Tampa Bay Estuary Program
Technical Report #0l-01

Survey of the Distribution of the Marine Slime Mold *Labyrinthula* sp. in the seagrass *Thalassia testudinum* in the Tampa Bay Area, Fall 1999-Fall 2000

FINAL REPORT

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Tampa Bay Estuary Program

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Investigative Team: Barbara Blakesley, Penny Hall, Donna Berns, Jitka Hyniova, Manuel Merello, Rebecca Conroy

Florida Fish and Wildlife Conservation Commission
Florida Marine Research Institute
100 8th Ave. S.E., St. Petersburg, FL 33701

INTRODUCTION

The protist, *Labyrinthula*, is a parasite of the subtropical seagrass *Thalssia testudinum* Banks ex Konig (turtle grass). Data from 3 different studies were used to evaluate the distribution of this marine slime mold in Florida Bay, the Eastern Gulf of Mexico, and the Tampa Bay area as well as to predict potential impacts on its host populations. This parasite had been proposed as a possible cause of a massive acute seagrass die-off in Florida Bay that began in the summer and fall of 1987. No definitive cause(s) of this acute die-off was ever determined, but many possible etiologies were proposed, including high temperatures and salinities, overdeveloped seagrass beds, elevated sediment sulfide levels, hypoxia, and disease (Porter and Muehlstein, 1989; Robblee et al., 1991; Durako and Kuss, 1994; Carlson et al., 1994). 'Wasting disease" in the eelgrass *Zostera marina*, which decimated the seagrass beds of Europe and North America in the 1930's and 1940's, had previously been shown to be caused by a species of *Labyrinthula*. Some of these seagrass beds took 40 years to recover. Such a possible outcome in Florida made it important to attempt to determine the distribution pattern of *Labyrinthula* in *Thalassia* populations in different Florida estuaries with varying environmental conditions and try to understand this parasite's potential impacts on the health and survival of those seagrass beds.

Data were collected in three separate studies of *Labyrinthula* distribution in different geographical areas. The most extensive data set was collected over the last 5 years in a study initiated in Florida Bay in 1995. Two other preliminary studies were also done, one in the eastern Gulf of Mexico, and the other in the Tampa Bay area. The same field methods were used in all 3 studies so that results could be easily compared. Results from the Florida Bay studies were extrapolated to propose potential impacts of this slime mold on *Thalassia* populations in the other areas of Florida where less extensive studies of *Labyrinthula* have been done.

Florida Bay Studies (1995-present)

Four principal questions were asked during our Florida Bay studies:

1. Did *Labyrinthula* have a role in the initial acute *Thalassia* die-offs (summer, 1987)?
2. Is *Labyrinthula* involved in the chronic die-off that we have been monitoring since the beginning of this study (1995-present)?
3. If it is involved, what role does *Labyrinthula* play in the chronic die-off ?
4. Does *Labyrinthula* have a role in the current (first noticed in summer, 1999) acute die-off in Barnes Key?

Data from four years of biannual sampling in Florida Bay were examined to determine the relationship between the distribution and abundance of the seagrass *Thalassia testudinum* and *Labyrinthula*. Ten basins with varied physical characteristics were studied intensively, including microscopic examination of thousands of *Thalassia* blades from more than 2,500 sites within these basins. We used ArcView's extension Spatial Analyst and the Inverse Distance Weighted (IDW) method to visualize the pattern of and changes in distribution and abundance of infection in *Thalassia* (Blakesley et al., public communication, The American Society of Limnology and Oceanography Conference Abstracts, Feb 1-5, 1999, Santa Fe, NM, p. 26). Both lab and field studies show that ongoing low salinities prevent *Labyrinthula* from infecting *Thalassia.* Field studies also suggest that a drop in salinity to below 15 ppt will reduce the existing level of infection.

The data collected both during this field study and from associated laboratory studies (Blakesley et al., public communication, Proceedings of the Florida Bay Conference, May 12-14, 1998, Miami, FL) resulted in the formulation of a preliminary hypothetical model (see Figure 1 and 2) describing the effects of *Labyinthula* on *Thalassia* populations in Florida Bay (Blakesley et al., public communication, 15th Biennial International Conference of the Estuarine Research Federation Proceedings, Sept. 25-30, 1999, New Orleans, LA, p. 10) Where seagrass densities are low, *Labyrinthula* does not cause major mortality. In moderate to high salinities and high seagrass densities, *Labyrinthula* plays a major role in seagrass mortality. With optimal conditions for seagrass, *Labyrinthula* can be a primary pathogen controlling seagrass densities. In suboptimal conditions for seagrass, such as lowered light levels, stressed seagrass may be weakened by opportunistic *Labyrinthula* that further contributes to chronic seagrass die-off.

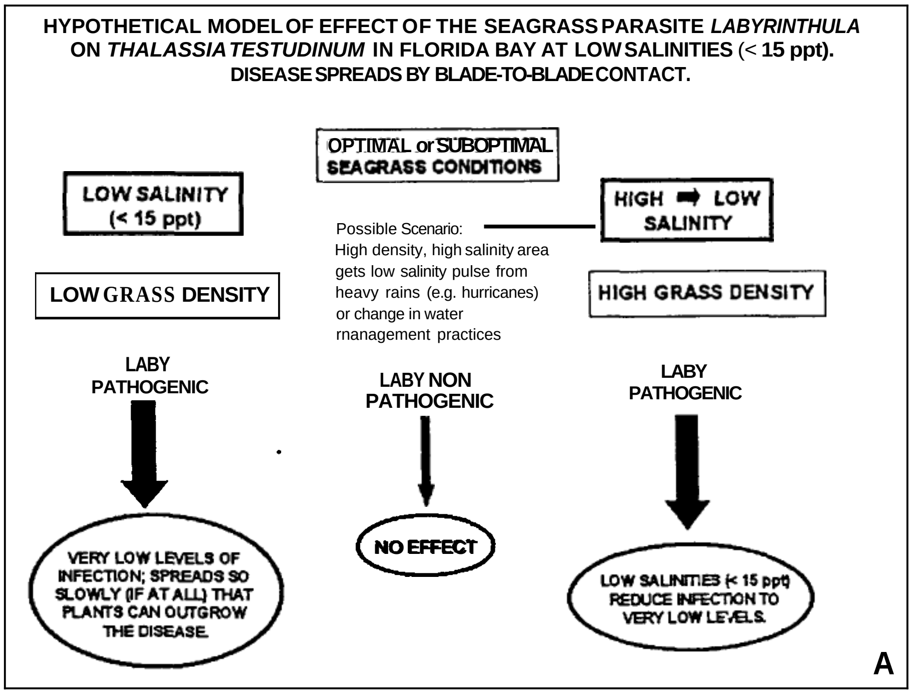

Figure 1. A hypothetical model developed using data from a 1995-1999 study of *Labyrinthula* on *Thalassia* in Florida Bay showing effects of the seagrass parasite at low salinities,

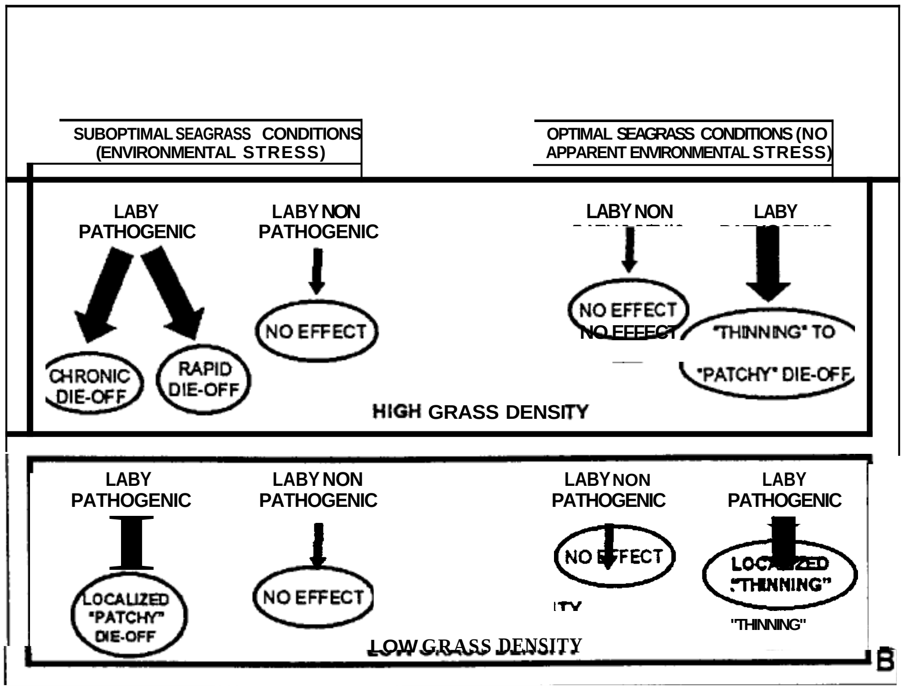

Figure 2. A hypothetical model developed using data from a 1995-1999 study of *Labyrinthula* on *Thalassia* in Florida Bay showing effects of the seagrass parasite at high salinities.

The theoretical model suggests 3 different roles that *Labyrinthla* might play in Florida Bay under different environmental conditions. These include: (1) a nonpathogenic parasite; (2) an opportunistic secondary pathogen; and (3) a primary pathogen. Five different factors are considered to be critical elements in determining the role(s) of *Labyrinthula* in seagrass health at a particular site in Florida Bay (Blakesley et al., public communication, Florida Bay and Adjacent Marine Systems Science Conference, Nov. 1-5, 1999, Key Largo, FL., pp. 12-14). Salinity controls infection (infection does not occur at < 15 ppt). Seagrass density determines the extent to which *Labyrinthula* infection spreads because the slime mold transmission is thought to depend on blade-to-blade contact (Muehlstein, 1992). Pathogenicity of a particular strain of *Labyrinthula* will determine severity of infection. Environmental stressors (abiotic factors) such as low light or high temperatures may weaken *Thalassia* and, in combination with the infection by pathogenic *Labyrinthula*, cause seagrass die-off. Resistance to disease due to genetic factors or production of phenolic compounds may be important in determining the health of *Thalassia* in Florida Bay. The model predicts that in areas with high seagrass density, high salinity, "suboptimal seagrass conditions (environmental stress)", and presence of pathogenic *Labyrinthula*, the slime mold could contribute to either chronic or acute die-off acting as an opportunistic secondary pathogen. With the same conditions, but without environmental stress, it suggests that *Labyrinthula* can still cause "thinning" or patchy die-off acting as a primary pathogen (Blakesley et al., public communication, Florida Bay and Adjacent Marine Systems Conference, Nov. 1-5, 1999, Key Largo, FL, pp. 12-14).

In late summer 1999, a new seagrass die-off was first noticed north of Barnes Key that resembled the acute die-off of 1987, but was very different fiom the chronic die-off we had been studying since 1995. As in the acute event of 1987-89, *Thalassia* appeared to be the only seagrass affected and the die-offs were occurring in dense, apparently "healthy" seagrass beds. Carlson reported (personal communication, Fl. Bay Conference, 1999) that seagrass affected by the new acute die-off exhibited symptoms like those of the 1987 event i.e. the lateral meristem tissue appeared to be the tissue most immediately affected. Meristem tissue seemed mushy and smelled like "mustard" while the rest of the blade looked green and healthy. Beginning in early winter 1999, an investigation of the new acute seagrass die-off in Barnes Key was initiated to try to readdress the question first asked and unresolved 12 years before; why did the *Thalassia* suddenly start dying in Florida Bay?

Preliminary data fiom our investigation of this event show that *Labyrinthula* is probably not the initial cause of the acute die-off but instead appears to be an opportunistic secondary pathogen. There may be a seasonal component; our extensive data from Florida Bay, both field and laboratory, indicates that *Labyrinthula* activity is related to temperature. When we first evaluated the site, in January, *Labyrinthula* was rarely present anywhere in the Barnes Key area and this remained the case in repeated sampling through July. In September, we first noticed *Labyrinthula* lesions, but only around old die-off patches. In November, we recorded the highest levels of *Labyrinthula* infection since our studies began in Florida Bay in 1995...in the margins of both active and inactive die-off patches as well as in the dense beds around those patches. Additional studies of this area are needed to follow the progression of the infection and die-off through time.

Apparent impacts of *Labyrinthula* on *Thalassia* seagrass beds in Florida Bay are summarized below:

- In low salinity areas (<15 ppt): **NO IMPACT**
- In dense beds with no apparent environmental stress (and salinity >15 ppt): **THINNING, PATCHY DIE-OFF**
- In dense beds with environmental stress (and salinity >15 ppt): **CHRONIC PATCHY DIE-OFF**
- In areas that have experienced an acute die-off (environmental stress present, and salinity >15 ppt): **SEVERE LOSS**

Eastern Gulf of Mexico Studies (1997-1999)

In this study of 10 sites in the eastern Gulf, 10 *Thalassia* shoots fiom 30 stations at each site were evaluated annually for lesion coverage and *Labyrinthula* infection. The sites were chosen based on perceived environmental stress such as salinity fluctuations, thermal stress fiom power plants, and urban stresses, including several sites categorized as pristine. Results are seen in Figure 3. Infection levels were high at all sites except

Mean % Shoots Infected, EPA sites, 1997-1998

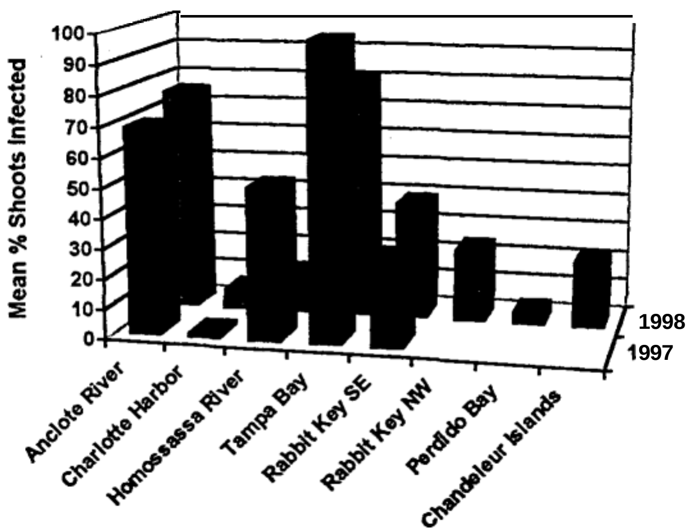

Figure 3. The mean percent of *Thalassia* shoots infected with *Labyrinthula* at 8 sites sampled in the eastern Gulf of Mexico during an EPA-funded study in the summer and fall of 1997 and 1998.

Charlotte Harbor and Perdido Bay; these sites had salinity fluctuations. Most other sites (including Tampa Bay) had infection levels higher than those found in the one Florida Bay site in this study (Rabbit Key basin) where seagrass losses have been substantial in the past. These results led to a request by the Tampa Bay Estuaries Program to begin a monitoring study of *Labyrinthula* in the Tampa Bay area in the fall of 1999.

Potential impacts of *Labyrinthula* on *Thalassia* in the Eastern Gulf of Mexico were extrapolated based on our data from these 10 sites and the information gained in the Florida Bay studies. The impacts are not now known, but the few data from this study suggest that the role(s) of *Labyrinthula* in these areas may be similar to that found in Florida Bay. In summary:

- Low salinity sites (Charlotte Harbor, Perdido Bay): **NO IMPACT?**
- Environmentally stressed sites (Anclote River, Homossassa River, Tampa Bay, Rabbit Key, Sarasota Bay): **ACUTE OR CHRONIC DIE-OFF?**
- Pristine sites (Chandeleur Islands, St. Joe Bay?): **THINNING TO PATCHY DIE-OFF?**

The St. Joe Bay site, which is categorized as pristine, may actually be experiencing some stress from elevated sediment sulfide levels caused by sea urchin grazing in the area (Paul

Carlson, personal communication). Alternately, the *Labyrinthula* may have been able to invade the blade tissue more easily because of mechanical damage caused by the urchins, with a subsequent indirect increase in sediment sulfide levels (microbial activity produces sulfide during the decay of below ground tissue fiom sick and dying plants). The Chandeleur Islands site may be a site similar to the Sunset Cove site in Florida Bay, where we hypothesize that *Labyrinthula* plays the role of primary pathogen, thinning the dense seagrass beds and causing patchy die-off.

METHODS

Samples were collected during the regular fall monitoring of established transects in Tampa Bay and the surrounding area. In 1999 samples were collected in Tampa Bay (Lower, Middle, and Old Tampa Bay), Clearwater Harbor, Boca Ciega Bay, and Sarasota Bay. In 2000 samples were also collected from Charlotte Harbor, Hillsborough Bay, and Egmont Key. Ten short shoots of *Thalassia testudinum* were randomly collected fiom each transect unless the transect had so few shoots that the field team felt that collecting ten would be harmful to the site. The shoots were individually bagged in labeled zip lock bags and put in a cooler on ice with an isulating layer between the samples and the ice. Coolers were either delivered or shipped to the Florida Marine Research Institute for processing. All samples were processed within 72 hours of collection, most within 24 hours. Each blade of every shoot was evaluated for lesion coverage by estimating the amount of blade covered with lesions using a modified wasting index (Burdick et al., 1993). A small piece (≤ 5 $mm2$) of the most-lesioned blade of each shoot was then split open on a glass slide using a dissecting knife and coverslipped. Both pieces fiom each lesion were then examined at 400X with light microscopy to determine the presence/absence of *Labyrinthula*. Data were compared fiom two years of sampling to estimate the extent of *Labyrinthula* distribution and occurrence in *Thalassia* in the Tampa Bay area. These limited data were then compared to larger data sets from the eastern Gulf of Mexico and Florida Bay to try and predict potential impacts of *Labyrinthula* on *Thalassia* populations in the Tampa Bay area.

RESULTS

Tampa Bay Area (1999)

Samples were collected during the fall 1999 Tampa Bay Estuaries monitoring program to begin looking at the distribution of *Labyrinthula* and severity of lesions in *Thalassia* in this area. The data are very limited, but our analysis of 5-10 shoots randomly collected From 32 transects showed that 75% of all transects with *Thalassia* were positive for *Labyrinthula* and all bay segments (sites) were positive except Sarasota Bay (see Figure 4). Results of our monitoring studies show that the highest percentage of infected transects was in Old Tampa Bay (l00%, N=2) and Lower Tampa Bay (90.9%, N=1l) (see Table l), but the highest percentage of shoots infected was Clearwater Harbor (44%, N= 50). The most severe lesion coverage was also found in Clearwater Harbor (2.09%; the average percent lesion cover of all blades at the site, with or without lesions). The 3 transects in Sarasota Bay were negative for *Labyrinthula* and were nearly lesion-free (average lesion severity = 0.28%).

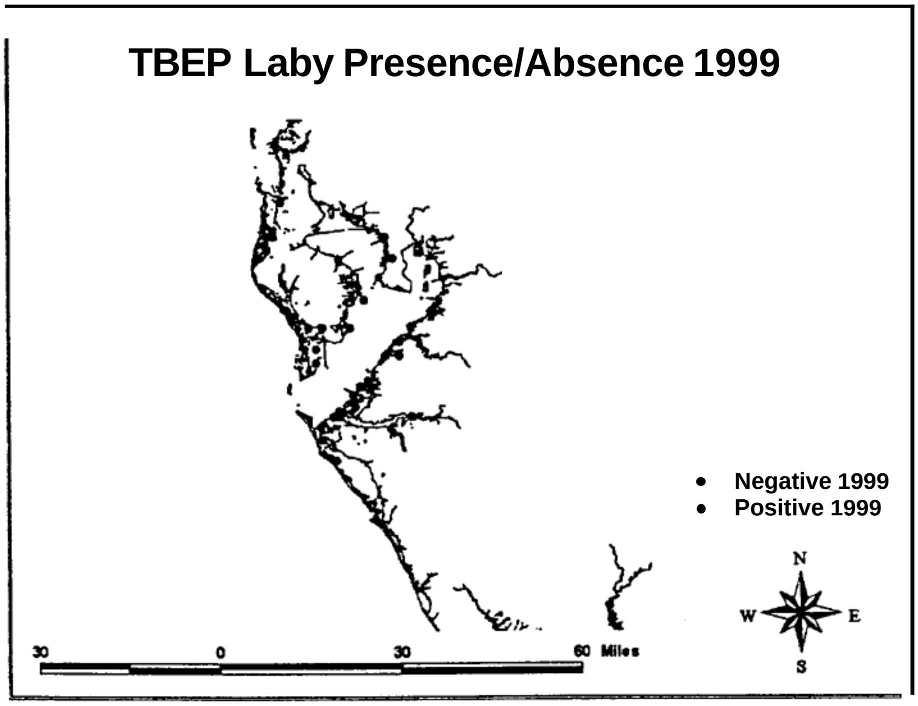

Figure 4. Map of the Tampa Bay area showing sites in fall 1999 where *Thalassia* was infected with *Labyrinthula* (in red) and negative sites in blue.

1999	
transects sampled	32
shoots examined	268
leaves examined	891
leaves microscopically examined	201
leaves(shoots) w/Laby=positive	96
lesioned leaves	335
negative transeds	6
positive=infected transeds	24

	infected t-sects in 1999	N=	%	infected shoots in 1999	N=	%	avg lesion severity/leaf in 1999	N=
BCB (Boca Ciega Bay)	3	4	75	8	20	40	0.64	73
CLW (Clearwater)	6	7	85.7	22	50	44	2.09	170
LTB (Lower Tampa Bay)	10	11	90.9	41	104	39.4	1.71	329
MTB (Middle Tampa Bay)	3	5	60	14	44	31.8	2	123
OTB (Old Tampa Bay)	2	2	100	8	20	40	0.92	61
SAR (Sarasota)	0	3	0	0	30	0	0.28	93
	24	32		93	268			849

Table 1. Data from all 1999 TBEP monitoring sites where *Thalassia* was collected for disease evaluation.

Tampa Bay Area (2000)

In fall, 2000 *Thalassia* shoots were collected at almost twice as many transects (59) as in the fall of 1999 (32). Eighteen of these additional transects were in Charlotte Harbor. In fall2000 78% of the transects with *Thalassia* were infected with *Labyrinthula* and all bay segments (sites) were infected except Sarasota Bay (see Figures 5 and 6).

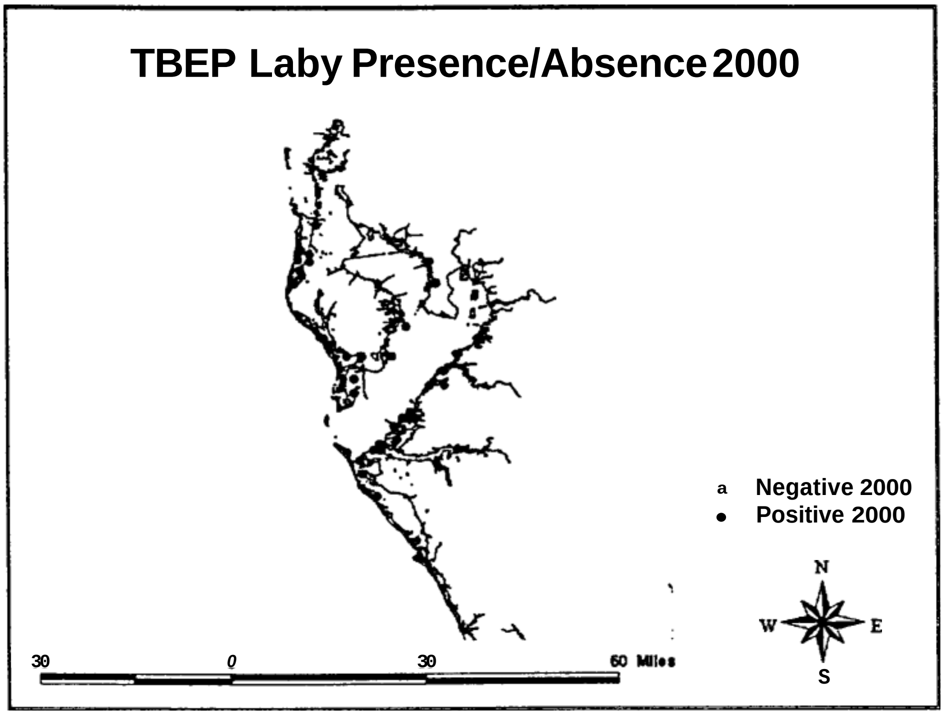

Figure 5. Map of the Tampa Bay area showing sites in fall 2000 where *Thalassia* was infected with *Labyrinthula* (in red) and negative sites in blue.

Data from the fall 2000 sites are found in Tables 2a and 2b. This was the first time that samples fiom Charlotte Harbor were evaluated for *Labyrinthula* infection and lesion severity since the preliminary study in the eastern Gulf of Mexico in 1997-1998. At that time infection levels at the EPA sites in Charlotte Harbor were very low compared to the other sites in the eastern Gulf of Mexico (see Figure 3). In fall 2000 the percent of transects infected at each Charlotte Harbor site ranged fiom 66.7-100 % and the percent of the shoots infected at each site ranged from 16.6-56.7%. The Intercoastal Waterway site (ICW) and the Pine Island site (PI) had the highest lesion severities of any of the sites in the Tampa Bay area in fall, 2000 (1.57 and 1.77 respectively).

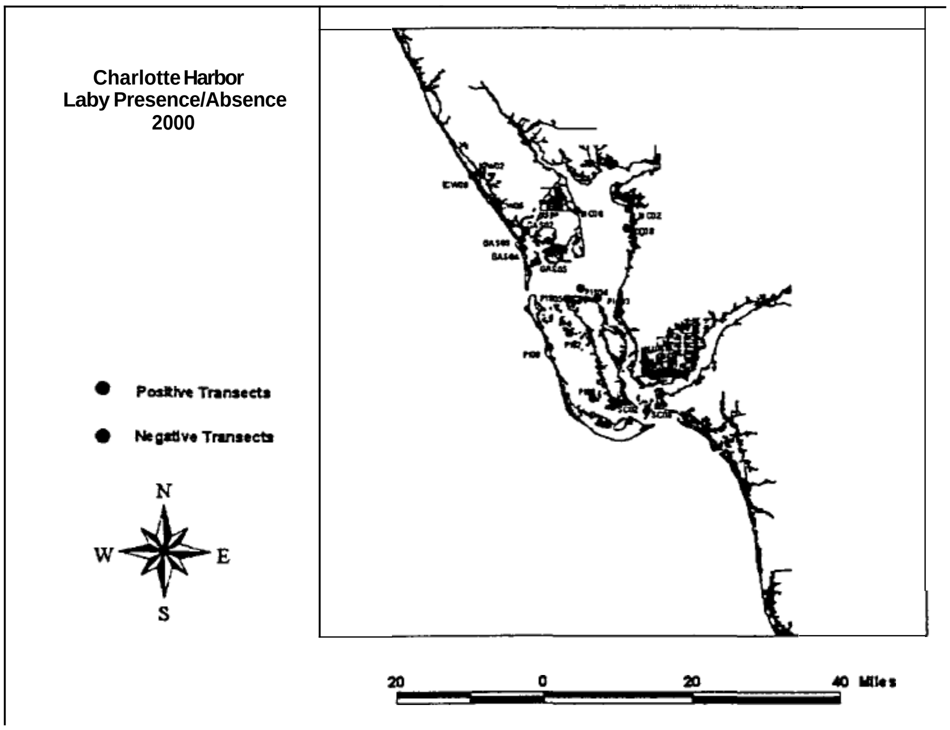

Figure6. Map of the Charlotte Harbor area showing sites in fall 2000 where *Thalassia* was infected with *Labyrinthula* (in red) and negative sites in blue.

2000	
transects sampled	59
shoots examined	579
leaves examined	1674
leaves microscopically examined	350
leaves(shoots) w/Laby=positive	193
lesioned leaves	594
negative transects	13
positive=infectedtransects	46

Table 2a. Data from fall 2000 sampling of all transects in the Tampa Bay area where *Thalassia* was collected for disease evaluation.

2000

	infected t-sects in 2000	N=	%	infected shoots in 2000	N=	%	avg lesion severity/leaf in 2000	N=
BCB (Boca Ciega Bay)	5	6	83.3	17	60	28.3	0.69	176
CLW (Clearwater)	6	8	75	25	79	31.6	1.37	214
LTB (Lower Tampa Bay)	9	10	90	31	100	31	1.06	262
MTB (Middle Tampa Bay)	7	10	70	32	95	33.7	1.21	279
OTB (Old Tampa Bay)	2	2	100	10	20	50	1.4	45
SAR (Sarasota)	0	3	0	0	30	0	0.1	91
EG(Egmont Key)	1	1	100	2	23	8.7	0.53	59
GAS (Charlotte)	3	4	75	15	38	39.5	0.89	120
HIB (Hillsborough Bay)	1	1	100	2	4	50	0.33	12
ICW (Charlotte)	3	3	100	17	30	56.7	1.57	98
MC (Charlotte)	2	3	66.7	5	30	16.6	0.28	101
PI (Charlotte)	2	3	66.7	12	30	40	1.77	83
PIS (Charlotte)	3	3	100	8	30	26.7	0.89	84
SC (Charlotte)	2	2	100	6	20	30	0.5	50
	46	59		182	589			1674

Table 2b. Data (by bay segment or site) from fall 2000 sampling of all transects in the Tampa Bay area where *Thalassia* was collected for disease evaluation.

Results from sites sampled in both 1999 and 2000 show that a higher percent of the sites were infected in fall 2000 than in fall 1999 and the percentage of infected shoots was higher at Middle Tampa Bay and Old Tampa Bay in the second year of sampling. However, the percentage of infected shoots was slightly lower at Boca Ciega Bay, Clearwater Harbor, and Lower Tampa Bay in 2000 than in 1999 (see Table 3b). Average lesion severity decreased in Clearwater Harbor, Lower Tampa Bay, and Middle Tampa Bay, and increased in Boca Ciega Bay and Sarasota Bay.

TBEP - Results of Labyrinthula Monitoring for Transects Sampled Both in 1999 and 2000		
#of Bay Segments sampled both years	6	
#of T-sects sampled both years	29	
shoots examined	267	289
leaves examined	801	781
leaves microscopically examined	180	175
leaves(shoots) w/Laby	90	104
lesioned leaves	298	309
negative transects	6	4
positive=infected transects	23	25

Table 3a. Data from sites sampled in fall 1999 and resampled in fall 2000.

	1999							
	infected t-sects in 1999	N=	%	infected shoots	N=	%	avg lesion severity/leaf in 1999	N=
BCB (Boca Ciega Bay)	3	4	75	11	35	31.4	0.5	100
CLW (Clearwater)	6	7	85.7	22	59	37.3	2.11	181
LTB (Lower Tampa Bay)	9	9	100	35	89	39.3	1.53	273
MTB (Middle Tampa Bay)	3	5	60	14	44	31.8	2	123
OTB (Old Tampa Bay)	2	2	100	8	20	40	0.92	61
SAR (Sarasota)	0	2	0	0	20	0	0.16	63
	23	29		90	267			801
	2000							
	infected t-sects in 2000	N=	%	infected shoots	N=	%	avg lesion severity/leaf in 2000	N=
BCB (Boca Ciega Bay)	4	4	100	14	40	35	0.84	122
CLW (Clearwater)	6	7	85.7	25	69	36.2	1.59	184
LTB (Lower Tampa Bay)	9	9	100	31	90	34.4	1.16	231
MTB (Middle Tampa Bay)	4	5	80	24	50	48	1.72	143
OTB (Old Tampa Bay)	2	2	100	10	20	50	1.4	45
SAR (Sarasota)	0	2	0	0	20	0	0.09	56
	25	29		104	289			781

Table 3b. Data from sites sampled in fall 1999 and resampled in fall 2000.

SUMMARY

Potential impacts of *Labyrinthula* on *Thalassia* populations in the Tampa Bay area could range from no impact, through seagrass losses resulting in beneficial thinning of overdeveloped beds, to chronic or acute patchy die-off

In summary, the distribution and potential impacts of *Labyrinthula* infection on *Thalassia* populations depend on a suite of interacting factors (salinity, seagrass density, pathogenicity, environmental stressors, and seagrass resistance to disease). All of these factors, as well as others that may as yet not have been identified, need to be taken into consideration before the potential impacts of *Labyrinthula* infections on *Thalassia* populations in any particular geographic area can be predicted. The roles of *Labyrinthula* in Seagrass health in Florida Bay have been studied for 5 years. We are presently testing our model in Florida Bay in two areas (Barnes Key and Sunset Cove) with different environmental conditions, one with acute die-off and the other with chronic die-off. Only very preliminary *Labyrinthula* and lesion distribution data have been collected elsewhere in the state.

Long-term careful monitoring of *Labyrinthula* should be carried out in estuaries other than Florida Bay, especially in those with environmental stresses. The dynamics of *Labyrinthula* distribution must be more clearly understood before the impacts of this slime mold on seagrass populations can be predicted. Seagrass recovery in urban estuaries must include health evaluations of seagrass beds to insure that gains in seagrass coverage can be maintained over time.

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