P.S.Z.N. I: Marine Ecology, 7 (4): 333–343 (1986)
Accepted: July 3, 1986

ISSN 0173-9565

On the Occurrence of Marine Fungi in the Diet of *Littorina angulifera* and Observations on the Behavior of the Periwinkle*

J. KOHLMEYER & B. BEBOUT

Institute of Marine Sciences, University of North Carolina at Chapel Hill;
3407 Arendell St., Morehead City, N. C. 28557, U. S. A.

With 16 figures

Key words: Marine fungi, *Littorina angulifera, Rhizophora mangle,* mangrove, behavior, feeding, dormancy.

Abstract. The feeding and resting patterns of *Littorina angulifera,* the southern periwinkle, were observed in mangrove habitats of Belize (Central America). The snails feed predominantly on the surface of prop roots of *Rhizophora mangle* in a narrow zone at and above the mean high water mark. This area contains large numbers of hyphae and chlamydospores of an unidentified marine fungus *(Deuteromycetes)* and filaments of a chlorophyte (*Chlorochytrium* sp.). Both organisms are ingested by snails whose digestive tracts and fecal pellets contain ground-up cork cells, tricho-sclereids, tracheids, calcium oxalate crystals, fungal hyphae and chlamydospores, as well as undigested cyanobacteria. Most fungal particles pass through the gut unchanged. During dry periods, *L. angulifera* is in a dormant state, usually attached with dried mucus to leaves high in the tree, causing necrotic, crescent-shaped marks. The leaf tissue under the area of shell attachment becomes meristematic, separating dead tissues from healthy mesophyll. The snails detach during rainfall and move downward to the feeding sites on the prop roots.

Problem

Although *Littorina angulifera* (LAMARCK) is a common species in the mangal on the North and South American East Coast, from Florida to Brazil (ABBOTT, 1974), very little is known about the activities of this species. Publications on *L. angulifera* are primarily concerned with the morphology, anatomy, sex ratio, breeding habits, spawning, or taxonomy (*e. g.,* LENDERKING, 1951, 1954; BANDEL, 1974). To our knowledge, the only reference to the feeding habits of *L. angulifera* was made by PLAZIAT (1984) who stated: "The species probably

* Contribution No. 202, Caribbean Coral Reef Ecosystem Program, Reef and Mangrove Study-Belize, Smithsonian Institution; partly supported by the Exxon Corporation.

feeds on lichens and fungi of the wet, splash- and rain-wetted level", without providing any evidence for this assumption. Observations of, and experiments performed on a related species, *L. irrorata* SAY have shown that fungi are regularly ingested and assimilated by this snail (BEBOUT, 1986). Fecal pellets produced by *L. angulifera* collected on *Rhizophora mangle* L. on various islands near Carrie Bow Cay, Belize contained fungal propagules over 80 % of the time. This paper presents observations on the occurrence of fungi in the diet of *L. angulifera,* on the source of the fungi, on the feeding behavior and dormancy of the snails, and on the reaction of the leaves of *R. mangle* to the adhesion of the resting periwinkles.

Material and Methods

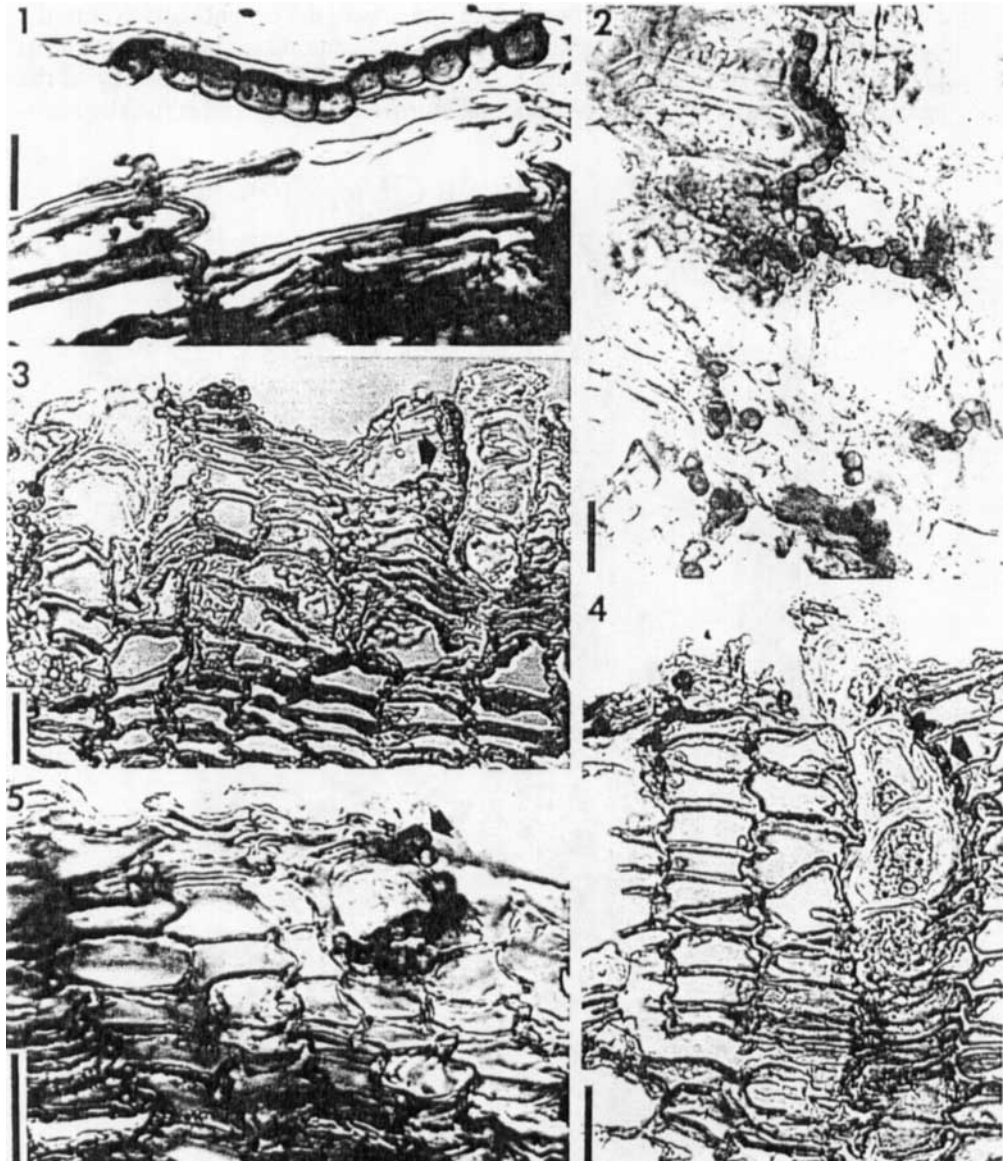
The populations of *Littorina angulifera* described herein are located on mangrove-covered islands in Belize, viz., Man-of-War Cay (16°53'N, 88°06'15"W), Tobacco Range (16°52'45"N, 88°05'30"W), and Twin Cays (16°50'N, 88°06'W). General informations (climatology, geomorphology, *etc.*) on the barrier reef complex and its vicinity are included in RÜTZLER & MACINTYRE (1982). An unidentified hyphomycete associated with roots and stems of *Rhizophora mangle* was collected on the same islands and on Torch Key, Florida (24°41'N, 81°25'W). Voucher specimens of the fungus are deposited in the herbarium of the Institute of Marine Sciences (IMS): Torch Key (20 Nov. 1965, J. K. 2404), Twin Cays (30 Mar. and 1 April 1985, J. K. 4703, 4706, 4707). Specimens of *L. angulifera* were collected on Twin Cays on 3 Apr. 1985 (J. K. 4773, 4774), preserved in 5 % formalin-seawater, and dissected to check for the presence of fungi in the digestive tract. Individuals of *L. angulifera* were collected randomly on the islands and kept in closed jars in the laboratory. Subsequently they produced over 300 fecal pellets that were examined under a compound microscope (400 X). A pure culture of the fungus was obtained by streaking out chlamydospores from bark of *R. mangle* (collection J. K. 4703) on the surface of antibiotic seawater agar (KOHLMEYER & KOHLMEYER, 1979). Sections of bark, intact and damaged leaves of *R. mangle* were made on an I. E. C. International cryostat. To observe the behavior of *L. angulifera,* we marked attached periwinkles with paint and returned at night to check if they had moved and were possibly feeding on roots at the high waterline.

Results

1. Fungi and algae from intertidal parts of *Rhizophora*

After discovering fungal propagules regularly in the feces of *Littorina angulifera,* we searched on roots and branches of *Rhizophora mangle* for the actively growing stages of the fungus. We observed that the fungus, an undescribed hyphomycete, grows on the surface of prop roots and stems of *R. mangle,* in a relatively narrow zone just above the mean high water level. In contrast to the normal brownish tissue, the outermost bark cells occupied by the fungus are hyaline and partly collapsed. Air in the lumina of the outer cells and the hyaline color of their walls give air-dried roots a whitish appearance. This whitish zone of 15–20 cm length begins just above thick growths of cyanobacteria or other fouling organisms and is usually not covered by water for an extended period of time. Thus far, we have found the inconspicuous fungus on *R. mangle* in Belize and on the Florida Keys, but it is likely to occur throughout the Caribbean and, possibly, throughout the range of its host.

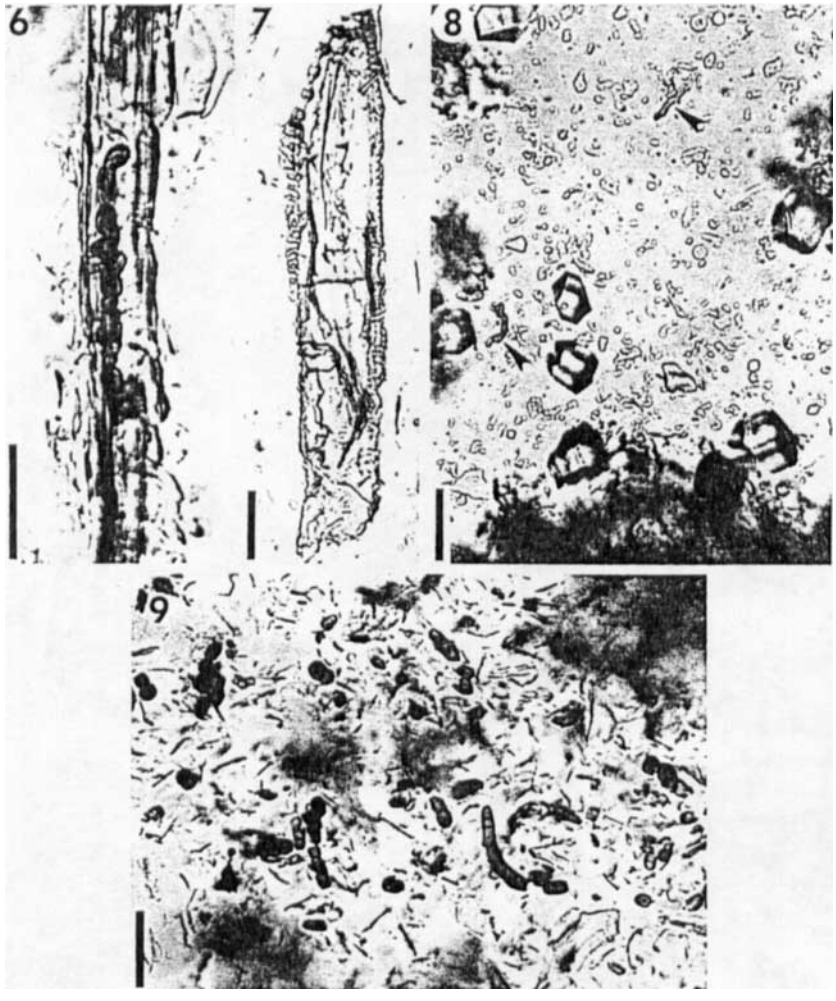
Hyphae and chlamydospores of the hyphomycete can be observed in tangential and cross sections through the outer layers of *R. mangle* bark in the whitish zone of prop roots (Figs. 1 and 2). The brown chlamydospores are usually formed in chains and measure 3.5 to 7.5 µm in diameter. In pure culture, hyphae



Figs. 1–5. Cork cells of *Rhizophora mangle* prop roots with unidentified marine fungus, Belize. Fig. 1. Cross-section through bark with chain of chlamydospores in outer cells; scale bar: 10 µm. Fig. 2. Tangential section of the bark with chlamydospores; scale bar: 15 µm. Figs.3–5. Filamentous chlorophyte (*Chlorochytrium* sp.) with closely attached fungal chlamydospores (arrows) in cross-section of the bark; scale bars: 25 µm. All in brightfield, from J. K. No. 4703 and 4707.

are 3–4 μm and chlamydospores 5.5–11 μm in diameter. Chains of chlamydospores are strongly constricted and may break up into single cells or groups of two or more cells. Occasionally, a longitudinal septum may occur also in chlamydospores grown in pure culture.

The whitish zone on roots of *Rhizophora* harboring the hyphomycete regularly contains filaments of a green alga. This chlorophyte grows between the outer cells of the bark and also penetrates like a wedge, perpendicularly into the bark tissue (Figs. 3 and 4). The diameter of the filaments is about 18 μm. Most probably, the alga is the alternate sporophytic stage in the life history of the genus *Spongomorpha (Acrosiphoniaceae, Acrosiphoniales)* and is usually refer-



Figs. 6–9. Unidentified marine fungus and root components of *Rhizophora mangle* from intestinal tract and feces of *Littorina angulifera,* Belize. Fig. 6. Chlamydospores in trichosclereid; scale bar: 25 μm. Fig. 7. Tracheid with hyphae; scale bar: 50 μm. Fig. 8. Calcium oxalate crystals, hyphal fragments (arrowheads), and cell remains in feces; scale bar: 25 μm. Fig. 9. Chlamydospores and fragments of *R. mangle* from feces; scale bar: 25 μm. All in brightfield; from J. K. No. 4773.

red to as *Chlorochytrium* sp. (HOMMERSAND & FREDERICQ, personal communication). The hyphomycete and the alga are often found in close association. Whereas the fungus normally occurs in the outer one to three cells of the bark, it penetrates deeper into the tissue when growing alongside the algal filaments (Figs. 3 and 4). Often, chains of chlamydospores are found coiled around the algal cells (Fig. 5).

2. Contents of digestive tract and feces of *Littorina angulifera*

Four main types of food materials are found in the gut, viz., cork cells, cellulose fibers, fungal propagules, and algae. The contents of the digestive tract of *L. angulifera* collected on different trees and locations vary only in the proportions of these components, depending on the substrate the snails had been feeding upon. The bulk of the contents consists of ground up cork cells of the periderm, but trichosclereids, long H-shaped sclerenchymatic fibers (GILL & TOMLINSON, 1971, 1977), occur occasionally. In some collections, the contents are comprised exclusively of trichosclereids, indicating that the periwinkles had been grazing on cortical tissues below the periderm. Because trichosclereids from the intestine of *L. angulifera* are corroded and covered with chlamydospores or hyphae, and often contain fungal elements in their lumina (Fig. 6), it can be assumed that the cork layer had been damaged, exposing the cortex for some time before the snails ingested the fibers. Other elements of the host plant found in the gut of *L. angulifera* are tracheids, also filled with fungal hyphae or chlamydospores (Fig. 7). Intestinal contents composed mainly of trichosclereids contain large numbers of calcium oxalate crystals as well (Fig. 8). These crystals pass unchanged through the digestive tract of the snail (Fig. 8). They are mostly rhomb-shaped or in form of double crystals and are soluble in concentrated HCl.

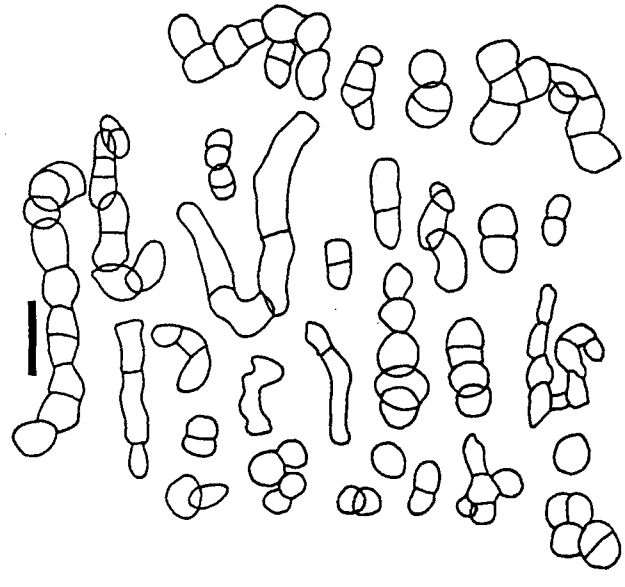
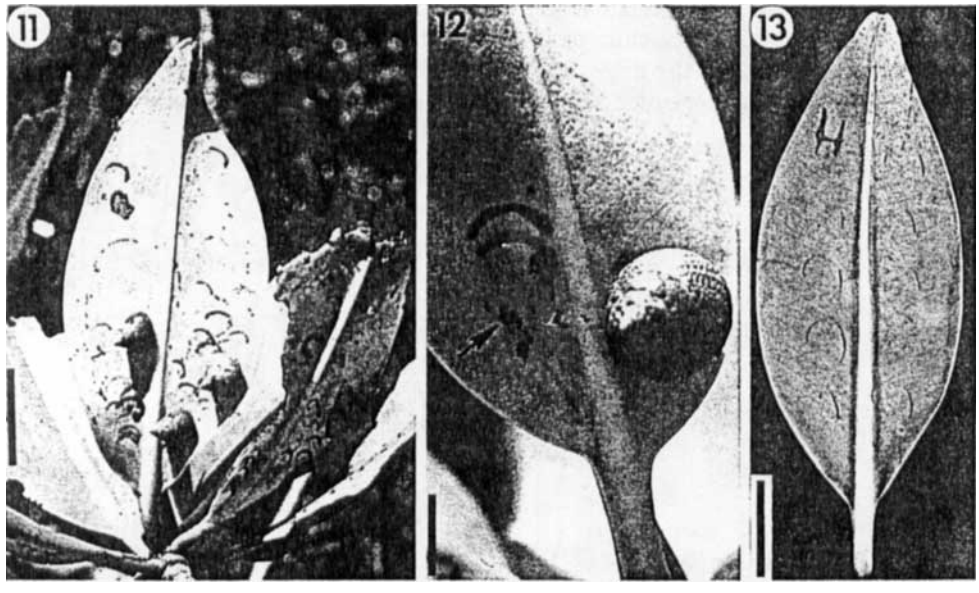


Fig. 10. Chlamydospores and hyphal fragments of an unidentified marine fungus from feces of *Littorina angulifera*, Belize; scale bar: 10 µm.

During passage through the digestive tract the ingested food changes appearance. Near the radula there are long trichosclereids (up to 330 μm), single cork cells, but also larger cell complexes (up to 240 μm in diameter), some single-celled or filamentous, thick-walled cyanobacteria (up to 280 μm), and many hyphal fragments and chlamydospores of the hyphomycete. Near the anus the contents are formed into ellipsoidal pellets that contain primarily fragmented *R. mangle* bark and wood fibers, and, in 80% of over 300 fecal pellets examined, chlamydospores and hyphal fragments of the hyphomycete (Figs. 9 and 10). Some fecal pellets also contained small amounts of filamentous and unicellular green algae and cyanobacteria, which pass through the digestive tract unchanged. Snails were collected on *Rhizophora mangle* trees near the waterline on prop roots, branches, leaves (Figs. 11 and 12), and from high on the trunk of the tree. Fecal pellets produced by these snails, however, contained the same materials, suggesting that the animals were feeding, to a large extent, in the same lower areas of the trees.

3. Activity and dormancy of *Littorina angulifera*

Feeding seems to occur only during wet periods, whereas the snails enter a resting stage when trunks and branches of the trees are dry. In March 1985, during five days without precipitation, the majority of the snails were found firmly attached to leaves (Figs. 11 and 12) and branches of *R. mangle,* in some cases more than three meters above the high tide level. In the resting stage, the animals are retracted into the shell, with tightly closed operculum, and the outer

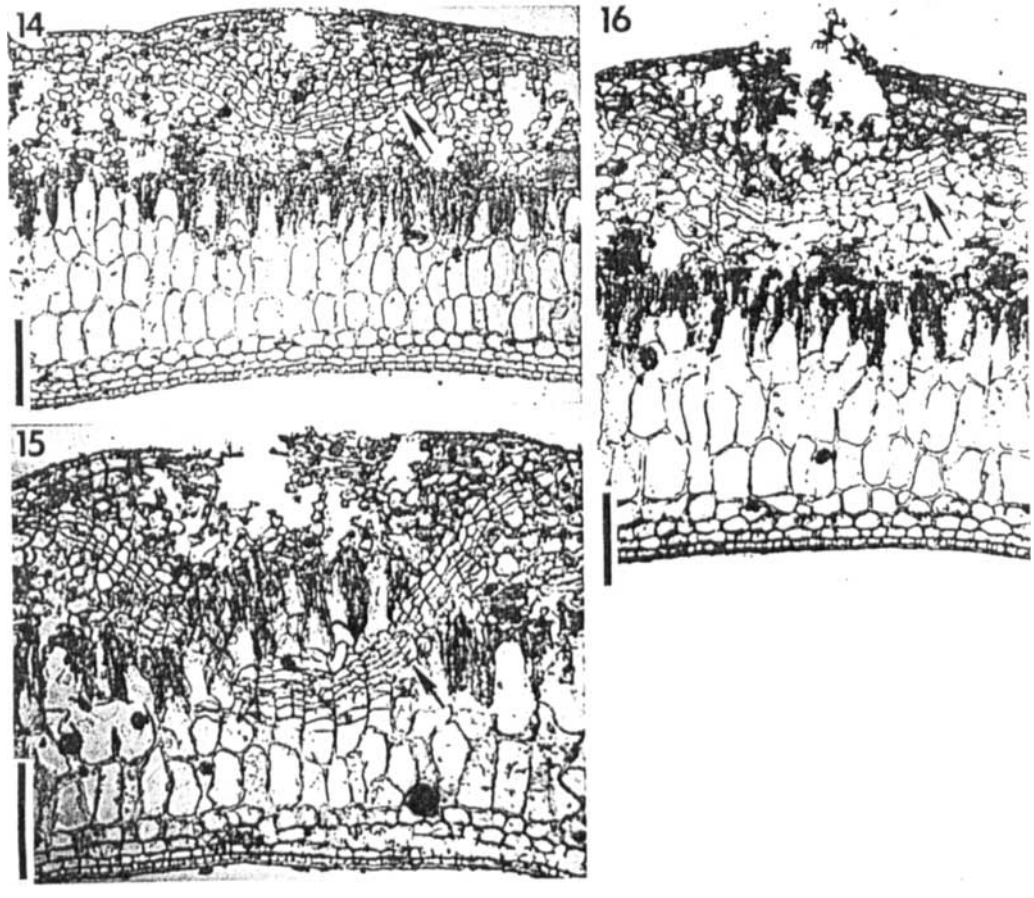


Figs. 11–13. Leaves of *Rhizophora mangle,* Belize. Fig. 11. Dormant *Littorina angulifera* attached to the lower side of a leaf; crescent-shaped marks show the sites of former attachment; scale bar: 50 mm. Fig. 12. Dormant *L. angulifera,* necrotic marks and fecal pellets (arrow); scale bar: 10 mm. Fig. 13. Experimentally produced necrotic marks on a leaf; scale bar: 50 mm.

lip of the shell is glued to the substrate by dried mucus. This attachment can be so strong that part of the shell may break off when the snail is pried loose. The snails remained in the same locations on leaves during this period of dryness. After the first rainfall, however, all snails became detached, moved downwards to the water level and started feeding. Water makes the dried mucus soft and gelatinous and permits *L. angulifera* to leave their resting places. Dormant snails also become active when they are sprayed with distilled water or seawater.

4. Reaction of *Rhizophora* leaves to the attachment of *Littorina*

Dormant snails attached by dried mucus to leaves of *R. mangle* cause a necrotic crescent-shaped mark on the surface of the substrate (Figs. 11 and 12). In most cases, *L. angulifera* are affixed to the lower sides of leaves. Cross-sections through the marks and underlying tissues show that the lower epidermis and one



Figs. 14–16. Cross-sections through leaves of *Rhizophora mangle* with necrotic marks caused by the attachment of dormant *Littorina angulifera;* meristematic cells (arrows) separate dead (above) and healthy (below) tissues. All in brightfield; scale bars: 200 µm.

or more cell layers of the adjoining mesophyll have turned brown, and new meristematic divisions cause a separation of the necrotic tissues from the healthy mesophyll (Figs. 14–16). Due to this initiation of meristematic activity, forming 5 to 6 layers of flattened cells (Figs. 14–16), the brown mark is raised slightly above the leaf surface (Fig. 14). When snails adhere near a vascular bundle, palisade, mesophyll cells and the entire bundle are cut off from the healthy part of the leaf by meristematic cells (Fig. 15). Recently attached snails that are sprayed with water move away and leave a crescent-shaped wall of mucus behind, having exactly the shape and size of the brown scars. Attempts to reproduce these characteristic marks by pressing outer lips of *L. angulifera* against leaves caused crescent-shaped brown lines (Fig. 13). Sections showed that the outer cell layers had been damaged, but no meristematic growth was initiated in the underlying tissues. Snail mucus applied to leaves and permitted to dry had no effect at all.

Discussion

A name cannot be assigned to the polymorphic hyphomycete from *Rhizophora* roots at the present time because similar mycelia with chlamydospores are formed by a number of marine ascomycetes (KOHLMEYER & KOHLMEYER, 1979). It is possible that it belongs to one of the fungi known to occur on *Rhizophora mangle* bark, *e.g.*, the ascomycete *Keissleriella blepharospora* KOHLM. & KOHLM. which occurs in the same area of the roots as the hyphomycete in collection J. K. 4703. The habit of the hyphomycete reminds one of members of the genus *Scytalidium* PESANTE (ELLIS, 1976).

The importance of microscopic fungi in the nutrition of freshwater animals is well known (BÄRLOCHER & KENDRICK, 1973; ROSSI & FANO, 1979; SUPERKROPP & ARSUFFI, 1984) whereas examples from marine habitats include only a few invertebrates that have fungi in their diet, namely *Limnoria tripunctata* MENZIES (KOHLMEYER *et al.*, 1959), *Orchestia grillus* Bosc. (BOYD, 1981), and *Littorina irrorata* (BEBOUT, 1986), or need them for settlement (*Teredo pedicellata* QUTRF., KAMPF *et al.*, 1959). Our investigations on *L. angulifera* have demonstrated that fungi are regularly ingested by the snail. As in *Limnoria, Orchestia* and *Littorina irrorata,* the fungal chitin appears not to be digested by *Littorina angulifera* because chlamydospores and hyphal fragments pass through the digestive tract unchanged. Most likely, only the contents of those fungal cells that are damaged during the rasping process are available as nutrients to the snail. Since a related species, *L. irrorata,* assimilates fungal material (BEBOUT, 1986), the same can be expected for *L. angulifera.* The fungus may even be involved in the breakdown of cellulose by producing cellulases in the digestive tract. The hyphomycete must be able to attack the trichosclereids, because fibers with hyphae and chlamydospores (Fig. 6) already show corrosion in the form of "soft rot" upon ingestion. During the passage through the gut the plant cells are partly digested, and the fungal propagules appear more concentrated in the feces than in the buccal part of the gut. The long chains of chlamydospores and hyphae are broken down into smaller portions, each of which is able to propagate the fungus (Figs. 9 and 10).

The wide filaments of *Chlorochytrium* that grow together with the hyphomycete in the same area of the *Rhizophora* roots are usually broken up by the snail's radula, and intact cells appear rarely in the feces. Therefore, most of the ingested chlorophyte material – cell walls and contents – may be digested by *L. angulifera*. In contrast, cyanobacteria, protected by thick sheaths, pass through the intestine intact, except for damaged cells that may become digested.

The intimate association between fungus and *Chlorochytrium* sp. could indicate a symbiotic relationship, similar to primitive lichenizations known from other marine habitats (KOHLMEYER & KOHLMEYER, 1979).

The crystals found in digestive tracts and feces of *L. angulifera* (Fig. 8) are known to occur in sclerenchymatous cells of *R. mangle* (KARSTEDT, 1971; KARSTEDT & PARAMESWARAN, 1976). Root tissues of *R. mangle* also contain druse-shaped oxalate crystals (GILL & TOMLINSON, 1971; KARSTEDT, 1971; KARSTEDT & PARAMESWARAN, 1976), but these are rarely found in the gut or feces of *L. angulifera*.

Our observations in the mangal of Belize suggest that *L. angulifera* is feeding and resting exclusively on *Rhizophora mangle*, even when other mangroves [*Avicennia germinans* (L.) L.] are growing next to it. It seems likely that the inactive state, sometimes assumed high in the trees, is a moisture conserving and predator avoidance behavior, and that snails feed most actively after rainfall or tidal wetting of the roots. Individual snails appear to return repeatedly to the same leaf for attachment and survival of dry periods, because numerous crescent-shaped marks, mostly corresponding in size to an attached shell, can be observed on a single leaf (Fig. 11). Most likely, several days of firm attachment of the shell are necessary to form a mark. Constant pressure of the shell, caused by the drying mucus probably prevents normal functioning of the outer cell layers that subsequently die.

The necrotic scars on *R. mangle* leaves caused by resting *L. angulifera* can be compared to cork warts formed on leaves of many mangroves (CHAPMAN, 1976). A cork cambium is formed on these leaves, the external tissue dies and is sloughed off (Fig. 16). We observed similar crescent-shaped marks on leaves of *Conocarpus erecta* L. on Stewart Island in Belize, but found these to be caused by leaf-mining insects.

Summary

Littorina angulifera is a frequent inhabitant in the mangal of the Western Atlantic Ocean, where it is found primarily on red mangrove *(Rhizophora mangle)*. Investigations in Belize showed that the snails feed mainly on prop roots of the upper intertidal zone, ingesting propagules and hyphae of an unidentified imperfect fungus and strands of the chlorophyte *Chlorochytrium* sp. Both of the microorganisms are restricted to this area, growing between and within the outer cork cells of *R. mangle*, where they are consumed by snails that rasp along the surface. Because most chlamydospores and hyphal fragments pass through the gut unchanged, probably only the contents of damaged fungal cells are digested by *L. angulifera*. Ingested cells of the mangrove are partly corroded enzymatically, whereas calcium oxalate crystals, one-celled and filamentous cyanobacteria are excreted unchanged with the feces.

Observations of marked *L. angulifera* showed them to be dormant for several days and possibly weeks during dry seasons, mostly attached to leaves up to 2 m above the waterline. The outer lip of the shell is glued to the leaf by dried mucus, causing a necrotic crescent-shaped mark. Leaf cells of epidermis and mesophyll under the attached shell die and are separated from healthy tissues by new meristematic growth. The dormant state of the snail is terminated by rain or experimental spraying with water, whereupon the animals travel along the prop roots to the water line for feeding. The attachment and dormancy in the tree canopy benefits the snails in conserving moisture and possibly avoiding marine predators.

Acknowledgements

Supported in part by National Science Foundation Grant No. BSR-8407155 to J. K. We are grateful to K. RÜTZLER for making our work on Carrie Bow possible, and to colleagues and staff for their support and friendship during our stay on this island. Thanks are due to E. A. CHORNESKY for supplying leaves with snail marks, and to M. H. HOMMERSAND and S. FREDERICQ for informations on the chlorophyte from *Rhizophora* roots. W. R. BURK kindly carried out a literature search on *Littorina angulifera,* K. C. SALESSES assisted in the laboratory, and H. E. PAGE helped with photographic work. We thank B. KOHLMEYER for reading the manuscript and for valuable suggestions.

References

ABBOTT, R. T., 1974: American Seashells. Van Nostrand Reinhold, New York; 633 pp.

BÄRLOCHER, F. & B. KENDRICK, 1973: Fungi in the diet of *Gammarus pseudolimnaeus (Amphipoda).* Oikos, **24:** 55–59.

BANDEL, K., 1974: Studies on *Littorinidae* from the Atlantic. Veliger, **17:** 92–114.

BEBOUT, B., 1986: The role of marine fungi in the nutrition of the salt marsh periwinkle, *Littorina irrorata.* Thesis, University of North Carolina, Chapel Hill.

BOYD, P. E., 1981: Ecology of three arenicolous marine fungi: I. Their role in the diet of the beachhopper *Orchestia grillus* and II. The influence of temperature on the seasonal and geographic distribution of *Asteromyces cruciatus, Sigmoidea marina* and *Varicosporina ramulosa.* Thesis, University of North Carolina, Chapel Hill; 59 pp.

CHAPMAN, V. J., 1976: Mangrove vegetation. J. Cramer, Vaduz; 447 pp.

ELLIS, M. B., 1976: More dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew; 507 pp.

GILL, A. M. & P. B. TOMLINSON, 1971: Studies on the growth of red mangrove (*Rhizophora mangle* L.) 2. Growth and differentiation of aerial roots. Biotropica, **3:** 63–77.

– – & P. B. TOMLINSON, 1977: Studies on the growth of red mangrove (*Rhizophora mangle* L.) 4. The adult root system. Biotropica, **9:** 145–155.

KAMPF, W.-D., G. BECKER & J. KOHLMEYER, 1959: Versuche über das Auffinden und den Befall von Holz durch Larven der Bohrmuschel *Teredo pedicellata* QUTRF. Z. Angew. Zool., **46:** 257–283.

KARSTEDT, P., 1971: Untersuchungen über Eigenschaften der Sproßachse von atlantischen Mangrovenarten mit Berücksichtigung ihrer Verwendung. Dissertation, Hamburg; 234 pp.

– – & N. PARAMESWARAN, 1976: Beitrag zur Anatomie und Systematik der atlantischen *Rhizophora*-Arten. Bot. Jahrb. Syst., **97:** 317–338.

KOHLMEYER, J., G. BECKER & W.-D. KAMPF, 1959: Versuche zur Kenntnis der Ernährung der Holzbohrassel *Limnoria tripunctata* und ihre Beziehung zu holzzerstörenden Pilzen. Z. Angew. Zool., **46:** 457–489.

– – & E. KOHLMEYER, 1979: Marine mycology: The higher fungi. Academic Press, New York; 690 pp.

LENDERKING, R. E., 1951: Observations on *Littorina angulifera* LAM. from Biscayne Key, Florida. Q. J. Fla Acad. Sci., **14:** 247–250.

– –, 1954: Some recent observations on the biology of *Littorina angulifera* LAM. of Biscayne and Virginia Keys, Florida. Bull. Mar. Sci. Gulf Caribb., **3:** 273–295.

PLAZIAT, J. C., 1984: Mollusk distribution in the mangal. In: F. D. POR & I. DOR (Eds.), Hydrobiology of the mangal. Dr. W. Junk, The Hague: 111–143.

RÜTZLER, K. & I. A. MACINTYRE, Eds., 1982: The Atlantic barrier reef ecosystem at Carrie Bow Cay, Belize, 1: Structure and Communities. Smithson. Contrib. Mar. Sci., **12:** 1–539.

ROSSI, L. & A. E. FANO, 1979: Role of fungi in the trophic niche of the congeneric detritivores *Asellus aquaticus* and *A. coxalis (Isopoda)*. Oikos, **32:** 380–385.

SUBERKROPP, K. F. & T. L. ARSUFFI, 1984: Degradation, growth, and changes in the palatability of leaves colonized by aquatic hyphomycete species. Mycologia, **76:** 398–407.