

A FUNGAL SURVEY OF THREE PONDS ON NORTH PADRE ISLAND

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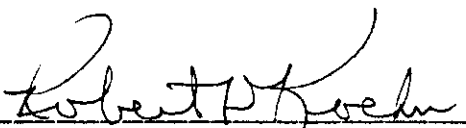
Florence Marie Oxley, B.S.

San Marcos, Texas

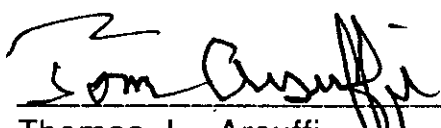
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Approved:


Robert D. Koehn


Paula S. Williamson


Thomas L. Arsuffi

Dean of the Graduate School

ABSTRACT

A fungal survey of three brackish water ponds on North Padre Island, Texas was conducted. Plant material, *Typha domingensis* in Pond A, *Andropogon glomeratus* in Pond B, and *Fimbristylis castanea* in Pond C, that was submerged or had been submerged was collected on a monthly basis (July 1990-June 1991) and examined for fungi. *Baptisia leucophaea* plants, which were blown into the ponds, were also collected and examined. Additionally, a six month study was done to determine the relative abundance of fungi colonizing *T. domingensis*, a major source of biomass in Pond A. Culms were randomly selected, sectioned, and cleared so that the ascomata could be quantified. A species of *Pestalotia* isolated from *T. domingensis* was compared to an airborne species of *Pestalotia* to determine the effect of salinity on fungal growth.

The three ponds support a diverse assemblage of mycoflora dominated by Deuteromycetes and Ascomycetes. Fungi colonizing *T. domingensis* reach their peak abundance in February, which corresponds to an observed increase in senescent plant material in the pond. Based on its ability to grow in media of increasing salinities, the *Pestalotia* species isolated from *T. domingensis* appears to have adapted to a saline habitat.

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INTRODUCTION

Many studies have been conducted to determine substrate-fungus relationships in terrestrial (Dickinson, 1965; Dickinson and Morgan-Jones, 1966; Dickinson and Pugh, 1965a, 1965b), freshwater (Cunnell, 1956; Apinis, et al., 1972; Panwar and Sharma, 1983) and marine or estuarine habitats (Goodman, 1959; Anastasiou and Churchland, 1969; Sivanesan and Manners, 1970; Pugh and Mulder, 1971; Koehn and Garrison, 1981; Koehn, 1985). These relationships may be dependent on the available surface area, nutrient content of the substrate, and the physical environment in which the substrate is located (Apinis, et al., 1972). Substrate-fungus associations may be very specific, i.e., fungi which colonize algae are restricted to a single class of algae, but may attack a number of genera within one class (Kohlmeyer and Kohlmeyer, 1979). Fungi may also be associated with a particular plant (Cunnell, 1956; Pugh and Mulder, 1971; Apinis, et al., 1972; Gessner, et al., 1972; Gessner and Goos, 1973a, 1973b; Gessner and Kohlmeyer, 1976; Gessner, 1977; Gessner, 1978; Panwar and Sharma, 1983;) and may be limited to specific organs, such as stems, leaves, or seeds, of the plant body.

Fungal colonization of substrates also provides information on patterns of seasonality or succession. Seasonality may be produced by factors such as substrate nutrient exhaustion (Cooke and Rayner, 1984) or an increase in the habitat water content (Manoharachary and Ramarao, 1981). Cooke and Rayner (1984) recognize two types of fungal succession. Seral succession involves the replacement of an entire community of fungi by another, different fungal community, while substratum succession involves the replacement of only one or a few species of the resident fungi (Cooke and Rayner, 1984).

Studies of *Dactylis glomerata* , a terrestrial macrophyte, by Webster (1956, 1967) and Webster and Dix (1960) revealed a seral succession during a two year study in which there was a general change in the abundance of the whole fungal flora rather than a change in the frequency of a few species. Similar studies done in marine and estuarine habitats (Borut and Johnson, 1962; Jones, 1963; Koehn, 1982), as well as freshwater ecosystems (Manoharachary and Ramarao, 1981) have demonstrated the occurrence of seasonality, while others have not. While Jones (1963) and Koehn (1982) reported no apparent fungal succession during their studies, Borut and Johnson (1962) and Manoharachary and Ramarao (1981) did observe fungal successions during their studies and

attributed them to a number of factors, including temperature, type of substrate, and available nutrients.

Among the features that promote the survival of many higher fungi is their capacity to tolerate and adapt to environmental extremes (Tresner and Hayes, 1971). By the same token, other fungi are equally influenced by their inability to tolerate and adapt to a less than optimum environment. According to Tresner and Hayes (1971), one of the factors that may limit the distribution of some terrestrial and marine fungi may be the salinity of their surroundings.

Many studies have been conducted to determine the effect of salinity on the reproduction and growth of freshwater, terrestrial, and marine and estuarine fungi (Gray, et al., 1963; Jones, et al., 1971; Shearer, 1972; Davidson, 1974b; Harrison and Jones, 1975; Amon, 1976; Padgett, 1978; Amon and Yei, 1982; Adler, 1986). These studies have shown that even fungi considered to be strictly terrestrial or aquatic can grow and reproduce at low salinity levels, thus increasing their distribution and range (Gray, et al., 1963; Jones and Jennings, 1964; Ristanovic and Miller, 1969; Harrison and Jones, 1971; Tresner and Hayes, 1971). In addition, studies comparing the effect of salinity on reproduction of terrestrial and marine species show that terrestrial species, while capable of

reproducing in low to moderate salinities, are not as reproductively successful as their marine counterparts at higher salinity concentrations and this may limit their numbers in saline environments (Byrne and Jones, 1975a, 1975b). Additionally, other abiotic factors, such as pH and temperature, may collectively work to decrease salinity tolerances (Norkrans, 1966; Hobot and Jennings, 1981; Wethered and Jennings, 1985).

In some habitats, direct observation of fungal development on the substrate is possible and a measure of the amount and distribution of fungal material present can be obtained (Cooke and Rayner, 1984). In many cases, measurement of mycelial growth or quantification of the number of fungal spores and/or ascomata can provide a reasonably accurate measurement of the relative abundance of and extent of colonization by the fungal species present. This type of approach is useful for locating sites of fungal activity, detecting fluctuations in community structure, and following changes in populations of individual species (Cooke and Rayner, 1984).

According to Cooke and Rayner (1984), the size of mycelia, spores, and ascomata are restricted by the physiochemical limits of the resources in which they are growing, the anatagonistic effects of competing organisms, and competition from other members of the same species. As

smaller mycelia, spores, or ascomata are produced, the surface area to volume ratio increases which, in turn increases the amount of substrate surface available for colonization. Therefore, size can also be an important indicator of the degree and efficiency of substrate colonization.

The three ponds selected for this investigation present interesting ecosystems for study since they are self-contained environments located within a mile of the Gulf of Mexico and have no physical connection with the adjacent marine habitat. This study was designed to determine what fungi inhabit the ponds, what substrate-fungus relationships exist, and if "true" marine fungi can move into and adapt to habitats that are not completely marine. The relative abundance of fungi colonizing *Typha domingensis* and the effect salinity has on the production of fungal biomass were also studied

SITE DESCRIPTION

This study focuses on the mycoflora associated with three ponds located on North Padre Island, Kleburg County, Texas. According to Rafferty (1986), Padre Island is the longest of the barrier islands that protect the Texas coast (Figure 1). It extends 130 km from Corpus Christi Bay south to Brazos Santiago Pass near the mouth of the Rio Grande River (Drawe, et al., 1981). The region has a littoral climate that is influenced by prevailing, moisture laden, southeasterly winds that carry salt spray inland (Drawe and Kattner, 1978). Average annual rainfall is approximately 76 cm per year, with peaks occurring during the spring and fall months. Moderate temperatures are the norm, although there are many foggy days and high humidity during the winter and early spring.

Pond A is located approximately one half-mile inside the gate of the park and is north of Malaquite Beach (Figure 2). When full, the pond has a maximum surface area of 1542 m² and a maximum volume of 623 m³. This pond is actually two ponds separated by a shallow sill that becomes exposed during the summer dry-down (Sissom, 1990) and it is surrounded by low brush (Figure 3). A large population of *Typha domingensis*, a cat-tail, is located at the east end of the pond (Figure 4).



Figure 1. Map of Padre Island, the longest barrier island protecting the Texas coast.

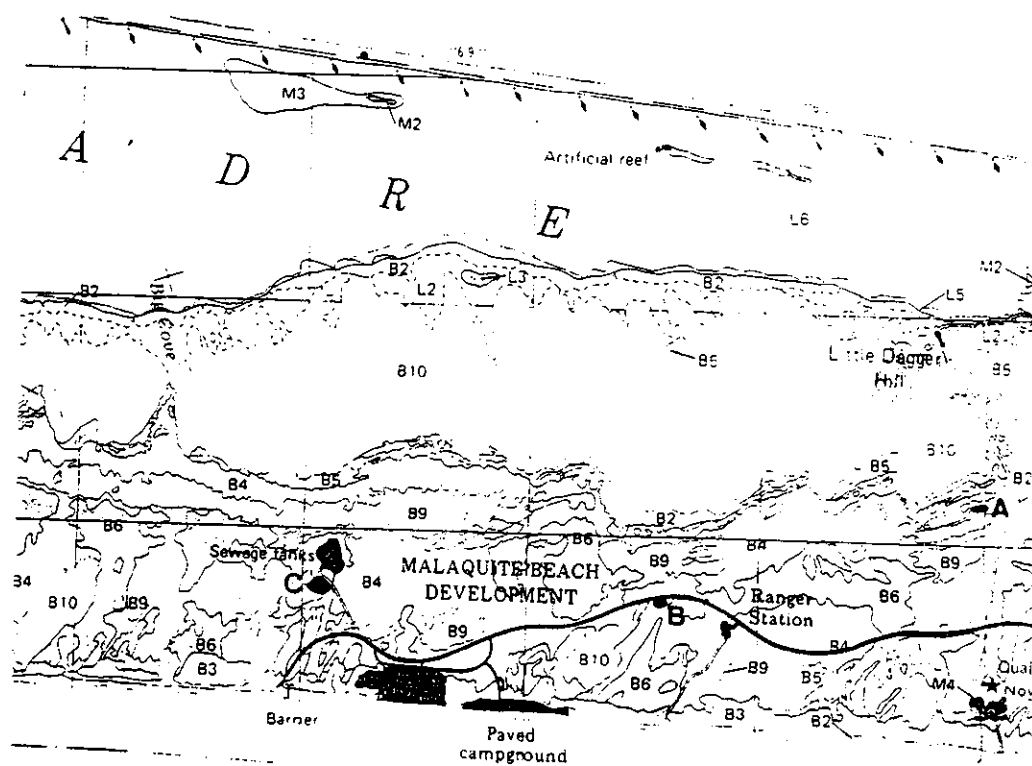


Figure 2. Location of the three study sites, Pond A, Pond B, and Pond C, within the National Seashore.

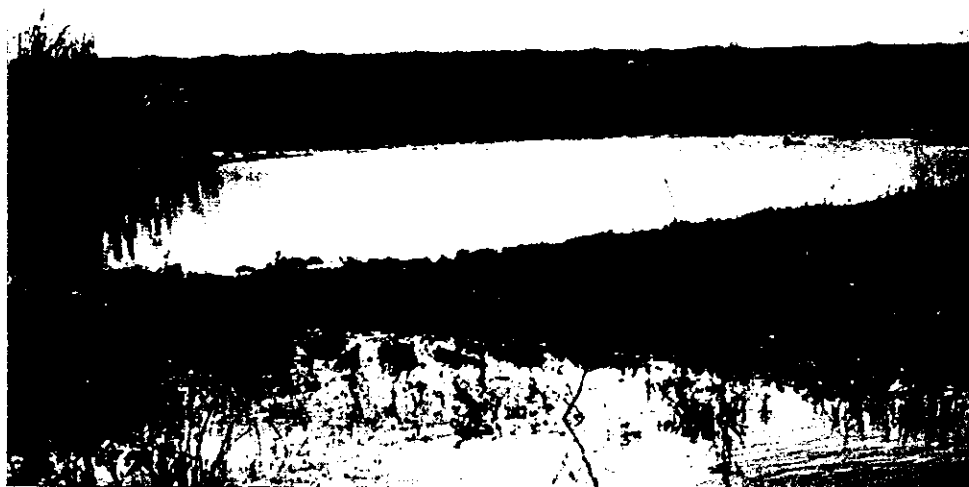


Figure 3. Pond A during the summer dry-down. Note the two distinct ponds separated by a narrow sill.

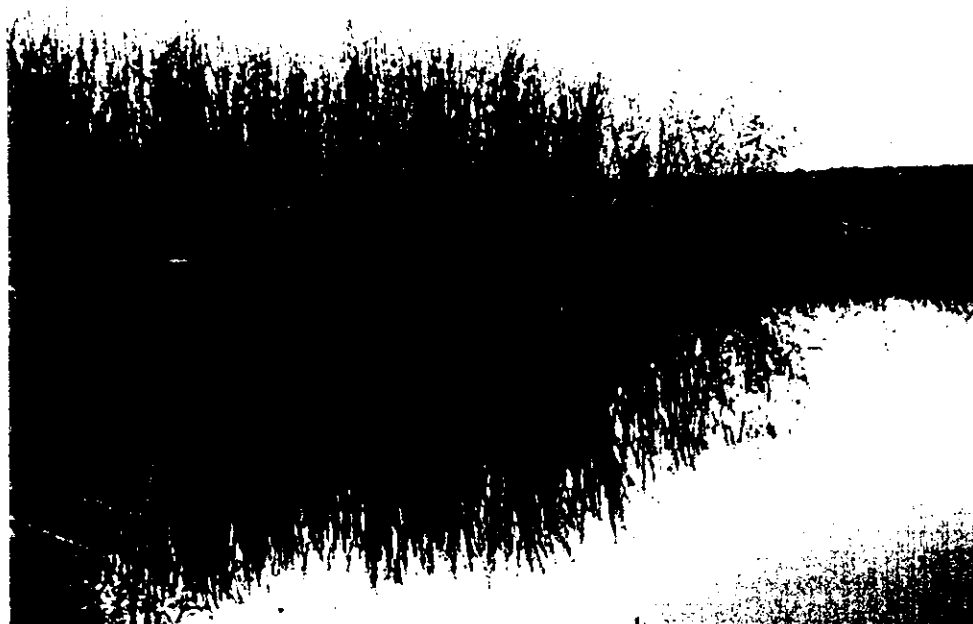


Figure 4. Pond A, showing the large population of *Typha domingensis* at the east end of the pond.

Pond B is one-half mile east-southeast of Pond A on the south side of the main road (Figure 2). This pond has a maximum surface area of 3167 m² and a maximum volume of 4532 m³. It is roughly rectangular in shape, with a clay-like bottom overlaid with soft mud (Sissom, 1990) and is surrounded by weedy monocots and dicots (Figure 5).

Pond C is located one mile west of Pond A (Figure 2) next to a hazardous waste disposal site. This is the largest of the three ponds with a maximum surface area of 11053 m² and a maximum volume of 7732 m³. The perimeter of Pond C is surrounded by grasses and, unlike the other two ponds, the bottom of this pond is covered by a dense population of *Chara* sp., a stonewort (Figure 6).



Figure 5. Pond B which is rectangular in shape and surrounded by weedy monocots and dicots.



Figure 6. Pond C during the summer dry-down showing the large population of *Chara* sp. that covers the pond's bottom.

METHODS AND MATERIALS

Plants that were unique to each pond and that spent at least part of the study period immersed in the water were collected. A large population of *Typha domingensis*, a cattail, at one end of pond A was sampled; *Andropogon glomeratus*, a grass, was collected at pond B; and *Fimbristylis castanea*, a sedge, was the substrate studied in pond C. In addition, *Baptisia leucophaea*, which acts like a tumbleweed and is blown into all the ponds from an outside source, was collected and studied as a substrate common to all three ponds.

Ponds were sampled at monthly intervals from July 1990 through June 1991. At each pond, dead plant material that was immersed, or had been immersed, was randomly collected, placed into plastic trash bags, and transported back to the laboratory. The water in each pond was analyzed using a LaMotte water testing kit. Dissolved oxygen, pH, and salinity, as well as water temperatures, were determined on site for each pond.

The plant material was examined using a dissecting microscope to locate fungal reproductive structures, which were removed using dissecting needles and mounted on slides in clear lactophenol. The coverslips were sealed with clear fingernail polish. Fungi were identified

using a light microscope and current available keys (Barnett and Hunter, 1987; Hanlin, 1990).

To determine the effect varying salinities have on fungal growth and biomass production, two experiments were performed. The fungi used in these experiments were two species of *Pestalotia*. *Pestalotia* sp. was isolated from the *T. domingensis* growing in pond A and *P. sydowiana* was isolated from the air in San Marcos, Texas. Both species were maintained on plain cornmeal agar and cornmeal agar containing malt and yeast extracts.

Koehn's liquid medium (Koehn, 1971) was the base medium used in both experiments and was prepared in the following way:

KH ₂ PO ₄ 0.50 g	ZnSO ₄≈0.0088 g
K ₂ HPO ₄ 0.60 g	MnCl ₂≈0.0014 g
MgSO ₄ 0.50 g	EDTA*.....4.0 ml
NaCl.....0.10 g	Casein.....0.10 g
CaCl ₂ 0.10 g	Dextrose.....10.0 g
FeSO ₄≈0.0049 g	Vitamins†.....10.0 ml
	Distilled H ₂ O.....1000 ml

* EDTA was prepared using 50 g EDTA, 31 g KOH, and 1000 ml distilled H₂O

† Vitamin solution was prepared using 0.10 g biotin, 0.10 g thiamin, and 1000 ml distilled H₂O

In the first experiment, 250 ml portions of Koehn's medium were poured into seven 500 ml Erlenmeyer flasks and mixed with total NaCl concentrations of 0.0%, 0.8%, 1.5%, 2.3%, 3.0%, 6.0%, and 9.0%, respectively. These concentrations represent approximately zero, one quarter, one half, three quarters, full strength, two, and three times the concentration of natural seawater. The pH of each treatment was adjusted to 7.2 using 1M KOH. Each solution was then cold filtered through 0.45 μ m Milipore filters and 50 ml portions were measured into 125 ml Erlenmeyer flasks. Each flask was sealed with cotton/cheesecloth stoppers and autoclaved at 121°C and 19 pounds of pressure for five minutes. The flasks were then allowed to stand at room temperature for one day to ensure that no contamination was present.

Each flask was inoculated with an agar plug measuring 4 mm in diameter and bearing hyphal tips and/or spores. The flasks were recapped with cotton/cheesecloth stoppers, placed on a shaker table, and incubated at room temperature (20-25°C) for seven days. A total of five replicates were done for each treatment with each fungus.

After seven days, the cultures were placed into 50 ml Nalgene centrifuge tubes and centrifuged at 4000 rpms and 22°C for 40 minutes in

a Beckman Acuspin FR centrifuge. The liquid was decanted, replaced with 50 ml of distilled water and centrifuged again at 4000 rpms and 22°C for an additional 40 minutes. The liquid was decanted and the remaining fungal biomass placed into pre-weighed aluminum cups and dried at 80°C for 24 hours. The dried fungal biomass was brought to room temperature in a dessicator, and weighed. In some cases, the cultures were frozen to prevent additional growth until equipment was available for use.

The second experiment was similar to the first, but 6.25 g of Noble's agar was added to the liquid medium which was autoclaved at 121°C and 19 pounds of pressure for 15 minutes. Approximately 25 ml of each medium was poured into each of 10 plastic Petri dishes (100 x 20 mm). The dishes were allowed to sit at room temperature for three days to ensure that there was no contamination.

Plugs were made as described earlier and used to inoculate the center of each Petri dish. There were five replicates for each treatment with each isolate. Each plate was sealed with Parafilm and allowed to incubate at room temperature for seven days. At the end of the seven day incubation period, the diameters of the colonies produced were measured.

A six month study, beginning January 1991 and ending June 1991,

was done to determine the relative abundance of fungi colonizing the *Typha* found in pond A. Two to three dead *Typha* plants were randomly collected every month, placed into plastic bags, and transported to the laboratory. Only plants that were immersed in the water at the time of sampling were collected.

Each plant was measured and roughly divided into quarters. Using a razor blade, 10 to 20 sections were randomly cut from each of the four quarters. These sections were then thinly sectioned lengthwise and excessive cortex material was removed leaving the ascomata bearing surface tissues. The sections were trimmed to measure approximately 1 cm x 1 cm and put into 10% KOH (the KOH was changed once) overnight. In the morning, they were rinsed three times in distilled water and acidified in glacial acetic acid for one to two minutes. Following acidification, they were rinsed once in distilled water and placed into full strength Clorox until they turned white. They were rinsed three more times in distilled water and stained for 20-30 minutes using 0.05% (w/v) trypan blue in lactophenol which had been diluted 50/50 with lactic acid. After staining, the sections were rinsed three times with distilled water and placed into a chloral hydrate (5 g chloral hydrate in 2 ml water) solution overnight, or, until the sections cleared. Because the sections tended to

fall apart during the clearing process, only those sections still measuring 1 cm x 1 cm (12-60 per plant) were rinsed and stored in clear lactophenol until examination.

Individual sections were placed on a slide without a coverslip and examined for ascomata using a light microscope. The low power (10X) objective, equipped with a micrometer, was used to count and measure the ascomata observed. Twenty random counts were made for each section and the number of ascomata, as well as the size of each, were recorded. Because the size of each plant sampled varied, ascomata counts were standardized to represent plants with 50 cm lengths.

RESULTS

Physical Properties of Pond Water

Water temperatures in the ponds varied throughout the study period. Temperatures were the highest during the summer and early spring and reflected a decrease in pond volumes. Low temperatures were recorded during the late fall and early winter months and corresponded to an increase in the volume of the ponds (Figure 7).

Dissolved oxygen also varied in the ponds during the study period. High concentrations of dissolved oxygen were observed in Ponds A and B during January and in Pond C during December. These high concentrations reflect the cooler water temperatures in the ponds at these times. Low concentrations were observed in Ponds A and B during May and in Pond C during October (Figure 8).

The pH reached a maximum for Pond A in October, Pond B in November, and Pond C in December. Following these peaks, pH remained relatively constant throughout the remainder of the study period (Figure 9).

Of the three ponds, Pond C had the highest salinities, with a maximum salinity occurring in October (Figure 10). Ponds A and B had relatively little differences in salinity, although slightly lower salinities

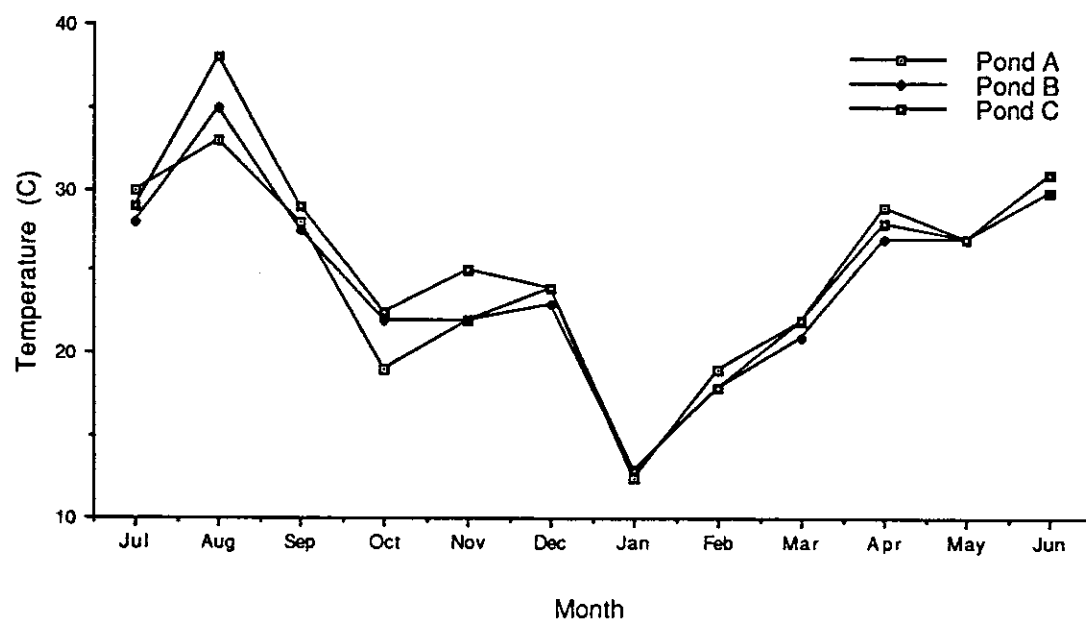


Figure 7. Water temperatures during the study period, July 1990-June 1991.

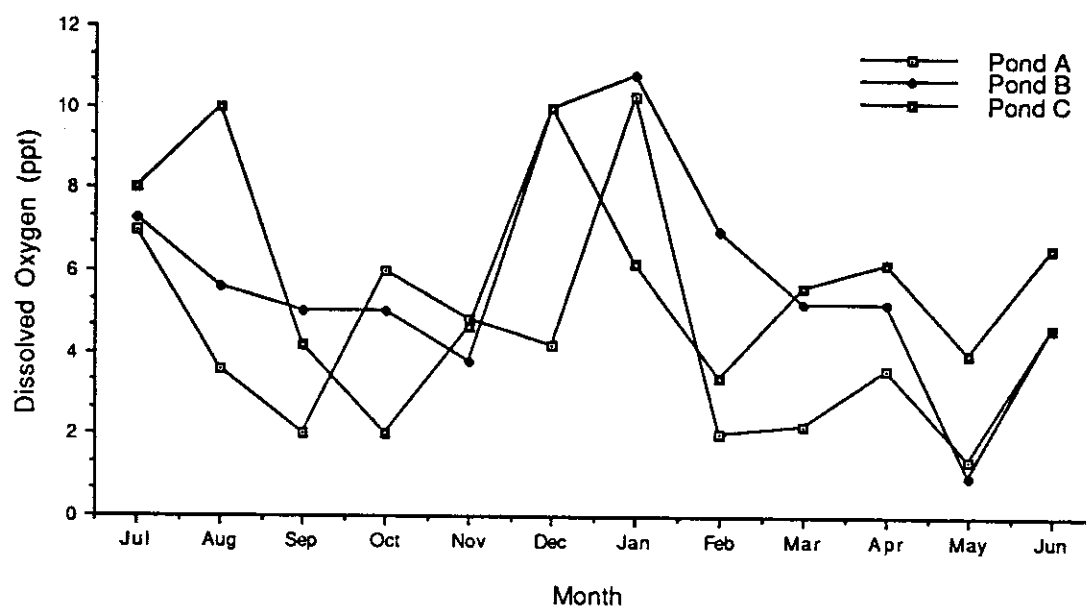


Figure 8. Dissolved oxygen during study period, July 1990-June 1991.

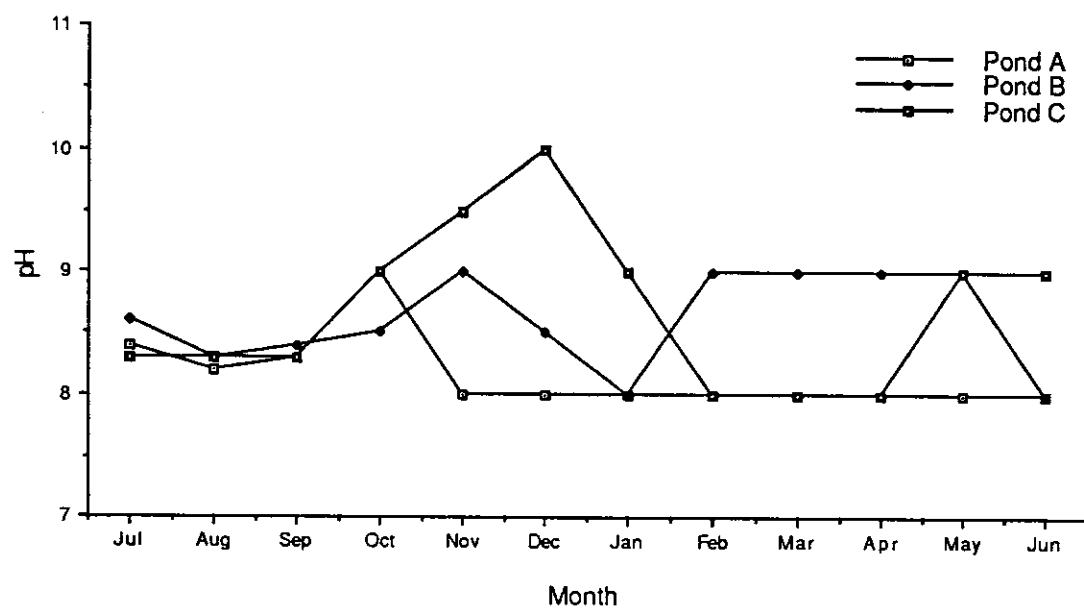


Figure 9. pH during study period, July 1990-June 1991.

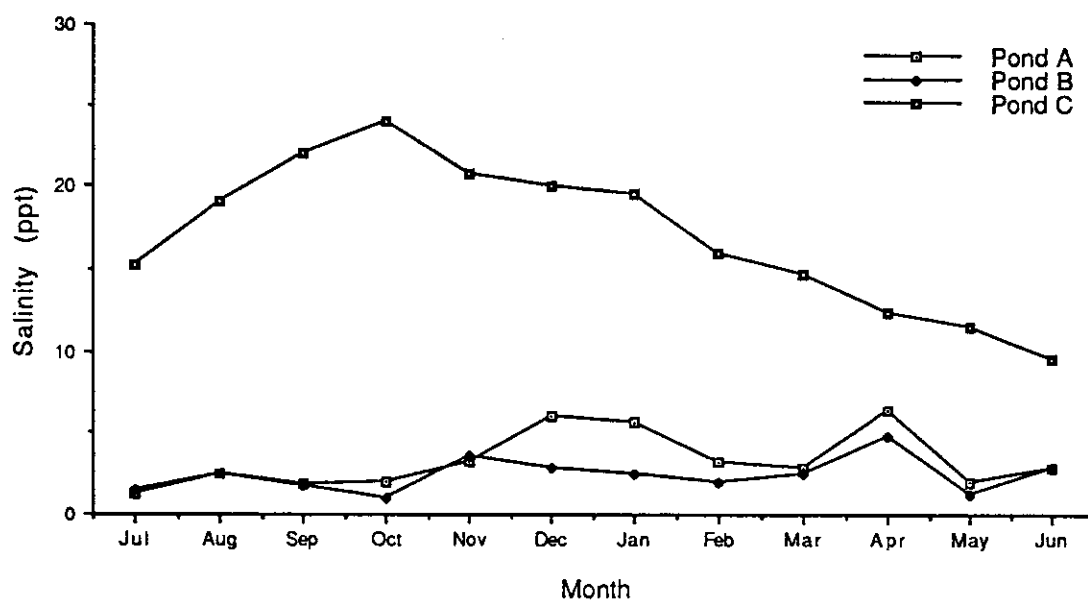


Figure 10. Salinity during study period, July 1990-June 1991.

were recorded for Pond B during December, January, and February. Both Pond A and Pond B had peak salinities in April.

Fungi Isolated

The dominant fungi isolated from the substrates collected from the ponds belong to the class Deuteromycetes, commonly referred to as the Fungi Imperfecti. Eighteen taxa of Hyphomycetes and nine taxa of Coelomycetes were isolated from the three ponds (Table 1). Of the 27 Deuteromycete taxa isolated, 14 have described marine representatives, 12 were common terrestrial/airborne species, and one "aquatic" species, *Lunulospora*, was isolated from Pond C during August. In addition, *Papulospora* was isolated from all three ponds.

A total of 12 Ascomycetes were isolated from the ponds. Loculoascomycetes dominated with ten genera, including four *Leptosphaeria* species, while *Lignincola laevis* and *Meliola* were the two Pyrenomycete species isolated (Table 2). All species except one, *Meliola*, are described by Kohlmeyer and Kohlmeyer (1979) as marine representatives (Table 2).

Pond A exhibited the highest fungal diversity. Twenty-nine species of fungi were isolated from Pond A and included 19 Deuteromycetes and

Table 1. Deuteromycetes isolated from each pond.

Deuteromycetes	Pond A	Pond B	Pond C
Hyphomycetes			
<i>Alternaria</i> Nees	X	X	X
<i>Botrytis</i> Pers.	X		
<i>Cirrenalia</i> Meyers et Moore		X*	
<i>Cladosporium</i> Link.	X	X	
<i>Curvularia</i> Boedijn.	X	X	X
<i>Dendryphiella</i> Bubak et Ranojevic in Ranojevic		X	X
<i>Dictyosporium pelagicum</i> (Linder) G.C. Hughes ex Johnson et Sparrow	X*	X*	X*
<i>Drechslera</i> Ito.		X	X
<i>Epicoccum</i> Link.		X	X
<i>Humicola</i> Traaen emend. Fassatova	X*		
<i>Lunulospora</i> Ingold			X
<i>Nigrospora</i> Zimm.	X	X	X
<i>Penicillium</i> Link.	X		
<i>Periconia</i> Bon.		X	X
<i>Pestalotia</i> de Not.	X		
<i>Stemphyllium</i> Wallr.		X	
<i>Tetraploa</i> Berk & Vr.	X	X	X
<i>Trichoderma</i> Pers.	X		
Coelomycetes			
<i>Ascochyta</i> Lib.	X		
<i>Camarosporium</i> Schulz.	X	X	
<i>Diplodia oraemaris</i> Linder in Barg- hoorn et Linder		X*	
<i>Diplodina</i> Westend.		X	X
<i>Hendersonia</i> Sacc.	X	X	X
<i>Hendersonula</i> Speg.	X	X	X
<i>Macrophoma</i> Berl. & Vogl.	X	X	X
<i>Phoma</i> Desm.	X	X	X
<i>Phylctaena</i> Mont. & Desm.		X	
Mycelia Sterilia			
<i>Papulospora</i> Preuss.	X	X	X
Total Number Deuteromycetes Isolated	19	20	17

* Marine Representatives

Table 2. Ascomycetes isolated from each pond.

Ascomycetes	Pond A	Pond B	Pond C
Pyrenomycetes			
<i>Lignincola laevis</i> Hohnk	X		
<i>Meliola</i> Fr.	X*	X*	X*
Loculoascomycetes			
<i>Didymella fucicola</i> (Sutherland) Kohlm.	X	X	X
<i>Didymosphaeria danica</i> (Berlese) Wilson et Knoyle		X	
<i>D. enalia</i> Kohlm.	X	X	X
<i>Leptosphaeria albopunctata</i> (Westend.) Sacc.	X	X	X
<i>L. discors</i> Sacc. et Ellis in Sacc.	X		X
<i>L. marina</i> Ellis et Everh.	X	X	
<i>L. oraemaris</i> Linder in Barghoorn et Linder		X	
<i>Mycosphaerella ascophylli</i> Cotton	X		X
<i>Sphaerulina oraemaris</i> Linder in Barghoorn et Linder	X		
<i>S. pedicellata</i> Johnson	X		
Total Number Ascomycetes Isolated	10	7	6

* Terrestrial Representatives

ten Ascomycetes. Table 3 lists the fungi identified from Pond A and the substrates from which they were isolated. Seven Deuteromycetes and three Ascomycetes were unique to Pond A.

Pond B had the second highest number of species isolated. Of the twenty-seven species of fungi identified, 20 represented Deuteromycetes and seven were Ascomycetes. Pond B fungi and the substrates from which they were isolated are listed in Table 4. Only three Deuteromycetes and two Ascomycetes were unique to this pond.

The least number of species were isolated from Pond C. Twenty-three species, including 17 Deuteromycetes and six Ascomycetes, were identified during the study. Table 5 lists the fungi and the substrates that supported them in Pond C. *Lunulospora* was the only taxon unique to this habitat.

Species richness and seasonal occurrence of fungi in each pond varied from month to month. The greatest number of species in Pond A, nine, was isolated during July, February, and June (Table 6). *Leptosphaeria albopunctata*, *Alternaria* sp., and *Didymella fucicola* were the most frequently isolated species. In Pond B, eight species were identified during October, January, and April and seven species in July (Table 7). The most frequently occurring species in this pond were *Tetraploa* and *L.*

Table 3. Fungi isolated from *Baptisia leucophaea* (B) and *Typha domingensis* (T) in Pond A, July 1990-June 1991.
(* denotes fungi unique to Pond A.)

Fungus	Month	J	A	S	O	N	D	J	F	M	A	M	J
<i>Alternaria</i>		B	BT		T		B	BT	T				BT
<i>Ascochyta</i> *						BT	B			B			
<i>Botrytis</i> *						B							
<i>Camarosporium</i>									BT		B	B	
<i>Cladosporium algarum</i>		B	T										
<i>Curvularia</i>				B								T	
<i>Dictyosporium pelagicum</i>				B						T			T
<i>Didymella fucicola</i>		T	B		B				B		T	B	B
<i>Didymosphaeria enalia</i>		B			B								BT
<i>Diplodia oraemaris</i> *			T										
<i>Hendersonia</i>										B	B	T	BT
<i>Hendersonula</i>									B				
<i>Humicola</i> *								T	T		BT	T	
<i>Leptosphaeria albopunctata</i>		T	BT		T	T	BT	T	T	T	B		T
<i>L. discors</i>		T											T
<i>L. marina</i>		T	B										
<i>Lignincola laevis</i> *						B							
<i>Macrophoma</i>					B								
<i>Meliola</i>										BT			
<i>Mycosphaerella ascophylli</i>							T						
<i>Nigrospora</i>			T										
<i>Papulospora</i>									T	T			B
<i>Penicillium</i> *								B	B				
<i>Pestalotia</i> *								T					
<i>Phoma</i>							T	T		T		B	
<i>Sphaerulina oraemaris</i> *												B	
<i>S. pedicellata</i> *		T											
<i>Tetraploa</i>		T				T	T					B	BT
<i>Trichoderma</i> *						B			B				

Table 4. Fungi isolated from *Baptisia leucophaea* (B) and *Andropogon glomeratus* (A) in Pond B, July 1990-June 1991. (* denotes fungi unique to Pond B).

Fungus	Month	J	A	S	O	N	D	J	F	M	A	M	J
<i>Alternaria</i>		BA	B	B	BA		B						
<i>Camarosporium</i>										B			
<i>Cirrinalia</i> *								A		A			A
<i>Cladosporium</i>									B				
<i>Curvularia</i>					A	B							
<i>Dendryphiella</i>						A							
<i>Dictyosporium</i>											A		B
<i>Didymella fucicola</i>								A	B				
<i>Didymosphaeria danica</i> *		A											
<i>D. enalia</i>					B								
<i>Diplodina</i>												A	
<i>Drechslera</i>				B	A	A							
<i>Epicoccum</i>										A			
<i>Hendersonia</i>									B		A	A	B
<i>Hendersonula</i>										A			
<i>Leptosphaeria albopunctata</i>		BA			A	A		BA		B			B
<i>L. marina</i>		BA											
<i>L. oraemaris</i> *		BA			BA			A					
<i>Macrophoma</i>									B				
<i>Meliola</i>							B		B				
<i>Nigrospora</i>		A									A		
<i>Papulospora</i>							B	B	B				
<i>Periconia</i>					A								
<i>Phoma</i>				B		B				B		A	B
<i>Phylctaena</i> *										B			
<i>Stemphyllium</i> *								B					
<i>Tetraploa</i>		A			BA	A		BA		BA	A	A	BA

Table 5. Fungi isolated from *Baptisia leucophaea* (B) and *Fimbristylis castanea* (F) in Pond C, July 1990-June 1991. (* denotes fungi unique to Pond C).

Fungus	Month	J	A	S	O	N	D	J	F	M	A	M	J
<i>Alternaria</i>		BF		B		F	BF	B		F		B	
<i>Camarosporium</i>												F	
<i>Curvularia</i>		F				F	F						
<i>Dendryphiella</i>						F							
<i>Dictyosporium pelagicum</i>		BF							B				
<i>Didymella fucicola</i>													B
<i>Didymosphaeria enalia</i>							B						
<i>Diplodina</i>									F				
<i>Drechslera</i>								B					
<i>Epicoccum</i>								B					
<i>Hendersonia</i>										B			B
<i>Hendersonula</i>											B		
<i>Leptosphaeria albopunctata</i>												F	
<i>L. discors</i>							F						
<i>Lunulospora*</i>			F										
<i>Macrophoma</i>					B								
<i>Meliola</i>							B			F	F		
<i>Mycosphaerella ascophylli</i>			F										
<i>Nigrospora</i>			BF	B		F					F		
<i>Papulospora</i>						BF		BF	BF	BF	B		
<i>Periconia</i>		BF		B		BF	B	F		B			
<i>Phoma</i>				B			BF	F					
<i>Tetraploa</i>		F			B			B					F

Table 6. Species richness and seasonal occurrence of fungi isolated from Pond A during the study, July 1990-June 1991.

Fungus	Month	J	A	S	O	N	D	J	F	M	A	M	J	Freq.
<i>Alternaria</i>		X	X		X		X	X	X				X	7
<i>Ascochyta</i>						X	X			X				3
<i>Botrytis</i>						X								1
<i>Camarosporium</i>									X		X	X		3
<i>Cladosporium algarum</i>		X	X											2
<i>Curvularia</i>				X								X		2
<i>Dictyosporium pelagicum</i>				X					X				X	3
<i>Didymella fusicola</i>		X	X		X			X		X	X	X	X	7
<i>Didymosphaeria enalia</i>		X			X								X	3
<i>Diplodia oraemaris</i>			X											1
<i>Hendersonia</i>										X	X	X	X	4
<i>Hendersonula</i>									X					1
<i>Humicola</i>								X	X		X	X		4
<i>Leptosphaeria albopunctata</i>		X	X		X	X	X	X	X	X	X		X	10
<i>L. discors</i>		X											X	2
<i>L. marina</i>		X	X											2
<i>Lignicola laevis</i>						X								1
<i>Macrophoma</i>					X									1
<i>Meliola</i>										X				1
<i>Mycosphaerella ascophylli</i>							X							1
<i>Nigrospora</i>			X											1
<i>Papulospora</i>									X	X			X	3
<i>Penicillium</i>								X	X					2
<i>Pestalotia</i>								X						1
<i>Phoma</i>							X	X		X		X		4
<i>Sphaerulina oraemaris</i>												X		1
<i>S. pedicellata</i>		X												1
<i>Tetraploa</i>		X				X	X					X	X	5
<i>Trichoderma</i>						X			X					2
Total Number of Fungi		9	7	2	5	6	6	6	9	8	5	7	9	

Table 7. Species richness and seasonal occurrence of fungi isolated from Pond B during the study, July 1990-June 1991.

Fungus	Month	J	A	S	O	N	D	J	F	M	A	M	J	Freq.
<i>Alternaria</i>		X	X	X	X		X							5
<i>Camarosporium</i>										X				1
<i>Cirrinalia</i>								X		X			X	3
<i>Cladosporium</i>									X					1
<i>Curvularia</i>					X	X								2
<i>Dendryphiella</i>						X								1
<i>Dictyosporium pelagicum</i>											X		X	2
<i>Didymella fucicola</i>								X	X					2
<i>Didymosphaeria danica</i>		X												1
<i>D. enalia</i>					X									1
<i>Diplodina</i>												X		1
<i>Drechslera</i>				X	X	X								3
<i>Epicoccum</i>										X				1
<i>Hendersonia</i>									X		X	X	X	4
<i>Hendersonula</i>										X				1
<i>Leptosphaeria albopunctata</i>		X			X	X		X		X			X	6
<i>L. marina</i>		X												1
<i>L. oraemaris</i>		X			X			X						3
<i>Macrophoma</i>										X				1
<i>Meliola</i>							X		X					2
<i>Nigrospora</i>		X										X		2
<i>Papulospora</i>						X	X	X						3
<i>Periconia</i>					X									1
<i>Phoma</i>				X		X				X		X	X	5
<i>Phylctaena</i>										X				1
<i>Stemphyllium</i>								X						1
<i>Tetraploa</i>		X			X	X		X		X	X	X	X	8
Total Number of Fungi		7	1	3	8	6	3	8	6	8	4	4	5	

albopunctata. The greatest diversity was observed in Pond C during the months of November, December, and January. Seven species of fungi were isolated during each of these months (Table 8). The most frequently occurring species were *Alternaria*, *Periconia*, and *Papulospora*.

Nine species of fungi, which were isolated from the substrates growing in the ponds, did not occur on *Baptisia leucophaea*. Of these, *Didymosphaeria danica*, *L. albopunctata*, *L. oraemaris*, *Mycosphaerella ascophylli*, and *Sphaerulina pedicellata* were marine Ascomycetes and *Cirrinalia* sp., *Dendryphiella* sp., *Diplodia* sp., *Diplodina oraemaris*, *Lunulospora* sp., and *Pestalotia* sp. were terrestrial/airborne Deuteromycetes. Of the substrates surveyed for each pond, *Typha domingensis* supported three unique species, *Fimbristylis castanea* supported one unique species, *Andropogon glomeratus* supported three unique species, and *Baptisia leucophaea* supported seven unique species (Figure 11).

Seven species of fungi occur only on *B. leucophaea*. Two species, *Lignincola laevis* and *Sphaerulina oraemaris*, are marine Ascomycetes, while the remaining species, *Botrytis* sp., *Macrophoma* sp., *Penicillium* sp., *Stemphyllium* sp., and *Trichoderma* sp., are common terrestrial/airborne Deuteromycetes.

Table 8. Species richness and seasonal occurrence of fungi isolated from Pond C during the study, July 1990-June 1991.

Fungus	Month	J	A	S	O	N	D	J	F	M	A	M	J	Freq.
<i>Alternaria</i>		X	X		X	X	X		X			X		7
<i>Camarosporium</i>												X		1
<i>Curvularia</i>		X			X	X								3
<i>Dendryphiella</i>					X									1
<i>Dictyosporium pelagicum</i>		X							X					2
<i>Didymella fucicola</i>													X	1
<i>Didymosphaeria enalia</i>							X							1
<i>Diplodina</i>									X					1
<i>Drechslera</i>								X						1
<i>Epicoccum</i>								X						1
<i>Hendersonia</i>										X			X	2
<i>Hendersonula</i>											X			1
<i>Leptosphaeria albopunctata</i>												X		1
<i>L. discors</i>							X							1
<i>Lunulospora</i>			X											1
<i>Macrophoma</i>					X									1
<i>Meliola</i>							X			X	X			3
<i>Mycosphaerella ascophylli</i>			X											1
<i>Nigrospora</i>			X	X		X						X		4
<i>Papulospora</i>					X			X	X	X	X			5
<i>Periconia</i>		X		X		X	X	X		X				6
<i>Phoma</i>				X			X	X						3
<i>Tetraploa</i>		X			X			X					X	4
Total Number of Fungi		4	3	4	2	7	7	7	2	6	4	3	3	

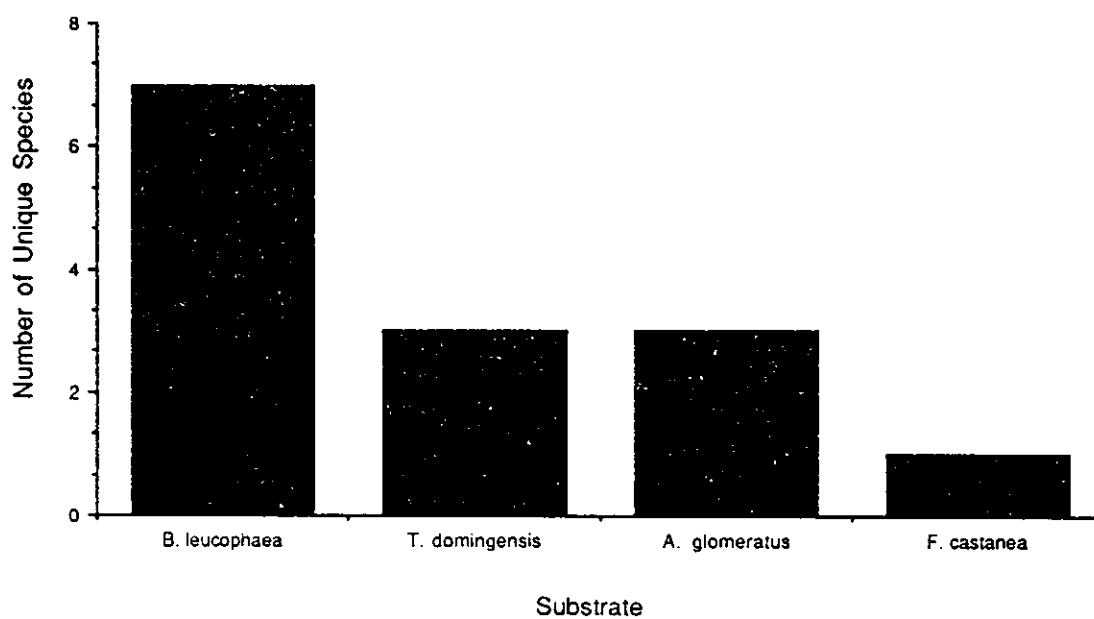


Figure 11. Number of unique species isolated from each substrate.

Fungi that were common to all the ponds are listed in Table 9. Four Ascomycetes and ten Deuteromycetes were isolated. In addition, *Papulospora*, representing the Mycelia Sterilia, was also isolated from all the ponds.

Effects of Varying Salinities on Biomass Production

When grown in liquid medium, the brackish water isolate, *Pestalotia* sp., produced more biomass with increasing salinity concentrations than did the airborne isolate, *P. sydowiana* (Figure 12). The brackish water isolate produced the greatest amount of biomass at low or no salinity, while the greatest amount of biomass produced by the airborne isolate was produced at 0% salinity. Both isolates produced less biomass as the salinity concentrations increased.

Growth on solid agar medium produced results similar to those with the liquid medium. As salinity concentrations increased, the brackish water isolate produced colonies with larger diameters than did the airborne isolate (Figure 13). Both isolates produced the largest colonies on agar containing 0% salinity. Colony diameters decreased with increasing salinity concentrations for both isolates.

Table 9. Fungi common to all the ponds.

Ascomycetes**Pyrenomycetes***Meliola* sp.**Loculoascomycetes***Didymella fucicola**Didymosphaeria enalia**Leptosphaeria albopunctata***Deuteromycetes****Hyphomycetes***Alternaria* sp.*Curvularia* sp.*Dictyosporium pelagicum**Nigrospora* sp.*Tetraploa* sp.**Coelomycetes***Camarosporium* sp.*Hendersonia* sp.*Hendersonula* sp.*Macrophoma* sp.*Phoma* sp.**Mycelia Sterilia***Papulospora* sp.

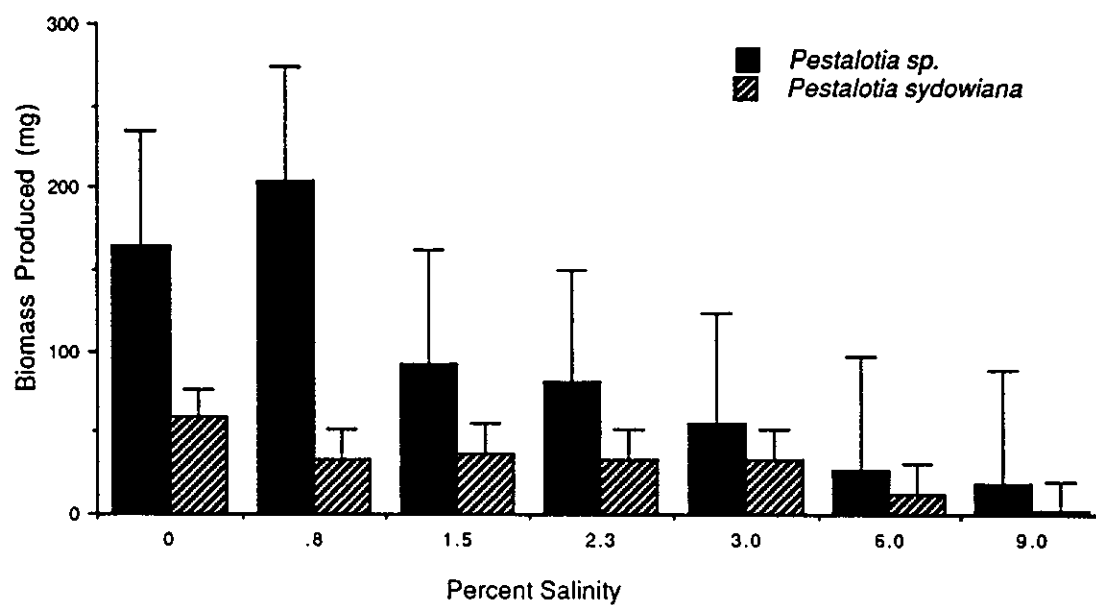


Figure 12. Biomass produced in liquid medium of varying salinities.
Y-bar errors reflect the standard deviation of the data.

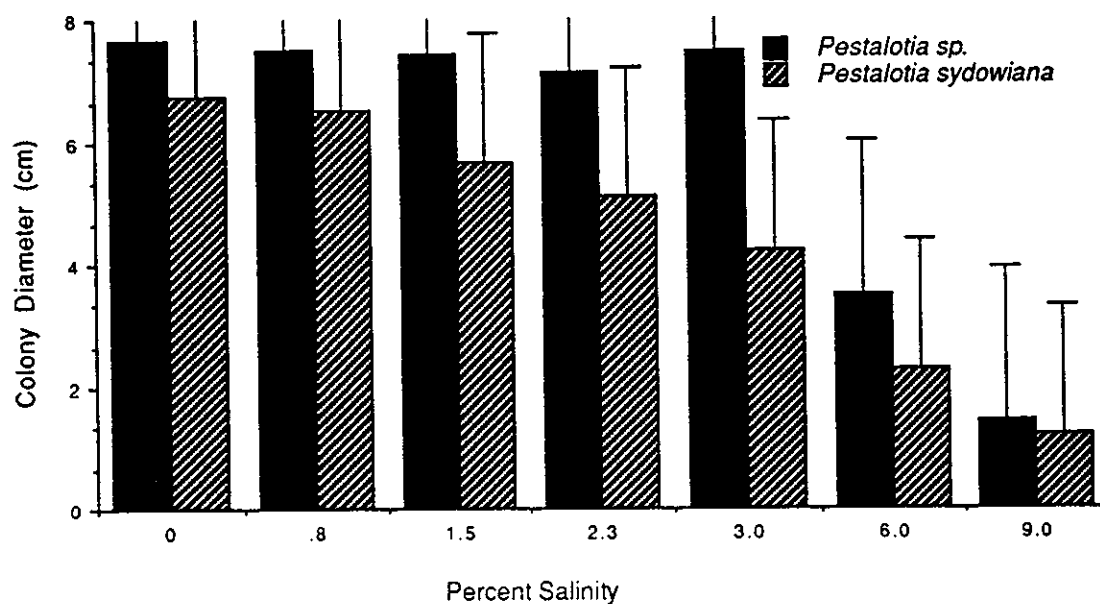


Figure 13. Colony diameters (cm) produced on solid agar medium with varying salinities. Y-bar errors reflect the standard deviation of the data.

Relative Abundance of Ascomata on *Typha domingensis*

The peak reproductive month for fungi colonizing *T. domingensis* was February, with ascomata present at lower abundances during other times of the year (Figure 14). Ascomata size was maximum during the months of April and June (Figure 15) and were the smallest during January and February.

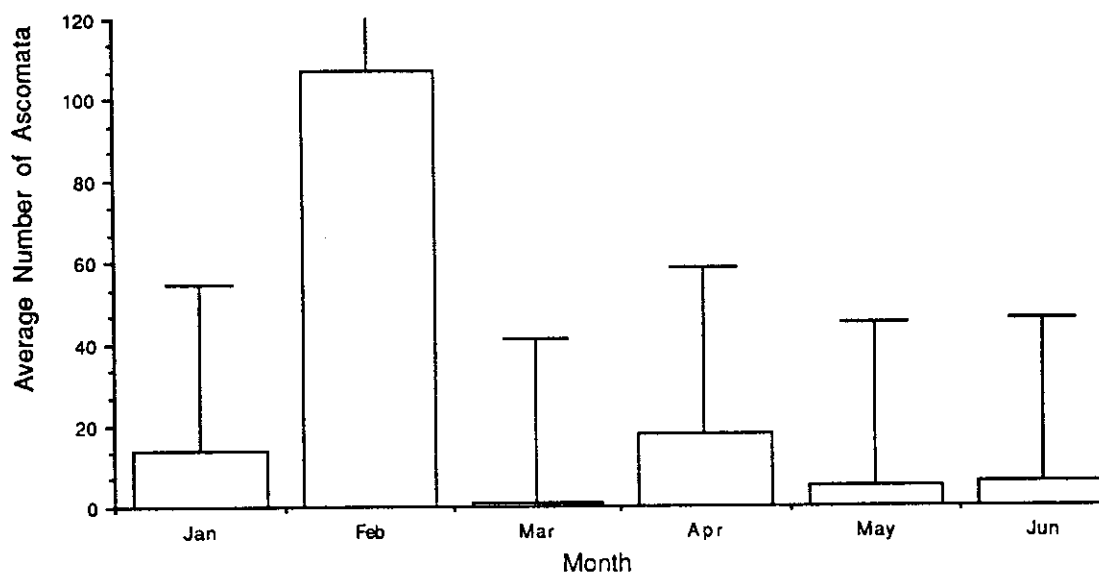


Figure 14. Average number of ascomata by month (based on 50 cm lengths).
Y-bar errors reflect the standard deviation of the data.

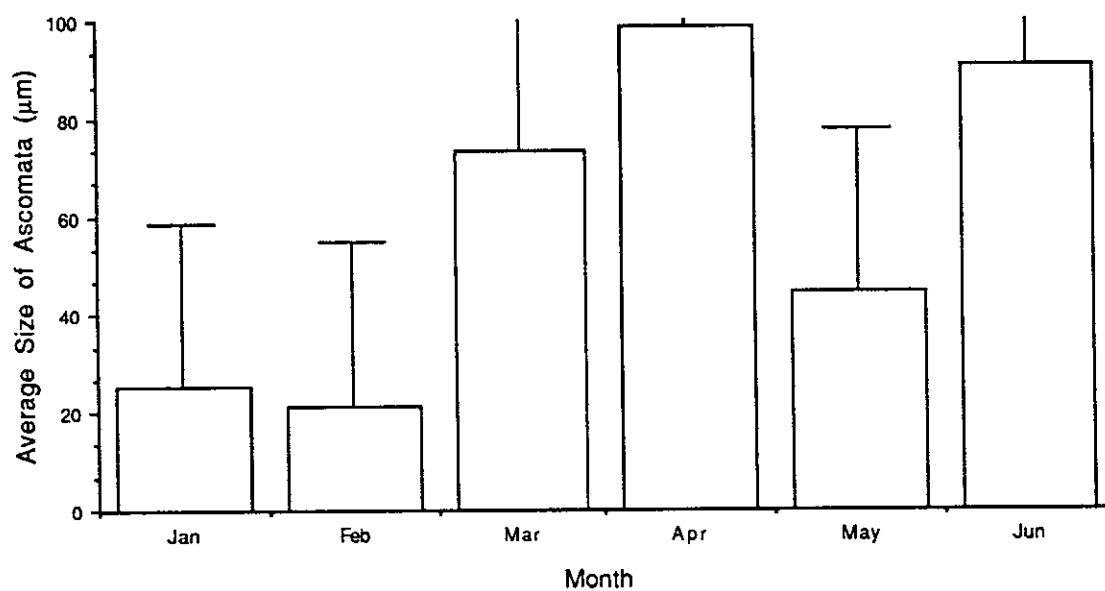


Figure 15. Average size of ascomata (μm) by month. Y-bar errors reflect the standard deviation of the data.

DISCUSSION

Physically, these ponds are best characterized by their salinities, which range from low in Ponds A and B, averaging 3.3 ppt and 2.4 ppt, respectively, to moderate in Pond C, averaging 17.1 ppt. Because of their varying salinity concentrations, the ponds can be best described as brackish water habitats. In this respect, the ponds closely resemble Johnson and Sparrow's (1961) "positive" estuarine habitat because the water in the ponds is fresher than that found in the open ocean.

The generic composition of the mycoflora isolated from the three ponds resembles that of similar studies done on the beaches and estuaries of the Texas Gulf coast (Koehn and Garrison, 1981; Koehn, 1982, 1985) and correlates well with studies done to determine the mycoflora inhabiting estuaries throughout North, Central, and South America (Johnson, 1956; Gold, 1959; Borut and Johnson, 1962; Steele, 1967; Ritchie, 1968; Anastasiou and Churchland, 1969; Shearer, 1972; Gessner and Goos, 1973a; Gessner and Kohlmeyer, 1976; Gessner, 1977, 1978; Gessner and Lamore, 1978), Europe (Goodman, 1959; Jones, 1963; Sivanesan and Manners, 1970; Pugh and Mulder, 1971; Apinis, et al., 1972; Henningsson, 1974), and landlocked bodies of saline water such as the Salton Sea in

California (Anastasiou, 1963) and Twin Buttes Lake in Wyoming (Davidson, 1974a).

By far the most dominant group of fungi isolated from the three ponds were Deuteromycetes, which accounted for 67% of the taxa, while Ascomycetes accounted for only 33%. This agrees with patterns observed in estuarine habitats. A majority of the studies on estuaries reveal that the mycoflora isolated from a variety of substrates is dominated by Deuteromycete representatives (Goodman, 1959; Borut and Johnson, 1962; Jones, 1963; Steele, 1967; Ritchie, 1968; Anastasiou and Churchland, 1969; Sivanesan and Manners, 1970; Pugh and Mulder, 1971; Shearer, 1972; Apinis, et al., 1972; Gessner, 1978; Koehn and Garrison, 1981; Koehn, 1982). Most, if not all, of these Deuteromycetes appear to be terrestrial or airborne species rather than true marine representatives. This holds true for the Deuteromycetes isolated from the three ponds. Although many of the genera observed in this study occur in the marine habitat and have been described by Kohlmeyer and Kohlmeyer (1979), it appears that the majority of the imperfect fungi may have entered the ponds via airborne propagules from the surrounding terrestrial habitat and these propagules have successfully germinated to produce new generations of fungi on the colonized substrates.

According to Johnson and Sparrow (1961) and Kohlmeyer and Kohlmeyer (1979), the majority of marine Ascomycetes belong to the series Pyrenomycetes. This does not hold true for the Ascomycetes isolated from these three ponds. Of the 11 marine genera identified, only one was a Pyrenomycete and the rest were Loculoascomycetes. This suggests that, perhaps, Loculoascomycetes are more successful adapting to the salinity gradients characteristic of these particular habitats.

Pond A and Pond B had the lowest salinities observed, although salinities in Pond A were slightly higher than those in Pond B. The large numbers of terrestrial fungi that were isolated from these ponds suggests that these organisms have adapted to the low salinities in the ponds (Table 1). Four Ascomycetes and one Deuteromycete isolated from Pond A were not found in Pond B and this indicates that these particular fungi may be able to adapt to slightly higher salinities than those fungi common to both ponds. In addition, the eight taxa described as facultative marine fungi by Kohlmeyer and Kohlmeyer (1979) isolated from Pond B suggests these fungi may have broader ranges of salinity tolerance.

Pond C had the highest salinities of all the ponds and supported the least number of genera. While this pond supports 23 taxa, a majority of these were also isolated from either Pond A and/or Pond B. This indicates

that this common mycoflora can tolerate broad ranges in salinity. The only fungus unique to this habitat was *Lunulospora*, an organism which has been described only from fresh, free flowing aquatic systems. The origin of this isolate is unknown as there are no freshwater streams located on the island. And, since it was isolated only once during the study, it would appear that it was not a *Lunulospora* species that had no tolerance for the high salinities characteristic of this pond.

Of the substrates surveyed in all the ponds, *Baptisia leucophaea*, a common terrestrial angiosperm which is blown into the ponds from outside sources, supported the greatest number of taxa. The majority of these were terrestrial Deuteromycetes, suggesting that the plants had been colonized by their mycoflora prior to entering the ponds and that these fungi had adapted to the environmental gradients in the ponds. Similar observations were reported by Chaturvedi, et al. (1990), who speculated that terrestrial fungi isolated from leaves and plant debris in freshwater ponds had colonized the substrates before they were washed into the ponds. Ascomycetes isolated from *B. leucophaea* were predominately marine genera, with colonization most likely occurring after the plants entered the ponds.

Plant species indigenous to each pond supported fewer species than

B. leucophaea. *Typha domingensis* in Pond A supported 18 taxa, *Andropogon glomeratus* in Pond B supported 17 taxa, and *Fimbristylis castanea* in Pond C supported 13 taxa. Once again the majority of the species isolated were terrestrial Deuteromycetes. This observation is supported by the results of other studies done on similar plants in estuaries (Goodman, 1959; Sivanesan and Manners, 1970; Gessner and Goos, 1973a, 1973b; Gessner, 1978; Koehn, 1985) and freshwater habitats (Cunnell, 1956; Apinis, et al., 1972; Panwar and Sharma, 1983). This suggests that aerial portions of the plants may be colonized prior to immersion in the ponds and that these fungi, like those colonizing *B. leucophaea*, have adapted to the environmental conditions in the ponds. Marine Loculoascomycetes were the dominant representatives, with colonization possibly occurring on plant parts already immersed, or following immersion, in the water.

Some of the fungi isolated were unique to only one pond. This would indicate that organisms, such as *Lignincola laevis* and *Diplodia oraemaris* in Pond A and *Leptosphaeria oraemaris* and *Cirrenalia* sp. in Pond B, may have adapted to the narrow range of conditions and/or substrates found in a particular pond and were unable to successfully adapt to the different conditions found in the other habitats. However, the large number of fungi, especially terrestrial fungi such as *Hendersonia*, *Meliola*,

Curvularia, and *Tetraploa*, that were common to all of the ponds seems to indicate that these particular organisms are very opportunistic and have little difficulty moving into and adapting to a wide range of habitat conditions.

The increasing numbers and descriptions of fungi from marine and estuarine habitats (Kohlmeyer and Kohlmeyer, 1991; Kohlmeyer and Kohlmeyer, 1979) raises the question of what factors influence their presence and distribution in these environments. According to Tresner and Hayes (1971), it is possible that the distribution of at least some terrestrial and some marine fungi in brackish water habitats is influenced by the salinity of the environment. Many studies have shown that salinity, together with other environmental factors, plays an important role in the distribution of fungi in aquatic and marine habitats (Gustafsson and Fries, 1956; Ritchie, 1957, 1959; Vishniac, 1960; Gray, et al., 1963; Jones and Jennings, 1964; Norkrans, 1966; Kirk, 1967; Ristanovic and Miller, 1969; Harrison and Jones, 1971; Jones, et al., 1971; Davidson, 1974b; Harrison and Jones, 1975; Byrne and Jones, 1975a, 1975b; Amon, 1976; Padgett, 1978; Hobot and Jennings, 1981; Amon and Yei, 1982; Wethered and Jennings, 1985; Adler, 1986) and it appears that salinity may be a major factor in the distribution of fungi in the ponds.

The results of salinity studies done with two species of *Pestalotia*, one isolated from Pond A and the other an airborne isolate, appear to indicate that salinity may play a part in the distribution of the fungi inhabiting the ponds. The greater biomass production and colony size with increasing salinities of *Pestalotia* sp., isolated from Pond A versus that produced by the airborne isolate under the same conditions, suggests that this fungus has adapted to the brackish conditions in the pond. That *Pestalotia sydowiana* produced any biomass or colonies of any size with increasing salinities indicates that this particular species may have some adaptive potential for low saline habitats.

Studies by Ristanovic and Miller (1969), Tresner and Hayes (1971), Jones, et al. (1971), and Byrne and Jones (1975a, 1975b) demonstrate the salinity tolerance of various freshwater and terrestrial Ascomycetes and Deuteromycetes. This adaptive ability may explain the large numbers of terrestrial Deuteromycetes isolated from the ponds during this study.

The relative abundance of fungi colonizing *T. domingensis* reached its peak in February. This peak in abundance corresponds to an observed increase in the input of senescent plant material into the pond. The peak in abundance is also reflected in the smaller ascomata produced during this month. This may be the result of decreased substrate surface area

and competition for available resources. Or, because ascomata are spore producing structures, it may be an example of the inverse relationship between spore size and spore number (Mauseth, 1991) in which a given mycelium can either produce a few large spores (ascomata?) or many small ones. Additionally, the larger ascomata observed during the other months may also represent ascomata that have matured during the preceding months.

It is obvious from the results that terrestrial Deuteromycetes are the dominant mycoflora inhabiting the ponds. According to Kohlmeyer and Kohlmeyer's (1979) definition, these Deuteromycete fungi may be considered as facultative, rather than obligate, marine fungi. These organisms appear to have easily moved from a terrestrial setting and successfully adapted to the low to moderate salinity gradients found in these brackish water habitats.

Do the marine Deuteromycete and Ascomycete genera isolated from the ponds represent "true" marine fungi that have successfully "crossed over" from a marine environment and adapted to these habitats that are not completely marine? Like their terrestrial counterparts, these "truly" marine fungi appear to have easily and successfully adapted to the salinity gradients found in the ponds. Rather than being considered as

obligate, or "true", marine organisms, these fungi may best be considered as facultative marine fungi.

Physiological studies by Kirk (1967) and Jones and Jennings (1964) have shown that marine and terrestrial fungi are capable of tolerating broad ranges of salinity. The fungi that inhabit these ponds apparently also demonstrate this ability to adapt to varying salinity gradients. These fungi may not represent strictly marine or terrestrial species. Rather, they may represent the "estuarine adapted" species defined by Ferguson-Wood (in Jones, et al., 1971).

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