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Transmittance Electron Microscopy: A Comparison of the Rotifers
Asplanchna herricki and Asplanchna brightwelli

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College of St. Benedict/St. John’s University

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In the Department of Biology

by
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Transmittance Electron Microscopy: A Comparison of the Rotifers
Asplanchna herricki and Asplanchna brightwelli

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SUMMARY:
The rotifers, *A. brightwelli* and *A. herricki* were surveyed under SEM and TEM. The overall external features were compared under SEM to determine significant differences in morphology. The widths of the coronal surface and midsection were larger in *A. herricki* than that of *A. brightwelli*. *A. herricki* was prepared for view under TEM, and integument was compared to *A. brightwelli*. The integument of *A. brightwelli* was substantially thicker than that of *A. herricki*, which disagreed with the hypothesis. Further studies must be conducted to determine the nature of this difference.
Acknowledgments:

I would like to thank my advisor, Dr. Wurdak, for the patience and time that she expended during this project. Without her guidance, prodding, and calmness, this project would have concluded prematurely. I have conquered my fear of the dark and have learned the value of cleanliness.

My readers, Dr. Jeanne-Marie Lust and Dr. Holly Adrian, also have been integral in the completion of this project. I thank them for the time they spent reading and marking drafts.

I would like to thank my parents who gave me the opportunity to complete a project. Even though they didn’t know exactly what I was doing, they always sent me back to the lab with enthusiasm and luck.

My friends also have been very important in completing this project. I thank everyone who has shown concern and interest. At least I know that they know the importance rotifers have in the world.
Look up in *Sky and Telescope*, one of last summer's issues I think it was, a helluva funny piece in this connection they reprinted from some book in which a bunch of rotifers--you know what rotifers are, don't you?--microscopic aquatic doohickeys with an anterior retractile disc of cilia that makes them look like their head are spinning--of course they aren't really, any more than owls can turn their heads clear around, it just gives that impression--*anyway*, a bunch of these rotifers are imagined in learned conversation concerning why their puddle had to be exactly the way it was--temperature, alkalinity, mud at the bottom sheltering methane-producing bacteria, all the rest of it--it was clever as hell like I said--and from the fact that if any of these things were even a little bit different--if the heat necessary to vaporize water was any lower, for example, or the freezing temperature of water any higher--this Little Puddlian Philosophical Society, I think it was called, but you can check that when you look it up, deduced that the whole operation was providential and obviously the universe existed to produce their little puddle and *them!* That's more or less what you're trying to tell me, young fella, except you ain't no rotifer (Updike 299).
INTRODUCTION

Rotifers are small aquatic animals first discovered by Leewenhoek, the founder of the microscope. Rotifers received their name as a result of the cilia located at the anterior of the animal, which moves in a pattern resembling two wheels turning. In Greek, *Rotifera* translates to “wheel bearer” hence the animal has acquired the name rotifer or wheel animal (Pechenik 1988). Since their discovery around the year 1703, rotifers have become an interest of many invertebrate and ecological biologists. They are important to the balance of an aquatic system serving as an integral part of the food chain by ingesting algae and other organisms and by serving as prey for a few protozoa, larger cladocerans, cyclopoid and calaniod copepods, the phantom midge, and some small fish (Pennak 1978).

Approximately 2500 species of rotifers have been described; they range in size between 0.5 and 1.0 mm. Rotifers are divided into two classes: the *Digononta* and the *Monogononta*. The female *Digononta* are characterized by having paired ovaries whereas the female *Monogononta* have a single ovary. Ninety percent of all rotifers belong to the class *Monogononta* and most are free swimming. Although rotifers inhabit
primarily freshwater, they may live under various conditions. Rotifers are found from the pelagic zone of lakes to the interstitial water within mosses, lichens, and soil (Pennak 1978). In order to survive under such conditions, rotifers have adaptations to respond to extreme changes within their environment. One such adaptation is based upon their reproductive cycle (Figure 3). Employing parthenogenesis, diploid females produce eggs that will hatch into other diploid females. These females are referred to as amictic because the genes do not mix. This process continues until environmental conditions occur to trigger the mictic response. When such conditions are present, diploid (mictic) females produce haploid eggs that hatch into males. The males’ purpose is to fertilize haploid eggs that other mictic females produce. The fertilized eggs or resting eggs, are dormant and have the capacity to overwinter. When conditions improve, the resting eggs hatch into diploid amictic females and another cycle begins.

Usually rotifers possess the basic body regions of head, trunk, and foot (see figure 1). The head region is normally distinct from the large trunk region. The head region contains the mouth, trophi (jawlike processes that serve to grab or crush food), and the ciliated corona located at the anterior. The trunk contains the bulk of the animal including the stomach, gastric
glands, ovary, yolk gland, bladder, and developing eggs in the female. The male rotifer usually does not develop as many internal structures as the female. Rotifers are pseudocoelomates; their interior cavity is not lined with mesoderm. The foot is posterior and usually tapers with the shape of the animal and houses the pedal gland. The pedal gland secretes mucous to enable the sessile rotifers to adhere to a substrate. The foot has other uses and takes on a variety of shapes. In some species it is absent altogether.

In this survey, the corona and the midsection widths of the rotifers *Asplanchna herricki* and *A. brightwelli* were compared using scanning electron microscopy and the integument was compared using transmission electron microscopy. *Asplanchna* belong to the class *Monogononta* and are omnivorous. Their diet includes invertebrates smaller than themselves such as the smaller rotifer species *Keratella quadrata, Philodina,* and *Branchionus* (Figure 2). *A. herricki* was first described in 1888 by De Guerne. Females range from 500-2000 μm in length while males reach a length of 250-350 μm (Koste 1978). They inhabit the pelagic zone of oligotrophic to lightly eutrophic lakes in Europe and North America. Rujinschi (1972-73) found *A. herricki* in Lake Bizal, Romania, from May to August in conditions of 19.5° C, up to 8.3 pH, and dissolved oxygen of 9.99 mg/l. *A. herricki* lack a foot
and have a pedal gland that is an evolutionary remnant of the foot. Their body is rounded and the trunk region is the dominant feature of the animal. Because *A. herricki* has not been previously studied by electron microscopy, general characteristics of *A. herricki* were compared to *A. brightwelli*, a close relative. In contrast to *A. herricki*, *A. brightwelli* are smaller rotifers, and females range from 500 to 1500 μm in length. *A. brightwelli* are found earlier in the season in Lake Stumpf, Collegeville, MN, than *A. herricki*. They are omnivorous like all *Asplanchna*. They lack a pedal gland at the posterior of the animal and the trophi are markedly more slender than the trophi of *A. herricki*. *A. brightwelli* have a horseshoe shaped yolk gland whereas *A. herricki* have a round yolk gland. The resting eggs of the two species also differ in external appearance.

The integument, outer body surface, of these two rotifers was surveyed using TEM. The rotifers are covered with a cuticle that is secreted from the hypodermal bulbs of the syncytial integument. The thickness of the integument varies with the region of the animal and the species. In some species, but not in *Asplanchna*, the cuticle is thick, rigid, and is called the lorida. In *Asplanchna, Synchaeta*, and others, the lorida is poorly developed and consequently the integument is very elastic. Gradations of these two
extremes have been observed in many rotifers. Since A. *brightwelli* and A. *herricki* inhabit similar aquatic environments and are members of the same genus, the integument of these two rotifers was expected to have a similar appearance. These two rotifer species were examined under SEM to characterize their exterior morphological characteristics. The morphology of the two rotifers was hypothesized to differ in length and coronal width under SEM, but the morphology of the integument was hypothesized to be similar under TEM.

**MATERIALS AND METHODS**

Using a plankton net, rotifers were harvested from Lake Stumpf located in Stearns County, Minnesota. The rotifers were identified and the A. *herricki* were fixed in 2% glutaraldehyde, pH 7.4, and stored at 4º C overnight. The following day the rotifers were rinsed in 2% cacodylate buffer for three 1 hour rinses and postfixed in 1% OsO₄ for 1 hour. The specimens were then dehydrated according to the procedure found in Hunter (1984) and embedded in Spurr’s epoxy resin. This resin was made according to the manufacturer’s instructions for medium hardness.
The specimens were sectioned with outfitted glass knives at 1 μm for light microscopy and at 60 nm for electron microscopy. The 1 μm sections were stained with either toluidine blue stain or Neal's dye and viewed under light microscopy. The silver colored 60 nm sections were gathered on 200 or 400 mesh grids covered with 0.05% formvar prepared according to Hunter (1984) and were stained with uranyl acetate and lead citrate (Hunter 1984). The grids were examined using a JEM 120CX Jeol electron microscope.

Photographs of the integument were taken at various magnifications. Integument thickness was measured at various areas of the animal. *A. brightwelli* was also viewed under TEM from previously prepared grids. The same sectioning and staining procedure was followed for the viewing of these animals.

SEM photomicrographs provided by Wurdak (1994) were used to measure the midsection width and the corona width of *A. herricki* and *A. brightwelli*. A mean was taken from the measurements of the different animals and the data were statistically compared using a T-test. The trophi of the two animals were photographed and compared in morphological features and length.
RESULTS

The measurements of the rotifers taken under SEM agreed with those reported in the literature for *A. herricki* and *A. brightwelli* (Koste 1978). *A. herricki* had a wider coronal and midsection width than *A. brightwelli*. *A. herricki* had a mean coronal width of 236 μm whereas the *A. brightwelli* had a mean width of 169 μm (Table 1.1). A t-test rejected the hypothesis that the measurements of the coronal surface could have occurred by chance (p < 0.05). Similarly, the midsection width also showed substantial variation between the two species. The *A. herricki* had an average width of 399 μm whereas the *A. brightwelli* had an average width of 231 μm. According to a t-test, these measurements did not occur by chance (p < 0.05).

In Figure 4A and B, *A. herricki* is shown under SEM. In figure 4A, the head region, with its ring of cilia, is in view and the corrugated neck region is clearly illustrated. In figure 4B, a full ventral body view of the rotifer with its ciliated head is shown. The large body is visible from the angle of this micrograph. Figure 5A presents a lateral full length image of *A. herricki* with the mouth region surrounded by cilia; the expanded trunk region is notable. In figure 5B the mouth region is magnified by 84X. The
trophi are visible within the mouth with the cilia located at either side.

Figures 6A and B are scanning electron micrographs of *A. brightwelli*. In figure 6A, a lateral view of the entire length of the animal is seen with the head region located at the left of the photo. The trunk region of the *A. brightwelli* is thinner and shorter than the *A. herricki*. In figure 6B the mouth region is magnified by 52X. The cilia are located on the lateral portion of the head and the trophi are slightly hidden.

Under the TEM, the integument of the *A. herricki* was much thinner than that of *A. brightwelli*. In figures 7A and B, the integument of the two rotifers is illustrated (X 20K). The cuticle, plasma membrane, and a hypodermal bulb of *A. herricki* are shown in figure 7A. Contrasting figure 7A, figure 7B illustrates the thicker cuticle and plasma membrane of *A. brightwelli*. In figure 8A, the integument of *A. herricki* is observed at 20 K. The membrane is thicker, and the cuticle and hypodermal bulb are also present. In figure 8B, the cuticle, plasma membrane, secretory bulbs, and mitochondria in the integument of the *A. brightwelli* are shown in greater detail at 30K. In figure 9A and B a magnified area of the integument with a hypodermal bulb of *A. herricki* is in view. In figure 9A the hypodermal bulb is located in the center of the photo (30 K). The two layers of the plasma
membrane are clearly visible along with the cuticle. The hypodermal bulb is magnified X50 K in 9B, the two layers of the plasma membrane are distinct, and the cuticle is present on the exterior of the membrane. From the data collected, the integument of the *A. brightwelli* is 2.5 times thicker than that of *A. herricki*.

**DISCUSSION**

Since *A. herricki* has the potential of becoming larger than the *A. brightwelli*, the advantages and the disadvantages of large body size were questioned. In previous studies, gigantism in the *Asplanchna* species was researched to determine the impact of body size on food uptake and reproductive efficiency (Gilbert and Stemberger 1986). In Gilbert and Stemberger’s study the larger form of *A. silvestrii* had an advantage when larger prey were present, but they had to ingest 5 times the prey biomass to maintain the same level of reproduction as a smaller form of *A. silvestrii*. The rotifers were cultured in the laboratory and sufficient food was given to the animals. These results were gathered in a laboratory environment. The impact of prey size in the natural environment was not assessed.
A. herricki and A. brightwelli coexist for a brief time in the early summer. A. brightwelli inhabits the lakes from early spring to early July. A. herricki inhabits the lake from mid June to October. The seasonal change from A. brightwelli to A. herricki may be the result of changes in food supply and of inherent differences in reproductive efficiency. The dissimilarities in size and reproductive efficiency could allow the animals to survive during different times of the year when conditions are slightly altered. Because they share the same genus, same habitat, and similar morphology, A. brightwelli and A. herricki should exhibit similar characteristics when viewed under electron microscopy.

The data for the SEM survey supported the hypothesis that A. herricki would be larger than the A. brightwelli. The statistical analysis performed indicates that the two rotifers’ mean size does not overlap. A. herricki females exceeded A. brightwelli in both the coronal width and the midsection width. Clearly, A. herricki has the ability to attain a larger size than A. brightwelli. These data agreed with Koste (1978).

The larger coronal surface of A. herricki could allow the rotifer a feeding advantage because the rotifer would cover more area while using the same amount of energy. The larger coronal surface of A. herricki could also
increase the number of prey encountered (Gilbert 1980). The rotifer may also have the capability to ingest larger prey, which would include the smaller *A. brightwelli*. A study conducted by Gilbert and Confer (1986) found that if the larger *A. silvestrii* was placed in culture with the smaller *A. brightwelli*, the *A. silvestrii* would ingest and eventually exclude the *A. brightwelli* from the culture. Interspecies competition in the lake could account for the seasonal distribution of the *Asplanchna* rotifers. Early in the season the available food is of lesser value and abundance. Stemberger and Gilbert (1987) found that *A. silvestrii* needed a greater amount of food in order to maintain the same reproductive efficiency as *A. brightwelli*. The ability to ingest larger prey because of a larger coronal surface may also compensate for the larger body size. The ability to ingest larger prey and an increase in chance encounter of prey may allow the larger rotifer to exist during certain times of the year. Therefore, low population of prey in the early season would favor *A. brightwelli* because the prey population would not be able to support a larger predator like *A. herricki*. Later in the season when food becomes more abundant, *A. herricki* would have an advantage because the prey population would increase, which would support a larger predator.
The larger midsection width of *A. herricki* would perhaps provide the rotifer with the ability to contain larger prey in its gut. Additional studies could be undertaken to determine if *A. herricki* has a larger stomach cavity than *A. brightwelli*. The larger midsection could be a disadvantage in swimming and maneuvering. Since the swimming speeds of *A. herricki* and *A. brightwelli* are not known, a future study could be conducted to survey how swimming speeds differ and if this might affect prey capture. Other physical structures such as cilia could also be surveyed to determine if discrepancies are found in the number and length of these structures.

The data for the TEM survey did not support the hypothesis that the integument of the two rotifers would be similar in appearance. The integument of *A. brightwelli* was considerably thicker than that of *A. herricki*. These results could be attributed to the different seasonal appearances of the two rotifers. Perhaps the thicker integument is an advantage earlier in the season and thinner integument is an advantage later in the season. The cooler water of the spring would provide extra buoyancy to the thicker integumented *A. brightwelli*. As the water warms, its ability to suspend the thicker integumented rotifer may be reduced. The thinner integumented *A. herricki* may have an advantage later in the season because its density would
be compatible with the density of the water in the epilimnion. This adaptation could be a further morphological feature that differentiates the two rotifers. The issue of swimming speed also could also be another reason why the integument is thinner in *A. brightwelli* compared to *A. herricki*. *A. herricki* is bigger than *A. brightwelli*, and the thinner integument could be an adaptation to increase swimming speed by facilitating oxygen uptake by diffusion.

The time of year that the rotifers were collected should not cause differences in the width of the integument. Some rotifers have the ability to adapt body shape over generations (Wurdak and Gilbert 1976). Smaller rotifers may produce eggs that can develop into larger body shaped rotifers in a process called polymorphism. Polymorphism is related to gigantism. A small saccate rotifer may give rise to eggs that develop into cruciform shaped rotifers. The cruciform shaped rotifers may give rise to a campanulate shaped offspring that have a wider and longer body than either the cruciforms or the saccates. A study conducted by Wurdak and Gilbert (1976) found that regardless of the morphotype of the rotifer, the thickness of the integument remains the same for a given species. Even if the two rotifers were experiencing seasonal variation, the thickness of the integument would
be expected to remain constant within a species.

The width of the hypodermis varied from region to region in the polymorphs in the study conducted by Wurdak and Gilbert (1976). In the present study, examination of different regions may have altered the results. Although this is a possibility, the stark differences between the appearance of the integument of *A. herricki* and *A. brightwelli* under the TEM support the idea that, overall, the integument differs in these two species. A further study should be conducted to determine if similar regions have comparative morphology. The thickness of the integument of the two rotifers could be compared to determine if thickness varies in relation to body region in a given species or if thickness is species dependent. This study could also determine if the integument thickness is related to swimming speed, prey capture, or species uniqueness. Also, if the integument varies as a function of water density, other related rotifers and plankton should be examined.
Figure 1. A ventral view of a digonant rotifer, Philodina. x375, b, brain; eb excretory bladder; i, intestine; m, mastax; pg, pedal gland; pt, protonephridial tubule; s, spur; sg, salivary gland; st, stomach; v, vitellarium. (Pennak, 1978).

Figure 2 (below): Two planktonic monogonont rotifers. Asplanchna ingesting a Branchionus. Note the absence of a foot region on the Asplanchna. eb, excretory bladder; de, developing egg; gg, gastric gland; st, stomach; t, trophi (pointed structures); v, vitellarium (yolk sac). (Pennak, 1978).
Figure 3: The reproductive cycle for monogononta rotifers. Without an external stimulus, reproduction is exclusively asexual (Pechnik, 1985).
Table 1.1 Statistical Analysis of *A. herricki* and *A. brightwelli* under SEM

Null hypothesis: Mean of *A. herricki* equals mean of *A. brightwelli*

<table>
<thead>
<tr>
<th></th>
<th>Coronal width</th>
<th>Midsection width</th>
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<tr>
<td><em>A. herricki</em></td>
<td>236 μm</td>
<td>169 μm</td>
</tr>
<tr>
<td><em>A. brightwelli</em></td>
<td>399 μm</td>
<td>231 μm</td>
</tr>
<tr>
<td>Probability of null hypothesis</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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<tr>
<td>t-value</td>
<td>6.74</td>
<td>13.5</td>
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Figure 4A and B. The head region of *A. herricki* with the corrugated neck is in view in 4A. The cilia is located at the edges of the coronal surface. In 4B, a full view of *A. herricki* is shown with the head region anterior.
Figure 5A and B. A lateral view of A. herricki is shown with the dominating body region in full view. The proportion of the body region is distinct in comparison to the head region. Figure 5B contains a magnified view of the mouth region on the coronal surface of A. herricki. The edges of the trophi are in view (t).
Figure 6A and B. A lateral view of *A. brightwelli* is shown in figure 6A. Note the difference in proportion of the body region to the head region from the *A. herricki*. Figure 6B presents a magnified view of the head region of *A. brightwelli*. 
Figure 7A (above) and 7B (below). In figure 7A, the integument of *A. herricki* is illustrated. Contrasting figure 7A, figure 7B contains the integument of *A. brightwellii*. Both micrographs are X20K. Note the difference in the thickness of the integument.
Figure 8A (above) and figure 8B (below). The integument of *A. herringi* is magnified X20K in figure 8A. At X30K, the integument of *A. brightwelli* is shown in greater detail in 8B.
Figure 9A (above) and figure 9B (below). In figure 9A, the integument is magnified X30K with the hypodermal bulb in the center of the photo. The hypodermal bulb secretes the cuticle. Figure 9B further magnifies the hypodermal bulb (X50K).
Works Cited


