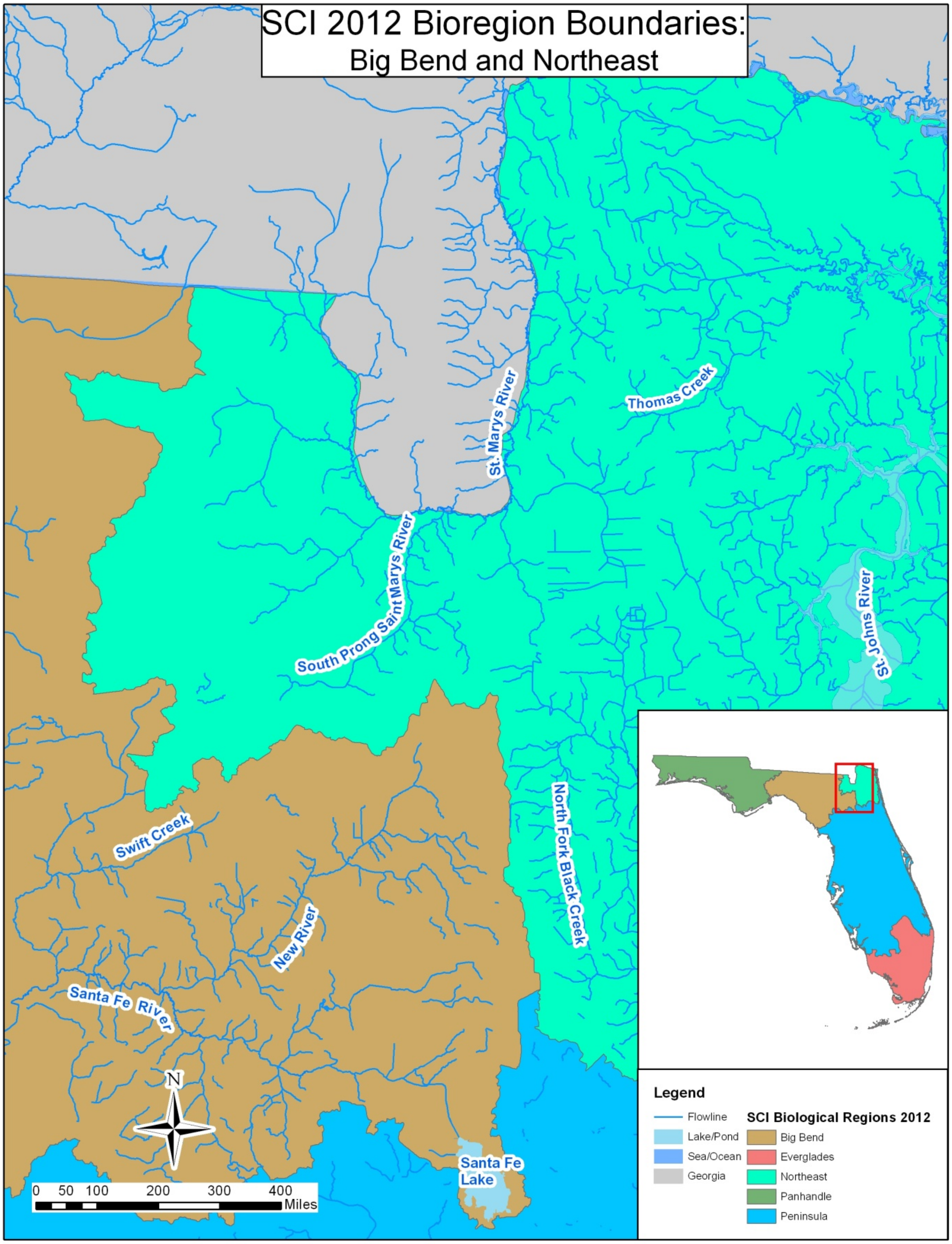


Figure B10. Detail view of boundary between Big Bend and Northeast SCI 2012 bioegions.

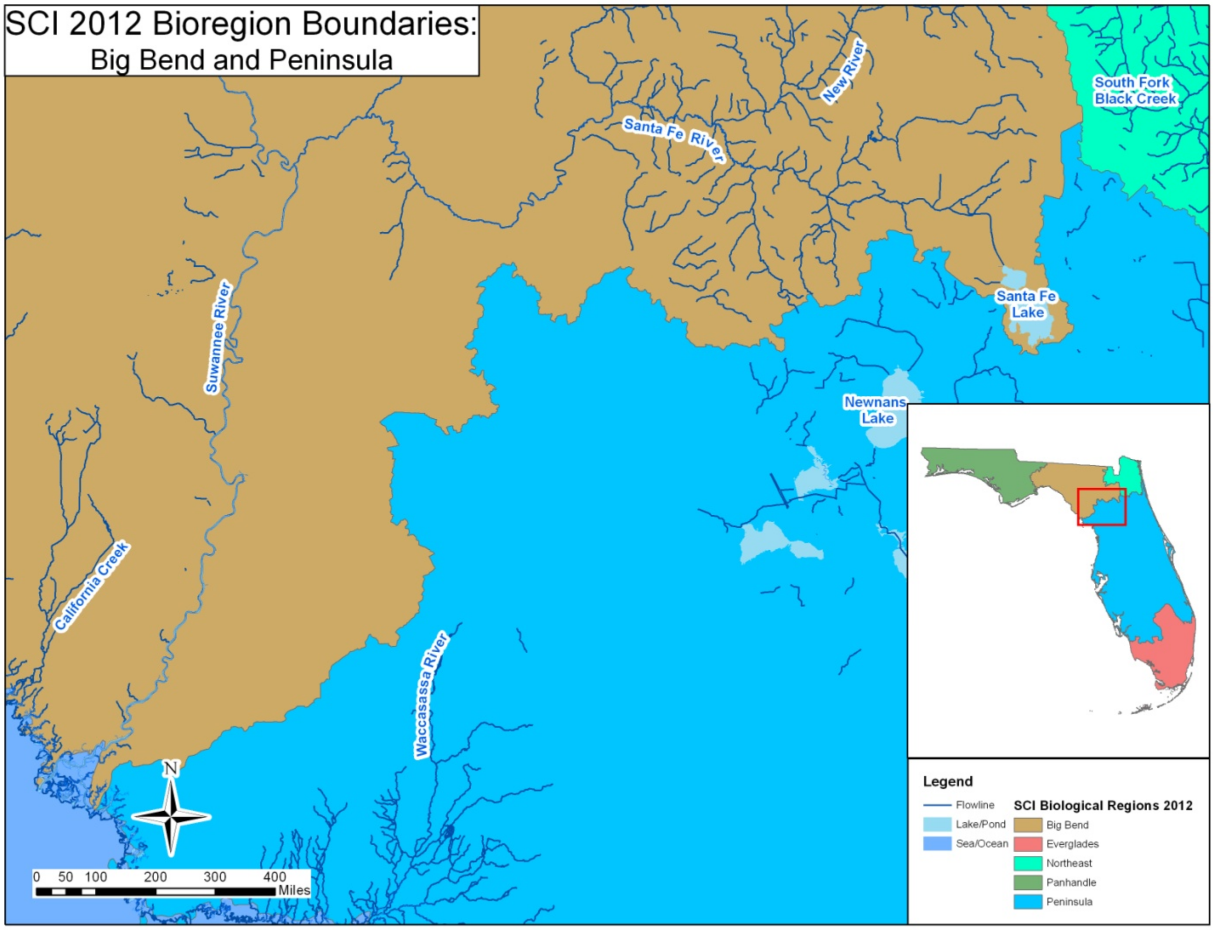


Figure B11. Detail view of boundary between Big Bend and Peninsula SCI 2012 bioegions.

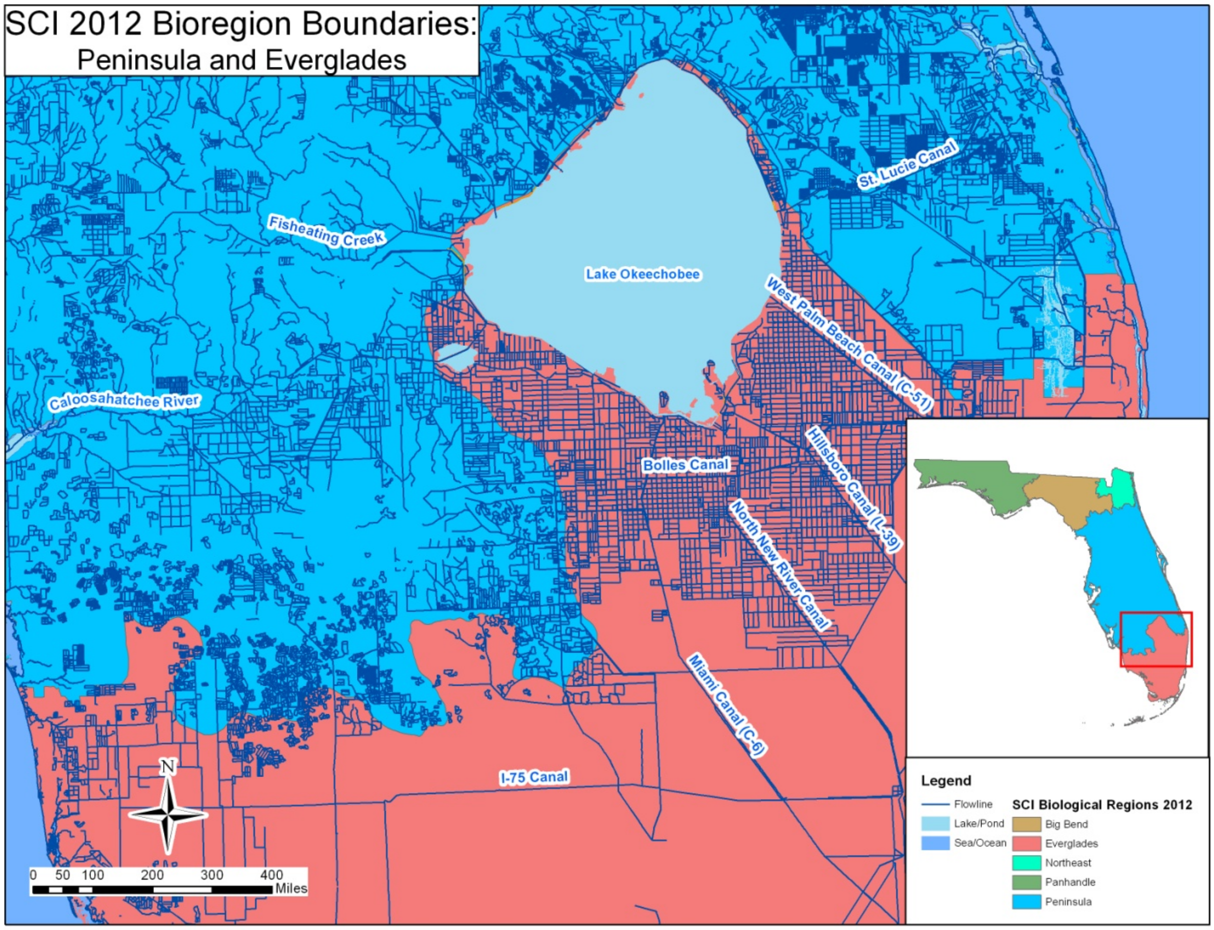


Figure B12. Detail view of boundary between Peninsula and Everglades SCI 2012 bioegions.

Because of these spatial changes, the ten SCI metrics were re-evaluated to determine what adjustments to SCI scoring would be needed based on the new regionalization scheme. Therefore, the equations for calculating the SCI for all 4 bioregions were examined based on data collected using the SCI 2007 method. Landscape Development Intensity Index scores showed that there was a wide range of human disturbance in each regional data set, from virtually no (or minimal) disturbance to high levels of human stress (1,072 sites, LDIs ranged from 1.0 to 8.7).

SCI metrics that **decrease** in response to human disturbance are generally scored using the following equation (note that natural logs are also used for some metrics):

$$10 * (X–L)/H$$

Where X is the metric value for an individual sample, L is the lower 5th percentile of the metric of the given bioregion, and H is the upper 95th percentile minus the lower 5th percentile (*i.e.*, the range between the 5th and 95th percentiles).

SCI metrics that **increase** in response to human disturbance are generally scored using the following equation:

$$10 – (10 * [(X–L)/H])$$

Where X is the metric value for an individual sample, L is the lower 5th percentile of the metric of the given bioregion, and H is the range between the 5th and 95th percentiles.

Adjustments to the metric scoring, comparing the SCI 2007 equations with the equations derived to be consistent with the new regionalization pattern (denoted as SCI 2012) are shown in **Table B1.**

Table B1. A comparison of the SCI 2007 SCI metric equations with the equations derived to be consistent with the new regionalization pattern (denoted as SCI-2012).

SCI_2007 Equations			
SCI metric	***Northeast***	***Panhandle***	***Peninsula***
Total taxa	10 * (X-16)/26	10 * (X-16)/33	10 * (X-16)/25
Ephem. taxa	10 * X /3.5	10 * X /6	10 * X /5
Trichoptera taxa	10 * X /6.5	10 * X /7	10 * X /7
% Filterer	10 * (X-1)/41	10 * (X-1)/44	10 * (X-1)/39
Long-lived taxa	10 * X /3	10 * X /5	10 * X /4
Clinger taxa	10 * X /9	10 * X /15.5	10 * X /8
% Dominance	10 - (10 * [(X-10)/44])	10 - (10 * [(X-10)/33])	10 - (10 * [(X-10)/44])
% Tanytarsini	10 * [ln(X + 1) /3.3]	10 * [ln(X + 1) /3.3]	10 * [ln(X + 1) /3.3]
Sensitive taxa	10 * X /11	10 * X /19	10 * X /9
% Very tolerant	10 - (10 * [ln(X + 1)/4.4])	10 - (10 * [ln(X + 1)/3.6])	10 - (10 * [ln(X + 1)/4.1])

SCI_2012 Equations				
SCI metric	***Northeast***	***BigBend***	***Panhandle West***	***Peninsula***
Total taxa	10 * (X-15)/27	10 * (X-17)/23	10 * (X-19)/28	10 * (X-15)/24
Ephem. taxa	10 * X /5	10 * X /5	10 * X /8	10 * X /5
Trichoptera taxa	10 * X /8	10 * X /7	10 * (X-1) /9	10 * X /7
% Filterer	10 * (X-0.7)/40.5	10 * (X-1)/53	10 * (X-2.7)/47	10 * (X-0.7)/43
Long-lived taxa	10 * X /4	10 * X /3	10 * X /5	10 * X /3
Clinger taxa	10 * X /10	10 * X /8	10 * (X-2) /10	10 * X /7
% Dominance	10 - (10 * [(X-11)/48])	10 - (10 * [(X-12.5)/54])	10 - (10 * [(X-10.5)/36])	10 - (10 * [(X-14)/50])
% Tanytarsini	10 * [ln(X + 1) /3.2]	10 * [ln(X + 1) /3.1]	10 * [ln(X + 1) /3.2]	10 * [ln(X + 1) /3.4]
Sensitive taxa	10 * X /13	10 * X /10	10 * (X-2) /15	10 * X /7
% Very tolerant	10 - (10 * [ln(X + 1)/4.1])	10 - (10 * [(ln(X + 1)-0.6)/3.6])	10 - (10 * [ln(X + 1)/3.3])	10 - (10 * [(ln(X + 1)-0.7)/4.0])

Calculate the metric scores, divide by 0.9
If an individual metric is greater than 10, set it equal to 10
If an individual metric is less than 0, set it equal to 0

Note that while changing the regional boundaries led to some differences in the equations, the biological expectations for the Big Bend were most affected. For example, instead of using a value of 33 in the denominator for the taxa richness calculation (the 95th - 5th percentile value for the aggregated Panhandle), a value of 23 taxa (the 95th - 5th percentile value for the Big Bend region) is used. Similarly, the 95th to 5th percentile range for long-lived taxa and sensitive taxa decreased from 5 to 3 and from 19 to 10, respectively, when evaluating the Big Bend separately from the entire Panhandle.

The effect of the new regions and different metric scoring calculations on vetted reference sites is summarized in **Table B2**. Note that while the reference site mean values for the Northeast and Panhandle West decreased by 4.7 and 3.1 points respectively, the reference site scores for the Big Bend and Peninsula increased by 10.6 and 6.1 points, respectively. In summary, this resulted in a 3.2 point increase in the reference site mean when all regions were averaged. However, the lower 2.5th percentile SCI value based on the average of the most recent two reference site samples, calculated using the new regionalization scheme, was 35 (a 5 point decrease from the former value).

Table B2. Average scores for 2007 most recent two visits for vetted reference sites using the 2012 regionalization scheme.

New Bioregion	Average of SCI 2012 Scores (New Regions)	Average of SCI 2007 Scores (Old Regions)	Number of Visits
Northeast	68.6	73.3	**14**
Big Bend	69.1	58.5	**20**
Panhandle West	64.4	67.5	**24**
Peninsula	70.2	64.1	**38**
Grand Average	**68.3**	**65.1**	**96**

2 Establishing Expectations for Aquatic Life Use: Stream Condition Index

2.1 *Application of the Reference Site Approach*

In 2007, DEP calibrated the SCI using primarily the Biological Condition Gradient approach (secondarily on the reference site approach), resulting in an "impairment" threshold of 34 points and "exceptional" threshold of 67 points. Subsequent EPA review resulted in the recommendation that Florida examine the lower distribution of reference sites as the principal line of evidence for establishing aquatic life use support thresholds, in combination with the Biological Condition Gradient approach.

In response to this request, DEP conducted statistical interval and equivalence tests with SCI data from 55 reference streams (predominantly consisting of the recently verified nutrient benchmark sites with additional data from the Fore *et al.* (2007a) analysis). This analysis was performed to determine the lower bounds of the reference site distribution of SCI scores while balancing type I errors (falsely calling a reference site impaired) and type II errors (failing to detect that a site is truly impaired) (**Table B1**). The examination of the two most recent visits at 55 reference streams showed that the 2.5th percentile of reference data was in the range of 35-44 points. The middle of this range was 40 points, which represents an impairment threshold that balances Type I and Type II errors.

When calibrating an impairment threshold for an index, the amount of human disturbance inherent at the reference sites is a major issue. Some states select reference sites based on the "best available condition" (may have substantial disturbance), using a Best Professional Judgment approach. Florida has employed a rigorous reference site selection approach, which objectively demonstrates the "minimally disturbed" (limited human influence) nature of Florida's reference sites. When establishing an impairment threshold using a lower distribution of reference sites, a rigorous reference site selection process provides greatly increased confidence that the reference site population is minimally disturbed, thereby significantly reducing Type II errors (*i.e.,* classifying impaired sites as healthy). This increased confidence also allows for establishing the impairment threshold at a low level of the reference site distribution to minimize Type I errors (classifying healthy sites as impaired**).**

Florida's Impaired Waters Rule (62-303, F.A.C.) assesses biological impairment based on the average of the two most recent site visits, so the threshold determined from the interval and equivalence tests (which was 40, based on an average of two site visits) is closely aligned with the assessment methods. An impairment threshold of 40 would result in approximately 2.5 % of reference sites (known to be minimally disturbed) to be deemed impaired. DEP believes that this threshold is consistent with the CWA aquatic life use support goal and complies with Florida law. The slight differences in scores due to changes in regionalization do not significantly affect the "impairment threshold" (**Table B3**). An analysis of the same 55 sites that were used to derive the lowest acceptable biological life support value showed that the lower 2.5 percentile changed from 40 to 42 points when using the new regionalization system. However, a reanalysis of the reference site lower 2.5th percentile, using the two most recent samplings (n = 51) and calculated with the new 2012 regional equations, yielded a score of 35. Because reference site results from the new regional equations bracket the existing lowest acceptable score of 40, this is evidence that the existing threshold should not be changed.

Table B3. Results of interval and equivalence tests conducted on 55 reference sites with 2 SCI results, using both the 2007 and 2012 regionalization scheme. Shown are site mean and impairment threshold at the 2.5th percentile of reference sites ($p < 0.05$; N = 55 reference sites with two SCI values for each site). The 2007 reference site values are from Fore *et al.* (2007a) and comprehensively verified nutrient benchmark sites. The 2012 reference sites with the most recent data (n = 51) were extracted from the Statewide Biological Database in 2012 and the SCI scores were calculated using the new 2012 Regions.

Parameter	Original 55 Reference Sites Using 2007 Regions	Original 55 Reference Sites Recalculated Using New 2012 Regions	Reference Sites with Most Recent Data (n = 51) Calculated Using New 2012 Regions
Mean	65	67	66
2.5th percentile of reference (Lowest Acceptable Average SCI Value)	40	42	35

2.2 *Biological Condition Gradient Approach*

The U.S. EPA has outlined a tiered system of aquatic life use designation, along a Biological Condition Gradient (BCG), that illustrates how ecological attributes change in response to increasing levels of human disturbance. The BCG is a conceptual model that assigns the relative health of aquatic communities into one of six categories, from natural to severely changed (**Figure B13**). It is based in fundamental ecological principles and has been extensively verified by aquatic biologists throughout the U.S.

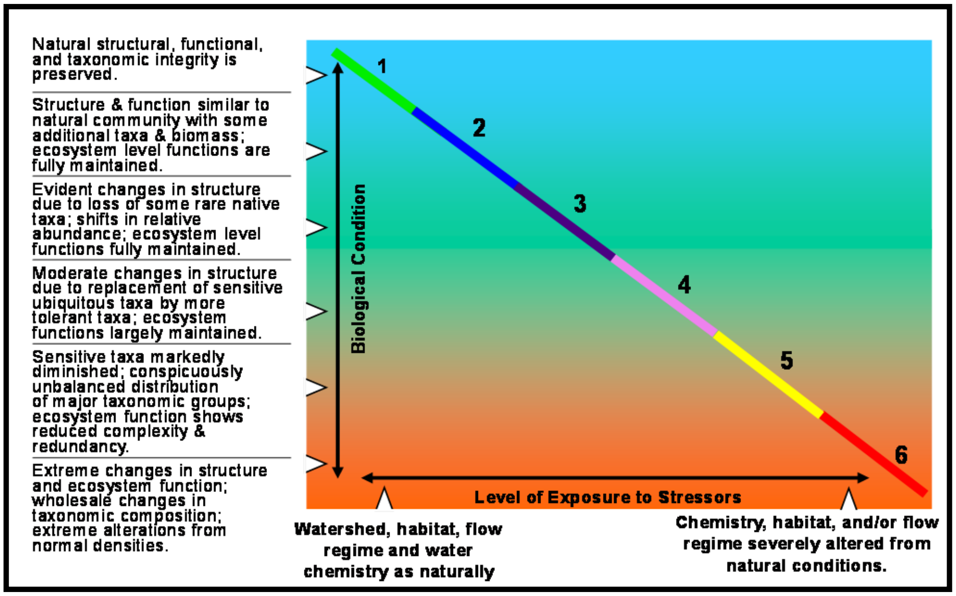


Figure B13. The Biological Condition Gradient Model (from Davies and Jackson 2006).

The BCG utilizes biological attributes of aquatic systems that predictably respond to increasing pollution and human disturbance. While these attributes are measurable, some are not routinely quantified in monitoring programs (*e.g.*, rate measurements such as productivity), but may be inferred via the community composition data (*e.g.*, abundance of taxa indicative of organic enrichment).

The biological attributes considered in the BCG are:

1. Historically documented, sensitive, long-lived or regionally endemic taxa;
2. Sensitive and rare taxa;
3. Sensitive but ubiquitous taxa;
4. Taxa of intermediate tolerance;
5. Tolerant taxa;
6. Non-native taxa;
7. Organism condition;
8. Ecosystem functions;
9. Spatial and temporal extent of detrimental effects; and
10. Ecosystem connectance.

The gradient represented by the BCG has been divided into six levels (tiers) of condition that were defined via a consensus process (Davies and Jackson 2006) using experienced aquatic biologists from across the U.S., including Florida representatives. The six tiers are:

1) Native structural, functional, and taxonomic integrity is preserved; ecosystem function is preserved within range of natural variability;
2) Virtually all native taxa are maintained with some changes in biomass and/or abundance; ecosystem functions are fully maintained within range of natural variability;
3) Some changes in structure due to loss of some rare native taxa; shifts in relative abundance of taxa but sensitive–ubiquitous taxa are common and abundant; ecosystem functions are fully maintained through redundant attributes of the system;
4) Moderate changes in structure due to replacement of some sensitive–ubiquitous taxa by more tolerant taxa, but reproducing populations of some sensitive taxa are maintained; overall balanced distribution of all expected major groups; ecosystem functions largely maintained through redundant attributes;
5) Sensitive taxa are markedly diminished; conspicuously unbalanced distribution of major groups from that expected; organism condition shows signs of physiological stress; system function shows reduced complexity and redundancy; increased buildup or export of unused materials; and
6) Extreme changes in structure; wholesale changes in taxonomic composition; extreme alterations from normal densities and distributions; organism conditioning is often poor; ecosystem functions are severely altered.

The six levels described above are used to correlate biological index scores with biological condition, as part of calibrating the index. Once the correlation is well established, a determination is made as to which biological condition represents attainment of the CWA goal according to paragraph 101(a)(2) related to aquatic life use support, "protection and propagation of fish, shellfish, and wildlife."

During the development of the BCG model at National BCG Workshops, each of the break-out groups independently reported that the ecological characteristics conceptually described by tiers 1–4 corresponded to how they interpret attainment of the CWA's interim goal for protection and propagation of aquatic life (Davies and Jackson 2006). Two panels of Florida experts (one for the SCI, and one for the Lake Vegetation Index) arrived at conclusions that were independent from, but identical to, the national expert groups (that a BCG category of 4 represented healthy, well balanced communities). Additionally, the State of Maine has adopted a policy that aquatic communities conceptually aligned with BCG Category 4 meets the CWA's interim goal for protection and propagation of aquatic life, and this was subsequently approved by EPA.

As mentioned above, DEP conducted a BCG exercise to calibrate scores for the SCI. Twenty-two experts "blindly" examined taxa lists from 30 stream sites throughout Florida, 10 in each Bioregion, that spanned the range of SCI scores. Without any knowledge of the SCI scores, they reviewed the data and assigned each macroinvertebrate community a BCG score from 1 to 6, where 1 represents natural or native condition and 6 represents a condition severely altered in structure and function from a natural condition. Experts independently assigned a BCG score to each site, and then were able to discuss their scores and rationale, and could opt to change their scores based on arguments from other participants. At the conclusion of the workshop, DEP regressed the mean BCG score given to each stream against the 2007 SCI score for that site (**Figure B14**), and subsequently, the 2012 SCI scores (adjusted for the 2012 regions), were also calculated and overlaid on the original graph. Note that there were virtually no differences in the regression slopes or confidence intervals between the old and new regional score calculations. This clearly shows that the new regional equations did not change the BCG results.

The experts were also asked to identify the lowest BCG level that still provided for the propagation and maintenance a healthy, well-balanced aquatic community (the interim goal of the Clean Water Act) and the BCG category (and higher) represented exceptional conditions (the ultimate goal of the Clean Water Act, also referred to as "biological integrity"). All of 22 participants thought category 2 SCI scores should be considered exceptional, which corresponds to an SCI score of 64. The median response from the expert group indicated that category 4, which corresponds to an SCI score of 34, was the lowest acceptable condition.

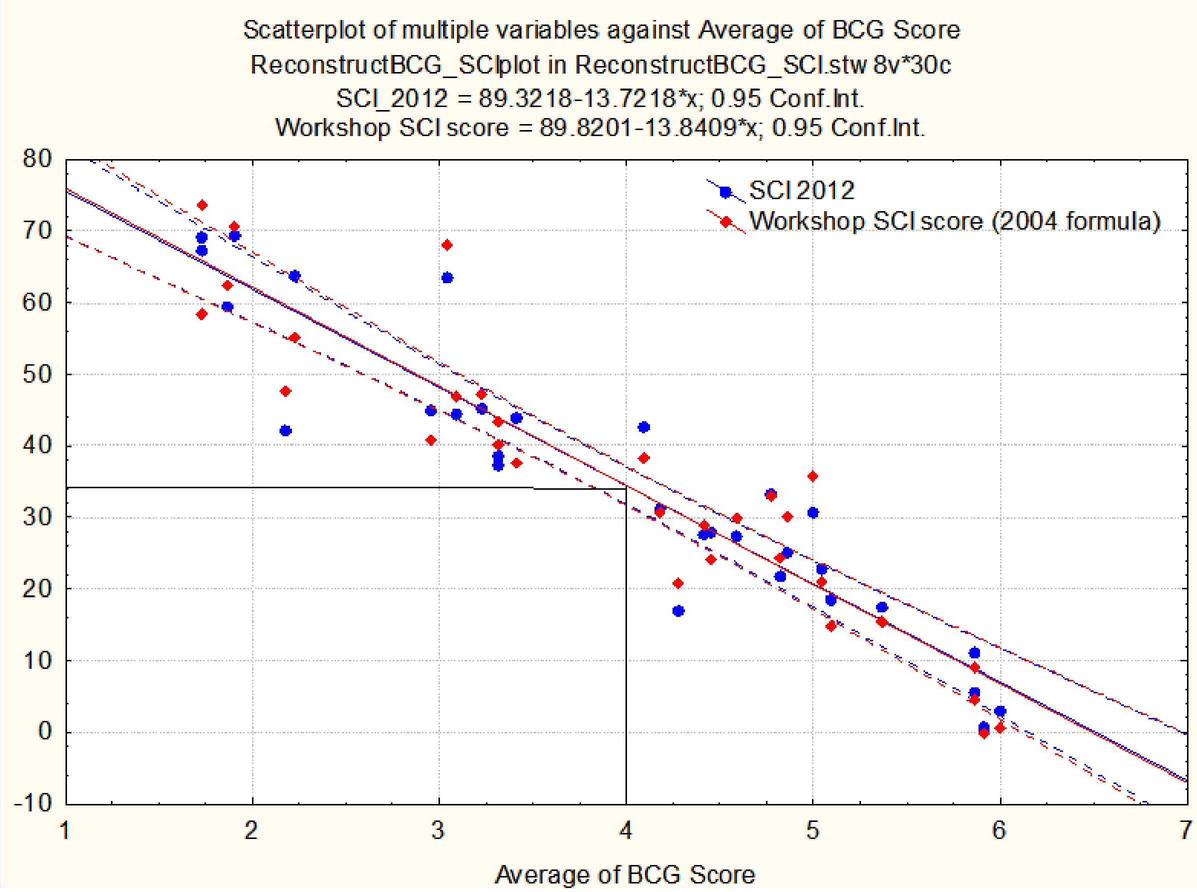


Figure B14. Regression line with 90% confidence interval showing the relationship between the mean BCG score and both the SCI 2007 and SCI 2012 scores (after regional revisions). The median BCG value the expert group considered meeting a healthy, well balanced community corresponded to a BCG tier of 4 and an SCI score of 34 (this subsequently changed based upon a proportional odds analysis). The "exceptional" threshold was established at 64 and above, based on the score associated with a BCG 2.

2.3 Evaluation of the Reference Site Approach Coupled with BCG

EPA noted the variability in the expert responses within each BCG category, and conducted an additional analysis of the BCG results to further define an acceptable aquatic life use threshold. EPA calculated a proportional odds logistic regression model (Guisan and Harrell, 2000) to better describe the relationship between a continuous variable (SCI scores) and a categorical variable (BCG categories). This model is based on the cumulative probability of a site being assigned to a given tier (*e.g.*, Tier 3) or to any higher quality tier (Tiers 1 and 2). Thus, five parallel models are fit, modeling the probability of assignment to Tiers 5 to 1, Tiers 4 to 1, Tiers 3 to 1, Tiers 2 to 1, and Tier 1 only. Once these five models are fit, the probability of assignment to any single tier can be extracted from the model results.

In **Figure B15**, the mean predictions of the proportional odds logistic regression models are plotted as solid lines. Lines are color-coded and labeled by different tiers, and each line can be interpreted as the proportion of experts that assigned samples with the indicated SCI value to a particular tier. For example, approximately 90% of experts assigned a sample with the lowest SCI score to Tier 6 (brown line), while the remaining 10% of experts assigned the sample to Tier 5 (purple line). In the figure, the solid circles represent the actual expert assignments recorded from the workshop for each SCI value. The size of the circle is proportional to the number of experts that assigned a sample to a particular tier, and the circles are color-coded by tier. There is some variability among experts in their assignment of BCG scores, but there is a clear central tendency at any given SCI score.

EPA recommended that the threshold be set at an SCI score where there is an approximately equally low probability of assignment to Tier 5 (*i.e.,* impaired) and a low probability of assignment to Tier 2 (*i.e.,* reference conditions). The resultant threshold of 42 balances the probability of mistakenly assessing a degraded site as meeting aquatic life use goals with the probability of mistakenly assessing a reference site as impaired. This score is consistent with the impairment threshold of 40 as determined by the reference site approach.

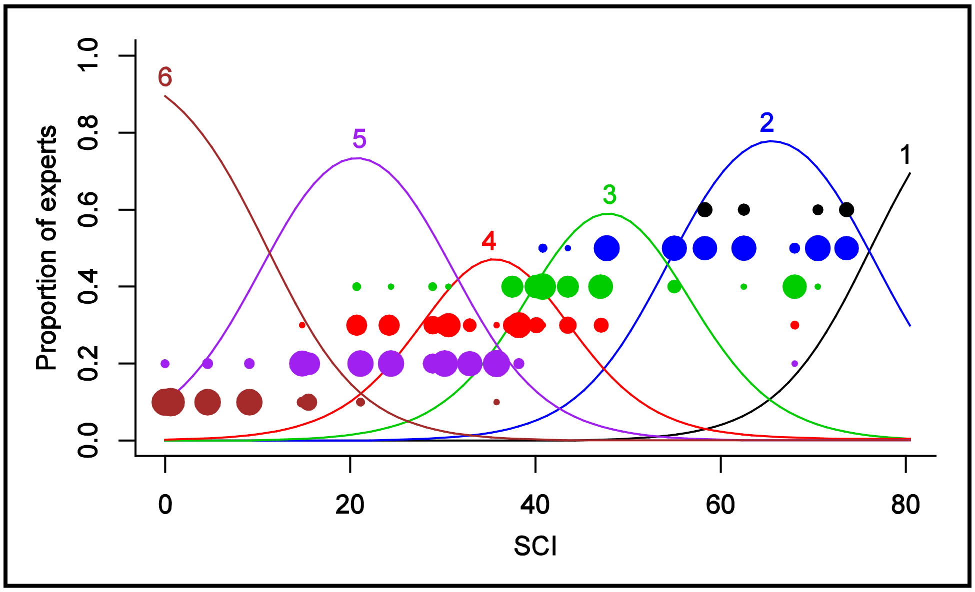


Figure B15. BCG tier assignments modeled with a proportional odds logistic regression.

2.4 Setting and Evaluating an SCI Impairment Threshold

Weighing these multiple lines of evidence, the DEP has determined that an average SCI score of 40 indicates that the designated use is being met, and n average score of 39 is impaired. This impairment threshold is supported by the distribution of benchmark site scores and corresponds with a BCG category midway between Tiers 3 and 4. The proportional odds analysis provides assurance that stream communities deemed exceptional (BCG category 2) will not be considered impaired at a threshold of 40.

The DEP evaluated recent data for the individual metrics of the SCI to determine what range of macroinvertebrate attributes would be considered healthy using this impairment threshold. Since DEP conducted the SCI calibration in 2007, the State has collected approximately 700 additional SCI samples from a variety of sites, including minimally disturbed reference sites (for nutrient and DO criteria development), sites located along a nutrient gradient, and randomly chosen sites for the status and trends network. Based upon the relationship described in **Figure B14**, the SCI values from this data set were subdivided into increments representing half-step BCG Categories, and the individual metrics associated with each half step interval were averaged. The metric data bracketing BCG category 2 were averaged to demonstrate metric values associated with exceptional conditions. Data within the range of the impairment threshold of 40 were also averaged to provide an example of the stream condition that Florida's SCI biological criterion will protect (**Table B4**). Note that although there are moderate differences between metrics associated with exceptional biological communities and those near the range of the impairment threshold, the attributes associated with communities near the threshold are still considered to be indicative of healthy, well balanced communities by more than half of the Florida stream experts who participated in the BCG exercise.

Table B4. Average values for metrics at an SCI score equivalent to a Biological Condition Gradient of category 2, and average values for metrics near the SCI score of impairment. Data was based upon the DEP's data collection effort since 2007 (total N = 696 SCI samples).

SCI Metric	Metric Average at BCG 2 (Exceptional)	Metric Average Near Impairment Threshold
Number of Total Taxa	32.0	28.7
Number of Clinger Taxa	5.6	3.3
Number of Long Lived Taxa	1.5	1.1
Percent Suspension Feeders and Filterers	22.0	15.8
Number of Sensitive Taxa	5.4	2.7
Percent Tanytarsini	13.3	9.5
Percent Very Tolerant	6.5	14.3
Number of Ephemeroptera Taxa	3.5	2.3
Number of Trichoptera Taxa	4.5	2.6
Percent Dominant	22.6	26.2
Number of Sites in Average	134	64

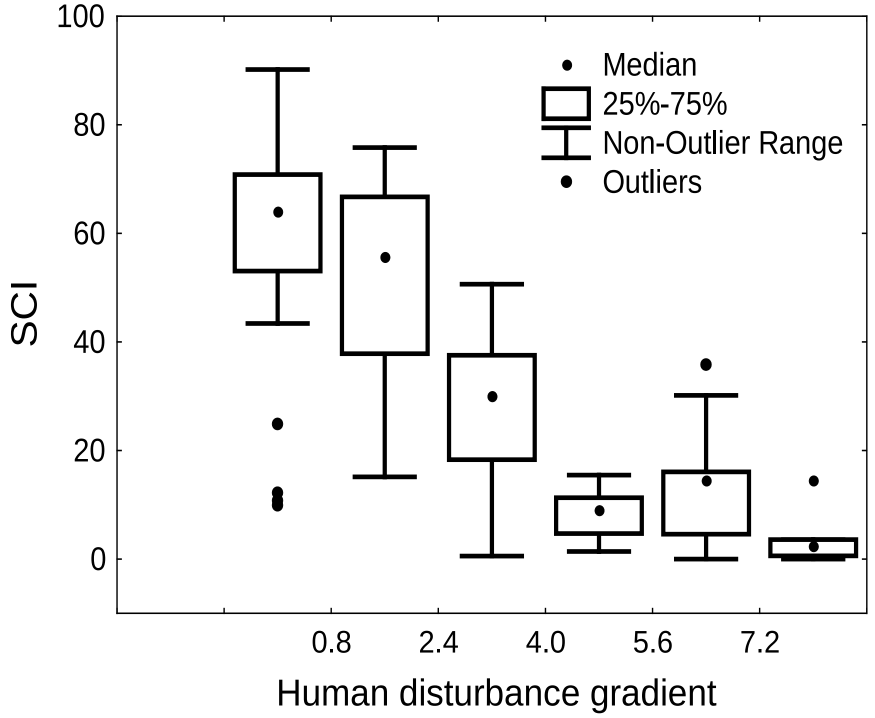


Figure B16. Relationship between the SCI (2004 data) and the Human Disturbance Gradient (from Fore *et al.*, 2007a).

During the development of the Stream Condition Index, DEP observed a clear and consistent relationship between the SCI and the Human Disturbance Gradient (**Figure B16**). Note the highest range of actual SCI scores were observed in the two groups of lowest human disturbance gradient sites (left most boxes in **Figure B16**). This wide range needs to be considered when establishing the threshold to limit the probability of falsely identifying unimpacted sites as not attaining an aquatic life use. However, the range of scores in the higher human disturbance gradient sites (expected to result in a BCG category 5-6) are low. Therefore, the risk is low (virtually non-existent for the SCI) in applying the biological assessment tool and falsely identifying impacted sites as attaining an aquatic life use.

This variability of the SCI scores within a given range of the human disturbance gradient is generally caused by changes in biological community relative to natural occurrences (droughts, floods, etc.), as well as the inherent limitation of the biological assessment methods.

Biological field observations can be influenced by natural conditions that may have occurred prior to the sampling event. Changes in hydrology, particularly high and low flow events that result in differential water velocities and habitat availability, will affect the biological community in a stream, potentially resulting in lower scores. The variability in low human disturbance gradient sites also reflects the fact that the biological communities in these systems are able to rapidly recover because the habitat and health of the stream is conducive to recovery. In high HDG sites, natural hydrologic events (along with human disturbance) can affect the biology, but any recovery is slow due to the human disturbance impacts and lack of recruitment of organisms from surrounding areas. Therefore, in high human disturbance gradient sites, SCI scores always tend to be low, and the range of values remains small.

The other factor leading to higher variability in scores for low disturbance sites relates to sampling issues. DEP's SCI collection methods follow EPA rapid bioassessment guidance, but do not result in a complete ecological census of all taxa present at a site. Instead, they provide a practical level of effort that can be used to distinguish healthy from impaired sites. Therefore, the sampling method is inherently conducted in a manner that may result in a high range of results where taxa are present and a low range of results where taxa are diminished. In other words, when taking a sample, it is possible to *fail to catch taxa that exist* in the water body, but it is not possible to *catch taxa that do not exist* in the water body.

In statistical terms, undisturbed sites have a higher probability of Type I error (falsely concluding that the site was impaired). Because the variability in the SCIs decreases as human disturbance increases, the disturbed sites fundamentally are subject to much lower occurrence rate of a Type II error (falsely concluding that the site was unimpaired) when compared to undisturbed sites. From a theoretical standpoint, since the error of the method used to collect representative taxa can only fail to capture and count taxa, and only 2 of the 10 metrics result in an improved SCI when specific organisms are missed, it is likely that Type I errors are of greater concern (occur more frequently) with the SCI methodology.

3 Additional Analysis of Rigorously Verified Benchmark Site SCI Data

The Stream Condition Index (SCI) scores from DEP's field-verified nutrient benchmark (*i.e.,* reference) site dataset were also evaluated to determine the range and variability of biological condition found in Florida's minimally-disturbed sites. Theoretically, these sites would be expected to have an SCI score reflective of a BCG category 1 or 2. In reality, as indicated previously, there is more variability in the actual scores. This benchmark dataset consists of sites determined by experienced DEP scientists to be influenced by only very low levels of anthropogenic stressors. Additional selection criteria included a Landscape Development Intensity index score of < 2, absence of upstream point source discharges, examination of aerial photographs, direct observations of watershed land use and hydrologic conditions during site visits, and habitat assessment. The dataset included 69 sampling events at a total of 53 stations across the state (16 stations were sampled twice during the verification process).

The mean SCI score from all 69 sampling events was 65.1, and the median was 65. The standard deviation from the mean was 15.8, and the range of scores was 80, spanning from 100 to 20. The one nutrient benchmark site that scored below the impairment threshold of 40 occurred at a Steinhatchee River site (at CR 357), which scored 20 on the SCI on August 12, 2008, after an extended period of low flow conditions (see **Figures B17, B18, and B19**). However, when this site was subsequently re-sampled on January 14, 2009 (after a period of higher flows), it scored a 53. Note that another minimally disturbed Steinhatchee River site located approximately 8 miles downstream, with slightly more flow (at Canal Road), scored 41 and 62 on the SCI during the same time period. Based on direct observations, the flow regime was the dominant factor for the variability in the SCI scores (**Figure B19**). DEP SOPs provide clear guidance regarding appropriate conditions during which to sample, including a minimum velocity of 0.05 m/sec. Although the Steinhatchee at CR 357 achieved this velocity and was not dry prior to sampling, the sluggish flows and less than optimal inundated habitat appeared to be responsible for the low SCI scores, not any human disturbance (the upstream basin is almost 100% forested). This is an example of the type of hydrologic conditions that occur randomly throughout the state, prompting DEP, in an attempt to minimize Type I errors, to select the lower 2.5% distribution of reference sites as the impairment threshold.

Figure B17. Steinhatchee River at CR 357, August 2008.

Figure B18. Steinhatchee River at CR 357, January 2009.

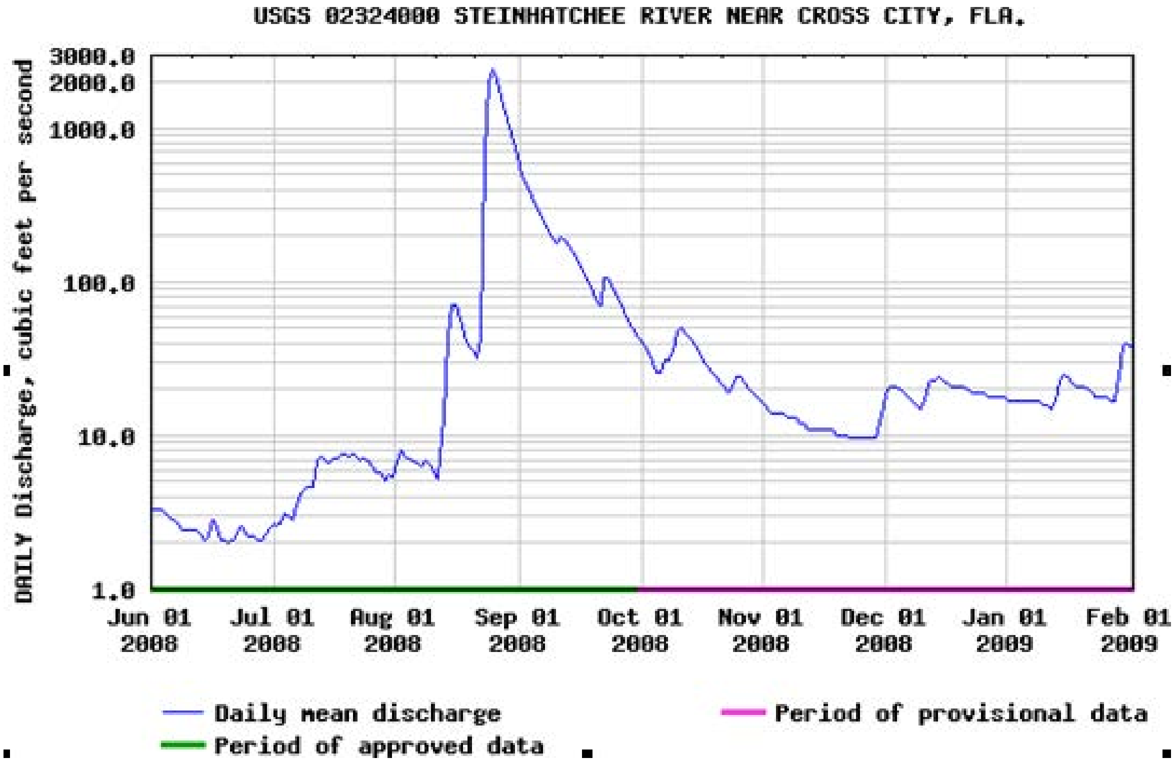


Figure B19. USGS hydrograph for the Steinhatchee River during the period of the two sampling events. The mean discharge rate for the Steinhatchee River near Cross City was 7.4 ft^3/sec on 8/12/2008 and 23 ft^3/sec on 1/14/2009.

4 Evaluation of Benchmark (Reference) Site Replicate Data: SCI

The 16 benchmark sites with replicate data were analyzed to determine the variability that can occur in SCI scores at the same sampling location. The benchmark sites with replicate data are shown below in **Table B5**. The mean difference in SCI scores from this sub-dataset was 17.1, with a standard deviation of 13.3. The median difference was 18. The largest difference in scores occurred at the St. Marys River at SR 2, which received SCI scores of 50 in June 2008, and 100 in November 2008.

Table B5. Minimally disturbed stream benchmark sites with replicate SCI data.

Benchmark Site	Date sampled	SCI score	Difference between replicates
Blackwater River at Highway 4	3/26/2007	56	14
	7/9/2008	70	
Cypress Branch	11/3/2008	66	3
	12/16/2008	63	
Escambia River at Highway 4	9/19/2007	57	6
	7/10/2008	51	
Manatee River at 64	5/16/2007	81	17
	12/17/2008	64	
Orange Creek upstream of Highway 21	2/26/2007	74	8
	5/1/2008	82	
Peters Creek at CR 315	5/28/2008	92	19
	10/28/2008	73	
Sopchoppy River	6/19/2008	41	23
	11/13/2008	64	
Steinhatchee River at CR 357	8/12/2008	20	33
	1/14/2009	53	
Steinhatchee River at Canal Road	8/12/2008	41	21
	1/14/2009	62	
St. Marys River at SR 2	6/18/2008	50	50
	11/12/2008	100	
Telogia Creek at CR 1641	6/10/2008	78	20
	11/20/2008	58	
Suwannee River at CR 6	10/10/2006	53	2
	12/12/2007	51	
Withlacoochee River above River Dr.	5/7/2008	44	2
	10/8/2008	42	
Withlacoochee River at Stokes Ferry	2/20/2007	68	21
	11/7/2007	47	
Yellow River at Hwy 2	5/15/2007	54	25
	7/9/2008	79	
Yon Creek at SR 12	6/13/2008	81	7
	11/20/2008	74	

Differences in SCI scores between replicates can be caused by the natural variability of environmental factors such as recent hydrologic conditions resulting in changes in habitat availability, as well variability associated with laboratory sub-sampling. Based on field observations, it was natural factors (water level and flow), and not changes in human disturbance, that were the main drivers of the differences in SCI scores between replicates taken at different times. Note that sampling visits to the sites with duplicate data were not separated by more than fourteen months (most were sampled less than six months apart).

Another indication that human disturbance was not associated with this variability was that no correlation was found between Landscape Development Intensity Index score and SCI score within the entire benchmark site dataset (**Figure B20**). This is in contrast to the strong relationship between the LDI and SCI scores across the entire range of human disturbance (in **Figure B16**, the LDI is a prominent influence on the HDG).

In conclusion, the SCI provides a reliable and predictable assessment of human disturbance. The calibration of the index involved identifying a score that could differentiate between biologically healthy sites and those impaired by human activities. Using multiple lines of evidence (BCG and reference site approaches), it was determined, in conjunction with EPA, that the threshold between impaired and unimpaired sites is a SCI score of 40, with a score of 40 or above indicating that the designated use is being met, and a score of 39 or below being indicative of impairment. At an SCI threshold of 40, approximately 2.5% of the minimally disturbed reference sites will be incorrectly categorized as impaired.

Greater detail concerning the development and application of the SCI can be found in the DEP guidance document "*Sampling and Use of the Stream Condition Index (SCI) for Assessing Flowing Waters: A Primer*" (FDEP 2011) which can be found at: http://www.dep.state.fl.us/water/sas/qa/docs/62-160/sci-primer-102411.pdf.

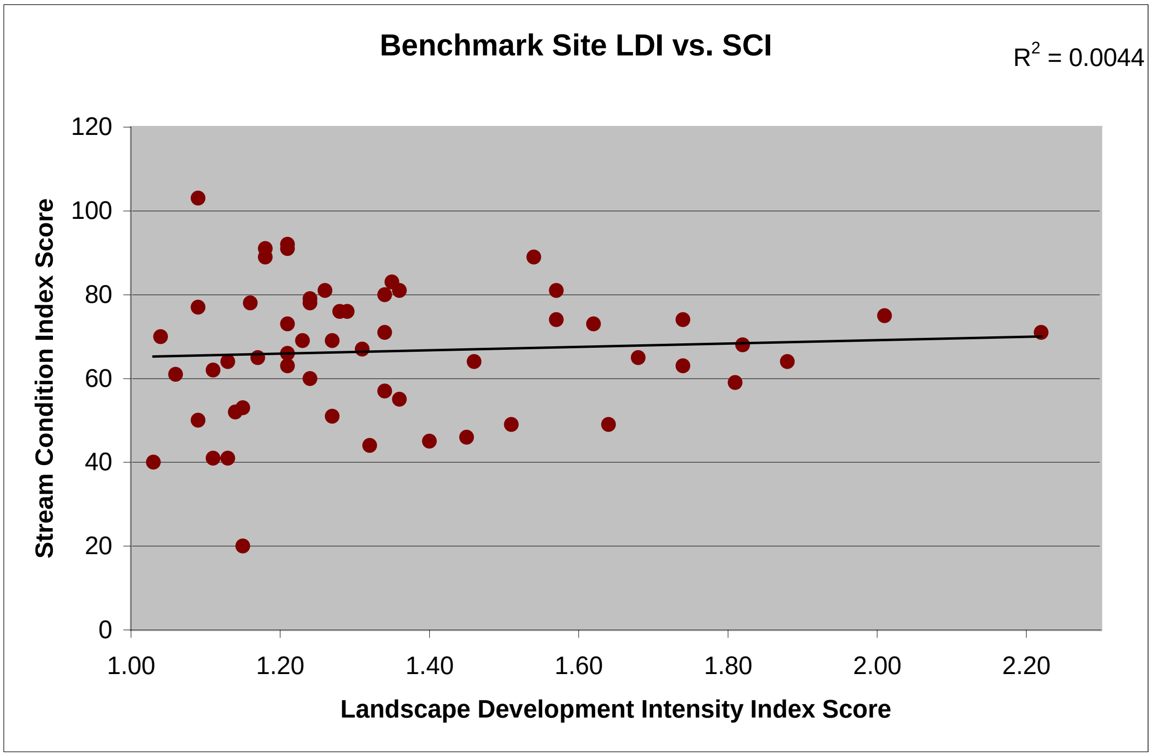


Figure B20. Minimally disturbed benchmark sites plotted against the Landscape Development Intensity Index (LDI). Direct observations indicated that the LDI reflected current land use and disturbance conditions.

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Appendix C:

Response of the Ten SCI Component Metrics to DO Levels

Response of the Ten SCI Component Metrics to DO Levels

1 Background

As described previously, the Stream Condition Index (SCI) is a ten metric index developed to be indicative of the health of the macroinvertebrate community in a stream and provide a predictable assessment of anthropogenic impacts on the biologic health if a waterbody. The 10 metrics composing the SCI represent an array of indicators of biological integrity that provide a comprehensive assessment of the species composition, diversity, and functional organization of the macroinvertebrate community. The metrics were selected based on their ability to respond predictably to anthropogenic disturbance and to avoid excessive redundancy of the community attribute being assessed.

The 10 SCI metrics are:

- Number of Total Taxa
- Number of Long Lived Taxa
- Number of Sensitive Taxa
- Percent Tanytarsini Individuals
- Percent of Individuals in the Dominant Taxon
- Number of Clinger Taxa
- Number of Ephemeroptera Taxa
- Number of Trichoptera Taxa
- Percent Very Tolerant Individuals
- Percent Suspension Feeder and Filterer Individuals

Since the response of the SCI to DO levels is the basis of the derivation of the proposed freshwater DO criteria presented in this Technical Support document, it is important to establish that there are significant predictable relationships between at least some of the component metrics and DO levels that result in the overall response of the SCI to DO used to derive the proposed criteria.

The relationships between the 10 individual macroinvertebrate metric that comprise the SCI and DO levels were examined and are presented in **Figures 1 - 10**. Because of the known regional differences in biological expectations that are incorporated into the SCI as well as observed spatial differences in DO levels, the relationships between the SCI metrics and DO were examined separately for the Panhandle West, Panhandle East + Big Bend, and Peninsula bioregions.

Predictably, the observed relationships varied across regions and by metric. Generally, the strongest responses to DO levels were for metrics that were measures of the pollution sensitive portion of the macroinvertebrate community such as; number of sensitive taxa, number of clinger taxa, and number of Ephemeroptera (mayfly) taxa (**Figure 1, 2, and 3**). All of these metrics exhibited a positive response to DO, as expected, with the number of sensitive taxa increasing with increasing DO levels. Spatially, the strongest relationships between the metrics indicative of the pollution sensitive taxa and DO levels were generally found in the Panhandle West bioregion where the biological expectation is higher and a greater number of sensitive organisms are typically found in conjunction with higher DO levels. In contrast, metrics that describe portions of the community that are more pollution tolerant such as percent very tolerant individuals, and percent dominant taxa exhibited less significant responses to DO and tended to decrease with increasing DO levels (**Figures 5 and 10**).

The results of the evaluation of the individual SCI metrics followed expected patterns and confirms that the macroinvertebrate community is responding to DO levels and that the relationships between the SCI scores and DO levels are not the random result of a combination of the individual metrics. This finding supports the use of the SCI versus DO relationships in the derivation of the proposed freshwater DO criteria described in this document.

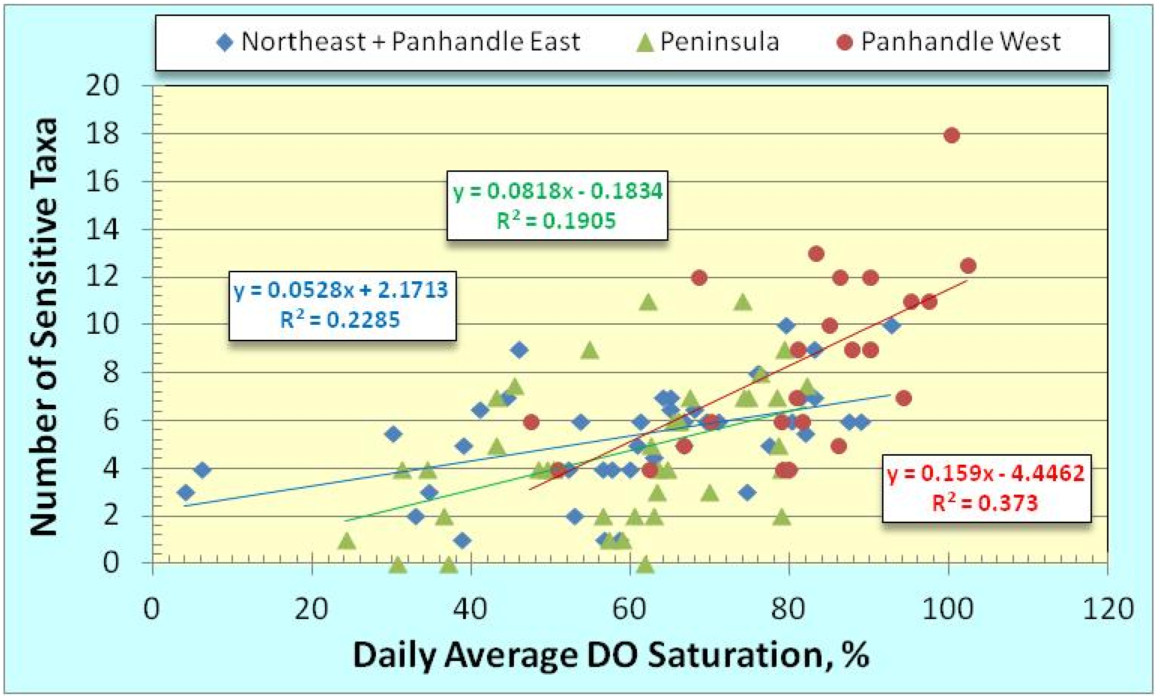

Figure 1. Regional relationships between the number of sensitive taxa SCI metric and daily average DO percent saturation for Northeast + Panhandle East, Peninsula, and Panhandle West bioregions. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment score ≥ 110, NOx ≤ 0.35 mg/L).

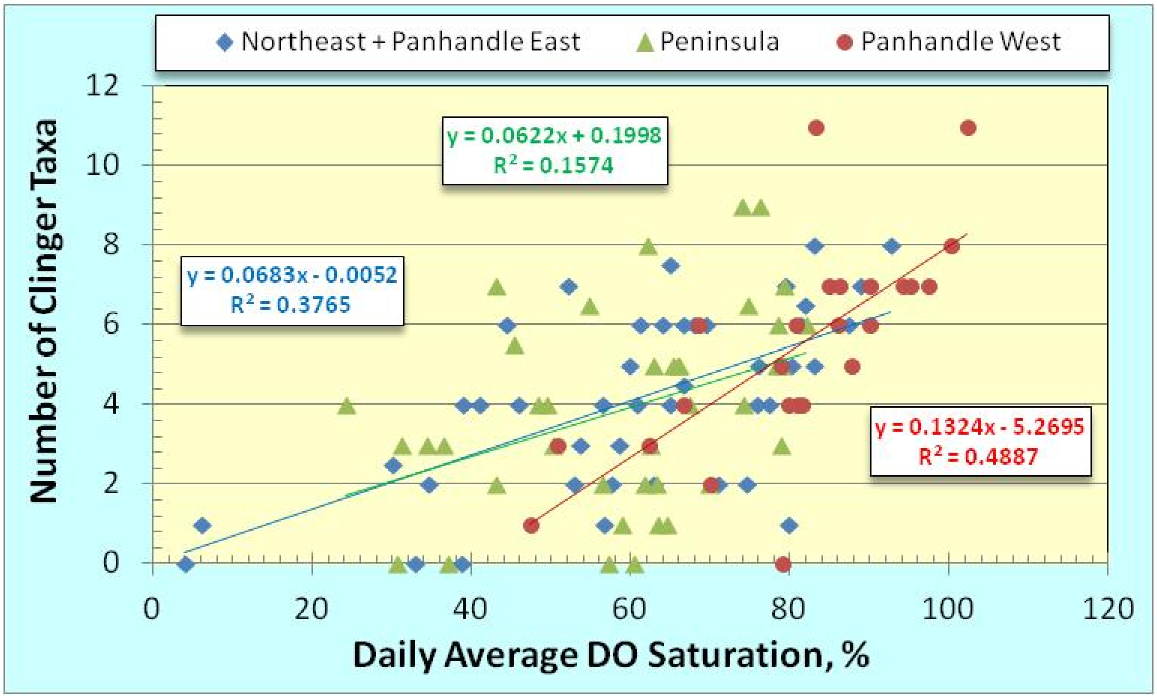

Figure 2. Regional relationships between the number of clinger taxa SCI metric and daily average DO percent saturation for Northeast + Panhandle East, Peninsula, and Panhandle West bioregions. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment score ≥ 110, NOx ≤ 0.35 mg/L).

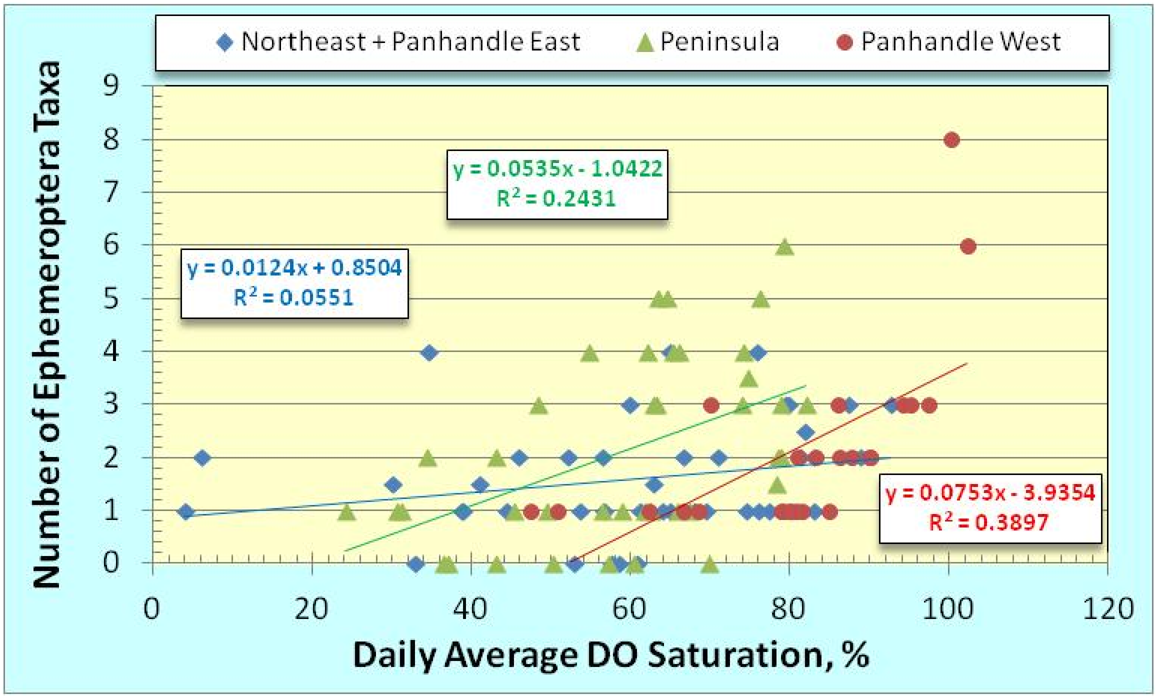

Figure 3. Regional relationships between the number of ephemeropterar taxa SCI metric and daily average DO percent saturation for Northeast + Panhandle East, Peninsula, and Panhandle West bioregions. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.,* LDI ≤ 2, conductivity ≤ 300, habitat assessment score ≥ 110, NOx ≤ 0.35 mg/L).

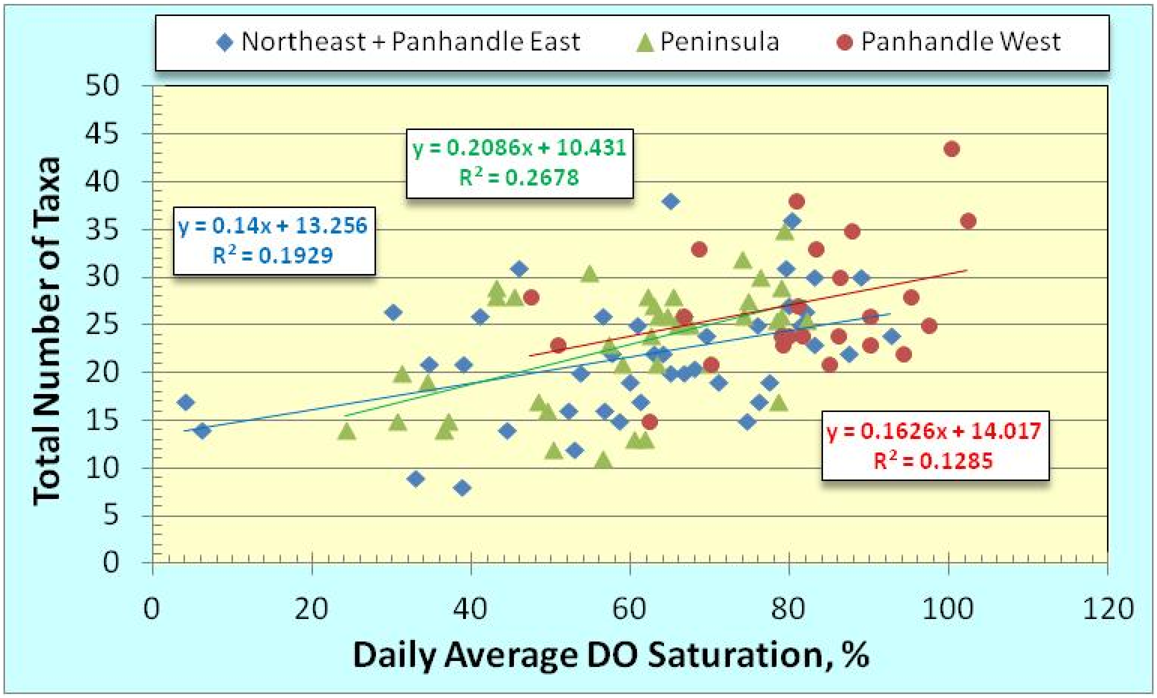

Figure 4. Regional relationships between the total number of taxa SCI metric and daily average DO percent saturation for Northeast + Panhandle East, Peninsula, and Panhandle West bioregions. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.,* LDI ≤ 2, conductivity ≤ 300, habitat assessment score ≥ 110, NOx ≤ 0.35 mg/L).

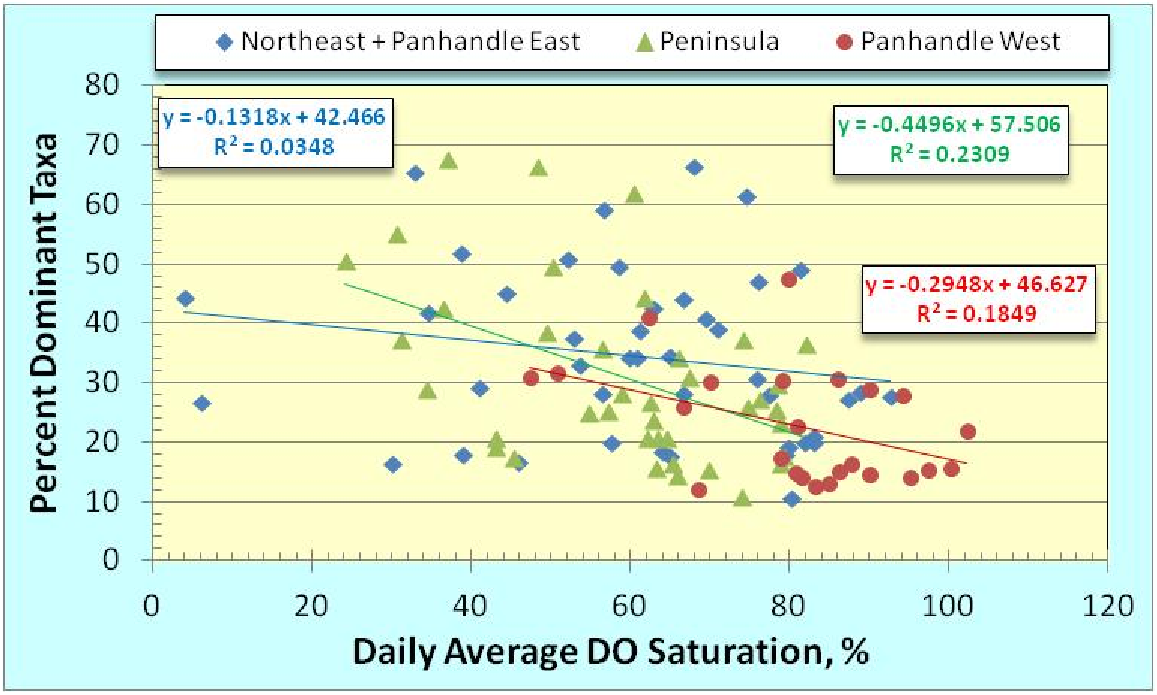

Figure 5. Regional relationships between the percent dominant taxa SCI metric and daily average DO percent saturation for Northeast + Panhandle East, Peninsula, and Panhandle West bioregions. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment score ≥ 110, NOx ≤ 0.35 mg/L).

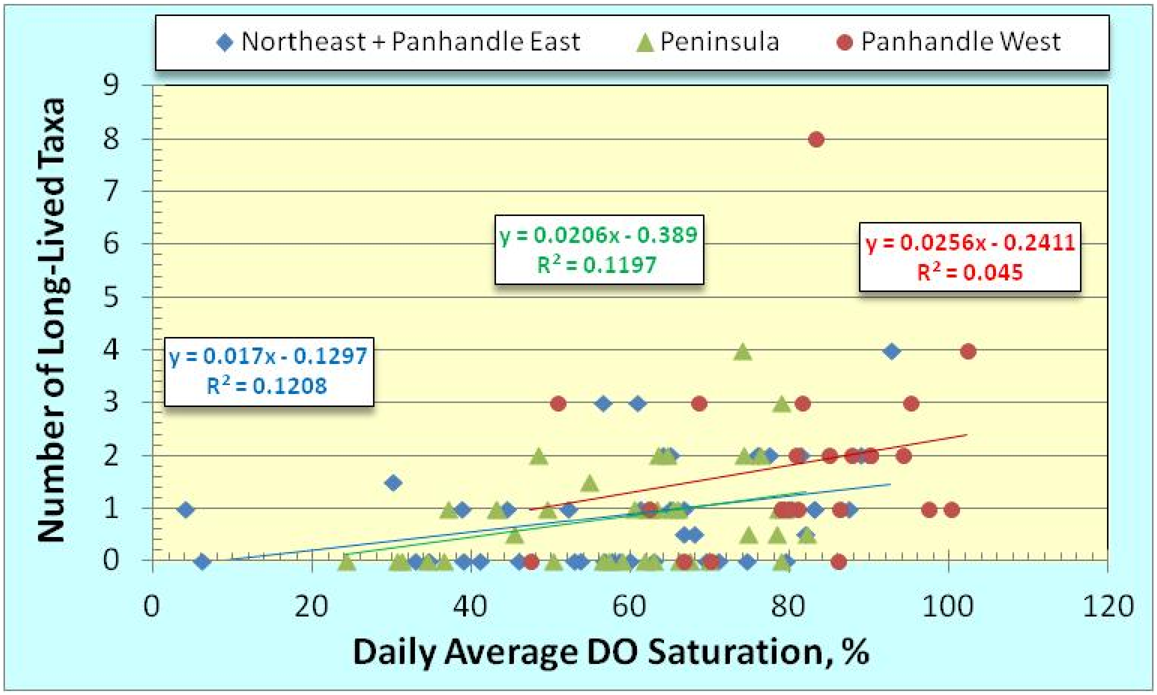

Figure 6. Regional relationships between the number of long-lived taxa SCI metric and daily average DO percent saturation for Northeast + Panhandle East, Peninsula, and Panhandle West bioregions. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment score ≥ 110, NOx ≤ 0.35 mg/L).

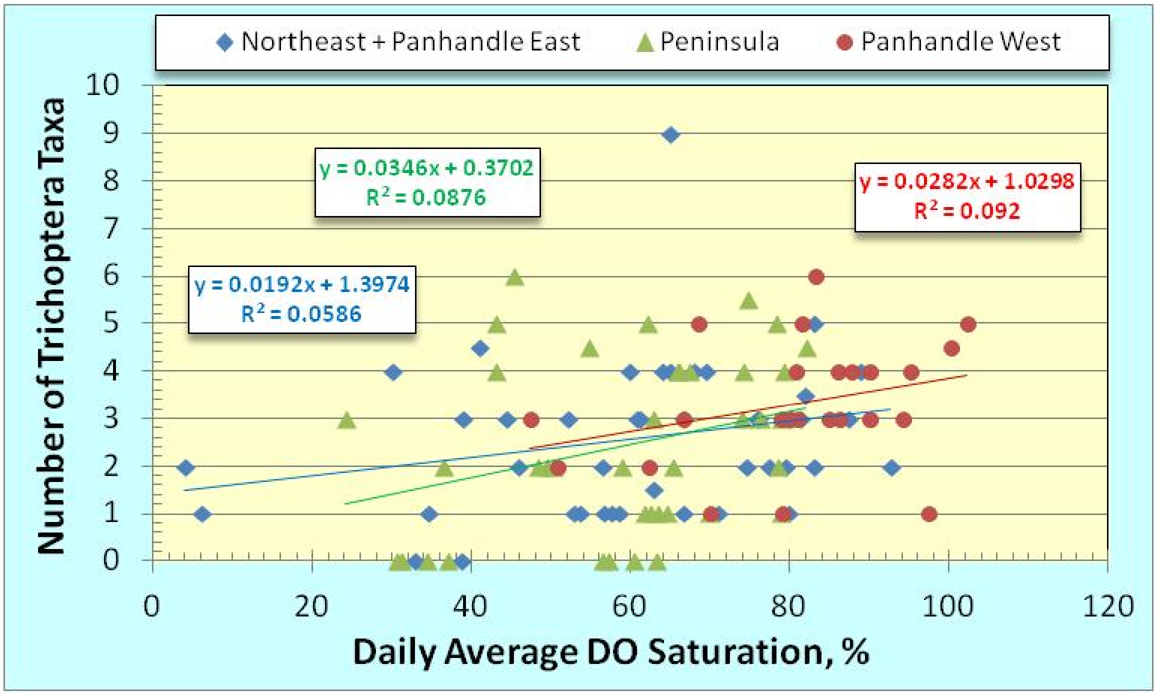

Figure 7. Regional relationships between the number of trichoptera taxa SCI metric and daily average DO percent saturation for Northeast + Panhandle East, Peninsula, and Panhandle West bioregions. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment score ≥ 110, NOx ≤ 0.35 mg/L).

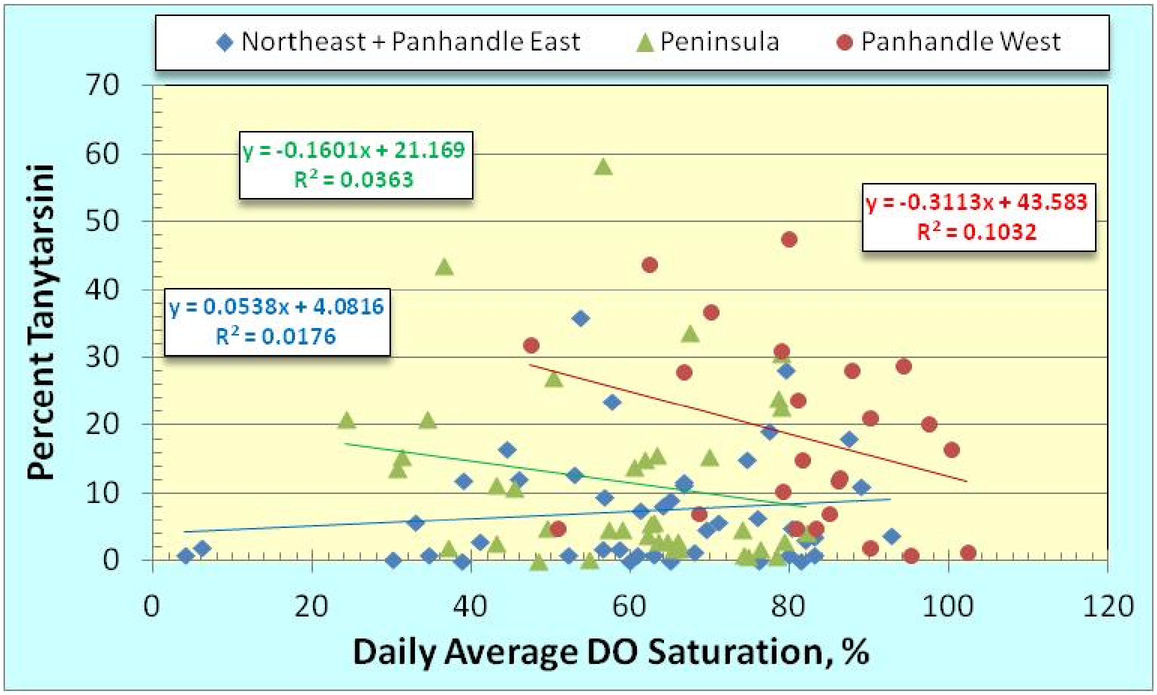

Figure 8. Regional relationships between the percent tanytarsini SCI metric and daily average DO percent saturation for Northeast + Panhandle East, Peninsula, and Panhandle West bioregions. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment score ≥ 110, NOx ≤ 0.35 mg/L).

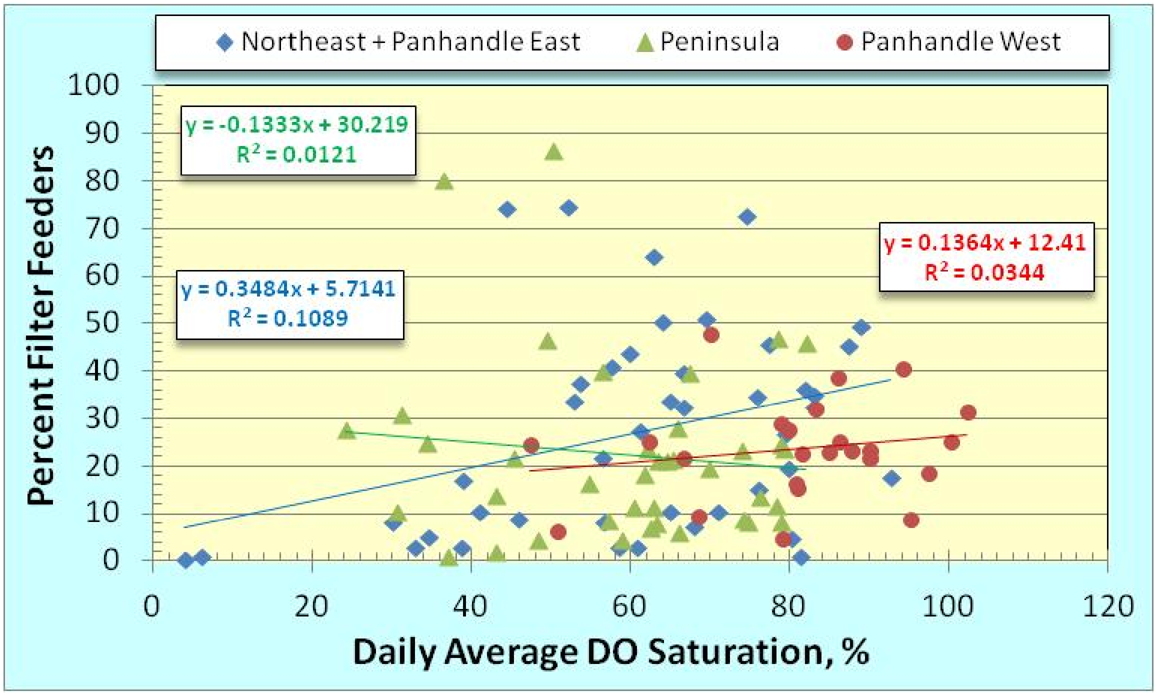

Figure 9. Regional relationships between the percent filter feeders SCI metric and daily average DO percent saturation for Northeast + Panhandle East, Peninsula, and Panhandle West bioregions. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment score ≥ 110, NOx ≤ 0.35 mg/L).

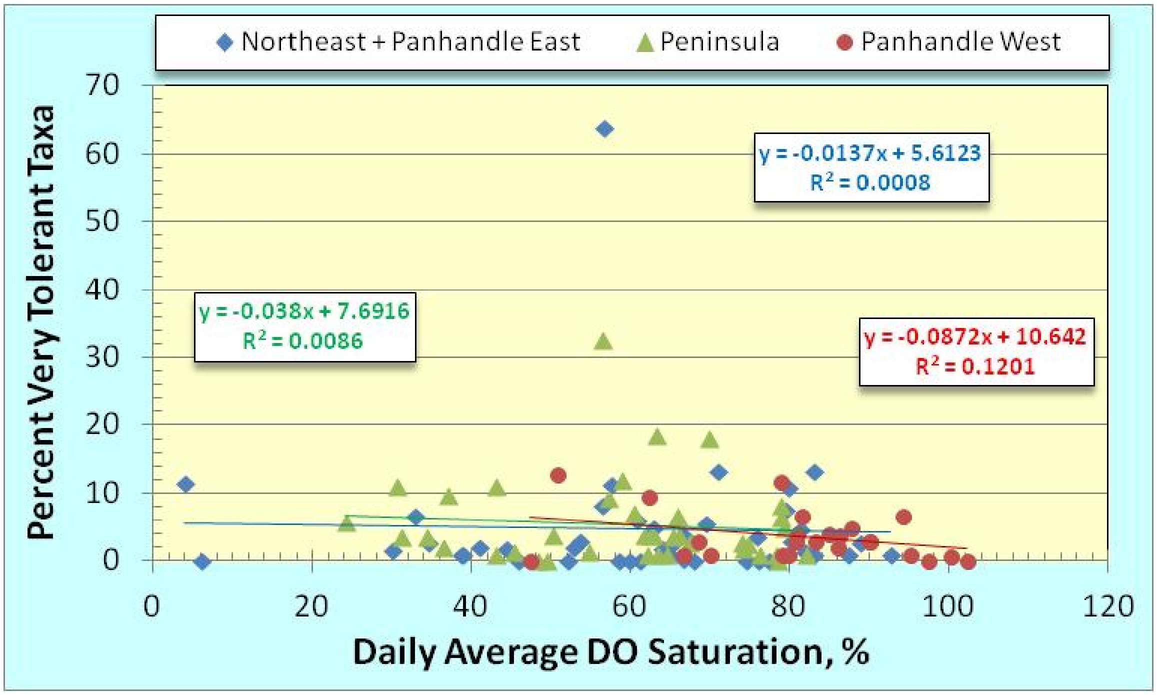

Figure 10. Regional relationships between the percent very tolerant SCI metric and daily average DO percent saturation for Northeast + Panhandle East, Peninsula, and Panhandle West bioregions. Based on data collected during 2005-2006 and 2010 DO studies screened to remove other potential influences on SCI scores (*i.e.*, LDI ≤ 2, conductivity ≤ 300, habitat assessment score ≥ 110, NOx ≤ 0.35 mg/L).

Appendix D:

Overview of the USEPA Virginian Province Approach to Developing DO Criteria

Overview of the EPA Virginian Province Approach to Developing DO Criteria

1. Introduction

The EPA Virginian Province document (EPA 2000) recommends an approach for deriving DO levels necessary to protect coastal and estuarine organisms in the Virginian Province (*i.e.,* between Cape Cod, Massachusetts and Cape Hatteras, North Carolina) based on laboratory dose-response data similar to the approach routinely used to set criteria for toxics (Stephan et al, 1985). The method is accepted by the United States Environmental Protection Agency (USEPA or EPA) Region 4 and has been used by several coastal States (including Florida) to develop DO criteria.

The EPA Virginian Province methodology represents a synthesis of current knowledge regarding biological responses to hypoxic stressors in aquatic ecosystems. This approach considers the response to both continuous and cyclic exposures to low DO levels to derive criteria that are protective of aquatic life. The aquatic life based approach utilized for the Virginian Province (EPA 2000) identifies three important DO concentration levels as follows:

- The Criterion Continuous Concentration (CCC), which is defined as a **mean daily** DO concentration above which continuous exposure is not expected to result in unacceptable chronic effects to sensitive biological communities.
- The Criterion Minimum Concentration (CMC), which is defined as a daily DO concentration below which any exposure for a 24-hour period would result in unacceptable acute effects (mortality) to sensitive organisms.
- The FRC, which is a function that defines the maximum allowable exposure duration at DO concentrations between the CMC and CCC necessary to prevent unacceptable reductions in seasonal larval recruitment for sensitive species. Since the effects of low DO depend on both the duration and intensity of exposure, the FRC allows shorter exposure durations as the DO level decreases.

Aquatic life and its uses are assumed to be fully supported as long as DO concentrations remain at or above the (CCC) chronic criterion for growth (EPA value = 4.8 mg/L). Conversely, if DO concentrations fall below the juvenile/adult survival criterion (CMC) (EPA value = 2.3 mg/L) low DO would be expected to result in an unacceptable rate of mortality to some sensitive species. When DO conditions are between these two values (2.3 to 4.8 mg/L), further evaluation of the duration and intensity of low DO is needed to determine whether the level of oxygen can support the most sensitive members of a healthy aquatic community (EPA 2000). This evaluation is conducted by comparing the monitored data in a given waterbody to the FRC developed for the species expected to be present.

A description of the derivation of the CCC, CMC, and FRC components of the criteria using USEPA's recommended Virginian Province method, for the Virginian Province, is provided below. A more detailed discussion of the Virginian Province method and the derivation of the various components of the criteria can be found in *Ambient Aquatic Life Water Quality Criteria for Dissolved Oxygen (Saltwater): Cape Code to Cape Hatteras* (referred to as the Virginian

Province) (USEPA 2000) and *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan *et al.* 1985).

2. Criterion Minimum Concentration Derivation (Survival)

Data regarding the acute sensitivity of juvenile and adult saltwater organisms to continuous low DO exposures, ranging from 24 to 96 hours, were used to derive the Criterion Minimum Concentration (CMC) in USEPA's Virginian Province method (USEPA, 2000). Acute response data were available for 12 invertebrate and 11 fish species. Many, but not all, of the 23 species used by EPA for the Virginian Province are also known to inhabit estuarine/coastal waters of Florida based on previous FDEP sampling and expert knowledge (Hendrickson *et al.* 2005; FMRI 2002; CSA, Inc. 1993; Frydenborg 2005). The species known to be indigenous in Florida generally span the range of acute DO sensitivities and include the most sensitive species (pipe fish, *Syngnathus fuscus*) used by EPA (**Table D1**).

USEPA calculated the criteria for exposure to continuous low DO by using a modified version of the procedure for the derivation of a final acute value (FAV) for toxicants presented in Stephen *et al.* (1985). The standard procedure was modified to account for the fact that organisms respond to DO in a manner opposite than responses to toxicants; that is, the greatest negative response to DO is to low levels rather than high levels. The FAV is calculated using the following series of equations modified from Stephen *et al.* (1985) (USEPA, 2000):

$$S^2 = \frac{\sum((\ln GMAV)^2) - ((\sum(\ln GMAV))^2 /4)}{\sum(P) - ((\sum \sqrt{P}))^2)/4)}$$

$$S = \sqrt{S^2} \qquad P = R/(n+1)$$

$$L = (\sum(\ln\ GMAV) - S(\sum(\sqrt{P})))/4$$

$$A = S(\sqrt{0.95}) + L$$

$$FAV = e^A$$

Where:
R = rank of sensitivity
GMAV = Genus mean acute value
FAV = Final Acute Value

The FAV for the Virginian Province was calculated to be 1.64 mg/L, which is the value representative of the LC_{50} for the 95th percentile genus (as ranked in order of sensitivity to low DO levels). The FAV was then adjusted to a CMC of 2.27 mg/L by multiplying by the average LC_5 to LC_{50} ratio (*i.e.,* 1.38) for juveniles so that the allowable loss is limited to five percent (not the 50 percent described by the LC_{50}). The CMC of 2.3 mg/L represents the DO concentration below which could potentially result in acute effects (*i.e.,* mortality) for a species at the 95th percentile based on the four most sensitive species represented in the data set. Therefore, DO concentrations below the 2.3 mg/L CMC should be avoided to prevent unacceptable acute effects to sensitive organisms.

3. Criterion Continuous Concentration Derivation (Growth)

To protect against chronic effects of exposure to low DO levels, the Virginian Province DO method also included an assessment of the effect of low DO levels on the growth of marine organisms. EPA (2000) noted that growth is generally more sensitive to low DO than survival, although the document does mention exceptions for *Menidia menidia* and *Dyspanopeus sayi*, where survival was the more sensitive endpoint in some tests.

EPA (2000) evaluated the effects of low DO on the growth of 11 species (4 fish and 7 invertebrates) from a total of 36 tests. The species used in the derivation of the chronic (CCC) and acute (CMC) portions of the criteria are not usually the same because (1) chronic and acute data are rarely available for the same species, and (2) even if results from both tests were available, it is not guaranteed that the genera would exhibit the same level of sensitivity in both chronic and acute tests.

Geometric mean chronic values (GMCV) for the 11 species ranged from 1.97 mg/L (sheepshead minnow, *Cyprinodon variegatus*) to 4.67 mg/L (longnose spider crab, *Libinia dubia*). By applying the equations provided above for the CMC, a CCC of 4.8 mg/L was determined to be protective of marine organism growth. The CCC of 4.8 mg/L represents the DO concentration above which would not result in chronic effects (*i.e.,* significant reduction in growth) for a species at the 95th percentile, based on the four most sensitive species represented in the data set. Therefore, long-term, continuous exposures at or above this level should not cause unacceptable chronic effects to marine organisms.

4. Larval Recruitment Curve

U.S. EPA (2000) developed a generic model to evaluate the cumulative effect of low DO between the acute value (CMC) of 2.3 mg/L and the CCC (4.8 mg/L) on early life stages of aquatic animals. The larval recruitment model generates a final curve that describes the number of days that larva (or other sensitive life stages) of sensitive organisms can be exposed to DO concentrations between the CMC and CCC without negatively affecting the total population. As described in the Virginian Province document, a maximum acceptable reduction in seasonal recruitment (due to low DO conditions) was defined as five percent (USEPA 2000).

The model developed by the USEPA uses laboratory dose-response data along with data that characterizes each genus, their developmental periods, and the duration that the sensitive life stage is available for exposure to low DO conditions. The additional information required for each genus includes:

- Length of spawning period,
- Larval development time,
- Natural attrition rate, and
- Percent population exposed to a hypoxic event (*e.g.,* vertical distribution)

Initially, for each genus for which the appropriate dose-response data are available, a line of best fit is generated using a standard mathematical expression for inhibited growth. The resulting equation describes the observed response of the different organisms in equivalent manner and provides the necessary coefficients for input into the Larval Recruitment Model. The equation used is:

$$P(t) = \frac{P_0 L}{P_0 + e^{-Lkt}\left(L - P_0\right)}$$

where: $P(t)$ = the DO concentration at time t
P_0 = the y-intercept
L = the upper DO limit
k = a rate constant, and
t = time in days, the number of days over which P(t) may be tolerated

The P_0, L, and k coefficients (which describe the response of the genus) derived from the response curve (line of best fit) for each genus along with the additional information listed above are input into the Larval Recruitment Model. The model then uses this information to generate a Larval Recruitment Curve for each genus.

The four most sensitive genera are then selected and used to generate a Final Recruitment Curve (FRC). The model generates the FRC based on the response of a species at the 95th percentile of sensitivity based on these four most sensitive species. The FRC is fitted using the standard equations for inhibited growth provided above.

In USEPA's application of the model to the Virginian Province, the four most sensitive genera used to generate the FRC were *Morone*, *Homarus*, *Dyspanopeus*, and *Eurypanopeus*. The larval recruitment curves for each of the four most sensitive species along with the FRC for the Virginian Province is provided in **Figure D1.**

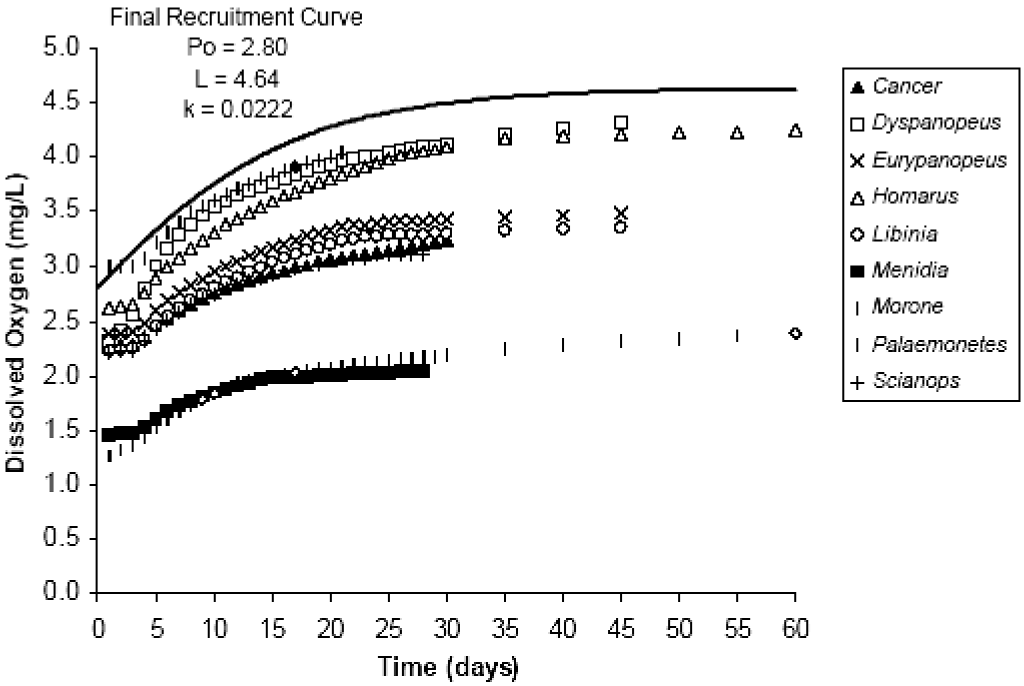

Figure D1. Plot of model outputs that protect against greater than a 5% cumulative impairment of larval recruitment. The solid line is regression of best fit for the FRC based on the 4 most sensitive species. Figure taken from EPA (2000).

Table D1. Acute sensitivity of juvenile and adult saltwater animals to low dissolved oxygen. Exposure durations ranged from 24 to 96 hr. (Recreated from EPA 2000).

Species	Common Name	Life Stage	SMAV LC50[a]	SMAV LC5	SMAV LC5/LC50	GMAV LC50	GMAV LC50[a]	GMAV LC5	GMAV LC5/LC50	GMAV Rank[b]
Carcinus maenus	Green Crab	Juvenile/Adult	<0.34			<0.34	0.34			1
Spisula solidissima	Atlantic Surf Clam	Juvenile	0.43	0.7	1.63	0.43	0.43	0.70	1.63	2
Rithropanopeus harrisii	Harris Mud Crab	Juvenile	0.51			0.51	0.51			3
Prionotus carolinus	Northern Sea Robin	Juvenile	0.55	0.8	1.45	0.55	0.55	0.80	1.45	4
Eurypanopeus depressus	Flat Mud Crab	Juvenile	0.57			0.57	0.57			5
Leiostomus xanthurus	Spot	Juvenile	0.7	0.81	1.16	0.7	0.7	0.81	1.16	6
Tautoga onitis	Tautog	Juvenile	0.82	1.15	1.40	0.82	0.82	1.15	1.40	7
Palaemonetes vulgaris	Marsh Grass Shrimp	Juvenile	1.02	1.4	1.37	0.86	0.86	1.24	1.44	8
Palaemonetes pugio	Daggerblade Grass Shrimp	Juvenile	0.72	1.1	1.53					
Ampelisca abdita	Amphipod	Juvenile	<0.9			<0.9	0.9			9
Scopthalmus aquosus	Windowpane Flounder	Juvenile	0.81	1.2	1.48	0.9	0.9	1.20	1.33	10
Apeltes quadracus	Fourspine Stickleback	Juvenile/Adult	0.91	1.2	1.32	0.91	0.91	1.20	1.32	11
Homarus americanus	American Lobster	Juvenile	0.91	1.6	1.76	0.91	0.91	1.60	1.76	12
Crangon septemspinosa	Sand Shrimp	Juvenile/Adult	0.97	1.6	1.65	0.97	0.97	1.60	1.65	13
Callinectes sapidus	Blue Crab	Adult	<1.0			<1.0	1			14
Brevoortia tyrannus	Atlantic Menhaden	Juvenile	1.12	1.72	1.54	1.12	1.12	1.72	1.54	15
Crassostrea virginica	Eastern Oyster	Juvenile	<1.15			<1.15	1.15			16
Stenotomus chrysops	Scup	Juvenile	1.25			1.25	1.25			17
Americamysis bahia	Mysid	Juvenile	1.27	1.5	1.18	1.27	1.27	1.50	1.18	18
Paralichthys dentatus	Summer Flounder	Juvenile	1.32	1.57	1.19	1.32	1.32	1.57	1.19	19
Pleuronectes americanus	Winter Flounder	Juvenile	1.38	1.65	1.20	1.38	1.38	1.65	1.20	20
Morone saxatilis	Striped Bass	Juvenile	1.58	1.95	1.23	1.58	1.58	1.95	1.23	21
Syngnathus fuscus	Pipe Fish	Juvenile	1.63	1.9	1.17	1.63	1.63	1.90	1.17	22

[a] SMAVs (Species Mean Acute Values) and GMAVs (Genus Mean Acute Values) are all geometric mean values (Stephen et al, 1985)

[b] Ranked according to LC50 GMAV values

Final Acute Value =	1.64 mg/L
Mean LC5/LC50 Ration =	1.38 mg/L
CMC = 1.64 mg/L x 1.38 =	2.27 mg/L

5. Final DO Criteria Based on EPA's Virginian Province Approach

The final marine DO criteria for the Virginian Province are summarized in **Figure D2**. Below the survival level (CMC=2.3 mg/L), DO would not fully support aquatic life uses unless it could be demonstrated that lower levels were a natural condition. At DO levels above the CCC growth level (4.8 mg/L) adverse effects are not expected. It is possible to establish fully protective DO criteria at levels between the survival and chronic protection levels by comparing the FRC and measured cumulative DO exposure durations.

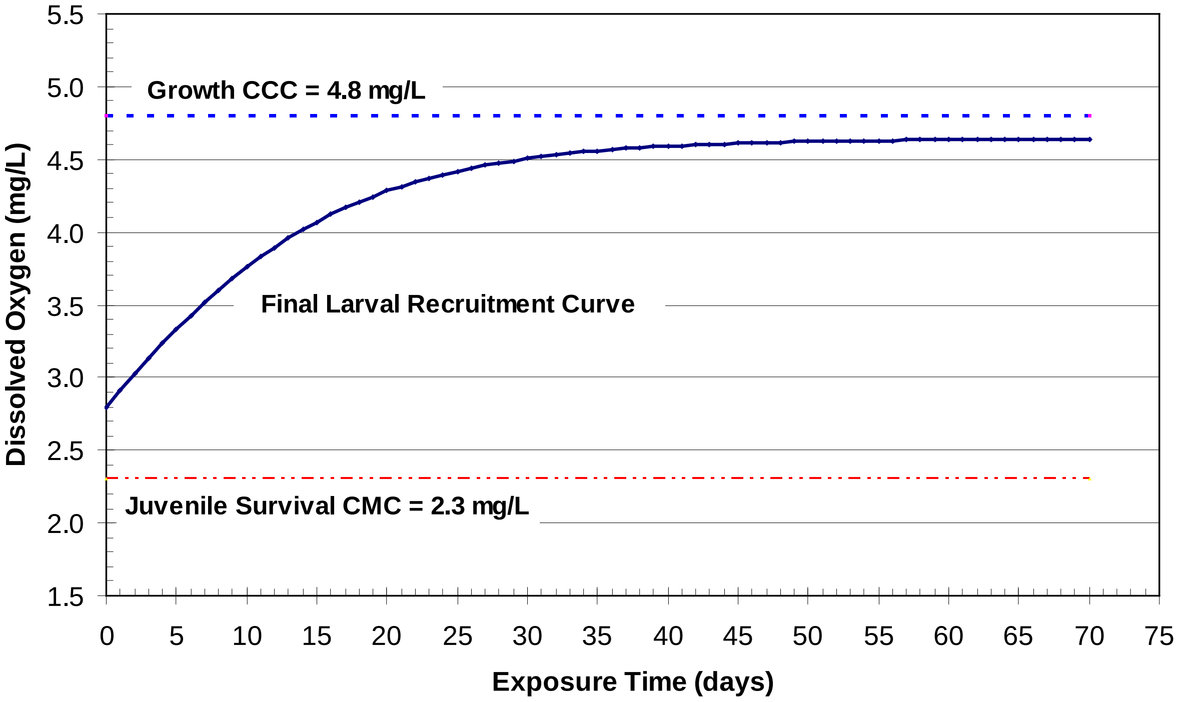

Figure D2. Plot of the final Virginian Province DO criteria for marine animals continuously exposed to low dissolved oxygen.

Appendix E:

Acute Toxicity Data Utilized in the Derivation of Proposed DO Criteria for Florida's Marine Waters

Species	Common name	Life stage	Duration	Salinity	Temp.	LC$_{50}$	LC$_{5}$	LC$_{5}$ / LC$_{50}$	Florida Species	Use	Exclusion Code	Reference
Acartia tonsa	copepod	10 to 13.5 hr old eggs	2.5		20	0.17			Yes	Yes		Lutz, et al., 1994 (as reported in USEPA, 2000)
Acartia tonsa	copepod	0 to 3.5 hr old eggs	2.5		20	0.21			Yes	Yes		Lutz, et al., 1994 (as reported in USEPA, 2000)
Acipenser brevirostrum	shortnose sturgeon	juvenile, 77 days	1	2	24.8	2.70			Yes	No	1	Campbell and Goodman, 2004
Acipenser brevirostrum	shortnose sturgeon	juvenile, 100 days	1	2	28.8	3.10			Yes	No	1	Campbell and Goodman, 2004
Acipenser brevirostrum	shortnose sturgeon	juvenile, 104 days	1	4	22.1	2.20			Yes	No	1	Campbell and Goodman, 2004
Acipenser brevirostrum	shortnose sturgeon	juvenile, 134 days	1	4.5	26.2	2.20			Yes	No	1	Campbell and Goodman, 2004
Acipenser brevirostrum	shortnose sturgeon	juvenile, 134 days	2	4.5	26.2	2.20			Yes	No	1	Campbell and Goodman, 2004
Acipenser brevirostrum	shortnose sturgeon	juvenile, 134 days	3	4.5	26.2	2.20			Yes	No	1	Campbell and Goodman, 2004
Acipenser brevirostrum	shortnose sturgeon	juvenile, 64 days	0.25	3	22.5	2.50			Yes	No	20	Jenkins, et al., 1993
Americamysis bahia	mysid shrimp	juvenile	4		26	1.20			Yes	No	2	Miller, et al., 2002
Americamysis bahia	mysid shrimp	juvenile, < 24	4	31.5	26	1.29	1.50	1.16	Yes	Yes		Poucher and Coiro, 1997
Americamysis bahia	mysid shrimp	juvenile, < 24	4	31.5	26	1.25			Yes	Yes		Poucher and Coiro, 1997
Americamysis bahia	mysid shrimp	3 day old	1	20	25	1.51			Yes	Yes		Goodman and Campbell, 2007
Americamysis bahia	mysid shrimp	10 day old	1	20	24	1.56			Yes	Yes		Goodman and Campbell, 2007
Ampelisca abdita	amphipod	juvenile	4	31.5	20.5	< 0.9			Yes	Yes		Poucher and Coiro, 1997
Anchoa mitchilli	bay anchovy	larvae	1	14	26	< 2.1			Yes	No	3	Breitburg, 1994
Anchoa mitchilli	bay anchovy	12 hr old eggs	0.5		26.5	2.80			Yes	Yes		Chesney and Houde, 1989 (as reported in USEPA, 2000)
Anchoa mitchilli	bay anchovy	12-24 hr yolk sac larvae	0.5	16.5	26.5	1.60			Yes	Yes		Chesney and Houde, 1989 (as reported in USEPA, 2000)
Apeltes quadracus	four-spined stickleback	juvenile/adult	4	31	19.4	0.91	1.20	1.32	No	Yes	14	Poucher and Coiro, 1997
Apeltes quadracus	four-spined stickleback	juvenile	4		19	0.90			No	No	2, 14	Miller, et al., 2002
Brevoortia tyrannus	Atlantic menhaden	juvenile	4		19	1.20			Yes	No	2	Miller, et al., 2002
Brevoortia tyrannus	Atlantic menhaden	juvenile	2	6.9	28	0.94			Yes	No	4	Burton, et al., 1980 (as reported in USEPA, 2000)
Brevoortia tyrannus	Atlantic menhaden	juvenile	3	6.9	28	0.96			Yes	No	4	Burton, et al., 1980 (as reported in USEPA, 2000)
Brevoortia tyrannus	Atlantic menhaden	33.8 mm long	0.25	31	20	1.90			Yes	No	5	Voyer and Hennekey, 1972
Brevoortia tyrannus	Atlantic menhaden	juvenile	4	30	19.5	1.21	1.90	1.57	Yes	Yes		Poucher and Coiro, 1997
Brevoortia tyrannus	Atlantic menhaden	juvenile (131.9 mm TL)	4	6.9	28	1.04	1.55	1.49	Yes	Yes		Burton, et al., 1980
Brevoortia tyrannus	Atlantic menhaden	juvenile	0.5	15	25	0.89			Yes	Yes		Shimps, et al., 2005

Species	Common name	Life stage	Duration	Salinity	Temp.	LC_{50}	LC_5	LC_5 / LC_{50}	Florida Species	Use	Exclusion Code	Reference
Brevoortia tyrannus	Atlantic menhaden	juvenile	0.5	15	30	1.04			Yes	Yes		Shimps, et al., 2005
Callinectes sapidus	blue crab	juvenile/adult	4	10	20	3.56			Yes	No	6	Stickle, 1988; Stickle, et al., 1989
Callinectes sapidus	blue crab	juvenile/adult	4	20	20	3.49			Yes	No	6	Stickle, 1988; Stickle, et al., 1989
Callinectes sapidus	blue crab	juvenile/adult	4	30	20	3.29			Yes	No	6	Stickle, 1988; Stickle, et al., 1989
Callinectes sapidus	blue crab	juvenile/adult	4	10	30	2.54			Yes	No	6	Stickle, 1988; Stickle, et al., 1989
Callinectes sapidus	blue crab	juvenile/adult	4	20	30	4.11			Yes	No	6	Stickle, 1988; Stickle, et al., 1989
Callinectes sapidus	blue crab	juvenile/adult	4	30	30	3.83			Yes	No	6	Stickle, 1988; Stickle, et al., 1989
Callinectes sapidus	blue crab	juvenile	2.56		23.4	> 0.5			Yes	No	7	Sagasti, et al., 2001
Callinectes sapidus	blue crab	adult	1	30		< 1			Yes	No	8	Carpenter and Cargo, 1957
Callinectes sapidus	blue crab	juvenile	1	32.5	24.5	> 1.4			Yes	Yes		Tankersley and Wieber, 2000
Callinectes sapidus	blue crab	megalopae	1	32.5	24.5	> 1.4			Yes	Yes		Tankersley and Wieber, 2000
Cancer irroratus	rock crab	larvae, stage 1-2	1	31	21	2.20			Yes	No		Poucher and Coiro, 1997
Cancer irroratus	rock crab	larvae, stage 3-4	1	30.5	20	2.14			Yes	No		Poucher and Coiro, 1997
Cancer irroratus	rock crab	larvae, stage 3-4	1	30	20.5	< 1.75			Yes	No		Poucher and Coiro, 1997
Cancer irroratus	rock crab	larvae, stage 3-5	1	31.5	20.5	< 1.72			Yes	No		Poucher and Coiro, 1997
Cancer irroratus	rock crab	megalopae-crab	1	31	20	1.85			Yes	No		Poucher and Coiro, 1997
Cancer irroratus	rock crab	stage 5, megalopae	1	30.5	20.5	< 1.89			Yes	No		Poucher and Coiro, 1997
Cancer irroratus	rock crab	larvae, stage 1-2	4	31	21	3.09			Yes	No		Poucher and Coiro, 1997
Cancer irroratus	rock crab	larvae, stage 3-4	4	30.5	20	2.80			Yes	No		Poucher and Coiro, 1997
Cancer irroratus	rock crab	larvae, stage 3-5	4	30	20.5	2.22			Yes	No		Poucher and Coiro, 1997
Cancer irroratus	rock crab	larvae, stage 3	4	31.5	20.5	2.17			Yes	No		Poucher and Coiro, 1997
Cancer irroratus	rock crab	megalops to 1st crab	4	31	20	2.20			Yes	No		Poucher and Coiro, 1997
Carcinus maenas	green crab	juvenile/young adult	4	30.5	20	< 0.54			No	Yes	14	Poucher and Coiro, 1997
Carcinus maenas	green crab	adult	2	15	10	< 0.21			No	Yes	11, 14	Theede, et al., 1969
Chasmodes bosquianus	striped blenny	newly hatched	1	20.5	21	2.50			Yes	Yes		Saksena and Joseph, 1972
Clupea harengus	Atlantic herring	yolk-sac larvae	0.5			2.80			No	Yes	14	DeSilva and Tytler, 1973
Crangon septemspinosa	sand shrimp	young adult	3	29.5	20.5	0.91			No	Yes	14	Poucher and Coiro, 1997
Crangon septemspinosa	sand shrimp	juvenile/young adult	4	31	19.9	0.97	1.60	1.65	No	Yes	14	Poucher and Coiro, 1997
Crangon septemspinosa	sand shrimp	juvenile	4		20	1.00			No	Yes	14	Miller, et al., 2002
Crassostrea virginica	eastern oyster	juvenile	4	20	30				Yes	No	9	Stickle, 1988
Crassostrea virginica	eastern oyster	juvenile	4	10	30				Yes	No	9	Stickle, 1988
Crassostrea virginica	eastern oyster	juvenile	4	30	20				Yes	No	9	Stickle, 1988
Crassostrea virginica	eastern oyster	juvenile	4	20	20				Yes	No	9	Stickle, 1988

Species	Common name	Life stage	Duration	Salinity	Temp.	LC_{50}	LC_5	LC_5 / LC_{50}	Florida Species	Use	Exclusion Code	Reference
Crassostrea virginica	eastern oyster	juvenile	4	10	20				Yes	No	9	Stickle, 1988
Crassostrea virginica	eastern oyster	juvenile	6.25	12	22	< 0.1			Yes	No	19	Widdows, et al., 1989
Crassostrea virginica	eastern oyster	larval	2	12	22	< 0.1			Yes	No	19	Widdows, et al., 1989
Crassostrea virginica	eastern oyster	juvenile	6	21	25	< 1.5			Yes	Yes		Baker and Mann, 1992
Crassostrea virginica	eastern oyster	larvae	4	21	25	1.50			Yes	Yes		Baker and Mann, 1992 (as reported in USEPA, 2000)
Crassostrea virginica	eastern oyster	postlarvae	4	21	25	1.50			Yes	Yes		Baker and Mann, 1994 (as reported in USEPA, 2000)
Crassostrea virginica	eastern oyster	larvae	1	21	25	1.50			Yes	Yes		Baker and Mann, 1992 (as reported in USEPA, 2000)
Crassostrea virginica	eastern oyster	juvenile	4	30	30	0.88			Yes	Yes		Stickle, 1988
Cynoscion nebulosus	spotted seatrout	juvenile	1	28	28	1.89			Yes	Yes		Goodman and Campbell, 2007
Cynoscion nebulosus	spotted seatrout	juvenile	2	28	28	1.88			Yes	Yes		Goodman and Campbell, 2007
Cyprinodon variegatus	sheepshead minnow	juvenile, adult	1	30	30	< 2.6			Yes	No	18	Peterson, 1990
Cyprinodon variegatus	sheepshead minnow	24 hr old larvae	4	31.5	20.5	< 0.4			Yes	Yes		Poucher and Coiro, 1997
Cyprinodon variegatus	sheepshead minnow	24-48 hr old larvae	4	21	30.5	< 1.45			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	3 day old larvae	4	29.5	27.75	2.77			Yes	Yes		Coiro, 2000
Dyspanopeus sayi	Say mud crab	8 day old larvae	1	30	27.5	2.06			Yes	Yes		Coiro, 2000
Dyspanopeus sayi	Say mud crab	8 day old larvae	4	30	27.5	2.22			Yes	Yes		Coiro, 2000
Dyspanopeus sayi	Say mud crab	larval, stage 1-3	1	30.5	25.5	1.95			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 1	1	31	25	< 1.55			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 3	1	31	20.5	< 1.18			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 3	1	29	21	1.61			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 3	1	31.5	24.5	1.88			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 3	1	31	24.5	< 1.83			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 4	1	30.5	20	1.66			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 1	4	30.5	25.5	1.97			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 1	4	31	25	1.57			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 3	4	31	24.5	2.40			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 3	4	31.5	24.5	2.13			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 3	4	29	21	1.73			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 3	4	31	20.5	1.73			Yes	Yes		Poucher and Coiro, 1997
Dyspanopeus sayi	Say mud crab	larval, stage 4	4	30.5	20	2.50			Yes	Yes		Poucher and Coiro, 1997
Eurypanopeus depressus	flat mud crab	larval, stage 3	1	31	20.5	2.09			Yes	Yes		Poucher and Coiro, 1997
Eurypanopeus depressus	flat mud crab	larval, stage 2	4	29.5	20.5	2.20			Yes	Yes		Poucher and Coiro, 1997
Eurypanopeus depressus	flat mud crab	larval, stage 3	4	31	20.5	2.10			Yes	Yes		Poucher and Coiro, 1997
Eurypanopeus depressus	flat mud crab	juvenile	4	10	30	0.57			Yes	Yes		Stickle, 1988

Species	Common name	Life stage	Duration	Salinity	Temp.	LC$_{50}$	LC$_{5}$	LC$_{5}$ / LC$_{50}$	Florida Species	Use	Exclusion Code	Reference
Farfantepenaeus duorarum	pink shrimp	Adult	1	20	27.8	1.36			Yes	Yes		Goodman and Campbell, 2007
Farfantepenaeus duorarum	pink shrimp	Adult	2	20	27.8	1.46			Yes	Yes		Goodman and Campbell, 2007
Fundulus heteroclitus	mummichog	embryo	1	30	20	< 2.4			Yes	No	10	Voyer and Hennekey, 1972
Gobiesox strumosus	skilletfish	newly hatched	1	20.5	21	1.00			Yes	Yes		Saksena and Joseph, 1972
Gobiosoma bosc	naked goby	postflexion	1	14	26	1.50			Yes	No	7	Breitburg, 1994
Gobiosoma bosc	naked goby	preflexion	1	14	26	> 1.2			Yes	No	7	Breitburg, 1994
Gobiosoma bosc	naked goby	newly hatched	1	20.5	21	1.30			Yes	Yes		Saksena and Joseph, 1972
Harengula jaguana	scaled sardines	juvenile	1	30.5	27	2.12			Yes	Yes		Goodman and Campbell, 2007
Harengula jaguana	scaled sardines	juvenile	1	30	28	2.22			Yes	Yes		Goodman and Campbell, 2007
Homarus americanus	American lobster	larval, stage 1	1	30	20.5	< 2.32			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 2	1	30.5	20.5	2.14			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 2	1	30.5	19.5	3.31			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 3	1	30	19.5	2.27			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 3	1	30.5	20	2.47			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 3	1	30	23	2.36			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 3	1	29.5	21	1.92			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	postlarval, stage 4	1	30	19	1.38			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 1	1	31	18	2.44			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 1	1	30	18.5	2.66			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 2	1	31	18.5	2.46			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 1	4	30	20.5	3.19			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 1	4	30	18.5	3.21			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 2	4	31	18.5	2.82			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 1	4	31	18	2.83			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 2	4	30.5	19.5	3.43			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 2	4	30.5	20.5	3.08			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 3	4	31.5	18.5	2.13			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 3	4	29.5	21	2.36			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	larval, stage 3	4	30	19.5	2.27			No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	juvenile	2	20	15	0.90			No	No	14	McLeese, 1956 (as reported in USEPA, 2000)
Homarus americanus	American lobster	juvenile	2	25	15	1.00			No	No	14	McLeese, 1956 (as reported in USEPA, 2000)
Homarus americanus	American lobster	juvenile	2	30	15	0.80			No	No	14	McLeese, 1956 (as reported in USEPA, 2000)
Homarus americanus	American lobster	juvenile, stages 5–6	1	31	20	0.94	1.60	1.70	No	Yes	14	Poucher and Coiro, 1997
Homarus americanus	American lobster	juvenile	4		20	1.00			No	No	2, 14	Miller, et al., 2002
Labidocera aestiva	copepod	0 to 3.5 hr old eggs	3		25	0.39			Yes	Yes		Lutz, et al., 1994 (as reported in USEPA, 2000)

Species	Common name	Life stage	Duration	Salinity	Temp.	LC_{50}	LC_5	LC_5 / LC_{50}	Florida Species	Use	Exclusion Code	Reference
Labidocera aestiva	copepod	20 to 23.5 hr old eggs	3		25	0.32			Yes	Yes		Lutz, et al., 1994 (as reported in USEPA, 2000)
Lagodon rhomboides	pinfish	juvenile	1	31	25.8	1.76			Yes	Yes		Goodman and Campbell, 2007
Lagodon rhomboides	pinfish	juvenile	1	28	26.1	1.34			Yes	Yes		Goodman and Campbell, 2007
Lagodon rhomboides	pinfish	juvenile	1	30	26.5	1.77			Yes	Yes		Goodman and Campbell, 2007
Leiostomus xanthurus	spot	88 mm	1	6.9	28	0.67			Yes	Yes		Burton, et al., 1980 (as reported in USEPA, 2000)
Leiostomus xanthurus	spot	88 mm	2	6.9	28	0.67			Yes	Yes		Burton, et al., 1980 (as reported in USEPA, 2000)
Leiostomus xanthurus	spot	88 mm	3	6.9	28	0.68			Yes	Yes		Burton, et al., 1980 (as reported in USEPA, 2000)
Leiostomus xanthurus	spot	juvenile (87.6 mm TL)	4	6.9	28	0.70	0.81	1.16	Yes	Yes		Burton, et al., 1980 (as reported in USEPA, 2000)
Leiostomus xanthurus	spot	juvenile	0.5	15	30	1.10			Yes	Yes		Shimps, et al., 2005
Libinia dubia	longnose spider crab	megalop to 1st crab	3	31.5	24.5	2.34			Yes	No	12	Poucher and Coiro, 1997
Libinia dubia	longnose spider crab	larval, stage 1	1	31.5	20.5	1.83			Yes	Yes		Poucher and Coiro, 1997
Libinia dubia	longnose spider crab	larval, megalop	1	31.5	24.5	1.97			Yes	Yes		Poucher and Coiro, 1997
Libinia dubia	longnose spider crab	larval, megalop	1	31	25	2.40			Yes	Yes		Poucher and Coiro, 1997
Libinia dubia	longnose spider crab	larval, stage 1	4	31.5	20.5	2.71			Yes	Yes		Poucher and Coiro, 1997
Libinia dubia	longnose spider crab	megalop to 1st crab	6	31	25	2.47			Yes	Yes		Poucher and Coiro, 1997
Libinia dubia	longnose spider crab	larval, stage 1	4	31	19.5	1.77			Yes	Yes		Poucher and Coiro, 1997
Libinia dubia	longnose spider crab	larval, stage 2 to megalop	4	31.5	20.5	1.81			Yes	Yes		Poucher and Coiro, 1997
Litopenaeus setiferus	northern white shrimp	postlarval	2	38	30	2.10			Yes	No	13	Martinez, et al., 1998
Litopenaeus setiferus	northern white shrimp	postlarval	2	15	30	2.40			Yes	No	13	Martinez, et al., 1998
Litopenaeus setiferus	northern white shrimp	juvenile	3	38	30	2.47			Yes	No	13	Martinez, et al., 1998
Litopenaeus setiferus	northern white shrimp	juvenile	3	15	30	2.37			Yes	No	13	Martinez, et al., 1998
Litopenaeus setiferus	northern white shrimp	postlarval	2	38	30	2.18			Yes	Yes		Martinez, et al., 1998
Litopenaeus setiferus	northern white shrimp	postlarval	2	15	30	1.27			Yes	Yes		Martinez, et al., 1998
Litopenaeus setiferus	northern white shrimp	juvenile	3	15	30	1.16			Yes	Yes		Martinez, et al., 1998
Litopenaeus setiferus	northern white shrimp	juvenile	3	38	30	1.86			Yes	Yes		Martinez, et al., 1998
Loligo pealii	long fin squid	newly hatched	1	30	19.5	< 1			No	No	14	Poucher and Coiro, 1997
Menidia beryllina	inland silversides	30 day old juvenile	1	30	23.55	1.24			Yes	No	15	Coiro, 2000
Menidia beryllina	inland silversides	30 day old juvenile	3	30	23.55	1.34			Yes	No	15	Coiro, 2000
Menidia beryllina	inland silversides	29 day old juvenile	1	30	28	1.51			Yes	Yes		Coiro, 2000
Menidia beryllina	inland silversides	7 day old larvae	1	30.5	27.95	1.34			Yes	Yes		Coiro, 2000
Menidia beryllina	inland silversides	7 day old larvae	1	30.5	28.35	1.36			Yes	Yes		Coiro, 2000
Menidia beryllina	inland silversides	embryo hatch	1	30.5	24.5	< 1.59			Yes	Yes		Poucher and Coiro, 1997
Menidia beryllina	inland silversides	larval (12 day old)	1	30.5	25	1.43			Yes	Yes		Poucher and Coiro, 1997
Menidia beryllina	inland silversides	newly hatched	1	30	28.5	1.25			Yes	Yes		Poucher and Coiro, 1997
Menidia beryllina	inland silversides	newly hatched	1	31.5	20	1.10			Yes	Yes		Poucher and Coiro, 1997

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Menidia beryllina	inland silversides	larval (12 day old)	4	30.5	25	1.44			Yes	Yes		Poucher and Coiro, 1997
Menidia beryllina	inland silversides	7 day old larvae	4	30.5	27.95	1.49			Yes	Yes		Coiro, 2000
Menidia beryllina	inland silversides	7 day old larvae	4	30.5	28.35	1.57			Yes	Yes		Coiro, 2000
Menidia beryllina	inland silversides	29 day old juvenile	3	30	28	1.49			Yes	Yes		Coiro, 2000
Menidia beryllina	inland silversides	adult	1	32	28	1.94			Yes	Yes		Goodman and Campbell, 2007
Menidia menidia	Atlantic silverside	54.6 mm	0.25			2.10			Yes	No	5	Voyer and Hennekey, 1972
Menidia menidia	Atlantic silverside	embryo-larval	4	31	21.5	< 1.71			Yes	Yes		Poucher and Coiro, 1997
Mercenaria mercenaria	northern quahog	1-4 day old veliger	1		22	< 1			Yes	Yes		Huntington and Miller, 1989
Mercenaria mercenaria	northern quahog	embryo-larval	1	29	25	< 0.5			Yes	Yes		Morrison, 1971 (as reported in USEPA, 2000)
Morone saxatilis	striped bass	juvenile	4		20	1.60			Yes	No	2	Miller, et al., 2002
Morone saxatilis	striped bass	postlarval	4	5.5	20.5	1.96			Yes	Yes		Poucher and Coiro, 1997
Morone saxatilis	striped bass	juvenile	4	30.25	21.5	1.53	2.00	1.31	Yes	Yes		Poucher and Coiro, 1997
Morone saxatilis	striped bass	juvenile	4	32	19	1.63	1.90	1.17	Yes	Yes		Poucher and Coiro, 1997
Morone saxatilis	striped bass	postlarvae	1	5.5	20.5	1.96			Yes	Yes		Poucher and Coiro, 1997
Morone saxatilis	striped bass	postlarvae	1	5.5	19	3.15			Yes	Yes		Poucher and Coiro, 1997
Morone saxatilis	striped bass	postlarvae	1	4.5	18.5	2.22			Yes	Yes		Poucher and Coiro, 1997
Morone saxatilis	striped bass	postlarvae	4	4.5	19	2.18			Yes	Yes		Poucher and Coiro, 1997
Morone saxatilis	striped bass	larvae	4	4.5	18.5	2.34			Yes	Yes		Poucher and Coiro, 1997
Morone saxatilis	striped bass	larvae	4	5.5	19	3.46			Yes	Yes		Poucher and Coiro, 1997
Mugil cephalus	striped mullet	Larvae (2.42-2.88 mm)	1	30	23.5	4.80			Yes	No	16	Sylvester, et al., 1975
Mugil cephalus	striped mullet	juvenile	1	29	28	1.39			Yes	Yes		Goodman and Campbell, 2007
Mugil cephalus	striped mullet	juvenile	2	29	28	1.38			Yes	Yes		Goodman and Campbell, 2007
Octopus burryi	Burry痴 octopus	embryo-hatch	2	31	24.75	> 3.43			Yes	Yes		Poucher and Coiro, 1997
Octopus burryi	Burry痴 octopus	embryo-hatch	1	31	25	2.54			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes pugio	daggerblade grass shrimp	juvenile	4		20	0.70			Yes	No	2	Miller, et al., 2002
Palaemonetes pugio	daggerblade grass shrimp	juvenile	4	10	30	1.72			Yes	No	9	Stickle, 1988
Palaemonetes pugio	daggerblade grass shrimp	<24 hr old larvae	1	30.5	24.5	1.24			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes pugio	daggerblade grass shrimp	<24 hr old larvae	4	30.5	24.5	1.58			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes pugio	daggerblade grass shrimp	juvenile	4	30.5	20	0.72	1.10	1.53	Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	juvenile	4		24	1.00			Yes	No	2	Miller, et al., 2002
Palaemonetes vulgaris	marsh grass shrimp	postlarval	1	31.5	18.5	< 0.48			Yes	No	17	Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	postlarval	4	31.5	18.5	0.98			Yes	No	17	Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<24 hr old larvae	1	30	27.95	1.73			Yes	Yes		Coiro, 2000
Palaemonetes vulgaris	marsh grass shrimp	<24 hr old larvae	4	30	27.95	2.08			Yes	Yes		Coiro, 2000

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Palaemonetes vulgaris	marsh grass shrimp	larval, stage 1	1	30.5	30	< 1.4			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	larval, stage 1-2	1	32	25	1.89			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<16 hr old larvae	1	30.5	26	< 1.79			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<24 hr old larvae	1	30.5	24.5	1.50			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<16 hr old larvae	1	30.5	25	< 2.05			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<24 hr old larvae	1	32	25	< 1.56			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<24 hr old larvae	1	31	29.5	< 1.54			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<20 hr old larvae	1	30	20.5	1.66			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<24 hr old larvae	1	32	26	< 1.59			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	larval, stage 1-4	1	30.5	25	1.95			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	larval, stage 1	1	31.5	25	1.89			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	larval, stage 3	1	31	25	1.77			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	larval, stage 6	1	31	25.5	1.70			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<24 hr old larvae	4	30.5	24.5	2.18			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<16 hr old larvae	4	30.5	26	< 1.79			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<16 hr old larvae	4	30.5	25	2.16			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<20 hr old larvae	4	30	20.5	2.15			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	larval, stage 1	4	30.5	25	2.10			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<24 hr old larvae	4	31.5	25	2.05			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	<24 hr old larvae	4	31	29.5	1.96			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	larval, stage 3	4	31	25	1.87			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	larval, stage 6	4	31	25.5	1.72			Yes	Yes		Poucher and Coiro, 1997
Palaemonetes vulgaris	marsh grass shrimp	juvenile	4	31	24.5	1.02	1.40	1.37	Yes	Yes		Poucher and Coiro, 1997
Paralichthys dentatus	summer flounder	juvenile	3		24	1.60			Yes	No	2	Miller, et al., 2002
Paralichthys dentatus	summer flounder	juvenile	4		20	1.10			Yes	No	2	Miller, et al., 2002
Paralichthys dentatus	summer flounder	newly metamorphosed juvenile	3	29.5	24.5	1.59			Yes	Yes		Poucher and Coiro, 1997
Paralichthys dentatus	summer flounder	metamorphosed juveniles	4	31.5	20.5	1.10	1.30	1.18	Yes	Yes		Poucher and Coiro, 1997
Paralichthys dentatus	summer flounder	metamorphosed juveniles	1	29.5	24.5	1.59	1.90	1.19	Yes	Yes		Poucher and Coiro, 1997
Pleuronectes americanus	winter flounder	metamorphosed juveniles	4	31.5	20.5	1.46	1.70	1.16	No	No	14	Poucher and Coiro, 1997
Pleuronectes americanus	winter flounder	metamorphosed juveniles	4	29.5	19.5	1.30	1.60	1.23	No	No	14	Poucher and Coiro, 1997
Pleuronectes americanus	winter flounder	juvenile	4		20	1.40			No	No	2, 14	Miller, et al., 2002
Poecilia latipinna	Sailfin molly	juvenile, adult	1	30	30	< 2.6			Yes	No	18	Peterson, 1990
Prionotus carolinus	northern sea robin	juvenile	4		19	0.60			Yes	No	2	Miller, et al., 2002
Prionotus carolinus	northern sea robin	juvenile	4	31.5	19.5	0.55	0.80	1.45	Yes	Yes		Poucher and Coiro, 1997
Rithropanopeus harrisii	Harris mud crab	juvenile	4	10	30	0.51			Yes	Yes		Stickle, 1988
Sciaenops ocellatus	red drum	juvenile	1	20	28	1.45			Yes	Yes		Goodman and Campbell, 2007

Species	Common name	Life stage	Duration	Salinity	Temp.	LC_{50}	LC_5	LC_5 / LC_{50}	Florida Species	Use	Exclusion Code	Reference
Sciaenops ocellatus	red drum	larval	1	31	28.5	1.76			Yes	Yes		Poucher and Coiro, 1997
Scophthalmus aquosus	windowpane flounder	juvenile	1		20	0.90			Yes	No	2	Miller, et al., 2002
Scophthalmus aquosus	windowpane flounder	juvenile	2	30	19.5	0.81	1.20	1.48	Yes	Yes		Poucher and Coiro, 1997
Spisula solidissima	Atlantic surfclam	juvenile	4		23	0.50			Yes	No	2	Miller, et al., 2002
Spisula solidissima	Atlantic surfclam	juvenile	4	31	23	0.43	0.70	1.63	Yes	Yes		Poucher and Coiro, 1997
Stenotomus chrysops	scup	juvenile	1		20	1.30			Yes	No	2	Miller, et al., 2002
Stenotomus chrysops	scup	juvenile	1	30.5	20.5	1.29			Yes	Yes		Poucher and Coiro, 1997
Stenotomus chrysops	scup	juvenile	1	31.5	20.5	1.22			Yes	Yes		Poucher and Coiro, 1997
Syngnathus fuscus	pipe fish	juvenile	1		20	1.50			Yes	No	2	Miller, et al., 2002
Syngnathus fuscus	pipe fish	juvenile	1	31	19	1.63	1.90	1.17	Yes	Yes		Poucher and Coiro, 1997
Tautoga onitis	tautoga	juvenile	4	31.5	24.2	0.82	1.20	1.46	No	Yes	14	Poucher and Coiro, 1997
Tautoga onitis	tautoga	juvenile	4	31.5	24.5	0.82	1.10	1.34	No	Yes	14	Poucher and Coiro, 1997
Tautoga onitis	tautoga	juvenile	4		24	0.80			No	No	2, 14	Miller, et al., 2002
Trachinotus carolinus	pompano	juvenile	1	31.75	25.8	1.74			Yes	Yes		Goodman and Campbell, 2007

Exclusion Code	Reason for Exclusion
1	DO requirements for *Acipenser* spp. are considered separately.
2	Data reported by Miller *et al.* was taken from other sources. Original source used.
3	An LC50 was not reported.
4	24- to 72-hour LC50 was lower than 96-hour LC50.
5	Investigators indicated that test results may be influenced by experimental set-up.
6	Effects concentrations for this test are much higher than other tests for this species.
7	LC50 not reported.
8	Experimental water temperature was not reported.
9	Inconsistentcies in reported effects concentrations.
10	High DO sensitivities reported are apparently flawed due to methodological issues.
11	Experimental water temperature outside Florida range.
12	High mortality in the control treatment suggests flawed experimental conditions.
13	Test results were confounded by pH effects.
14	Habitat range does not include Florida.
15	Specimens from Rhode Island utilized in tests. Data from tests using Florida specimens were used instead.
16	Specimens of *Mugil cephalus* larvae used were not typical of those found in Florida estuaries.
17	Postlarval life stage is less sensitive than the larval life stage.
18	100 percent survival at DO levels of 2.6 and 1.6 mg/L over 24 hour period.
19	LT50s of juv and larval life stages assessed.
20	Findings identified as "preliminary" until more rigorous testing can be conducted.

Appendix F:

Chronic Effects of Low Dissolved Oxygen on Saltwater Animals Utilized in the Derivation of Proposed DO Criteria for Florida's Marine Waters

Taxon	Common Name	Life Stage	Duration	Water Temp (°C)	NOEC (mg O2/L)	HOEC (mg O2/L)	Chronic Value	Florida Species	Use	Exclusion Code	Reference
Acartia tonsa	copepod	adult	28	25	2.14*	2.14	2.14	Yes	Yes		Richmond, et al., 2006
Acipenser oxyrinchus	Atlantic sturgeon	juveniles	10	26	3.00*	3.00	3.00	Yes	No	1	Secor and Gunderson, 1998
Acipenser oxyrinchus	Atlantic sturgeon	Young of year		20	6.70	3.47	4.82	Yes	No	1	Niklitschek and Secor, 2009
Americamysis bahia	Mysid Shrimp	< 48 hr	28	25.5	4.17	3.17	3.64	Yes	Yes		Poucher, 1998
Americamysis bahia	Mysid Shrimp	< 48 hr	10	26.5	2.40	1.60	1.96	Yes	Yes		Poucher, 1998
Brevoortia tyrannus	Atlantic Menhaden	juvenile	14	27.5	4.00	2.00	2.83	Yes	Yes		McNatt and Rice, 2004
Callinectes sapidus	Blue Crab	adult	25	22	2.4*	2.40	2.40	Yes	No	2	DeFur, et al., 1990
Callinectes sapidus	Blue Crab	juveniles	28	24	5.60	5.60	5.60	Yes	No	2	Das and Stickle, 1993
Cancer irroratus	Rock Crab	larval stage 5 to megalopa	7	20	3.42	2.41	2.87	Yes	Yes		Poucher and Coiro, 1999
Crassostrea virginica	Eastern Oyster	juveniles	6	25	1.50*	1.50	1.50	Yes	Yes		Baker and Mann, 1992
Cynoscion regalis	Weakfish	juvenile	7	25	2.00	2.00**	2.00	Yes	Yes		Stierhoff, et al., 2009
Cyprinodon variegatus	Sheepshead Minnow	larval	14	21	2.50	1.50	1.94	Yes	Yes		USEPA, 2000
Cyprinodon variegatus	Sheepshead Minnow	larval	7	21	2.00*	2.00	2.00	Yes	Yes		USEPA, 2000
Dyspanopeus sayi	Say Mud Crab	<48 hr old	8	25	6.81	4.21	5.35	Yes	Yes		USEPA, 2000
Dyspanopeus sayi	Say Mud Crab	larval stage 1 to 3	7	25.5	3.31	2.45	2.85	Yes	Yes		USEPA, 2000
Dyspanopeus sayi	Say Mud Crab	larval stae 1 to 3	7	20	7.65	3.39	5.09	Yes	Yes		USEPA, 2000
Dyspanopeus sayi	Say Mud Crab	larval stae 1 to 3	7	20	4.46	3.51	3.96	Yes	Yes		USEPA, 2000
Dyspanopeus sayi	Say Mud Crab	larval stage 3 to 4	7	20	6.27	5.00	5.60	Yes	Yes		USEPA, 2000
Dyspanopeus sayi	Say Mud Crab	larval stage 3 to megalopa	4	25	5.44	4.40	4.89	Yes	Yes		USEPA, 2000
Dyspanopeus sayi	Say Mud Crab	larval stage 3 to megalopa	8	24.5	5.78	4.68	5.20	Yes	Yes		USEPA, 2000
Dyspanopeus sayi	Say Mud Crab	larval stage 3 to megalopa	10	25	5.47	4.40	4.91	Yes	Yes		USEPA, 2000
Dyspanopeus sayi	Say Mud Crab	larval stage 3 to megalopa	11	20	7.54	3.23	4.93	Yes	Yes		USEPA, 2000
Fundulus grandis	Gulf killifish	juvenile/adult	30	27	1.34*	1.34	1.34	Yes	Yes		Landry, et al., 2007
Homarus americanus	American Lobster	larval stage 2 to 3	4	18	5.40	3.90	4.59	No	No	3	USEPA, 2000
Homarus americanus	American Lobster	larval stage 2 to 3	4	20	5.00	3.70	4.30	No	No	3	USEPA, 2000
Homarus americanus	American Lobster	larval stage 3 to 4	4	19	7.70	5.45	6.48	No	No	3	USEPA, 2000
Homarus americanus	American Lobster	larval stage 3 to 4	4	20	4.90	3.80	4.32	No	No	3	USEPA, 2000
Homarus americanus	American Lobster	larval stage 3 to 4	6	19	5.25	4.22	4.71	No	No	3	USEPA, 2000
Homarus americanus	American Lobster	postlarval stage 4 to 5	20	19	7.51	3.45	5.09	No	No	3	USEPA, 2000
Homarus americanus	American Lobster	juvenile stage 5 to 6	27	17	3.50	1.53	2.31	No	No	3	USEPA, 2000
Homarus americanus	American Lobster	juvenile stage 5 to 6	29	18	7.61	3.54	5.19	No	No	3	USEPA, 2000
Jordanella floridae	Florida flagfish	embryo	10	26	5.38	2.00	3.28	Yes	Yes		Hale, et al., 2003
Leiostomus xanthurus	Spot	juvenile	14	27.5	2.00	1.50	1.73	Yes	Yes		McNatt and Rice, 2004
Libinia dubia	Longnose Spider Crab	larval stage 1 to 2	7	21	5.30	4.11	4.67	Yes	Yes		USEPA, 2000; Poucher and Coiro, 1999*

Taxon	Common Name	Life Stage	Duration	Water Temp (°C)	NOEC (mg O2/L)	HOEC (mg O2/L)	Chronic Value	Florida Species	Use	Exclusion Code	Reference
Litopenaeus setiferus	Northern White Shrimp	postlarvae	1	28	4.50	4.50	4.50	Yes	No	4	Rosas, et al., 1997
Litopenaeus setiferus	Northern White Shrimp	juvenile	50	28	4.00	3.00	3.46	Yes	Yes		Rosas, et al., 1998
Menidia menidia	Atlantic silverside	embryo to larva	28	21.5	3.90	2.80	3.30	Yes	Yes		USEPA, 2000
Mercenaria mercenaria	Northern quahog	embryo	14	25	4.20	2.40	3.17	Yes	Yes		Morrison, 1971*
Micropogonias undulatus	Atlantic croaker	adult	70	23.5	2.70*	2.70	2.70	Yes	Yes		Thomas and Rahman, 2009
Micropogonias undulatus	Atlantic croaker	adult	70	23.5	2.70*	2.70	2.70	Yes	Yes		Thomas, et al., 2007
Morone saxatilis	Striped Bass	juvenile	10	23	4.00*	4.00	4.00	Yes	Yes		Brandt, et al., 2009
Morone saxatilis	Striped Bass	juvenile	21	21	2.80	2.80**	2.80	Yes	Yes		USEPA, 2000
Palaemonetes pugio	Daggerblade Grass Shrimp	adult	28	27	1.50*	1.50	1.50	Yes	Yes		Brouwer, et al., 2007
Palaemonetes vulgaris	Marsh Grass Shrimp	newly hatched	8	25	6.71	3.42	4.79	Yes	Yes		USEPA, 2000
Palaemonetes vulgaris	Marsh Grass Shrimp	<16 hrs	7	25	5.40	3.77	4.51	Yes	Yes		USEPA, 2000; Poucher and Coiro, 1999*
Palaemonetes vulgaris	Marsh Grass Shrimp	<16 hrs	8	25.5	6.94	3.20	4.71	Yes	Yes		USEPA, 2000
Palaemonetes vulgaris	Marsh Grass Shrimp	larval stage 1 to 3	7	29.5	2.30	1.56	1.89	Yes	Yes		USEPA, 2000
Palaemonetes vulgaris	Marsh Grass Shrimp	postlarval	14	25	3.57	2.59	3.04	Yes	Yes		USEPA, 2000; Poucher and Coiro, 1999*
Palaemonetes vulgaris	Marsh Grass Shrimp	postlarval	14	24	3.42	2.17	2.72	Yes	Yes		USEPA, 2000; Poucher and Coiro, 1999*
Palaemonetes vulgaris	Marsh Grass Shrimp	postlarval	14	25.5	2.50	1.51	1.94	Yes	Yes		USEPA, 2000
Paralichthys dentatus	Summer Flounder	newly met. juvenile	14	20	4.53	3.53	4.00	Yes	Yes		USEPA, 2000
Paralichthys dentatus	Summer Flounder	newly met. juvenile	14	20	4.39	3.39	3.86	Yes	Yes		USEPA, 2000; Poucher and Coiro, 1999*
Paralichthys dentatus	Summer Flounder	newly met. juvenile	14	20	7.23	4.49	5.70	Yes	Yes		USEPA, 2000; Poucher and Coiro, 1999*
Paralichthys dentatus	Summer Flounder	newly met. juvenile	10	19.5	4.40	1.80	2.81	Yes	Yes		USEPA, 2000; Poucher and Coiro, 1999*
Paralichthys lethostigma	Southern Flounder	juvenile	14	29	6.00	4.00	4.90	Yes	Yes		Del Toro-Silva, et al., 2008
Paralichthys lethostigma	Southern Flounder	juvenile	21	25	4.74	2.79	3.64	Yes	Yes		Taylor and Miller, 2001
Pseudopleuronectes americanus	winter flounder	juvenile	70	20.5	2.20	2.20	2.20	No	No	3	Bedja, et al., 1992

* NOEC set to HOEC, ** HOEC set to NOEC.

Exclusion Code	Reason for Exclusion
1	DO requirements for *Acipenser* spp. are considered separately.
2	Results potentially affected by handling of organisms during tests.
3	Species not found in Florida.
4	Test conducted to determine alternative endpoint (*i.e.*, critical oxygen level, COL).

Appendix G:

Responses to Initial Comments Received During the August 11, 2011 Peer-Review Workshop

DO Peer Review Verbal Recommendations for Improving the Dissolved Oxygen (DO) Technical Support Document (TSD)

Meeting Date: August 11, 2011

Peer Review Committee Members:

Dr. Jim Heffernan- Florida International University
Dr. Kyeong Park- University of Alabama
Dr. Tom Frazer- University of Florida
Dr. Matt Cohen- University of Florida
Dr. Douglas McLaughlin- National Council Air and Stream Improvement
Dr. Robert Diaz- Virginia Institute of Marine Science
Dr. Rich Batiuk- Environmental Protection Agency
Dr. Michael Kaller- Louisiana State University

Comments for Chapter 1

Comment 1. *Doug McLaughlin and Matt Cohen suggested that we expand section 1.3 to include freshwater examples of naturally low DO, such as Turkey Creek. Noted that we may want to reference conclusions from Statewide DO study, which are provided in Chapter 2.*

Response 1: Section 1.3 was expanded to provide freshwater examples of naturally low DO systems and a reference to the findings from the Statewide DO study was added.

Comment 2. *Matt Cohen noted that the document doesn't really address springs and suggested we may want to add text.*

Response 2: The discussion of springs in Chapter 1 of the document was expanded with available data from selected springs being summarized and discussed.

Comment 3. *Rich Batiuk suggested that we examine Windsor (1985) and include pertinent information.*

Response 3. Reference to Windsor (1985) along with the pertinent information was added to the discussion of naturally low DO conditions exhibited by many estuarine waters in Florida.

Comments for Chapter 2 (Statewide Freshwater DO Study)

Comment 1. *Mike Kaller noted there are two "axes" for evaluating reference conditions (LDI ≤ 2, and SCI ≥ 40), and suggested we look at the case where LDI ≤ 2 and SCI < 40 and take into account both axes when evaluating DO versus SCI response, including Type I Errors associated with LDI≤2, but SCI <40 at a range of DO concentrations. Based on further* *discussion, it was suggested to average two temporally independent SCIs and calculate the associated average percent saturation to provide better fit and reduce Type I error of criteria.*

Response 1: The final regression analysis used to derive the proposed DO criteria utilized average SCI scores for each site as the response variable paired with the daily average percent DO saturation based on the three full days of measurements during each deployment. The averaging of the SCI scores is more consistent with the development and application of the SCI minimum acceptable threshold of 40 points, which is based on the average of two temporally independent samples. Additionally, averaging the SCI scores reduced variability in the data and improved the SCI versus DO relationship, but did not significantly change the final proposed criteria.

Comment 2. *Doug McLaughlin and Jim Heffernan suggested the Department add more detailed analysis, including amending Table 2 to include other parameters (chlorophyll a, TN and TP) and an expression of diel variation. Investigate regional differences in the DO regime.*

Response 2. Both Table 1 and 2 were revised to include DO range as well as other parameters as suggested.

Comment 3. *Jim Heffernan suggested we investigate the proximity to headwater wetlands or suitability of adjacent lands for human development as a variable to explain differences or natural systems subject to low DO.*

Response 3. The Department did attempt to visually evaluate the proximity to wetlands as a potential explanation for the observation that undisturbed waterbodies commonly had lower DO levels than those observed for sites with more anthropogenic inputs, however, the spatial resolution of the maps produced was not sufficient to draw any definitive conclusions. To further evaluate potential reasons for the observed phenomena, Table 1 was expanded to include additional parameters.

The additional data supported the previous conclusion that the higher DO levels observed for sites with more anthropogenic input resulted from greater photosynthetic production. Higher chlorophyll-a concentrations and larger average daily DO ranges were found at the "non-reference" sites compared to "reference" sites (Table 1). Additionally, reference sites consistently exhibited higher color and TOC (total organic carbon from natural leaf litter fall) levels that may inhibit photosynthetic oxygen production and result in greater oxygen demand for the reference sites compared to non-reference systems. The additional findings are discussed in greater detail in the text.

Comment 4. *Address the decision errors associated with uncertainty in the relationship between DO and aquatic life use support, try to quantify and minimize the errors to provide the best prediction possible.*

Response 4. The Department has concluded that minimally disturbed sites subject to a natural DO regime, in general, have a higher probability of Type I error (falsely concluding that the site was impaired) than Type II errors. Because the variability in the SCI (the primary measure of biological health) decreases as human disturbance increases, disturbed sites fundamentally are subject to much lower occurrence rate of a Type II error (falsely concluding that the site was unimpaired) when compared to undisturbed sites. From a theoretical standpoint, since the error of the SCI sampling method used to collect representative taxa can only fail to capture and count taxa, and since only 2 of the 10 metrics result in an improved SCI when specific organisms are missed, it is likely that Type I errors were of greater concern (occurred more frequently) during development of the SCI/DO relationship. Although the Department is unable to fully quantify the errors associated with the proposed DO criteria, the evidence suggests that the new criteria are not only protective, but potentially remain over-protective. The Department also concludes that the proposed DO criteria are supported by the best currently available science, and that they represent a considerable improvement over the existing DO criteria. All water quality criteria are routinely addressed during triennial reviews of water quality standards, and may be improved as new scientific information becomes available.

Comment 5. *Several members suggested we provide an explanation for the observation that DO tended to be higher in disturbed sites, such as nutrient enrichment/production or wetlands.*

Response 5. Based on the additional analyses and data summaries presented in Tables 1 and 2, the likely explanation for the observation that disturbed sites have higher DO is provided and discussed in Chapter 2 of the document. Also see response to comment 3.

Comment 6. *Jim Heffernan noted that we need to ensure that the assessment process is designed to avoid designating a system as impaired when it has naturally low DO.*

Response 6. The Department agrees, and as with any criteria and associated assessment process, the goal of the proposed DO criteria is to accurately distinguish systems that are truly impaired from those with naturally low DO. Greater detail concerning the planned application of the proposed criteria is now provided in the document.

Comment 7. *Mike Kaller noted that DO measured in the lower water column of lakes is naturally low. Emphasize measuring DO in top 2 meters of water column and describing the differential between top and bottom layer. If bottom layer hypoxia moves higher in water column (above 2 meters), especially if levels below 2 mg/L occur, this would have adverse effects on lake biota.*

Response 7. The Department agrees that the DO levels near the bottom of lakes is naturally low and that DO monitoring performed near the bottom is generally not a good indicator of the biological health of the system. A recommendation that ambient monitoring for criteria assessment purposes be collected in the upper portion of the water column (upper 2 meters or upper half of water column, depending on depth) is provided and discussed with appropriate references.

Recommendations for Chapter 4: Development of Revised Freshwater DO Criteria

Comment 1. *Mike Kaller suggested we add a provision to conduct a third SCI if the variability between the two most recent exceeds some agreed upon value (for example, the Minimum Detectable Difference, which is plus or minus 13 points for individual samples, so this would apply to two samples that were 26 or more points apart). Consider flows, rainfall, etc., to assure representative samples.*

Response 1. The SCI is currently a component of the Department's assessment process, with a passing SCI score being based on an average of at least two temporally independent samples. In the SCI primer and SOPs there is currently a requirement for additional data if the available data is highly variable as suggested above. The Department has also proposed a new requirement for the Impaired Waters Rule (Chapter 62-303, F.A.C.) that requires a third SCI if there are only two available and the difference between the two is greater than 20 points.

For the application of the SCI in the derivation of revised freshwater DO criteria, an additional screening step was added to identify sites at which the available SCI sores were more than 20 points apart. The sites identified were examined to determine if the conditions (water level, flow, rainfall, etc.) during the SCI sampling events were suitable for an accurate SCI determination. Any SCI data that were collected under inappropriate conditions as specified in the SCI Primer and SOPs were omitted from the analyses.

Comment 2. *There were several questions about how the SCI data were used in the overall assessment process, and several members asked for a description of DEP actions in response to failing the criterion so that context of criteria in regulatory proceedings can be assessed.*

Response 2. The SCI is a part of the Department's overall assessment in determining if a waterbody meets its designated use. If the average of two temporally independent SCIs is below 40, the Department conducts a stressor identification study to determine the causative pollutant (or other factors) responsible for the failures. Similarly, sites failing the DO criteria (current or proposed) undergo an additional assessment to determine the causative pollutant (*e.g.*, BOD) responsible for the low DO conditions. In either case, if a causative pollutant is identified, the Total Maximum Daily Load (TMDL) of the pollutant is established and the Department takes actions to reduce the pollutant and restore the waterbody.

Comment 3. *Several members agreed that stream invertebrates are generally more sensitive than stream fish or lake biota, but recommended that we provide additional justification. They suggested we review the literature for more recent freshwater bioassay data and eliminate the "personal communication" reference.*

Response 3. Additional justification for the conclusion that stream invertebrates are generally more sensitive than stream fish or lake biota was added to the document including literature citations. The personal communication reference was removed.

Comment 4. *Jim Heffernan suggested we change the wording to say the most sensitive invertebrates are more sensitive than fish.*

Response 4. Wording in the document was changed as suggested.

Comment 5. *Matt Cohen requested that we show the data distribution for sites <2 LDI.*

Response 5. Table 1 was expanded to provide more information on the DO distribution for sites <2 LDI as requested.

Comment 6. *Rich Batiuk suggested that we add text to the early part of the chapter to describe what analyses were conducted and better explain the logical sequence of events leading up to the DEP recommendation.*

Response 6. A summary was added to the beginning of the document and the document was reorganized and simplified to initially specify the proposed criteria and to explain the sequence of events and analyses leading up to the recommend criteria.

Comment 7. *Matt Cohen said that if diurnal range in DO is important, then DEP should develop a management strategy that involves diel DO Sonde deployment if a site fails both the SCI and DO criteria, and follow up with a stressor identification study.*

Response 7. Once the revised DO criteria are adopted, sites failing the criteria will undergo an additional assessment to determine the causative pollutant responsible for the low DO conditions. A stressor identification study is part of that assessment. Since the details concerning the stressor identification study and the factors that will be evaluated is currently under internal DEP development, more information concerning those studies is not available for inclusion in this document.

In many cases, the diurnal DO range was found to be better correlated to the SCI scores than the absolute DO concentration, with SCI scores generally decreasing with increasing DO range. However, it is unlikely that the biological organisms are responding directly to the range in DO, especially since the greater range results more from increased daily maximum concentrations than lower minimum concentrations. Instead, the DO range is probably serving as a surrogate for other pollutants/stressors (*e.g.*, physical or hydrologic changes, nutrients, etc.) or of conditions conducive to greater production. Therefore, an evaluation of other factors coincident with a high DO range would likely be useful in explaining why a site fails the SCI. The Department may also deploy DO Sondes where needed during stressor identification studies.

Comment 8. *Mike Kaller recommended that we describe the origin of the 3rd order polynomial function in Figure 32, and Bob Diaz suggested that we provide the formula for percent saturation.*

Response 8. The polynomial equations included in Figure 32 were included as a simplified expression of the complex empirical DO versus temperature relationship. The equations were derived by fitting a polynomial curve to the actual DO versus temperature relationships at 41.9 and 39.1 percent saturation, respectively. The curves in Figure 32 were only provided as a means of comparison of the proposed criteria to the current criteria and because the proposed criteria will be expressed as a percent saturation, the polynomial equations are not needed and were removed in the current version of the document.

Comment 9. *Rich Batiuk recommended that we add an appendix with more details. FDEP will rearrange the document to include recommended approaches in the body of the document and other analyses in the Appendix.*

Response 9. A summary was added to the beginning of the document and the document was reorganized with much of the information not directly related to the derivation of the recommended criteria being moved to appendices. These changes should more directly present the proposed criteria in an easier to follow manner.

Comment 10. *Matt Cohen suggested we provide more discussion on regional differences and consider regional criteria if region is found to be a significant variable.*

Response 10. Additional analysis revealed that there were statistically significant differences in the SCI versus DO relationship in some bioregions, therefore, the proposed freshwater DO criteria are now regionalized with different criteria for the Panhandle and the Peninsula + Northeast. Greater detail concerning the regional differences and the derivation of the regional criteria is provided in Section 4 of the document.

Comment 11. *Doug McLaughlin agreed that stressor-response approaches are best, but noted that regression alone is not enough. Doug McLaughlin and Rich Batiuk recommended a weight of evidence approach that could be used to determine role of DO, as well as other factors, that are responsible for biological response. Link criteria to biology.*

Response 11. The Department agrees that a weight of evidence approach is better than any single line of evidence. In the development of the proposed freshwater and marine DO criteria, the Department utilized observed field (freshwater) and laboratory (marine) biological responses to derive criteria that are protective of the designated use of Florida's waters. For freshwater, the proposed criteria are relatively consistent with the 10th percentile of the distribution observed at the reference sites, which suggests that the criteria are protective of the reference condition while providing an acceptable Type I error rate. While the proposed criteria are more accurate than the current criteria, it is recognized that some waterbodies will still require SSACs.

Comment 12. *Mike Kaller suggested that we eliminate confounding variables in analysis by assessing fixed vs. random effects, and setting confounding variables as random effects.*

Response 12. As indicated in the response to comment 13, the type of analyses suggested in the comment is currently being conducted by Dr. Curt Pollman (UF) as part of his development of a stressor identification procedure under contract to the Department. Initial efforts to utilize this type of analysis to develop DO criteria were ineffective, because the DO model was very sensitive to the levels of the confounding variables used in the model.

Comment 13. *Bob Diaz suggested we put all variables in multiple regression analysis and see what is or is not significant.*

Response 13. The type of analyses suggested in the comment is currently being conducted by Dr. Curt Pollman as part of his development of a stressor identification procedure under contract to the Department. His preliminary work has confirmed that a number of factors, in addition to DO, have a significant influence on the SCI score in streams. Biplot analyses conducted using environmental variables clearly indicate that DO dynamics are a major determinant of SCI status. The analyses also indicate that the key environmental drivers are specific conductance, trophic state (N status in particular), and temperature, with the most important habitat drivers including artificial channelization, riparian buffer width, riparian buffer vegetation quality, water velocity, and substrate diversity.

Additional results obtained from preliminary logistic regression and MLR modeling are consistent with the results from the ordination/biplot analysies. The dominant environmental drivers are specific conductance, trophic state (N status in particular), and temperature with the most important habitat drivers including riparian buffer width, water velocity, and substrate diversity.

During the derivation of the proposed freshwater criteria, the data were screened to minimize the influence of the factors found to have a strong influence on the SCI score. Dr. Pollman's work is ongoing and when finished, will be used as the basis of a stressor identification model that can be used to help more accurately identify the causative pollutant(s) in instances of exceedances of the DO criteria.

Comment 14. *Mike Kaller also suggested we evaluate non-linear fits.*

Response 14. Non-linear fits for the SCI versus DO relationships were examined as suggested in the comment. The additional analyses performed revealed that there was not a reasonable non-linear fit that was consistently better than the linear fit utilized in the original document. Therefore, a linear fit continued to be used in the derivation of the recommended criteria as described in Section 4 of the document.

Comment 15. *Several members suggested that we avoid individual SCI vs. DO measurements, and instead, do several analyses using average SCI versus deployment average DO, minimum (10%) DO, and daylight hour (8:00 AM- 3:00 PM) saturations.*

Response 15. The final regression analysis used to derive the proposed DO criteria paired average SCI scores for each site as the response variable paired with the daily average percent DO saturation based on the three full days of measurements during each deployment. The averaging of the SCI scores is more consistent with the development and application of the minimum acceptable SCI threshold of 40 points, which is based on the average of two samples. Additionally, averaging the SCI scores reduced variability in the data and improved the SCI versus DO relationship, but did not significantly change the final proposed criteria.

During the more in-depth analysis of the time of day issue it was found that work day was approximately centered around the mean and included both the daily minimum and maximum. Additionally, the full-day daily average and the work-day average DO concentrations (and saturations) were highly correlated ($r^2 = 0.98$) with a slope near one and an intercept of 0.03. Therefore, the full day statistics are representative of the workday, so the regression between the workday average did not yield a significantly different result or a better correlation than that obtained using the full day average presented in the document.

Preliminary analyses indicated that other measures of the daily DO regime (*e.g.*, minimum, 10th percentile, *etc.*) did not consistently provide significantly better relationships with the SCI scores. Because the potential criteria derived on these other DO measures presented more restrictions relative to their application, they were not pursued further.

Comment 16. *Doug McLaughlin suggested that we determine the time period during the day that best approximates the average DO vs. SCI relationship.*

Response 16. A more in-depth analysis and discussion of the time of day issue and a proposed method to address the expected diel fluctuations are provided in the revised document.

Comment 17. *Mike Kaller suggested that we should consider excluding data from sites if the two SCIs significantly exceeded the MDD (26 points) and recalculating the regressions.*

Response 17. See response to comment #1 above.

Comment 18. *Doug McLaughlin suggested we provide text on diurnal changes in DO and address whether time of day is important.*

Response 18. A more in-depth analysis and discussion of the time of day issue and a proposed method to address the expected diel fluctuations are provided in the revised document.

Comment 19. *Several members agreed that a percent saturation of 41.9% was fully protective of healthy, well balanced aquatic communities and recommended that FDEP incorporate this into the criteria.*

Response 19. The Department agrees that the statewide analysis suggested that 41.9% is fully protective of healthy, well balanced aquatic communities. However, the results of the most current analyses presented herein indicate that there are significant regional differences in DO levels necessary to fully protect the sensitive aquatic communities. The current analyses indicate that a higher DO level of 58.5 percent saturation is required in the Panhandle to support the expected greater number of sensitive organisms. In contrast, a slightly lower level of 33.2 percent saturation was found to be protective of the naturally less sensitive biological community expected in the northeast and peninsula bioregions. These findings are consistent with the regional calibration of the SCI which requires a greater number of sensitive organisms in the panhandle to achieve a passing score. Details of the current regional analyses can be found in the document.

Comment 20. *Matt Cohen reiterated that we should add text addressing the magnitude of the diurnal variation in DO.*

Response 20. In many cases, the diurnal DO range was found to be better correlated to the SCI scores than the absolute DO concentration, with SCI scores generally decreasing with increasing DO range. However, it is unlikely that the biological organisms are responding directly to the range in DO, especially since the greater range results more from increased daily maximum concentrations than lower minimum concentrations. Instead, the DO range is probably serving as a surrogate for other pollutants/stressors (*e.g.*, physical or hydrologic changes, nutrients, etc.) or of conditions conducive to greater production. Therefore, an evaluation of diurnal DO range would likely be more useful in differentiating truly impaired sites from sites with naturally low DO levels or to explain why a site fails the SCI.

Comment 21. *Rich Batiuk noted we should include the assessment method in our regulations (Daryll Joyner noted this is addressed in the Impaired Waters Rule, Chapter 62-303, Florida Administrative Code), and Matt Cohen noted that we might miss impairment if someone only sampled in the afternoon.*

Response 21. Greater detail concerning the planned application of the proposed DO criteria is included in this version of the document. However, these details will not be included in this rule. Instead, the assessment method for the DO criteria will be addressed in the Impaired Waters Rule, Chapter 62-303, Florida Administrative Code.

Comment 22. *Reviewers generally agreed that time of day needed to be further addressed in the document, and reiterated the recommendation to conduct regressions of average SCI versus all DO metrics, such as deployment minimum, average, range, and work hour DO, etc. Doug McLaughlin reiterated that we should try to identify the time of day that is representative of the average.*

Response 22. A more detailed discussion of the time of day issue is provided in the revised document.

Comment 23. *Mike Kaller suggested that, for lakes, the criteria should focus on the top 2 meters. He added that collection of vertical data in lakes is important, but critical that top 1 m meet criteria, and Jim agreed that the DO in bottom waters not appropriate for criteria purposes.*

Response 23. The Department agrees that the DO levels near the bottom of lakes is naturally low and that DO monitoring performed near the bottom is generally not a good indicator of the biological health of the system. A recommendation that ambient monitoring for criteria assessment purposes be collected in the upper portion of the water column (upper 2 meters or upper half of water column, depending on depth) is provided and discussed with appropriate references. If a site fails the proposed criteria, the vertical DO profile data will be examined, as well as determining the causative pollutant responsible for the exceedance.

Comment 24. *Matt Cohen noted that the position that the stream criteria are protective of lakes is not adequately supported in the document and suggested we elaborate. Jim Kaller suggested we determine how many lakes will be deemed impaired based on DO criteria derived to protect streams.*

Response 24. Additional information supporting the position that the stream criteria will be protective of lakes is provided and discussed in the document as suggested.

Comment 25. *Matt Cohen said that based on the Statewide DO study results, it seems compelling to consider different criteria for Panhandle.*

Response 25. Additional analysis revealed that there were statistically significant differences in the SCI versus DO relationship across the three bioregions, therefore, the proposed freshwater DO criteria are now regionalized with different criteria for the Panhandle and the Peninsula + Northeast. Greater detail concerning the regional differences and the derivation of the regional criteria is provided in Section 4 of the document.

Recommendations for Chapter 5 (Development of Revised Marine DO Criteria)

Comment 1. *Rich Batiuk suggested that we review EPA Gulf Breeze's (Jim Hagy) related work, which used data from 43 species to develop CMC and CCC values. FDEP needs to get the data first.*

Response 1. Data and information from additional sources, including that from EPA Gulf Breeze, were obtained and the additional data were incorporated into the calculations presented in the revised document.

Comment 2. *Doug McLaughlin suggested we add the number of tests and replicates associated with data for an individual organism.*

Response 2. The requested information was summarized and provided in the appendices added to the document.

Comment 3. *Rich Batiuk noted the EPA document provided some of these details and suggested that they be added to the document.*

Response 3. See response to comment #2.

Comment 4. *Bob Diaz and Rich Batiuk recommended that we consider the Endangered Species Act consultation for sturgeon, which can occur in tidal, fresh, and marine waters, and involve the Fish and Wildlife staff early in the process.*

Response 4. The Department agrees that Fish and Wildlife staff need to be involved early in the DO criteria development/adoption process to assure that the proposed DO criteria will be protective of threatened and endangered species, including the sturgeon as well as other fresh and marine water species. The Department has discussed this issue with USEPA Region 4 and they have initiated informal coordination with the U.S. Fish and Wildlife Service. Additionally, this draft of the technical support document has been revised to provide a more in depth discussion of the threatened and endangered species that occur in Florida and their DO requirements, as well as steps that will be taken to assure that these species are fully protected. This version of the support document has been provided to Fish and Wildlife for their review.

Comment 5. *There was general support for the Virginian Province method, and Florida's application of it, although Doug McLaughlin encouraged DEP to build marine assessment tools, such as the Chesapeake Bay IBI.*

Response 5. The Department agrees that the Virginian Province approach provides a defensible method (that has been used by other states and in Florida) to derive revised DO criteria that are protective of sensitive species found in Florida's marine waters. Although a marine assessment tool would be very useful in assessing Florida's waters, there is insufficient time and data available to develop such a tool during this rule revision. The comment will be addressed during future criteria development efforts and triennial reviews of water quality standards.

Comment 6. *Bob Diaz asked if we planned to cover the full range of estuarine and coastal areas, and Rich Batiuk asked if we considered breaking out the criteria by salinity. There seemed to be a general suggestion that FDEP should discuss application of the marine DO criteria in estuary versus open coastal systems.*

Response 6. The Department currently plans for the proposed marine DO criteria to apply to all estuarine and coastal areas. While the Department agrees that ideally the criteria would be tailored to specific habitats (taking into account salinity and species expected for different area), the sufficient information is unavailable at this time to allow the development more habitat specific criteria.

Comment 7. *Several members suggested that, as was suggested for freshwaters, we should compare grab sample DO data to diel data in various marine locations.*

Response 7. A more detailed analysis of the diel DO fluctuations observed in marine waters and the sampling time of day issue as it relates to the application of the proposed criteria was conducted with the results being provided and discussed in Chapter 5 of the document.

Comment 8. *Rich Batiuk stated that using 7-day and 30-day averages from the larval recruitment curve was defensible and represented a workable approach.*

Response 8. The Department agrees and has used the approach to develop the DO saturation criteria as presented in Chapter 5 of the document.

Comment 9. *Rich Batiuk noted that EPA used a temperature dependent threshold for protection of the short nosed sturgeon, and noted that we need to address how criteria are protective (an audience member noted that small-toothed sawfish may also be a T&E Species to address).*

Response 9. The Department is aware of the temperature dependent DO requirements for the sturgeon. In this draft of the technical support document, the Department has provided a more in depth discussion of the threatened and endangered species that occur in Florida and their DO requirements as well as steps that will be taken to assure that these species are fully protected.

Comment 10. *Given that shallow, wind driven estuaries with sediment re-suspension often have natural low DO, Kyeong Park noted that the mean DO may not be representative and suggested that DEP describe how unusual events get factored in DO criteria exceedances.*

Response 10. As specified in document, the Department intends to apply the criteria according to the State's Impaired Water Rule, which uses a binomial hypothesis test allowing a 10 percent exceedance frequency. The 10 percent exceedance frequency should account for most unusual events. Additionally, if a waterbody exceeds the criteria, an additional evaluation will be conducted to determine if the exceedance is due to natural causes and to determine the causative pollutant responsible for the low DO condition. If a waterbody consistently fails to meet the criteria due to natural causes, site specific alternative criteria (SSAC) can be developed.

Comment 11. *Bob Diaz stated that while persistent, seasonal hypoxia is a serious issue, normal low nighttime DOs in grassbeds should not be considered exceedances by the DEP criteria. Matt Cohen suggested that additional monitoring be required if measured values exceed (are less than) certain thresholds.*

Response 11. The Department agrees that normal low nighttime DOs in grassbeds should not result in a waterbody being considered as being impaired. Since these areas are typically highly diverse biologically, they normally support high DO levels during the daylight hours. Basing the derivation and application of the criteria on daily average DO levels should minimize short-term naturally low DO levels being identified as being impaired.

Additionally, if a waterbody fails to meet the criteria, an additional assessment will be conducted to determine if the exceedance is due to natural causes and to determine the causative pollutant responsible for the low DO condition. If a waterbody consistently fails to meet the criteria due to natural causes, site specific alternative criteria (SSAC) can be developed.

Comment 12. *Rich Batiuk suggested that DEP describe the implementation of the DO criteria, including frequency, magnitude, duration, data sufficiency, and follow up actions (e.g., causative pollutant identification), including adaptive management. Jim noted that the criteria should be conservative given that measurements will mainly be grab samples.*

Response 12. The discussion of the planned application of the proposed criteria in the document has been revised to provide greater detail. Much of the detailed information concerning the application of the criteria will be provided in the Impaired Waters Rule (62-303, F.A.C.). The goal of the effort to revise the State's DO criteria was to develop criteria that more accurately differentiate truly impaired waterbodies from those with naturally low DO levels. The Department believes the methods used to develop the proposed DO criteria result in criteria that accomplish this goal and are fully protective of the designated uses of Florida waters, especially when combined with the planned implementation method.

Comment 13. *Suggested that DEP investigate temperature dependent or saturation based criteria for marine waters.*

Response 13. As suggested, the Department has recalculated the proposed marine criteria based on DO saturation to be consistent with the proposed freshwater criteria. The saturation based criteria would essentially correct the concentration-based criteria for the effects of temperature and salinity and are more representative of the expected condition than the strict concentration-based criteria. Both the concentration- and saturation-based criteria are provided and discussed in the document.

Comment 14. *Bob Diaz stated that diel hypoxia is a natural phenomenon. Aquatic life has developed strategies for tolerating and surviving diel hypoxia. Protracted (and unnatural) season hypoxia cause ecological impacted and is of regulatory concern.*

Response 14. The department agrees with the comment. Because the criteria are expressed as a daily average, diel hypoxia should not be identified as exceedances of the criteria. Additionally, it is expected that the criteria would be applied in accordance with the State's Impaired Waters Rule 62-303, F.A.C., which allows a 10% exceedance frequency to minimize the influence of abnormal events.

Comment 15. *Bob Diaz noted that the DEP approach is an order of magnitude improvement over the current criteria, but that they could be even better with more data. Matt Cohen suggested that the document include issues to be addressed for with additional study to continually improve criteria.*

Response 15. The Department agrees that the existing DO criteria are clearly inaccurate and are in need of revision. Additionally, the proposed DO criteria, which are based on biological responses observed in the field (freshwater) and laboratory (marine), are expected to much more accurately differentiate waterbodies that are truly impaired from those with naturally low DO conditions that are capable of fully supporting healthy biological communities. The Department will consider additional revisions to the criteria as new information is developed.

Comment 16. *Matt Cohen noted that determination of natural background will be helpful to address springs, and Rich Batiuk stated that DEP needs to develop a clear process to identify and characterize natural background. There is a need for developing a process for acknowledging natural conditions without getting into an overly burdensome process.*

Response 16. The Department agrees with the comment and is developing a process by which the natural background DO conditions for a waterbody can be characterized.

Comment 17. *Matt Cohen recommended that we use biological information to determine the allowable deviation for a given site.*

Response 17. The Department agrees with the comment and as specified in the response to comment 16 above, is working to develop and document a process by which the natural background DO conditions for a waterbody can be characterized. Additionally, to utilize the deviation from background concept to develop more site specific DO criteria, the USEPA requires a demonstration that the natural biological populations at the site will not be adversely affected. The Department is evaluating methods that could potentially be used to make such a demonstration and will include a discussion of appropriate methods in the process document.

Appendix H:

Determination of Acceptable Deviation from Natural Background Dissolved Oxygen Levels in Marine Waters

Determination of Acceptable Deviation from Natural Background Dissolved Oxygen Levels in Fresh and Marine Waters

March 2013

Prepared by:
Florida Department of Environmental Protection
Division of Environmental Assessment and Restoration

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Determination of Acceptable Deviation from Natural Background Dissolved Oxygen Levels in Fresh and Marine Waters

1 Introduction

This document describes a process for determining natural background dissolved oxygen (DO) levels for both fresh and marine waters and a detailed process for determining the allowable deviation from natural background for marine waters that will not cause adverse impacts on resident aquatic species. The Department plans to incorporate these processes into the proposed revisions to the DO criteria for both fresh and marine waters. While these revisions are designed to better address naturally low DO levels while ensuring protection of aquatic life, there are a number of natural conditions in both fresh and marine waters that can result in DO levels below the proposed DO criteria.

The USEPA guidance on fresh and marine DO criteria recognized that under some circumstances, natural conditions can result in DO concentrations below the generally applicable criteria:

> "*Where natural conditions alone create dissolved oxygen concentrations less than 110% of the applicable criteria means or minima, or both, the minimum acceptable concentration is 90% of the natural concentration* (EPA 1986, Ambient Water Quality for DO)"

and

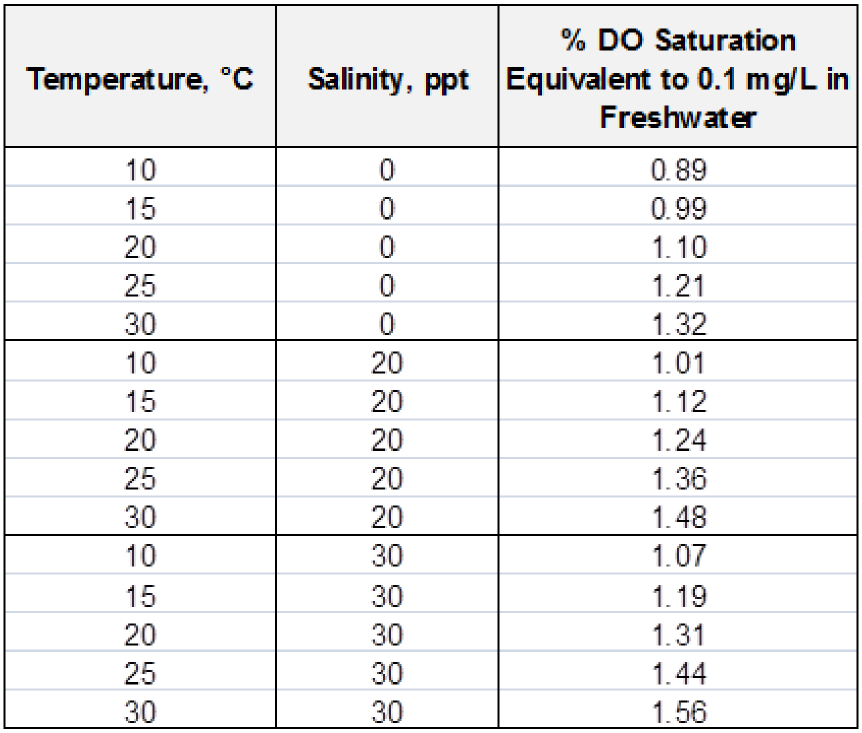
> *"If it is determined that the natural condition in the waterbody is less than the values stated above, then the criteria will revert to the natural condition and the water quality standard will allow for a 0.1 mg/L deficit from the natural dissolved oxygen value. Up to 10 percent deficit will be allowed if it is demonstrated that resident aquatic species shall not be adversely affected" (USEPA 1980)."*

To account for these situations, the proposed rule revisions will include a clause that allows the DO levels to be below the numeric criteria due to natural background conditions, and a clause that allows small deviations from natural background conditions as long as the deviations do not cause adverse impacts on resident aquatic species. Inclusion of such language in Florida standards would reduce the number of waterbodies that are incorrectly placed on the 303(d) list of impaired waters and provide more accurate, implementable Total Maximum Daily Load (TMDL) targets as well as reducing the need for Site Specific Alternative Criteria (SSACs).

To be consistent with the expression of the proposed DO criteria as a percent saturation, the allowed 0.1 mg/L deviation from natural background conditions can be converted to a percent saturation. Since the relationship between DO concentration and DO % saturation is dependent on temperature and salinity, these factors must be considered in making the conversion. As an example, 0.1 mg/L was converted to percent saturation under a range of temperatures expected in Florida's freshwater and saltwater. The results of the conversion are summarized in **Table 1**.

In freshwater (*i.e.*, 0 ppt salinity) the percent saturation corresponding to 0.1 mg/L ranges from 0.9% at 10°C to 1.3% at 30°C. Similarly, in marine water with a salinity of 20 ppt, a DO concentration of 0.1 mg/L corresponds to a DO percent saturation of 1.0% at 10°C to 1.5% at 30°C. Therefore, rounding the percent saturation results to the nearest percent indicates that the allowed 0.1 mg/L deviation from natural background conditions equated to an allowable deviation of 1 percent saturation under most conditions expected in Florida waters. In waters with salinities above 20 ppt with accompanying high temperatures, the conversion can result in percent saturations slightly greater than 1.5% saturation. In these circumstances, the use of the allowed deviation expressed as 1% saturation deviation would be slightly less than allowed by a 0.1 mg/L deviation. Based on these results, the allowed deviation from natural background DO conditions could be expressed as 1% saturation for the purpose of the proposed DO criteria.

Table 1. Results of the conversion of a DO concentration of 0.1 mg/L to percent saturation under various temperature and salinity conditions.



Temperature, °C	Salinity, ppt	% DO Saturation Equivalent to 0.1 mg/L in Freshwater
10	0	0.89
15	0	0.99
20	0	1.10
25	0	1.21
30	0	1.32
10	20	1.01
15	20	1.12
20	20	1.24
25	20	1.36
30	20	1.48
10	30	1.07
15	30	1.19
20	30	1.31
25	30	1.44
30	30	1.56

2 DEP Process for Determining Natural Background DO

All aquatic ecosystems are inherently influenced by natural factors, and many water quality parameters (*e.g*., pH, DO, turbidity, and transparency, etc.) exhibit a degree of spatial and temporal variability within the natural systems present in Florida. Since these same parameters may also be affected by human activities, a comparison to “natural background” conditions may be required to conclude that human activities have caused exceedances of the water quality criteria. The purpose of this chapter is to provide a procedure for establishing “natural background” DO conditions for Florida’s surface waters. Since DO concentrations naturally exhibit spatial and temporal variability, it is important to determine whether failure to achieve the DO criterion is due to anthropogenic pollutant loadings or is simply due to natural background conditions.

2.1 *EPA Policy on Natural Background*

On November 5, 1997, the EPA Office of Water provided the following EPA policy statement.

> “States and Tribes may establish site specific numeric aquatic life water quality criteria by setting the criteria value equal to *natural* background. Natural background is defined as background concentration due *only* to non-anthropogenic sources, *i.e.*, non-manmade sources. In setting criteria equal to natural background the State or Tribe should, at a minimum, include in their water quality standards:
>
> (1) a definition of natural background consistent with the above;
>
> (2) a provision that site specific criteria may be set equal to natural background; and
>
> (3) a procedure for determining natural background, or alternatively, a reference in their water quality standards to another document describing the binding procedure that will be used.”

2.2 *Magnitude, Duration, and Frequency*

Deriving background DO conditions should consider magnitude, duration, and frequency. The magnitude is related to the concentration of a parameter, the duration is related to the averaging period for measuring that concentration, and the frequency is related to the expected acceptable occurrence of deviations from the magnitude and duration. Expressing the background condition as a magnitude, duration, and frequency characterizes the central tendency of the data while acknowledging variability. This type of expression accounts for natural fluctuations in the waterbody condition and anomalous events, such as droughts and hurricanes.

2.3 *Determining Natural Background DO*

The three methods available to describe or estimate natural background conditions include the:

- Reference Condition Approach;
- Pre- vs. Post-Disturbance Approach; and
- Modeling Approach (including use of empirical and/or deterministic models).

2.3.1 Reference Condition Approach

Use of a reference condition is an excellent method for determining a natural background DO regime. Sites used to describe the reference condition should be demonstrated to be minimally disturbed by human activities, using the objective criteria below. Use of data from rigorously vetted reference sites is a common and useful method for determining natural background conditions, including for DO.

When comparing data from a potentially impaired test site to a population of data representative of the reference condition, the reference sites should be minimally disturbed by human activities and functionally similar to the test site (*e.g.*, a blackwater test stream should be compared with blackwater reference stream). For use in a natural conditions determination, the reference sites should also have a similar natural disturbance regime (*e.g.*, droughts, floods, hot temperatures) to that expected in the assessment watershed.

When documenting the appropriateness of a reference site to make a natural background condition determination at an assessment location, the following factors should be taken into account:

- Demonstrate that the proposed reference locations and the assessment locations are functionally comparable, including considerations such as geographic proximity, climate, watershed size, timing and quantity of flow, and other factors relevant to the parameter of concern;
- Demonstrate that the proposed reference watershed has been minimally affected by human activities, including point source dischargers and nonpoint source inputs.

EPA determined that DEP's objective approach for determining suitable reference conditions was a scientifically defensible method for developing protective nutrient criteria, and ultimately used DEP's methodology to promulgate TP and TN criteria for Florida streams (U.S. EPA 2010b). DEP and EPA identified stream and lake reference sites by application of the following criteria:

1. Landscape Development Intensity Index (LDI) (Brown and Vivas 2007) score < 2 for land use within the 100 meter corridor 10 km upstream of the sample site;
2. Watershed or near-field LDI scores < 3;
3. No land uses or nutrient sources, as discerned by using aerial photographs, field observations, and DEP Best Professional Judgment, that would remove them from consideration as minimally disturbed sites for nutrients;
4. Not in a waterbody segment (WBID) listed for nutrient impairment on the EPA-approved Florida 303(d) list of impaired waters;
5. Not within WBIDs with average SCI scores <40 or LVI scores <43;
6. Not in a waterbody segment (WBID) listed for DO impairment on the EPA-approved Florida 303(d) list of impaired waters; and
7. Average nitrate/nitrite concentrations < 0.35 mg/L;

Although these criteria were developed specifically for stream and lake nutrient criteria development purposes, the criteria may be modified to identify reference-quality waterbody segments for other parameters. For example, if the first five of the above criteria are met in a stream or lake, one could conclude that the DO regime in the waterbody would be representative of natural background conditions and would fully support aquatic life uses. Note that numbers 6 and 7 above would not be appropriate for determining natural background DO for Floridan Aquifer springs because it has been established that DO in these systems is naturally less than the existing water quality criterion (due to the age of the water), and that nitrate levels do not influence the DO in springs. Similarly, because there are no calibrated multi-metric indices for Florida marine systems, only one through four of the above criteria would apply for identifying reference quality marine segments for DO. Tides and natural salinity stratification should also be considered when identifying marine reference zones.

2.3.2 Pre- vs. Post- Disturbance (Historical Conditions) Approach

If DO data from a minimally disturbed watershed are available prior to the onset of a disturbance under investigation, one may use a "before" versus "after" comparison approach. For this approach to be scientifically defensible, the minimally disturbed (reference) nature of the historic conditions should be documented, the variability of the DO must be adequately characterized, and site conditions such as seasonality, hydrologic and weather conditions, or other influential factors should be described and linked to the natural background DO levels. Sampling conditions before and after the anthropogenic disturbance should be as similar as possible, so the primary difference at the site(s) between sampling events is the target disturbance. The central tendency (mean or median, as appropriate) and lower distribution of the data (*e.g.*, 10th percentile) should be determined, with confidence intervals, to adequately conclude that the post-disturbance data are significantly different from the pre-disturbance data (see section on Statistical Considerations below).

When using this approach, it is also important to ensure that sampling used to establish water quality of the "before" conditions is representative of the waterbody. A sample is considered representative if it has approximately the same distribution of characteristics as the population from which it was drawn. Since there may be spatial and temporal variations in the characteristics of DO at background (and test) sites, one should have a reasonable understanding of what the data from the background condition represents when using these data for determining compliance with water quality standards. Depending on a given environmental situation (and variability of the parameter in question), there may be a range of data sufficiency needs for confident determination of background conditions, and the following should be taken under consideration:

- The amount and quality of the data used to estimate natural, or minimally affected condition;
- The appropriateness of statistical treatment of the data and the rationale for its selection, including the handling of values less than the detection limit (generally, one half the detection limit is a good estimate if detection limits are consistent);
- Whether data were collected during the appropriate time period to evaluate the parameter of concern;

- The variability of the measurements and how this variability is taken into account in the analysis.

2.3.3 Modeling Approach

The term "model" simply refers to a technique for predicting a condition in a specific place in the environment. A water quality model is a mathematical tool used to estimate water quality conditions under a specific set of environmental conditions. Confidence in a model's results is dependent upon its ability to accurately predict the existing condition.

There are two basic types of models used to estimate water quality:

1. Statistical or empirical models, which are based on observed relationships between environmental variables and which are often used in conjunction with measurements from reference locations, and
2. Simulation or process-based deterministic models that attempt to quantify the natural and anthropogenic processes acting on the waterbody. Using deterministic models, anthropogenic inputs may be mathematically set to "zero" to estimate what environmental conditions would be in the absence of human pollution loading. This technique is an excellent option when no suitable reference locations or reference watersheds can be identified

Both types of models use equations to represent the key relationships among system components, but the ways they derive those equations are different.

Empirical models use measurements from target locations to describe relationships, using statistical techniques such as correlation or regression. The equations describe the observed relationships between the variables as they were measured at those specific locations. Statistical models have the advantage of being relatively simple, as they rely on actual data and statistics to develop correlations. In the case of modeling the natural condition, the correlations would involve a parameter of interest and the landscape or other water quality characteristics that control that parameter.

For example, previous analyses by FDEP have shown that color and Total Organic Carbon (TOC) are significantly and inversely correlated with DO in streams. Although correlations are not direct indications of cause and effect, a cause and effect relationship can be inferred by linking the statistical model to a conceptual model that describes the known relationships between the environmental processes affecting the parameter of interest. In the color/TOC example, the conceptual model would predict that color and TOC are related to the decomposition of leaf litter, which in turn is related to the spatial coverage of wetlands in a watershed. Therefore, the conceptual model would predict that a non-anthropogenically affected land cover (wetlands), which is known to be associated with naturally low DO, would influence the DO in downstream receiving waters.

Another example of an empirical model involves the known relationship between river discharge, salinity, stratification, and naturally low DO in estuaries. Therefore, a statistical relationship between river stage and salinity could be used to predict the naturally low DO concentrations in an estuary. The comparability between a reference location and an assessment location affects the results from empirical models, and therefore, use of statistical models to estimate the natural condition should also describe the uncertainties associated with the estimates.

Simulation modeling, which may also be referred to as process modeling, numeric modeling, deterministic modeling, or mechanistic modeling, can be used to estimate water quality under natural conditions using a two-step process. The first step is to simulate the existing condition and calibrate the model based on comparisons between measurements and model estimates for the parameter in question. The second step is to remove the model inputs that represent the human-caused sources of the pollutant from the model of the existing condition. While the resulting output from the model is a representation of natural background pollutant loading conditions, it may not always represent all historic natural conditions (*e.g.,* hydrologic modifications may still be present).

2.3.3.1 Estimating Natural Background Land Uses from Modeling

There are several approaches that can be used to establish natural background conditions using a model calibrated to existing conditions. These approaches are not intended to re-establish natural background hydrology, but rather to establish the total anthropogenic pollutant loading delivered to a waterbody under existing hydrologic conditions and estimate the impact of anthropogenic loads on surface water quality conditions. As applied for the development of Total Maximum Daily Loads (TMDLs), this estimate of total anthropogenic load has been used to ensure that load reductions required by TMDLs do not result in reductions in excess of the anthropogenic component, assuring that the TMDL is not established at a load that is less than the watershed load estimate for natural land cover. For this type of background condition model simulation, the impacts of channelization, weirs, control structures, dams, or other types of physical alterations to the water bodies are not removed (hydrology stays the same as in the existing condition).

There are various methods that can be used to establish the natural background pollutant loading estimates for a natural land use condition. The following are examples of methods that have been used by DEP to estimate natural landuse within a watershed:

1. Ratio Method.

   Where the Water Management Districts or other entities have developed GIS information for natural landuse conditions (with and without changes in hydrology), this information should be preferentially used. In cases where this information is not available, the ratio of forest to wetland cover under current conditions is applied to the total watershed acreage to establish a background condition of forest and wetland.

2. Soil Information Method.

   This method can be applied in cases where local historic land use information is not available. The investigator should utilize soil information to calculate the human land uses based on the soil hydrological group information included in the Natural Resource Conservation Service (NRCS)'s SSURGO soil coverage. Four hydrological soil groups are generally used in classifying the hydrological characteristics of soils, including:

a. **Type A soil (low runoff potential):** Soils having high infiltration rates even if thoroughly wetted and consisting chiefly of deep, well-drained to excessively drained sands or gravels. These soils have a high rate of water transmission.

b. **Type B soil:** Soils having moderate infiltration rates if thoroughly wetted and consisting chiefly of moderately deep to deep, moderately well-drained to well-drained soils with moderately fine to moderately coarse textures. These soils have a moderate rate of water transmission.

c. **Type C soil:** Soils having slow infiltration rates if thoroughly wetted and consisting chiefly of soils with a layer that impedes the downward movement of water, or soils with moderately fine to fine texture. These soils have a slow rate of water transmission.

d. **Type D soil (high runoff potential):** Soils having very slow infiltration rates if thoroughly wetted and consisting chiefly of clay soils with a high swelling potential, soils with a permanent high water table, soils with a clay pan or clay layer at or near the surface, and shallow soils over nearly impervious materials. These soils have a very slow rate of water transmission.

Dual soil groups also exist, including A/D, B/D, and C/D, which are soils that, when dry, show A, B, and C soil characteristics, but when flooded, showed D soil characteristics.

To use the hydrological soil group information to estimate natural land cover from the existing human land uses, the SSURGO soil GIS coverage is overlaid with the land use GIS coverage to create a combined hydrological group-land use coverage, and human land uses that are associated with the C, D, A/D, B/D, and C/D soil groups are assigned to wetland areas and the remaining human land uses are assigned to upland forest. When using this method, it should be noted that some existing soil coverage may not represent the historical coverage. However, in many cases, the soil coverage is the best available information for developing a natural land cover condition.

2.3.3.2 Deriving an Estimate of the Background Conditions for Pollutant Loads

After the natural land uses have been estimated, the background landuse estimates can then be characterized using the existing characteristics, *e.g.*, event mean concentrations, for natural forest and wetland areas. Depending on the type of model used, estimates of background/natural concentrations of pollutants may also be needed for rainfall, interflow, and groundwater. Once this information has been incorporated into the model, a model simulation is performed using the same “weather data” as applied in the existing condition model to derive an estimate of the background conditions for pollutant loads and surface water quality.

Unlike statistical models, process models do not rely upon data from specific reference locations, so these models can be used for aquatic systems that have no suitable natural reference comparisons available. For example, it is often difficult to establish minimally disturbed reference conditions for larger rivers and estuaries, so this method is especially useful in those cases.

Any model used to estimate natural background conditions should be appropriate for the scale and type of system being assessed, and there should be adequate data available to use for the model input parameters. In addition, it is important to document that the uncertainties in the estimate of the natural background condition are within a reasonable range.

Since no model can include all processes that may affect water quality, the following questions should be addressed when describing the model results:

- What factors or important processes does the model include?
- What factors or processes does it omit? Are the un-modeled factors likely to be significant?
- What input parameters have the strongest effect on the model results?
- What input parameters have the greatest and the least amounts of uncertainty?

2.3.4 Statistical Considerations

Statistics are useful when evaluating differences between populations of data or when determining whether water quality changes are attributable to human influences. Ideally, monitoring and assessment should be of sufficient rigor to detect significant differences in ambient water quality caused by humans if in fact they exist (referred to as having a low Type II error). Simultaneously, the assessment should not falsely indicate there is a human-induced difference when in fact there is none (low Type I error). The analytical detection limit is important when making statistical comparisons, especially relating to the concept of a minimum detectable difference. The detection limit should be sufficiently low to quantify environmentally relevant concentrations or levels and to allow for differences between sites to be observed, if in fact differences actually exist.

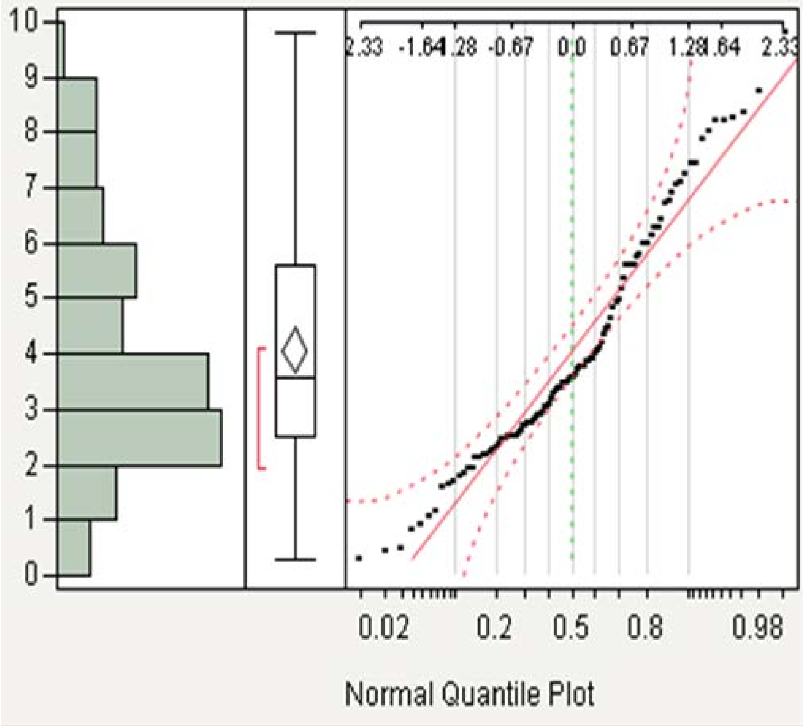
The ability to statistically determine exceedances of criteria from background depends upon five interacting factors: sample size, variability, level of significance, power, and minimum detectable effect (MacDonald *et al.* 1991):

1. *Sample size*: Larger sample size increases the ability to detect a difference between two groups of samples;
2. *Variability*: The more variable a measure, the less the ability to detect significant change;
3. *Level of significance*: This refers to the probability that an apparently significant difference is not real but simply due to chance. This is referred to as alpha (α) or a Type I error. An α of 0.10 means there is a 1 in 10 chance that an observed difference is due to chance, or a test is 90% “confident”;
4. *Power*: The probability of detecting a difference when in fact one exists; designated (1-β). β or a “Type II” error, is the probability of incorrectly concluding that two groups of samples are the same when in fact they are different. Significance and power values of $\alpha < 0.1$ and $\beta < 0.2$ are commonly used in environmental studies.
5. *Minimum detectable difference (MDD)*: Determining how much change is unacceptable should be linked to the inherent error associated with a given measurement system.

2.3.5 Natural Background DO Examples

Example 1: Natural Background Dissolved Oxygen Using the Reference Approach

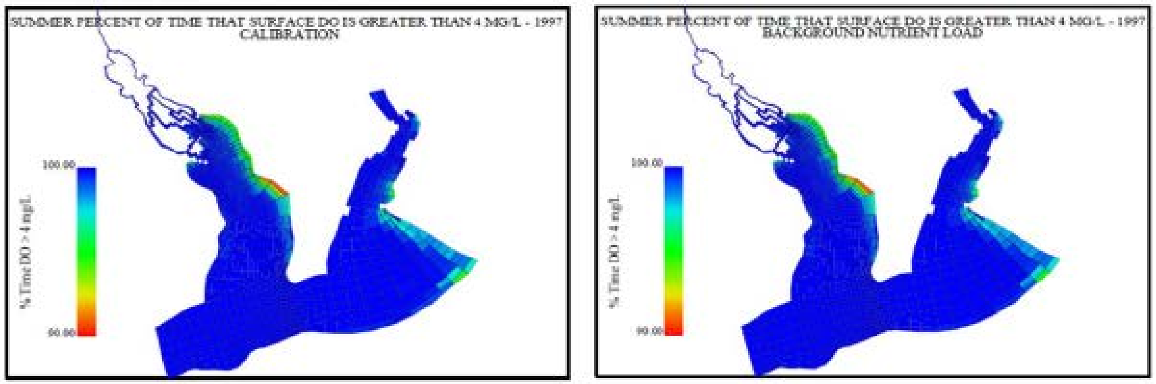
Thomas Creek, a minimally disturbed, blackwater stream in northeast Florida, meets all of the criteria discussed in the above reference approach section except that it does not meet the DO criterion (number 6). For example, the 100 meter buffer LDI was 1.9, the entire watershed LDI was 2.1, field observations indicated minimal human inputs, and the waterbody passed the SCI at two separate stations on two separate time periods. Therefore, the existing DO in the creek approximates natural background conditions. An analysis of the DO data showed that that annual average DO was 3.1 mg/L and that that lower 10th percentile of the individual DO measurements was 1.3 mg/L (see Figure below). Therefore, these DO levels serve as appropriate natural background targets, and would provide the basis for a Type I (Natural Background) SSAC for Thomas Creek itself, as well as for similar nearby streams**.**



Statistical distribution of DO data collected from Thomas Creek and its tributaries.

Example2: Natural Background Dissolved Oxygen Using Modeling

Due to natural stratification, Escambia/Blackwater/Pensacola Bay systems experience DO levels in some areas that are lower than the current criterion of 4 mg/L as an instantaneous measurement. To address this, a consultant developed calibrated three-dimensional, time variable hydrodynamic and water quality models to evaluate whether an alternate DO target, based on natural background conditions, could be established. Natural background conditions were estimated using the model by removing all point source discharges and converting all agricultural, range, urban, and barren land uses to forest land use. These changes resulted in a reduction in nutrient and Biochemical Oxygen Demand loads of approximately 42% to 44%. A comparison of the “current” and “natural background” modeling output showed essentially no differences for the DO regime, meaning the existing DO conditions are acceptable because they approximate natural background (see Figure below).



Existing Condition **Natural Background Condition**

Existing DO conditions compared to modeled natural background conditions in Pensacola Bay.

3 The USEPA Natural Background Larval Recruitment Model

One potential method for evaluating the potential effects of changes in the background DO regime on sensitive biological communities in marine waters is the use of a spreadsheet model developed by the USEPA. The model has been used by the USEPA to determine the allowable deviation from natural background DO levels (up to 10%) that would result in a less than 5% additional loss in larval recruitment (Tetra-Tech, 2005). This model can be used to make site-specific evaluations using information about natural background DO levels and those taxa expected in similar, minimally disturbed conditions to demonstrate that the deviation from natural background is protective. The use of the USEPA Natural Background Larval Recruitment Model and the data requirements for use of the model are described below. A more detailed discussion of the model and its application can be found in Tetra Tech, Inc. (2005).

3.1 *Data Requirements*

The USEPA model uses site specific information concerning the natural background DO regime and sensitive aquatic species that occur in the waterbody or region to estimate the effect that changes to the DO regime will have on larval recruitment of sensitive species or species of special concern (*e.g.*, threatened or endangered species). The Excel spreadsheet model developed by the USEPA is entitled, “Generic Implementation for % Impairment-REGION 4.xls”. There are three different worksheets visible in the file named “Parameters”, “D.O. time series-daily” and “Custom Spawning Season”. A description of the data requirements for each portion of the model is provided below.

3.1.1 Parameters sheet

The information required to evaluate each DO time series of interest is input on the Parameters worksheet. The items that require user input are inside the Input Parameters box (**Figure 1**). The parameters that must be entered include:

Number of Time Series: the number of DO regimes that the user provides and wants to assess in the model run. One DO regime should be the estimate of natural background conditions determined using one of the approaches described above.

Spawning Season information:

Length: The length in days of the spawning season of the species of concern (There is no need to enter units; the word “days” appears automatically);

Start Day: The Julian day that the first cohort appears or the spawning season starts;

Larval Development Time: The number of days it takes a larva of the species of concern to develop into a juvenile.

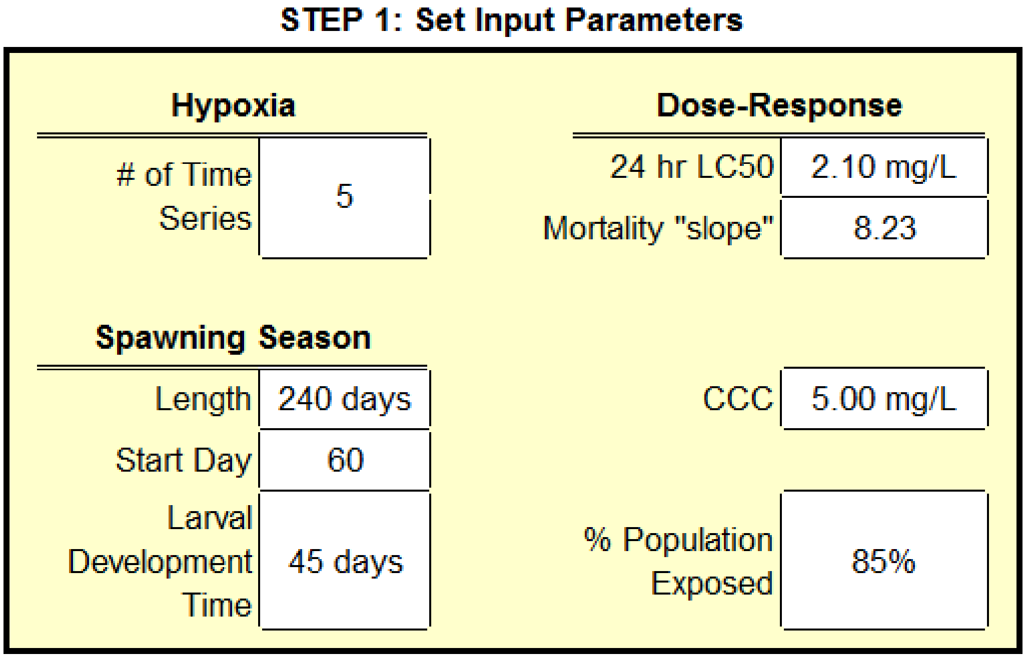
Dose-Response Information:

24-hour LC50: The acute effect concentration for the species of interest. The 24-hour LC50 for the hypothetical 95th percentile sensitive species from USEPA’s original application of the Virginian Province approach is 3.15 mg/L.

Mortality “slope”: The shape factor of the sigmoid dose-response curve. It is a constant (*i.e.*, 8.23) for DO no matter which species is being tested. It does not need to be altered, but can be changed if desired.

CCC: The Criterion Continuous Concentration. All DO values in a time series that are at or above this value are considered to have no effect. The CCC value for the hypothetical 95th percentile Florida specific sensitive species used in the derivation of the proposed criteria is 5.0 mg/L.

% Population Exposed: This represents the portion of the population of larvae that are exposed to low DO conditions. This can encompass both horizontal and vertical considerations. For example, if the low DO conditions are confined primarily to bottom waters, the % of the population exposed can be adjusted to account for the vertical distribution of the larvae in the water column. Additionally, if the hypoxia area only represents 20% of the range of the species at the site of concern, then this value would be 20%.



STEP 1: Set Input Parameters

Hypoxia

# of Time Series: 5

Spawning Season

Length: 240 days

Start Day: 60

Larval Development Time: 45 days

Dose-Response

24 hr LC50: 2.10 mg/L

Mortality "slope": 8.23

CCC: 5.00 mg/L

% Population Exposed: 85%

Figure 1. Screen capture of data entry portion of the Parameters worksheet showing spawning and dose-response information required by the model. An explanation of each component is provided above.

Cohort Distributions:

After entering the spawning parameters, the user must select one of the seven cohort distributions from the set of options located on the Parameters worksheet (Figure 2). The options include six pre-defined cohort distributions (uniform, wide peak, narrow peak, twin peaks, tall/short peaks, and short/tall peaks) and a customizable distribution that can be used if detailed site specific information is available. “Clicking” on one of the blue boxes below the spawning information input block will automatically update the spreadsheet with the appropriate cohort distribution. The six standard spawning distributions all assume a new cohort is created each day. The total for the season for each of these is adjusted so that the same numbers are created as would be for the uniform distribution using 1000 per cohort. For example, if the spawning season is 90 days, then the total created under uniform spawning conditions would be 90,000. Any other spawning distribution that also lasts 90 days would be adjusted such that the total for the season would be 90,000 (this does not apply to the custom spawning situation). This feature allows the direct comparison of different spawning strategies under the same DO conditions.

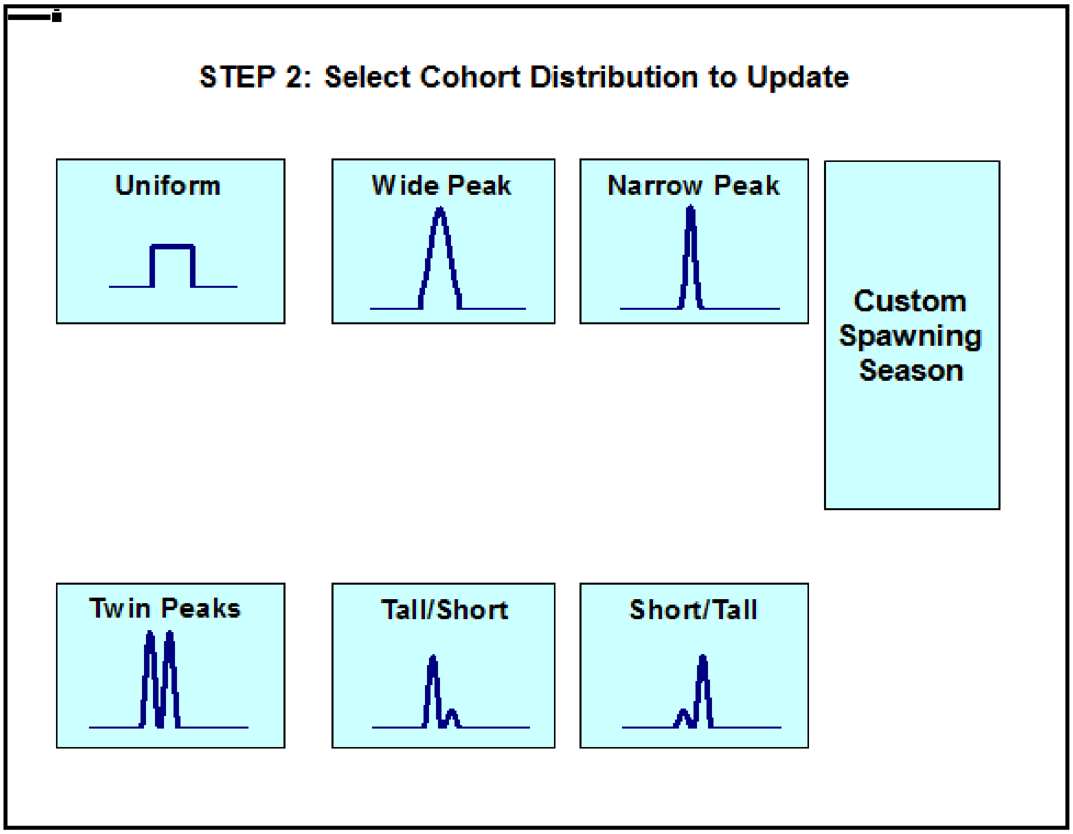


Figure 2. Screen capture of spawning season cohort distribution selection portion of the Paramaetrs worksheet showing six pre-established cohort distributions and the Custon Spawing Season selection.

3.2 *Custom Spawning Season Worksheet*

If the custom spawning season is selected on the Parameters worksheet, the cohort distribution specified on the Custom Spawning Season worksheet is utilized. The number of cohorts present is entered for each day of the year based on site specific information. A zero is entered for any day that is not a spawning day, including days before and after the spawning season, as well as any non-spawning days between the start and the end of the season. Also, the first non-zero day after January 1 should be entered as the spawning "Start Day" in the "Parameters" input table. Likewise, the number of days from the "Start Day" until the last nonzero cohort at the end of the season, regardless of the any zero days between successive cohorts, should be entered as the "Length" of the spawning season.

3.3 *DO Daily Time Series Worksheet*

To accurately predict the larval recruitment effects resulting from a deviation from natural background DO levels, an accurate estimate of the natural background DO daily time series must be entered on the "D.O. Daily Time Series Worksheet". The daily average natural background DO concentrations are entered in Column C of the worksheet. A number of additional time-series to be evaluated in comparison to the background condition can be entered in consecutive columns to the right of the natural background DO time series. There is no limitation on the number DO time series that can be entered. A daily average DO level is expected for each day, however, a value at or above the CCC can be entered for days outside of the hypoxia season. Since DO levels at or above the CCC will have no effect on recruitment, this will assure that any cohorts present outside of the hypoxia season are considered to have 100% survival. It should be noted that the first cell in each column in which the DO time series is evaluated is used as the identifier for the calculated results. Additionally, the "# of time series" entered in the "Parameters" input table controls the number of columns (*i.e.*, DO time series), starting with the background (column C), that are evaluated.

The natural background DO regime can be estimated in a number of ways including actual measurements at the site, if the site is minimally impacted, or from a nearby minimally disturbed reference site; or by results from an appropriate water quality model. The DO regime should be representative of the area being assessed as well as the habitat occupied by the DO sensitive species being evaluated.

To protect the juvenile and adult life stages of DO sensitive organisms, the alternative DO regimes should not be allowed to fall below the proposed Criteria Minimum Concentration (CMC) during more than 10 percent of the days. If the daily average DO levels fall below the CMC during natural background conditions, then no further reduction in DO levels during these periods should be allowed.

4 Estimating Change to Larval Recruitment

After all of the required data are entered, the model can be run to estimate changes to larval recruitment based on the DO time series entered. When the model is run, the graph on the "Parameters" worksheet is updated to show the selected spawning season cohort distribution (black line), the background DO time series (blue line), and the most recent DO time series used (red line) (Figure 3). Additionally, as the larval recruitment is evaluated for each DO time series, the results are placed in the "Most Recent Results" table on the "Parameters" worksheet (these data are automatically cleared at the start of each run). Figure 4 provides an example of the results from an evaluation of five DO time series (*i.e.*, background and four alternative DO regimes). In the example, the natural background DO condition would result in approximately 4.4 percent loss in recruitment. In this example, the four alternative DO regimes were created by multiplying the baseline daily DO concentrations by 95, 90, 85, and 80 percent to reduce the baseline DO levels by 5 to 20 percent. It should be noted that the alternative DO regimes to be evaluated can be derived in any manner that meets the objectives of the evaluation.

The results for the alternative DO regimes resulted in additional recruitment losses 1.8 to 18 percent over the natural background condition, for the 5 to 20% reductions in DO, respectively (Figure 4). Given that EPA generally considers a maximum of five percent increase in recruitment loss over natural background conditions an acceptable minimal change, the results in the example indicate that the natural background DO regime could be decreased by approximately 10 percent (*i.e.*, 10 percent of natural background DO regime) with a minimal loss in recruitment of the sensitive organisms of concern. It should be noted that an additional five percent loss in recruitment over natural background may not always be an acceptable result. For example, in cases where the effects of an altered DO regime on a threatened or endangered species are being accessed, no additional recruitment loss may be the goal.

Additionally, if the model results indicate abnormally high recruitment losses under the natural background condition, the model inputs should be checked and verified, especially the estimates of the natural background DO regime. Further, if the larval stage of the species is rare within the area being assessed, it may be naturally excluded from the area due to the natural conditions present and may not be a reliable indicator species. In this case, a species that is more common in that specific estuary should be selected and evaluated.

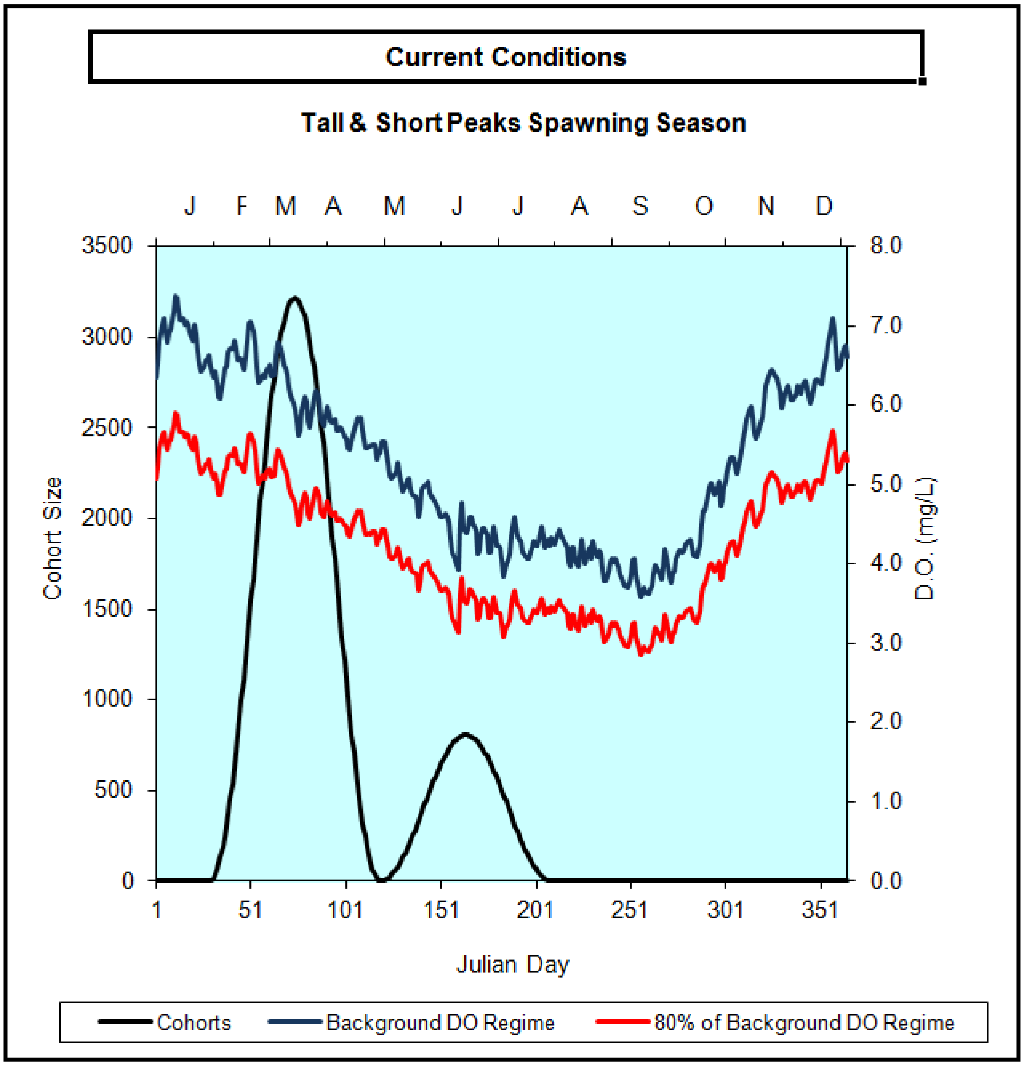
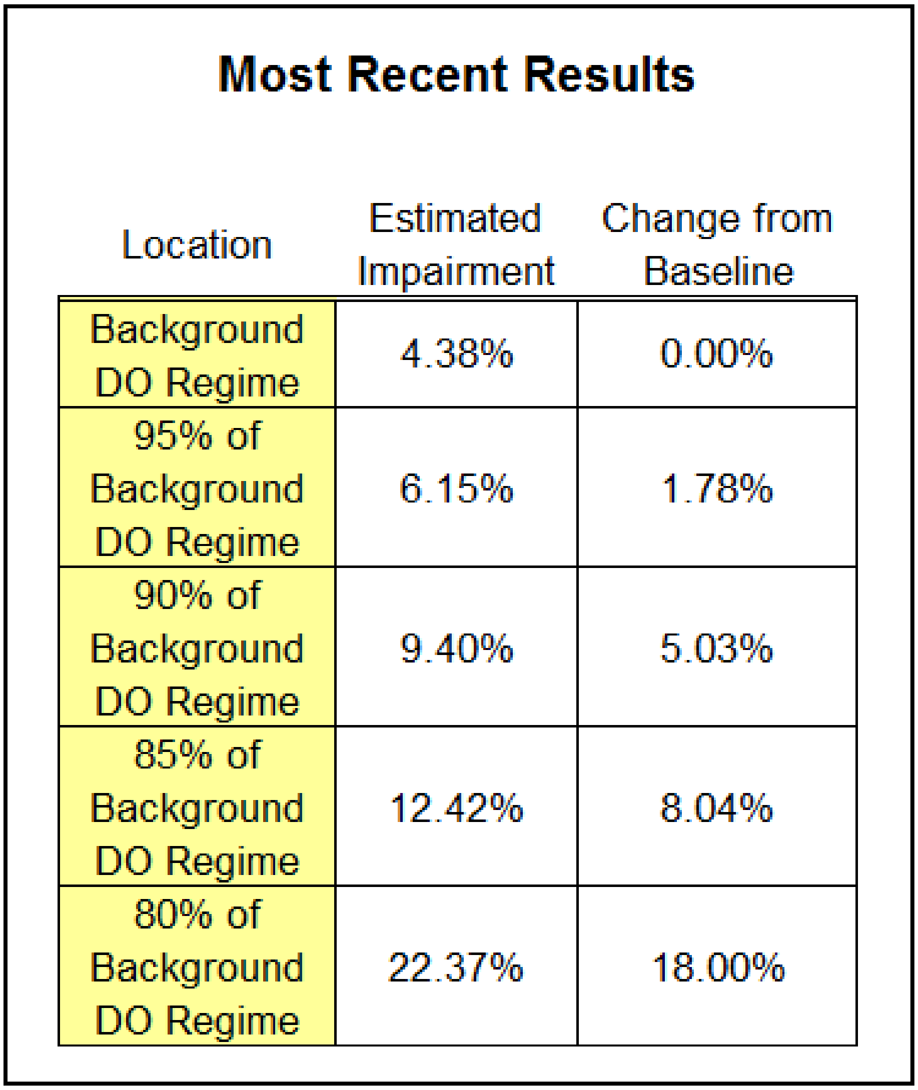


Figure 3. Screen capture of "Current Conditions" graph on the Parameters worksheet which is automatically updated as the required data is entered and the model is run. The graph displays the spawning season cohort distribution along with the natural background DO regime and the alternative DO regime being evaluated as the model is running or the final alternative after the model has finished running.



Most Recent Results

Location	Estimated Impairment	Change from Baseline
Background DO Regime	4.38%	0.00%
95% of Background DO Regime	6.15%	1.78%
90% of Background DO Regime	9.40%	5.03%
85% of Background DO Regime	12.42%	8.04%
80% of Background DO Regime	22.37%	18.00%

Figure 4. Screen capture of "Most Recent Results" table on the Parameters worksheet which is automatically updated as the model is run. The results table provides the predicted recruitment loss as well as the change in recruitment loss from the natural background condition for each alternative DO regime evaluated.

5 Spawning and Developmental information for Sensitive Species in Florida Marine Waters

As described above, the application of the USEPA's recruitment effects model requires acute and chronic hypoxia response criteria as well as information concerning the spawning period and length of larval development for each species of interest. The required information can be found from a number of sources including site specific observations and in scientific literature. The information required by the model has been assembled for a number of sensitive Florida species and is presented below. If detailed site specific information is not available for the area being evaluated, the recruitment effects can be estimated using the generic information provided below.

The acute and chronic DO effects concentrations provided below were taken from the data used to develop the revised DO criteria for Florida's marine waters (FDEP, 2012). The presence and spawning larval distribution and length of larval development for each species were determined from data collected as part of NOAA's Estuarine Living Marine Resources (ELMR) and Biogeography Programs database available at http://ccma.nos.noaa.gov/ecosystems/estuaries/elmr.aspx#products and NOAA Reports (Pattillo et al, 1997; Nelson *et al.* 1992; and 1991).

The NOAA database provides information on the distribution and relative abundance of 153 fishes and invertebrates in estuaries within the continental United States. The NOAA database and reports provide site specific data and observations for 15 estuaries covering both the Atlantic and Gulf coasts. Because species abundance can depend on salinity, the database provides monthly species abundance scores for each life stage for each species within various salinity ranges. In the NOAA database, spawning abundance is reported as a value from 0 to 5, where:

0 – Not present: species of life stage not found, questionable data as to identification of the species, or recent loss of habitat or environment degradation suggests absence.

2 – Rare: species is present but not frequently encountered.

3 – Common: species is generally encountered but not in large numbers; does not imply an even distribution over a specific salinity zone.

4 – Abundant: species is numerically dominant relative to other species.

5 – Highly Abundant: species is numerically dominant relative to other species.

NOAA's 1992 (Volume 1) and 1997 (Volume II) (Nelson *et al.* 1992; and Pattillo et al, 1997) studies of the "Distribution and Abundance of Fishes and Invertebrates in Gulf of Mexico Estuaries" were used to estimate the length of larval development. Volume I of the report describes the spawning abundance, and Volume II summarizes the species life history and includes descriptions of growth and development. Similarly, a 1991 NOAA report "Distribution and Abundance of Fishes and Invertebrates in Southeast Estuaries" (Nelson *et al.* 1992) covers the estuaries on Florida's Atlantic coast. The length of development was determined for the period from larvae to juvenile maturity. Where the length of development was given by a time range, the mid-point of the range is provided as an estimate.

5.1 *Species Selection*

During the derivation of the proposed revised DO criteria for Florida's marine waters, a comprehensive literature review was conducted to assemble the data necessary to evaluate the effects of low DO levels on various Florida specific coastal and estuarine animals. During this effort, DO concentrations protective of chronic and acute effects for a number of species found in Florida waters were tabulated and can be found in FDEP, 2012. While some information concerning the effects of low DO levels is available for a significant number of Florida organisms, far fewer have all of the information required by the model to estimate recruitment effects. **Table 2** lists the species mean acute and chronic values calculated in accordance with USEPA guidance for species occurring in Florida marine waters for which all of model required information is available. Where the chronic effects concentration (SMCV) is not available, the CCC derived as part of the statewide criteria development effort can be substituted as a conservative estimate. Because the model is designed to evaluate the low DO effects on individual species, multiple model runs using a number of sensitive species listed in FDEP, 2012 or **Table 2** will be required to assure no sensitive species are adversely affected. Unfortunately, not all of these species spawn in all portions of the state; therefore the list of species to be evaluated should be selected based on more site-specific data and observations wherever possible.

The habitat, range, and spawning characteristics of a number of DO sensitive Florida species are discussed in the following sections to provide some background information. Estimates of the specific information required by the recruitment effects model for each species are presented in **Table 3**. Because site specific conditions can significantly influence the spawning and growth of many of these species, this information should be modified based on site specific observations when possible.

5.1.1 Spotted Seatrout (*Cynoscion nebulosus*)

The spotted seatrout is an important species to commercial and recreational fishing interests. It occurs in coastal waters from Cape Cod to Mexico, but is most abundant from Florida to Texas (NOAA, 1997). The spotted seatrout is a top trophic level carnivore within coastal and estuarine ecosystems, and probably plays a significant role as a predator in the structure of estuarine communities. Seatrout complete their entire life cycle in near shore waters with seagrass areas being an important habitat area. Based on the presence of larval seatrout in the northern Gulf, spawning appears to occur from February through October, with a peak from April through August. Reports indicate that larval seatrout grow approximately 0.4 mm per day and require approximately 25 days to mature into juveniles. It should be noted that growth rates and spawning periods can vary significantly due to differences in temperature and other site specific conditions. Therefore, site specific information should be used to refine the spawning and developmental information input into the model whenever possible.

5.1.2 Summer Flounder (*Paralichthys dentatus*)

The summer flounder is another important commercial and recreational fishery species that occurs in southeastern Atlantic coastal waters from North Carolina to Florida and in the Gulf from Florida to northern Mexico. However, the range of the flounder does not appear to be continuous around the southern tip of Florida and is not commonly found in estuaries along the southwestern coast of Florida. Spawning occurs in marine waters in the northern Gulf from

September through April, with a peak from December to February. The transformation of the larval flounder to the post-larval stage is completed within approximately 50 days.

The flounder was originally identified as species sensitive to low dissolved oxygen from data gathered in the Virginian Province (EPA 2000) and is a species used in the derivation of the proposed Florida DO criteria for marine waters (FDEP, 2012).

5.1.3 Quahog (aka Hard Clam) (*Mercenaria species*)

The northern quahog was originally identified as a species sensitive to low dissolved oxygen based on their tolerance to dissolved oxygen during spawning, as shown in **Table 2**. The quahog or hard clam is a filter feeder that resides in the bottom sediments. They are widely distributed throughout Florida but are not generally abundant in the near shore waters, preferring intertidal shallow water in coastal bays, sounds, and estuaries.

The quahog is known to spawn in marine and estuarine subtidal seawater. Spawning appears to coincide with high algal concentrations, allowing ample food resources for larval stages. NOAA's Biogeography Program reports (NOAA, 1997) the quahog as commonly spawning in Florida waters throughout much of the year, with bimodal peaks occurring in the spring (February to June) and Fall (September to December), but can occur yearlong in warmer areas.

NOAA (1997) also describes the characteristics of the quahog's habitat and indicates that the development from larval to juvenile stages to be depended on temperature with an average developmental time of 30 days.

5.1.4 Silverside (*Menidia species*)

The silverside was also identified as a species sensitive to low dissolved oxygen based on tolerance to dissolved oxygen during spawning, as shown in **Table 2**. They are ubiquitous residents of shallow estuarine waters in the southeastern United States. Most silversides are typically collected in the top 30-45 cm of the water column and near vegetated shorelines.

NOAA (1997) indicates that spawning of the silverside in Gulf waters is typically bimodal with peaks in the spring and fall, but can occur all year long in some locations. Seasonal peaks often occur from May to June and September to January. Spawning is most prevalent in tidal freshwater or brackish water in the upper parts of estuaries, but larvae have been found in salinities ranging from 0 to 30 ppt.

NOAA's Biogeography Program reports the most abundant spawning of the silverside in Florida estuaries from March through October at salinities from 0.5 to 25 ppt. All the necessary modeling parameters are available to assess the influence of dissolved oxygen on the spawning of silversides. Growth estimates on the silverside are unreliable, but one study in Tampa Bay indicated silversides grew 5-7mm per month from June to November, and that early-spawned juveniles grew about 8 mm (Standard Length) per month from June to September (Springer and Woodburn 1960). These studies were used to estimate a 72 day developmental period for transition from larvae to juvenile.

5.1.5 Blue Crab (*Callinectes sapidus*)

The blue crab is a cosmopolitan species commonly found in coastal waters, primarily bays and brackish estuaries. The blue crab is in high demand as a commercial and recreational fishery and is commonly used in pollution studies because of its wide distribution in the nation's estuaries including Florida estuaries and its fishery. The blue crab has been characterized as an opportunistic benthic omnivore whose food habits are governed by availability of food items.

Blue crab females release their eggs near estuarine mouths so they can be carried offshore during the zoeal larval stage. They return to estuarine waters during the final of several larval stages. In the northern Gulf, larval crabs have been found year-round, with less frequent occurrences in December through April and with peaks typically occurring in late fall and late summer or early fall. In the St. Johns River, spawning typically occurs from February through October, with a peak from March through October. Laboratory studies indicate that 31 to 43 days are required to complete zoeal larval stages at typical temperature and salinity ranges. The length of development of 37 days is provided as a general estimate for the purpose of the recruitment model.

5.1.6 Red Drum (*Sciaenops ocellatus*)

The red drum is a highly valued game and food fish throughout its range in the Atlantic from Massachusetts to Florida and in the Gulf from Florida to Mexico. Red drum are estuarine-dependent. Eggs, larvae, and early juveniles are planktonic and pelagic. Red drum eggs are spawned in nearshore and inshore waters close to barrier island passes and channels. After hatching, larvae and post-larvae are carried by tidal currents into the shallow waters of bays and estuaries. Larvae seek grassy coves, tidal flats, and lagoons where the vegetation protects them from predators and currents, and where they can avoid rough waters until they are strong enough to swim actively.

The spawning season typically lasts from summer through early winter, but its onset and duration vary with photoperiod, water temperature, and possibly other factors (Holt *et al.* 1981a, Overstreet 1983). Spawning can start as early as August in some parts of the study area, but it usually begins in September and ends in early January, with peaks occurring in mid-September through October. Growth from larvae to juvenile requires approximately 30 days, but as with many other species, growth rate is highly dependent on temperature, food source, and other environmental factors.

5.1.7 Pink Shrimp (*Penaeus duorarum*)

The pink shrimp is a commercially important and valuable species throughout the Gulf. The range of the pink shrimp extends from the Chesapeake Bay to southern Florida and throughout the Gulf. The distribution of pink shrimp generally appears to coincide with the presence of seagrass, with the maximum abundance occurring in southwest Florida waters.

Larval shrimp occur most commonly from March through October. The time required for development to juvenile stage ranges from 15 to 25 days depending on temperature with an average of 20 days.

5.1.8 Sturgeon (*Acipenser oxyrinchus desotoi* and *Acipenser brevirostrum*)

Two sturgeon species occurring in Florida (*i.e.,* Gulf sturgeon, *Acipenser oxyrinchus desotoi* and shortnose sturgeon, *Acipenser brevirostrum*) have been shown to be particularly sensitive to low oxygen levels (Wakeford 2001) and are provided special consideration due to their listing as threatened and endangered, respectively, under the Endangered Species Act. The sturgeon are most sensitive to low DO levels during their early life stages. As the Gulf and shortnose sturgeon mature and become less sensitive to low DO, they migrate downstream to brackish and marine waters (McEnroe and Cech 1987). Because all modern sturgeon species spawn in fresh water, further information is not included for these species.

Table 2. Florida specific DO sensitive species with species mean acute (SMAV) and chronic (SMCV) DO concentrations. Where the SMCV is missing, the CCC of 4.9 mg/L derived during the development of the proposed DO criteria for Florida's marine waters using the Virginian Province approach.

Scientific Name	Common Name	Species Mean Acute DO concentration, mg/L	Species Mean Chronic DO concentration, mg/L
Cynoscion nebulosus	Spotted Seatrout	1.88	----
Paralichthys dentatus	Summer Flounder	1.41	3.97
Mercenaria species	Quahog (Hard Clam)	0.43	3.17
Menidia species	Silverside	1.63	3.30
Callinectes sapidus	Blue Crab	1.40	----
Sciaenops ocellatus	Red Drum	1.45	----
Penaeus duorarum	Pink Shrimp	1.41	3.46

5.2 *Estimating Larval Recruitment Effects*

To obtain an accurate estimate of the recruitment effects resulting from deviations from background DO levels, accurate information concerning the spawning and larval development must be entered into the spreadsheet model. Although generic spawning and developmental information is provided above for selected species, the information should be refined based on site specific knowledge of the system being evaluated because spawning and development of many species is highly dependent on specific site conditions (*e.g.,* temperature, salinity, food sources and availability, etc.). Additional species can also be added if all of the necessary information is available and data indicates that they are sensitive to decreasing DO levels. More detailed site specific information is available for a number of species in the NOAA (1991, 1992, and 1997) reports and the NOAA ELMR database described previously.

Once the required information is collected and entered into the model, estimating the effects on larval recruitment is relatively straight forward as described in Sections 2 and 3 above. To assure all sensitive species are protected, the model should be run with multiple species from different taxonomic groups with different spawning patterns and developmental times to evaluate the range of effects that could be expected.

Table 3. Relative monthly spawning abundance and length of larval development for selected Florida sensitive species based on NOAA ELMR database available at http://ccma.nos.noaa.gov/ecosystems/estuaries/elmr.aspx#products.

Scientific Name	Common Name	Length of Larval Development, Days	Relative Monthly Spawning Abundance*											
			J	F	M	A	M	J	J	A	S	O	N	D
Cynoscion nebulosus	Spotted Seatrout	25	0	0	4	5	5	5	4	4	4	0	0	0
Paralichthys dentatus	Summer Flounder	50	3	3	3	3	3	0	0	0	0	0	0	3
Mercenaria species	Quahog (Hard Clam)	30	3	3	3	0	0	0	0	0	0	0	0	3
Menidia species	Silverside	72	2	3	4	4	4	4	4	4	4	4	3	2
Callinectes sapidus	Blue Crab	37	5	5	5	5	5	5	5	5	5	5	5	5
Sciaenops ocellatus	Red Drum	30	3	0	0	0	0	0	0	0	3	3	3	3
Penaeus duorarum	Pink Shrimp	20	2	2	3	4	5	5	5	5	5	4	3	3

*Spawning abundance scores: 0 = Not present, 2 = Rare: species is present but not frequently encountered, 3 = Common: species is generally encountered but not in large numbers, 4 = Abundant: species is numerically dominant relative to other species, 5 = Highly Abundant: species is numerically highly dominant relative to other species.

6 References

FDEP. 2012. Technical support document: Derivation of dissolved oxygen criteria to protect aquatic life in Florida's fresh and marine waters. Draft July 2012. 190 p.

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USEPA. 2000. Ambient Aquatic Life Water Quality Criteria for Dissolved Oxygen (Saltwater): Cape Cod to Cape Hatteras. Environmental Protection Agency, Office of Water, Washington D.C. PA-822-R-00-012.

Appendix I:

Protection of Threatened and Endangered Species in Portions of the Suwannee, Withlacoochee, Santa Fe, New, and St. Johns Rivers

Protection of Threatened and Endangered Species in Portions of the Suwannee, Withlacoochee, Santa Fe, New, and St. Johns Rivers

March 2013

Prepared by:
Florida Department of Environmental Protection
Division of Environmental Assessment and Restoration

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Protection of Threatened and Endangered Species in Portions of the Suwannee, Withlacoochee, Santa Fe, New, and St. Johns Rivers

1 Introduction

The purpose of the Endangered Species Act (ESA) passed by Congress in 1973 is to protect and promote recovery of imperiled species and the ecosystems upon which they depend. To accomplish this objective, the ESA affords additional protection to threatened and endangered species to prevent: 1) damage to, or destruction of, a species' habitat; 2) overutilization of the species for commercial, recreational, scientific, or educational purposes; 3) disease or predation; 4) inadequacy of existing protection; and 5) other natural or manmade factors that affect the continued existence of the species.

During the development of the proposed dissolved oxygen (DO) criteria, FDEP has worked with the U.S. Fish and Wildlife Service (FWS) and NOAA's National Marine Fisheries Service (NMFS) to assure that the threatened and endangered species occurring in Florida are provided adequate protection. During their review of the proposed freshwater criteria, FWS and NMFS determined that four endangered species may not be fully protected by the proposed DO criteria. These species are the young of the year Gulf sturgeon (*Acipenser oxyrinchus desotoi*) that can be found in portions of the Suwannee, Santa Fe, and Withlacoochee Rivers, the oval pigtoe mussel (*Pleurobema pyriforme*) that inhabits portions of the Santa Fe and New Rivers, and young Atlantic (*Acipenser oxyrinchus*) and shortnose sturgeon (*Acipenser brevirostrum*) that can inhabit the St. Johns River. The specific areas where the Gulf sturgeon and mussel may be found are illustrated in **Figure 1**.

The St. Johns River represents the southern extent of the range for the Atlantic and shortnose sturgeon. Even though the evidence suggests that the sturgeon occurring in the St. Johns River are transient individuals that do not spawn in the St. Johns, the ESA still requires that the portions of the river where spawning may occur in the future be afforded additional protection. A map showing the portions of the St. Johns River where the sturgeon could potentially spawn is provided in **Figure 2**.

2 Summary of Existing DO Conditions in Portions of the Suwannee, Santa Fe, New, and Withlacoochee Rivers

Because relatively little information is available concerning the specific DO requirements of these species, especially for the mussel, and since the populations of the sturgeon and mussel are stable and may actually be increasing in these river systems, it is reasonable to assume that maintaining the existing DO conditions would provide adequate protection in the future.

To summarize the existing DO conditions, data for each river segment in the potential range of the young sturgeon and mussel were obtained from the Impaired Waters Rule (IWR) database for the period since 1966. After reviewing the data for the entire period of record (*i.e.,* 1966 – 2011), the period from 1991 through 2011 was chosen for use in summarizing the existing conditions. The 1991 to 2011 period was selected because the 21 year period is long enough to capture the expected range of temporal variability and covers a significant portion of the period when the sturgeon population in the region has been stable or increasing. Additionally, the monitoring conducted prior to 1991 was conducted less frequently and often only covered portions of the year. Data collection after 1990 was more consistent, with a greater amount of data being collected that generally covered all months of the year. Therefore, to avoid biasing the summary of the existing DO conditions, the data collected prior to 1991 were omitted from further data analyses.

A summary of the existing DO conditions during the period from 1991 through 2011 for the portions of the Santa Fe and New Rivers potentially utilized by the Oval Pigtoe mussel is provided in **Table 1** by river system and individual river segment (River km/WBID). Similarly, the summary statistics for the portions of the Suwannee, Santa Fe, and Withlacoochee Rivers potentially utilized by the gulf sturgeon are provided in **Table 2** by river system and individual river segment.

3 Determining Whether DO Values Have Decreased Below the Baseline Distribution

To evaluate whether DO values have decreased below the baseline distribution, it is recommended that a) no more than 10 percent of the DO measurements be below the 10th percentile of the existing data distribution for that river segment, b) no more than 50 percent of the measured values to be below the median of the existing data distribution for that river segment. The 10th percentiles and median DO values for each of the affected river segments are provided in **Table 3**.

The recommended rule language is:

> *In the portions of the Suwannee, Withlacoochee (North), and Santa Fe Rivers utilized by the Gulf Sturgeon, and in the portions of the Santa Fe and New Rivers utilized by the oval pigtoe mussel, DO levels shall not be lowered below the baseline distribution such that more than 50 percent of measurements are below the median of the baseline distribution or more than 10 percent of the daily average values are below the 10th percentile of the baseline distribution for the applicable waterbody. The baseline distributions are provided in Appendix I of the Technical Support Document for the Derivation of Dissolved Oxygen Criteria to Protect Aquatic Life in Florida's Fresh and Marine Waters, which is incorporated by reference.*

When assessing these waters in the future, compliance with both the 10th percentile and median DO values will be evaluated using a binomial hypothesis test at the 80 percent and 90 percent confidence levels necessary to place a water segment on the Planning List and Verified Lists, respectively, for TMDL development. The use of the binomial hypothesis test is consistent with the assessment for other water quality parameters conducted under Chapter 62-303, F.A.C. The number of exceedances required to have 80 percent and 90 percent confidence that more than 10 percent of the measurements are below the applicable 10th percentile value are provided in Chapter 62-303, F.A.C., Tables 1 and 3, respectively. The number exceedances required to have 80 percent and 90 percent confidence that more than 50 percent of the measurements are below the applicable median value for sample sizes up to 419 are provided in **Table 4**.

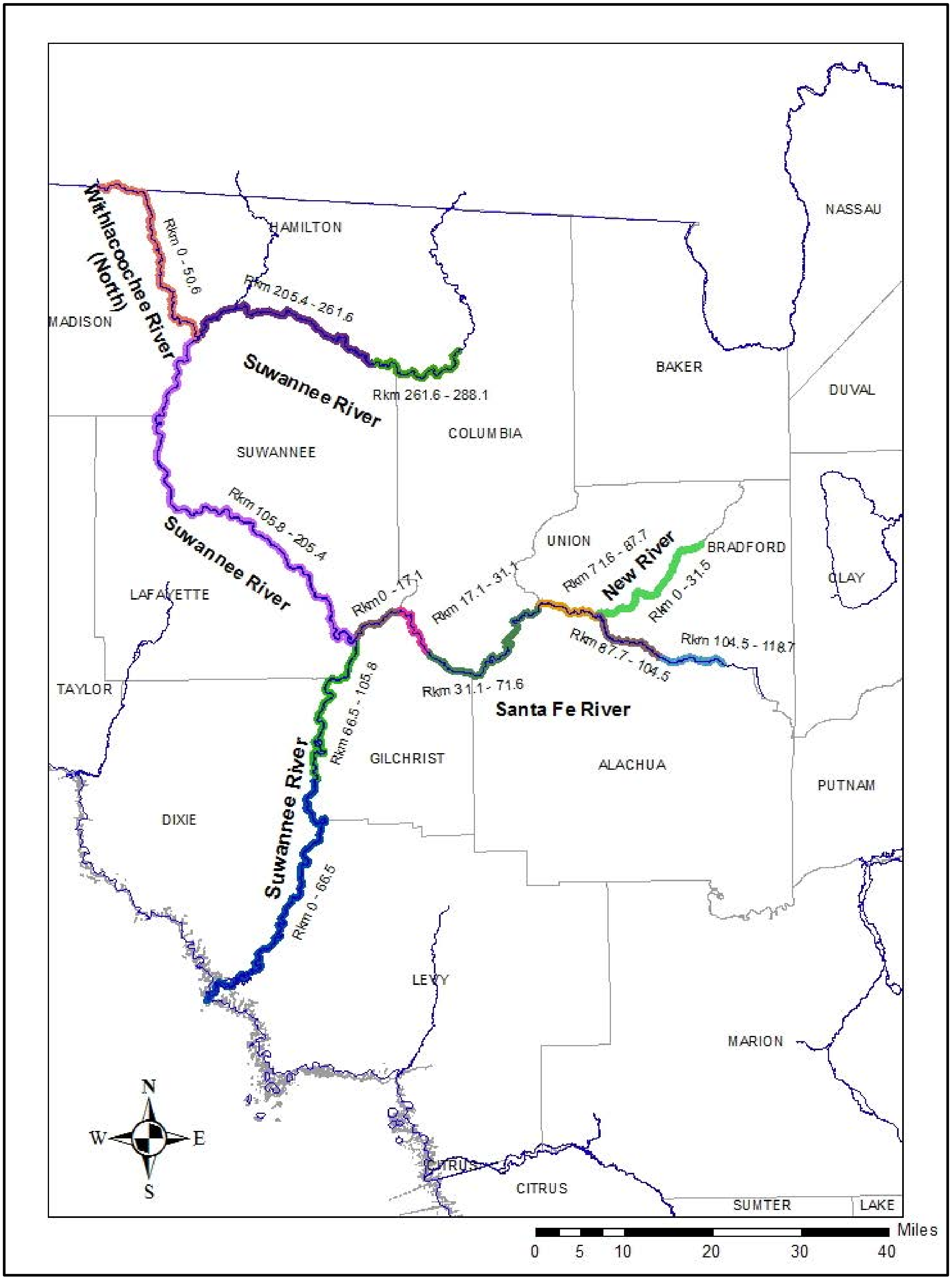

Figure 1. The portion of the Suwannee, Santa Fe, New, and Withlacoochee North Rivers utilized by the Gulf Sturgeon and oval pigtoe mussel requiring alternative DO criteria.

Table 1. Summary statistics for existing DO conditions in the portions of the Santa Fe and New Rivers utilized by the Oval Pigtoe mussel for the period from 1991 through 2011.

River System	WBID	River km	Statistic	DO Concentration, mg/L	DO Percent Saturation
New	**3506**	**0 - 31.5 km**	Count	406	404
New	**3506**	**0 - 31.5 km**	Avg	6.42	67.14
New	**3506**	**0 - 31.5 km**	Std Dev	1.77	13.80
New	**3506**	**0 - 31.5 km**	10th percentile	4.60	52.48
New	**3506**	**0 - 31.5 km**	25th percentile	5.30	60.20
New	**3506**	**0 - 31.5 km**	50th percentile	6.29	67.65
New	**3506**	**0 - 31.5 km**	75th percentile	7.50	74.76
New	**3506**	**0 - 31.5 km**	90th percentile	8.62	80.62
Santa Fe	**3605D**	**71.6 - 87.7 km**	Count	269	269
Santa Fe	**3605D**	**71.6 - 87.7 km**	Avg	6.77	72.54
Santa Fe	**3605D**	**71.6 - 87.7 km**	Std Dev	1.69	11.96
Santa Fe	**3605D**	**71.6 - 87.7 km**	10th percentile	5.00	59.51
Santa Fe	**3605D**	**71.6 - 87.7 km**	25th percentile	5.60	65.49
Santa Fe	**3605D**	**71.6 - 87.7 km**	50th percentile	6.50	72.95
Santa Fe	**3605D**	**71.6 - 87.7 km**	75th percentile	7.80	79.40
Santa Fe	**3605D**	**71.6 - 87.7 km**	90th percentile	9.00	86.58
Santa Fe	**3605E**	**87.7 - 104.5 km**	Count	239	237
Santa Fe	**3605E**	**87.7 - 104.5 km**	Avg	6.32	67.33
Santa Fe	**3605E**	**87.7 - 104.5 km**	Std Dev	1.89	18.35
Santa Fe	**3605E**	**87.7 - 104.5 km**	10th percentile	4.00	46.06
Santa Fe	**3605E**	**87.7 - 104.5 km**	25th percentile	5.00	54.65
Santa Fe	**3605E**	**87.7 - 104.5 km**	50th percentile	6.20	69.16
Santa Fe	**3605E**	**87.7 - 104.5 km**	75th percentile	7.40	78.00
Santa Fe	**3605E**	**87.7 - 104.5 km**	90th percentile	8.58	85.32
Santa Fe	**3605**	**104.5 - 118.7 km**	Count	83	83
Santa Fe	**3605**	**104.5 - 118.7 km**	Avg	6.30	65.66
Santa Fe	**3605**	**104.5 - 118.7 km**	Std Dev	2.23	19.27
Santa Fe	**3605**	**104.5 - 118.7 km**	10th percentile	3.17	37.14
Santa Fe	**3605**	**104.5 - 118.7 km**	25th percentile	5.40	60.40
Santa Fe	**3605**	**104.5 - 118.7 km**	50th percentile	6.23	69.30
Santa Fe	**3605**	**104.5 - 118.7 km**	75th percentile	7.81	77.14
Santa Fe	**3605**	**104.5 - 118.7 km**	90th percentile	8.89	84.00

Table 2. Summary statistics for existing DO conditions in the portions of the Suwannee, Santa Fe and Withlacoochee Rivers utilized by the Gulf Sturgeon for the period from 1991 through 2011.

River System	WBID	River km	Statistic	DO Concentration, mg/L	DO Percent Saturation
Santa Fe	3605A	0 - 17.1 km	Count	268	268
Santa Fe	3605A	0 - 17.1 km	Avg	5.85	66.17
Santa Fe	3605A	0 - 17.1 km	Std Dev	1.11	12.49
Santa Fe	3605A	0 - 17.1 km	10th percentile	4.50	50.90
Santa Fe	3605A	0 - 17.1 km	25th percentile	5.24	59.78
Santa Fe	3605A	0 - 17.1 km	50th percentile	5.90	66.04
Santa Fe	3605A	0 - 17.1 km	75th percentile	6.50	73.30
Santa Fe	3605A	0 - 17.1 km	90th percentile	7.13	80.82
Santa Fe	3605B	17.1 - 31.1 km	Count	52	49
Santa Fe	3605B	17.1 - 31.1 km	Avg	6.30	71.08
Santa Fe	3605B	17.1 - 31.1 km	Std Dev	1.52	16.46
Santa Fe	3605B	17.1 - 31.1 km	10th percentile	3.95	47.62
Santa Fe	3605B	17.1 - 31.1 km	25th percentile	5.56	61.00
Santa Fe	3605B	17.1 - 31.1 km	50th percentile	6.60	74.00
Santa Fe	3605B	17.1 - 31.1 km	75th percentile	7.34	85.00
Santa Fe	3605B	17.1 - 31.1 km	90th percentile	8.10	89.32
Santa Fe	3605C	31.1 - 71.6 km	Count	1201	1202
Santa Fe	3605C	31.1 - 71.6 km	Avg	4.79	53.70
Santa Fe	3605C	31.1 - 71.6 km	Std Dev	1.70	17.85
Santa Fe	3605C	31.1 - 71.6 km	10th percentile	2.66	30.69
Santa Fe	3605C	31.1 - 71.6 km	25th percentile	3.80	43.25
Santa Fe	3605C	31.1 - 71.6 km	50th percentile	4.70	53.56
Santa Fe	3605C	31.1 - 71.6 km	75th percentile	5.70	63.08
Santa Fe	3605C	31.1 - 71.6 km	90th percentile	7.05	76.96
Suwannee	3422	66.5 - 105.8 km	Count	290	290
Suwannee	3422	66.5 - 105.8 km	Avg	6.62	74.64
Suwannee	3422	66.5 - 105.8 km	Std Dev	1.29	13.75
Suwannee	3422	66.5 - 105.8 km	10th percentile	5.00	60.25
Suwannee	3422	66.5 - 105.8 km	25th percentile	5.62	65.22
Suwannee	3422	66.5 - 105.8 km	50th percentile	6.55	74.55
Suwannee	3422	66.5 - 105.8 km	75th percentile	7.60	81.70
Suwannee	3422	66.5 - 105.8 km	90th percentile	8.30	94.00
Suwannee	3422A	0 - 66.5 km	Count	1600	1598
Suwannee	3422A	0 - 66.5 km	Avg	6.71	76.40
Suwannee	3422A	0 - 66.5 km	Std Dev	1.43	14.88
Suwannee	3422A	0 - 66.5 km	10th percentile	4.90	58.90
Suwannee	3422A	0 - 66.5 km	25th percentile	5.80	68.40
Suwannee	3422A	0 - 66.5 km	50th percentile	6.76	76.69
Suwannee	3422A	0 - 66.5 km	75th percentile	7.62	83.90
Suwannee	3422A	0 - 66.5 km	90th percentile	8.40	93.16

Table 2. Continued.

River System	WBID	River km	Statistic	DO Concentration, mg/L	DO Percent Saturation
Suwannee	3422B	105.8 - 205.4 km	Count	1898	1894
Suwannee	3422B	105.8 - 205.4 km	Avg	6.31	69.96
Suwannee	3422B	105.8 - 205.4 km	Std Dev	1.51	15.30
Suwannee	3422B	105.8 - 205.4 km	10th percentile	4.60	53.31
Suwannee	3422B	105.8 - 205.4 km	25th percentile	5.20	60.61
Suwannee	3422B	105.8 - 205.4 km	50th percentile	6.16	68.95
Suwannee	3422B	105.8 - 205.4 km	75th percentile	7.26	77.30
Suwannee	3422B	105.8 - 205.4 km	90th percentile	8.30	86.57
Suwannee	3341	205.4 - 261.6 km	Count	599	599
Suwannee	3341	205.4 - 261.6 km	Avg	5.91	64.04
Suwannee	3341	205.4 - 261.6 km	Std Dev	1.94	17.04
Suwannee	3341	205.4 - 261.6 km	10th percentile	3.55	41.07
Suwannee	3341	205.4 - 261.6 km	25th percentile	4.50	51.93
Suwannee	3341	205.4 - 261.6 km	50th percentile	5.70	66.40
Suwannee	3341	205.4 - 261.6 km	75th percentile	7.20	76.35
Suwannee	3341	205.4 - 261.6 km	90th percentile	8.60	84.24
Suwannee	3341A	261.6 - 288.1 km	Count	350	350
Suwannee	3341A	261.6 - 288.1 km	Avg	7.08	77.46
Suwannee	3341A	261.6 - 288.1 km	Std Dev	1.62	10.78
Suwannee	3341A	261.6 - 288.1 km	10th percentile	5.49	65.45
Suwannee	3341A	261.6 - 288.1 km	25th percentile	5.90	71.55
Suwannee	3341A	261.6 - 288.1 km	50th percentile	6.60	78.16
Suwannee	3341A	261.6 - 288.1 km	75th percentile	8.30	84.90
Suwannee	3341A	261.6 - 288.1 km	90th percentile	9.40	90.01
Withlacoochee	3315	0 - 50.6 km	Count	986	986
Withlacoochee	3315	0 - 50.6 km	Avg	6.51	69.93
Withlacoochee	3315	0 - 50.6 km	Std Dev	1.64	12.70
Withlacoochee	3315	0 - 50.6 km	10th percentile	4.71	54.90
Withlacoochee	3315	0 - 50.6 km	25th percentile	5.30	61.70
Withlacoochee	3315	0 - 50.6 km	50th percentile	6.13	68.20
Withlacoochee	3315	0 - 50.6 km	75th percentile	7.50	78.28
Withlacoochee	3315	0 - 50.6 km	90th percentile	8.90	86.30

Table 3. Baseline DO conditions for portions of the Suwannee, Santa Fe, New, and Withlacoochee Rivers utilized by the Gulf Sturgeon and Oval Pigtoe Mussel. The 10th percentile and median percent DO saturation values were determined from data collected from 1991 through 2011.

Species	River System	River km	10th Percentile	Median
Oval Pigtoe Mussel	New River	0 - 31.5	52.5	67.7
Gulf Sturgeon	Santa Fe River	0 - 17.1	50.9	66.0
Gulf Sturgeon	Santa Fe River	17.1 - 31.1	47.6	74.0
Gulf Sturgeon	Santa Fe River	31.1 - 71.6	30.7	53.6
Oval Pigtoe Mussel	Santa Fe River	71.6 - 87.7	59.5	73.0
Oval Pigtoe Mussel	Santa Fe River	87.7 - 104.5	46.1	69.2
Oval Pigtoe Mussel	Santa Fe River	104.5 - 118.7	37.1	69.3
Gulf Sturgeon	Suwannee River	0 - 66.5	58.9	76.7
Gulf Sturgeon	Suwannee River	66.5 - 105.8	60.2	74.6
Gulf Sturgeon	Suwannee River	105.8 - 205.4	53.3	69.0
Gulf Sturgeon	Suwannee River	205.4 - 261.6	41.1	66.4
Gulf Sturgeon	Suwannee River	261.6 - 288.1	65.5	78.2
Gulf Sturgeon	Withlacoochee River	0 - 50.6	54.9	68.2

Table 4. Minimum number of samples not meeting applicable median criterion needed to put a water on the planning list with 80% confidence and on verified list with 90% confidence that more than 50% of measurements are below median.

Number of Samples	Number of exceedances required for 80% confidence that more than 50% of measurements are below median	Number of exceedances required for 90% confidence that more than 50% of measurements are below median
10	7	8
11	8	9
12	8	9
13	9	10
14	10	10
15	10	11
16	11	12
17	11	12
18	12	13
19	12	13
20	13	14
21	13	14
22	14	15
23	15	16
24	15	16
25	16	17
26	16	17
27	17	18
28	17	18
29	18	19
30	18	20
31	19	20
32	19	21
33	20	21
34	20	22
35	21	22
36	22	23
37	22	23
38	23	24
39	23	24
40	24	25
41	24	26
42	25	26
43	25	27
44	26	27
45	26	28
46	27	28
47	27	29
48	28	29
49	28	30
50	29	31
51	30	31
52	30	32
53	31	32
54	31	33
55	32	33
56	32	34
57	33	34
58	33	35
59	34	35
60	34	36
61	35	37
62	35	37
63	36	38
64	36	38
65	37	39
66	37	39
67	38	40
68	38	40
69	39	41
70	40	41
71	40	42
72	41	42
73	41	43
74	42	44
75	42	44
76	43	45
77	43	45
78	44	46
79	44	46
80	45	47
81	45	47
82	46	48
83	46	48
84	47	49
85	47	49
86	48	50
87	48	50
88	49	51
89	49	52
90	50	52
91	51	53
92	51	53
93	52	54
94	52	54
95	53	55
96	53	55
97	54	56
98	54	56
99	55	57
100	55	57
101	56	58
102	56	58
103	57	59
104	57	60
105	58	60
106	58	61
107	59	61
108	59	62
109	60	62
110	60	63
111	61	63
112	61	64
113	62	64
114	62	65
115	63	65
116	64	66
117	64	66
118	65	67
119	65	67
120	66	68
121	66	69
122	67	69
123	67	70
124	68	70
125	68	71
126	69	71
127	69	72
128	70	72
129	70	73
130	71	73
131	71	74
132	72	74
133	72	75
134	73	75
135	73	76
136	74	76
137	74	77
138	75	78
139	75	78
140	76	79
141	76	79

Table 4. Continued.

Number of Samples	Number of exceedances required for 80% confidence that more than 50% of measurements are below median	Number of exceedances required for 90% confidence that more than 50% of measurements are below median
142	77	80
143	78	80
144	78	81
145	79	81
146	79	82
147	80	82
148	80	83
149	81	83
150	81	84
151	82	84
152	82	85
153	83	85
154	83	86
155	84	86
156	84	87
157	85	88
158	85	88
159	86	89
160	86	89
161	87	90
162	87	90
163	88	91
164	88	91
165	89	92
166	89	92
167	90	93
168	90	93
169	91	94
170	91	94
171	92	95
172	93	95
173	93	96
174	94	96
175	94	97
176	95	97
177	95	98
178	96	99
179	96	99
180	97	100
181	97	100
182	98	101
183	98	101
184	99	102
185	99	102
186	100	103
187	100	103
188	101	104
189	101	104
190	102	105
191	102	105
192	103	106
193	103	106
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196	105	108
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218	116	119
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220	117	121
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237	126	129
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242	129	132
243	129	132
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245	130	134
246	131	134
247	131	135
248	132	135
249	132	136
250	133	136
251	133	137
252	134	137
253	134	138
254	135	138
255	135	139
256	136	139
257	136	140
258	137	140
259	137	141
260	138	141
261	138	142
262	139	142
263	139	143
264	140	143
265	140	144
266	141	144
267	141	145
268	142	145
269	142	146
270	143	147
271	143	147
272	144	148
273	144	148
274	145	149
275	145	149
276	146	150
277	147	150
278	147	151
279	148	151

Table 4. Continued.

Number of Samples	Number of exceedances required for 80% confidence that more than 50% of measurements are below median	Number of exceedances required for 90% confidence that more than 50% of measurements are below median
280	148	152
281	149	152
282	149	153
283	150	153
284	150	154
285	151	154
286	151	155
287	152	155
288	152	156
289	153	156
290	153	157
291	154	157
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314	165	169
315	166	170
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334	176	180
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338	178	182
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350	184	188
351	184	189
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417	218	223
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419	219	224

4 Protection of the Atlantic and Shortnose Sturgeon

Based on discussions with NOAA's National Marine Fisheries Service (NMFS) staff responsible for the protection of the Atlantic and shortnose sturgeon, the area in the St. Johns River between the U.S. Highway 17 Bridge in Palatka north to the Shands Bridge (U.S. Highway 16) bridge near Green Cove Springs (**Figure 2)** is an area where both species could potentially spawn in the future. According to the NMFS, any future spawning of the sturgeon in the St. Johns River would occur during the period from February through March.

To assure no adverse effects on the Atlantic and shortnose sturgeon juveniles, the current 5.0 mg/L DO criterion will be maintained in the St. Johns River between the U.S. Highway 17 Bridge in Palatka north to the Shands Bridge (U.S. Highway 16) bridge near Green Cove Springs during the months of February and March. During the other times of the year when the sturgeon are less sensitive, the DO criteria proposed for the Northeast and Big Bend bioregion would apply.

4.1 *Sturgeon in the St. Marys River*

Historically, Atlantic and shortnose sturgeon have occasionally been found in portions of the St. Marys River. According to NMFS staff, most of the sturgeon captures in the St. Marys have occurred between river km 26 and 44. However, there is no evidence that spawning has occurred in the St. Marys River due to natural conditions not being favorable. Even though the portions of the Marys River where sturgeon have been captured have very limited anthropogenic inputs, the DO levels are naturally low with significant portions of the river commonly exhibiting DO concentrations below 3 mg/L as a result of the natural conditions including wetland inputs, high color, high degree of shading/canopy cover, low flow, etc. Additionally, NMFS staff have indicated that sturgeon have been captured in the St. Marys at DO concentrations as low as 2.7 mg/L.

While the natural DO levels in the St. Marys may not be ideal for the widespread occurrence of the sturgeon, FDEP is prohibited by state statute from implementing regulations that would require natural background conditions to be ameliorated. Since the DO criteria proposed for the Northeast and Big Bend bioregion are protective of the natural DO levels found in the St. Marys River, no additional modification was deemed necessary.

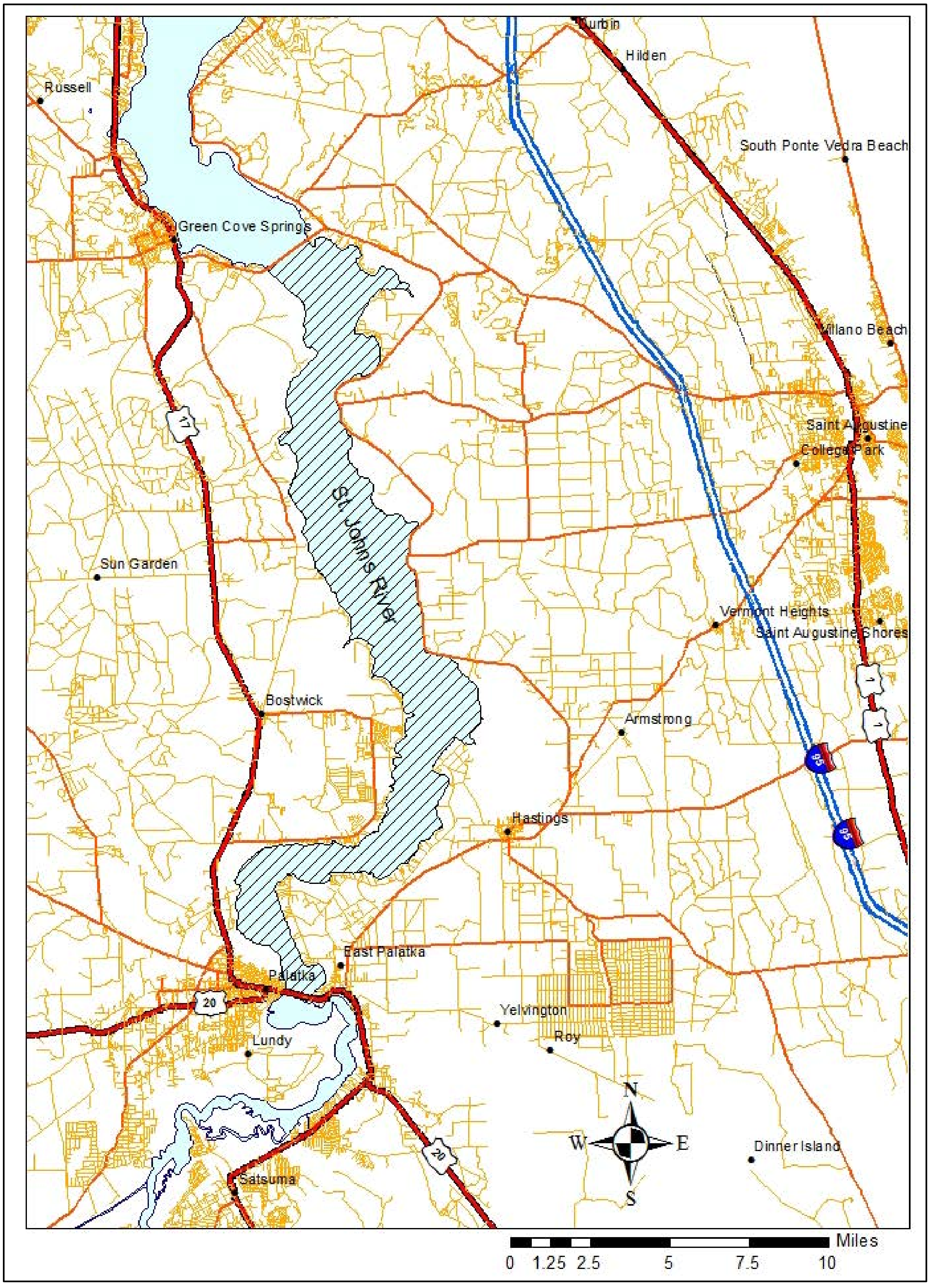

Figure 2. The portion of the St. Johns River between the U.S. Highway 17 Bridge in Palatka north to the Shands Bridge (U.S. Highway 16) bridge near Green Cove Springs (shown by hatching) requiring alternative DO criteria to assure potential sturgeon spawning habitat is protected.