A BIOECOLOGICAL STUDY OF THE FRESH-WATER SPONGES OF HAMMOND BAY, LAKE HURON

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ABSTRACT

A bioecological study of *Eunapius fragilis*, *Heteromeyenia tubisperma*, *Heteromeyenia baileyi*, *Ephydatia fluviatilis*, and *Ephydatia mulleri* was conducted in the vicinity of Hammond Bay (Michigan), Lake Huron, from September 1976 to November 1977. All five species were found encrusted on various wood substrates in a shallow, seasonally inundated pond adjacent to the bay. *E. fragilis* was the only species collected from the rock-cobble eulittoral zone of the lake.

A synopsis of the distribution of fresh-water sponges in the St. Lawrence Great Lake system; observations on the timing of life cycle events, spicule size-distributions, and associated organisms; and a revision of the physiological ranges of the above species are presented.

INTRODUCTION

Macan (1974) concedes "many workers comparing the fauna of two pieces of water or studying the range of species, have analysed the water in the hope of finding correlations. They have generally been disappointed." Old (1932a), in his study of sponges in Michigan, investigated numerous sponge habitats and compared their chemical and physical conditions to species occurrences. Similarly, Jewell (1935, 1939) collected fresh-water sponges from various lakes and streams in Wisconsin ultimately relating their distribution to the calcium content of the water. Many subsequent studies were similar in their approach to elucidate the preferred habitat of sponges. However, Moore (1953) and Strekal and McDiffet (1974) studied seasonal fluctuations of seemingly important parameters within one particular habitat and noted how these changes might affect species distribution. Harrison (1974) summarized sponge collection data from much of the available literature to ascertain their "tolerance" to certain physicochemical conditions. Despite these various efforts to categorize distributioal patterns of fresh-water sponges with environmental variables, few correlations can reliably be made.

The objectives of this study were to (1) expand the knowledge of the ecological requirements of certain fresh-water sponges, (2) document their presence in Lake Huron, (3) provide a synopsis of their occurrence in the St. Lawrence Great Lakes system, and (4) collect and document certain base-line data on the inshore environment of Hammond Bay, the importance of which will be realized as development accelerates in this area of high recreational potential.

STUDY AREA DESCRIPTION

Hammond Bay is on the Michigan shoreline of Lake Huron about 70 km southeast of the Straits of Mackinac (Figure 1). The Ocqueoc and Black Mallard Rivers are tributary to the bay at about 4 and 8 km, respectively, from the study area. The watersheds of these two rivers are predominantly forested (aspen/birch) and have sloping, well-drained sandy soils or nearly level, poorly-drained organic soils of the Roscommon-Eastport-Rubicon association (USDA, 1974). Geologic formations in this region consist of Pleistocene glacial deposits over gently dipping beds of limestone and shale of Mississippian and Devonian age (USDA, 1974). Land uses within the watersheds and along the bay are limited to seasonal recreational use and occasionally farming.

Six study sites were established in the eulittoral zone of the rock-cobble southeast corner of Hammond Bay (Figure 2). Site delineations were based on shoreline morphology, sizes of available substrates, and orientation of the shore to prevailing winds.

The eulittoral zone of Site 1 was generally sandy with occasional igneous and sedimentary stones (1,000 to 20,000 cm^3). At Site 2, the stones were of a more uniform size (2,000 to 6,000 cm^3) and randomly placed one on top of another (perched) forming a strata which decreased in thickness towards shore. The prevailing winds were such that Sites 1 and 2 were frequently exposed to wind-induced waves.

Site 3 was a quiet-water area protected from violent wave action by a submerged offshore barrier. Silt and clay-type materials were deposited along shore. The igneous and sedimentary stones at this site were of a smaller size (800 to 4,000 cm^3).

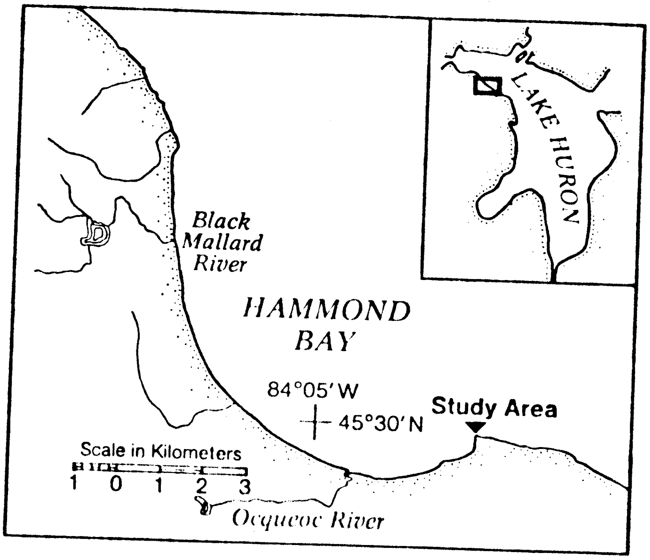

Fig. 1. Location map of Hammond Bay.

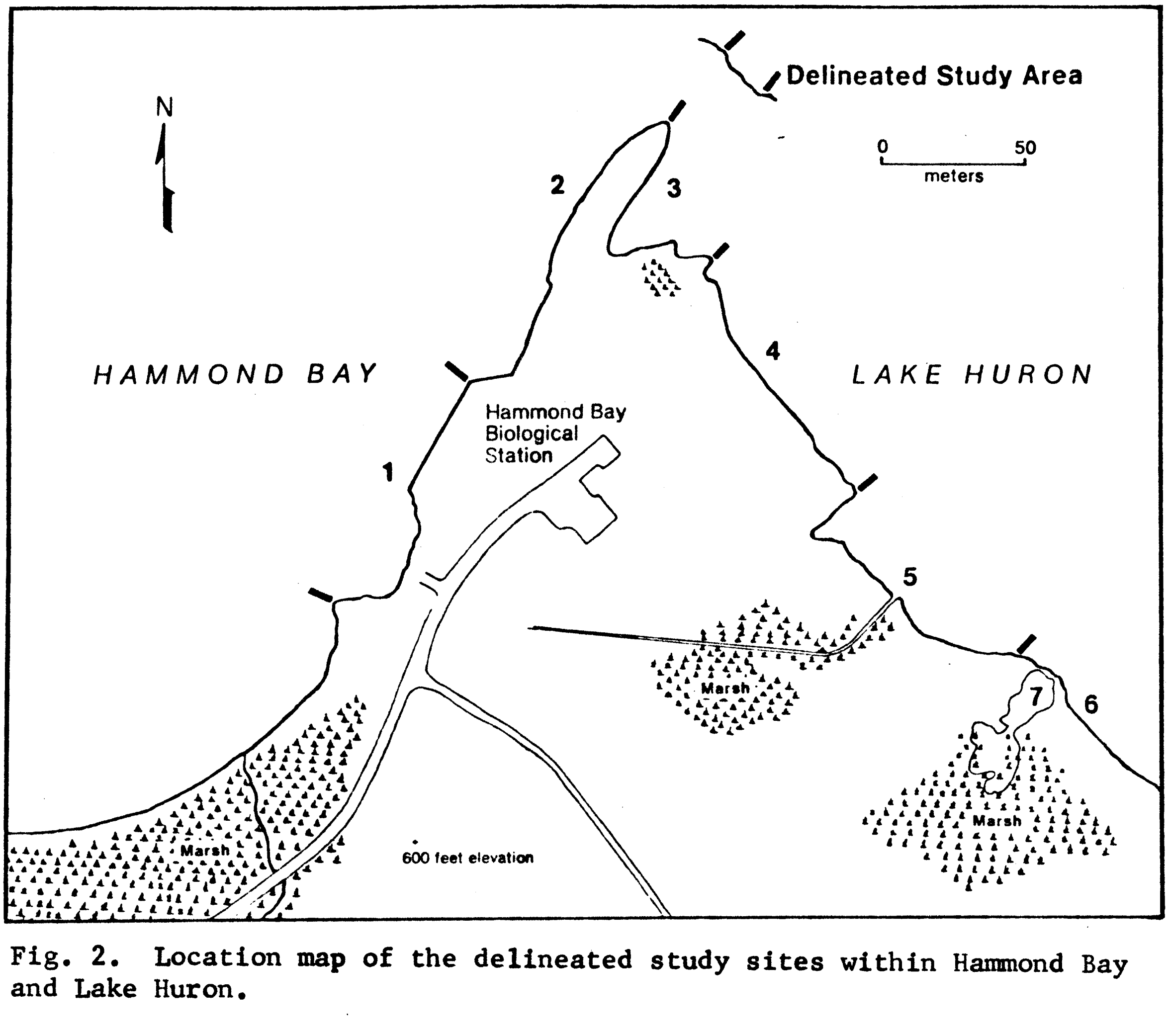

Fig. 2. Location map of the delineated study sites within Hammond Bay and Lake Huron.

With the degree of wave exposure increasing from Site 4 to Site 6, the approximate size of igneous and sedimentary stones also increased. Their sizes ranged from 50 to 2,000 cm^3 at Site 4, 50 to 10,000 cm^3 at Site 5, and 25 to 25,000 cm^3 at Site 6. A narrow, intermittent drainage ditch bisected the shoreline of Site 5.

A small (<1 ha), shallow pond (<1 m) immediately adjacent to Hammond Bay at Site 6 was established as Study Site 7. During low water levels the pond had an hour-glass shape with numerous igneous stones (1,000 to 3,000 cm^3), both solitary and perched, occurring near the lake. This area was inundated by Lake Huron from May to August 1976 at which time numerous logs, boards and stumps were washed into the pond.

METHODS

Biological

Sponges were collected at irregular intervals from September to November, 1976 from three square-meter sample plots randomly established at each of the six lake sites and from randomly selected stones (12 per lake site). Since time and weather prohibited a more thorough investigation of the lake, this sampling design optimized randomization and still provided semi-quantitative data. In the shallow pond, essentially every potential sponge substrate was investigated, hence, the entire sponge population in this area was censused.

At the time of collection, the depth at which the sponge was growing, its color, size (in cm^2), position on the substrate, the substrate type and its approximate size (in cm^3) were recorded. Because of the seasonal fluctuation in the Lake Huron water level, actual observed water depths were corrected to the mid-June water levels reported by the U.S. Army Corps of Engineers (Figure 3). This period of high water coincides with the production of free-swimming larvae in certain fresh-water sponges (Simpson and Gilbert, 1973, 1974) and thus, may best represent the time sponges colonized the Hammond Bay region.

In May, July, September, and October, 1977, additional scalpel-cut fragments of sponges growing in Lake Huron were collected and immediately fixed in either glycerine alcohol (80% ethanol and 5% glycerine) or Bouin's fixative, then rinsed and stored in 75% ethanol for subsequent histological examination. Sponge fragments were processed (dehydrated then infiltrated with paraffin) with a Fisher Tissuematon, sectioned at 10 to 15 microns, and differentiated with Mayer's hematoxylin (3 to 4 minutes) and eosin Y (25 seconds), Weigert's iron hematoxylin (7 minutes) and Gomori's Trichrome (10 minutes), methylene blue (3 minutes) and eosin Y (25 seconds), or standard hematoxylin (1 to 2 minutes) and eosin Y (25 seconds).

Sponge spicules were isolated for species identification by boiling sponge fragments and gemmules in concentrated nitric acid until disintegrated (Pennak, 1953). Each sample was then diluted with distilled water, centrifuged, and the supernatant siphoned off. The centrifugate was dehydrated in absolute ethanol then resuspended in xylene. A drop of this final suspension was mounted in Permount on a microscope slide.

Permanent slides of intact gemmules were also used to confirm identifications by the above method. Gemmules were prepared for mounting by placing them in hot nitric acid until translucent, after which they were dehydrated and mounted like the spicules.

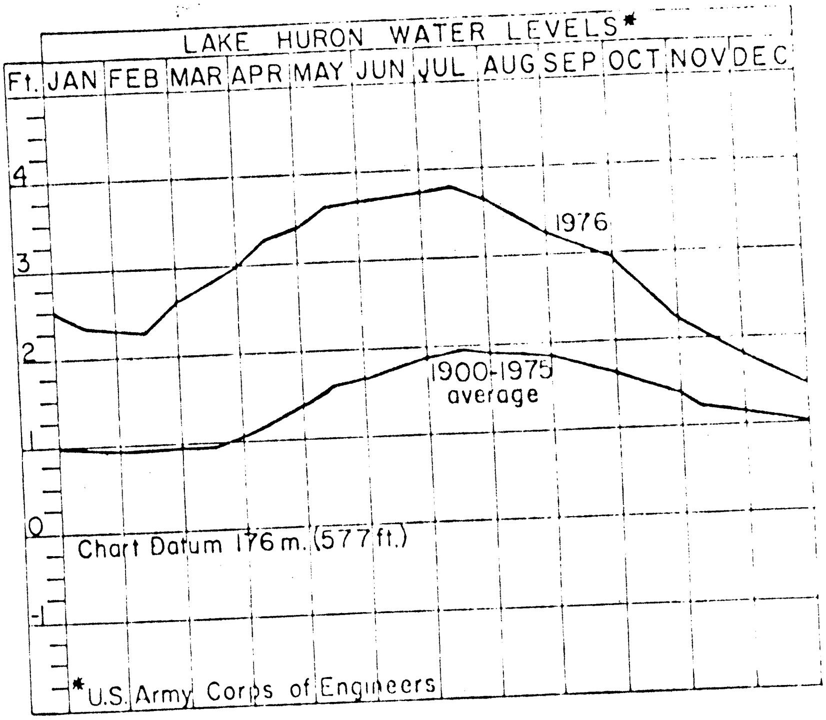

Fig. 3. Lake Huron water levels (Army Corps of Engineers, 1976).

Length and width measurements were made on the spicules of those sponges subsequently identified as Eunapius fragilis (Leidy). Fifty megascleres and 50 gemmoscleres from each of seven sponges from each habitat were measured to the nearest micron at 250X magnification. The mean, standard deviation, standard error, skewness, and kurtosis of the spicule size-distributions of each sponge were computed with the Hewlett/Packard HP-65 programmable calculator. In addition, size variations within habitats were tested by a one-way analysis of variance also using the HP-65.

Physicochemical

Lake Huron water temperatures were obtained with a continuously recording thermograph placed at the nearshore water intake of the nearby Hammond Bay Biological Station. Water temperatures were taken with a mercury bulb thermometer at Site 7.

All water chemistry data to be presented here, except calcium and magnesium hardness, bicarbonate, and carbon dioxide, were provide by Dr. B. Manny of the Great Lakes Fishery Laboratory, U.S. Fish and Wildlife Service. Lake Huron water samples were collected weekly between July 1975 and July 1976 and again in October 1976 from the water intake of the Hammond Bay Biological Station. Water samples from Site 7 were collected about twice a week during September and October 1976. Measurements of alkalinity and pH were performed usually within one hour after collection according to standard methods (APHA, 1971). Specific conductance was measured with a conductance meter at 25 C (YSI, Model 31). Subsamples of filtered (Reeve Angle 984 H glass fiber filter) raw water were frozen for no longer than three months, then analyzed colorimetrically for chloride by ferric thiocyanate formation, for soluble silica by heteropoly blue formation, for nitrate and nitrite by copper-cadmium reduction, and for ammonium by indolphenol blue formation (EPA, 1971). Sodium, potassium, calcium, and magnesium concentrations were analyzed with an atomic absorption spectrophotometer (Perkin-Elmer, Model 403). Calcium and magnesium hardness were determined by calculation according to standard methods (APHA, 1971). Both carbon dioxide (as mg/l CO_2) and bicarbonate (as mg/l $CaCO_3$) were determined using a computer program (University of Wisconsin- Stevens Point) based on equilibrium relationships between alkalinity, pH, and temperature.

RESULTS AND DISCUSSION

Distributional Records

Reports on the occurrence of fresh-water sponges in the St. Lawrence Great Lakes system are few. Apparently, the earliest record of these animals is that of Reighard (1894), who found an unidentified species of Ephydatia Lamaroux in Lake St. Clair in 1893. Later, Landacre (1901) reported Spongilla fragilis[1] (Leidy) and Radiospongilla cinerea[2] (Carter) in Lake Erie. An unidentified species of Spongilla Lamarck was also found in Lake Erie during studies by Krecker and Lancaster (1933). In

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[1] Penney and Racek (1968) relegate Spongilla fragilis into synonomy under Eunapius fragilis.

[2] Inadequate collection data and sparse sy type material on Radiospongilla cinerea confuses its generic positioning (Penney and Racek, 1968) and indicates Landacre's finding of this species was a misidentification (Harrison, 1974).

1974, J.K. Hiltunen (personal communication) collected E. fragilis and Spongilla lacustris (Linnaeus) off tips of quillwort (Isoetes macrospora) in the St. Marys River, Chippewa County, Michigan. Elsewhere in the Great Lakes system, sponges remain unreported.

During the course of this study and as summarized in Table 1, E. fragilis was the only sponge species found in Lake Huron (Sites 1-6). However, E. fragilis, Heteromeyenia baileyi (Leiberkuhn), Heteromeyenia tubisperma (Mills), Ephydatia mulleri (Leiberkuhn), and Ephydatia fluviatilis (Linnaeus) were collected from the pond.

The relative abundance of E. fragilis between Lake Huron sites was quite variable. Despite an extensive search for sponges at Sites 1 and 2, none were found.[3] At Site 3, 28 specimens of E. fragilis were found encrusted on the side or underside of sedimentary (limestone) and igneous stones in 43 to 67 cm water. The sponges were either white, pallid, or green with tan or brown gemmules arranged in a basal layer.

At Site 4, 10 specimens of E. fragilis were collected from the underside of sedimentary and igneous stones in 33 to 36 cm water. All sponges collected at this site were green with brown gemmules arranged in a uniform basal layer or in randomly distributed clusters.

Two sponges were found at Site 5 and three at Site 6 on the underside of igneous stones in about 41 cm and 48 to 65 cm water, respectively. At Site 5, the sponges were either white or pallid with brown gemmules forming a basal layer while the sponges from Site 6 were green with pallid gemmules irregularly distributed along the base.

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[3] An extensive search in addition to the three square-meter sample plots and 12 random spot checks was conducted to insure the lack of sponges at these sites was real and not a result of sampling design.

Table 1

RESULTS OF SELECTED PARAMETERS

	Study area	Species	Number of sponges	Mean size (cm^2)	Size range (cm^2)	Mean depth (cm)	Depth range (cm)	Preferred substrate	Wave action
Lake Huron	1	None	0	-	-	-	-	-	Direct
	2	None	0	-	-	-	-	-	Direct
	3	Eunapius fragilis	28	1.1	0.1-7.1	51.5	43-67	Granite	Limited
	4	Eunapius fragilis	10	0.2	0.1-0.4	35.3	33-36	Granite	Indirect
	5	Eunapius fragilis	2	1.1	1.1	40.7	40-41	Granite	Indirect
	6	Eunapius fragilis	3	0.7	0.3-1.1	55.3	48-65	Granite	Indirect
Pond	7	Eunapius fragilis	24	18.3	0.1-90.0	19.1	15-36	Wood	Limited
		Heteromeyenia tubisperma	16	11.0	0.8-75.0	20.5	16-38	Wood	
		Heteromeyenia baileyi	2	3.0	0.1-6.0	27.5	16-39	Wood	
		Ephydatia fluviatilis	35	4.2	0.1-32.0	18.2	16-35	Wood	
		Ephydatia mulleri	4	1.6	0.5-4.3	25.8	16-38		

The distribution of E. fragilis in the eulittoral zone of Hammond Bay and Lake Huron indicates the intensity of waves is an important factor regulating the relative abundance of this species in wave-exposed habitats. This is supported by the fact that no sponges were found at Sites 1 or 2 where the wave action was usually direct and intense. At Site 3, where the eulittoral zone is substantially protected from wave action, the relative abundance of E. fragilis was the greatest. Indeed, as wave action respectively increased from Site 3 to Sites 4, 5, and 6, the relative abundance of sponges decreased.

The mechanisms of regulation by excessive wave activity might be the dislodgement of sponge parts or all of it, deformation with possible internal injury, or death of the sponge by smothering sediments (de Laubenfels, 1947). Waves and currents also influence sponge distribution by controlling the dissemination of asexually-produced gemmules and probably less so the distribution of sexually-produced free-swimming sponge larvae. Gemmules carried by waves or currents will eventually settle in response to gravity. Those above or below certain limits would either not germinate or would germinate but die before maturation. Larvae on the other hand may: (1) seek out a particular level for settlement, (2) settle at all levels but those above or below certain limits would subsequently die, (3) seek out areas of low density, or (4) settle in response to a tropism (McDougall, 1943).

It has been found that certain marine sponges undergo behavioral modifications which aid them in survival in intertidal or wave-swept areas (Berquist, et. al., 1970). There is appparently an increased incidence of asexual reproduction in intertidal species which helps circumvent the difficulties of fertilization associated with intertidal existence. Whether fresh-water sponges occurring in similar habitats also undergo this behavioral modification is not known. However, certain fresh-water sponges, such as E. fragilis, have a propensity for growing on the underside of objects (Harrison, 1974), thus being able to temporarily survive periods of violent wave action. This particular behavior must certainly be a modification which enables it to survive in habitats not suitable for other sponges.

It is important to note that waves promote sponge survival by bringing in food and oxygen, removing waste products, and washing the sponge free of sediment (de Laubenfels, 1947). In wave exposed habitats, successful sponge colonization can occur when the beneficial effects of wave activity equal or exceed the detrimental effects.

Of the five different sponge species collected in the pond (Site 7), E. fluviatilis was the most abundant accounting for 43% of the total (Figure 4). All but one of these were found encrusted on the underside of boards and logs in 16 to 35 cm water. They were typically clear or white with crystal-white gemmules randomly distributed throughout the mesogloea.

E. fragilis, the next most common species in the pond, was found almost exclusively on the underside of logs and boards in 20 to 36 cm water. These were usually green with brown gemmules forming a basal layer within the sponge. Two specimens were massive upright forms (1.5 cm high) growing out of the sediment in 15 cm water. Both sponges were green with brown gemmules in axial clusters. Another specimen was encrusted on the side of an igneous stone. This sponge was also green with brown gemmules though the gemmules were arranged in basal clusters.

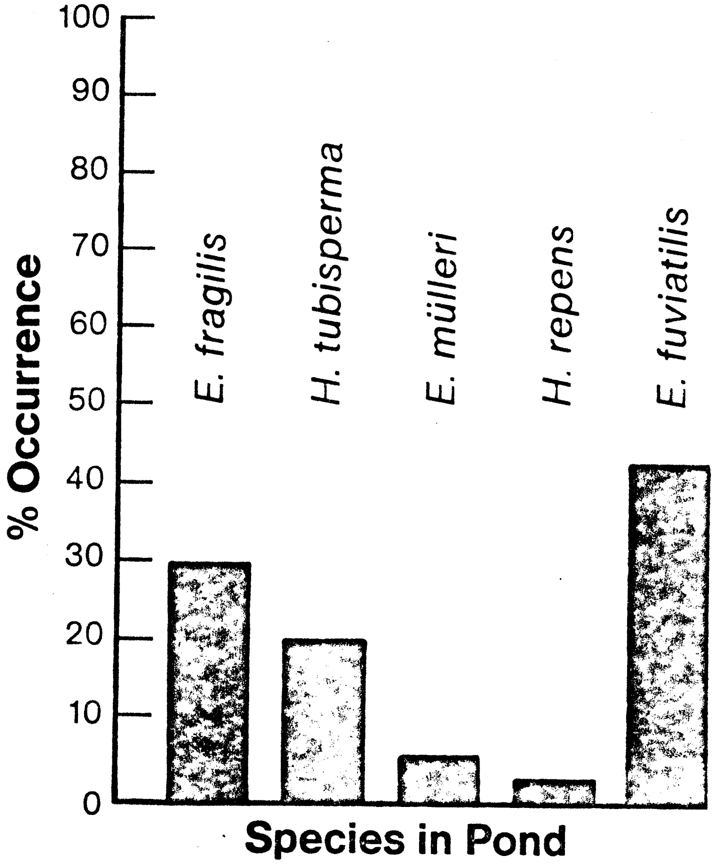

Fig. 4. The populations of the various species which occur in the pond are compared.

Nearly 20% of the sponges collected in the pond were *H. tubisperma*. All were found encrusted on the underside of boards and logs in 16 to 38cm water. Four of the sponges were green while the rest were whitish to translucent. All had crystal-white gemmules irregularly distributed throughout the sponge.

E. mulleri and *H. baileyi* were the least common species found at Site 7 comprising only about 5% and 2%, respectively, of the total. The sponges representing these two species were found encrusted on the underside of boards and logs in 16 to 39 cm water. Specimens of *E. mulleri* were translucent or whitish with crystal-white gemmules irregularly distributed throughout the sponge. Those of *H. baileyi* were green with pallid gemmules irregularly distributed along the base of the sponge.

It is important to note that one timber near the western edge of the pond had nearly 50 sponges, representing all five species, growing on its underside. Most of these were colonized at one end of the timber creating keen competition for space. In fact, one sponge (*H. tubisperma*) was actually epizoic on another (*E. fragilis*). This phenomenon has also been observed in marine sponges during the absence of available substrates for colonization (Rutzler, 1970). An attempt was made in 1977 to further study the spatial distribution and epizoism phenomenon of the sponges on the timber, but low pond water levels in the spring and a continued drought into summer caused the pond to dry completely by July.

A survey of the dried pond produced several dessicated sponges which were devoid of gemmules. Similarly, sponges in Lake Huron that were dessicated as the water level receded were also lacking gemmules. This evidence suggests dehydration-induced physiological stress on fresh-water sponges does not stimulate gemmulation. Perhaps other exogenous factors, alone or in combination with certain endogenous factors, influence sponge gemmulation (Simpson and Gilbert, 1973).

Fresh-water sponges have been known to colonize a variety of substrates (Old, 1932a; Jewell, 1935; Neidhoefer, 1940) though many seem to prefer wood as a base for attachment (Old, 1932a). In the pond, 76 of the 81 sponges collected were colonized on various wood substrates. Perched stones similar to those colonized by sponges in Lake Huron were devoid of sponges. Old (1932a) hypothesized that a paucity of wood and an abundance of stones led to the preferral of colonization on the former, More likely, the large number of sponge-food organisms, such as bacteria and protozoans, associated with decomposing wood makes it an ideal substrate for sponges which are sessile passive feeders.

Boards, stumps, fallen trees, or similar wood objects were absent from the Lake Huron sites. Consequently, sponge colonization was limited to sedimentary and igneous stones, the sizes of which were found to be an unimportant factor for colonization. This confirms similar findings by Old (1932a, 1932b).

Considerable differences in the mean sizes and size ranges of sponges occurred between the wave-protected and exposed study sites. Sponges collected from Site 3 were generally larger than those found at the other lake sites, whereas, sponges in the pond were significantly larger than those found in Lake Huron. The mean size of E. fragilis in the pond was several orders of magnitude greater than sponges of the same species in the lake. Variations in the sizes of sponges within a habitat have in part been attributed to age (Old, 1932b) and/or the number of gemmules from which the sponges were derived (Rasmont, 1970). If the latter is true, the propensity of certain sponges to form clustered or layered gemmules may very well be an adaptation which increases their sexual reproductive capacity in subsequent years.[5] However, excessive wave activity can break up gemmule clusters, possibly reducing the reproductive potential of sponges in an area by reducing their size. This hypothesis seems to explain the generally smaller sizes and lower relative abundance of E. fragilis in the wave-exposed lake sites. Though a weak correlation between sponge size and depth of occurrence existed in the lake ($r=0.26$, $P<0.05$), it is not likely related to wave activity as the depth range occupied by E. fragilis was quite narrow with respect to the depth range of wave influence.

Comparison of Histological Techniques

In preparing the histological sections of sponges for observation, Weeigert's iron hematoxylin with Gomori's Trichrome was found to give excellent differentiation between sponge embryos (orangish-red) and surrounding cellular material (yellow-green). The methylene blue/ eosin Y combination consistently understained sections, probably as a result of the low density of sponge tissue and the extreme solubility of methylene blue in the alcohol used for tissue dehydrations. Conversely, the standard hematoxylin method generally overstained sections though a few trials produced sections of fair quality (pinkish amoebocytes, violet-

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[5] Gilbert and Hadzisce (1977) found that large specimens of the fresh-water sponge Ochridaspongia rotunda Arndt produced more sexual elements than smaller ones.

blue embryonic material). Mayer's hematoxylin with eosin Y as a counterstain produced sections of varying quality. Generally though, amoebocytes were stained pinkish-orange with violet nuclei while gemmular platelets and the blastomeres of developing embryos were stained blue-violet to violet.

Timing of Life Cycle Events

Fresh-water sponges are monocyclic in noth-temperate climates. They usually hatch from overwintered gemmules in the spring, produce gametes by sexual reproductive processes in early summer, produce gemmules by asexual reproductive processes in the late summer or early fall, then degenerate and die before winter (Pennak, 1953). The life-cycle events of sponges collected from Lake Huron during 1977 did not vary considerably from this developmental time schedule.

Collections made in mid-May produced recently hatched sponges in which little differentiation of cellular material was observed (Figure 5). In a few sponges however, flagellated chambers had already formed. Even in these, many undifferentiated archeocytes were still present (Figure 6).

Sponges collected in July were highly differentiated though still in a growth phase (Figure 7). In one sponge, an embryo in the late cleavage stage was observed (Figure 8). However, the lack of oocytes and embryos in other sponges, and the fact that spermatogenic masses were found in only one specimen (Figure 9) suggests their sexual reproductive processes cease by mid-summer. This finding is similar to those of Simpson and Gilbert (1973) in which sexual reproductive processes in S. lacustris and

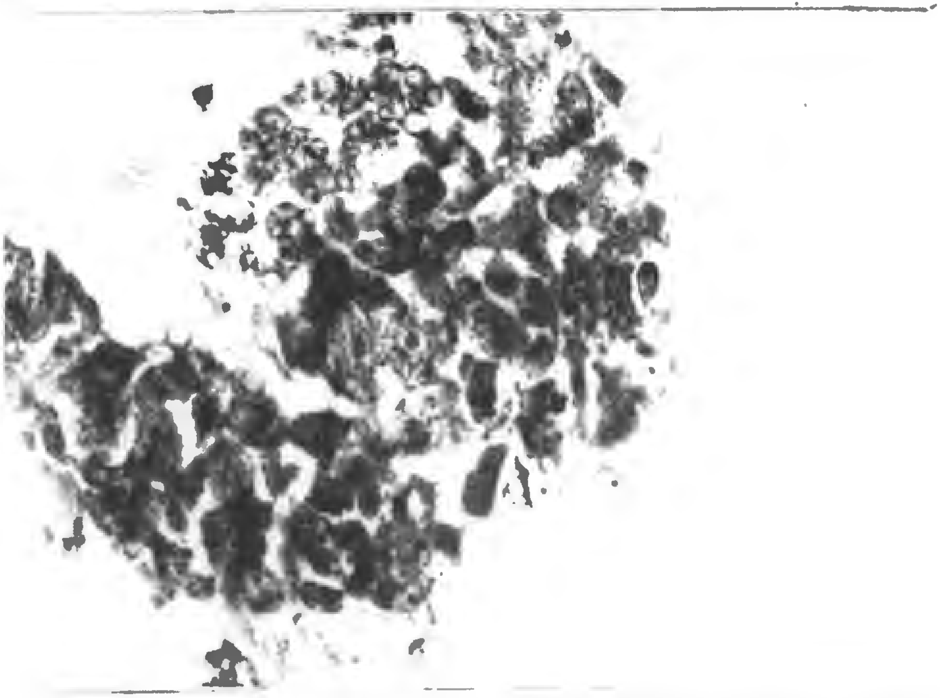

Figure 5. An undifferentiated, recently-hatched sponge. Note the formation of amoebocytes from archeocytes (200x, Methylene Blue/Eosin Y).

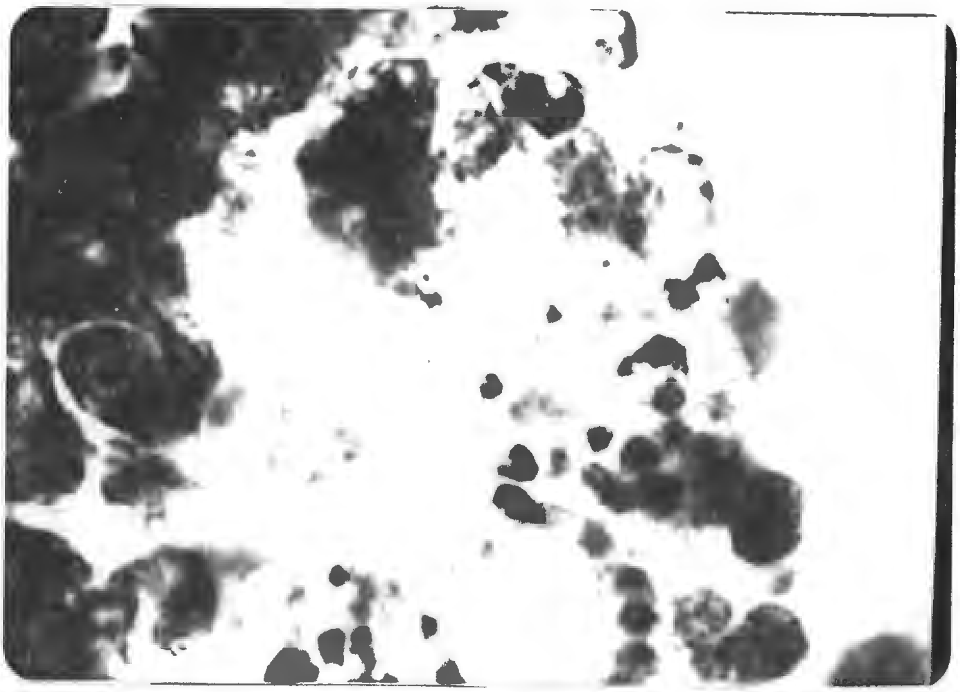

Figure 6. A maturing sponge. Flagellated chambers appear in center-right of photograph (500x, Methylene Blue/Eosin Y).

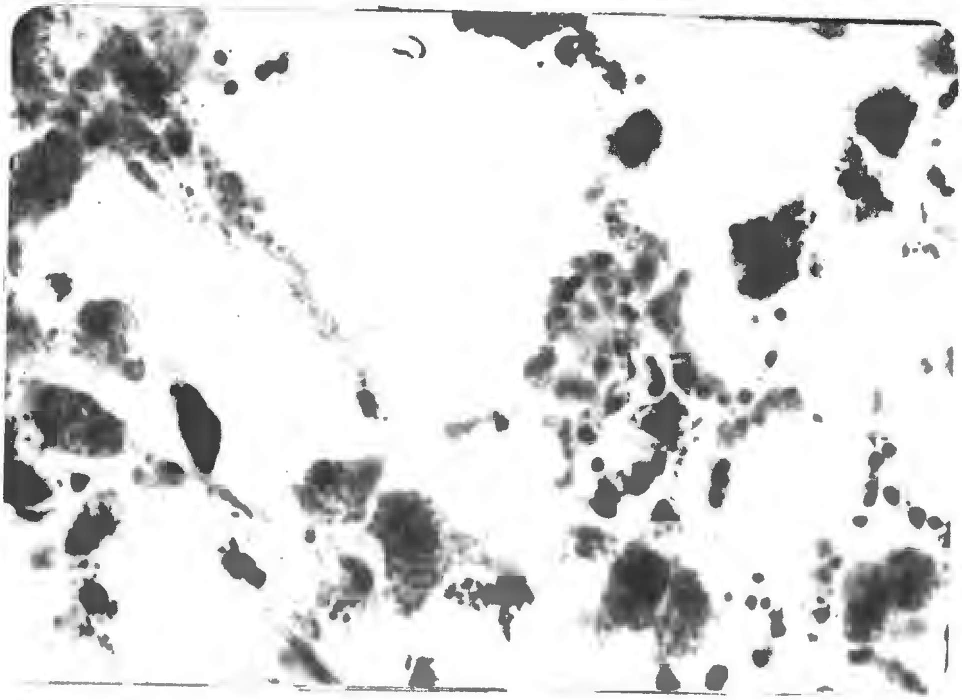

Figure 7. Flagellated chambers are completely developed. Metamorphosing amoebocytes are still present (200x, Methylene Blue/Eosin Y).

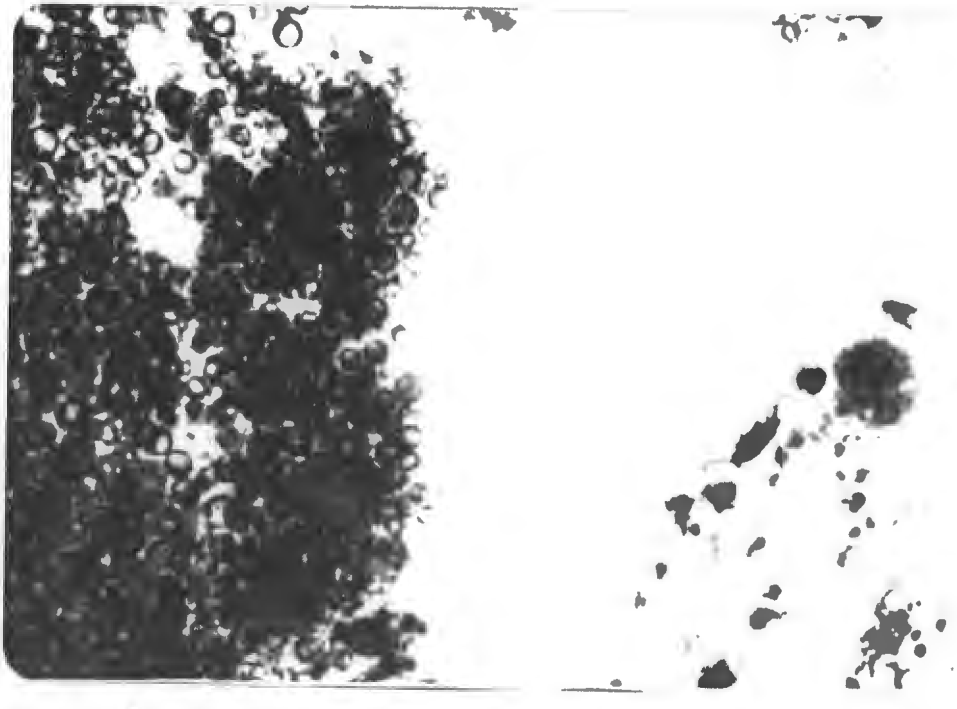

Figure 8. Developing embryo (200x, Mayer's Hematoxylin/ Eosin Y).

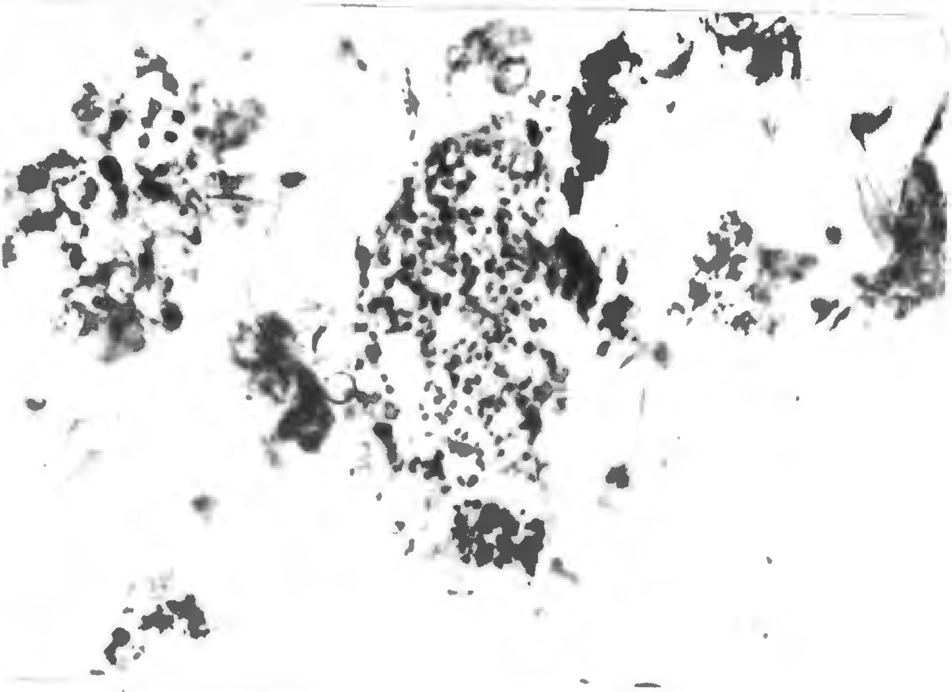

Figure 9. One of two sperm masses observed in a sponge (200x, Standard Hematoxylin/Eosin Y).

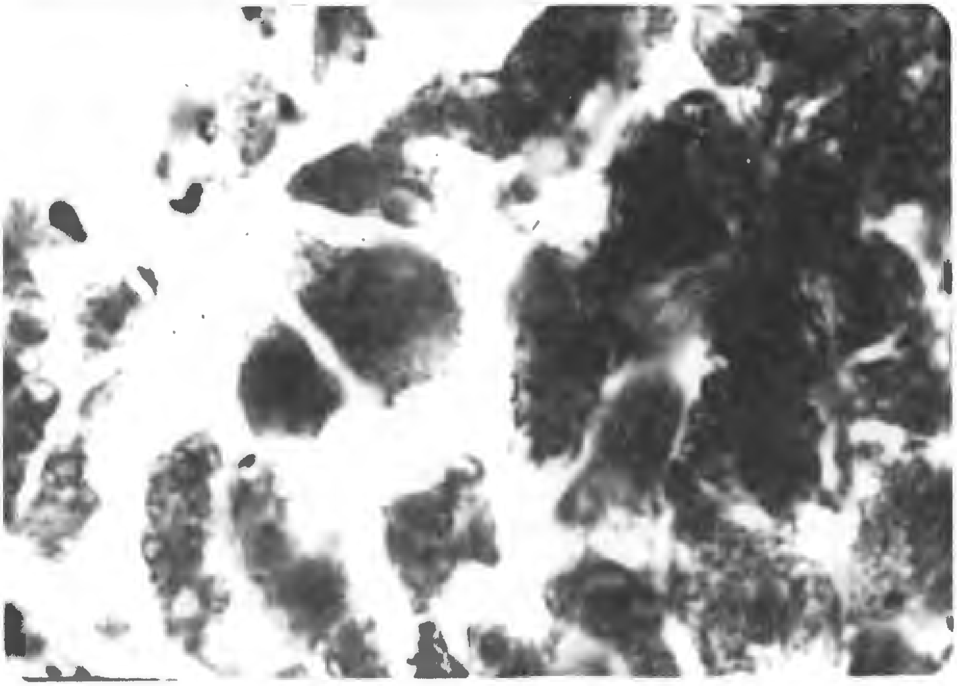

Figure 10. Formation of gemmule by the breakdown of amoebocytes into archeocytes (500x, Methylene Blue/Eosin Y).

Tubella pennsylvanica[6] Potts were found to cease by mid-July.

Most of the sponges collected in September and October were in the process of forming gemmules. Systematically, the amoebocytes congregate and apparently produce or break down into archeocytes with vitteline platelets (Figure 10). Columnar cells subsequently develop around the aggregating archeocytes, then a hard gemmule layer is formed around this condensed cell material. Concurrent with gemmule formation, the sponge continues to degenerate until just the gemmules remain.

Spicule Size Distributions

Potentially valuble eocological information is lost by neglecting spicule size-frequency distributions of sponges within and between specific habitats or by restricting such descriptions to values of their first two moments (Fry, 1970). Fry (1970) has shown that the spicules of the marine sponge *Ophlitaspongia seriata* (Grant) exhibit distinct habitat-related size distributions. Such spicule size-variation phenomena have not been heretofore examined in the Spongillidae.

As reported by Penney and Racek (1968), *E. fragilis* is a cosmopolitan fresh-water sponge with invariably smooth, slighty curved, fusiform, amphioxea megascleres, 180 to 270 microns long and 5 to 12 microns wide. Gemmoscleres of this species are slender to stout and are inconspicuously curved amphioxea or amphistrongyla, typically covered with prominent spines. Their lengths and widths range from 75 to 140 microns and 2 to 7 microns, respectively. Microscleres are absent in this species.

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[6] Penney and Racek (1968) relegate *Tubella pennsylvanica* into synonomy under *Trochospongilla pennsylvanica*.

Spicules from sponges found in Lake Huron and the pond were morphologically similar to those just described. Their spicule size-ranges however, were considerably lower (Table 2).

The silicic acid concentration of the water is thought to be an important factor in the growth of fresh-water sponge spicules (Jewell, 1935; Jorgensen, 1944; Pennak, 1953; Strekal and McDiffet, 1974). Harrison (1974) reviewed much of the literature used by Penney and Racek (1968) and found that E. fragilis occurs in waters with 0.3 to 24.9 mg SiO_2/l. The SiO_2 concentration of Lake Huron at Hammond Bay was 0.72 to 1.26 mg/l from May through October, 1976 (Manny and Owens, manuscript in review). During September and October of the same year, the SiO_2 concentration of the pond was 4.59 to 7.16 mg/l. Since the SiO_2 concentration of Lake Huron and the pond were within the range established by Harrison (1974), and the spicules from sponges found in these habitats were shorter on the average than those reported by Penney and Racek (1968), it follows that factors other than or in combination with SiO_2 affect spicule size-variability.

To exemplify within and between habitat spicule size-variations, the spicule size-distributions of E. fragilis from Lake Huron and the pond are graphically compared (Figures 11 and 12). The Pearsonian coefficients of skewness and kurtosis were calculated to assist in the interpretations of the histograms (Table 3).

Though not so apparent in the histograms, gemmosclere size-distributions of E. fragilis from both habitats were considerably skewed and slightly leptokurtic (peaked). The megasclere size-distribution of pond sponges was also considerably skewed but somewhat platykurtic (flattened). That

POND	Meg (1)	106-234u
	Meg (w)	3-8
	Gem (1)	26-104
	Gem (w)	2-6
LAKE HURON	Meg (1)	114-198
	Meg (w)	3-6
	Gem (1)	16-110
	Gem (w)	2-7
PENNEY & RACEK	Meg (1)	180-270
	Meg (w)	5-12
	Gem (1)	75-140
	Gem (w)	2-7

Tab. 2. Megasclere (Meg) and gemmosclere (Gem) length (1) and width (w) ranges of *Eunapius fragilis* collected from Lake Huron and the pond as compared to the size ranges established by Penney and Racek (1968) from previous studies on this species.

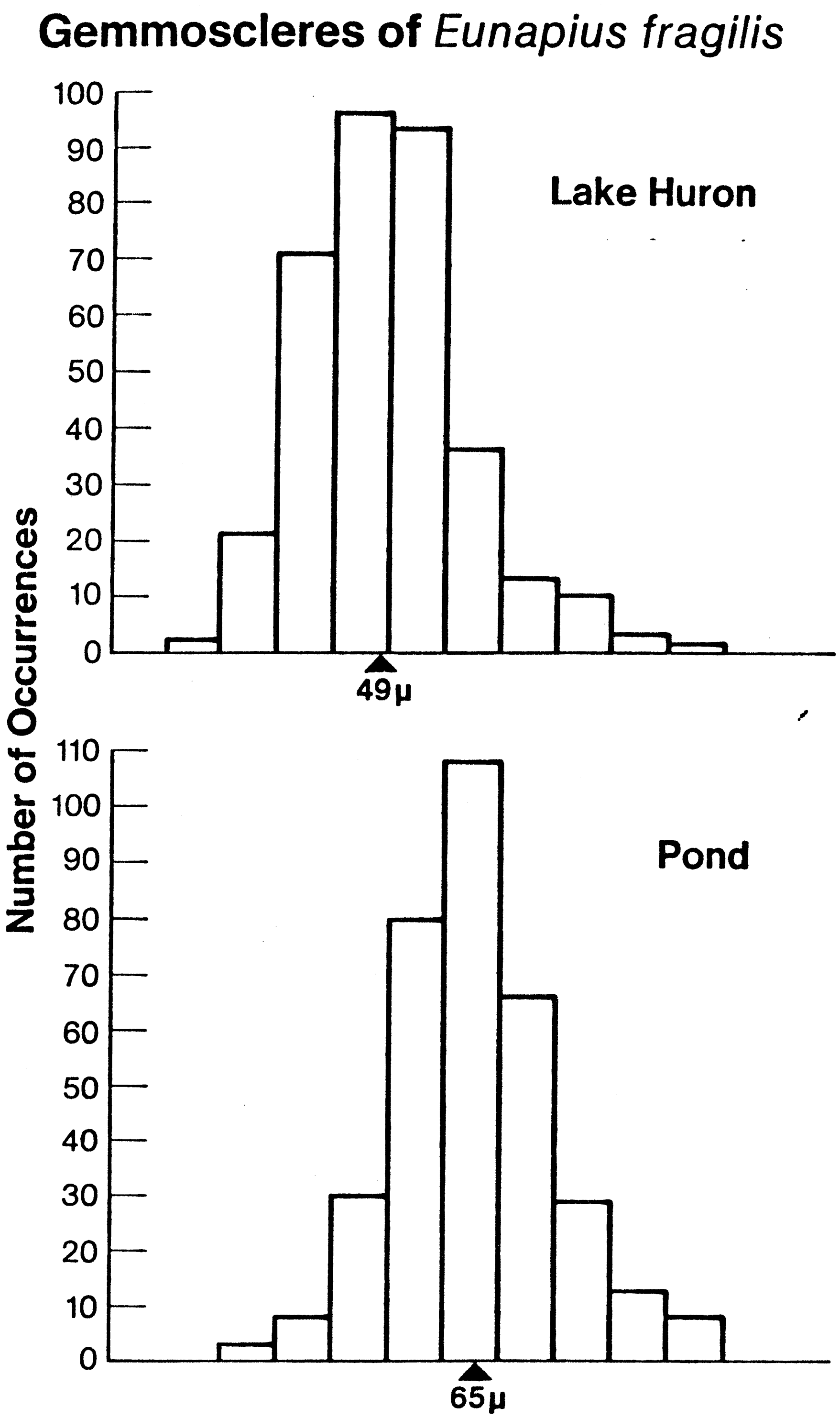

Fig. 11 . Gemmosclere size distributions of Eunapius fragilis. Size class interval is 10 microns. The mean sizes of the two populations are indicated.

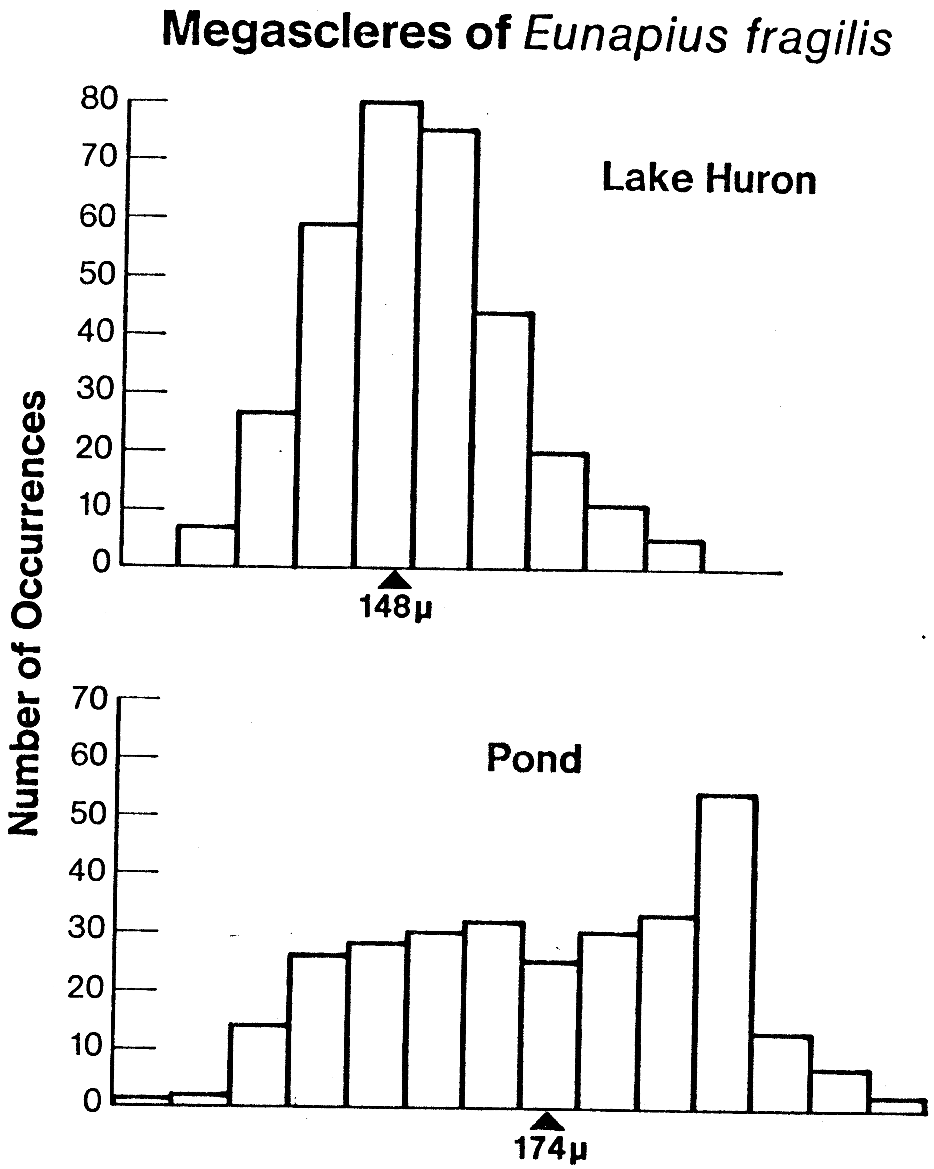

Fig. 12. Megasclere size distributions of Eunapius fragilis. Size class interval is 10 microns. The mean sizes of the two populations are indicated.

		LAKE HURON		POND	
		Megascleres	Gemmoscleres	Megascleres	Gemmoscleres
$\bar{x}_s$	$\bar{x}$	148	48	174	65
	s_x	7.18	9.55	18.75	9.83
	s_x	2.72	3.61	7.09	3.71
m_3	x	0.16	0.62	0.56	0.40
	s_x	0.19	0.56	0.48	0.35
	s_x	0.07	0.21	0.18	0.13
m_4	x	2.90	3.58	2.92	3.53
	s_x	0.74	1.33	0.91	0.70
	s_x	0.28	0.50	0.34	0.26

Tab. 3. Summary table of the mean (x), standard deviation (s_x), and standard error (s_x) of the spicule size distributions of Eunapius fragilis. Table includes calculated values of mean skewness (m_3) and kurtosis (m_4) computed at the 95% confidence level. An m_3 greater than 0.5 indicates considerable skewness. An m_4 greater than 3.0 indicates the curve is leptokurtic. An m_4 less than 3.0 indicates the curve is platykurtic.

of the Lake Huron sponges was nearly symmetrical, though once again, somewhat platykurtic.

Megasclere and gemmosclere size-distributions of sponges from both habitats showed a lack of homogeniety of variance as determined by a one-way ANOVA. In conjunction with the asymmetrical size-distributions displayed by the histograms and calculated values of skewness and kurtosis, this finding clearly supports Fry's (1970) assertion that "we have no right to assume normal distributions of size frequency of sponge spicules."

Spicule size-variations within habitats may be related to substrate type, depth of sponge occurrence, genotype, time of year, presence of intracellular zoochlorellae, etc. Upon a more intense investigation, the single or multiple effect of these conditions on spicule size-distributions can be determined.

A problem inherent in using spicule size-distributions to compare sponges within and between habitats is the amount of time and labor necessary to properly prepare the slides and take accurate size measurements of spicules. These two factors alone may preclude its practical application. In any event, this technique should be investigated further to determine its usefulness as an ecological tool.

Associated Organisms

There have been few studies on the microscopic plants and animals associated with fresh-water sponges. In his early ecological studies, Old (1932b,1932c) compiled a list of organisms collected from various sponges. Gilbert and Allen (1973) observed numerous chironomid larvae and hemipteran embryos within *S. lacustris* during their investigations of symbiotic zoochlorellae. Larvae of Sisyridae (Spongilla flies) are known to be entirely dependent upon certain sponges as a food source (Pennak, 1953).

Aquatic invertebrates collected from *E. fragilis* during 1977 are presented in Table 4. Several *Chaetogaster* were found preying upon sponges (histological sections confirmed the presence of sponge cells in their digestive tracts). Other *Chaetogaster* were found but it is not known whether they were on the sponge surface or within the numerous canals and pores of the sponge's aquiferous system. The same is true for the other organisms listed.

Numerous diatoms, both pennate and centric forms, were observed in the histological sections (Figure 9) and spicule slide preparations. Their presence within sponges has apparently been overlooked by other investigators. Gilbert and Allen (1973) made no mention of the presence of diatoms when they studied the primary productivity, organic matter, and the chlorophyll content of a green sponge.

The green color of many sponges is imparted by the phycobiont *Zoochlorellae parasitica* (Prescott, 1973). This symbiosis is not clearly understood though it is thought to be mutualistic. As stated by Rasmont (1968), the "symbiosis between a primary producer and a heterotroph provides such an efficient shortcut in the cycles of oxygen, carbon dioxide, phosphorus, and nitrogen, that both organisms must draw some benefit by it." Gilbert and Allen (1973) hypothesize that sponges with symbiotic zoochlorellae may be largely independent of external carbon for an energy source because of the release of carbon compounds into sponge

- Rotatoria

- Coelenterata
  - C. Hydrozoa
    - *Hydra*

- Nematoda

- Annelida
  - C. Oligochaeta
    - *Chaetogaster*

- Arthropoda
  - C. Crustacea
    - S.C. Ostracoda
      - O. Podocopa
    - S.C. Copepoda
      - O. Eucopepoda
        - S.O. Cyclopoida
    - S.C. Branchiopoda
      - O. Cladocera
        - F. Chydoridae
  - C. Insecta
    - O. Trichoptera
      - F. Hydropsychidae
      - F. Unidentified (early instar stage)

Tab. 4. Listing of aquatic invertebrates found on or within *Eunapius fragilis* collected from Lake Huron during 1977.

cells by the zoochlorellae. They also state that under certain conditions, zoochlorellae in the sponge would produce more oxygen than that required for the respiration of both the sponge and the zoochlorellae.

In addition, the presence of symbiotic zoochlorellae has been indicated as being important in prolonging the onset of gemmulation, possibly by providing the necessary nutrients or oxygen during normally critical periods (Rasmont, 1968; Simpson and Gilbert, 1973). However, in this study, all the sponges encountered seemed to be maturing at the same time irrespective of the presence or absence of zoochlorellae.

Chemical

The results of the chemical analyses performed on the Lake Huron and pond water samples are summarized in Table 5. This information has been incorporated with the sponge collection data compiled by Harrison (1974) into Table 6, expanding in some cases the "tolerance ranges" of the five species involved in this study.

E. fluviatilis, the most abundant species in the pond, has been reported to prefer brackish environments (Harrison, 1974). This species has only been found in waters where conductivities exceed 350 micromhos/cm or nearly three times that of the pond. Conversely, findings of E. mulleri have been limited to waters of comparatively low conductivities (30 to 100 micromhos/cm) (Harrison, 1974). E. fragilis and H. baileyi have been reported from waters with conductivities of 16 to 760 micromhos/cm (Old, 1931; Jewell, 1935, 1939; Wurtz, 1950; Penney, 1954) and 14 to 300 micromhos/cm (Harrison, 1974), respectively. There have been no reports on the occurrence of H. tubisperma with respect to waters of varying conductivities.

SELECTED CHEMICAL PARAMETERS-1976

	Lake Huron		Pond	
	Range	Mean	Range	Mean
Alkalinity (ppm $CaCO_3$)	76-119	-	178-198	187
Conductivity (micromhos/cm)	178-273	-	107-112	111
pH	7.0-8.3	-	7.4-7.6	-
SiO_2 (ppm)	0.72-1.26*	-	4.59-7.16	5.85
NO_3-NO_2 (ppb-N)	174-232	-	T-46	16
NH_4 (ppb-N)	N.D.-10	-	N.D.-49	15
K (ppm)	0.9-1.1	1.0	0.5-0.9	0.7
Na (ppm)	3.2-4.1	3.6	5.2-5.4	5.3
Ca (ppm)	23.4-30.5	25.6	42.8-46.2	44.7
Mg (ppm)	6.4-8.6	7.0	12.3-13.0	12.3
Ca Hardness (ppm $CaCO_3$)	58.5-76.3	64.0	107.0-115.5	111.7
Mg Hardness (ppm $MgCO_3$)	26.2-35.3	28.7	50.4-53.3	50.4
Cl (ppm)	5.9-6.4	-	11.0-16.9	13.5
HCO_3 (ppm $CaCO_3$)	75.2-105.8	-	177.7-197.6	186.3
CO_2 (ppm)	0.8-28.0	-	12.6-17.3	15.5

T Trace
N.D. Not Detectable

Table 6

	Ephydatia mulleri	Ephydatia fluviatilis	Heteromeyenia tubisperma	Heteromeyenia baileyi	Eunapius fragilis
Alkalinity ($ppmCaCO_3$)	178-198***	19.0-230.0	110.0-198**	18.0-198**	14.7-230
Conductivity (micromhos/cm)	31-112**	107-2,950*	107-112***	14-3,000	16-760
pH	6.1-8.5	5.9-8.3	6.6-8.5	4.2-8.4	4.2-9.2
SiO_2 (ppm)	0.7-11.6	4.59-7.16***	4.59-7.16***	0.25-13.0	0.3-24.9
NO_3/NO_2-N (ppb)	<2-51***	<2-51***	<2-51***	<2-51***	1-385
NH_4-N (ppb)	<2-49***	<2-49***	<2-49***	<2-49***	1-169
K (ppm)	0.5-0.9***	0.5-0.9***	0.5-0.9***	0.5-0.9***	0.5-1.1***
Na (ppm)	5.2-5.4***	5.2-5.4***	5.2-5.4***	5.2-5.4***	3.2-5.4***
Ca (ppm)	5.6-46.2**	42.8-46.2***	8.0-46.2**	1.5-72.2	1.6-46.2**
Mg (ppm)	12.3-13.0***	12.3-13.0***	12.3-13.0***	0.2-38.0	1.0-13.0**
Cl (ppm)	11.0-16.9***	11-2,600*	11.0-16.9***	1-1,100	0.6-16.9**
HCO_3 (ppm $CaCO_3$)	177.7-197.6***	177.7-197.6***	177.7-197.6***	43-197.6**	43-197.6**
CO_2 (ppm)	0.0-28.0	6.5-17.3**	0.0-17.3**	2.1-26.5	0.0-28.0**
D.O.	-	1.0-4.3	-	-	2.5-16**

* = new minama established
** = new maxima established
*** = range established

The pH of water samples from Lake Huron and the pond were within previously reported ranges for all sponge species collected (Harrison, 1974). Silicon dioxide concentrations of both habitats were within the ranges established for E. mulleri, H. baileyi, and E. fragilis (Harrison, 1974). Such ranges for E. fluviatilis and H. tubisperma have not heretofore been established. Silicon dioxide is physiologically important in the formation of spicules (Jewell, 1935; Jorgensen, 1944; Pennak, 1953; Strekal and McDiffet, 1974; Elvin, 1971). Its relation to spicule size was discussed in the Spicule Size Distributions section.

Sodium and potassium, though not likely to occur in high concentrations in typical fresh-water environments (Hem, 1975), have been neglected as factors important to the distribution of fresh-water sponges. It is known that certain environmental conditions can markedly alter the permeability of cells; divalent ions, such as calcium or magnesium, tend to reduce permeability while the monovalent ones, such as sodium or potassium, increase it (Hoar, 1975). A proper balance is essential, and a lack or excess of any of these cations in the environment may greatly reduce the survival time in otherwise innocuous habitats (Cuthbert, 1970). Also, the oxides of sodium and potassium are present in trace amounts in the spicules of sponges (Pennak, 1953) possibly acting as cementing agents of the siliceous crystallite units of which spicules are composed (Fjerdingstad, 1970). As indicated in Table 5, there was little fluctuation in the concentrations of either sodium or potassium in Lake Huron or the pond during the course of this study.

The importance of nitrate, nitrite, or ammonium nitrogen in the physiology of fresh-water sponges has not been demonstrated. In view of the fact that both ammonium and nitrate-nitrite nitrogen were barely, if at all detectable at times (minimum level of detection was 2 µg/l), indicates concentrations of these nitrogen fractions in excess of 2 µg/l are not essential for sponge survival. Likely, the nitrogen they use in protein synthesis is derived from strictly organic sources as is done in their probable evolutionary antecedents, the zooflagellates (Kidder, 1967).

In Jewell's (1939) classic study on the importance of calcium in the distribution of fresh-water sponges, E. mulleri was found only in waters of 5.6 to 16.3 mg/l calcium. Similarly, H. tubisperma has been collected only from waters with less than 15 mg/l calcium (Old, 1931; Wurtz, 1950) while E. fragilis has been reported occurring in habitats with 1.6 to 45.6 mg/l calcium (Harrison, 1974). Contrary to these reports, these three species were growing in the pond where the calcium concentration was as high as 46.2 mg/l during the course of this study. As indicated by the occasional colonization of E. fragilis on limestone (Stephens, 1920; Cheatum and Harris, 1954), this species probably can tolerate considerably higher calcium concentrations. H. baileyi has been found in waters of 1.5 to 72.2 mg/l calcium indicating it can withstand an extremely wide range of this cation (Harrison, 1974). There are no reports relating the occurrence of E. fluviatilis to various concentrations of calcium in natural waters.

The importance of calcium with respect to sodium and potassium in controlling the permeability of cells has been discussed above. However, calcium is also important in that it appears to be essential for the formation of cell membranes which are presumably formed by the precipitation of the cell material in its presence (Giese, 1968). With respect to cell division, it has been found that agents which release calcium facilitate cell division whereas agents which bind calcium inhibit it. A possible explanation is that cell division is initiated by coagulative processes similar to the formation of a clot in blood, and any stimulus which induces clot formation is likely to initiate formation of spindles and hence, cell division (Giese, 1968). Laboratory studies on *E. fragilis* (Strekal and McDiffet, 1974) have shown that development of young sponges was supported in calcium and magnesium concentrations of at least 0.2 mg/l and, in the absence of these two cations, development was inhibited. Humphreys (1970) also recognized the importance of calcium in the coalescence and reaggregation of sponge cells and the subsequent effect of calcium on regeneration and growth.

The importance of magnesium in regulating cell permeability and as a factor facilitating sponge development has been discussed. Jewell (1939) found *E. mulleri* and *H. baileyi* in waters of 2.45 to 5.44 and 0.2 to 38.0 mg/l magnesium, respectively. The latter finding suggests *H. baileyi* can tolerate nearly the entire range of magnesium concentrations one would expect to find in typical North American lakes and streams (Hem, 1975). *E. fragilis* has been reported only from waters of 1.05 to 10.0 mg/l magnesium (Jewell, 1939; Harrison, 1974) which is within the magnesium range of Lake Huron but slightly below that of the pond. Similar ranges for *H. tubisperma* and *E. fluviatilis* have not been established.

The range of chlorides in both the pond and Lake Huron were within previously reported ranges for *E. fragilis*, *E. fluviatilis*, and *H. baileyi* (Harrison, 1974). There are no reports relating the occurrence of *E. mulleri* and *H. tubisperma* to waters of varying chloride concentrations.

Free carbon dioxide, bicarbonate, and dissolved oxygen concentrations do not seem to be important as limiting or controlling factors in sponge distribution (Jewell, 1935, 1939; Neidhoefer, 1940; Moore, 1950). And, as already discussed, the presence of symbiotic zoochlorellae may very well limit the sponges's need for external carbon and possibly oxygen.

SUMMARY

1. The occurrence of fresh-water sponges in Lake Huron is reported for the first time. Eunapius fragilis was collected from the rock-cobble eulittoral zone of Lake Huron near Hammond Bay in 33 to 67 cm water.

2. The sizes and relative abundance of E. fragilis within the lake sites is apparently related to wave exposure. As wave exposure increased, the sizes and relative abundance of sponges decreased.

3. E. fragilis, Heteromeyenia tubisperma, Heteromeyenia baileyi, Ephydatia fluviatilis, and Ephydatia mulleri were collected from a seasonally inundated pond adjacent to Hammond Bay. The sponges were generally found encrusted on the underside of wood substrates in 15 to 33 cm water.

4. The phenomenon of epizoism was observed in one specimen of H. tubisperma during high sponge-density conditions. Among Porifera, this behavior has been observed only in marine species.

5. Physiological stress induced by dehydration does not stimulate gemmulation in fresh-water sponges. Other exogenous stress factors and probably certain endogenous factors are responsible.

6. The oligochaete Chaetogaster was found preying upon several sponges in Lake Huron. Other invertebrate organisms as well as various diatoms and the symbiotic zoochlorellae were found on or within various sponges.

7. Spicule size distributions of fresh-water sponges can deviate significantly from normality. Such variations may in part be related to environmental conditions.

8. The timing of life-cycle events of sponges in Lake Huron was similar to that reported for Spongilla lacustris and Trochospongilla pennsylvanica.

9. The establishment of or changes in the physiological range of E. mulleri with respect to alkalinity, conductivity, nitrate/nitrite nitrogen, ammonia nitrogen, potassium, sodium, calcium, magnesium, chlorides, and bicarbonate are presented.

10. The establishment of or changes in the physiological range of E. fluviatilis with respect to conductivity, silicon dioxide, nitrate/ nitrite nitrogen, ammonia nitrogen, potassium, sodium, calcium, magnesium, chlorides, bicarbonates, and carbon dioxide are presented.

11. The establishment of or changes in the physiological range of H. tubisperma with respect to alkalinity, conductivity, silicon dioxide, calcium, nitrate/ nitrite nitrogen, ammonia nitrogen, potassium, sodium, magnesium, bicarbonates, and carbon dioxide are presented.

12. The establishment of or changes in the physiological range of H. baileyi with respect to alkalinity, nitrate/ nitrite nitrogen, ammonia nitrogen, potassium, sodium, and bicarbonate are presented.

13. The establishment of or changes in the physiological range of *E. fragilis* with respect to potassium, sodium, calcium, magnesium, chlorides, bicarbonate, carbon dioxide, and dissolved oxygen are presented.

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