Made in United States of America
Reprinted from FLORIDA SCIENTIST
Vol. 62, No. 2, Spring 1999

EVIDENCE OF CELLULOSE DIGESTION IN THE WOOD BORING ISOPOD SPHAEROMA TEREBRANS

LORI K. BENSON[1], STANLEY A. RICE[2] AND BRUCE R. JOHNSON[3]

[1]Museum of Natural Science, Louisiana State University, Baton Rouge, LA 70803
[2]Department of Biology, University of Tampa, Tampa, FL 33606
[3]Forest Products Laboratory, U.S. Dept. of Agriculture, Madison, WI

ABSTRACT: Sphaeroma terebrans *Bate is a widespread estuarine, wood-boring, isopod found in red mangrove* (Rhizophora mangle) *forests of tropical and subtropical estuarine waters. Because it causes extensive damage to man-made structures in marine coastal zones and is resistant to common methods of wood preservation it is important to understand fully how* S. terebrans *uses the wood into which it bores. Previous studies indicate that* S. terebrans *is primarily a filler-feeder and does not utilize bored wood as a nutritive substrate. However, laboratory feeding data obtained in this study indicate that juvenile* S. terebrans *specimens can survive on a diet of pure cellulose signifcantly longer than individuals given no food. In addition, enzyme assays show that digestive caeca homogenates from* S. terebrans *have active cellulose-digesting enzyines. Furthermore, SEM rind TEM studies of this organism indicate that cellulose-like material is present in the hindgut of these organisms. These data taken collectively indicate that S. terebrans has the ability to use the wood as food source.*

BEFORE the introduction of man-made wooden structures into the marine environment, mangroves and various types of driftwood were the only regularly available intertidal substrata for estuarine wood-boring organisms in Florida (Estevez, 1978). Wooden structures introduced into the marine environment in more recent times have been besieged by wood-borers. Many methods of wood preservation have been implemented to combat the destructive activities of marine boring organisms. In spite of attempts at controlling wood borers, damage estimates of $50 million each year have been attributed to borer activity along U.S. coasts (Menzies and Turner, 1957; Hochman, 1973). The most common marine wood borers are bivalve mollusks (shipworms and mussels) and crustaceans (amphipods and isopods). The importance of wood to the borers varies among species, since some use the burrows only for habitat and protection while others use the wood as a source of food. (For a review of the marine borer literature prior to 1958, see Clapp and Kenk, 1957.)

The focus of the present study is on *Sphaeroma terebrans* Bate, a widespread estuarine, wood-boring isopod found in tropical and subtropical habitats. It flourishes in estuarine mangrove forests, such as those of the Tampa Bay area (the study site for this report). In a state-wide survey of more than 100 sites along the Florida coast, Rice and co-workers (1990) found active wood borers or historical evidence of attack by *S. terebrans* at 56% of

freshwater sites and 33% of marine sites. Under natural conditions, *S. terebrans* may inhabit burrows in cypress, cedar, palm, and pine. It has been reported to attack *Juncus roemerianus* (black needle rush) and *Acrostichum aureum* (leatherfern) under estuarine and brackish conditions (Rice et al., 1990). *S. terebrans* is particularly common in the intertidal prop roots of *Rhizophora mangle* (red mangrove) under low salinity conditions. They can withstand several hours of exposure to air during low tide and typically will retreat into their burrows and remain inactive during these periods (Estevez, 1978). According to Siniberloff and co-workers (1978), *S. terebrans* may indirectly aid the mangroves into which they burrow by helping them to survive wave action, as burrowing activity appears to initiate additional branching of aerial prop roots. Unfortunately *S. terebrans,* and the isopod *Limnoria tripunctata* (Menzies) also tend to bore into piers and pilings causing widespread damage in man-made structures of marine coastal zones (Boyle and Mitchell, 1978).

Wood-boring animals may use the wood they bore into in a variety of ways. For an animal to gain nutrition from the wood itself, it must be able to digest cellulose by using either symbiotic microorganisms (such as intestinal bacteria or protozoans) or endogenous cellulases. Endogenous cellulase activity for metabolic purposes is widespread in marine invertebrates (Elyakova, 1972; Foulds and Mann, 1978) including the obligate wood-boring teredinid ship worms *Bankia gorildii* (Dean, 1978) and *Teredo bartschi* (Turner, 1966), the wood-boring isopods of the genus *Limnoria* (Ray and Julian, 1952), and the omnivorous marine mysid *Mysis stenolepis* (Friesen et al., 1986).

Previous studies indicate that S. terebrans is primarily a filter-feeding isopod that does not require a nutritive wood substrate (Estevez and Simon, 1975; Rotramel, 1975). In addition, evidence indicates that the guts of *S. terebrans* and the obligate wood borers *Limnoria* spp. have no microorganisms that would assist them in the digestion of wood (Boyle and Mitchell, 1978). According to Estevez (1978), gut cellulases that are present in *S. terebrans* are thought to serve only for facultative activity, and even its anatomy suggests a filter-feeding lifestyle. Research on the digestive processes of this organism will allow an understanding of food requirements and feeding behavior of individuals and populations (Brunet et al., 1994). Furthermore, the question of whether or not *S. terebrans* uses its wood substrate as food is very important, as it is relevant to the development of better methods for wood preservation. Common methods of treating immersed wooden structures have proven ineffective against the boring activities of *S. terebrans,* and it is imperative to develop better wood treatment methods to reduce degradation of wooden piers and pilings.

Wood is a complex and variable substance depending upon its species of origin and environmental history (Parham and Gray, 1984). Natural wood is a complex material consisting of cellulose, hemicellulose, pentosans and lignin. The chemical composition and proportions of these substances varies

however, between tree species and even within a given tree (Pettersen, 1984). Cellulose, the most abundant component of wood, is a polymer of b(1-4)-bonded anhydroglucose units arranged in strong, coiled microfibrils that provide support to plant cell walls (Pettersen, 1984). Few organisms have the enzymes necessary to digest cellulose since the b(1–4) bonds are not affected by most carbohydrate-digesting enzymes. Many marine borers use their mouth parts or other body parts to grind the wood particles to a digestible size and then use enzymes in their gut to digest the exposed polysaccharides (Kirk and Cowling, 1984). The enzymes necessary to break the strong beta linkages between the glucose units in cellulose are called cellulases (Lehninger et al., 1993). These are the enzymes of interest in this research.

To observe cellulolytic activity for wood digestion, we compared S. terebrans tissue extracts to commercial preparations of cellulolytic enzymes from fungi and to homogenates of *Limnoria tripunctata* for their relative activity on two substrates. Sigma Cell microcrystalline cellulose and carboxymethyl cellulose were used in cellulase assays and are both documented in previous studies (Dean, 1978; Elyakova, 1972) as suitable substrates for measuring cellulase activity. In addition to the enzyme assays, feeding studies were performed to determine if *S. terebrans* juveniles could survive on cellulose particles alone. Finally, a scanning electron microscope was used to examine isopods mouth parts, and a transmission electron microscope was used to detect cellulose-like particles in the digestive tract of these organisms.

MATERIALS AND METHODS–*specimen collection* — Specimens of *Sphaeroma terebrans* were obtained from prop roots of a natural stand of *Rhizophora mangle* lining the North side of the Gandy Causeway in Old Tampa Bay, Florida. The prop roots were split with scissors and animals were gently lifted out with forceps. Adult animals, including brooding females, were cultured in the laboratory to provide newly released juveniles for the feeding studies. The isopods were placed in polypropylene containers (35mm diam.) with Nytex screen sides (0.10-0.25 mm mesh) to allow water flow. These containers were placed on plastic grids in 40 1 aquaria that contained recirculating filtered natural seawater with a salinity ranging from 22 to 25 ppt, comparable to the salinity at the Gandy collection site. The isopods held in these aquaria were fed about every 3 d with a mixture of cultured *Tetraselmis* (green alga), organic matter from naturally occurring sediment, and Roti-rich food supplement (Florida Aqua Farms. Inc.). This food mixture 15 referred to as "regular food" below.

Feeding and growth experiments – Animals for the feeding experiments were newly released juveniles collected from adult culture containers. Two separate feeding experiments were performed. In the first experiment, which lasted for 10 wk, the juveniles were raised in six-well tissue culture dishes (one animal per well) with water changes every other day. The first six–well container held isopods that were given no food: these were the starvation control animals. The second six-well dish contained individuals that were fed a mixture of food identical to that given to the adults in the laboratory cultures (regular food). The third six-well container held individuals that were fed a 5% solution (w/v) of Sigma Cell 20 (microcrystalline cellulose in 20 µm particles. Sigma Chemical Co.) in deionized water. The isopods were fed and monitored for mortality, fecal pellet production, and molts every other day

Because water quality was a problem in the small wells of the six-well containers, a second

feeding experiment was attempted in which five isopods per treatment were replaced in individual cylindrical polypropylene containers measuring 7 cm high by 4.5 cm in diameter with Nytex-screened windows. These cylindrical containers were, in turn, placed inside 8-1 battery jars that containing 1 1 of seawater. The seawater in the battery jars was monitored for temperature, which remained between 19.5° C and 21.5° C, and for salintiy, which ranged from 20 to 25 ppt. The water was aerated and changed every 6 to 8 d. One battery jar contained five individuals that were given no food. A second battery jar contained five animal that were given regular food. A third battery jar contained five individuals that were fed 5% solution of Sigma Cell 20 suspended in deionized water. The Sigma Cell 20 used in this trial was repeatedly rinsed with dilute hydrochloric acid to remove as much of the free glucose as possible and then frozen in 1.5 mL aliquots until needed. The isopods in the regular food and Sigma Cell 20 treatments were fed every 2 d, and all treatments were monitored tor feca1 pellets, molts, and mortalities. Each container was cleaned every two days to remove fecal pellet, molts, algae, dead animals and food build-up. This experiment lasted for 20 wk.

Cellulose enzyme assay – For the cellulase experiments, large adult animals were used to prepare tissue homogenates. Isopods were cold-narcotized and placed under a stereoscopic dissecting microscope. The head was held down, and the rest of the body was quickly pulled from the head with a pair of forceps. The digestive caeca were then removed and transferred to deionized water. These were homogenized in deionized water and, the supernatant obtained after centrifugation was used as the crude enzyme extract. In addition, standard cellulase enzyme (EC 3.2.1.4) from the fungus *Aspergillus niger* and homogenized *Limnoria tripunctata* (from whole animals) were prepared to run simultaneously for comparison in the cellulase activity assays.

Two substrates were exposed to the tissue homogenates and the standard cellulase: a 1% solution of carboxymethyl cellulose and a 1% solution of Sigma Cell 20. The cellulose substrates were repeatedly dialyzed and frozen prior to use to minimize the amount of glucose initially present. An assay for cellulase activity was performed using Nelson's method for reducing sugars (Nelson 1944, Alexander, et al., 1985) with a Spectronic 20-D spectrophotometer. A glucose control for all experiments was run to ensure that no reagents were contaminated with glucose. Any glucose detected in the controls was subtracted from the experimental values prior to data analysis. In addition, sodium azide was added to all tubes in each assay to inhibit the growth of bacteria. The spectrophotometric assay was performed on the tubes after incubations of 1–. 4-, and 8-hour time intervals. All absorbances from the spectrophotometric data were then converted to micromoles of sugar produced by using a glucose standard curve, according to Nelson (1944). The amount of sugar produced in each assay was converted to micromoles sugar per mg protein. Amounts of protein were determined following the Sigma procedure for protein determination (Sigma Chemical Co. Kit 690). This procedure involved mixing a protein sample with a diluted biuret reagent. followed with a Folin and Ciocalteu's Phenol Reagent. The protein concentration was then determined from a calibration curve formulated from spectrophotometric data obtained from a known protein (bovine serum albumin) dilution series.

Electrom micorscopy – Appendages, mouth parts, and digestive systems of *Sphaeroma terebrans* were dissected out and fixed in 2.5% glutaraldehyde for the scanning and transmission electron micropscopes. After being dehydrated and critical-point dried, the body parts were sputter coated with gold and mounted whole on a specimen holder for examination on a scanning electron microscope (SEM). Samples for transmission electron microscopy (TEM) were postfixed in 2% osmium tetroxide, dehydrated to acetone and embedded in epon using propylene oxide as the transition solvent. Sections were cut on a Reichert-Jung Ultracut E using a diamond knife. Thin sections were stained in uranyl acetate and lead citrate and examined on a Hitachi 500 transmission electron micoscope. Micorgraphs were examined for cellulose-like substances in the gut and caeca of thses organisms. *Limnoria tripunctata* was prepared in a similar manner and used as a control, as they are known to be digesters of cellulose.

RESULTS – *Feeding and growth experiments* – The initial feeding experiment resulted in the death of five of the six unfed individuals by the fourth week. After 6 wk. all of the unfed animals, along with one each of those fed regular food and Sigma Cell 30 were dead. At the end of 10 wk, five of the animals fed regular food and five fed Sigma Cell 20 were still alive (Fig. 1 A). Molting rates between the different treatments also varied. Figure 2A represents the mean number of molts per individual over the 10-week experiment. The control animals with no food produced no molts; the animals given regular food had an average of 2.5 molts per individual; and the animals fed Sigma Cell 20 had an average of 1.0 molts per individual.

In the second feeding experiment, using larger containers, all control organisms (no food) died between 3 and 6 wk after the experiment began. One of the Sigma Cell fed animals died after 45 d, and a second Sigma Cell treatment death occurred after 69 d. All individuals fed regular food were still alive after 20 wk (Fig. 1B). The mean number of molts per individual over the 30-week period is presented in Figure 2B. The control organisms with no food showed a mean of 0.2 molts per individual while the animals fed Sigma Cell 20 produced 3.4 molts per individual; and the animals that were fed regular food produced 4.8 molts per individual after 20 wk. The molting rates for the latter two treatments were significantly higher ($P = 0.001$, F-test) than the rate for the unfed animals in the 20-wk experiment. In a combined analysis of the 10-wk and 20-wk experiments, both the regular food and Sigma Cell-fed treatments had molting rates that were significantly higher ($P = 0.0001$, F-test) than the unfed treatment, but the former two treatments were not significantly different from each other ($P = 0.095$, F-test).

Cellulase enzyme assay – In these experiments, carboxymethyl cellulose (CMC) and Sigma Cell 20 substrates were exposed to a standard cellulase enzyme and to *Limnoria tripunctata* and *Sphaeroma terebrans* tissue homogenates. The cellulase control was included to demonstrate that the substrates were digestible and to provide a standard with which to compare the activities of the isopod cellulases.

The cellulase assays produced variable results for the *Sphaeroma terebrans* and *Limnoria tripunctata* tissue homogenates but more consistent results with the purified cellulase used as a standard. In addition, there was some free glucose present in the standard cellulase, tissue homogenates, Sigma Cell 20, and carboxymethyl cellulose substrates. Corrections were made in the experimental results to account for this extra glucose (see METHODS). The data presented below represent two separate experiments with two replicates of each control and experimental solution.

The cellulose substrates (Sigma Cell 20 and CMC) were progressively broken down by the *Aspergillus* cellulase (Fig. 3A) with the Sigma Cell 20 being more resistant to digestion than the CMC. Digestion of CMC by isopod cellulases (Fig. 3B) was rapid during the first 4 h of exposure but slowed

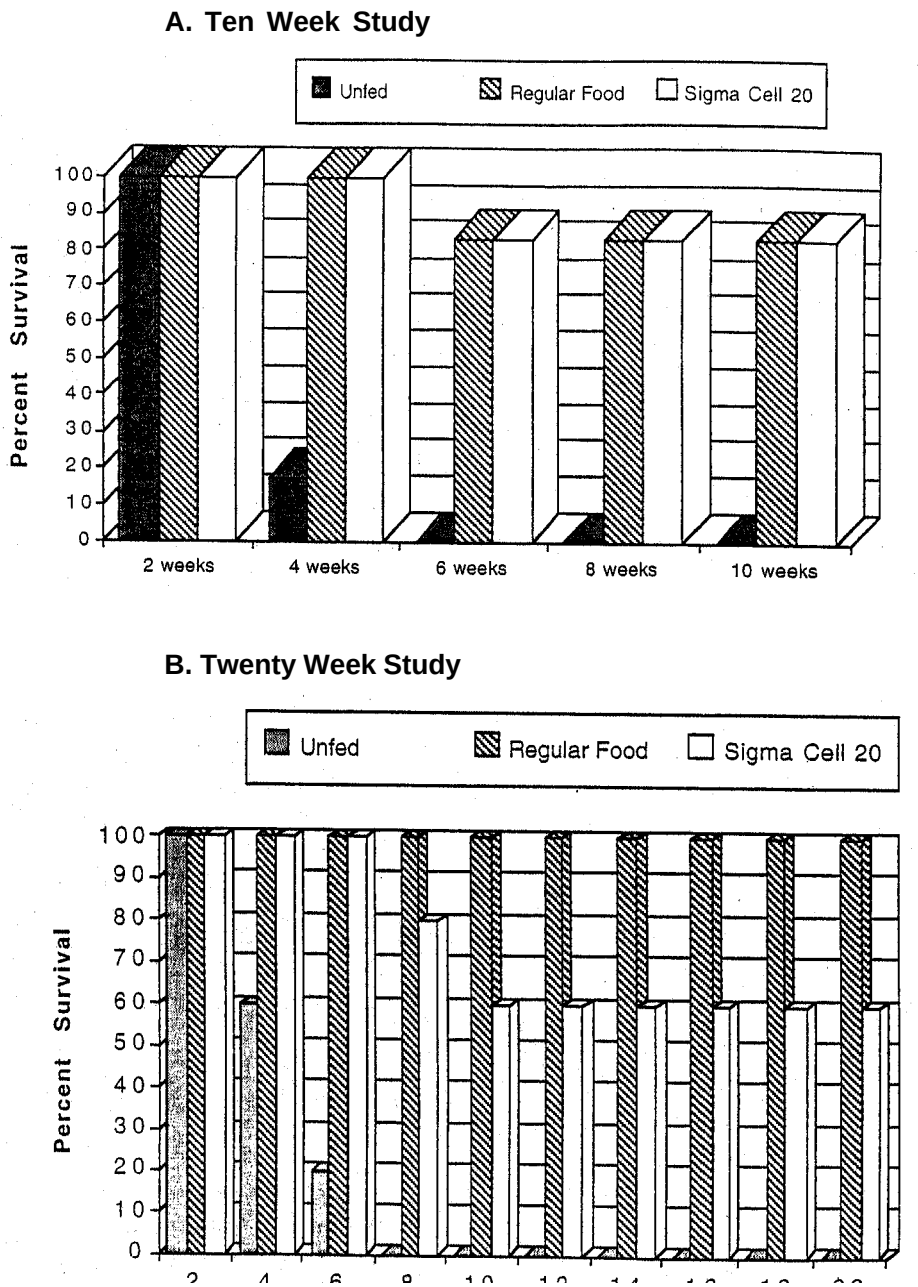

FIG. 1. Survival of *Sphaeroma terebrans* juveniles. A. 10-week feeding experiment (n = 6 per treatment); B. 20-week feeding experiment (n = 5 per treatment).

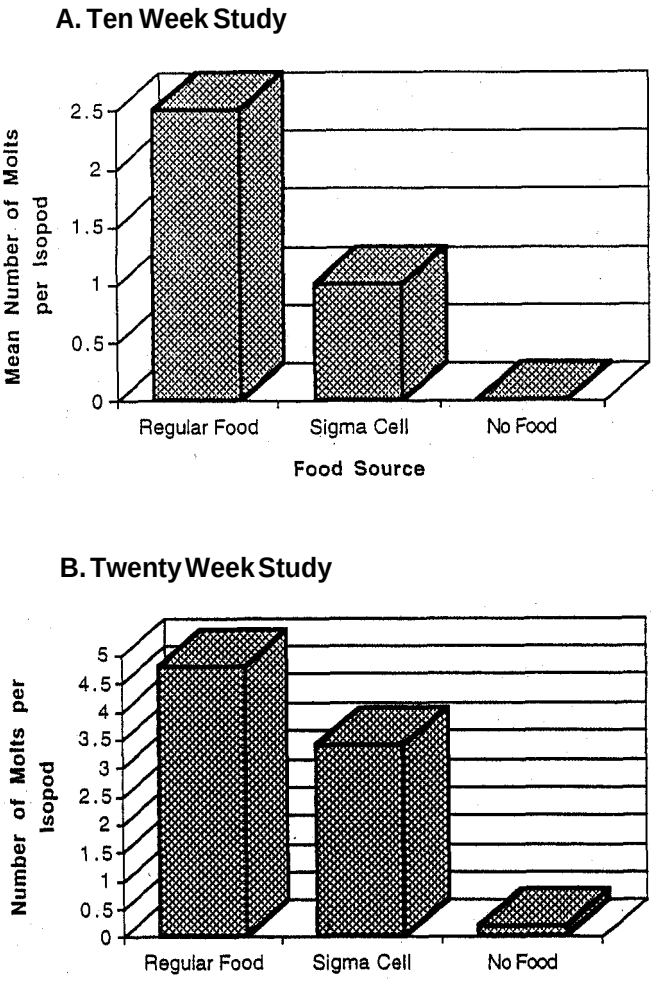

FIG. 2. Mean molting rate for *Sphaeroma terebrans* juveniles. A. 10-week feeding experiment: B. 20-week feeding experiment.

thereafter. Figure 4 presents a summary of the mean rates of digestion of the two cellulose substrates by the three enzyme preparations. CMC was broken down by all three enzyme preparations, with *L. tripunctata* showing the highest amount of activity, liberating about twice as much glucose from the cellulose polymers as the standard cellulase. *S. terebrans* showed the lowest enzyme activity. All of the mean values for CMC are significantly different from each other (P = 0.0003, F-test). Sigma Cell 20 was only broken down by the standard cellulose and *L. tripunctata* tissue homogenates during the 8 hr incubations. The *S. terebran* homogenate did not produce digestion products above background levels during the initial 8 hr of incubation. Due

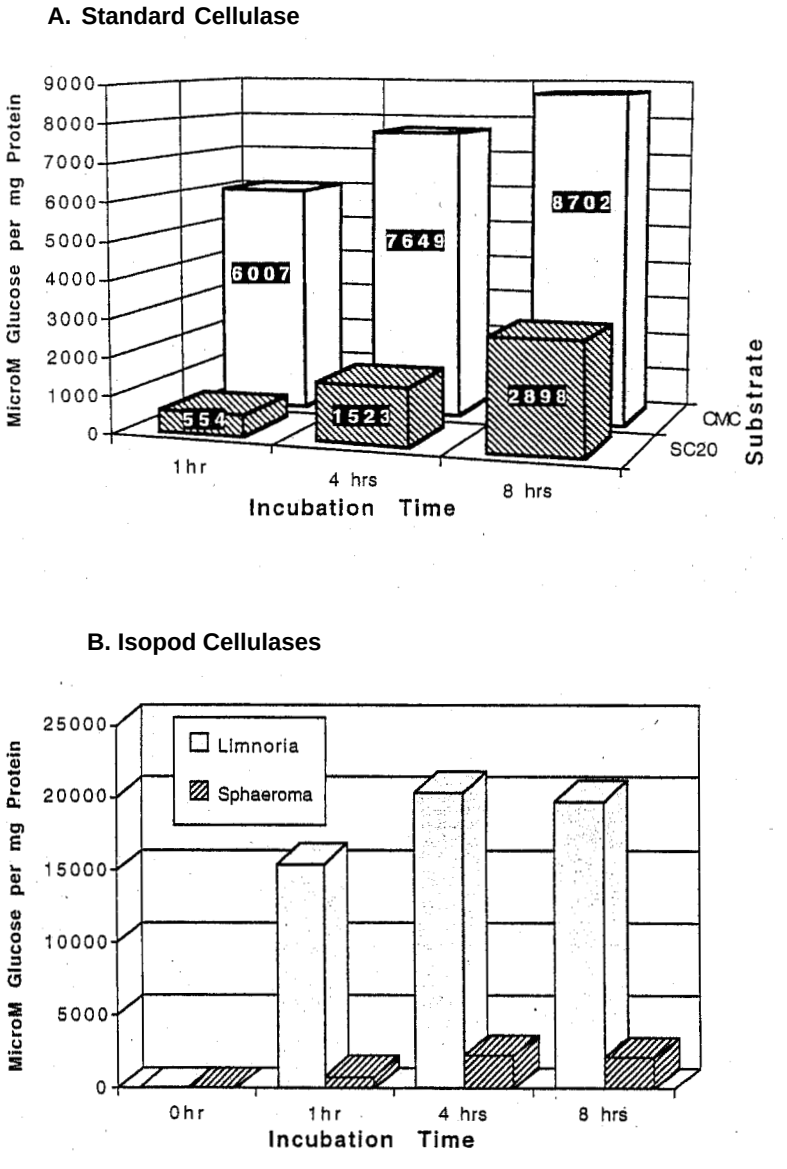

FIG. 3. A. Digestion rates from a representative experiment on two cellulose substrates. carboxymethyl cellulose (CMC) and Sigma Cell 20 (SC20), by standard fungal cellulase: B. Digestion rates of carhoxymethyl cellulose by isopod cellulases.

to the large degree of variability in the Sigma Cell 20 results, the amounts of glucose released by the standard cellulase preparation and the *L. tripunctata* homogenate were not significantly different from each other (P = 0.098. F-test).

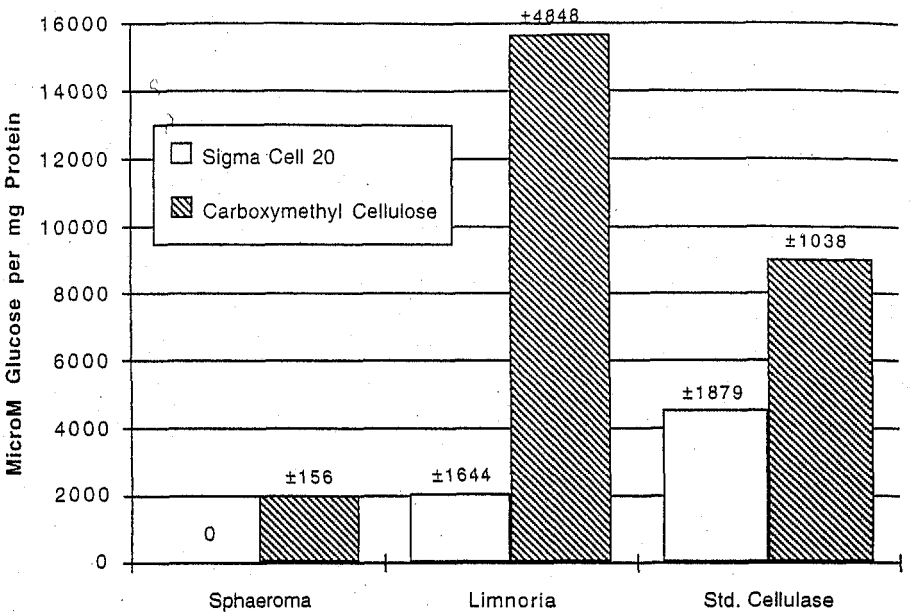

FIG. 4. Comparison of mean cellulose digestion rates (from two separate experiments) after 8 h of incubation among enzymes from *Sphaeroma terebrans*, *Limnoria tripunctata*, and commercially available fungal cellulose. Numbers represent one standard deviation.

Morphology of appendages and digestive system – The anterior appendages of Sphaeroma terebrans are adapted for collection of particulate matter that enters the burrow along with the respiratory/feeding current produced by the pleopods. The mandibles are armed with strong cylindrical teeth as seen in Figure 5, that are used to excavate burrows in wooden structures. The two pairs of maxillae and single pair of maxillipeds are endowed with abundant setae (Fig. 6) that aid in transfer of trapped particulate matter from the setae on the pereopods into the mouth. Initial particle capture is accomplished by the first three pairs of pereopods, which have long, plumose setae directed ventrally from the ischium and merus of each appendage (Figs. 7 and 8). Trapped particulate material is removed from the setae of the pereopods by the maxillipeds at intervals during feeding.

Once particulate material enters the mouth, it travels through a short esophagus into a complex stomach containing multiple channels with spines which sort and direct the food particles depending upon size (Wägele, 1992). Large particles are directed toward the hindgut while finer particles are channeled toward the ventral filter system. The ventral filter system is composed of an anterior filter (clatri setarum anteriores) followed by a finer posterior filter (clatri setarum posteriores) that guards the entrance to the digestive caeca. Figures 9 and 10 depict representative sections through the posterior ventral filter system. In order to enter the ducts leading to the digestive caeca, ingested material must pass between the lateral elastic cushions and

the inferomedianum posterium (imp in Figs. 9 and 10). The spinose border of the elastic cushion and the filtering apparatus of the clatri setarum posteriores are shown in Figures 11 and 12. What appears to be the walls of algal cels are visible between the spines in Figure 11. No particulate material was observed in sections of the digestive caeca or the ducts leading to them, suggesting that only moleculur-size material is allowed to pass into these structures.

Electron micrographs of the hindgut contents of *Sphaeroma terebrans* revealed fibrous material that resembles wood fragments (Figs. 15 and 16). These animals were fixed immediately after removal from mangrove root tips and within 1 hr of collecion from the field. Sections of the hindgut of *Limnoria tripunctata* showed numerous heterogeneous fragments of the wood on which they had been feeding (Figs. 13 and 14). No symbiotic bacteria or protozoans were seen in hindgut sections of either species.

DISCUSSION–Both feeding experiments performed in the laboratory on juvenile isopods resulted in the animals fed Sigma Cell 20 living longer than the unfed isopods (Figs. 1A,B). these results indicate that some nutrition is being gained from the Sigma Cell 20 food, and that these isopods are capable of using particulate cellulose as a food source under laboratory conditions. Although the molting rate of isopods given regular food was more rapid than those given only Sigma Cell 20, there was significant growth (as indicated by molting rate) in the animals with the Sigma Cell 20 diet when compared to the molting rate in the no food treatment. This suggests that the isopods were gaining enough nutrition from their cellulose food source to grow, although some essential ingredients needed for optimal growth were lilkely missing from their diet. No attempt was made to control or inhibit microbial growth in the laboratory experiments, and it is possible that microbial breakdown of the cellulose substrate might have been occurring.

John (1968) conducted feeding experiments with *S. terebrans* from the Kerala Coast (India) in which test animals were given either: 1) no food in artificial seawater; 2) filter paper in artificial seawater; 3) filter paper in natural seawater; or 4) seawater plus algae. He found that the unfed animals survived for an average of 12 d while the second and third treatments survived for an average of 43 d and 26 d, respectively. The seawater plus algae treatment of 70% survival after 120 d. John (1968, pg 4) concluded from these experiments that *S. terebrans* "... are able to subsist on cellulose." Kühne (1972) conducted feeding experiments using Australian wood-borers including *S terebrans.* In these tests, juvenile *S. terebrans* were used in static dishes and provide with one of four combinations of natural celluloses and algae. Mean survival times in decreasing order were: 36 d for algae alone: 26 d for pinewood plus algae; 23 d for pine sapwood; 16 d for the starvation control: and 6 d for shipworm feces. Statistical analyses were not presented, but it was concluded that *S. terebrans* survives longer when given algae rather than wood as a food source (Kühne, 1972).

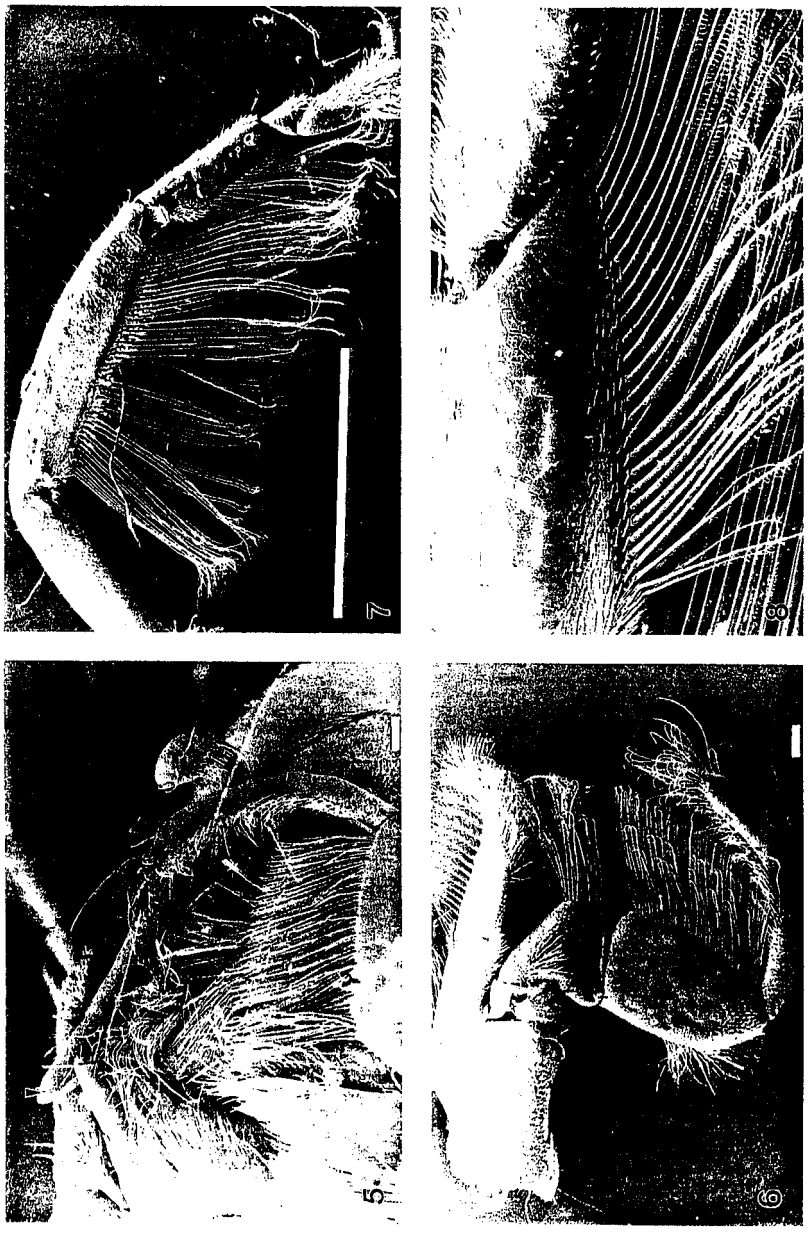

FIG 5. *Sphaeroma terebrans*, mouth area of intact animal viewed from a posterio-ventral aspect. The tips of the mandibles are visible at the arrow. The maxilliped is located in the lower middle of the micrograph. Magnification marker = 1.10 mm.

FIG 6. *Sphaeroma terebrans*, excised maxilliped. The proximal article of the appendage is located in the upper left with the most distal article at the lower right. Tthe setae on this appendage are used to clean material off the more posterior appendages. Magnification marker = 0.10 mm.

FIG 7. *Sphaeroma terebrans*, excised first pereopod (walking leg) showing long setae used to collect suspended material from the water. The most proximal article of the appendage is to the left. Magnification marker = 1.0 mm

FIG 8. *Sphaeroma terebrans*, close-up of the joint between the merus and carpus of a pereopod showing details of the food collection setae. Magnification marker = 0.10 mm.

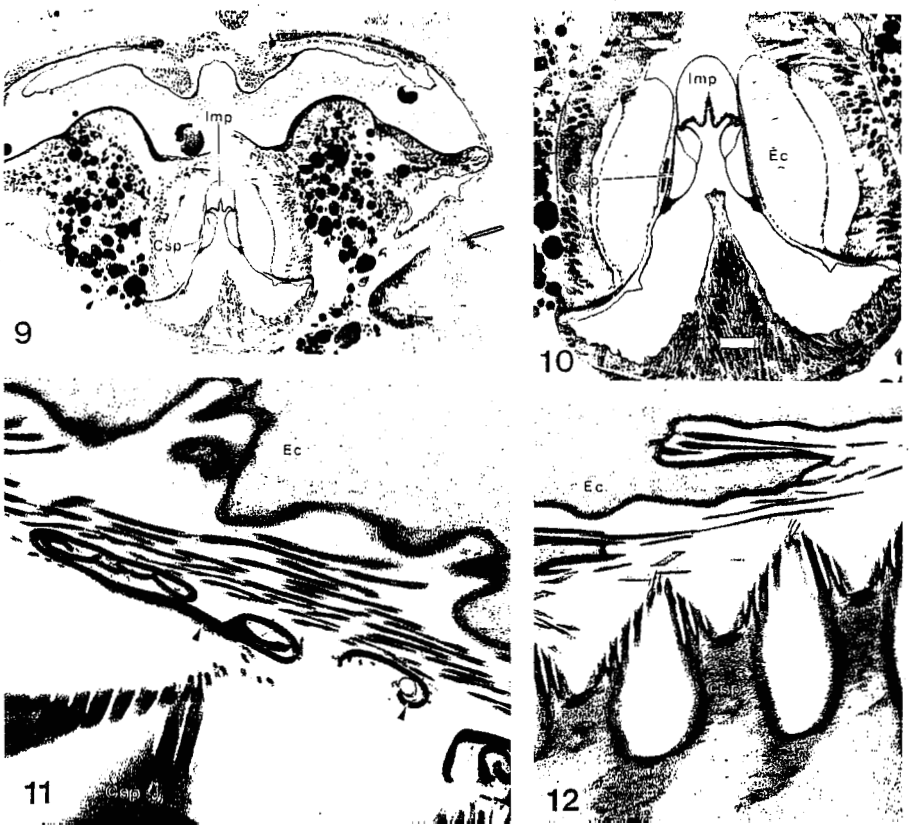

FIG. 9 *Sphaeroma terebrans,* light micrograph of a section through the stomach at the level of the inferomedianum posterium (Imp) and catri setarum posteriores (Csp), where food material must pass to enter the hepatopancreas. Magnificaton marker = 0.05 mm.

FIG. 10. *Sphaeroma terebrans,* light micrograph of the posterior stomach filter machanism showing the inferomedianum posterium (Imp), clatri setarum posteriores (Csp) and the elastic cushions (Ec). Magnification marker = 0.01 mm.

FIG 11. *Sphaeroma terebrans,* electron micrograph of the filter mechanism that makes up the clatri setarum posteriores (Csp). The cell walls of algal cells (arrows) are visible diagonlly across the middle of the micrograph between the Cps and the elastic cushion (Ec). Magnification marker = 0.50 µm.

FIG. 12. *Sphaeroma terebrans,* electron micrograph of the filter mechanism that makes up the clatri setarum posteriores (Csp) where it presses against the elastic cushion (Ec). Material that passes through this filter is transported to the hepatopancreas. Magnification marker = 0.50 µm.

Estevez (1978) tested the effects of burrow availability and food availability on the survival of adult *S. terebrans* from Florida. He found that animals survived longer when burrows (either natural or artificial) were provided and when food material was kept in suspension by aeration. This combination of conditions (burrows and suspended particulate matter) is

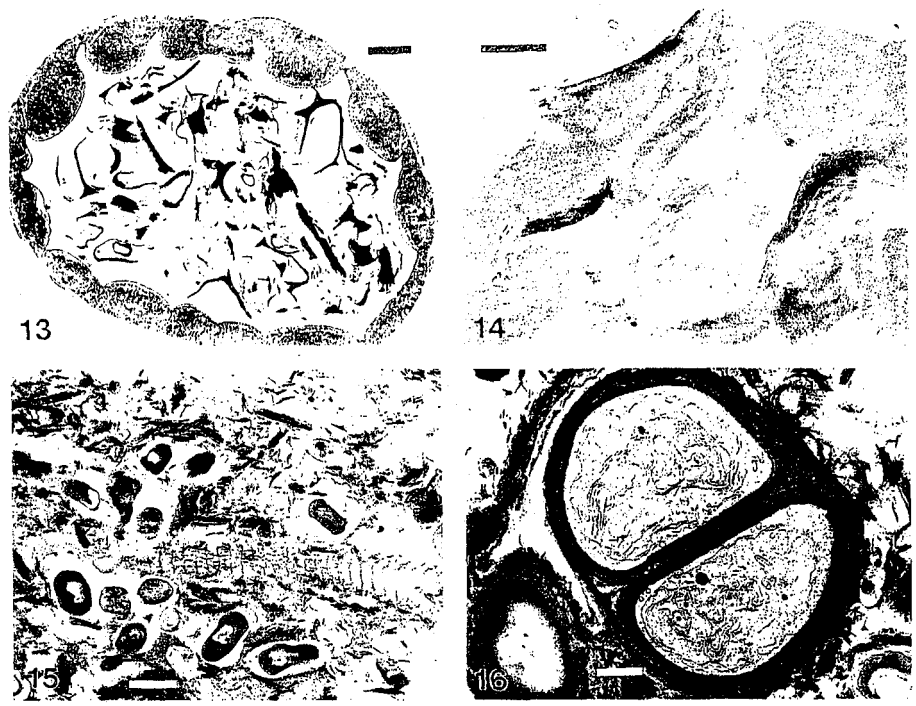

FIG. 13. *Limnoria tripunctata* light micrograph of a section through the hindgut showing pieces of injested wood. Magnification marker = 0.02 mm.

FIG. 14. *Limnoria tripunctata*, electron micrograph of injested wood material from the hindgut of a well-fed animal. Magnification marker = 1.0 μm.

FIG. 15. *Sphaeroma terebrans*, electron micrograph of the hindgut contents of an animal removed from a mangrove root tip. Fibrous material that resembles wood fragments is visible throughout the micrograph. Magnification marker = 0.50 μm.

FIG. 16. *Sphaeroma terebrans*, electron micrograph of algal cells and fibrous material from the hindgut of an animal fresh from the field. Magnification marker = 0.50 μm.

found at field sites where *S. terebrans* establishes successful populations. In the present study, food material was not kept in suspension: and animals were not provided with burrows yet were able to survive and grow for extended periods of time (20 wk) if provided with cellulose or natural organic material. For juvenile animals under laboratory conditions, individuals seem to be capable of foraging successfully outside of a burrow.

El-Shanshoury and co-workers (1994) found that naturally occurring bacteria and fungi associated with the exoskeletons and burrows of *Limnoria lignorum* and *Sphaeroma serratum* had the ability to produce cellulases and that these enzymes might be important to the nutrition of these wood-borers.

John (1968) reported cellulose digestion by extracts prepared from the gut and the hepatopancreas of *S. terebrans* and concluded that this species

may digest cellulose but needs additional nutrients from algae or diatoms for normal growth. In the present study, carboxymethyl cellulose was broken down rapidly by isopod and fungal cellulases, with enzymatic activity from the S. *terebrabs* homogenate being the lowest of the three preparations tested. Sigma Cell 20 was digested more slowly than CMC by the standard cellulase and the *Limnoria tripunctata* enzymes whereas the *S. terebrans* homogenate showed no activity on this substrate after 8 h of incubation. Preliminary tests using longer incubation times (24 h) resulted in some Sigma Cell 20 digestion by the *S. terebrans* extract, but the experimental results were variable and need to be repented using a more purified enzyme extract than that used in the present study. The fact that the cellulose digestion capabilities of *L. tripunctata* proved to be superior to those of *S. terebrans* supports the observation that wood is the primary food source of *L. tripunctata* (Ray, 1958; 1959), while *S. terebrans* also feeds on other suspended materials. These experimental data also indicate that further study is necessary to understand the importance of cellulose to *S. terebrans* in its natural environment.

The morphology of the digestive system in *Sphaeroma terebrans* from Florida appears to be similar to that described for the "generalized isopod" by Wägele (1992) and for *S. terebrans* from Kerala (India) by Johns (1968). Regardless of whether individuals feed on cellulose or other food, the ingested food is sorted in the stomach, only the finest colloidal or dissolved portion passing through the posterior filter and entering the hepatopancreas where endogenous enzymes are released (Brunet et al. 1994). The present study found no evidence from TEM micrographs for symbiotic bacteria in the digestive systems of *S. terebrans* or *Limnoria tripunctata.* Sections of hindgut from field-collected *S. terebrans* revealed cellulose-like material similar in morphological detail to the wood particles present in the hindgut of *L. tripunctata.* It is unclear if the cellulose-like particles in the gut of *S. terebrans* originated from bored mangrove root or from suspended material filtered from the water. Tracer studies using labeled mangrove wood might help to answer this question.

In conclusion, these data taken collectively suggest that S. *terebrans* has the capability to use the wood it bores as a food source. Further, it appears that this species can survive and grow for extended periods of time on a cellulose diet and that field specimens have cellulose-like material in their digestive systems. Further analysis of *S. terebrans* feeding behavior and digestive capabilities is warranted and would be useful in designing a wood preservative to will help minimize boring damage by this isopod.

ACKNOWLEDGMENT–The authors would like to acknowledge the assistance of several undergraduate research assistants who contributed to this project including Maria Okuniewski, Karen Davis, and Joe Abdella. We thank E. Estevez for thoughtful comments on an earlier version of the manuscript. This research was suppored by a grant to SAR from the Forest Products Laboratory, U.S. Department of Agriculture (FP-92-1805) and by a Faculty Development Grant from the University of Tampa.

LITERATURE CITED

ALEXANDER, R. R., J. M. GRIFFITHS, AND M. L. Wilkinson. 1985. Basic Biochemical Methods. John Wiley & Sons, New York, NY.

BOYLE, P. J. AND R. MITCHELL. 1978. Absence of Microorganisms in Crustacean Digestive Tracts. Science 200:1157–1159.

BRUNET, M., J. ARNAUD, AND J. MAZZA. 1994. Gut structure and digestive cellular processes in marine crustacea. Oceanogr. and Mar. Biol. 32:335–367.

CLAPP, W. F. AND R. KENK. 1957. Marine borers: A preliminary bibliography. Part II. Technical Information Division, Library of Congress, Washington, D.C. 350 pp.

DEAN, R. C. 1978. Mechanisms of wood digestion in the shipworm *Bankia gouldi*. Bartsch: enzyme degradation of celluloses, hemicelluloses, and wood cell walls. Biol. Bull. 155: 297–316.

EL-SHANSHOURY, A. R., M. H. MONA, F. A. SHOUKR, AND A. M. EL-BOSSERY. 1994. The enumeration and characterization of bacteria and fungi associated with marine wood-boring isopods, and the ability of these microorganisms to digest cellulose and wood. Mar. Biol. 119:321–326.

ELYAKOVA, L. A. 1972. Distribution of cellulases and chitinases in marine invertebrates. Comp. Biochem. Physiol. 43:67–70.

ESTEVEZ, E. D. 1978. Ecology of *Sphaeroma terebrans* Bate, a Wood Boring Isopod in a Florida Mangrove Forest. Ph. D. Dissertation. Univ. of South Florida, Tampa, FL.

——— AND J. L. SIMON. 1975. Systematics and ecology of *Sphaeroma* (Crustacea: Isopoda) in the mangrove habitats of Florida. Pp 286–304. *In*: WALSH, E. G., S. C. SNEDAKER, AND H. J. TEAS (eds.), Biology and Management of Mangroves; Proceedings of International Symposium, Honolulu, HI. Vol. 1.

FOULDS, J. B. AND K. H. MANN. 1978. Cellulose digestion in *Mysis stenolepis* and its ecological implications. Limnol. Oceanogr. 23 (4):760–766.

FRIESEN, J. A., K. H. MANN, AND J. A. NOVITSKY. 1986. *Mysis* digests cellulose in the absence of a gut microflora. Can. J. Zool. 64:442–446.

HOCHMAN, H. 1973. Degradation and protection of wood from marine organisms. Pp. 247–275. *In*: NICHOLAS, D. D. (ed.), Wood Deterioration and Its Prevention by Preservative Treatments. Syracuse University Press, Syracuse, NY.

JOHN, P. A. 1968. Habits, structure and development of *Sphaeroma terebrans* (a wood-boring isopod). University of Kerala Publications 1:1–73. Trivandrum, S. India.

KIRK, T. K. AND E. B. COWLING. 1984. Biological decomposition of solid wood. Pp. 455–488 *In:* ROWELL, R. (ed.), The Chemistry of Solid Wood. Adv. Chem. Ser. 207 American Chemical Society, Washington, D. C.

KÜHNE, H. 1972. On the nutritional requirements of wood-boring crustacea. Pp. 814–821. *In*: Proc. Third International Congress on Marine Corrosion and Fouling. National Bureau of Standards, Washington, D.C.

LEHNINGER, A. L., D. L. NELSON, AND M. M. COX. 1993. Principles of Biochemistry. 2nd. Ed. Worth Publishers, New York, NY. p. 311.

MENZIES, R. J. AND R. D. TURNER. 1957. Distribution and importance of marine wood borers in the United States. Symp. on Wood for Marine Use and Protection from Marine Organisms. Am. Soc. for Testing and Materials, Special Tech. Publ. 200, Philadelphia, PA.

NELSON, N. 1944. A photometric adaptation of the somogyi method of determination of glucose. J. Biochem. 153:375–381.

PARHAM, R. A. AND R. L. GRAY. 1984. Formation and structure of wood. Pp. 3–56. *In*: ROWELL, R. (ed.). The Chemistry of Solid Wood, Adv. Chem. Ser. 207, American Chemical Society, Washington, D. C.

PETTERSEN, R. C. 1984. The chemical composition of wood. Pp.57–126. *In*: The Chemistry of Solid Wood. Adv. Chem. Ser. 207, American Chemical Society, Washington, D. C.

RAY, D. L. 1958. Recent research on the biology of marine wood borers. Am. Wood Pres. Assoc. Proc. 54:120–128.

——— 1959. Some properties of cellulase from *Limnoria*. Pp. 372–396 *In*: RAY, D. L. (ed.), Marine Boring and Fouling Organisms. University of Washington Press, Seattle, WA.
——— AND J. R. JULIAN. 1952. Occurrence of cellulase in *Limnoria*. Nature 169:32–33.
RICE, S. A., B. R. JOHNSON, AND E. D. ESTEVEZ. 1990. Wood Boring Marine and Estuarine Animals in Florida. Florida Sea Grant College Program, Extension Bulletin No. 15, University of Florida, Gainesville, FL.
ROTRAMEL, G. 1975. Filter-feeding by the marine boring isopod, *Sphaeroma quoyanum* H. Milne Edwards. Crustaceana 28 (1):7–10.
SIMBERLOFF, D., B. J. BROWN, AND S. LOWNE. 1978. Isopod and insect root borers may benefit Florida mangroves. Science 201:630–32.
TURNER, R. D. 1966. A survey and illustrated catalogue of the Teredinidae (Molluscs: Bivalvia). Museum of Comparative Zoology, Cambridge, MA. 265 pp.
WÄGELE, J.-W. 1992. Isopoda. Pp 529–617. *In*: HARRISON, F. W. AND A. G. HUMES (eds.), Microscopic Anatomy of Invertebrates, Wiley-Liss, Inc. New York, NY.

Florida Scient. 62(2): 128–144. 1999
Accepted: October 14, 1998.