

ALGAL SPECIES COMPOSITION ON TURTLE CARAPACES IN SPRING LAKE,  
SAN MARCOS, TEXAS

THESIS

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in Partial Fulfillment of the Requirements

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By

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## ABSTRACT

Algae are found in a wide variety of habitats on the planet. They are found in the depths of the oceans, in freshwater lakes and streams, in different types of soil, in aerial habitats, and living in or on other organisms. One notable habitat is the carapaces of freshwater turtles. Historically, species of algae have been reported using turtles as their main substrate; however no study has statistically proven a difference in algal species composition among turtle species or if algae colonize specific substrates. This study, conducted in Spring Lake, San Marcos, Texas, determined significant differences in algae species, *Basicladia chelonum* ( $DF = 3$ ,  $G^2 = 195.53$ ,  $P < 0.0001$ ), *Basicladia crassa* ( $DF = 3$ ,  $G^2 = 24.38$ ,  $P < 0.0001$ ), and *Cladophora glomerata* ( $DF = 3$ ,  $G^2 = 250.59$ ,  $P < 0.0001$ ) among turtle species in Spring Lake. This study also determined that significant differences in algae species, *C. glomerata* ( $DF = 3$ ,  $G^2 = 32.43$ ,  $P < 0.0001$ ), *Coleochaete scutata* ( $DF = 3$ ,  $G^2 = 42.60$ ,  $P < 0.0001$ ), *Rhizoclonium hieroglyphicum* ( $DF = 3$ ,  $G^2 = 17.70$ ,  $P < 0.001$ ), and *Lyngbya* spp. ( $DF = 3$ ,  $G^2 = 20.84$ ,  $P < 0.0001$ ) among substrate types in Spring Lake. Factors that may influence the colonization of algae on freshwater turtles include turtle habit, light availability, and desiccation rates. The patterns in algal composition among turtle species and substrate types found in this study suggest a relationship between some algae species and freshwater turtles. The relationship can be considered to be commensalism; however the results from this study suggest mutualism.

## INTRODUCTION

Algae are ubiquitous. Essentially two thirds of the earth is covered by water, and algae are found abundantly in these marine and freshwater habitats. Algae are important primary producers in aquatic systems and probably account for more than half the primary production worldwide (Hoek et al., 1995). The productivity of some coastal algal communities exceeds that of tropical rain forests (Bell and Hemsley, 2000). Algae not only are important as a food source in aquatic systems, but also play an important role for humans as well. Many Pacific and Asian countries farm red and brown algae for human consumption. Some macroalgal species are used in animal feed, fertilizer, and soil conditioners for agricultural production. In addition, microalgae are used as feed for aquaculture species and in wastewater treatment to remove nutrients and metals (Sze, 1998).

Algae grow attached to the substrate (benthic) or occur free floating in the water column (planktonic). However, algae can also be found in terrestrial habitats growing in soils and epiphytically on other living organisms. Algae also are highly variable in size and morphology, ranging from microscopic unicellular species to giant kelp species.

Plasticity of algae in their habitat and structure make them difficult to classify.

Researchers are using a combination of morphological and molecular techniques to identify and classify algae. Hillis (1987) reported that there were pros and cons for each method and a combination of methods was the better way to classify a specimen. Many

researchers place the algae in the kingdom Protista (e.g. Graham and Wilcox, 1999) however; others place them in kingdom Plantae (Bell and Hemsley, 2000). Regardless of which kingdom the divisions of algae are placed, they are not a monophyletic group; thus, systematists have suggested classifying the divisions of algae into new kingdoms (Graham and Wilcox, 1999).

Algae in the division Chlorophyta (green algae), class Chlorophyceae, are considered the precursors to terrestrial plants (Bell and Hemsley, 2000; Campbell and Reece, 2002). Chlorophyta is one of the largest divisions of algae with about 500 genera and 8000 species (Hoek et al., 1995). There are species represented from marine, freshwater, and even terrestrial habitats. Green algae were observed on a variety of different substrates, including the carapaces of turtles.

Algal growth on turtle carapaces is a common occurrence in freshwater streams and lakes. The relationship between epizoophytic algae and turtles is postulated to be a type of commensalism (Harper, 1950; Edgren et al., 1953; Dixon, 1960). Neill and Allen (1954) concluded that the turtles most frequently epizoized were the ones that profit from algal camouflage, those that often stalk active prey. Instead of commensalism, this relationship could be considered mutualistic. The algae, too, may gain some benefit from growing on the carapace of aquatic turtles. A moving substrate, may allow them access to nutrients or a possible escape from herbivory.

Dixon (1960) studied the epizootic algae of turtles in Texas and Mexico. All turtles examined had *Basycladia chelonum* growing on the carapace, along with other species of *Basycladia*, *Cladophora* and some species of cyanobacteria. Edgren et al. (1953) found *B. chelonum* and *B. crassa*, to be the prevalent species on *Chrysemys picta*, *Graptemys geographica*, *Chelydra serpentina*, *Sternotherus odoratus*, and *Pseudemys* (= *Trachemys*) *scripta*. *Cladophora glomerata*, *Rhizoclonium hieroglyphicum*, *Dermatophyton radians*, and *Entophysalis rivularis* were found also. The turtles examined were collected primarily from midwestern areas, although a few were collected in other scattered areas of North America. Edgren et al. (1953) also looked at differences among turtle species with regard to the proportion of epizootism. They found that *Sternotherus odoratus*, *Chelydra serpentina*, *Emys blandingii*, and *Kinosternon* sp. possessed the densest algal growth. Vinyard (1953) found that *Emys blandingii*, *Chelydra s. serpentina*, *Sternotherus odoratus*, *Chrysemys picta marinate*, *C. p. belly*, *Graptemys geographica*, *Pseudomys* (sic.) *scripta elegans*, and *Graptemys pseudogeographica* examined from Oklahoma also had *Basycladia* and *Dermatophyton* growing on their carapaces. *Dermatophyton* was found also growing on the legs of *Amyda ferox* (soft-shelled turtle) (Vinyard, 1953). The majority of these researchers focused on the turtle species and what species of algae colonized the carapace. However, none of the researchers applied any quantitative statistical analyses to test differences in algal species composition among the turtles. Although the occurrence of algae on turtle carapaces has been studied in numerous ecosystems throughout the world (Gardner, 1937; Yoneda, 1952; Edgren et al., 1953; Ducker, 1958; Proctor, 1958; Dixon, 1960; Islam and Hameed, 1982; Semir et al., 1988; Arif, 1991; Colt et al., 1995), no studies of turtles and their epizootic algae had

been conducted in Spring Lake and the San Marcos River ecosystem prior to my investigation.

The most prevalent algae reported growing on turtles belong to the genus *Basicladia* (Hoffmann and Tilden, 1930; Edgren et al., 1953; Vinyard, 1953; Neill and Allen, 1954; Ducker, 1958; Proctor, 1958; Ernst and Norris, 1978; Colt et al., 1995). This taxon was first described by Collins (1907) as a new species, *Chaetomorpha chelonum*. However, Kützing's (1843) description of the genus *Chaetomorpha* stated that it is characterized by unbranched filaments. Hoffmann and Tilden (1930) found the taxon to have a distinct basal branching habit and erected a new genus, *Basicladia*. Hoffmann and Tilden (1930) reclassified *C. chelonum* as *Basicladia chelonum*. The validity of *Basicladia* as an accepted genus was reviewed by Hoek (1963). He stated that there are morphological overlaps in the genera *Basicladia* and *Cladophora* and that the species in the genus *Basicladia* are most closely related to the unbranched species of *Cladophora*. However, most researchers have accepted the genus *Basicladia* as described by Hoffmann and Tilden (1930).

Proctor (1958) found that the genus *Basicladia* grew prolifically on the carapaces of turtles and was uncommon on other substrata. He found that the genus could be cultured indefinitely in the laboratory in the absence of turtles or turtle extracts. He noted that in nature the alga occasionally attached to substrata other than turtles suggesting that physical factors of the carapace, not the chemical composition of the shell, were crucial in limiting the distribution of the alga (Proctor, 1958). Turtle carapaces are composed of

$\alpha$ -keratin and  $\beta$ -keratin (Zug, 1993). During this study, I predicted that there would be no difference in algal species composition among turtle species since the substrate framework for algae is the same among turtle species. In the soft-shell and leatherback turtles, the entire shell surface is an  $\alpha$ -keratin skin and in the vast majority of the hard-shelled turtles, the scutes and sutures contain only  $\beta$ -keratin (Zug, 1993). Hoffmann and Tilden (1930) described the attachment of *Basicladia* to the turtle carapace by creeping, rhizome-like filaments fastened by holdfasts. However, Vinyard (1953) noted that two species of algae were found on fish, in all cases where the algae were attached, the bone of the fish was deeply penetrated by the holdfast of the algae. One of the species of algae was identified in the genus *Cladophora*, which is in the same family, Cladophoraceae, as *Basicladia*. The other species was an unidentified alga in the family Chaetophoraceae. A study by Ducker (1958) depicted the attachment of *Basicladia ramulosa* to the carapace of *Chelodina longicollis*. However I found no literature that depicted (through drawings or micrographs) the mode of attachment of *Basicladia chelonum* or *B. crassa* to turtle carapace. I assumed attachment would be similar to that of *B. ramulosa* illustrated by Ducker (1958). A few authors noted that members of the genus *Basicladia* seemed to grow mainly on turtles (Edgren et al., 1953; Ducker, 1958; Proctor, 1958; Ernst and Norris, 1978), but no substrate preference experiments were conducted in the natural environment. Normandin and Taft (1959) described a new species of *Basicladia*, *B. vivipara*, found growing on the snail *Viviparus malleatus*. They conducted laboratory tests and found that *B. vivipara* grew well on snail shell, but did not exhibit growth on turtle carapace or agar. Based on surveys from past literature, I predicted there would be



a difference in algal species composition among substrates (i.e., *Basicladia* would colonize the turtle shell substrate in higher abundance than the other substrates).

To understand the relationship between algae and turtles, first it must be determined if there are patterns in occurrence between the organisms. This study ① determined the species of algae that occur on the carapaces of turtles in Spring Lake, San Marcos, Texas; ② determined there were differences in the algae species composition among species of turtle across season; ③ determined there were differences in algal composition on different substrates (i.e., turtle carapace, glass, wood, brick); ④ determined the attachment of *Basicladia chelonum* to the carapace of *Trachemys scripta elegans*. This study is a beginning to revealing the patterns of certain algal species growing on freshwater turtles

## MATERIALS AND METHODS

### Study Site

This study was conducted in Spring Lake (29° 53' N 97° 55' W) at Aquarena Center (Southwest Texas State University) in San Marcos, Hays County, Texas (Figure 1a, b). The San Marcos Springs were dammed in 1849 (Brune, 1981) to form Spring Lake. Spring Lake is approximately an 8-hectare reservoir (Aguirre, 1999) of extremely clear water and is the headwaters for the San Marcos River. It is fed by some 200 springs coming from the Edwards Aquifer (Brune, 1981). The San Marcos Springs are the second largest springs in North America west of the Mississippi River (Ogden et al., 1986). The water temperature of the lake is 21°C and fluctuates less than 3°C throughout the year (Groeger et al., 1997). Spring Lake is bordered on the northwest by a steep rocky slope formed by the Balcones Escarpment and on the east and southeast side by the Blackland Prairie (Bruchmiller, 1973). The slough of Spring Lake is formed by backwater from the dam and Sink Creek flowing into the lake (Figure 1a).

Spring Lake and the San Marcos River are unique habitats and have a rich history. Many artifacts have been found from archeological sites around Spring Lake dating back 10,000 – 12,000 years and the area is thought to be one of the oldest continuously inhabited sites in North America (Arsuffi et al., 2000). In the 1940s the site was made into the resort, Aquarena Springs. Attractions such as swimming mermaids, Ralph the swimming pig, amusement rides, and glass bottom boat rides were added in later years

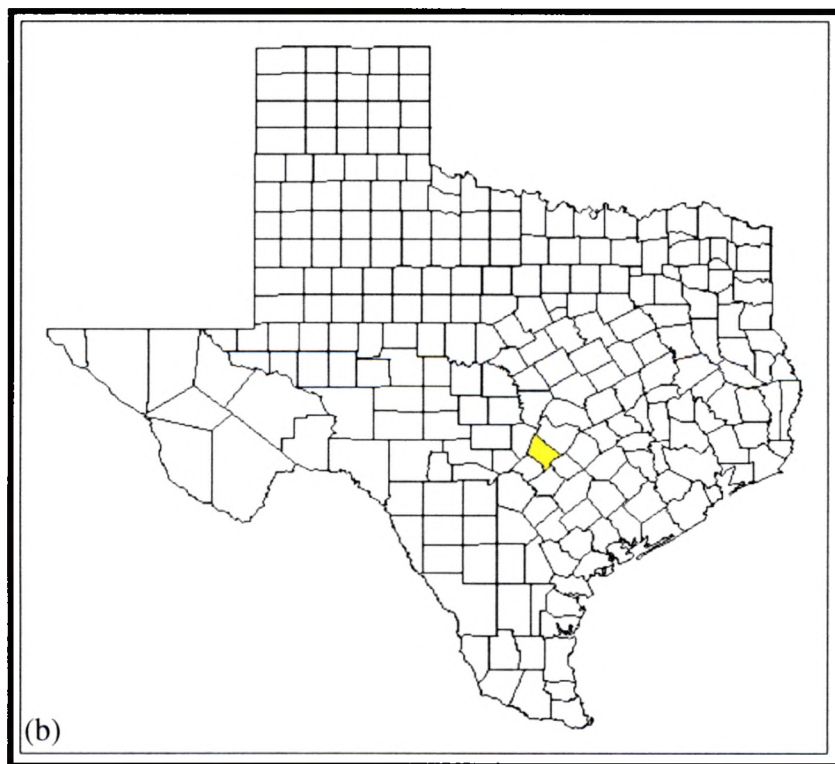
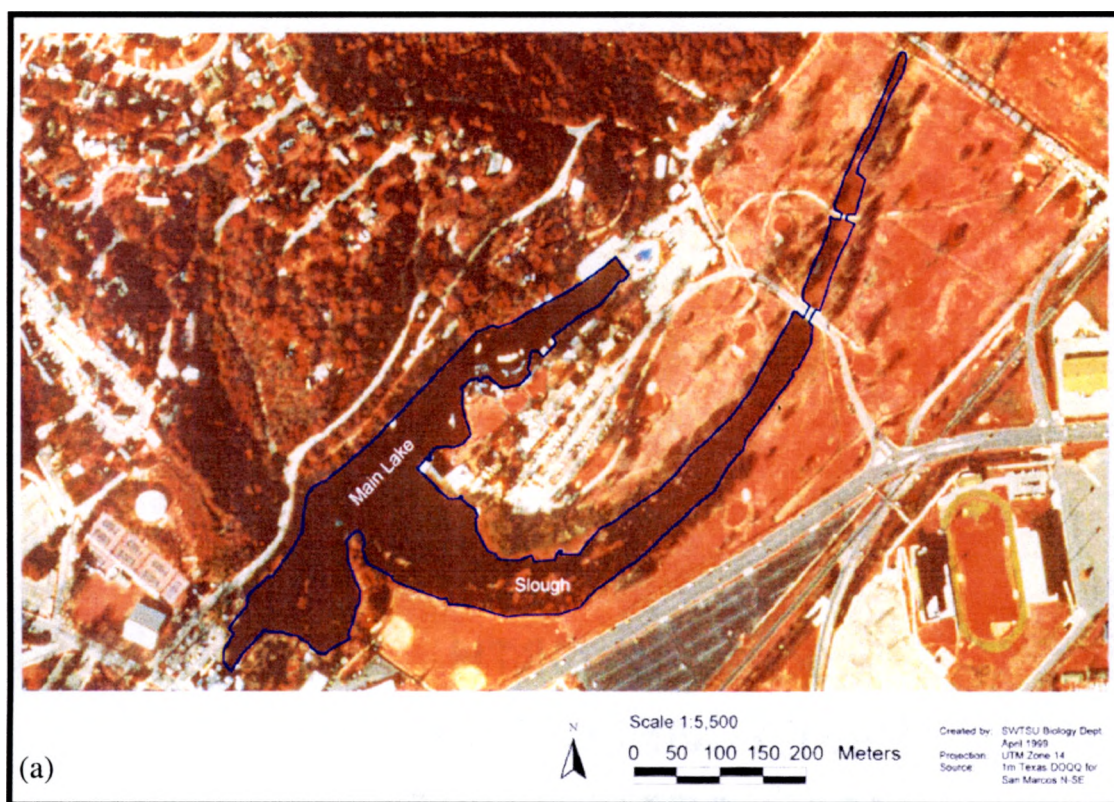


Figure 1. (a) Aerial photograph of Spring Lake, Aquarena Center. The main lake and slough are indicated on the map. (b) Map of Texas showing Hays County in yellow.

(Arsuffi et al., 2000). Much of the natural environment of Spring Lake was altered to form Aquarena Springs. Cement bulkheads were put into place along the north and east sides of the lake, buildings were constructed, and various parts of the lake were dredged. Many invasive and exotic organisms were introduced to Spring Lake. Some of the introduced aquatic macrophytes include *Hydrilla verticillata* (hydrilla), *Colocasia esculenta* (elephant's ear), *Eichhorina crassipes* (water hyacinth), *Myriophyllum spicatum* (Eurasian watermilfoil), and *Ceratopteris thalictroides* (water sprite). Some of the introduced animals include *Myocastor coypus* (nutria), *Cygnus olor* (mute swan), *Marisa cornuarietis* (giant ramshorn snail), *Tilapia aurea* (blue tilapia), and *Cichlasoma cyanoattutum* (Rio Grande cichlid). Destruction of natural habitat during the formation of Aquarena Springs and the introduction of exotic species has altered the ecosystem. Southwest Texas State University purchased Aquarena Springs in 1994. In partnership with U.S. Fish and Wildlife Service and Texas Parks and Wildlife Department, the University plans to restore the natural habitat and focus on community awareness and continuing education of the San Marcos River ecosystem (Arsuffi et al., 2000). All of the rides have been dismantled and removed. Many of the buildings and impervious cover are to be removed and the land is to be revegetated with native plants. As this study was being conducted a wetlands boardwalk was constructed in the wetland area of the slough to educate the public on the delicate nature of wetlands and the severe impact humans and exotic species can have on them. Despite alterations, the ecosystem supports a rich diversity of organisms.

Spring Lake and the San Marcos River are home to five threatened or endangered species, including *Eurycea nana* (San Marcos salamander), *Zizania texana* (Texas wild rice), *Heterelmis texanus* (Comal Springs water beetle), *Typhlomolge rathbuni* (Texas blind salamander), and *Gambusia georgei* (San Marcos gambusia) (U.S. Fish & Wildlife Service, 1996).

Spring Lake contains many autotrophic species including the divisions Chlorophyta (green algae), Cyanophyta (cyanobacteria), Rhodophyta (red algae), Xanthophyta (yellow algae), Bacilliophyta (diatoms), Dinophyta (dinoflagellates), and a vast amount of aquatic macrophytes. These autotrophic taxa provide food, housing, and refuge to many of the animals that live in the lake.

Aquatic turtles that commonly occur in Spring Lake include *Sternotherus odoratus* (common musk turtle), *Chelydra s. serpentina* (common snapping turtle), *Trachemys scripta elegans* (red-eared slider), *Pseudemys texana* (Texas river cooter), *Pseudemys nelsoni* (Florida red-bellied turtle), and *Apalone spinifera* (spiny soft-shell) (F.L. Rose, personal communication, 2002). *Sternotherus odoratus*, *C. s. serpentina*, *T. scripta elegans* and *P. texana* are the most commonly occurring aquatic turtles in Spring Lake and were the turtles collected for this study.

### **Turtle Collection**

Samples of turtles were collected seasonally for one year. The turtles were captured using dip nets, hoop traps and basking traps since the turtles have different habits, calling

for different trapping methods. *Sternotherus odoratus*, *Chelydra s. serpentina*, and *Trachemys scripta elegans* are carnivorous and were trapped with hoop traps (1.0 m x 0.8 m) baited with fish or chicken (Figure 2). *Pseudemys texana*, an herbivore, basks often and was trapped with basking traps (1.85 m x 1.25 m x 0.4 m) (Figure 3). Each species of turtle was also captured by using dip nets (Figure 4). Each turtle was given an unique mark by drilling or filing into the marginal scutes. This mark was used to identify the turtle in case of recapture.

### **Algae Collection and Identification**

Algae were removed from the carapace by scrapping three areas (approximately 1.0 cm<sup>2</sup> each) with a field knife and preserved in a glass vial of 4% formalin. The date, time, location, trapping method, species of turtle, and the turtles' unique mark were recorded. The algae were taken to the lab, identified using a Meiji dissecting scope and Zeiss compound light microscope, and micrographs were taken using Kodak Select Elite Chrome 100 speed film with a Labophot – 2 Nikon light microscope and camera setup. For this study, the algae were identified by morphological characteristics using the most detailed descriptions published (Kützing, 1843; Collins, 1909; Hoffmann and Tilden, 1930; Tiffany, 1937; Transeau, 1951; Krishnamurthy, 1962; Hoek, 1963; Flint, 1970; Vis and Sheath, 1996). A few species of cyanobacteria (Cyanophyta) were encountered during this investigation. These organisms were grouped under the broad term 'algae' for this study.



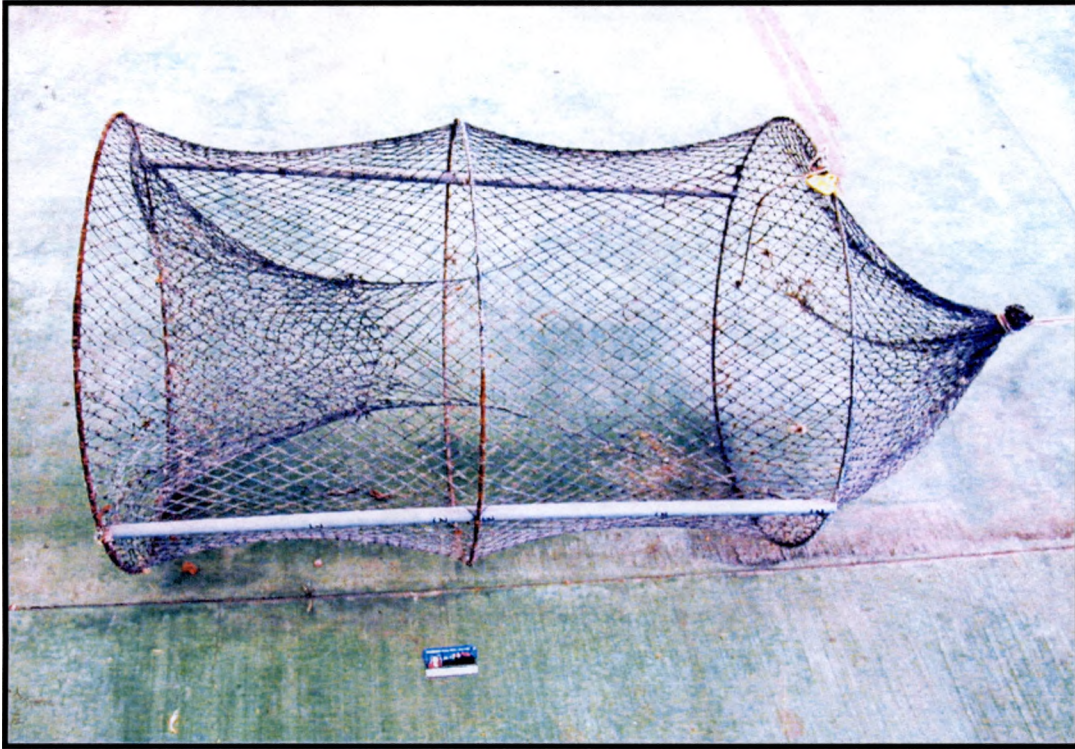


Figure 2. Hoop trap (1.0 m x 0.8 m) used to collect *Chelydra s. serpentina*, *Sternotherus odoratus*, and *Trachemys scripta elegans*. Credit card for scale.

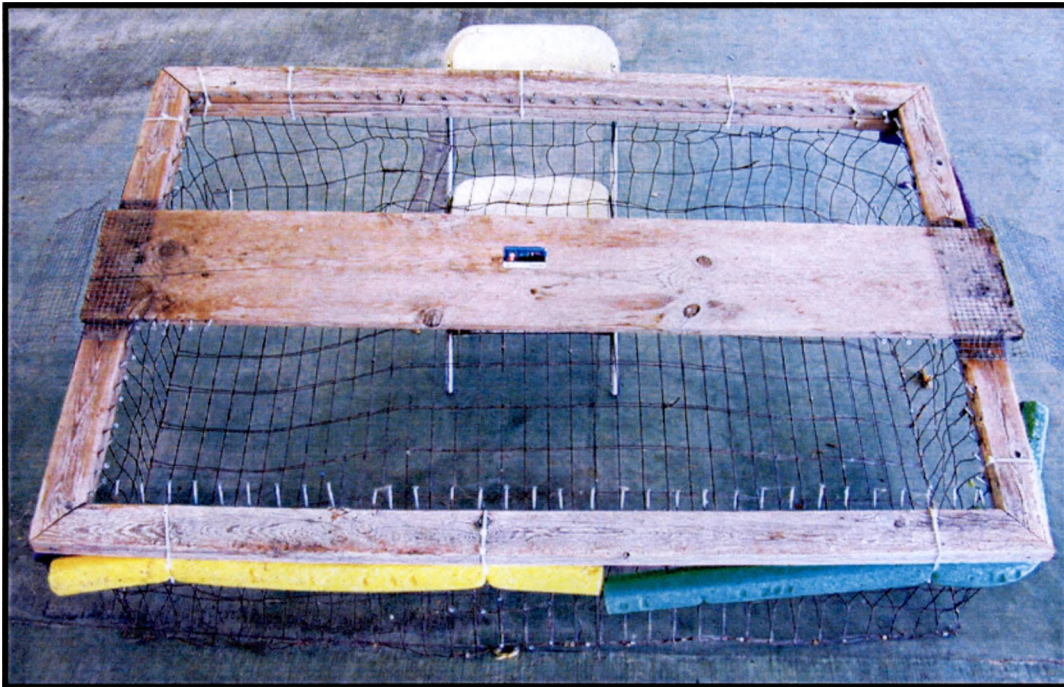


Figure 3. Basking trap (1.85 m x 1.25 m x 0.4 m) used to collect *Pseudemys texana*. Credit card for scale.

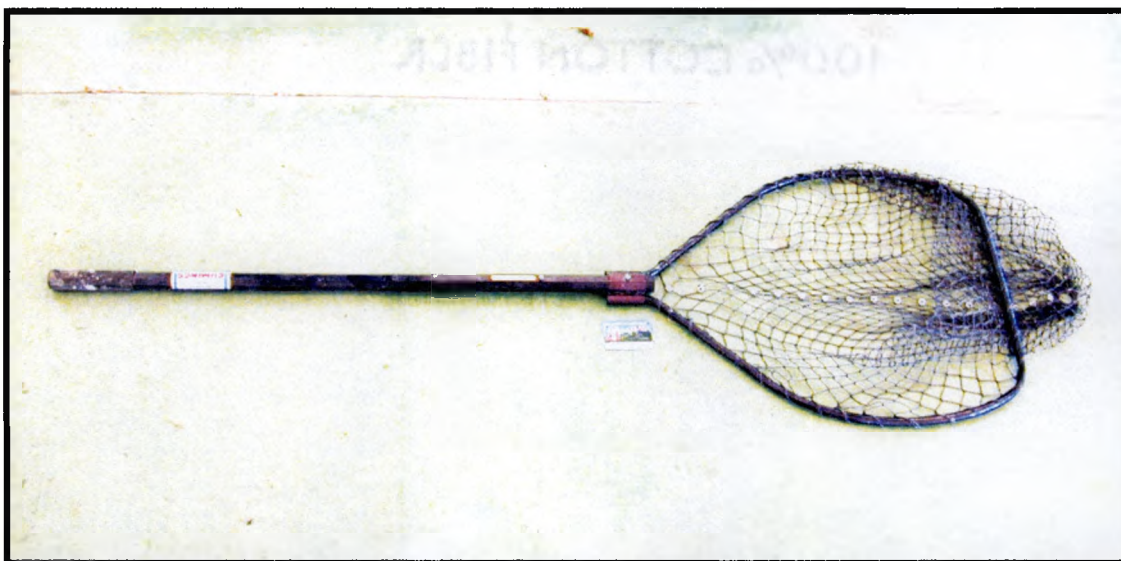


Figure 4. Dip net used to collect *Chelydra s. serpentina*, *Sternotherus odoratus*, *Trachemys scripta elegans* and *Pseudemys texana*. Credit card for scale.



## Substrate Experiment

Algal substrate colonization was investigated by mounting 3.0 cm<sup>2</sup> pieces of turtle scute, glass, wood, and brick on a 22 cm x 27 cm polystyrene board. One of each substrate type was randomly placed in three separate rows on the board (Figure 5). Ten replicates of this design were suspended in the water column at depths of 0.5 m and 2.0 m (Figure 6). The replicates were placed in the main channel and the slough of Spring Lake in November 2001 (Figure 7). Each apparatus was mapped using a Global Positioning Satellite unit. They were left in position until April 2002. Algae were removed from each substrate by scrapping with a field knife and preserved in a glass vial in 4% formalin. Algae from each substrate at each depth from the separate replicates were preserved in separate vials. The algae were identified using a Meiji dissecting scope and Zeiss compound light microscope. Micrographs were taken using Kodak Select Elite Chrome 100 speed film with a Labophot – 2 Nikon light microscope and camera.

## Algal Attachment

To investigate algal attachment to turtle carapace, scutes with algal growth were collected in summer 2001 from *Trachemys scripta elegans* and preserved in 4% formalin. This species was selected because it sheds its scutes annually in the summer. The algae and scute tissues were fixed in formalin. The scutes, with attached algae, were cut into small pieces and air-dried. The tissues were mounted on scanning electron microscope stubs with copper adhesive tape and sputter coated with 24-carat gold. Tissue pieces were examined using a Hitachi S4500 field emission Scanning Electron Microscope. Digital micrographs were taken.

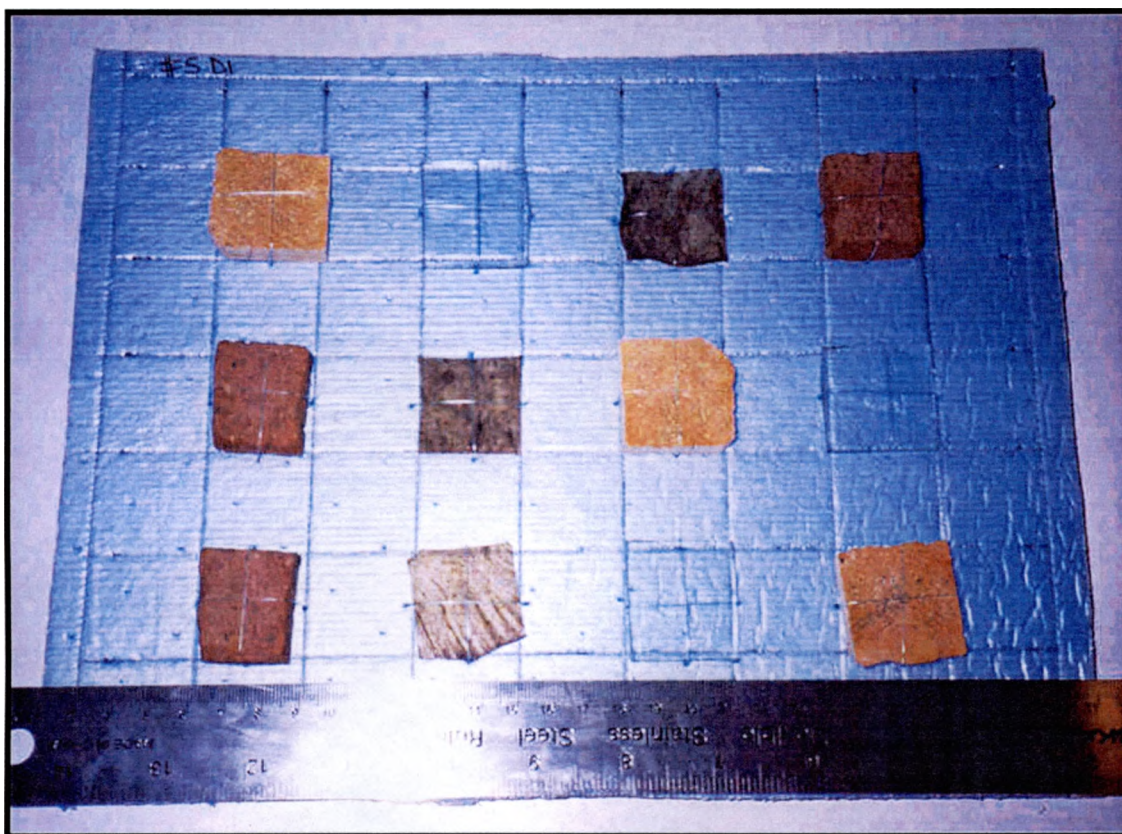


Figure 5. Polystyrene board with four 3 cm<sup>2</sup> substrates (turtle scute, glass, wood, and brick) mounted randomly in three rows.

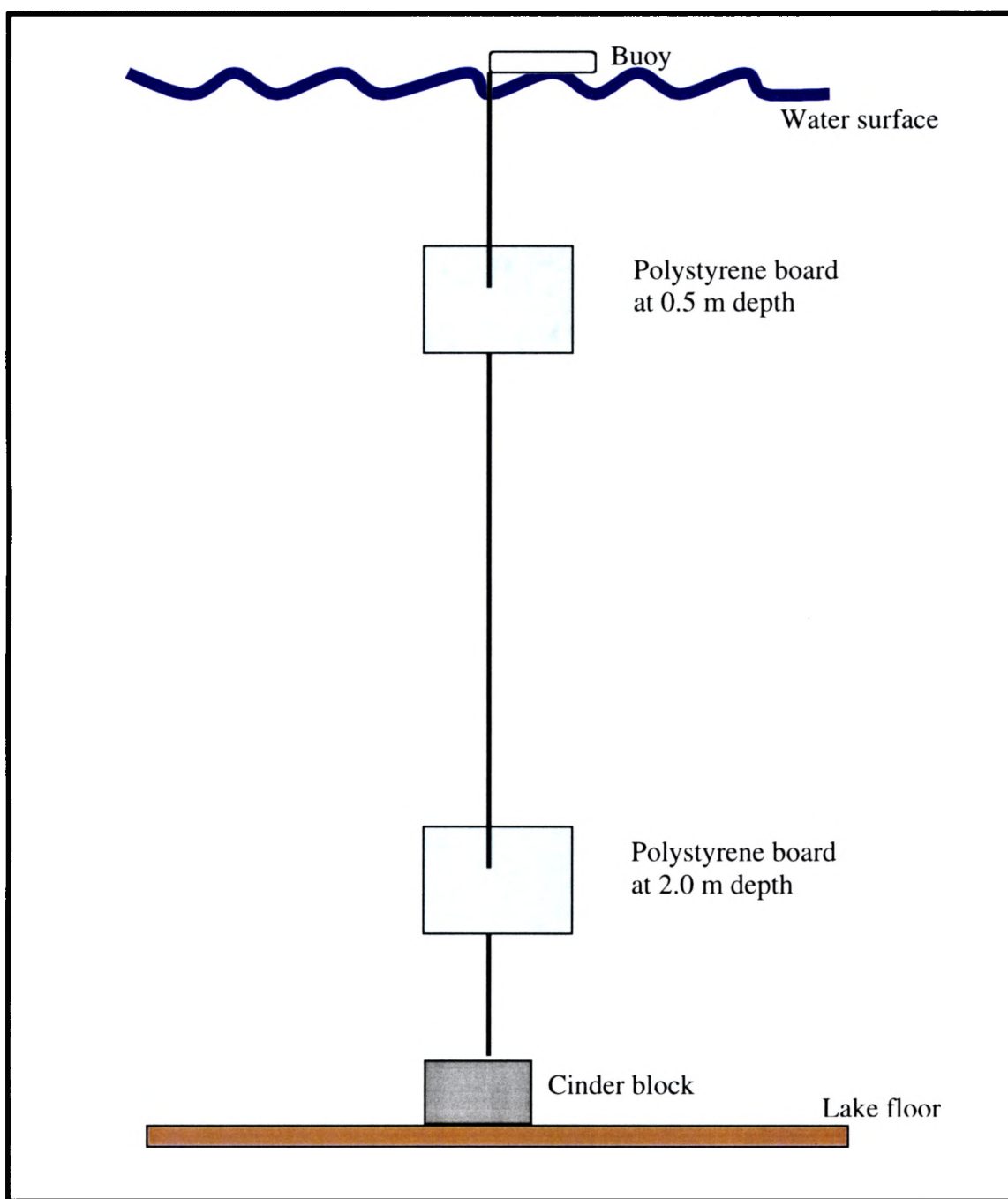


Figure 6. Diagram of substrate experiment apparatus with two polystyrene boards at depths of 0.5 m and 2.0 m.

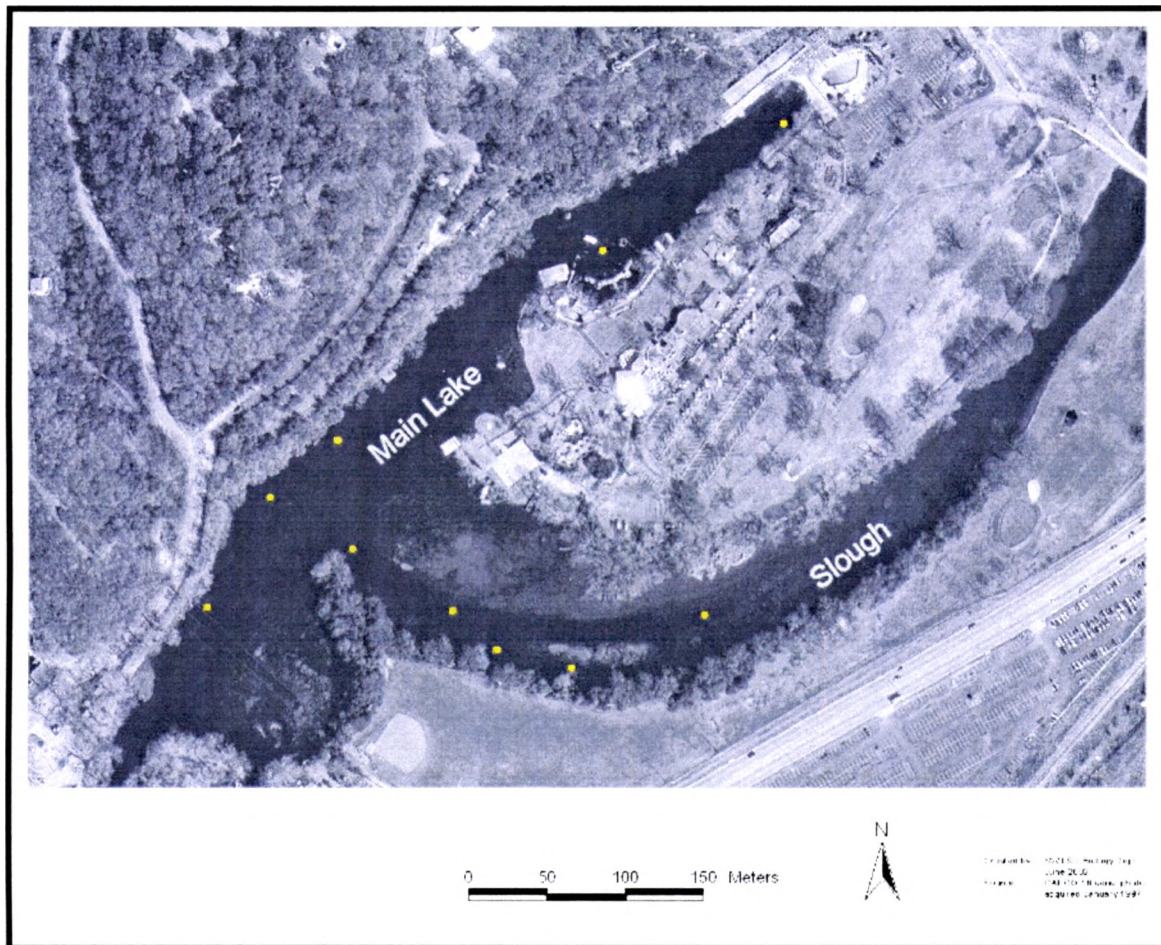


Figure 7. Aerial photograph showing location of each substrate experiment. Each apparatus was placed in the main lake (n=5) or the slough (n=5). Each apparatus is denoted by a yellow dot.

## **Data Analyses**

Data in this study were categorical and were represented by contingency tables. The SPSS® statistical program was used to analyze the data. The Loglinear Analysis procedure was used to determine significance between the interactions of turtle species, algae species, and season. The same procedure was used to analyze data from the substrate experiment. The Loglinear Analysis procedure estimates maximum likelihood parameters of hierarchical and nonhierarchical loglinear models using the Newton-Raphson method (SPSS Inc., 1999). Loglinear model analyses for categorical data are similar to factorial ANOVAs for continuous data. Loglinear model analyses are preferred over chi square analyses for multiway contingency tables because they allow the researcher to detect three-factor or higher order interactions and examine pairwise relationships (Stevens, 1996). The Crosstabs procedure in SPSS® was used to pinpoint which variables of the interactions contributed to the significance.

## RESULTS

### Algal Species Composition Among Turtle Species

During this study, algae samples were taken from 262 turtles. The number of each turtle species sampled from each season is shown in Table 1. The algae identified from the carapaces of the turtles are listed in Table 2. Light micrographs of these algae are shown in Figures 8-12. Statistical analyses indicated that there were no significant differences among turtle species across seasons for the algae identified (i.e., no three-way interactions). Statistical analyses, however, did indicate significant differences in the algae *B. chelonum*, *C. crassa*, *C. glomerata*, and *Symploca* sp. in the two-way interactions (i.e. algae x turtle and algae x season). Figures 13-16 show the two-way interactions of algae x turtle species and algae x season. There was no significant difference in either two-way interaction (i.e., algae x turtle and algae x season) for the alga *R. hieroglyphicum*.

Presence of *B. chelonum* differed significantly across turtle species ( $DF = 3$ ,  $G^2 = 195.53$ ,  $P < 0.0001$ ) (Figure 13a). The adjusted residuals (z scores) of the data (Table 3.1) show that the dependence lies in the turtle species *S. odoratus* ( $z = 8.3$ ), *T. s. elegans* ( $z = 4.8$ ) and *P. texana* ( $z = -12.7$ ). *Basidiocladia chelonum* presence also differed significantly across season ( $DF = 3$ ,  $G^2 = 11.87$ ,  $P < 0.008$ ) (Figure 13b). Presence of this alga differed significantly in the fall ( $z = 2.8$ ) and winter ( $z = -2.2$ ) (Table 3.2).



Table 1. Number of each turtle species captured each season in Spring Lake, San Marcos, Texas. Algae samples were taken from the carapace of each turtle specimen.

	Spring	Summer	Fall	Winter	$\Sigma$
<i>Chelydra s. serpentina</i>	3	2	7	3	15
<i>Sternotherus odoratus</i>	19	24	30	14	87
<i>Trachemys s. elegans</i>	29	7	22	14	72
<i>Pseudemys texana</i>	20	22	21	25	88
$\Sigma$	71	55	80	56	262

Table 2. Algae species identified growing on turtle carapaces. Algae samples were taken from 262 turtles of four different species.

Algae species	Division
<i>Basicladia crassa</i> Hoffmann & Tilden	Chlorophyta
<i>Basicladia chelonum</i> Hoffmann & Tilden	Chlorophyta
<i>Cladophora glomerata</i> (L.) Kützing	Chlorophyta
<i>Rhizoclonium hieroglyphicum</i> (Agardh) Kützing	Chlorophyta
<i>Symploca</i> sp.	Cyanophyta



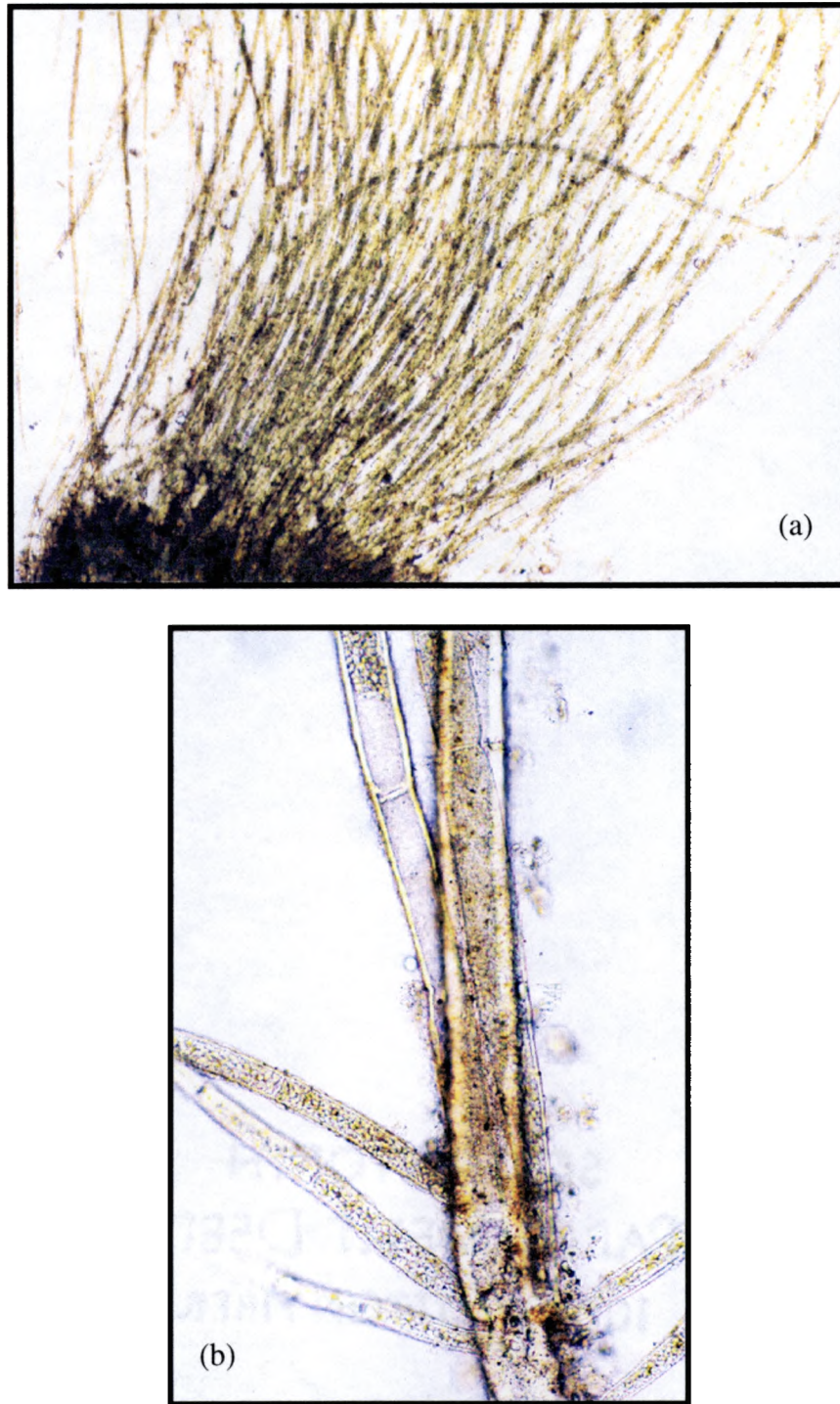


Figure 8. Light micrographs of (a) *Basicladia chelonum* thallus (magnification 40x).  
(b) Basal branching habit of *Basicladia* (magnification 200x).

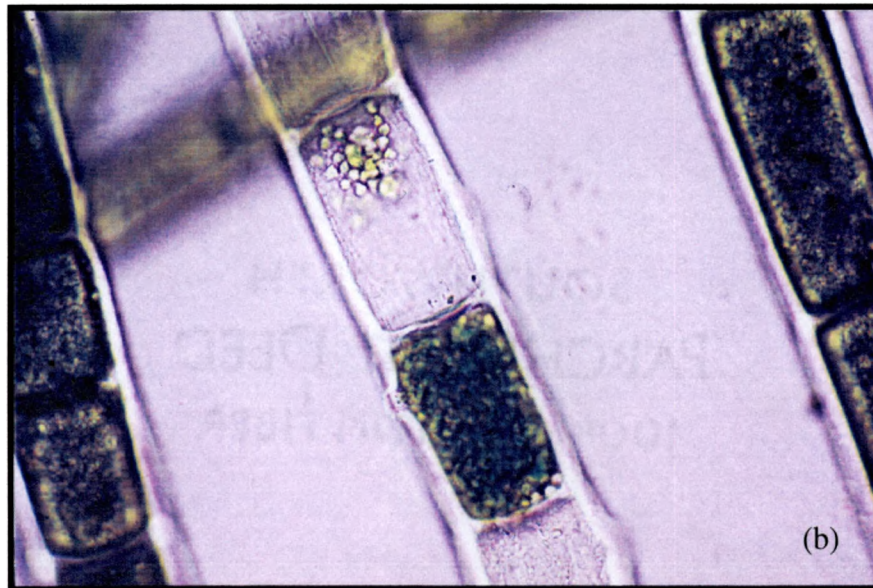


Figure 9. Light micrographs of (a) *Basicladia crassa* thallus (magnification 40x). (b) Formation and release of zoospores in *B. crassa* (magnification 400x).





Figure 10. Light micrograph of *Cladophora glomerata* thallus showing lateral branching at the apex of the cells (magnification 100x).

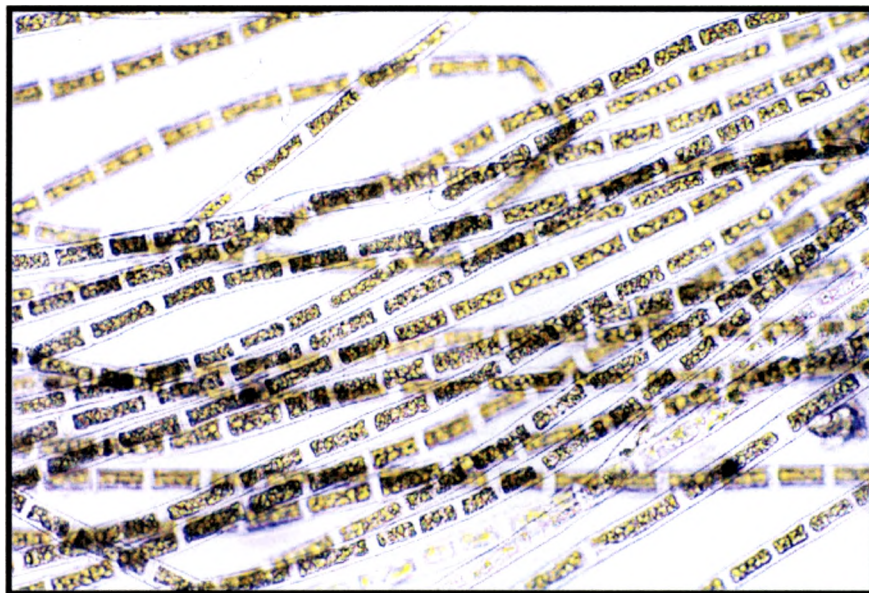


Figure 11. Light micrograph of *Rhizoclonium hieroglyphicum* filaments (magnification 100x).

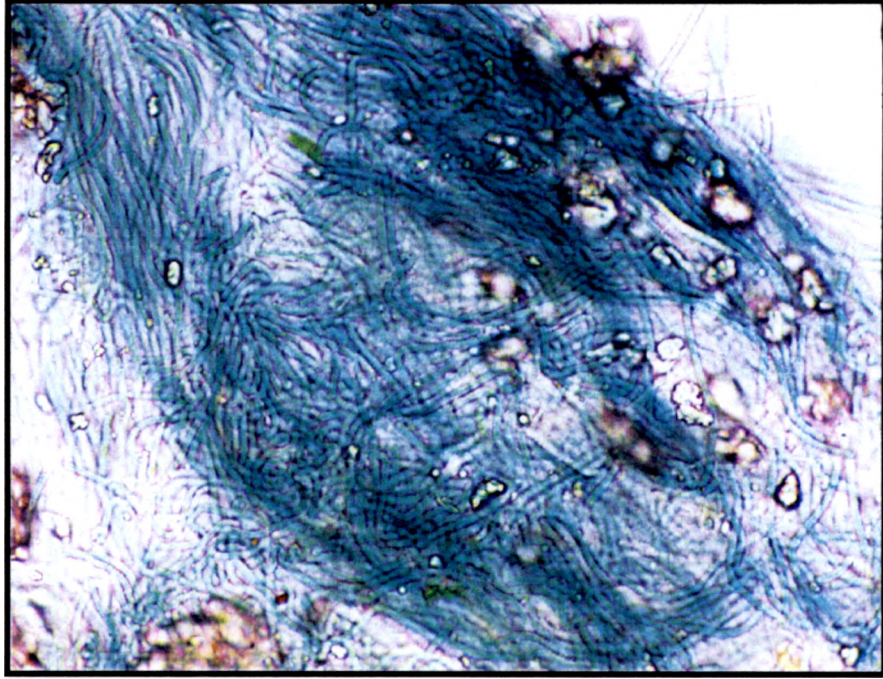


Figure 12. Light micrograph of *Symploca* sp. filaments growing intertwined to form a mat (magnification 200x).

Table 3.1. Presence (%) of *Basiacladia chelonum* on four turtle species in Spring Lake.

Turtle species	Alga Present (n = 164)	Alga Absent (n = 98)	% Presence	Adjusted Residual (z)
<i>Chelydra s. serpentina</i>	9	6	60.0%	-0.2
<i>Sternotherus odoratus</i>	85	2	97.7%	8.3**
<i>Trachemys s. elegans</i>	62	10	86.1%	4.8**
<i>Pseudemys texana</i>	8	80	9.1%	-12.7**

\*\* P &lt; 0.0001

Table 3.2. Presence (%) of *Basiacladia chelonum* across four seasons in Spring Lake.

Season	Alga Present (n = 164)	Alga Absent (n = 98)	% Presence	Adjusted Residual (z)
Spring	47	24	66.2%	0.7
Summer	29	26	52.7%	-1.7
Fall	60	20	75.0%	2.8**
Winter	28	28	50.0%	-2.2**

\*\* P &lt; 0.008

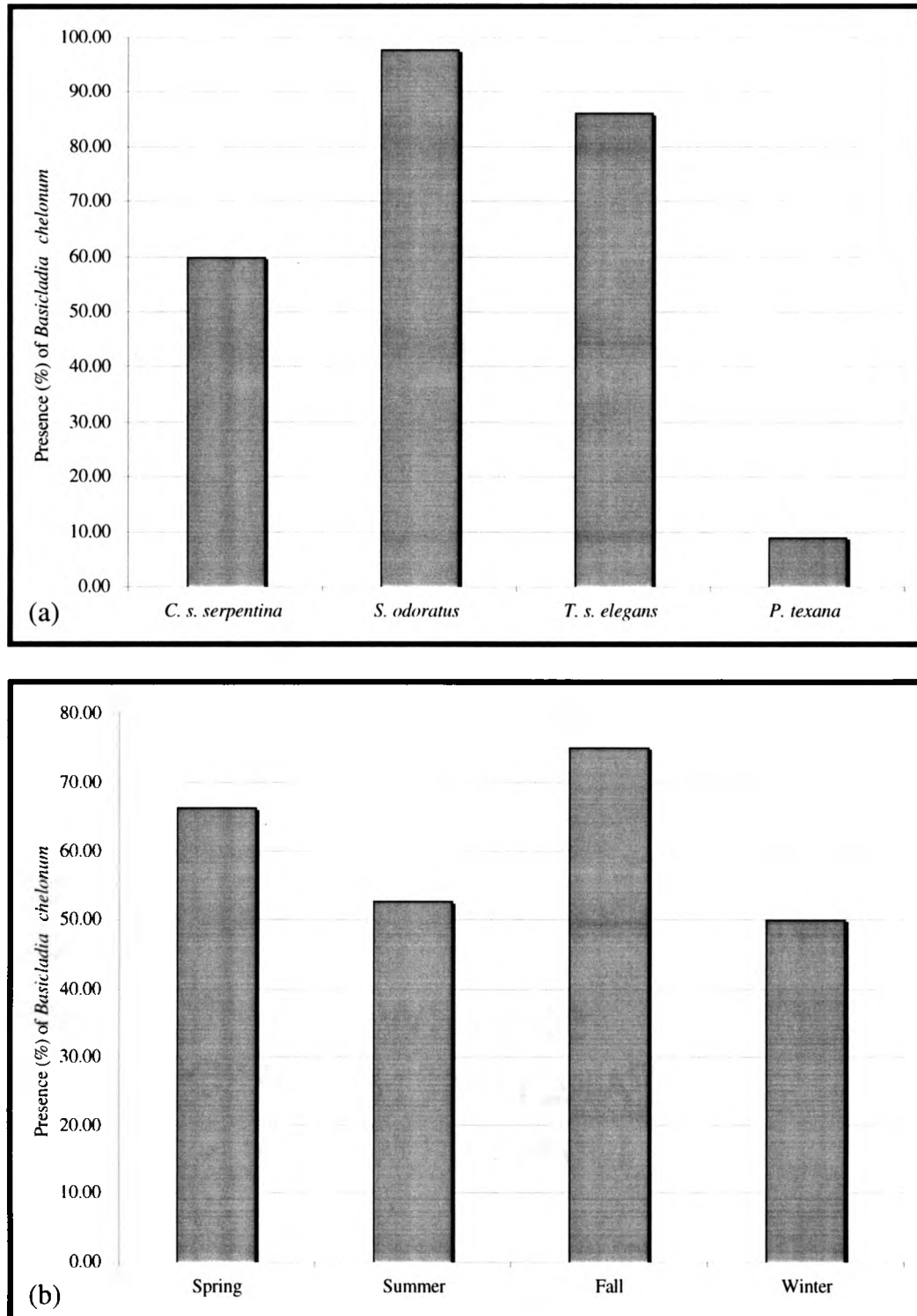


Figure 13. Graphs showing presence (%) of *Basicladia chelonum*. (a) Among four species of turtle (DF = 3,  $G^2 = 195.53$ ,  $P < 0.0001$ ). (b) Among seasons (DF = 3,  $G^2 = 11.87$ ,  $P < 0.008$ ).

Presence of *B. crassa* differed significantly across turtle species ( $DF = 3$ ,  $G^2 = 24.38$ ,  $P < 0.0001$ ) (Figure 14a). The z scores (Table 4.1) indicate that the dependence comes from the turtle species *T. s. elegans* ( $z = 2.7$ ) and *P. texana* ( $z = -3.8$ ). *Basiacladia crassa* presence also differed significantly across season ( $DF = 3$ ,  $G^2 = 17.50$ ,  $P < 0.001$ ) (Figure 14b). Table 4.2 shows that the presence of this alga differed significantly in the spring ( $z = 4.2$ ) and fall ( $z = -2.7$ ).

Presence of *C. glomerata* differed significantly across turtle species ( $DF = 3$ ,  $G^2 = 250.59$ ,  $P < 0.0001$ ) (Figure 15). The significance (Table 5) lies in all the turtle species *C. s. serpentina* ( $z = -4.2$ ), *S. odoratus* ( $z = -11.7$ ), *T. s. elegans* ( $z = 3.1$ ) and *P. texana* ( $z = 10.7$ ). *Cladophora glomerata* presence did not differ significantly across season.

Presence of *Symploca* sp. differed significantly across turtle species ( $DF = 3$ ,  $G^2 = 12.45$ ,  $P < 0.006$ ) (Figure 16a). The z scores (Table 6.1) show that the significance comes from *S. odoratus* ( $z = -2.9$ ) and *P. texana* ( $z = 2.7$ ). *Symploca* sp. presence also differed significantly across season ( $DF = 3$ ,  $G^2 = 11.51$ ,  $P < 0.009$ ) (Figure 16b). The presence of this alga differed significantly in the spring ( $z = -2.4$ ) and winter ( $z = 2.6$ ) (Table 6.2).

Table 4.1. Presence (%) of *Basicladia crassa* on four turtle species in Spring Lake.

<b>Turtle species</b>	<b>Alga Present (n = 26)</b>	<b>Alga Absent (n = 236)</b>	<b>% Presence</b>	<b>Adjusted Residual (z)</b>
<i>Chelydra s. serpentina</i>	3	12	20.0%	1.3
<i>Sternotherus odoratus</i>	10	77	11.5%	0.6
<i>Trachemys s. elegans</i>	13	59	18.1%	2.7**
<i>Pseudemys texana</i>	0	88	0.0%	-3.8**

\*\* P &lt; 0.0001

Table 4.2. Presence (%) of *Basicladia crassa* across four seasons in Spring Lake.

<b>Season</b>	<b>Alga Present (n = 26)</b>	<b>Alga Absent (n = 236)</b>	<b>% Presence</b>	<b>Adjusted Residual (z)</b>
Spring	16	55	6.1%	4.2**
Summer	4	51	1.5%	-0.7
Fall	2	78	0.8%	-2.7**
Winter	4	52	1.5%	-0.8

\*\* P &lt; 0.001



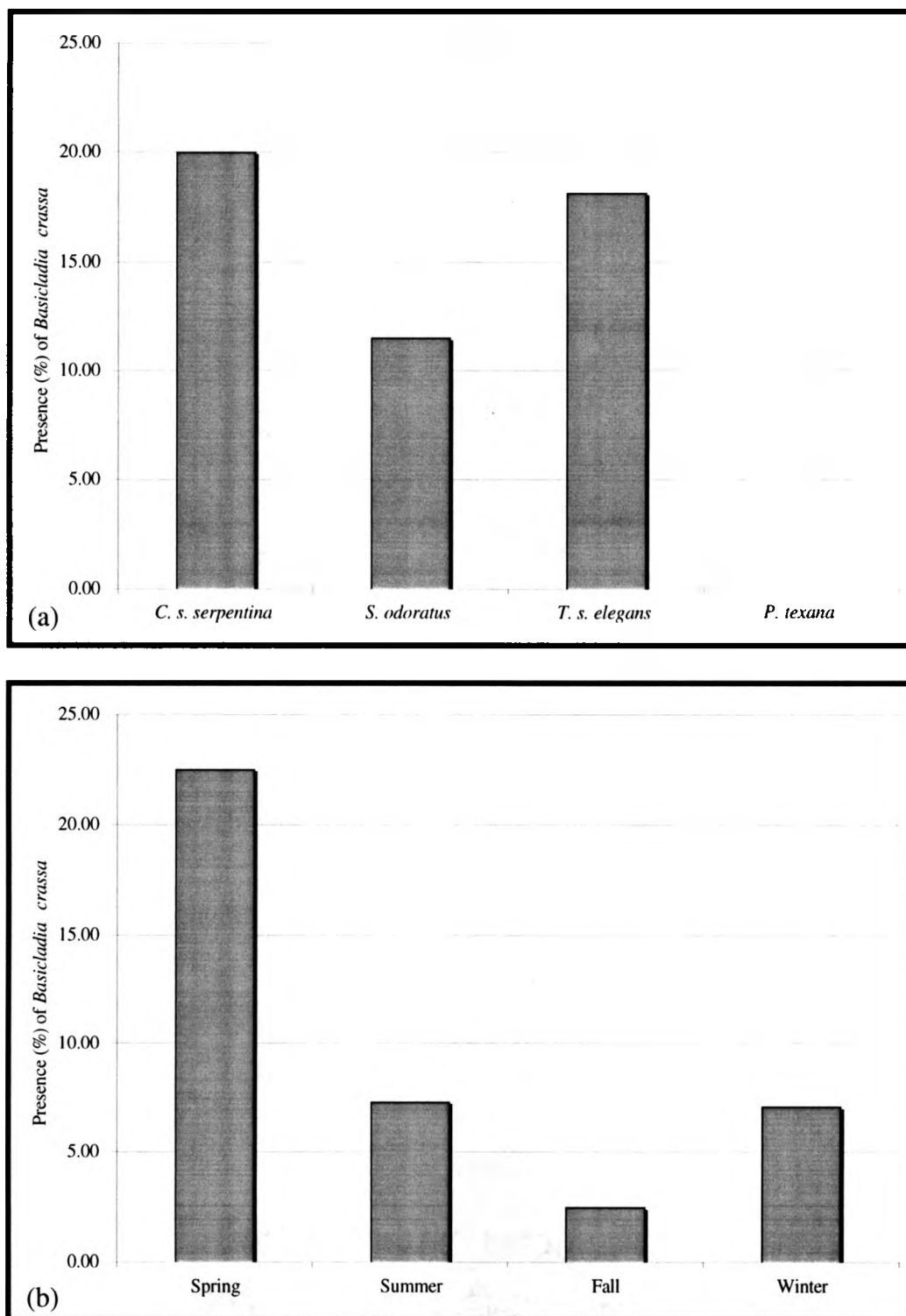


Figure 14. Graphs showing presence (%) of *Basicladia crassa*. (a) Among four species of turtle (DF = 3,  $G^2 = 24.38$ ,  $P < 0.0001$ ). (b) Among seasons (DF = 3,  $G^2 = 17.50$ ,  $P < 0.001$ ).

Table 5. Presence (%) of *Cladophora glomerata* on four turtle species in Spring Lake.

Turtle species	Alga Present (n = 137)	Alga Absent (n = 125)	% Presence	Adjusted Residual (z)
<i>Chelydra s. serpentina</i>	0	15	0.0%	-4.2**
<i>Sternotherus odoratus</i>	1	86	1.1%	-11.7**
<i>Trachemys s. elegans</i>	49	23	68.1%	3.1**
<i>Pseudemys texana</i>	87	1	98.9%	10.7**

\*\* P &lt; 0.0001

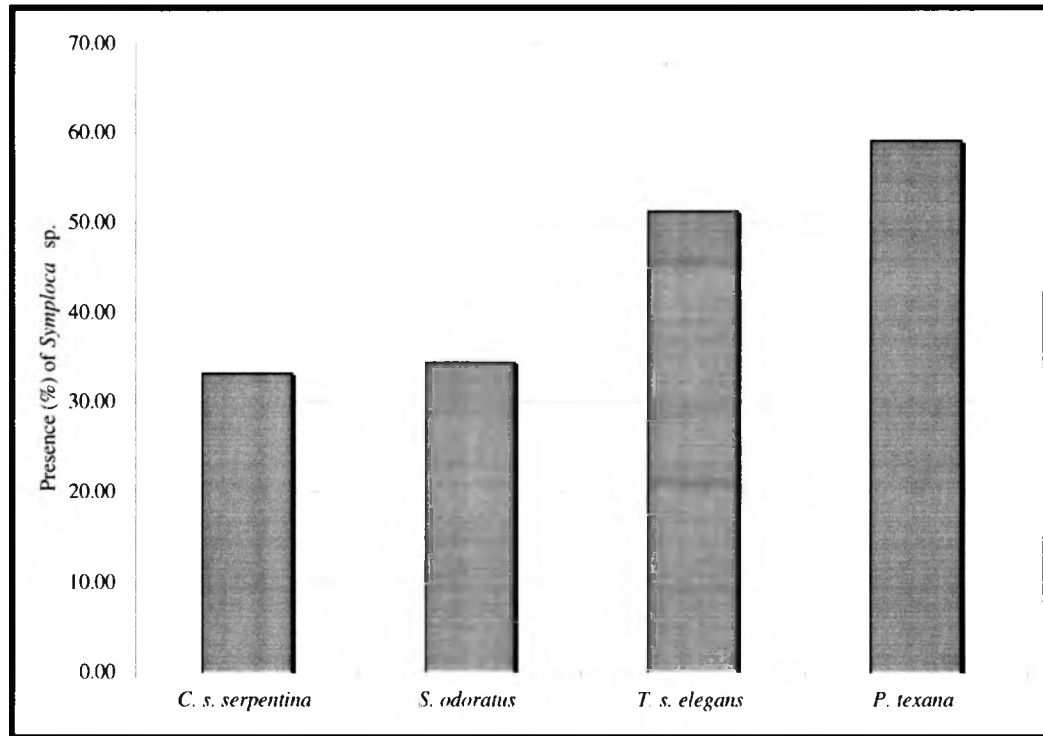


Figure 15. Graph showing presence (%) of *Cladophora glomerata* among four species of turtle (DF = 3,  $G^2 = 250.59$ ,  $P < 0.0001$ ).

Table 6.1. Presence (%) of *Symploca* sp. on four turtle species in Spring Lake.

<b>Turtle species</b>	<b>Alga Present (n = 124)</b>	<b>Alga Absent (n = 138)</b>	<b>% Presence</b>	<b>Adjusted Residual (z)</b>
<i>Chelydra s. serpentina</i>	5	10	3.8%	-1.1
<i>Sternotherus odoratus</i>	30	57	21.8%	-2.9**
<i>Trachemys s. elegans</i>	37	35	13.4%	0.8
<i>Pseudemys texana</i>	52	36	13.7%	2.7**

\*\* P &lt; 0.006

Table 6.2. Presence (%) of *Symploca* sp. across four seasons in Spring Lake.

<b>Season</b>	<b>Alga Present (n = 124)</b>	<b>Alga Absent (n = 138)</b>	<b>% Presence</b>	<b>Adjusted Residual (z)</b>
Spring	25	46	9.5%	-2.4**
Summer	22	33	8.4%	-1.2
Fall	42	38	16.0%	1.1
Winter	35	21	100.0%	2.6**

\*\* P &lt; 0.009

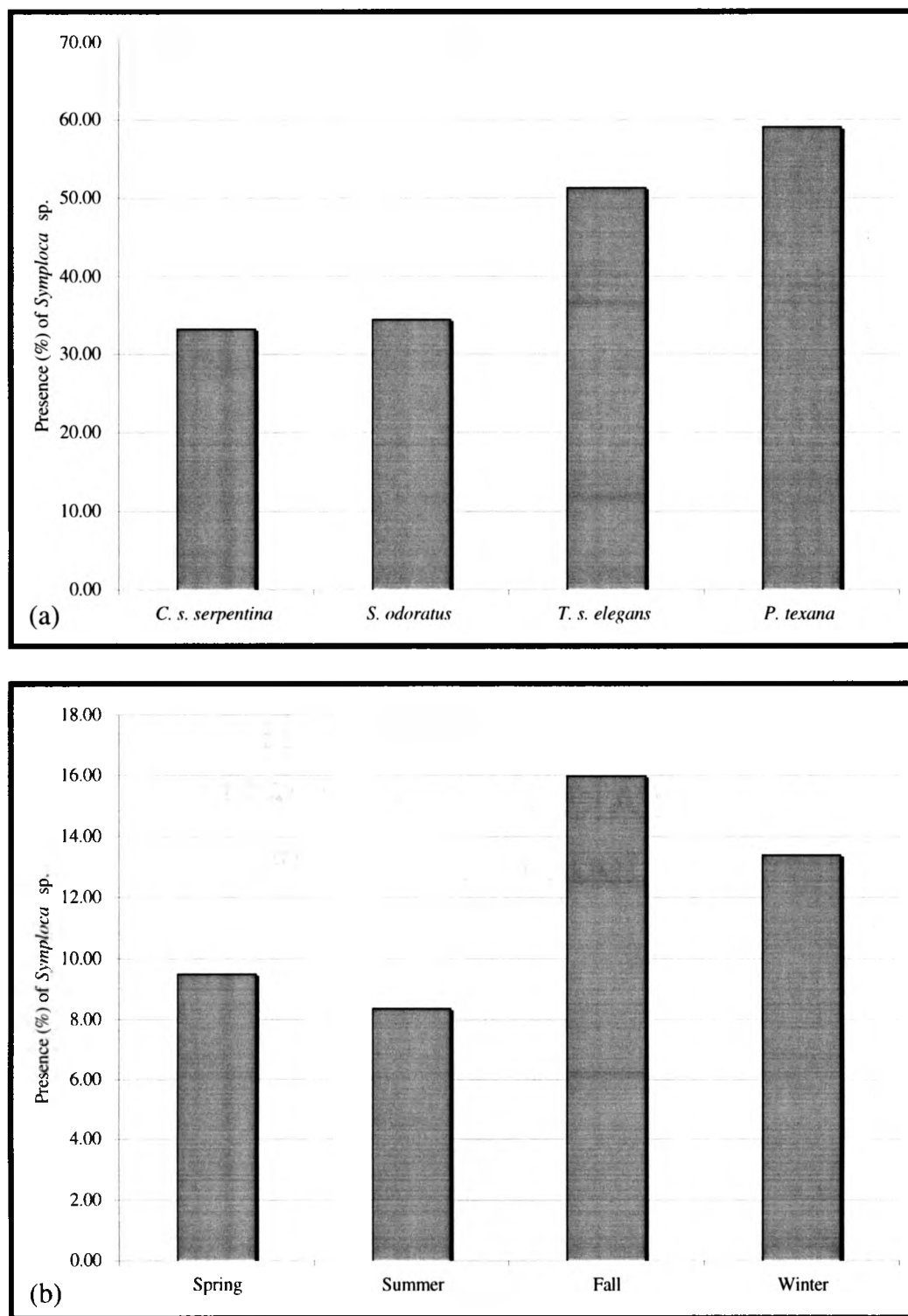


Figure 16. Graphs showing presence (%) of *Symploca* sp. (a) Among four species of turtle (DF = 3,  $G^2 = 12.45$ ,  $P < 0.006$ ). (b) Among seasons (DF = 3,  $G^2 = 11.51$ ,  $P < 0.009$ ).

## Substrate Experiment

Algae identified in the substrate experiment are listed in Table 7. Light micrographs of some of these algae are shown in Figures 17-25. Statistical analyses indicated that there were no significant differences in the three-way interactions (algae x substrate x depth) for any of the algae identified. There were four algae, *Cladophora glomerata*, *Coleochaete scutata*, *Lyngbya* spp., and *R. hieroglyphicum* that had significant differences in the two-way interactions (i.e. algae x substrate and algae x depth).

Presence of *C. glomerata* showed a significant difference among substrate types (DF = 3,  $G^2 = 32.43$ ,  $P < 0.0001$ ) (Figure 26). The z scores for the substrates (Table 8), indicate that the substrates brick ( $z = 4.2$ ) and glass ( $z = -4.2$ ) cause the significance of the algae x substrate interaction. There was no significant difference for *C. glomerata* and depth.

For the alga *Coleochaete scutata*, there was a significant difference in the algae x substrate interaction and in the algae x depth interaction. Presence of *C. scutata* showed a significant difference among substrate types (DF = 3,  $G^2 = 42.60$ ,  $P < 0.0001$ ) (Figure 27a). The substrates wood ( $z = -3.3$ ), glass ( $z = 5.8$ ), and brick ( $z = -3.5$ ) contribute to the significance for this two-way interaction (Table 9.1). *Coleochaete scutata* presence also showed a significant difference in depth (DF = 3,  $G^2 = 8.30$ ,  $P < 0.004$ ) (Figure 27b). The z scores for depth, 0.5 m ( $z = 2.9$ ) and 2.0 m ( $z = -2.9$ ), indicate significance among depths (Table 9.2).

Table 7. Algae species identified from substrate experiment. Algae samples were taken from 480 substrates of four different types (turtle scute, glass, wood, and brick).

Algae species	Division
<i>Bulbochaete</i> sp.	Chlorophyta
<i>Chaetophora elegans</i> (Roth) Agardh	Chlorophyta
<i>Cladophora glomerata</i> (L.) Kützing	Chlorophyta
<i>Closterium</i> sp.	Chlorophyta
<i>Coleochaete scutata</i> Brebisson	Chlorophyta
<i>Gloeocystis</i> sp.	Chlorophyta
<i>Oedogonium</i> sp.	Chlorophyta
<i>Rhizoclonium hieroglyphicum</i> (Agardh) Kützing	Chlorophyta
<i>Spirogyra</i> sp.	Chlorophyta
<i>Ulothrix zonata</i> (Weber & Mohr) Kützing	Chlorophyta
Unknown colonial chlorophyte	Chlorophyta
<i>Calothrix</i> sp.	Cyanophyta
<i>Lyngbya</i> spp.	Cyanophyta
<i>Batrachospermum involutum</i> Vis et Sheath	Rhodophyta
<i>Compsopogon coeruleus</i> (Balbis Ex Agardh) Montagne	Rhodophyta
<i>Sirodotia huillensis</i> Welw., W. & G.S. West	Rhodophyta
<i>Tribonema</i> sp.	Xanthophyta



Figure 17. Light micrograph of *Bulbochaete* sp. thallus (magnification 200x).

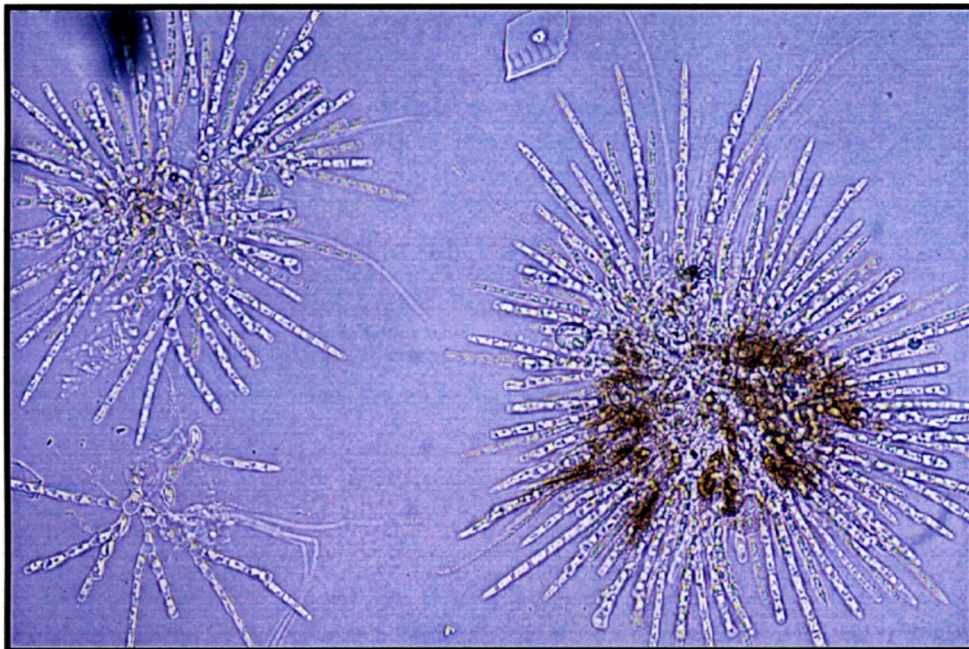


Figure 18. Light micrograph of *Chaetophora elegans* thallus with radiating filaments (magnification 200x).



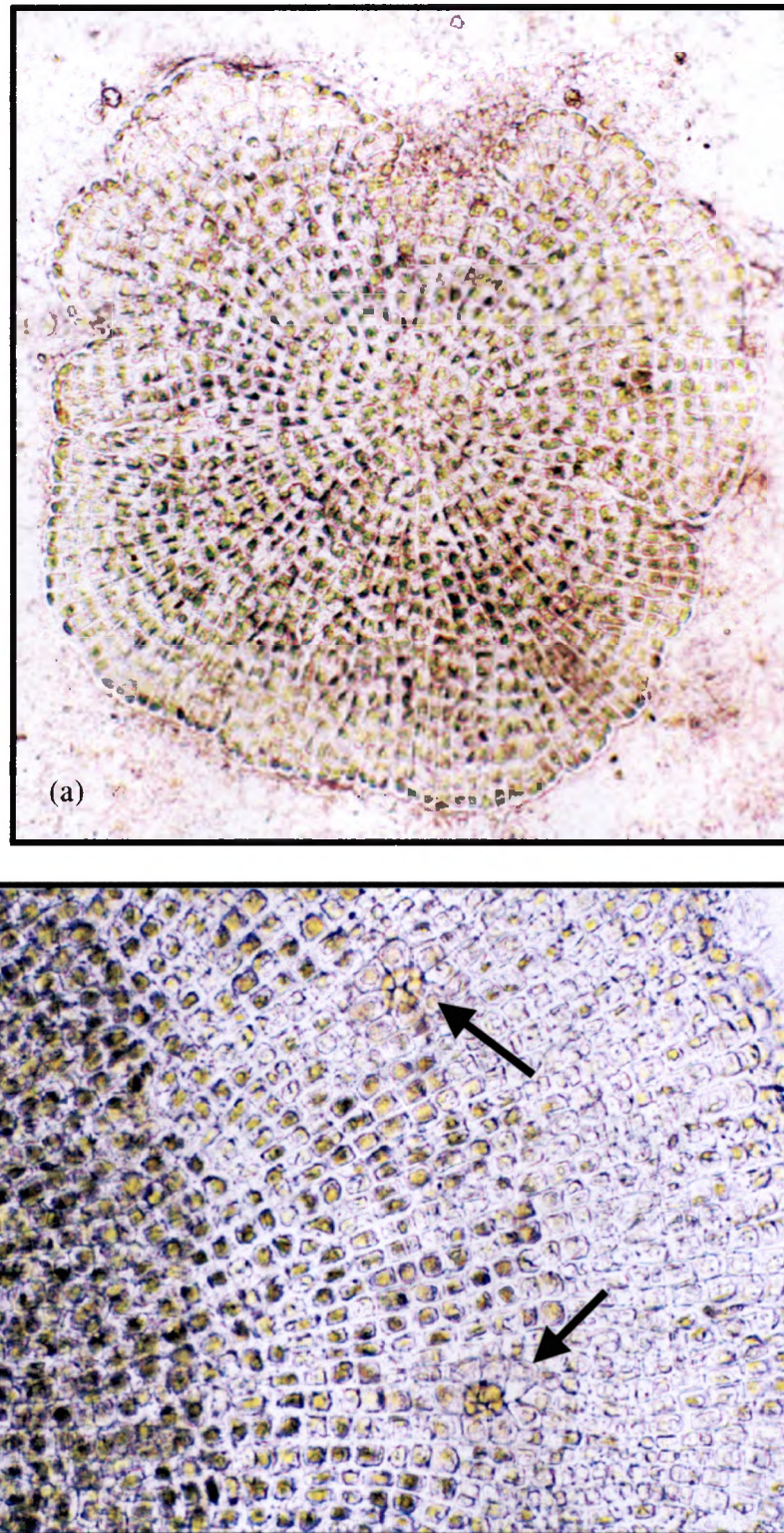


Figure 19. Light micrographs of (a) *Coleochaete scutata* thallus growing prostrate on a turtle scute (magnification 100x). (b) *C. scutata* thallus, arrows indicate the cortication of the zygotes (magnification 200x).



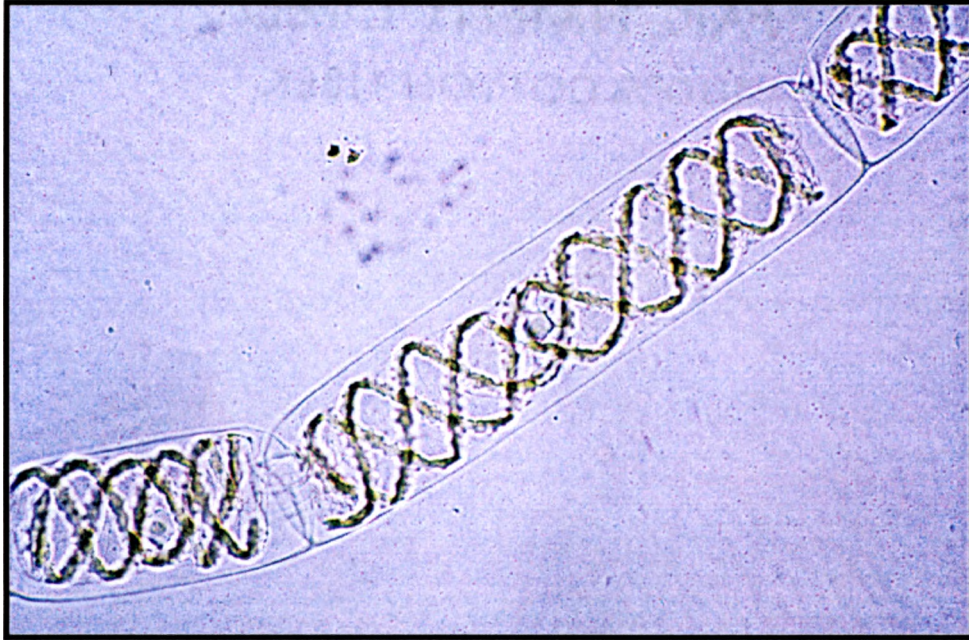


Figure 20. Light micrograph of *Spirogyra* sp. cell with distinct spiral shaped chloroplast (magnification 400x).

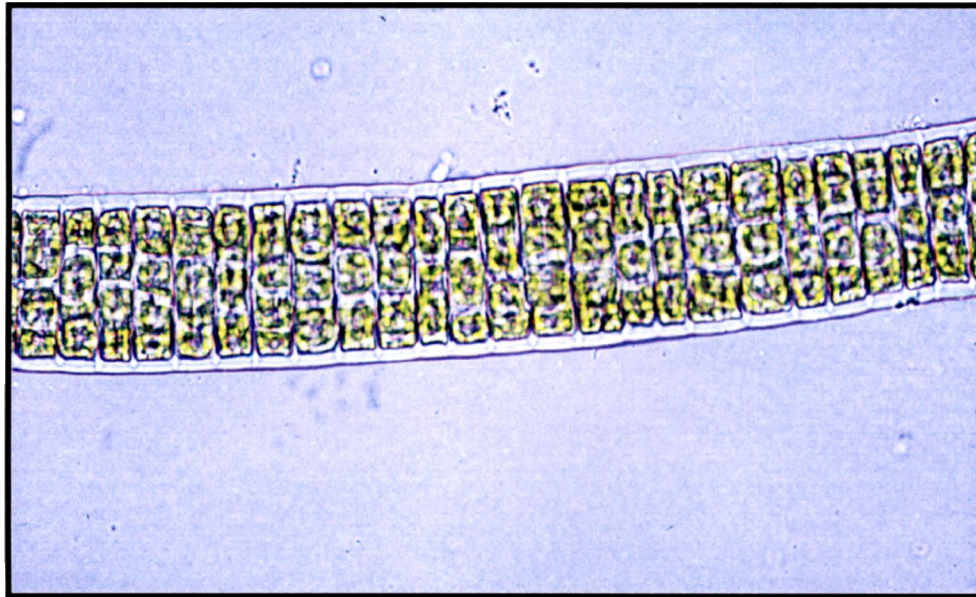


Figure 21. Light micrograph of a section of *Ulothrix zonata* filament with distinct bracelet shaped chloroplasts (magnification 400x).



Figure 22. Light micrograph of *Lyngbya* sp. filaments with distinct sheaths around each filament (magnification 400x).

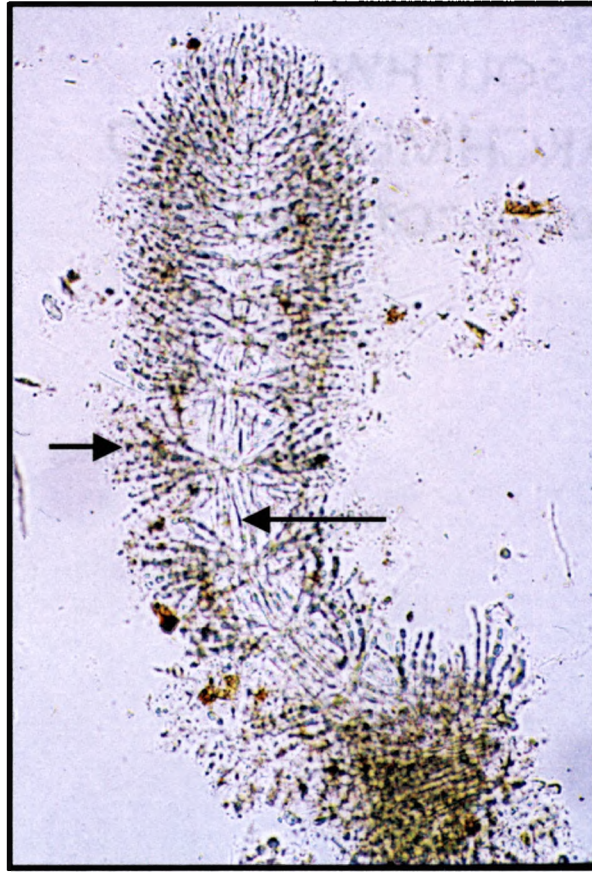


Figure 23. Light micrograph of a segment of *Batrachospermum involutum* thallus (magnification 200x). The long arrow indicates the axial filaments. The short arrow indicates the branching filaments arising from the axial filaments in whorls.



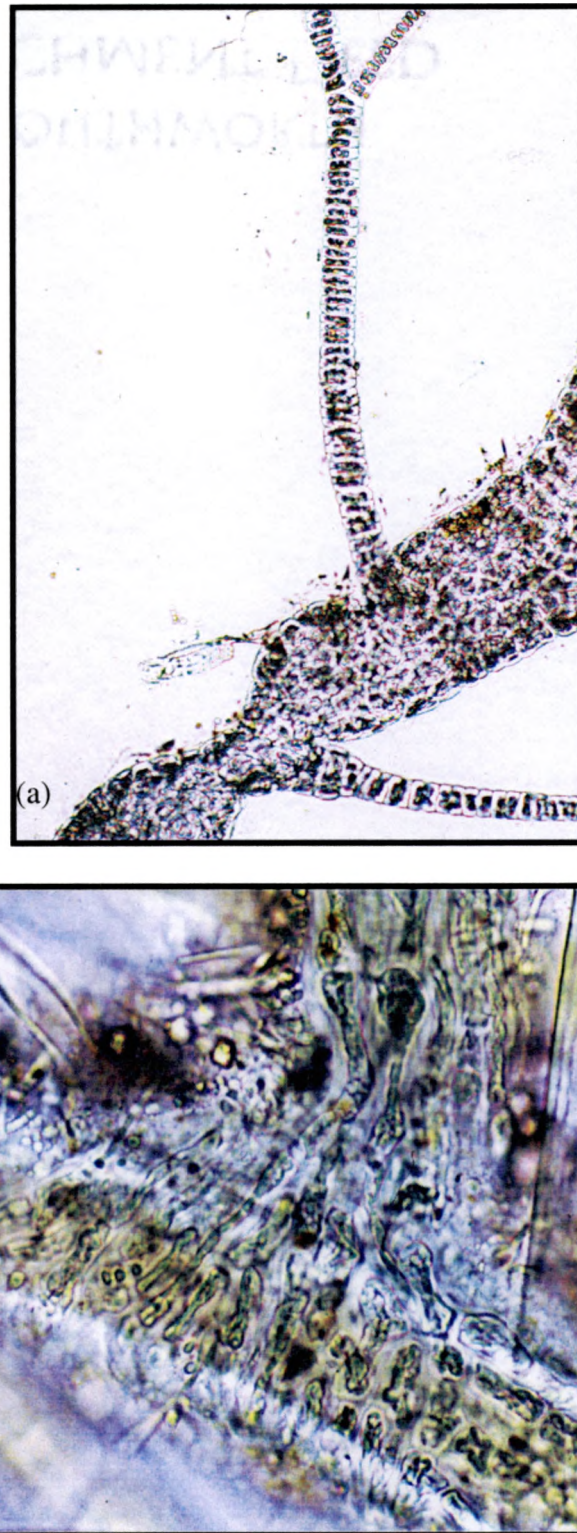


Figure 24. Light micrographs of (a) *Compsopogon coeruleus* showing multiseriate condition in older filaments and uniseriate condition in younger filaments (magnification 100x). (b) *C. coeruleus* holdfast (magnification 400x).

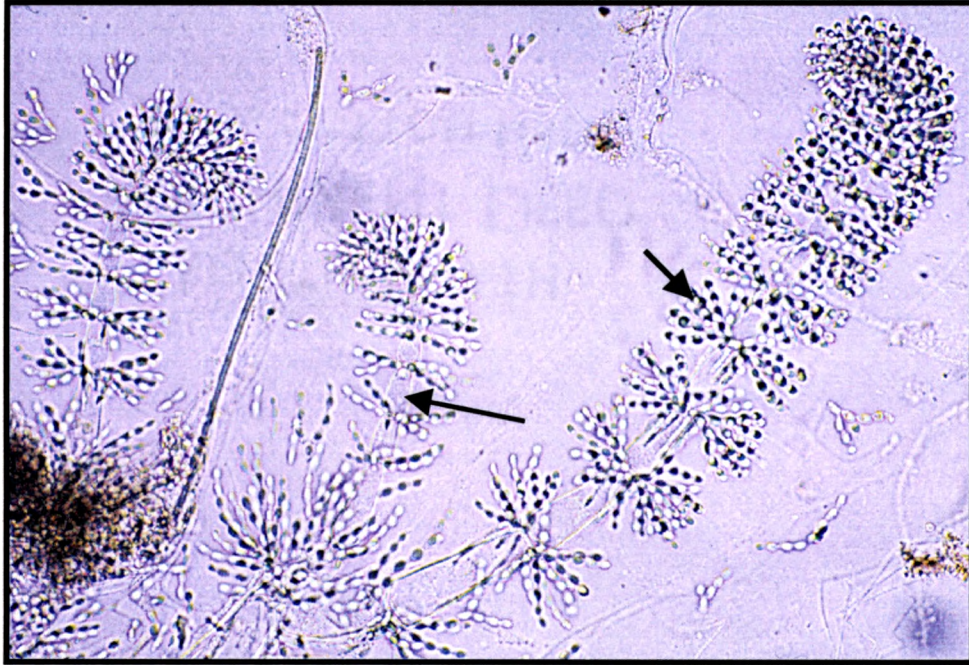


Figure 25. Light micrograph of a segment of *Sirodotia huillensis* thallus showing distinct curl at apex (magnification 200x). The long arrow indicates the axial filaments. The short arrow indicates the branching filaments that arise from the axial filaments in whorls.

Table 8. Presence (%) of *Cladophora glomerata* on four substrates in Spring Lake.

Substrate Type	Alga Present (n = 96)	Alga Absent (n = 384)	% Presence	Adjusted Residual (z)
Wood	18	102	3.8%	-1.6
Glass	8	112	1.7%	-4.2**
Brick	40	80	8.3%	4.2**
Turtle	30	90	1.7%	1.6

\*\* P < 0.0001

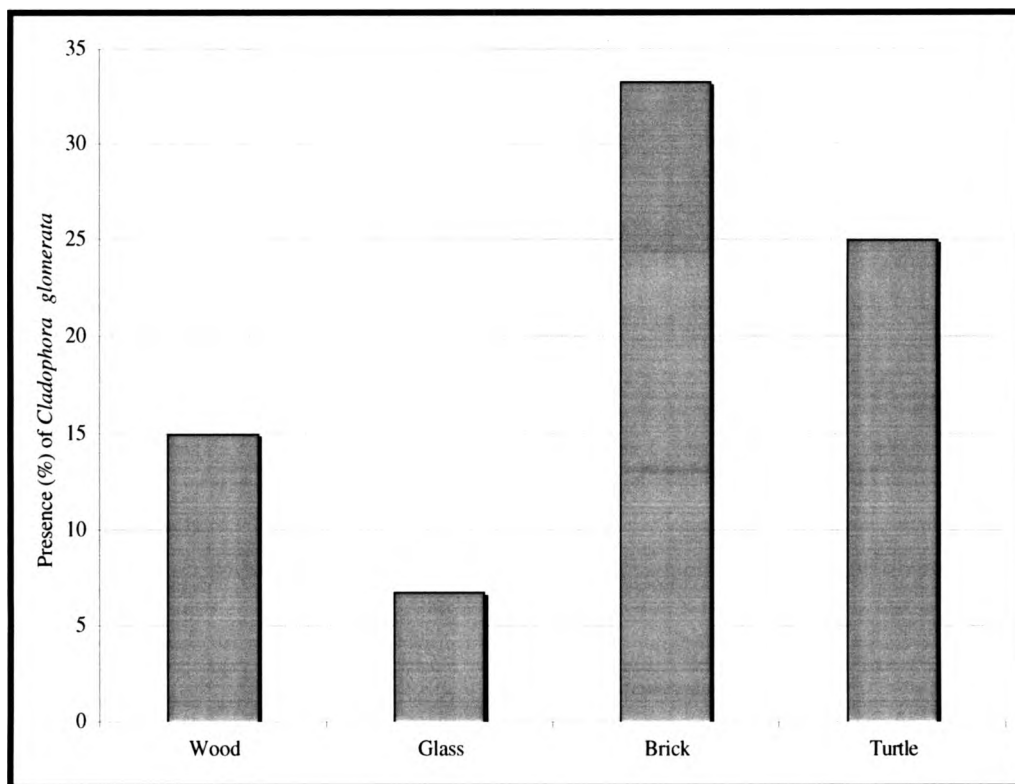


Figure 26. Graph showing presence (%) of *Cladophora glomerata* among four types of substrates (DF = 3,  $G^2 = 32.43$ ,  $P < 0.0001$ ).



Table 9.1. Presence (%) of *Coleochaete scutata* on four substrates in Spring Lake.

Substrate Type	Alga Present (n = 117)	Alga Absent (n = 363)	% Presence	Adjusted Residual (z)
Wood	16	104	13.3%	-3.3**
Glass	53	67	44.2%	5.8**
Brick	15	105	12.5%	-3.5**
Turtle	33	87	27.5%	0.9

\*\* P < 0.0001

Table 9.2. Presence (%) of *Coleochaete scutata* across two depths in Spring Lake.

Depth	Alga Present (n = 117)	Alga Absent (n = 363)	% Presence	Adjusted Residual (z)
0.5 m	72	168	15.0%	2.9**
2.0 m	45	195	9.4%	-2.9**

\*\* P < 0.004

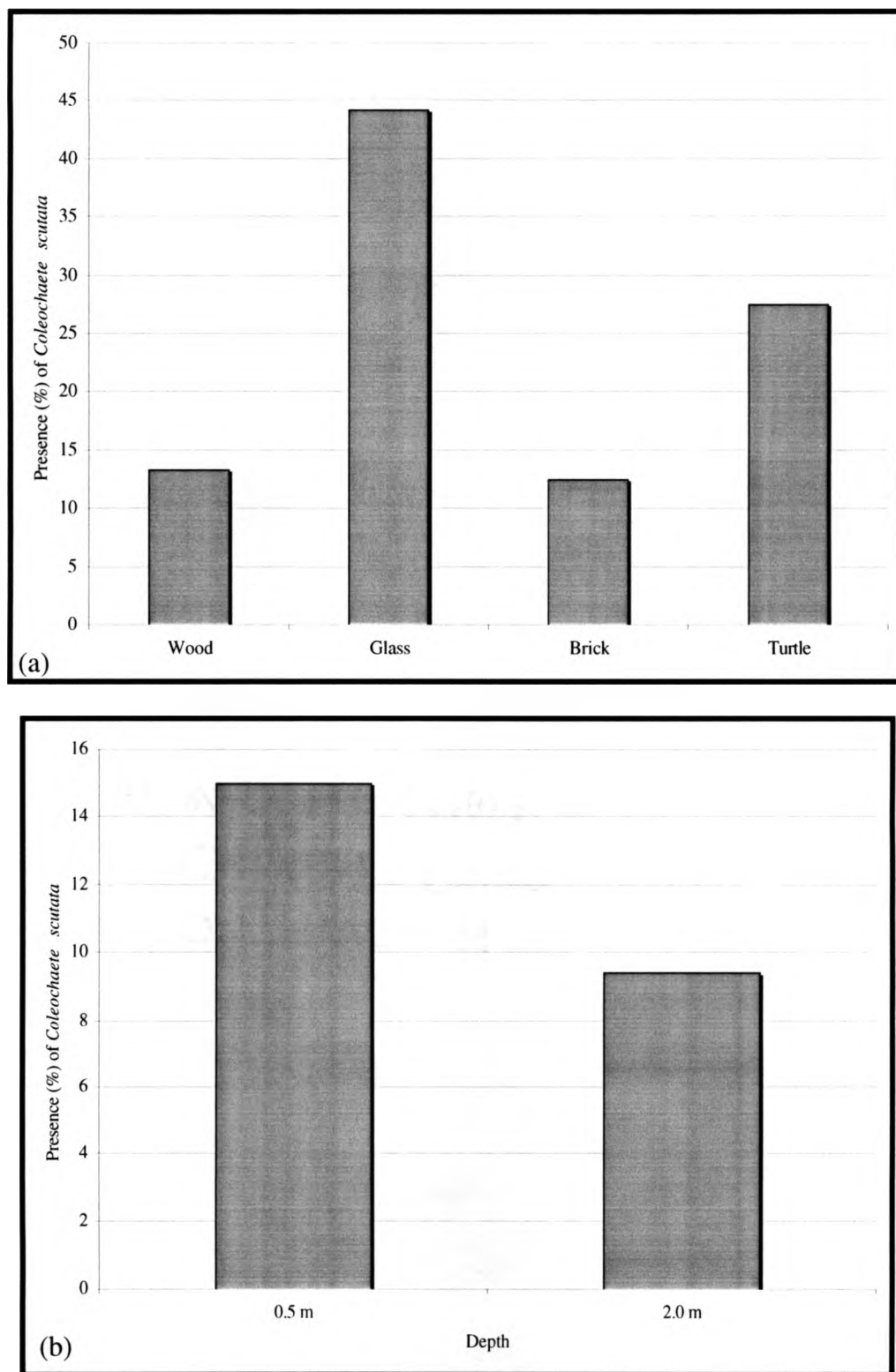


Figure 27. Graphs showing presence (%) of *Coleochaete scutata*. (a) Among four types of substrates (DF = 3,  $G^2 = 42.60$ ,  $P < 0.0001$ ). (b) Among depths (DF = 3,  $G^2 = 8.30$ ,  $P < 0.004$ ).

Presence of *Lyngbya* spp. showed a significant difference among substrate types (DF = 3,  $G^2 = 20.84$ ,  $P < 0.0001$ ) (Figure 28). The substrates wood ( $z = 3.7$ ) and brick ( $z = -3.6$ ) cause the significance of the algae x substrate interaction (Table 10). There was no significant difference for *Lyngbya* spp. and depth.

Presence of *R. hieroglyphicum* showed a significant difference among substrate types (DF = 3,  $G^2 = 17.70$ ,  $P < 0.001$ ) (Figure 29). The substrates wood ( $z = 4.0$ ), glass ( $z = -2.1$ ), and turtle ( $z = -2.1$ ) cause the significance of the algae x substrate interaction (Table 11). There was no significant difference for *R. hieroglyphicum* and depth.

The genus *Basicladia*, known to grow mainly on turtle carapaces, was found prevalently growing on the carapaces of the turtles in this study. However, neither *B. crassa* nor *B. chelonum* were identified from any of the four substrates in this experiment, suggesting that turtle habit may have an influence on the recruitment of these algae.

Table 10. Presence (%) of *Lyngbya* spp. on four substrates in Spring Lake.

Substrate Type	Alga Present (n = 133)	Alga Absent (n = 347)	% Presence	Adjusted Residual (z)
Wood	49	71	40.8%	3.7**
Glass	31	89	25.8%	-0.5
Brick	18	102	15.0%	-3.6**
Turtle	35	85	29.2%	0.4.

\*\* P < 0.0001

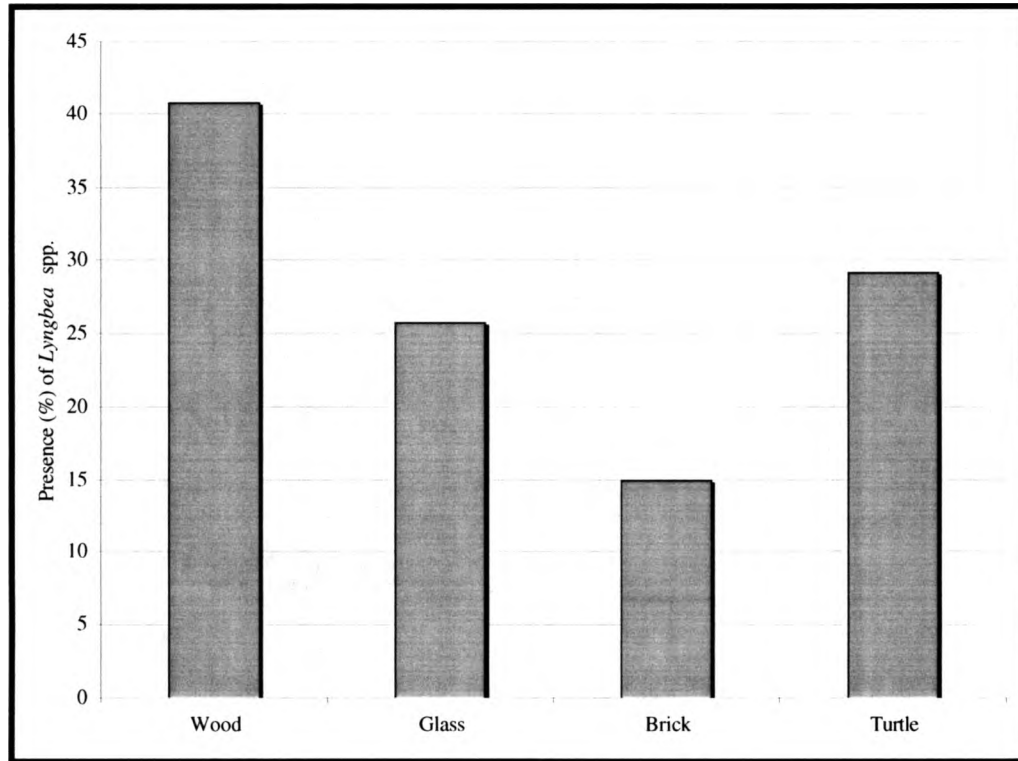


Figure 28. Graph showing presence (%) of *Lyngbya* spp. on four types of substrates (DF = 3  $G^2 = 20.84$ ,  $P < 0.0001$ ).

Table 11. Presence (%) of *Rhizoclonium hieroglyphicum* on four substrates in Spring Lake.

Substrate Type	Alga Present (n = 26)	Alga Absent (n = 454)	% Presence	Adjusted Residual (z)
Wood	15	105	12.5%	4.0**
Glass	2	118	1.7%	-2.1**
Brick	7	113	5.8%	0.2
Turtle	2	118	1.7%	2.1**

\*\*  $P < 0.001$

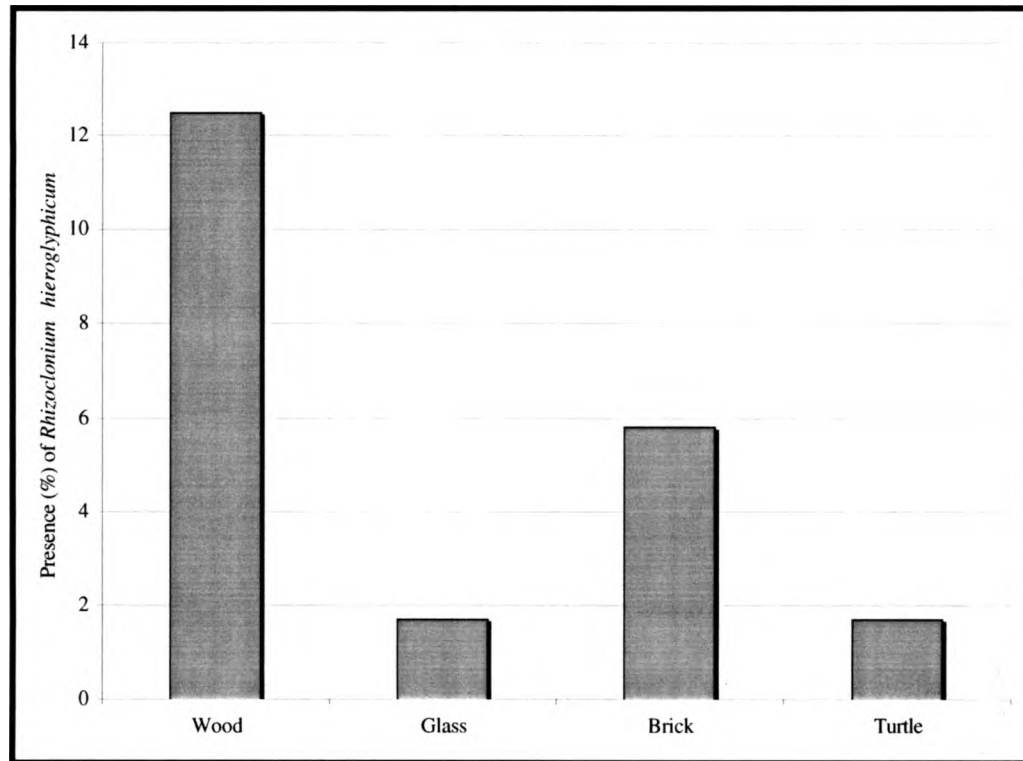


Figure 29. Graph showing presence (%) of *Rhizoclonium hieroglyphicum* on four types of substrates (DF = 3,  $G^2 = 17.70$ ,  $P < 0.001$ ).

**Algal Attachment**

*Basycladia crassa* and *B. chelonum* attach to turtle carapace by a holdfast of prostrate filaments (Figures 30, 31). These filaments do not penetrate the turtle's scute and therefore the algae are shed when the scute is shed from the turtle.



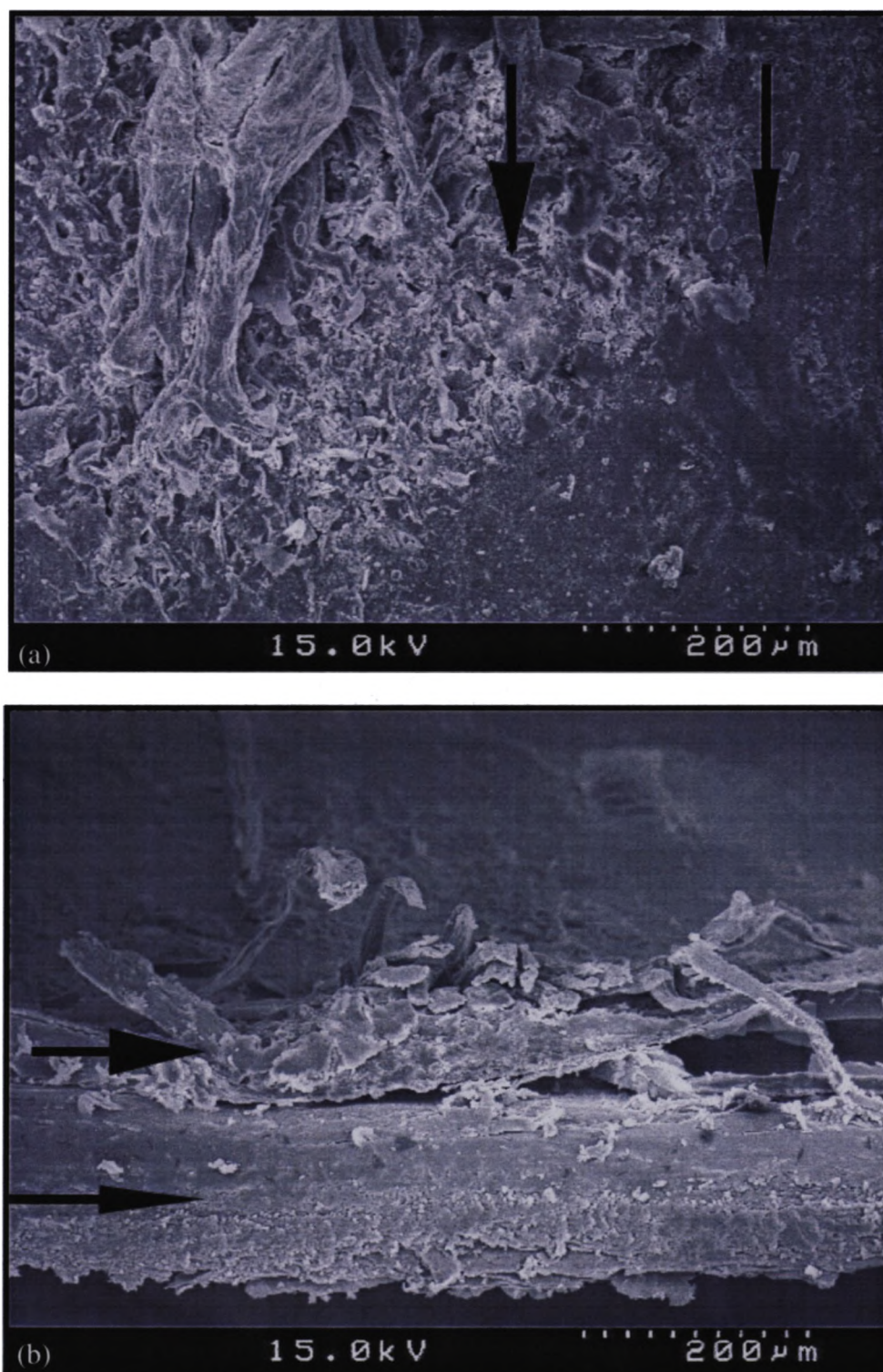


Figure 30. SEM micrographs of the attachment of *Basicladia chelonum* to turtle scute. (a) Top view. (b) Side view. Thick arrow indicates *B. chelonum* filaments and holdfast. Thin arrow indicates *Trachemys scripta elegans* scute.

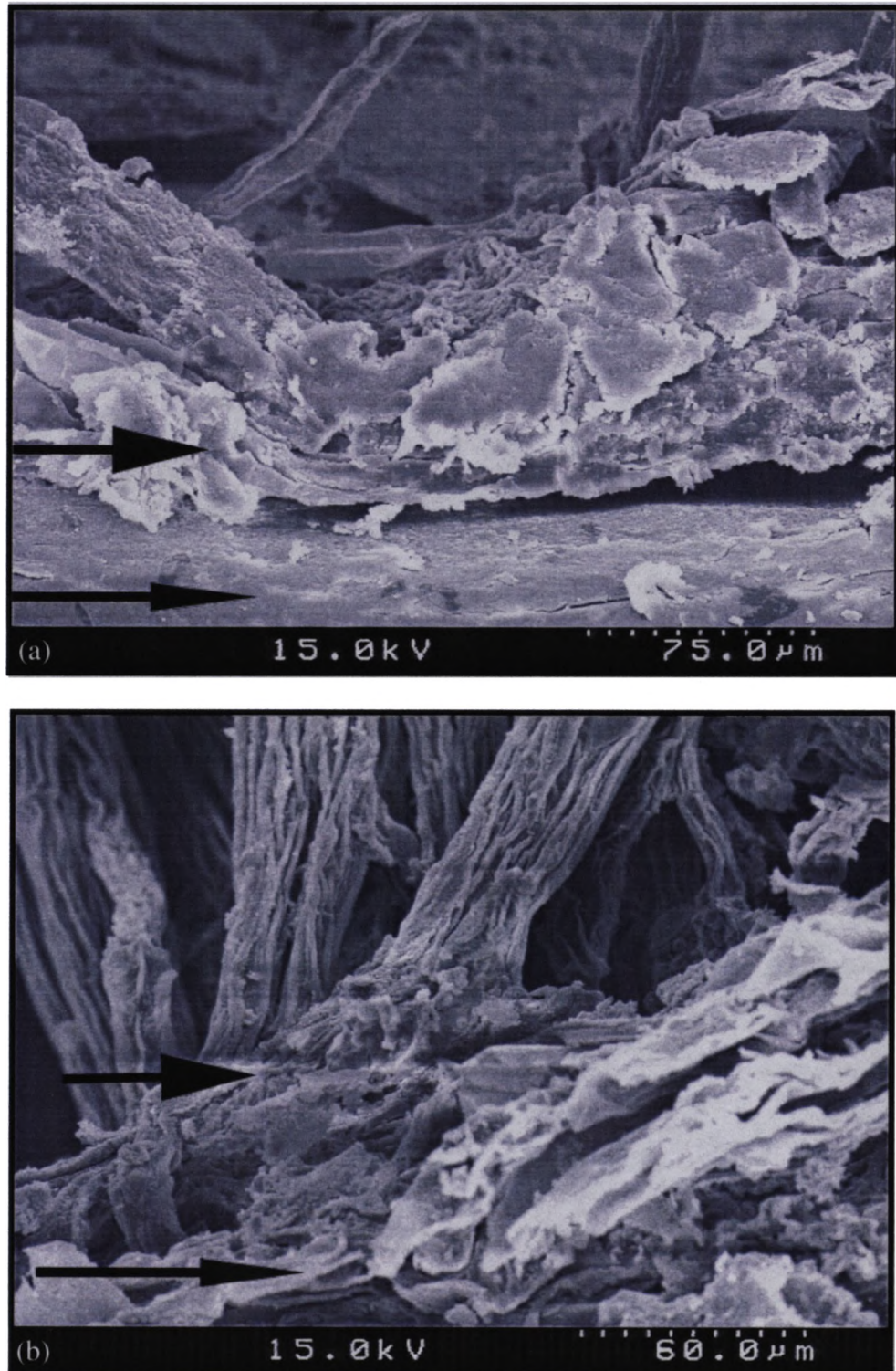


Figure 31. SEM micrographs of the attachment of *Basicladia crassa* to turtle scute. (a) Side view. (b) Oblique view. Thick arrow indicates *B. crassa* filaments and holdfast. Thin arrow indicates *Trachemys scripta elegans* scute.



## DISCUSSION

Green algae and turtles are considered ancient in their respective groups (Bold and Wynne, 1985; Zug, 1993). The association of these two ancient groups may be indicative of a relationship with a long history, and perhaps a coevolution between epizootic algae and habitat preference of the turtle hosts (Edgren et al., 1953). This study revealed certain patterns in algae composition among turtle species, among season, and among substrate types.

### Algal Species Composition Among Turtle Species

The results of this study support findings of Edgren et al. (1953) and Proctor (1958) that the genus *Basicladia* frequently inhabits the carapace of highly aquatic turtles. The results of this study also support Edgren et al. (1953) in that neither *B. chelonum* nor *B. crassa* colonize frequently sun-basking turtles. Statistically, season had no effect on algal species composition among turtle species, however algae did vary significantly among seasons. This study found that *B. chelonum* was rarely collected from *P. texana* (9.1%) and was collected in relatively high abundances on *Chelydra s. serpentina* (60.0%), *S. odoratus* (97.7%), and *T. s. elegans* (86.1%). The same pattern was observed for the other species of *Basicladia* identified, *B. crassa*. The opposite pattern was found for *Cladophora glomerata*, which was collected in a relatively high abundance on *P. texana* (98.9%) and *T. s. scripta* (68.1%) and a low abundance on *C. s. serpentina* (0.0%) and *S. odoratus* (1.1%).

The significant difference observed between *P. texana* and the other species of turtles for the algae *B. chelonum*, *B. crassa*, and *C. glomerata* could be due to turtle habit or environmental factors. *Pseudemys texana* is a gregarious basker, spending a large part of its day out of the water. Therefore algae colonizing the carapace of *P. texana* would be subjected to desiccation. Proctor (1958) found that *Basicladia* was able to withstand more desiccation than could most turtles. However, *Basicladia* was rarely found on *P. texana*. Rather, *C. glomerata* was the dominant alga colonizing *P. texana*. This is somewhat surprising because the literature indicates this taxon is not well adapted to desiccation. Shaver et al. (1997) found that *C. glomerata* grows best in continuously submerged, clear-water, stable habitats. *Cladophora glomerata* has been shown to not respond well to desiccation losing significant amounts of chlorophyll *a* and mass after  $\leq 6$  hours of atmospheric exposure in field and laboratory experiments (Usher and Blinn, 1990). Desiccation of two or more days significantly lowered *C. glomerata*'s recolonization rates (Blinn et al., 1995).

The species of *Basicladia* were found colonizing the carapaces of *C. s. serpentina* and *S. odoratus*, which spend most of their life submerged in the water and are rarely found on land. This suggests that there may be another reason, other than desiccation, to explain the low incidence of *Basicladia* on *P. texana*.

Light availability is another possible explanation for the significant difference in algal composition among turtle species. Light and nutrient availability are two factors that directly affect algal assemblages (Stevenson, 1997). Other authors found that light

availability and shading limit algae production and growth (Krause et al., 1996; Mosisch et al., 2001). *Chelydra s. serpentina* and *S. odoratus* spend most of their time at the lake bottom, whereas *T. s. scripta* and *P. texana* spend a majority of their time at the surface of the lake or basking. Species of *Basicladia* may be able to withstand lower light levels better than *C. glomerata*, which may explain the low occurrence of *C. glomerata* on *C. s. serpentina* and *S. odoratus*. *Cladophora* may be able to tolerate higher light levels and therefore may out compete *B. chelonum* and *B. crassa* on the carapaces of *T. s. scripta* and *P. texana*.

### **Algal Species Composition Among Substrate Types**

Algae that have been observed growing mainly on turtle carapaces rarely grow on other substrates (Proctor, 1958; Normandin and Taft, 1959). Proctor (1958) usually collected the algae on cement walls or other substrates from a turtle enclosure indicating these individuals came from turtles. This study tested whether algae species differ in colonization among four substrates (wood, glass, brick, turtle). Of the seventeen algae identified from the substrates, only four varied significantly among substrate types. *Rhizoclonium hieroglyphicum* and *Lyngbya* spp. had higher abundance on wood, *C. scutata* had a higher abundance on glass, and *C. glomerata* grew in higher abundance on brick. The texture of the substrate may influence the type of algae that colonizes its surface. The growth habit of *R. hieroglyphicum* is usually free-floating in slower moving waters and rarely forms holdfasts (Smith, 1950), so many of the specimens identified of this alga could have been fragments floating near or caught on the substrate instead of growing on the substrate. The growth habit of *C. scutata* is a flat discoid thallus that is

prostrate on the substrate (Smith, 1950). *Cladophora glomerata* is a cosmopolitan species and attaches to a substrate by a holdfast (Smith, 1950). It would make sense that algae that attach to a substrate by a holdfast (*C. glomerata*, *R. hieroglyphicum*) or by forming mats (*Lyngbya* spp., *Symploca* sp.), would grow on substrates with a rougher texture, which provides the algae with a suitable surface on which to adhere. Whereas algae that grow prostrate (*C. scutata*) would grow on a substrate that was flat or smooth, which allows for easier growth outward. These results support the prediction of Proctor (1958). He found that algae typically associated with turtles could be grown in absence of turtle carapace, suggesting that physical, not chemical, factors influenced growth. Neither species of *Basycladia* were found on any of the substrates in this experiment.

### **Algal Attachment**

The species of *Basycladia* examined in this study attach to a substrate using a holdfast, similarly to the other species in the family Cladophoraceae (Smith, 1950). However, Vinyard (1953) found that one species of *Cladophora* growing on a fish penetrated into the fish's bone. This study documented through scanning electron micrographs the attachment of *B. chelonum* and *B. crassa* to turtle carapace. These species do indeed attach by a holdfast of rhizoid filament that grow prostrate across the turtle scute. This finding supports Hoffmann and Tilden's (1930) description of *Basycladia*'s holdfast. Its holdfast does not penetrate into the turtle scute, but grows across the surface. Proctor (1958) found that *Basycladia* filaments were mainly restricted to the outer surface of the carapace, but he did find *Basycladia* cells between the loose layers of turtle laminae. As I had suspected *B. chelonum* and *B. crassa* show the same attachment on turtle carapace as

*B. ramulosa* described and illustrated by Ducker (1958). Therefore, when the turtle sheds its scutes, it also sheds the algae growing on those scutes. Based on Proctor's (1958) findings, *Basicladia* begins to colonize the new scutes before the old ones are shed.

### **Interspecific Interactions**

Interspecific interactions are relationships between different species of a community (Campbell and Reece, 2002). Examples of interspecific interactions are commensalism, mutualism, parasitism, and competition. Such interactions are often considered the result of coevolution. Coevolution refers to a change in one species acting as a selective force on another species; counteradaptation in turn acts as a selective force on the first species (Campbell and Reece, 2002). The results of this study can be used to better understand the relationship among algae and freshwater turtles. Previous researchers considered the relationship one of commensalism (Harper, 1950; Edgren et al., 1953; Dixon, 1960), whereby turtles benefit in gaining camouflage from prey, and the algae are unaffected (Neill and Allen, 1954). There is evidence, however, that the relationship can be considered mutualistic due to the patterns of occurrence of some algae (i.e., the presence/absence of *Basicladia* on certain turtles and substrates). *Basicladia chelonum* and *B. crassa* were frequently collected from *C. s. serpentina*, *S. odoratus*, and *T. s. scripta*. *Basicladia* was found to be a dominant colonizer of *T. s. scripta*. However, neither species of *Basicladia* was found growing *T. s. scripta* scutes in the substrate experiment. This suggests that elements of the actual living turtle and not just the turtle scute are influencing the colonization of *Basicladia*, and that *Basicladia* benefits in some way from this interaction with the turtle. Now that patterns in algal composition and

substrate colonization have been elucidated, detailed field and laboratory experiments should be conducted to determine the mechanism that regulates the observed patterns.



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