

Hillsborough Independent Monitoring Program: Pre-operational Characterization of Benthic Habitats of the Alafia & Little Manatee Rivers

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INTRODUCTION

The Environmental Protection Commission of Hillsborough County (EPCHC) has been collecting samples in the Alafia River since 1995 as part of a larger bay-wide benthic monitoring program initiated during in 1993 (Tampa Bay National Estuary Program 1996). The original objectives of this program were to discern the “health”—or “status”— of the bay’s sediments by developing a Benthic Index for Tampa Bay as well as evaluating sediment quality by means of Sediment Quality Assessment Guidelines (SQAGs. Beginning in 1998 and continuing through 2000, support for this monitoring was provided, in part, by the Southwest Florida Water Management District (SWFWMD).

In 1999, Tampa Bay Water developed a Master Water Plan to provide additional water resources for the Tampa Bay region while at the same time reduce dependence upon groundwater sources. Part of this plan calls for diverting freshwater inflows to an off stream reservoir during periods of high flow (PBS&J 1999). In response to the TBW proposal, the Hillsborough County Board of County Commissioners (BOCC) requested that Hillsborough County and EPCHC staff develop an independent monitoring program in 1999 to address concerns of potential environmental impacts of this and other proposed TBW projects.

As part of their response to this request, the EPCHC proposed an increase in benthic macroinvertebrate sampling in the Alafia River during the three “wet seasons” preceding the initiation of withdrawals, to be followed by at least three years of post-diversion sampling. Comparisons are to be made with a “reference” estuary, the Little Manatee River.

The Alafia River extends approximately 40 km and the estuary extends approximately 19-km (Parsons Engineering Science, Inc. 2002; Mote Marine Laboratory 2003) The watershed is approximately 1,100 km², with that of the main stem of the Alafia encompassing approximately 129 km² (Parsons Engineering Science, Inc. 2002). Major land uses in the estuarine portion include forest, residential, and agriculture (Parsons Engineering Science, Inc. 2002). Daily freshwater inflows at Lithia Springs have ranged from 4 to 5,050 cfs with the median daily flow of 115 cfs (USGS 2004).

Dames & Moore (1975) surveyed the Alafia River benthos during 1973 and 1974 and included two stations in the estuarine portion of the river. At the most bayward station, mollusk and amphipods were numerically dominant. At the more upstream station polychaete worms and a chironomid were abundant. Mote Marine Laboratory (2003) surveyed the river up to River kilometer (Rkm) 15 during both wet (September 2001) and dry seasons (May 1999). Among the more important findings of this study was that the estuary could be divided into two primary faunal zones for benthic macroinvertebrates: 0-15 and 16-24 ppt. They also suggested that within these zones, there were faunal assemblages characteristic of more narrow salinity ranges.

This report summarizes data collected from 1995 –2002 for the Alafia River and compares these data with those from the Little Manatee River during 1996-2002.

METHODS

Study Design

A “Before-After-Control-Impact” (BACI; Green 1979) approach was taken to assess putative impacts to benthic macroinvertebrates from the diversion of freshwater inflow to the Alafia River. It is imperative that baseline data be collected prior to any putative impact, thus providing a “temporal control” (Green 1979). The “before” impact sampling for the Alafia River was expected to include at least three “wet” season sampling periods. In fact, a fourth pre-operational sampling event was conducted during Fall 2002, although the biological data from this sampling are not yet available.

The BACI design requires that at least one “control” area be monitored. For this region, the Little Manatee River represented the best option for a control.

Data collected from 1995-1998 to determine the minimum sample size for the pre- and post-operational periods. The level of effort selected was that considered to be the minimum sample size necessary to detect a 20% change in mean ($\log_{10} n+1$) numbers of taxa (S) 80% of the time with a 5% ($p=0.05$) likelihood that any detected change was due to chance. This analysis (SPSS 2000) indicated that at least 110 samples should be collected during the “wet” season prior to increasing the amount of freshwater withheld from the Alafia River and again during the first three years after operation (Figure 2). The addition of the 2002 sampling event effectively increases the power to detect a 20% change in mean S . A similar process was undertaken for the Little Manatee River and the resultant minimum sample size was estimated to be 30 (Figure 1).

Field Collection and Laboratory Procedures:

A total of 192 stations were sampled in the Alafia River (1995-2002) and 100 stations were sampled in the Little Manatee River (1996-2002) (Table 1; Figures 2 and 3). Sample locations were randomly selected from computer-generated coordinates. Benthic samples were collected using a Young grab sampler following the field protocols outlined in Courtney *et. al.* (1993). Laboratory procedures followed the protocols set forth in Courtney *et. al.* (1995).

Data Analyses:

The degree of water column stratification was based on criteria suggested by NOAA (Hyland *et al.* 1996) for differences between surface and bottom water density (as sigma-t). Stratification was “low” where the difference was <1 and “high” when the difference is >2. Species richness (S), Shannon-Wiener diversity (H’), and Evenness (J) were calculated using PISCES Conservation Ltd.’s (2001) “Species Diversity and Richness II” software. Descriptive statistics, regression analyses, analysis of variance (ANOVA), multivariate analysis of variance (MANOVA), and the Kolmogorov-Smirnov (KS) “two-sample” test (used to compare frequency distributions by salinity zone, year, or “control” vs. “impact” areas), and graphs were generated using SYSTAT 10 (SSPS Inc. 2000). Maps were generated using GIS Arcview ver. 3.2 (ESRI 1999).

Where statistical tests are used, the criterion for a “statistically significant” difference will be $p \leq 0.2$. The choice for this higher p value is based on the need to protect a valuable resource and be able to detect a change even if it is not “real” rather than opt for a lower level of protection and perhaps miss “real” changes. This is consistent with the ‘Precautionary Principle’:

“where there are serious threats of irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost effective measures to prevent environmental degradation” (UNEP 1992).

A corollary of this principle is that non-statistically significant results should be recognized as early warning signs (Sanderson & Peterson 2002). *Delaying action until ecological effects are established with a high degree of certainty may very well increase the risk of ecological damage* (Buhl-Mortensen & Welin 1998).

Sediment type (*e.g.*, sand, mud) was determined by regressing %SC vs. mean grain (ϕ) size for Tampa Bay data collected by Long *et al.* (1994) using TableCurve 2D (AISN Software, 2000). These data were used to develop a relationship between %SC and mean grain size: %SC = $1/(0.0097 + 1.575 * e^{-\phi})$ adjusted $r^2 = 0.947$). Wentworth size classes for sediments (*cf.* Percival & Lindsay 1997) were then estimated for each %SC value.

Non-metric Multidimensional Scaling (MDS) is an ordination technique in which rank similarities of a large number of variables are expressed as a two-dimensional map (Clarke & Warwick 2001). In these analyses, taxa abundances were fourth root transformed $n+0.1$ and the similarity coefficient was Bray-Curtis (PRIMER-E Ltd. 2001).

PRIMER's ANOSIM and SIMPER (PRIMER-E Ltd. 2001) programs were used to compare the dissimilarity of benthic assemblages between the Alafia and Little Manatee rivers, between similar habitats (based upon Venice salinity zones and sand and mud sediments) in both tributaries, between adjacent habitats in the Alafia River, and between consecutive years in the Alafia River and Little Manatee rivers.

Relationships between benthic community structure and a suite of physical, hydrological, and hydrographic variables were carried out in two phases. First, the RELATE test in PRIMER (PRIMER-E Ltd. 2001) was used to compare the biotic (4th root transformed abundances; Bray-Curtis similarity) with an abiotic matrix (standardized variables; Euclidean distance). The variables used included temperature, salinity, dissolved oxygen, stratification, year, ENSO state, depth, %SC, and \log_{10} transformed cumulative flow over 7, 14, 28, 56, and 112 days preceding sample collection. If the RELATE test is significant, then the BIO-ENV procedure (Clarke & Ainsworth 1993) was used to determine which variables were most closely associated with the overall benthic structure.

RESULTS

Water-Mass Characteristics

MANOVA showed that water mass characteristics (near bottom temperature and salinity) were similar between the Control (Little Manatee River) and Impact (Alafia River) estuaries (Wilks Lambda $F_{2,218}=2.6$; $p=0.07$;) (Figure 4). The KS test, however, showed that the frequency distributions for the Alafia and Little Manatee rivers differed for both temperature ($p=0.02$) (Figure 5) and salinity ($p<0.01$) (Figure 6). Density stratification of the water column was frequent in the Alafia River than in the Little Manatee River (Figure 7).

Dissolved Oxygen

DO was generally lower in the Alafia River, where approximately 50% of the samples were hypoxic (Figure 8). Hypoxia was pervasive in the lower reaches of the Alafia River (Figure 9) and in the bayous of the Little Manatee River (Figure 10). The frequency distributions of near-bottom DO differed between the Alafia and Little Manatee rivers (KS test; $p < 0.001$). Using forward stepwise multiple regression, near-bottom DO was associated with depth, salinity, density stratification, temperature, %SC, and short-term (7 to 28 day) cumulative freshwater inflows (Table 2).

Sample Depths

Sample depths in the Alafia River ranged to approximately 5-m (Figure 11) and Alafia River sites were generally deeper than Little Manatee River sites. Sample depths differed for the Alafia and Little Manatee rivers (KS test; $p = 0.04$).

Sediment Types

Sand-sized sediments predominated in both rivers (Figure 12), although mud-sized sediments were more often encountered in the Alafia River. The %SC content differed between the Alafia and Little Manatee rivers (KS test $p < 0.001$).

Estuarine Habitats:

The predominant benthic habitats, defined by Venice salinity zone and sediment type (sand, mud), were mesohaline and polyhaline sands (Figure 13). Mesohaline and polyhaline muds were generally confined to the Alafia River. Overall, the habitat composition of these two rivers are >60% similar.

Benthic Community:

Species richness ranged from 0 to 48 taxa in the Alafia River (Figure 14), with the highest numbers generally in the polyhaline zone and fewest in the oligohaline zone. The frequency distributions in tidal freshwater was not significantly different from that of both the oligohaline and mesohaline zones (KS test; $p > 0.05$); the polyhaline and mesohaline zones also had similar frequency distributions. Species richness was generally higher in the Little Manatee River than in the Alafia (Figure 15) (KS test; $p < 0.001$).

Benthic abundance ranged from 0 to $>21,000 \text{ m}^{-2}$ in the Alafia River (Figure 16), with the highest numbers generally in the mesohaline and polyhaline zones and lowest in the oligohaline zone. The frequency distribution in tidal freshwater was significantly different from that of the polyhaline zone and that of the oligohaline zone was significantly different from both the mesohaline and polyhaline zones (KS test; $p < 0.05$). Total numbers of benthic macroinvertebrates m^{-2} was generally higher in the Little Manatee River than in the Alafia (Figure 17) (KS test; $p < 0.001$).

In the Alafia River, the benthic assemblages (Figure 18) within the tidal freshwater mud and oligohaline mud habitats were not significantly different (ANOSIM test; $p = 0.7$; Table 3) nor were the oligohaline mud and mesohaline mud assemblages ($p = 0.2$). The mesohaline mud and sand assemblages were significantly different at $p = 0.1$; all other habitat comparisons were significantly different at $p < 0.1$ (Table 3).

The comparison of similar habitats in the Alafia and Little Manatee rivers (Table 4) showed that the benthic assemblages of comparable mud habitats in both systems were not significantly different (ANOSIM test; $p > 0.14$).

Interannual comparisons during the period of enhanced sampling (1999-2002) showed that within the Alafia River, only 1999 and 2000 had a similar community structure (Figure 19; Table 5). Benthic community structure in the Alafia and Little Manatee rivers were significantly different in each of the four years of pre-impact monitoring (Figure 19; Table 5).

Association Between Biotic and Abiotic Variables:

The premise for the study design was that, baywide, there is a positive association between numbers of taxa (S) and salinity. Using all data available to date for the Alafia River, this relationship (Figure 19) can be expressed as: $S = 7.43 + 0.20 * \text{Salinity}^{-6.7}$ (adjusted $r^2 = 0.15$). Species richness was essentially unchanged over the 0-15 ppt range (Figure 20). This contrasts with the Little Manatee River where there is a general increase in S as salinity increases (Figure 21).

Inclusion of other measured abiotic variables (*e.g.*, temperature, DO, depth, and cumulative freshwater inflows, %SC) improved this association. Forward stepwise multiple regression, using transformed variables showed that S increases with salinity, DO and cumulative flow over 56 days and decreases as %SC increases and 14-day flows increase (Table 2). Total macroinvertebrate abundances were also associated with salinity, %SC, and cumulative freshwater inflows (Table 2). Cumulative freshwater inflows are shown in Appendix A.

The RELATE test showed that the biotic and abiotic data matrices for the Alafia River were similar ($Rho = 0.089$; $p = 0.001$). The BIO-ENV test showed that the abiotic variables which best explained the biotic structure were 56-day cumulative flow and ENSO state [El-Nino, neutral, La Nina; *cf.* NOAA 2004] ($r_s = 0.18$) and 28 and 56-day cumulative flows and ENSO state ($r_s = 0.18$).

The RELATE test comparing the resemblance between the biotic and the abiotic (excluding sediment contaminants) similarity matrices for the Little Manatee River was significant at 3.7% ($Rho = 0.085$). The four highest Spearman rank correlation coefficients (> 0.91) were for five variable combinations:

- 1- Stratification Index, Cumulative flow at 7 days, 56 days, 112 days, and year
- 2- Stratification Index, 112 day flow, %SC, temperature, and year
- 3- Stratification Index, 56 day flow, %SC, temperature, and year
- 4- 14 day cumulative flow, 28 day flow, 112 day flow, DO, and year.

Alafia River "Inset":

The portion of the Alafia River encompassing Rkm 6-9 is the reach of the estuary in which the *change* in the location of the 0.5 ppt isohaline is expected to be greatest under the proposed

withdrawal schedule (Coastal Environmental, Inc. and PBS&J 1998). It has been selected for enhanced sampling under TBW's Hydrobiological Monitoring Program for its Water Use Permit.

Data collected under the HIMP in this part of the Alafia River show that near-bottom salinities ranged from 0 to 23.5 ppt, with more than half of the observations <1 ppt (Figure 22). Stratification Index values mirrored the pattern for salinity (Figure 23). Sample depths range to 3-m, with the majority between 1 and 2-m (Figure 24). Sand-sized sediments predominated (Figure 25). More than 40% of the samples were hypoxic (Figure 26).

The benthic assemblage in this reach of the estuary was characterized by species richness values similar to those of the oligohaline and tidal freshwater zones of the estuary as a whole (Figures 27 and 14). Overall abundance (Figure 28) ranged to >20,000 organisms m⁻². Characteristic taxa within this reach included *Mytilopsis leucophaeata* (Bivalvia), tubificid oligochaetes, *Stenoninereis martini* (Polychaeta), and *Grandidierella bonnieroides* (Amphipoda) (Table 6).

Sample Power Analyses and Monitoring Issues

The original study design was based upon the decision to be able to detect a 20% change in estuarine mean species richness (S) during the 'wet' season. The above data (Figure 20) suggest that S is unlikely to change in association with any increase in salinity over the range of 0-15 ppt. Therefore it may be more instructive to examine changes in benthic community structure as well as the distribution of selected species common in the estuary.

The current sampling effort (162 samples during the four-year pre-impact period) is able to detect a 20% change in both species richness and overall abundance at P>0.9. Within the different salinity zones, P is lowest in the oligohaline zone and highest in the polyhaline zone.

DISCUSSION

The objective of the HIMP, with respect to diversion of freshwater from the Alafia River is two-fold: (1) to characterize baseline conditions prior to diversions from the Alafia River and then be able to detect ecological changes in the Alafia River area *should they occur* and (2) to compare these data

and conclusions with those collected by the permittee. This report summarizes the four year baseline period characterizing the structure and composition of the benthic community in the Alafia River prior to freshwater inflow diversion. Ultimately the determination of adverse “impact” to biotic communities, as distinguished from a statistical “change”, will be based upon a “weight of evidence” approach (Burton *et al.* 2002; Smith *et al.* 2002) incorporating multiple lines of evidence gleaned from abiotic (salinity) and biotic (*e.g.*, S, species abundances) variables.

For the purposes of the HIMP, the determination of adverse “impact” to biotic communities, as distinguished from a statistical “change”, will be based upon a “weight of evidence” (WOE) approach (Burton *et al.* 2002; Smith *et al.* 2002). The WOE approach will incorporate multiple lines of evidence gleaned from abiotic (salinity) and biotic (*e.g.*, S, distributions of selected species) variables as well as multivariate analyses of community structure, including association with abiotic variables such as freshwater inflow, and comparisons of these data with those from the Little Manatee River. Should different analytical methods yield generally similar results, the likelihood that measured changes are “real” is greater. The detection of statistical “changes” [in the benthos] is and of itself not necessarily evidence of adverse ecological change (Sanderson & Petersen 2002).

The primary approach then, will be to compare “pre-“ vs. “post-“ differences in S as well as estimates of the densities of selected taxa within the study area using ANOVA. These analyses will be supplemented by multivariate analyses. The study areas will be post-stratified (*e.g.*, by salinity zones, habitats) to permit comparisons, albeit with less power, on a smaller spatial scale.

The data collected to date demonstrate that although the Little Manatee is not an “ideal” control, it should be adequate. Underwood (1996) points out that it is not necessary to find an “identical” control site; rather, the control “must simply represent the range of habitats of the...impact location”.

The habitats sampled in both rivers, during the preoperational period are >60% similar, with mesohaline and polyhaline sand habitats predominant in both tributaries. The two rivers do demonstrate *statistical* differences in their temperature and salinity regimes, sediment type, and sample depths. Biotic assemblages of the two tributaries also differ, even within similar habitats (salinity zone and sediment type).

The benthic assemblages of the Alafia River were shown to differ by salinity zone as well as by habitat. Interannual differences were also evident during the 1999-2002 period when sampling was more intensive. Abiotic factors associated with univariate and multivariate measures of benthic community structure included, among other variables, cumulative freshwater inflow. Multivariate community structure was also affected by the ENSO state in the season preceding sampling (*cf.* Schmidt & Luther 2002; NOAA 2004). The effect of the antecedent ENSO state would also be associated with cumulative freshwater inflows. However, seasonal differences in the ENSO state could affect cumulative freshwater inflows over longer time periods not necessarily reflected by the ENSO state in the spring/summer preceding sampling.

CONCLUSIONS

Benthic samples were collected from Alafia River since the 1995 “wet” season, with the more spatially intensive sampling required for the HIMP starting in 1999. Sampling effort at the “control” estuary, the Little Manatee River, began in 1996, with the more intensive HIMP-based sampling also starting in 1999.

The estuarine portion of the Alafia River showed a wide-range of salinities and sediment types. The predominant habitats, however, were mesohaline and polyhaline sands. Much of the estuary was also hypoxic. Benthic assemblages in the Alafia River differed by habitat and by year. Within habitats, the assemblages of the Alafia River (“impact”) were also significantly different from those of the Little Manatee River (“control”).

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Table 1. Number of samples collected for analysis of benthic macroinvertebrates in the Alafia and Little Manatee rivers, by year, 1995-2002.

YEAR	ALAFIA	LITTLE MANATEE
1995	5	0
1996	5	4
1997	7	6
1998	5	6
1999	42	21
2000	43	21
2001	45	22
2002	40	20
TOTAL	192	100

Table 2. Results of forward stepwise multiple regression analyses. Association between selected abiotic variables and near-bottom dissolved oxygen concentrations, species richness and total abundance, Alafia River.

	DO	NUMBERS OF TAXA	ABUNDANCE
$F_{df} (p)$	$F_{8,180}=74 (<0.001)$	$F_{5,177} = 26 (<0.001)$	$F_{7,175}=22 (<0.001)$
Adjusted multiple r^2	0.76	0.41	0.37
Constant	-0.361	1.997	-5.99
$\text{Log}_{10}n+1$ Depth (m)	-0.319	NS	NS
$\text{Log}_{10}n+1$ Salinity (ppt)	-0.177	0.488	1.136
$\text{Log}_{10}N+1$ Temperature $^{\circ}\text{C}$	1.436	NS	NS
$\text{Log}_{10} n+1$ Stratification Index	-0.267	NS	NS
ASN % SC	0.089	-1.15	-2.93
$\text{Log}_{10}n+1$ 7-day Cumulative Flow (cfs)	-0.518	NS	2.604
$\text{Log}_{10}n+1$ 14-day Cumulative Flow (cfs)	0.986	-0.64	-4.455
$\text{Log}_{10}n+1$ 28-day Cumulative Flow (cfs)	-0.684	NS	NS
$\text{Log}_{10}n+1$ 56-day Cumulative Flow (cfs)	NS	1.136	1.503
$\text{Log}_{10}n+1$ 112-day Cumulative Flow (cfs)	NS	NS	2.05

Table 3. ANOSIM and SIMPER tests comparing benthic community structure within the Alafia River.

Global Test

Sample statistic (Global R): 0.297

Significance level of sample statistic: 0.1%

Pairwise Tests

Groups	Statistic	R	Significance Level %
1-ATM, ATS	0.279		0.4
2-AOS, AOM	0.122		9.6
3-AMS, AMM	0.099		11.9
4-APM, APS	0.2		0.1
5-AOS, ATS	0.257		0.1
6-AOS, AMS	0.154		0.7
7-APS, AMS	0.089		0.8
8-ATM, AOM	-0.065		71.1
9-AOM, AMM	0.064		22.3
10-APM, AMM	0.134		1.2

1-Groups ATM & ATS

Average dissimilarity = 81.51

Species	Group ATM Av.Abund	Group ATS Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mytilopsis leucophaeata	22.22	1209.68	6.68	0.92	8.19	8.19
Chironomus sp.	13.89	208.06	6.53	0.79	8.02	16.21
TUBIFICIDAE	16.67	220.16	6.43	0.98	7.89	24.10
Grandidierella bonnieroides	11.11	276.61	5.85	1.17	7.18	31.28

2-Groups AOS & AOM

Average dissimilarity = 79.37

Species	Group AOS Av.Abund	Group AOM Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Streblospio spp.	90.63	304.17	9.03	0.89	11.37	11.37
Stenoninereis martini	0.00	141.67	8.25	0.64	10.40	21.77
Mytilopsis leucophaeata	1129.69	533.33	5.33	0.55	6.72	28.49

3-Groups AMS & AMM

Average dissimilarity = 81.73

Species	Group AMS Av.Abund	Group AMM Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Streblospio spp.	688.97	211.11	4.89	0.89	5.98	5.98
Mytilopsis leucophaeata	123.53	705.56	4.29	0.62	5.25	11.24
Ampelisca abdita	811.03	175.00	3.76	0.87	4.60	15.83
TUBIFICIDAE	168.38	13.89	3.50	0.86	4.28	20.11
Stenoninereis martini	45.59	61.11	3.46	0.62	4.23	24.34
Paraprionospio pinnata	71.32	25.00	2.32	0.65	2.83	27.17

Table 3-CONTINUED. ANOSIM and SIMPER tests comparing benthic community structure within the Alafia River.

4-Groups APM & APS

Average dissimilarity = 85.27

Species	Group APM	Group APS	Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund				
Glottidia pyramidata	25.00	1338.73	3.57	0.93	4.18	4.18
TUBIFICIDAE	12.50	321.57	3.33	0.85	3.90	8.08
Paraprionospio pinnata	50.78	118.63	3.23	0.60	3.78	11.87
Streblospio spp.	32.81	396.08	3.17	0.66	3.72	15.59
Ampelisca abdita	221.09	210.29	3.16	0.53	3.71	19.29
Stenoninereis martini	5.47	32.35	3.12	0.40	3.65	22.95
Monticellina dorsobranchialis	13.28	819.61	2.86	0.86	3.35	26.30

5- Groups AOS & ATS

Average dissimilarity = 82.34

Species	Group AOS	Group ATS	Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund				
Mytilopsis leucophaeata	1129.69	1209.68	5.94	0.92	7.21	7.21
TUBIFICIDAE	120.31	220.16	5.52	0.97	6.71	13.92
Chironomus sp.	17.19	208.06	5.05	0.67	6.14	20.06
Streblospio spp.	90.63	91.13	4.86	0.78	5.90	25.96

6-Groups AOS & AMS

Average dissimilarity = 82.36

Species	Group AOS	Group AMS	Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund				
Streblospio spp.	90.63	688.97	5.90	0.90	7.17	7.17
TUBIFICIDAE	120.31	168.38	4.79	0.96	5.82	12.98
Stenoninereis martini	0.00	45.59	3.76	0.59	4.56	17.55
Ampelisca abdita	1.56	811.03	3.71	0.71	4.50	22.05
Mytilopsis leucophaeata	1129.69	123.53	3.20	0.53	3.89	25.94

7-Groups APS & AMS

Average dissimilarity = 82.63

Species	Group APS	Group AMS	Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund				
Streblospio spp.	396.08	688.97	3.72	0.82	4.50	4.50
TUBIFICIDAE	321.57	168.38	3.00	0.81	3.63	8.13
Glottidia pyramidata	1338.73	250.00	2.96	0.93	3.58	11.71
Ampelisca abdita	210.29	811.03	2.89	0.71	3.50	15.21
Stenoninereis martini	32.35	45.59	2.56	0.50	3.09	18.30
Monticellina dorsobranchialis	819.61	13.97	2.43	0.88	2.94	21.24
Paraprionospio pinnata	118.63	71.32	2.12	0.64	2.56	23.80
Mytilopsis leucophaeata	80.39	123.53	1.99	0.49	2.41	26.22

8-Groups ATM & AOM

Average dissimilarity = 69.82

Species	Group ATM	Group AOM	Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund				
Stenoninereis martini	19.44	141.67	13.10	0.92	18.76	18.76
Mytilopsis leucophaeata	22.22	533.33	8.61	0.63	12.33	31.09

Table 3-CONTINUED. ANOSIM and SIMPER tests comparing benthic community structure within the Alafia River.

9-Groups AOM & AMM

Average dissimilarity = 81.15

Species	Group AOM		Group AMM		Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund		Av.Abund					
Mytilopsis leucophaeata	533.33		705.56		8.21	0.70	10.12	10.12
Stenoninereis martini	141.67		61.11		7.33	0.65	9.03	19.15
Streblospio spp.	304.17		211.11		6.47	0.75	7.98	27.13

10-Groups APM & AMM

Average dissimilarity = 82.95

Species	Group APM		Group AMM		Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund		Av.Abund					
Mytilopsis leucophaeata	1.56		705.56		5.74	0.58	6.92	6.92
Streblospio spp.	32.81		211.11		5.06	0.72	6.10	13.02
Stenoninereis martini	5.47		61.11		4.81	0.60	5.80	18.82
Ampelisca abdita	221.09		175.00		4.36	0.69	5.25	24.08
Paraprionospio pinnata	50.78		25.00		3.95	0.73	4.76	28.83

Table 4. ANOSIM and SIMPER analyses comparing benthic community structure within similar habitats by study area (Alafia and Little Manatee rivers).

Global Test

Sample statistic (Global R): 0.297

Significance level of sample statistic: 0.1%

Pairwise Tests

Groups	R Statistic	Significance Level %
ATS, LTS	0.209	1.9
AOS, LOS	0.244	0.3
AMS, LMS	0.166	0.2
APS, LPS	0.167	0.5
AOM, LOM	0.778	14.3
APM, LPM	0.094	22.6

Groups ATS & LTS

Average dissimilarity = 81.50

Species	Group ATS Av.Abund	Group LTS Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
TUBIFICIDAE	220.16	282.69	4.65	1.02	5.71	5.71
Mytilopsis leucophaeata	1209.68	19.23	4.20	0.88	5.15	10.86
Grandidierella bonnieroides	276.61	28.85	3.86	1.06	4.73	15.59
Chironomus sp.	208.06	11.54	3.68	0.66	4.51	20.10
Cyathura polita	36.29	105.77	3.53	0.94	4.33	24.43
Laeonereis culveri	128.23	84.62	3.49	0.90	4.28	28.71

Groups AOS & LOS

Average dissimilarity = 82.06

Species	Group AOS Av.Abund	Group LOS Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
TUBIFICIDAE	120.31	364.29	5.46	1.16	6.65	6.65
Cyathura polita	21.88	196.43	5.42	1.00	6.60	13.25
Grandidierella bonnieroides	25.00	696.43	4.94	1.16	6.02	19.27
Ampelisca abdita	1.56	198.21	3.79	0.90	4.62	23.89
Laeonereis culveri	18.75	150.00	3.68	0.99	4.48	28.38

Groups AMS & LMS

Average dissimilarity = 83.02

Species	Group AMS Av.Abund	Group LMS Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
TUBIFICIDAE	168.38	827.98	4.19	0.69	5.05	5.05
Streblospio spp.	688.97	108.93	3.77	0.82	4.55	9.59
Ampelisca abdita	811.03	79.17	2.77	0.71	3.34	12.93
Cyathura polita	42.65	449.40	2.70	0.88	3.25	16.18
Grandidierella bonnieroides	24.26	1338.10	2.37	0.81	2.85	19.04
Stenoninereis martini	45.59	10.71	2.35	0.56	2.83	21.87
Xenanthura brevitelson	0.00	272.02	2.33	0.75	2.80	24.67
Laeonereis culveri	38.24	141.07	2.18	0.83	2.62	27.29

Table 4-CONTINUED. ANOSIM and SIMPER analyses comparing benthic community structure within similar habitats by study area (Alafia and Little Manatee rivers).

Groups APS & LPS

Average dissimilarity = 84.24

Species	Group APS	Group LPS	Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund				
Monticellina dorsobranchialis	819.61	984.38	2.51	0.99	2.97	2.97
Ampelisca holmesi	170.59	1281.25	2.36	1.24	2.80	5.77
Cerapus sp. C ("tubularis")	0.49	1796.88	2.12	0.90	2.51	8.29
Glottidia pyramidata	1338.73	410.42	2.07	1.01	2.46	10.74
TUBIFICIDAE	321.57	325.00	1.81	0.78	2.15	12.89
Aricidea philbinae	1.96	292.71	1.76	0.88	2.08	14.98
Mysella planulata	121.57	359.38	1.49	1.04	1.77	16.74
Streblospio spp.	396.08	34.38	1.44	0.84	1.71	18.45
Amygdalum papyrium	52.94	229.17	1.39	1.20	1.65	20.10
Ampelisca abdita	210.29	108.33	1.38	0.79	1.64	21.74
Prionospio perkinsi	288.73	37.50	1.29	0.85	1.53	23.27
Tubificoides brownae	98.53	178.13	1.27	0.48	1.51	24.78
Cyclaspis cf. varians	16.18	256.25	1.26	1.01	1.50	26.28

Groups AOM & LOM

Average dissimilarity = 92.69

Species	Group AOM	Group LOM	Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund				
Polypedilum halterale grp.	0.00	675.00	11.80	5.14	12.73	12.73
TUBIFICIDAE	0.00	475.00	10.81	5.14	11.66	24.39
Cyathura polita	0.00	200.00	8.70	5.14	9.39	33.78

Groups APM & LPM

Average dissimilarity = 77.56

Species	Group APM	Group LPM	Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund				
TUBIFICIDAE	12.50	462.50	6.43	1.03	8.30	8.30
Tubificoides brownae	31.25	425.00	6.15	1.00	7.93	16.23
Paraprionospio pinnata	50.78	12.50	5.15	0.68	6.63	22.86
Ampelisca abdita	221.09	0.00	4.08	0.44	5.26	28.12

Table 5. ANOSIM and SIMPER analyses compare benthic community structure, by year, in the Alafia and Little Manatee rivers, 1999-2002.

Global Test

Sample statistic (Global R): 0.134
 Significance level of sample statistic: 0.1%

Pairwise Tests

Groups	R Statistic	Significance Level %
AR00, AR99	0.014	18.3
AR01, AR00	0.046	2.9
AR02, AR01	0.097	0.4
AR99, LMR99	0.111	0.8
AR00, LMR00	0.142	0.1
AR01, LMR01	0.239	0.1
AR02, LMR02	0.145	0.5

Groups AR00 & AR99
 Average dissimilarity = 82.50

Species	Group AR00 Av.Abund	Group AR99 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Ampelisca abdita	332.32	706.71	4.56	0.70	5.53	5.53
Streblospio spp.	737.80	150.61	3.69	0.81	4.47	10.00
Mytilopsis leucophaeata	190.85	1357.32	3.69	0.59	4.47	14.46
TUBIFICIDAE	310.98	207.93	3.67	0.78	4.45	18.91
Stenoninereis martini	31.71	17.68	3.49	0.45	4.23	23.13
Glottidia pyramidata	170.12	628.66	2.51	0.71	3.05	26.18

Groups AR01 & AR00
 Average dissimilarity = 83.14

Species	Group AR01 Av.Abund	Group AR00 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Streblospio spp.	66.67	737.80	4.92	0.77	5.92	5.92
Stenoninereis martini	19.44	31.71	4.22	0.48	5.07	10.99
TUBIFICIDAE	88.89	310.98	3.85	0.75	4.63	15.62
Chironomus sp.	96.67	95.73	3.00	0.47	3.61	19.23
Ampelisca abdita	4.44	332.32	2.93	0.59	3.52	22.75
Mytilopsis leucophaeata	22.78	190.85	2.74	0.48	3.30	26.05

Groups AR02 & AR01
 Average dissimilarity = 84.62

Species	Group AR02 Av.Abund	Group AR01 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
TUBIFICIDAE	140.85	88.89	3.81	0.96	4.50	4.50
Streblospio spp.	201.83	66.67	3.73	0.77	4.41	8.91
Glottidia pyramidata	845.12	407.22	3.36	0.79	3.97	12.88
Stenoninereis martini	73.17	19.44	3.31	0.61	3.91	16.79
Monticellina dorsobranchialis	243.29	630.56	2.78	0.81	3.28	20.07
Paraprionospio pinnata	97.56	26.67	2.60	0.68	3.08	23.15
Cyathura polita	37.80	21.67	2.37	0.61	2.80	25.95

Table 5-CONTINUED. ANOSIM and SIMPER analyses compare benthic community structure, by year, in the Alafia and Little Manatee rivers, 1999-2002.

Groups AR99 & LMR99

Average dissimilarity = 86.62

Species	Group AR99 Av.Abund	Group LMR99 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
TUBIFICIDAE	207.93	273.75	4.23	0.68	4.89	4.89
Ampelisca abdita	706.71	163.75	3.62	0.86	4.18	9.07
Cyathura polita	9.15	117.50	3.51	0.89	4.06	13.13
Glottidia pyramidata	628.66	193.75	2.67	0.80	3.09	16.22
Laeonereis culveri	28.66	155.00	2.40	0.90	2.77	18.98
Xenanthura brevitelson	0.00	191.25	2.36	0.59	2.72	21.70
Polypedilum scalaneum group	0.00	83.75	2.22	0.62	2.57	24.27
Mytilopsis leucophaeata	1357.32	1.25	2.19	0.52	2.53	26.80

Groups AR00 & LMR00

Average dissimilarity = 84.57

Species	Group AR00 Av.Abund	Group LMR00 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
TUBIFICIDAE	310.98	182.14	4.19	0.62	4.96	4.96
Streblospio spp.	737.80	10.71	3.49	0.70	4.13	9.09
Stenoninereis martini	31.71	1.19	3.41	0.40	4.03	13.12
Ampelisca abdita	332.32	159.52	2.83	0.61	3.35	16.47
Paraprionospio pinnata	101.22	17.86	2.19	0.41	2.59	19.06
Ampelisca holmesi	196.95	983.33	2.10	0.71	2.48	21.54
Grandidierella bonnieroides	14.02	322.62	1.99	0.71	2.36	23.89
Mytilopsis leucophaeata	190.85	16.67	1.96	0.45	2.32	26.21

Groups AR01 & LMR01

Average dissimilarity = 88.25

Species	Group AR01 Av.Abund	Group LMR01 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
TUBIFICIDAE	88.89	434.09	4.53	0.67	5.13	5.13
Cerapus sp. C ("tubularis")	0.00	1432.95	2.74	0.93	3.10	8.23
Monticellina dorsobranchialis	630.56	646.59	2.44	0.80	2.76	10.99
Cyathura polita	21.67	110.23	2.41	0.61	2.73	13.72
Ampelisca holmesi	0.56	998.86	2.40	1.03	2.71	16.44
Grandidierella bonnieroides	63.33	405.68	2.29	0.70	2.60	19.03
Laeonereis culveri	26.11	92.05	2.22	0.66	2.51	21.55
Streblospio spp.	66.67	35.23	2.17	0.56	2.46	24.01
Xenanthura brevitelson	0.00	231.82	1.97	0.62	2.23	26.24

Groups AR02 & LMR02

Average dissimilarity = 84.46

Species	Group AR02 Av.Abund	Group LMR02 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
TUBIFICIDAE	140.85	572.22	2.83	0.96	3.35	3.35
Tubificoides brownae	108.54	259.72	2.53	0.76	3.00	6.35
Laeonereis culveri	35.98	291.67	2.41	1.01	2.86	9.21
Glottidia pyramidata	845.12	913.89	2.01	0.82	2.38	11.59
Grandidierella bonnieroides	42.07	356.94	1.91	0.77	2.26	13.85
Streblospio spp.	201.83	63.89	1.85	0.80	2.20	16.05
Cyathura polita	37.80	169.44	1.80	1.00	2.13	18.18
Apocorophium louisianum	1.22	8177.78	1.78	0.56	2.11	20.29
Polypedilum scalaneum group	21.34	97.22	1.76	0.88	2.08	22.37
Xenanthura brevitelson	0.00	245.83	1.64	0.75	1.95	24.32
Heteromastus filiformis	15.24	88.89	1.64	0.92	1.95	26.26

Table 6. SIMPER analysis of taxa characteristic of the Alafia River, Rkm 6-9, 1995-2002 (n=27): average abundance and contributions to dissimilarity.

Species	Av.Abund	Contrib%	Cum.%
Mytilopsis leucophaeata	1,633	23.8	23.8
TUBIFICIDAE	279	15.7	39.5
Stenoninereis martini	56	13.0	52.5
Grandidierella bonnieroides	77	10.1	62.6
Chironomus sp.	315	8.3	70.9
Laeonereis culveri	96	6.5	77.4
Littoridinops palustris	257	6.1	83.5
Streblospio spp.	238	4.2	87.7
Cyathura polita	26	2.4	90.1

Table 7. Estimated Power to detect 20% changes in mean $\log_{10} n+1$ S and mean $\log_{10} n+1$ total numbers m^{-2} in the Alafia River: estimated minimum sample sizes (n) based on data collected 1999-2002 and Power (P) for the actual sample sizes within Venice salinity zones.

RIVER	ESTIMATED TOTAL N for P=0.8 AND ACTUAL N (P)	Tidal Freshwater P (N)	Oligohaline P (N)	Mesohaline P (N)	Polyhaline P (N)
NUMBERS OF TAXA	97 (P=0.8) 162 (P=0.95)	0.41 (34)	0.14 (19)	0.59 (34)	0.74 (75)
ABUNDANCE	66 (P=0.8) 162 (P=0.99)	0.50 (34)	0.16 (19)	0.66 (34)	0.91 (75)

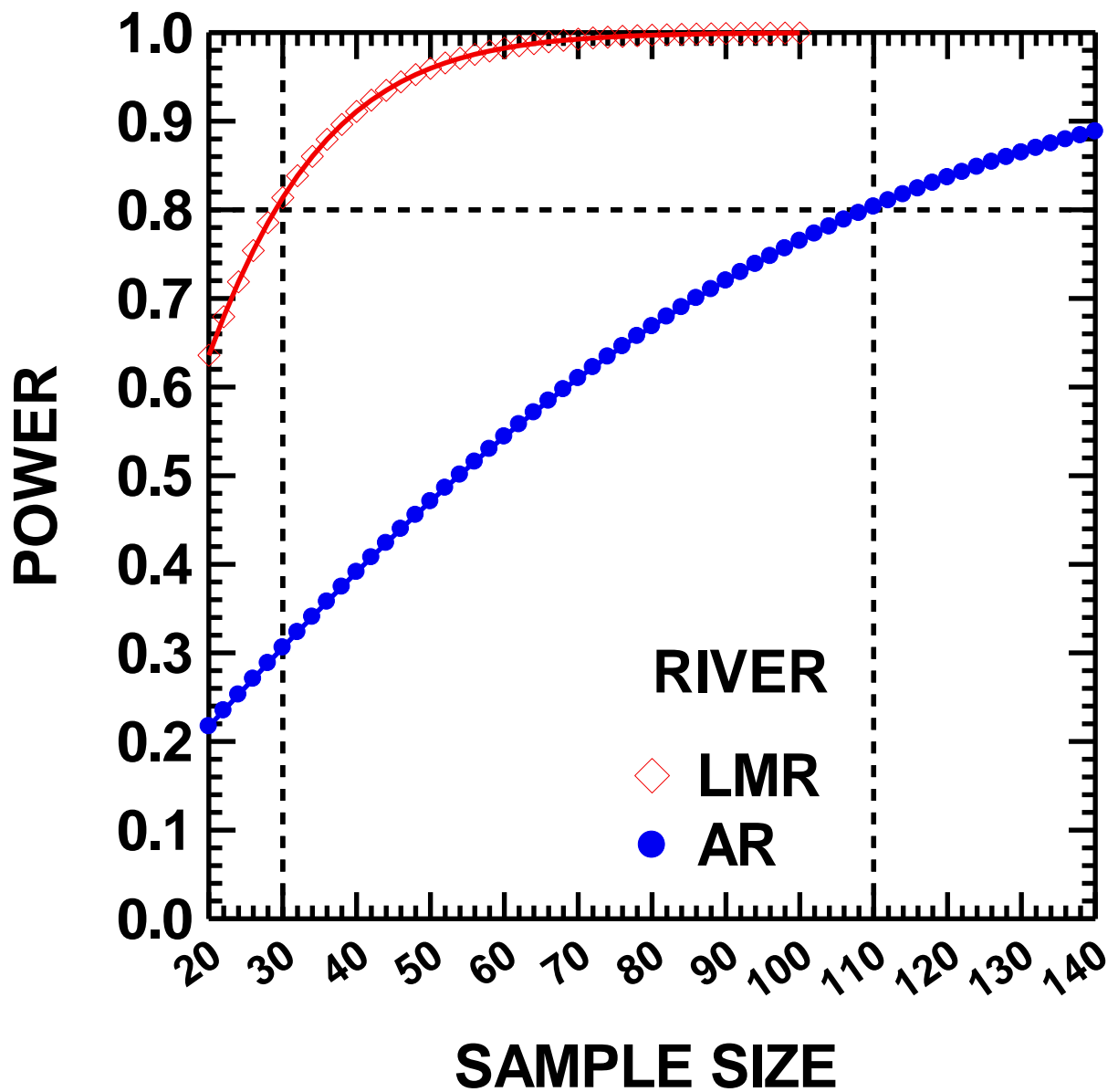


Figure 1. Estimated number of samples required to detect a 20% change in mean S ($\log_{10} n+1$) in the Alafia (110 samples) and Little Manatee rivers (30 samples) based upon data collected 1995-1998.

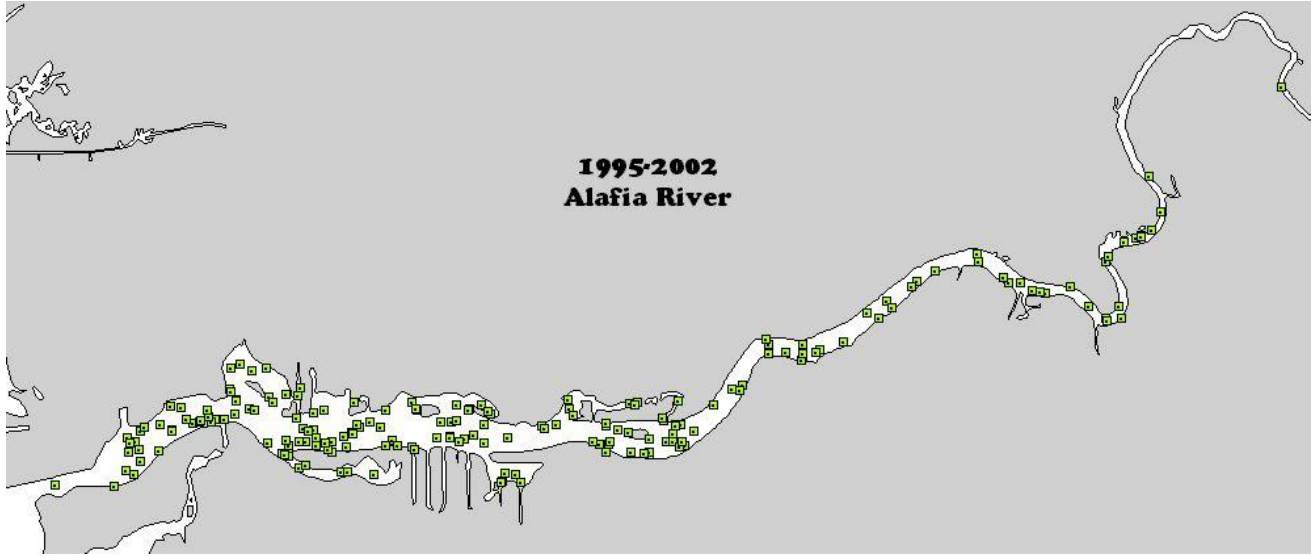


Figure 2. location of sampling stations for benthic macroinvertebrates in the Alafia river, 1995-2002.

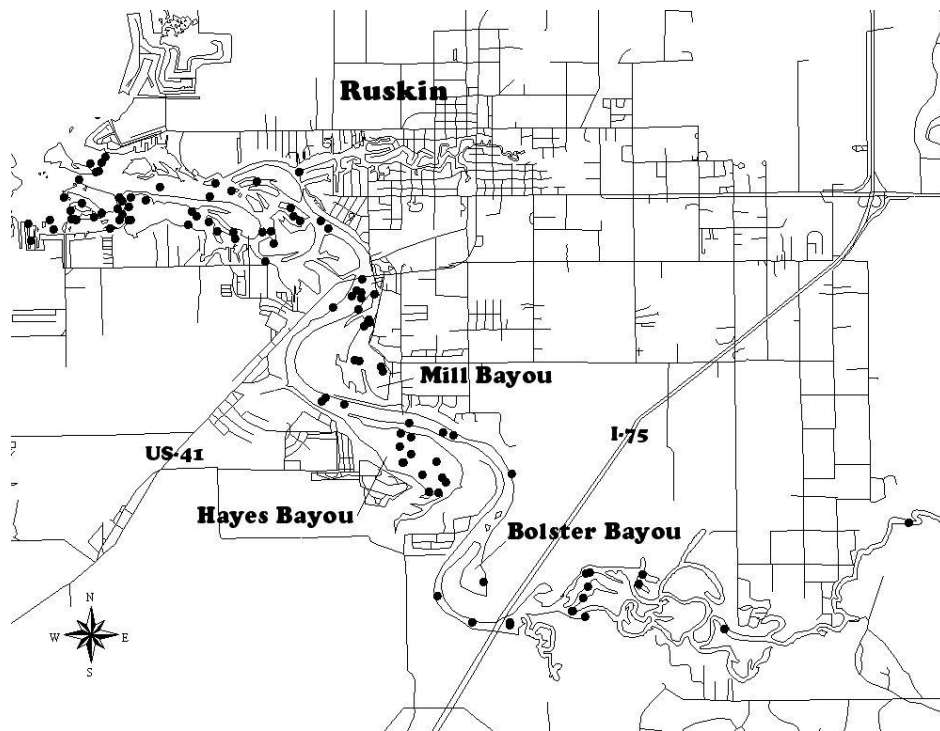


Figure 3. Location of sampling stations in the Little Manatee River, 1996-2002.

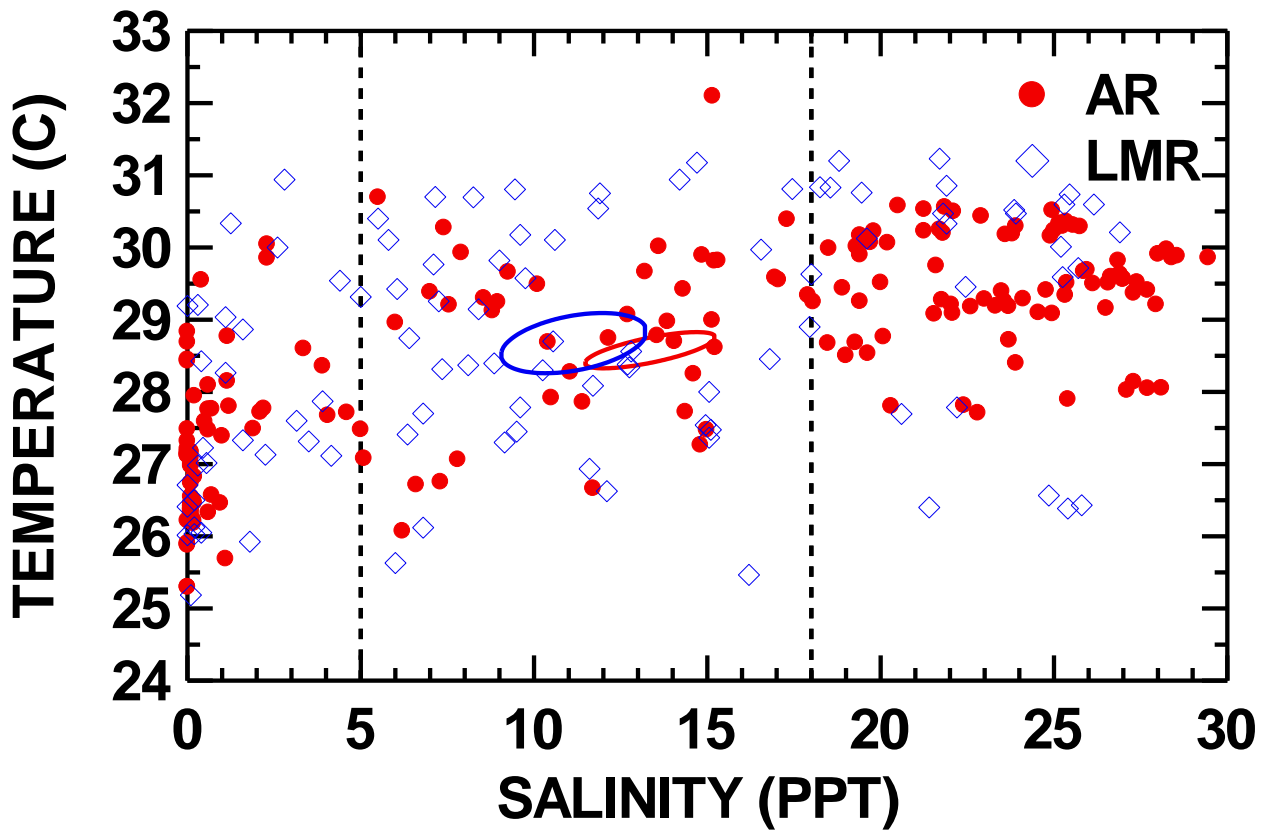


Figure 4. Temperature-salinity plot of near-bottom waters, by study area (Alafia River=Impact; Little Manatee River=Control), 1995-2002. Ellipses embrace ± 1 s.d. of the mean.

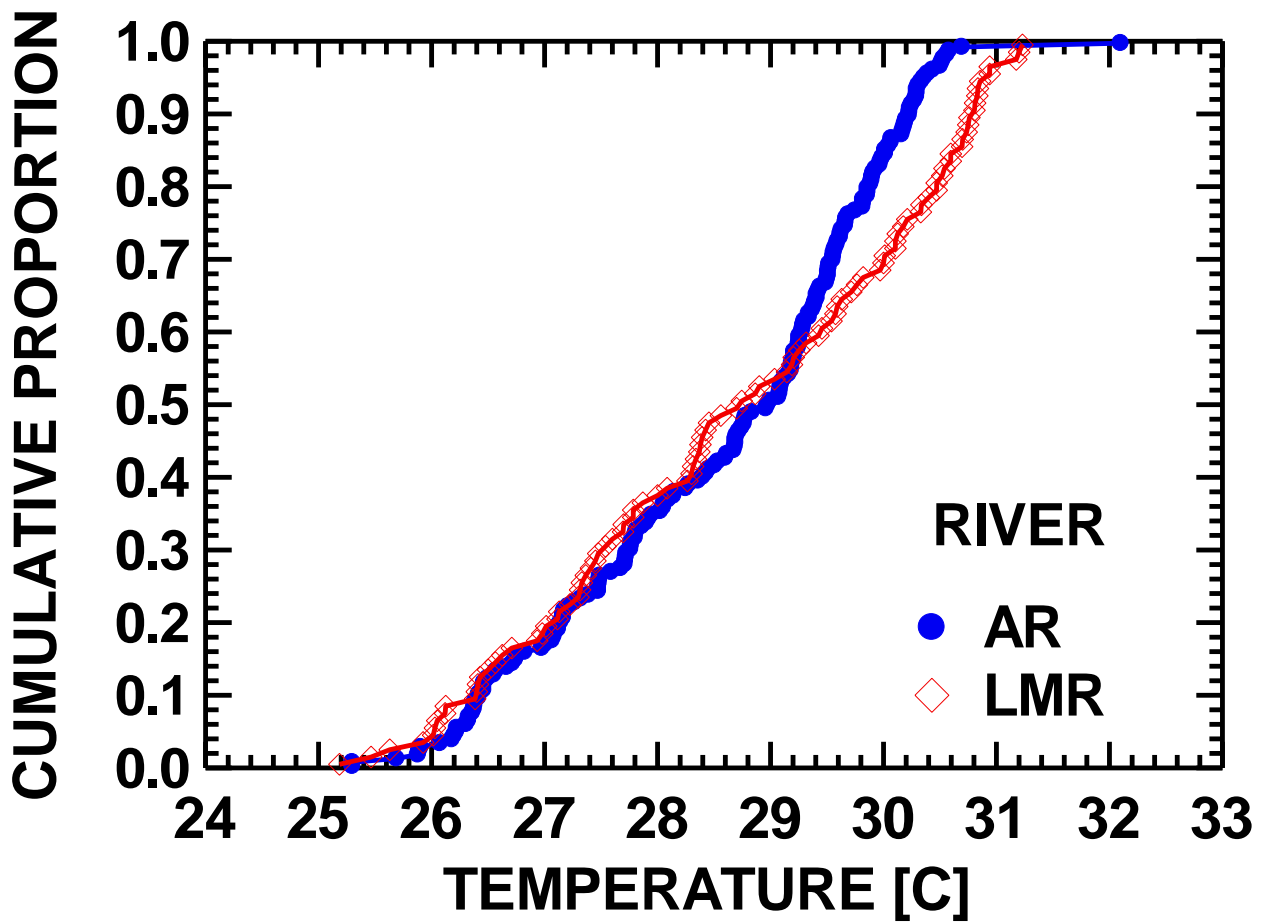


Figure 5. Cumulative distribution function plot of near-bottom water temperatures in the Alafia River “Impact” and the Little Manatee “Control” area: 1995-2002.

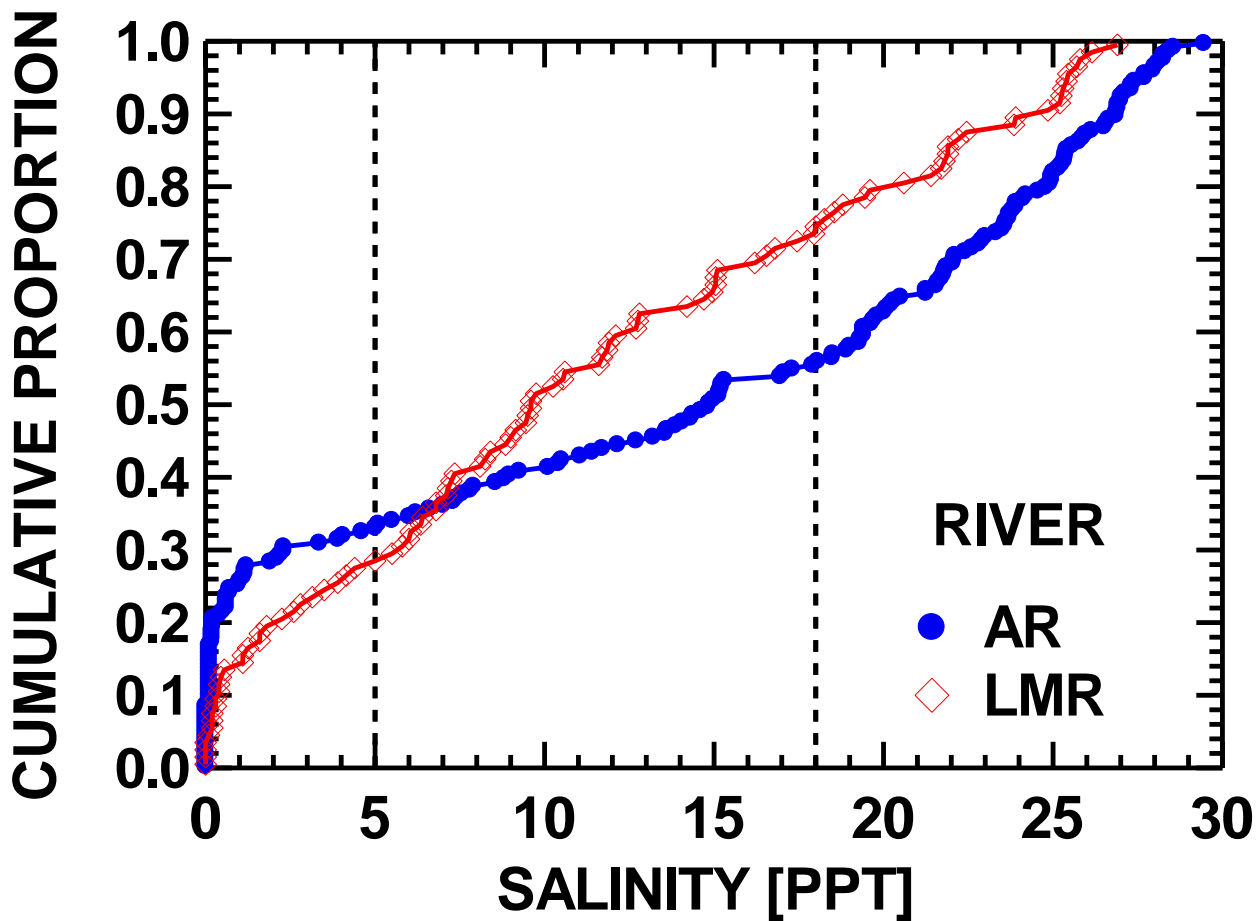


Figure 6. Cumulative distribution function plot of near-bottom salinities in the Alafia River “Impact” and the Little Manatee River “Control” areas (1995-2002).

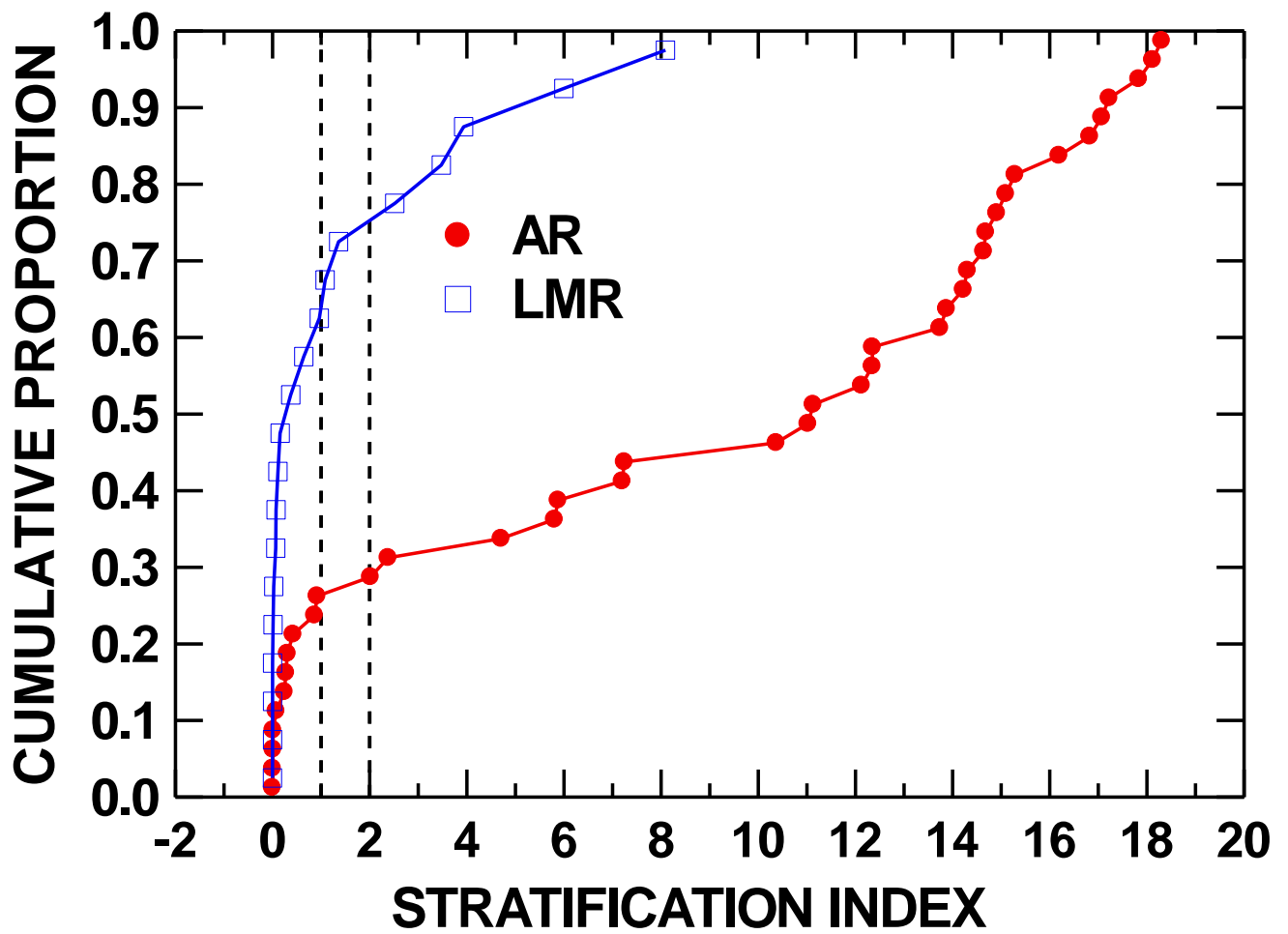


Figure 7. Cumulative distribution function plots of the Stratification Index: Alafia River (Impact) vs. Little Manatee River (Control) areas (1995-2002).

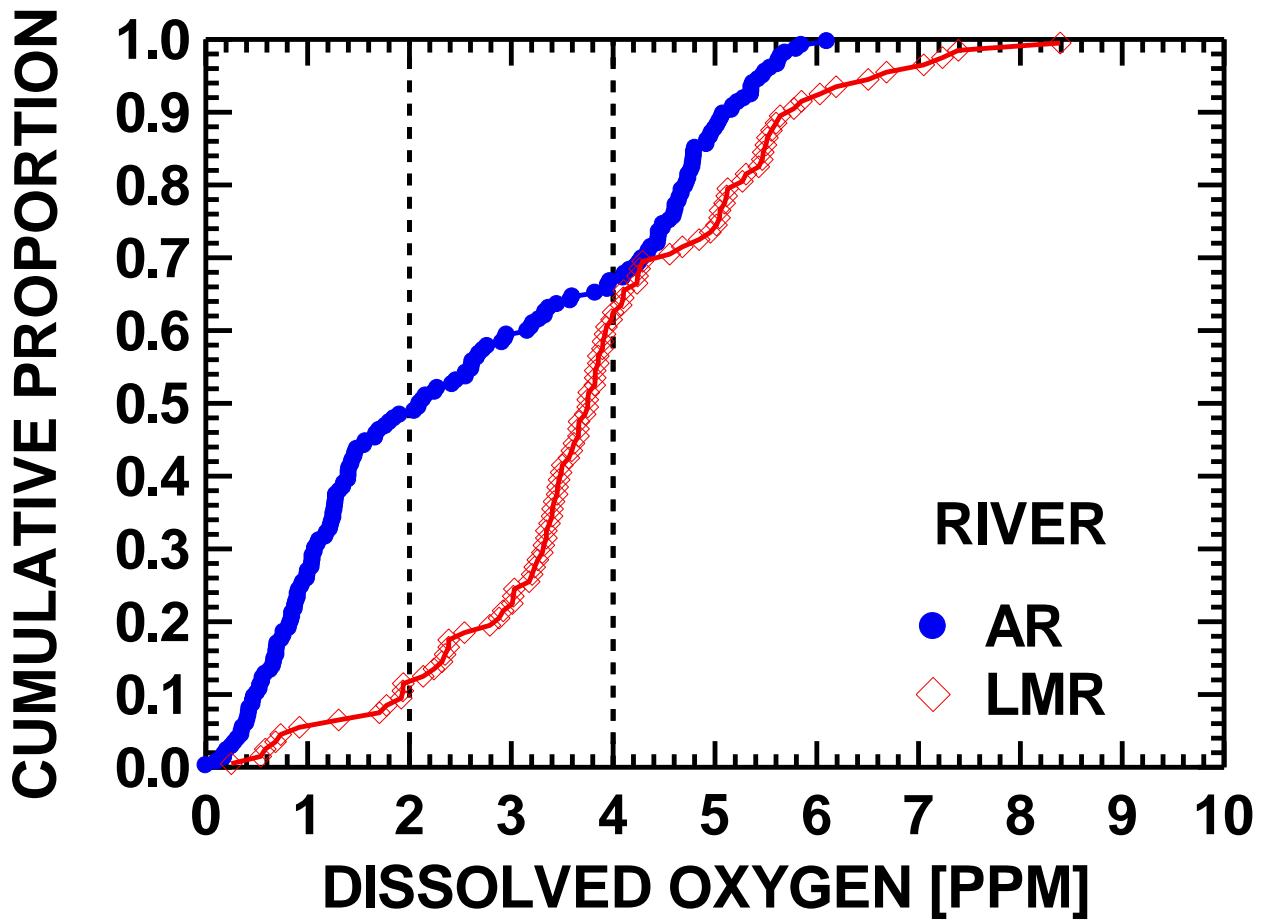


Figure 8. Cumulative distribution function plots of near-bottom dissolved oxygen: Alafia River (Impact) vs. Little Manatee River (Control) areas (1995-2002).

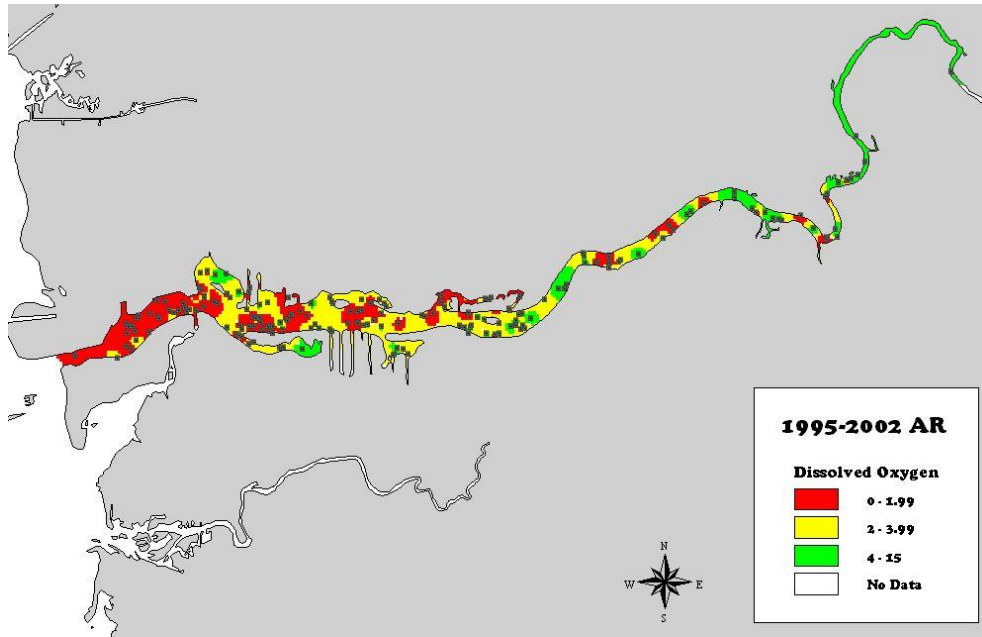


Figure 9. Map depicting the distribution of dissolved oxygen status of the Alafia River, 1995-2002 inclusive.

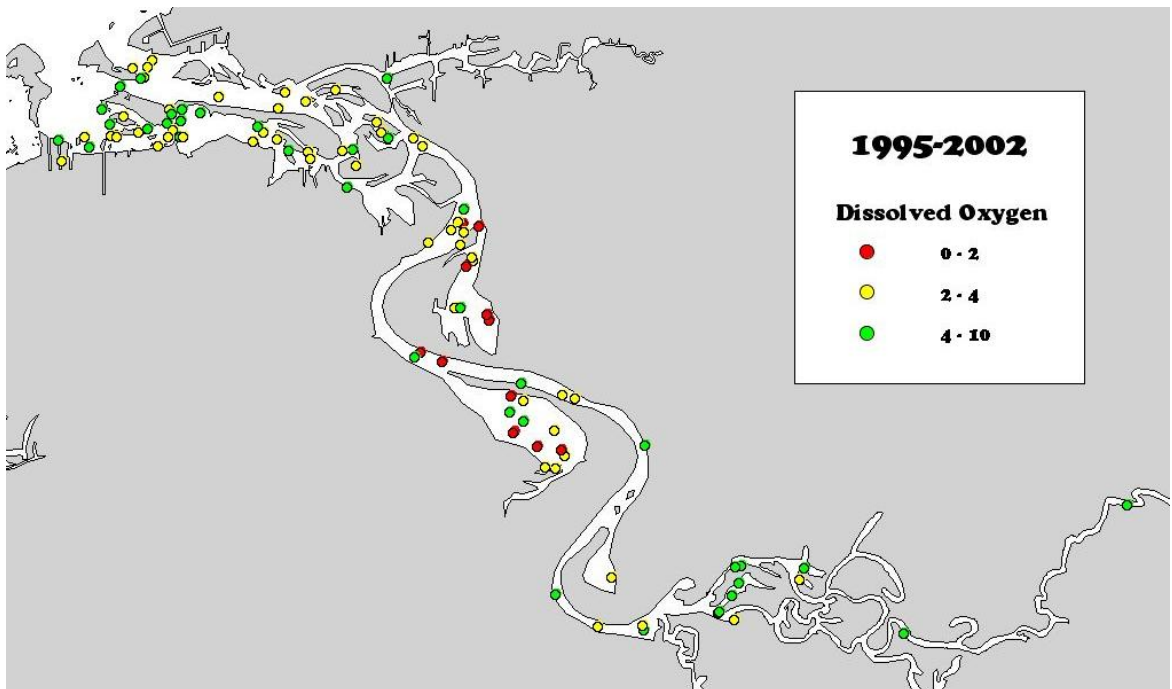


Figure 10. Map depicting the distribution of dissolved oxygen status of the Little Manatee River, 1996-2002 inclusive.

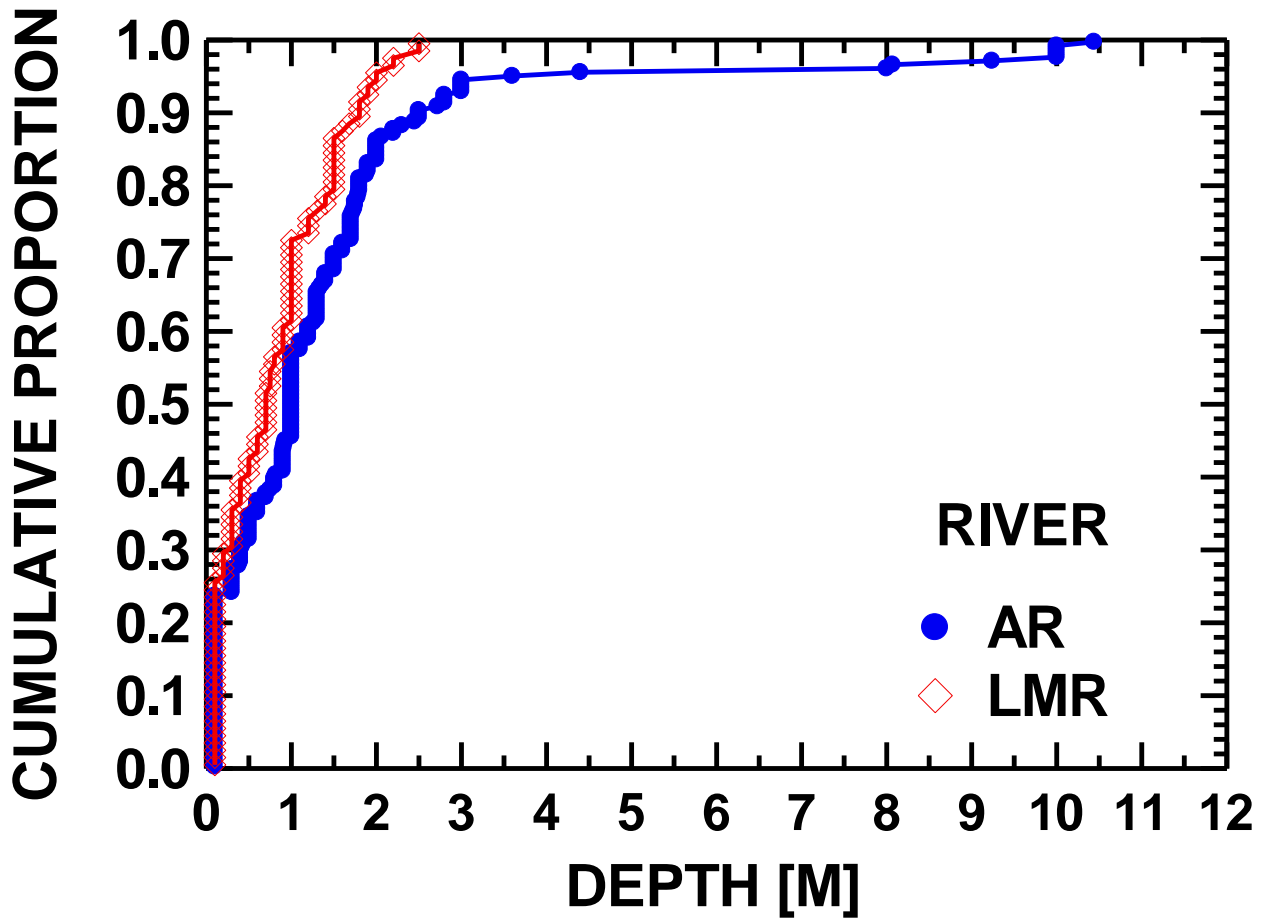


Figure 11. Cumulative distribution function plot of sample depths at the Alafia River (Impact) and Little Manatee River (Control) areas (1995-2002).

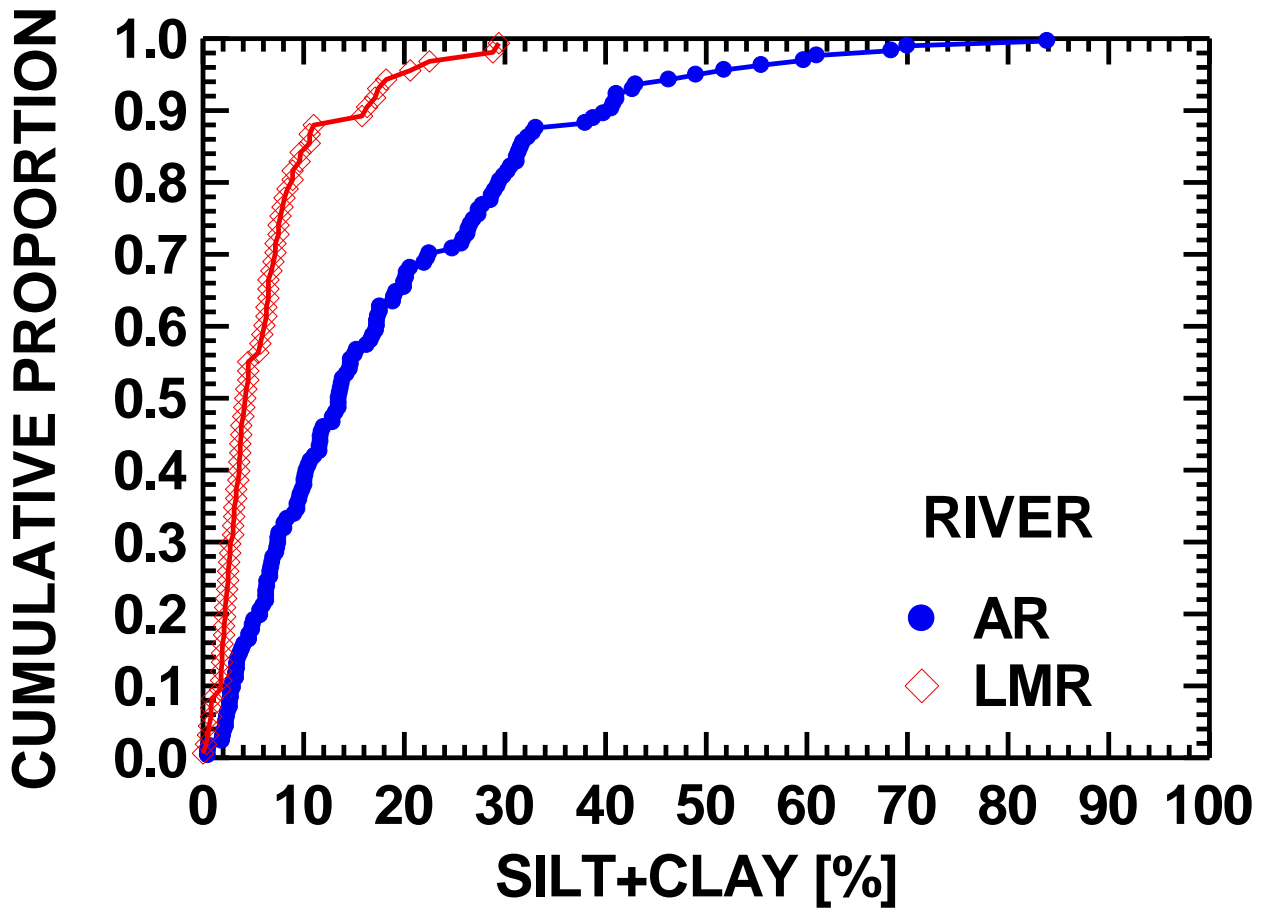


Figure 12. Cumulative distribution function plot of % silt+clay in sediments of the Alafia River (Impact) and Little Manatee River (Control) areas (1995-2002). The sand vs. mud fractions are demarcated at 25.95% SC.

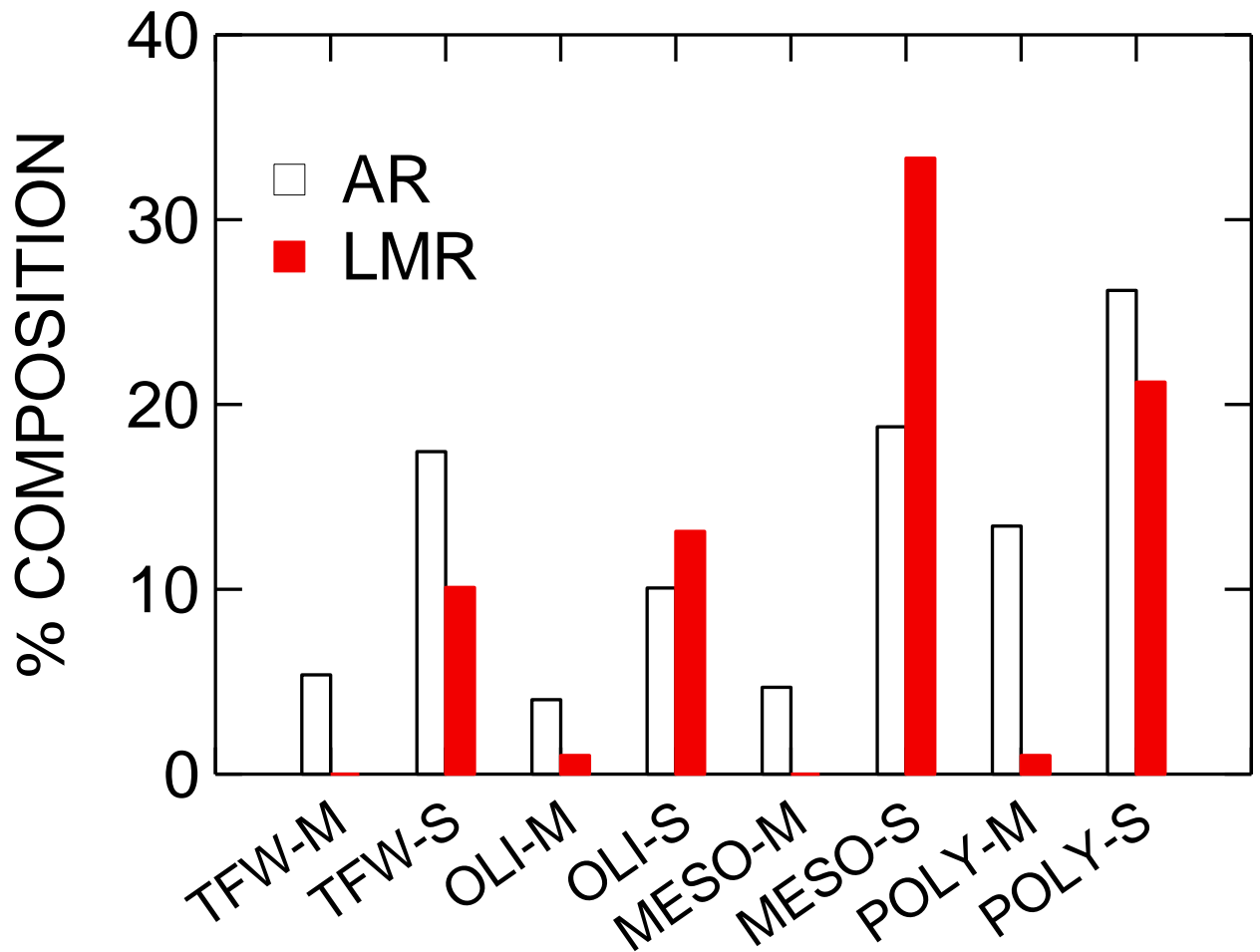


Figure 13. Distribution of habitats (salinity zone-sediment type) in the Alafia and Little Manatee rivers, 1995-2002. The areas are 63.6% similar in habitat composition (% similarity).

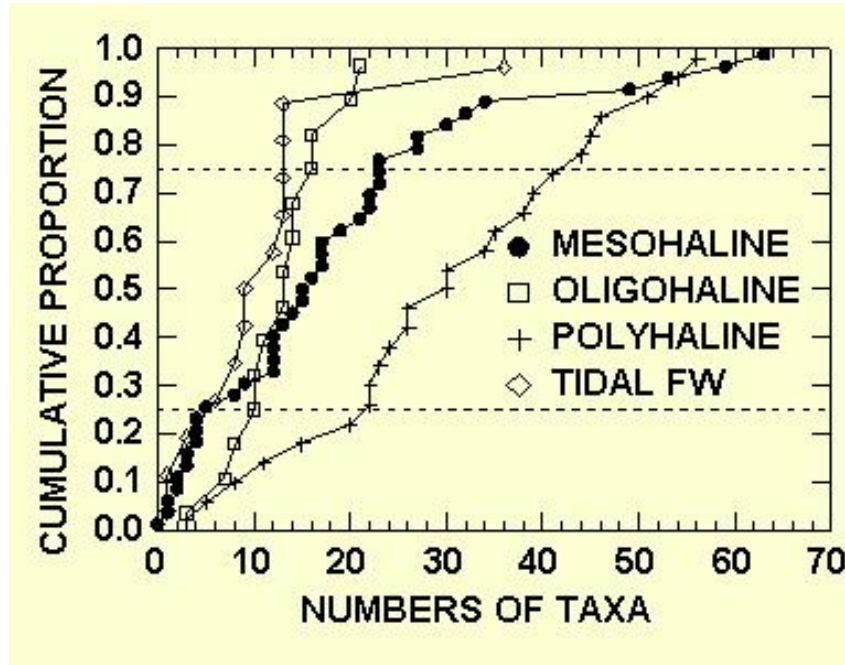
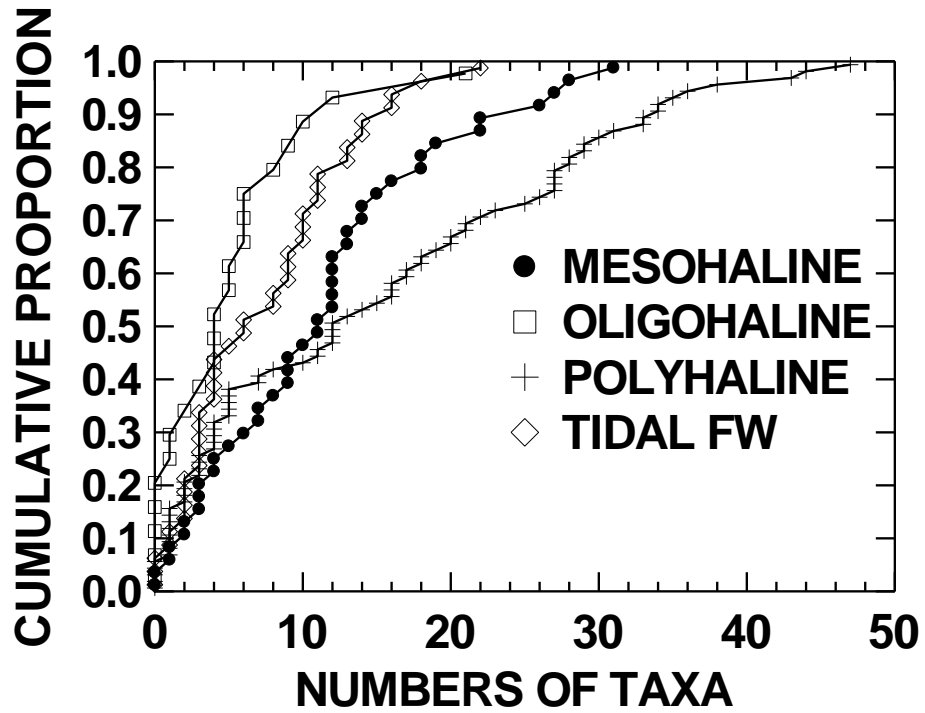


Figure 14. Cumulative function distribution plots of taxa richness (S), by salinity zone in the Alafia River, (1995-2002) and Little Manatee River (1996-2002).

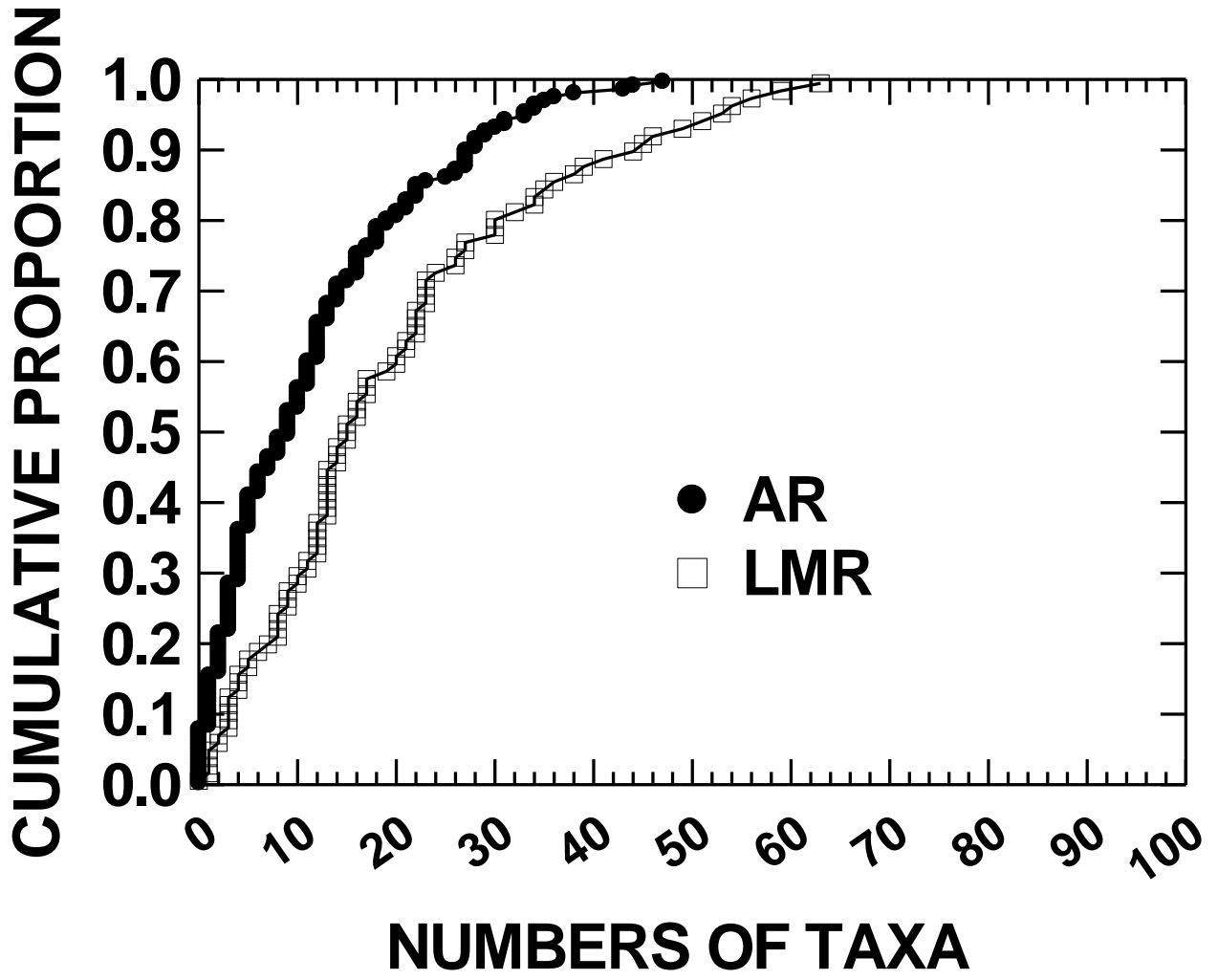


Figure 15. Cumulative function distribution plots of taxa richness (S): Alafia River and Little Manatee River (1995-2002).

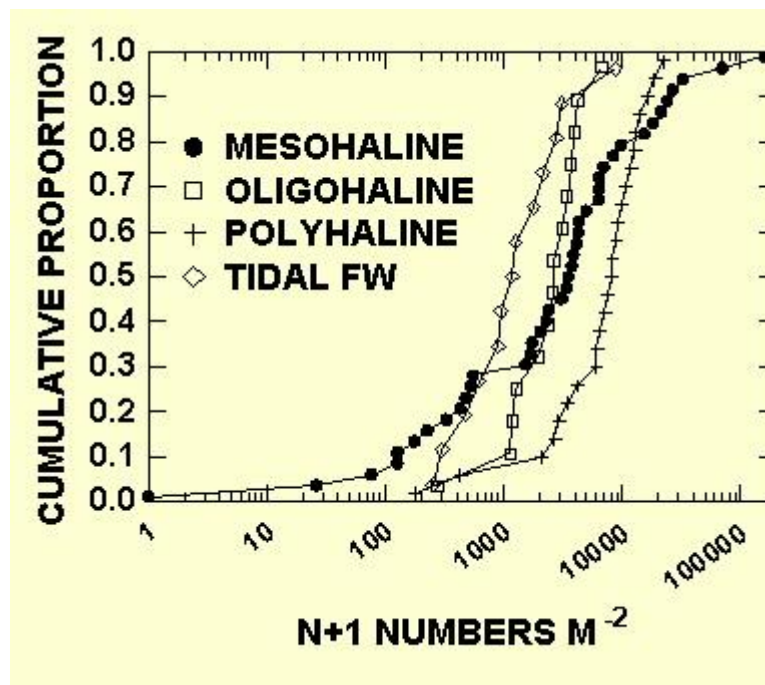
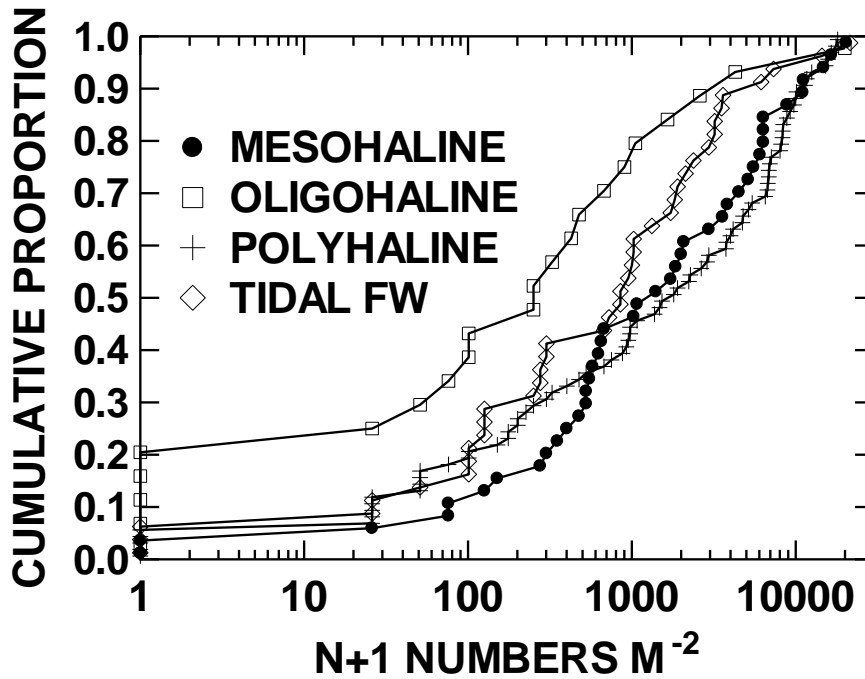


Figure 16. Cumulative function distribution plots of total abundance of benthic macroinvertebrates, by salinity zone, Alafia River (1995-2002) and Little Manatee River (1996-2002).

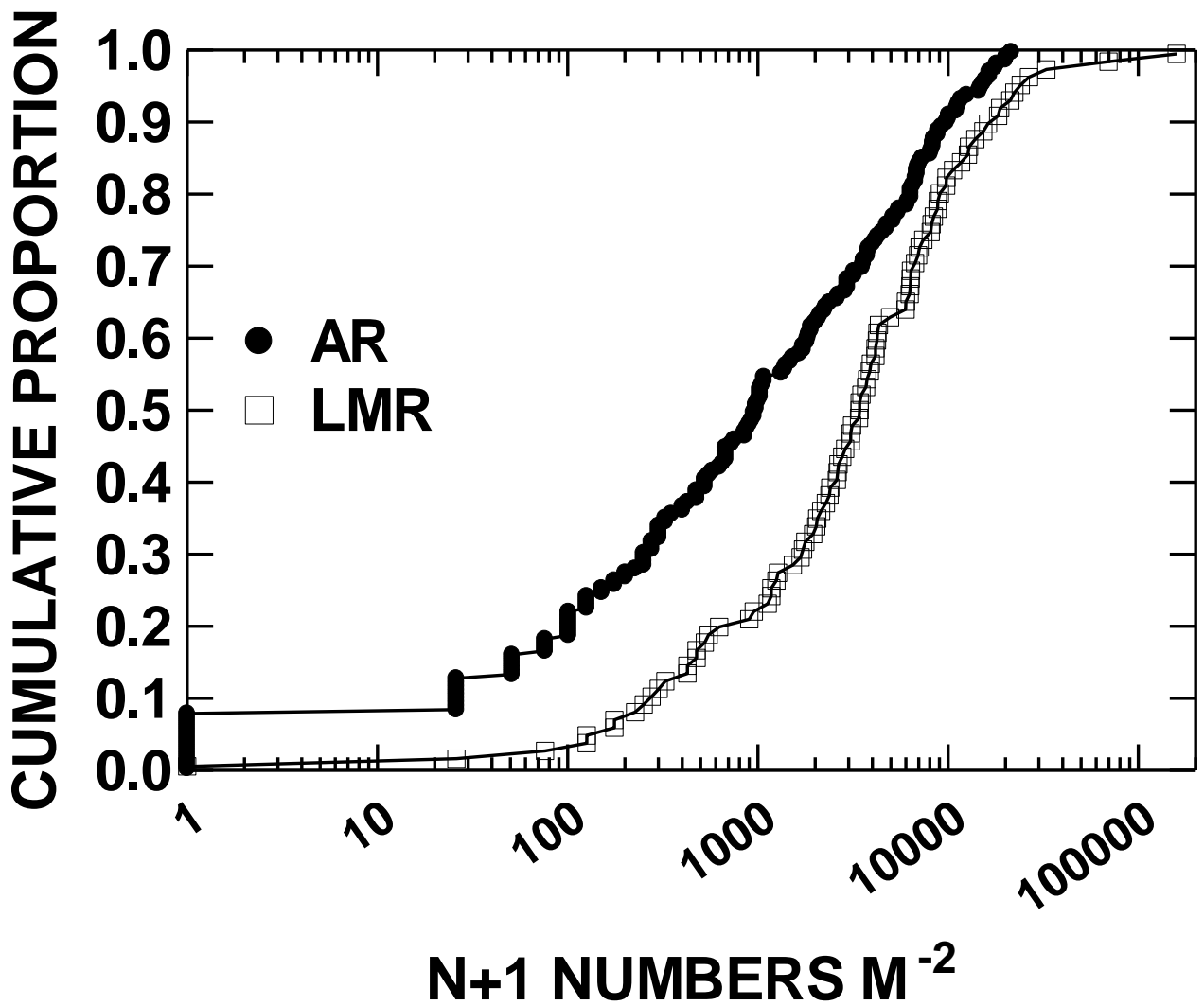


Figure 17. Cumulative function distribution plots of total abundance of benthic macroinvertebrates: Alafia River (1995-2002) and Little Manatee River (1996-2002).

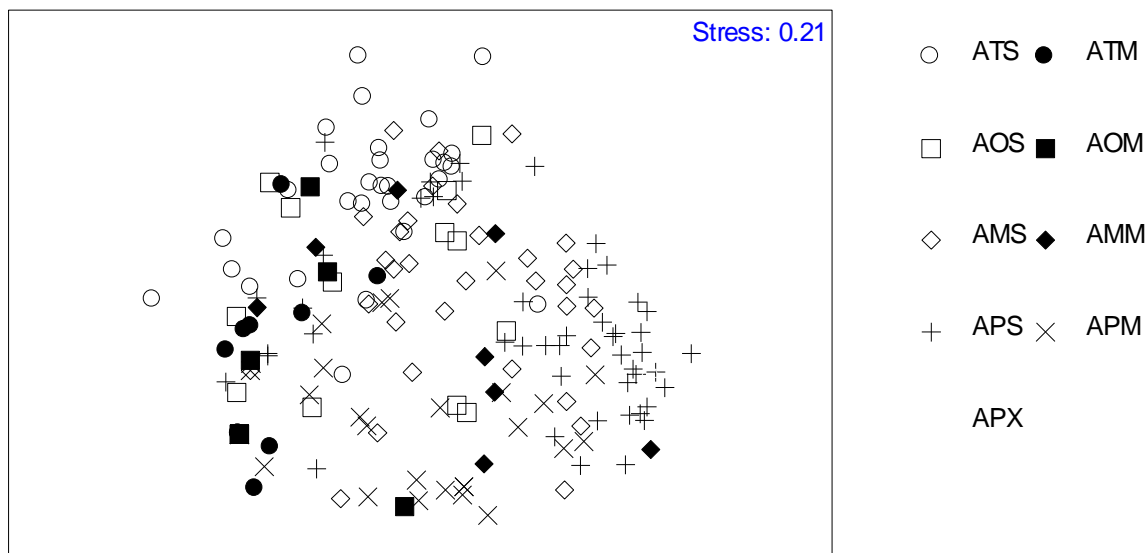


Figure 18. MDS plot of benthic community structure in the Alafia River, by habitat, 1995-2002. A= Alafia River; T= tidal freshwater; O=oligohaline; M=mesohaline; P=polyhaline; S=sand; M=mud.

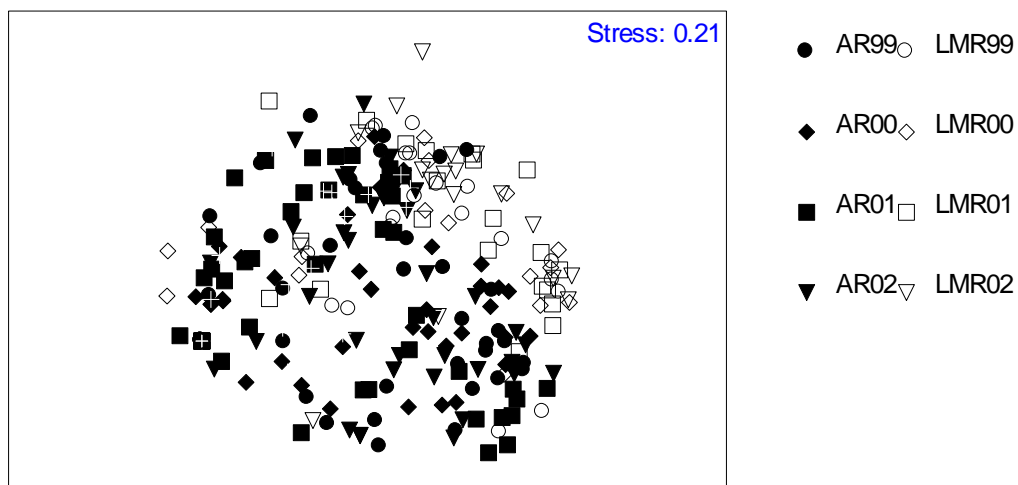


Figure 19. MDS representation of the similarity of benthic community structure between years in the Alafia River and Little Manatee River, 1999-2002.

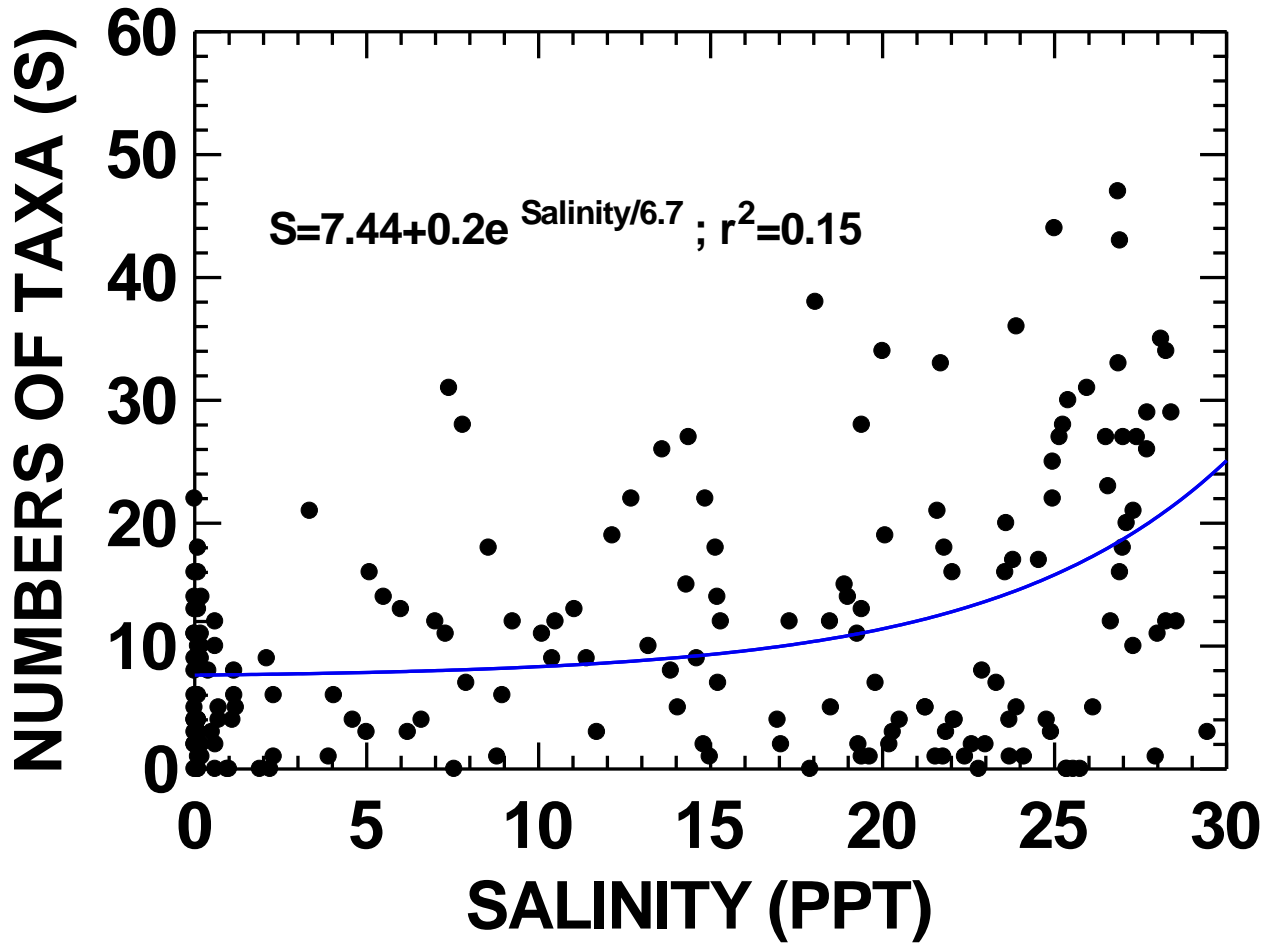


Figure 20. Association between species richness and near-bottom salinity, in the Alafia River, 1995-2002.

LITTLE MANATEE RIVER 1996-2002

Rank 1 Eqn 8010 Power(a,b,c)
 $r^2=0.2551418$ DF Adj $r^2=0.23003422$ FitStdErr=13.178512 Fstat=15.414183
a=10.171761 b=0.57559441
c=1.1300266

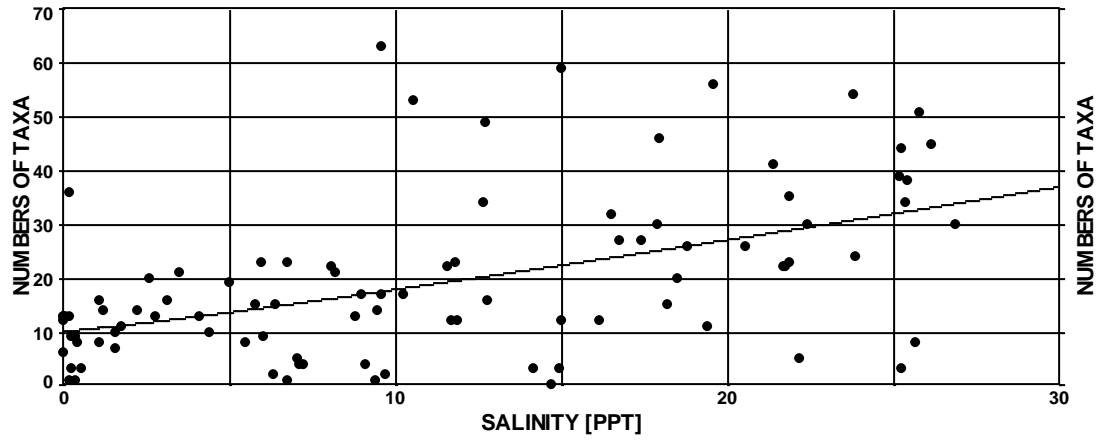


Figure 21. Association between numbers of species and near-bottom salinity in the Little Manatee River, 1996-2002.

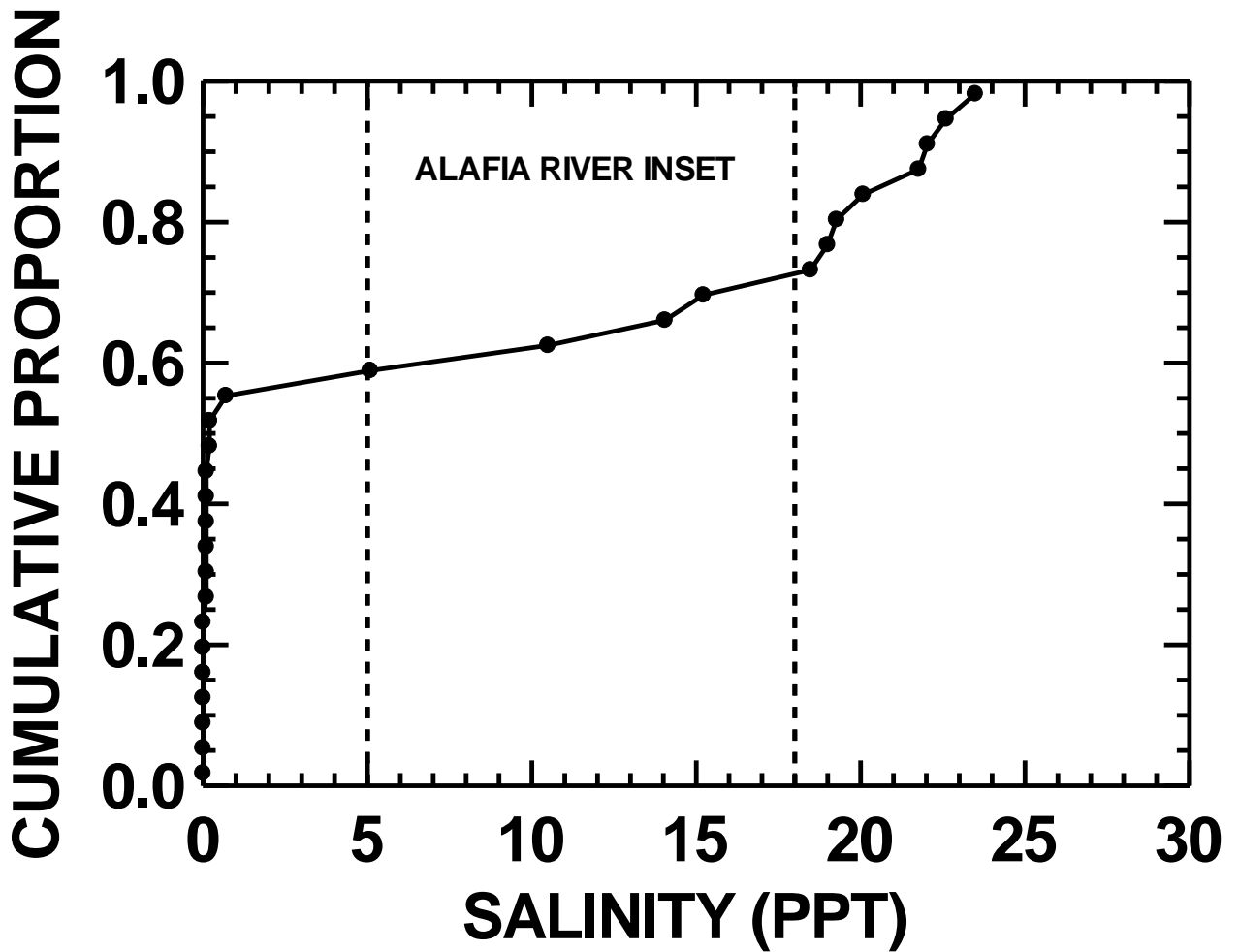


Figure 22. Cumulative distribution function plot of near-bottom salinity in the Alafia river “inset” zone (RKM 6-9), 1995-2002.

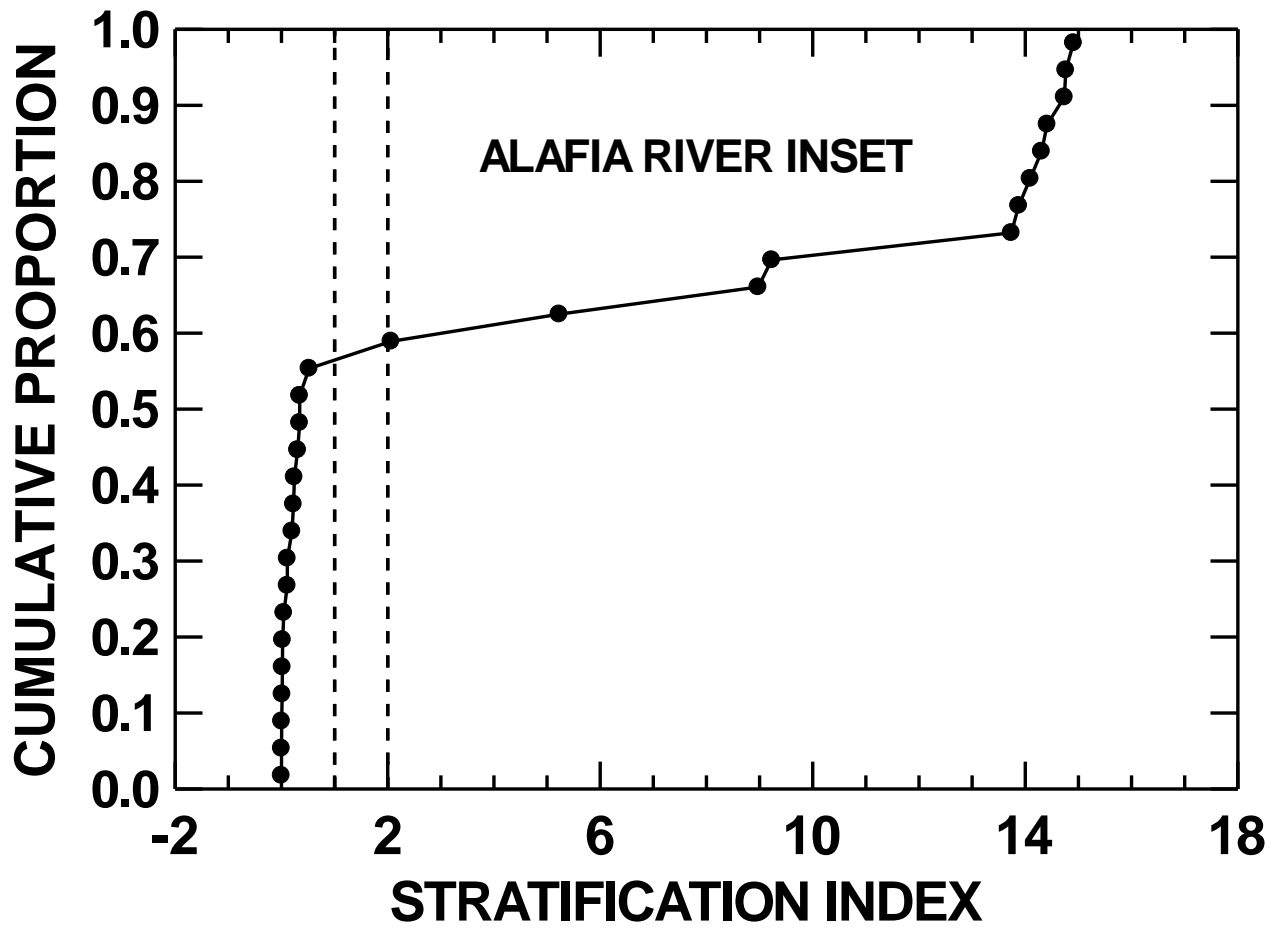


Figure 23. Cumulative distribution function plot of stratification in the Alafia river "inset" zone (RKM 6-9), 1995-2002.

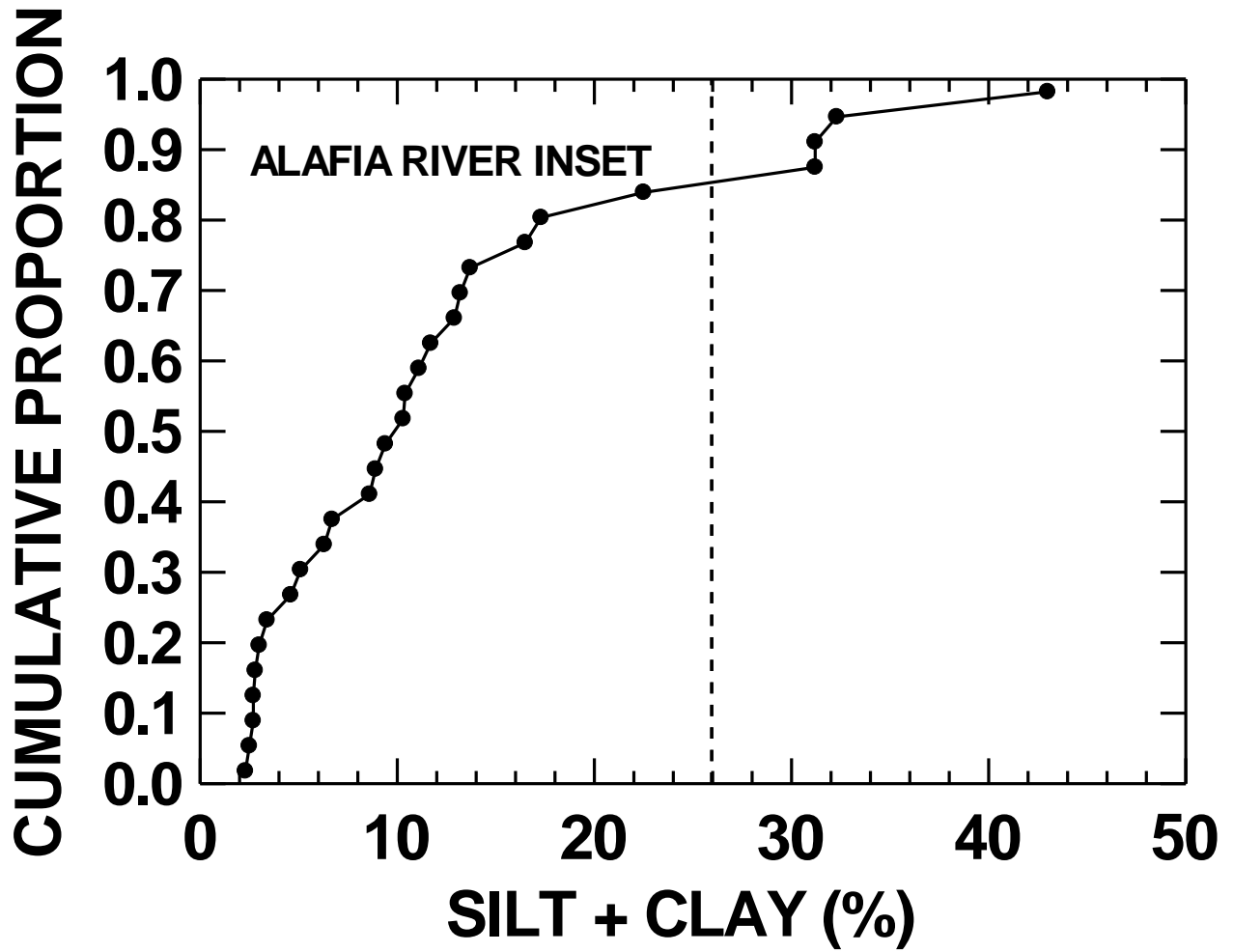


Figure 24. Cumulative distribution function plot of %SC in the Alafia river “inset” zone (RKM 6-9), 1995-2002.

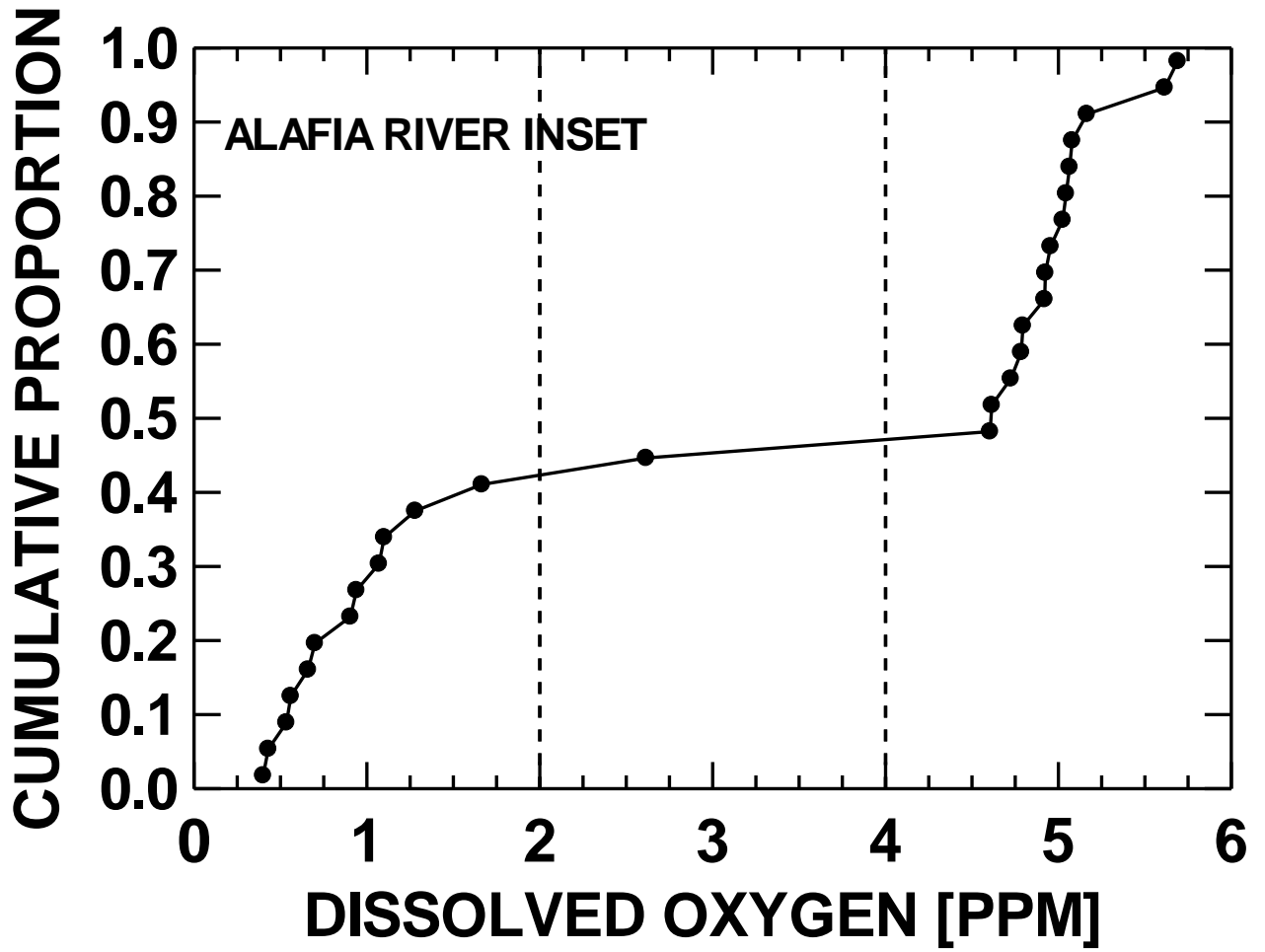


Figure 25. Cumulative distribution function plot of near-bottom DO in the Alafia river “inset” zone (RKM 6-9), 1995-2002.

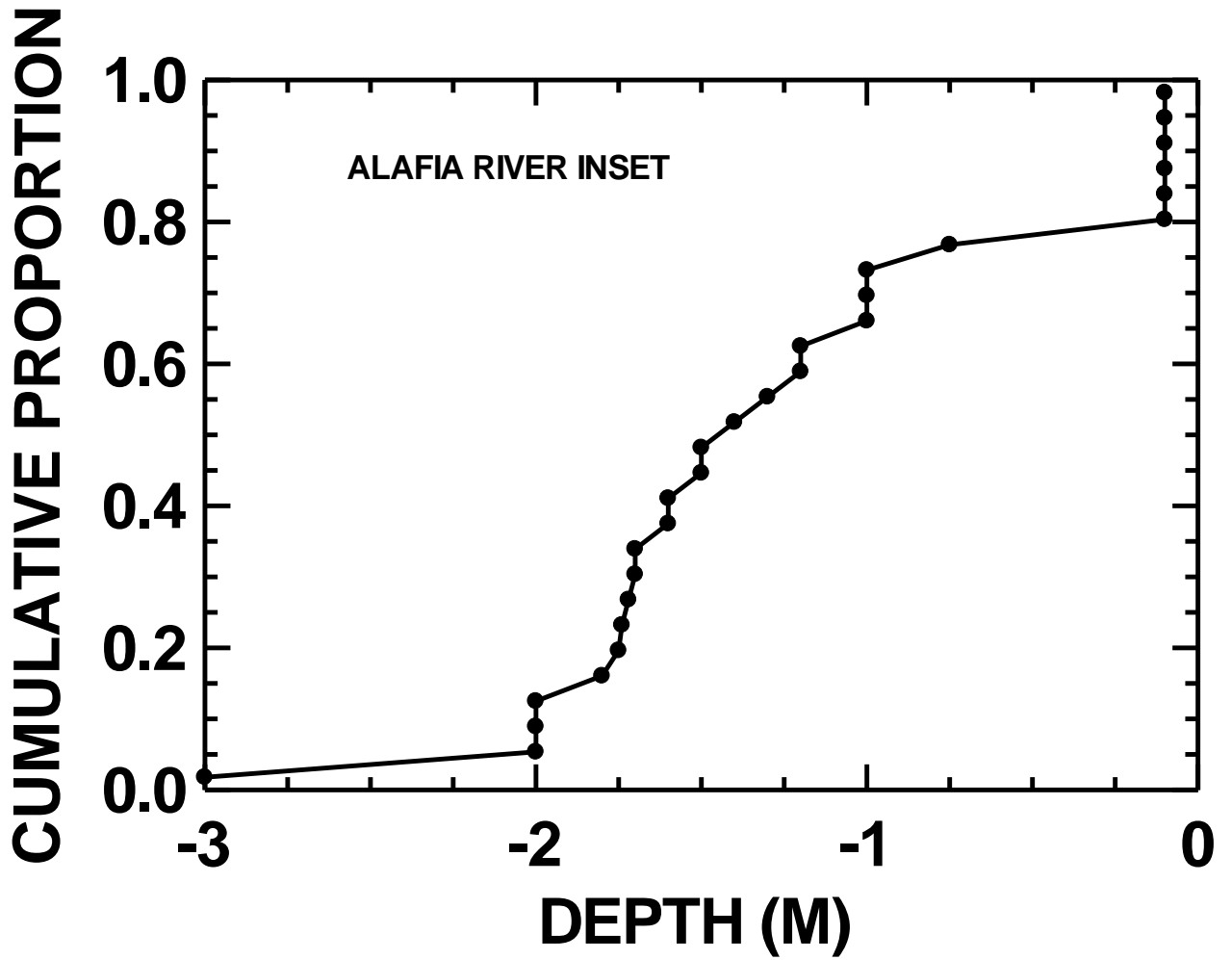


Figure 26. Cumulative distribution function plot of sample depths in the Alafia River “inset” zone (RKM 6-9), 1995-2002

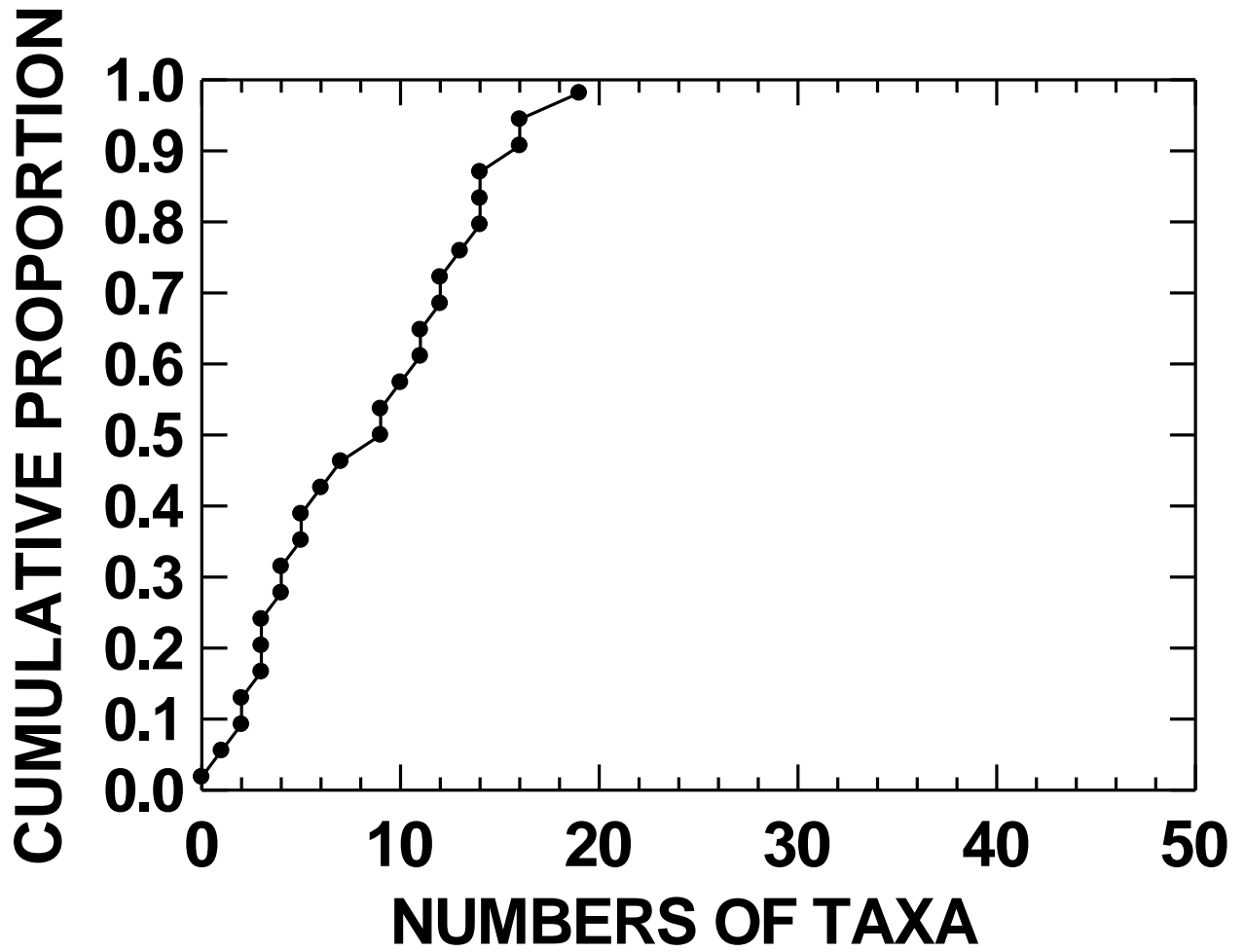


Figure 27. Cumulative distribution function plot of numbers of taxa in the Alafia river “inset” zone (RKM 6-9), 1995-2002.

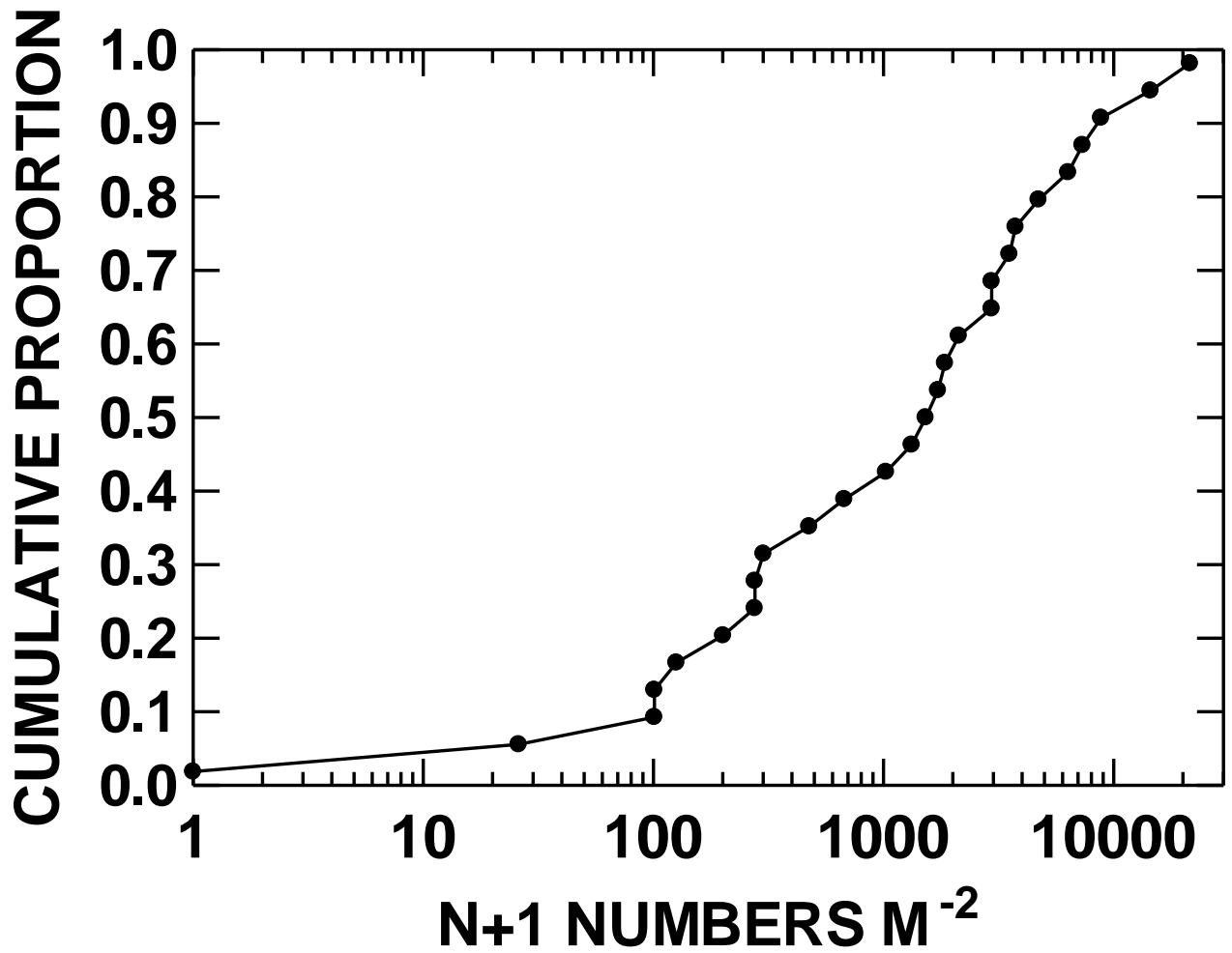
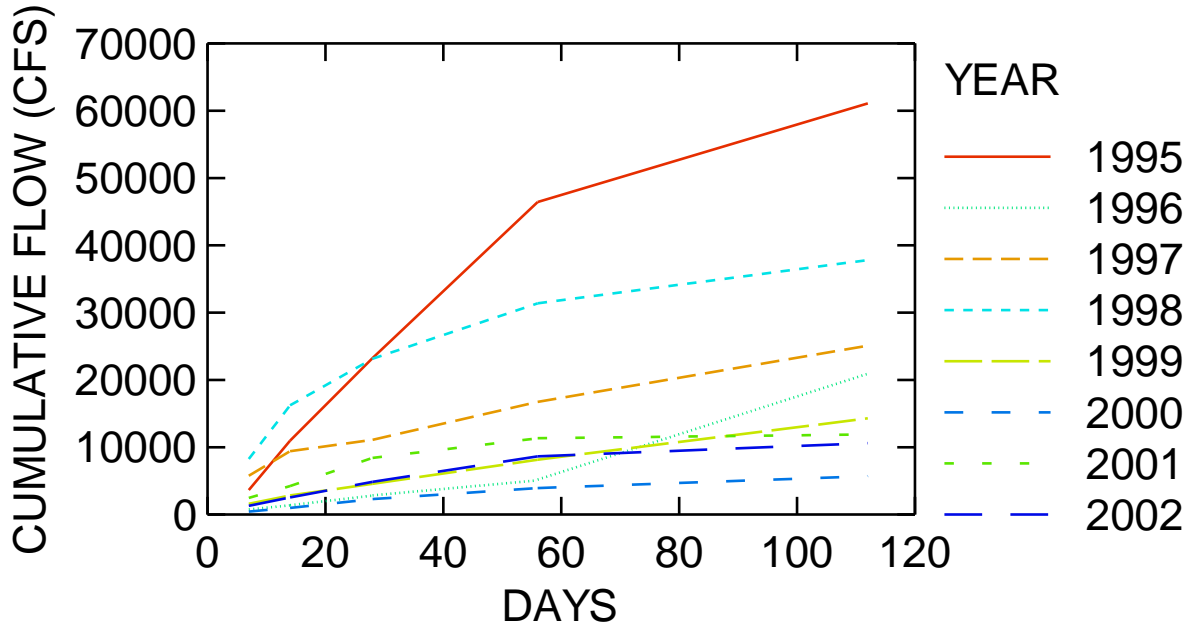
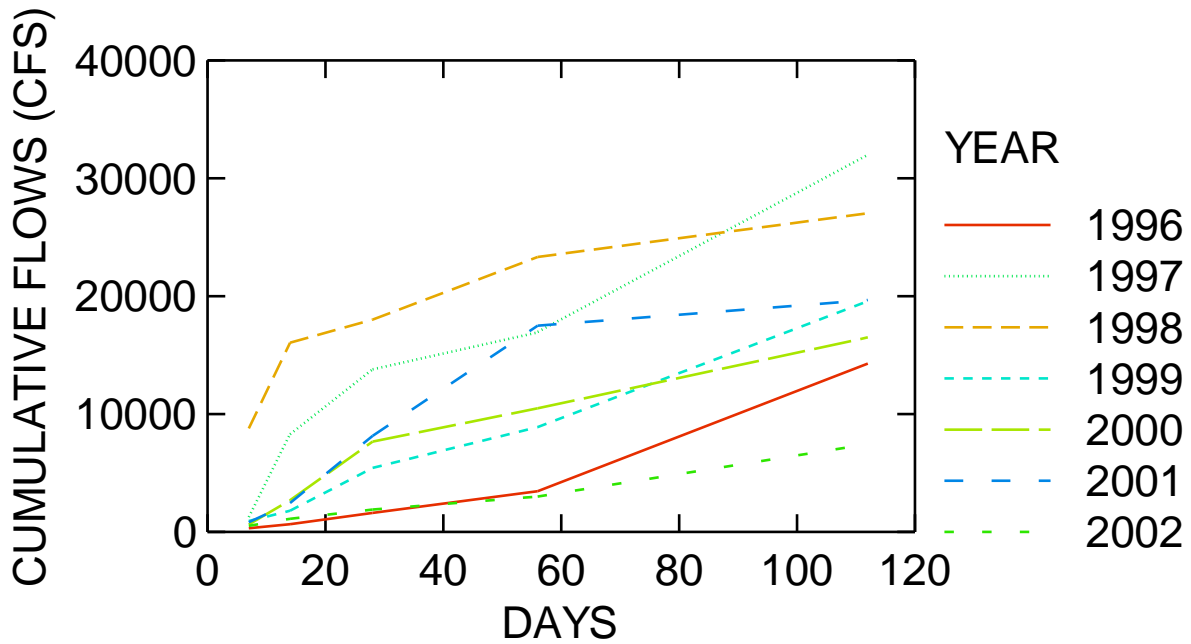


Figure 28. Cumulative distribution function plot of benthic abundance in the Alafia River “inset” zone (Rkm 6-9), 1995-2002.

APPENDIX A



Appendix Figure A-1. Cumulative freshwater inflow, by year, Alafia River.



Appendix Figure A-2. Cumulative freshwater inflow, by year, Little Manatee River.