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Sudden appearance of cysts and ellobiopsid parasites on zooplankton in a Michigan lake: a potential explanation of tumor-like anomalies

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Abstract: Cysts on calanoid and cyclopoid copepods, previously reported as tumor-like anomalies (TLAs) in Lake Michigan and Europe, appeared briefly in Patterson Lake, a small Michigan inland lake. Cysts were rare (4% maximum) in samples collected on September 11, 1999, but appeared with high frequency on calanoid adults (49%) and cyclopoid nauplii (73%) in samples collected on October 16. By October 30, cysts were again rare (0.4% maximum). Cysts most commonly appeared on the lateral surface of the animal at the articulation of the 1st and 2nd prosomal segments and often consisted of herniated copepod tissues. Transparent, pyriform cysts co-occurred in low frequency with other types of cysts and are believed to be the trophomeres and gonomeres of ellobiopsid parasites. Histologic manifestations of cysts were diverse; herniations consisted of acellular yolk-like material and apparent host tissue, while cysts thought to be *Ellobiopsis* contained cells with different degrees of nuclear staining and unusual spherical bodies. Hernias were experimentally induced on live calanoid copepods by piercing the carapace with a fine needle, suggesting that ellobiopsid parasites may cause the hernias by puncturing the carapace of their hosts. Ellobiopsid parasites are common on marine crustacean zooplankton but have been recorded only once before in freshwater.

Résumé : Des kystes affectant les copépodes calanoïdes et cyclopoïdes, déjà signalés comme anomalies pseudotumorales dans le lac Michigan et en Europe, ont fait une brève apparition dans le lac Patterson, petit lac intérieur du Michigan. Les kystes étaient rares (4% maximum) dans les échantillons recueillis le 11 septembre 1999, mais apparaissaient avec une forte fréquence chez les adultes de calanoïdes (49%) et les nauplius de cyclopoïdes (73%) dans les échantillons prélevés le 16 octobre. Le 30 octobre, les kystes étaient redevenus rares (0,4% maximum). Les kystes apparaissaient le plus souvent sur la surface latérale de l'animal, à l'articulation du 1er et du 2e segment prosomal, et consistaient souvent en hernies des tissus des copépodes. Des kystes transparents et piriformes, présents à faible fréquence avec d'autres types de kystes, pourraient être des trophomères et des gonomères d'ellobiopsidés parasites. Les manifestations histologiques des kystes étaient diverses; les hernies consistaient en matière acellulaire de type vitellin et en tissu apparent de l'hôte, tandis que les kystes attribués à *Ellobiopsis* contenaient des cellules présentant différents degrés de coloration du noyau et des corps sphériques inhabituels. Nous avons expérimentalement induit des hernies chez des copépodes calanoïdes vivants en perçant la carapace avec une aiguille fine, ce qui permet de penser que les ellobiopsidés parasites peuvent causer les hernies en perforant la carapace de leurs hôtes. Les ellobiopsidés parasites se retrouvent couramment sur les crustacés du zooplancton marin, mais n'avaient jusqu'ici été signalés qu'une fois en eau douce.

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Introduction

Recent reports of tumor-like anomalies (TLAs) on crustacean zooplankton in Lake Michigan (Omair et al. 1999) have aroused interest in the nature and distribution of the abnormalities and their effects on zooplankton populations. TLAs found on copepods in Lake Michigan and several other Laurentian Great Lakes (M. Tuchman, EPA Great Lakes National Program Office, Chicago, Ill., personal communication) are similar in appearance to "cysts" reported from Lake

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Maggiore, Italy (Manca et al. 1996), and the "protruded protoplasmatic masses" described from copepods in the Mediterranean Sea (Crisafi and Crescenti 1975). To avoid the suggestion of neoplasia associated with the word "tumor," we use "cyst" to describe the abnormalities in general and "hernia" for those consisting of extruded internal tissues.

We report an episodic occurrence of cysts among the copepods of a small inland lake, including a brief histologic description of the cysts. We present evidence that some types of cysts are ellobiopsid parasites and that many other cysts are hernias potentially caused by damage to the carapace.

Materials and Methods

Site description

Patterson Lake and a second lake sampled for comparison (Third Sister Lake) are located within the Huron River watershed in southeastern Michigan. Patterson Lake (42°26.18′N, 84°01.40′W) is similar in area (0.62 km^2) and depth (z_{max} = 20 m) to other lakes in the region. The lake is used for recreation and has a wooded shoreline except for approximately 100 residences. During the autumn sampling period, the lake was stratified and the hypolimnion was anoxic. Third Sister Lake (area = 0.68 km^2, z_{max} = 17.5 m) lies 25 km southeast of Patterson Lake (42°16.54′N, 83°48.35′W) in an area characterized by light industry and residential development.

Methods

Zooplankton samples were collected at Patterson Lake on September 11, October 16, and October 30, 1999, and February 2 and April 18, 2000, from a location about 100 m from the northeast shore (z = 12 m) by vertical hauls of a 153-μm-mesh plankton net with a 30-cm-diameter opening. The September sample was collected by hauling from 6 m (1 m below the oxycline, <1 $mg \cdot L^{-1}$ dissolved oxygen) to the surface and was preserved in 4% sugar formalin. Because the mixed layer deepened in October, all subsequent hauls were made from within 1 m of the bottom.

The October 16 sample was examined immediately after collection at which time cysts were first noted. This observation prompted subsequent collections on October 20 (Third Sister Lake, 12 m) and October 30 (Patterson Lake) and the examination of the September 11 sample from Patterson Lake. On October 16, 1 mL of 0.1% chloral hydrate and club soda were added before preservation to prevent the egestion of gut contents. The same procedure was followed in subsequent collections to maintain consistency.

To determine if our collection and preservation techniques induced the formation of cysts, four vertical tows were made at Patterson Lake on April 18. The first two tows were preserved as before and the 3rd and 4th tows were transferred to 5-L bottles containing lake water and transported live to the laboratory. Batches of live animals were transferred to a counting chamber and scanned for cysts under a dissecting microscope until 200 nauplii and 100 calanoid copepods had been examined. The live sample was then split, with half preserved as previously described and the second half subjected to approximately double the amount of chloral hydrate, club soda, and sugar formalin (final concentration 10%). Both laboratory- and field-preserved samples were then scanned for cysts under a dissecting microscope.

In an effort to induce cysts in live copepods, individual adult and immature calanoid copepods were isolated in drops of lake water. A fine hypodermic needle (insulin 28G) was used to pierce the carapace on the dorsal side. Nauplii were not used because of their small size.

Photographs were made using dissecting and compound microscopes equipped with a digital camera. Sizes of cysts were measured from the images using a PC with analytical software (SPOT V2.2, Diagnostic Instruments, Sterling Heights, Mich.).

Copepods processed for histology were transferred from 4% sugar formalin into 70% ethanol. Copepods were photographed individually to record gross morphology and coded for tracking for histologic manifestations. Specimens were dehydrated, infiltrated with paraffin, embedded, sectioned at 4–6 μm thickness and stained with Meyer's hematoxylin and eosin.

Results

Cysts occurred only on copepod species. Although cladoceran species, including *Daphnia retrocurva*, *Daphnia pulicaria*, *Bosmina longirostris*, *Ceriodaphnia* sp., and *Leptodora kindti*, were common in Patterson Lake, no cysts were observed on them. Cyclopoid copepods included *Diacyclops thomasi*, *Acanthocyclops* sp., *Tropocyclops prasinus*, and *Mesocyclops* sp. All adult calanoid copepods were *Skistodiaptomus oregonensis* in the autumn samples. The abundance of copepods and frequencies of cysts in Patterson Lake and Third Sister Lake are given in Table 1. Life stages are divided into three major groups: nauplii (larvae), copepodids (juveniles), and adults.

High frequencies of cysts occurred only in the October 16 sample from Patterson Lake. The two most acutely affected groups were the naupliar larvae of cyclopoid copepods and the adults of *S. oregonensis*. In the latter group, the frequency of cysts was equal for males and females (chi-square, $p > 0.50$). Juvenile stages of both cyclopoid and calanoid species were moderately affected, but no cysts were found on any adult cyclopoid. The zooplankton community of Third Sister Lake was similar in composition to that of Patterson Lake, but on October 20, cysts were rare in Third Sister Lake. No cysts were found in preserved samples from Patterson Lake in February and April 2000. Cysts were not found on live zooplankton collected in April and did not appear after the animals were preserved. Re-examination of all samples over several weeks did not reveal any obvious change in the frequency or appearance of cysts as the result of preservation in formalin.

Cysts most commonly appeared on the lateral surface of adult *S. oregonensis* at the articulation of the 1st and 2nd prosomal segments (Fig. 1*a*). Cysts ranged from small (7×10^{-4} mm^2 dorsal surface area) to large (5×10^{-2} mm^2, Fig. 1*b*) but were typically intermediate in size ($3–11 \times 10^{-3}$ mm^2, Fig. 1*a*) and rough-edged in appearance (Fig. 1*a*). Smooth, rounded cysts (Fig. 1*b*) and elongated, transparent cysts (Figs. 1*c*, 1g, and 1*i*) were present but rare (<1%).

Cysts had diverse histologic characteristics that are currently under detailed investigation. Some cysts were composed of a largely acellular mass, containing yolk-like material and a margin of host-derived cells defining the outer periphery. Many cysts appeared as herniated host tissue and often demonstrated communication between the cyst and the host (Fig. 1*f*). Other cysts consisted of masses of cells appearing to be host hemocytes that formed protrusions on the external surface of the copepod.

Elongated and pyriform transparent cysts closely resembled ellobiopsid parasites. These cysts contained granular material and numerous round bodies in various stages of spore formation. Round bodies were observed with no apparent nuclear material (Figs. 1*d* and 1*j*), numerous small

Table 1. Occurrence of cysts (all types) on copepods in Patterson Lake and Third Sister Lake.

	Patterson Lake									Third Sister	
	Sept. 11			Oct. 16			Oct. 30			Oct. 20	
	Ind.·L^{-1}	%	*N*	Ind.·L^{-1}	%	*N*	Ind.·L^{-1}	%	*N*	%	*N*
Cyclopoida											
Nauplii	2.6	3	119	3.1	73	55	1.4	0	27	8	61
Copepodids	15.1	0	618	11.8	28	103	14.7	0.4	231	0	200
Adults	4.2	0	203	1.7	0	39	0.4	0	14	0	43
Calanoida											
Nauplii	1.7	1	95	11.1	21	238	13.3	3	212	0	36
Copepodids	6.3	0	340	9.3	17	170	23.0	3	259	0	61
Adult *S. oregonensis*	1.9	4	72	1.1	49	63	3.9	1	79	0	43

Note: Ind., individuals; %, percentage of *N* individuals in which cysts occurred.

nuclei (Fig. 1*j*), nuclei of various sizes (Fig. 1*j*,), or undergoing cleavage (Fig. 1*j*). Round bodies varied in size and were occasionally present within the body of the copepod as well.

Piercing the carapaces of live adult calanoid copepods with a fine needle produced immediate hernias, often of gonadal tissue, that appeared to be identical with many of the cysts observed in the October 16 sample (Figs. 1*e* and 1*f*). Piercing immature calanoid copepods resulted only occasionally in the extrusion of internal tissues. Hernias could not be induced on formalin-preserved specimens.

Discussion

Live collections of zooplankton are rarely examined closely, and to date, all reports of TLAs or cysts have come from preserved samples. The absence of observations of cysts on live zooplankton raises a question of whether cysts might be an artifact of collection and preservation. Although we did not find cysts on live copepods, our normal preservation procedure or doubling the concentration of preservative did not induce cysts. None of the copepods or cladocerans in our preserved collections had carapace ballooning or loss of eggs that might result from the osmotic shock of death and preservation. Although piercing the carapace of copepods induced hernias very similar to those found in our collections, we do not believe we could have injured large numbers of animals in this way by collection alone.

Male and female calanoid copepods with mature gonads appeared to have an internal hydrostatic pressure or turgor that caused gonad and other tissues to extrude through the punctures. Copepodids had less turgor; therefore, piercing them produced hernias less often. Differences in turgor between mature and immature calanoid copepods may explain the higher frequency of hernias on adult calanoids than on calanoid copepodids observed in Patterson Lake on October 16, 1999.

In samples where hernias occurred, a few elongate and pyriform transparent cysts were also found. The transparent cysts were very similar in appearance to ellobiopsid parasites reported on calanoid copepods from Lake Midmar in South Africa (Rayner and King 1986).

The family Ellobiopsidae is a small, heterogeneous group of protist parasites on marine crustacean zooplankton thought to be allied with the dinoflagellates (Theodorides 1989). The life histories of the ellobiopsids are diverse and only partially understood. The genus *Thalassomyces* develops internally and then erupts through the carapace of the host (Mauchline 1966), while *Ellobiopsis* is believed to attack the carapace externally (Jepps 1937). A common feature of many ellobiopsids is the appearance on the host of a stalked or ellipsoid trophomere bearing a fruiting gonomere at the distal end. The transparent cysts in Figs. 1*c*, 1*g*, and 1*i* bear a strong resemblance to trophomeres of the genus *Ellobiopsis* illustrated in Boschma (1959) and Rayner and King (1986). The pyriform cysts in Figs. 1*h* and 1*i* have knurled distal ends and enclose a darker interior body similar to the cysts in Rayner and King (1986). The elongated cysts in Figs. 1*c* and 1*g* are more similar to the descriptions of mature *Ellobiopsis* by Jepps (1937), Boschma (1959), and Theodorides (1989), suggesting that the pyriform cysts are immature versions. The specimen in Fig. 1*g* bears cysts of both shapes. The internal structure of *Ellobiopsis* is generally granular, with no large nuclear structures (Jepps 1937). At times, however, small nuclei can be observed (Jepps 1937) and the granular material may occur in spherical globules (Boschma 1959).

Ellobiopsid parasites are fairly common in marine environments but Lake Midmar (Rayner and King 1986) is the only freshwater report of ellobiopsids. In some reports of TLAs or cysts, however, ellobiopsid parasites may have gone unrecognized. Among other cysts, Crisafi and Crescenti (1975) reported copepods with elongate, transparent cysts similar to our Fig. 1c. Photographs of TLAs on immature calanoid copepods from the Baltic Sea (T. Shchuka, Institute of Global Climate and Ecology of the Russian Academy of Science and Rosgidromet, Moscow, personal communication) include forms that are probably ellobiopsid parasites. Although Omair et al. (1999) did not report finding crustaceans with elongated, transparent cysts in Lake Michigan, several have recently been found. One specimen is clearly *Ellobiopsis*, having a distinct trophomere and gonomere separated by a constriction and septum (J. Cavaletto, GLERL, Ann Arbor, Mich., personal communication). TLAs with stalked attachments to the mouthparts or antennae of *Limnocalanus* in Lake Michigan (Omair et al. 1999) match the description of *Ellobiopsis* occurring on *Calanus* in the Clyde Sea (Jepps 1937).

We suggest that ellobiopsid parasites are responsible for many of the cysts found in Patterson Lake and TLAs reported elsewhere. The hernias on Patterson Lake copepods likely resulted from punctures in the carapace possibly

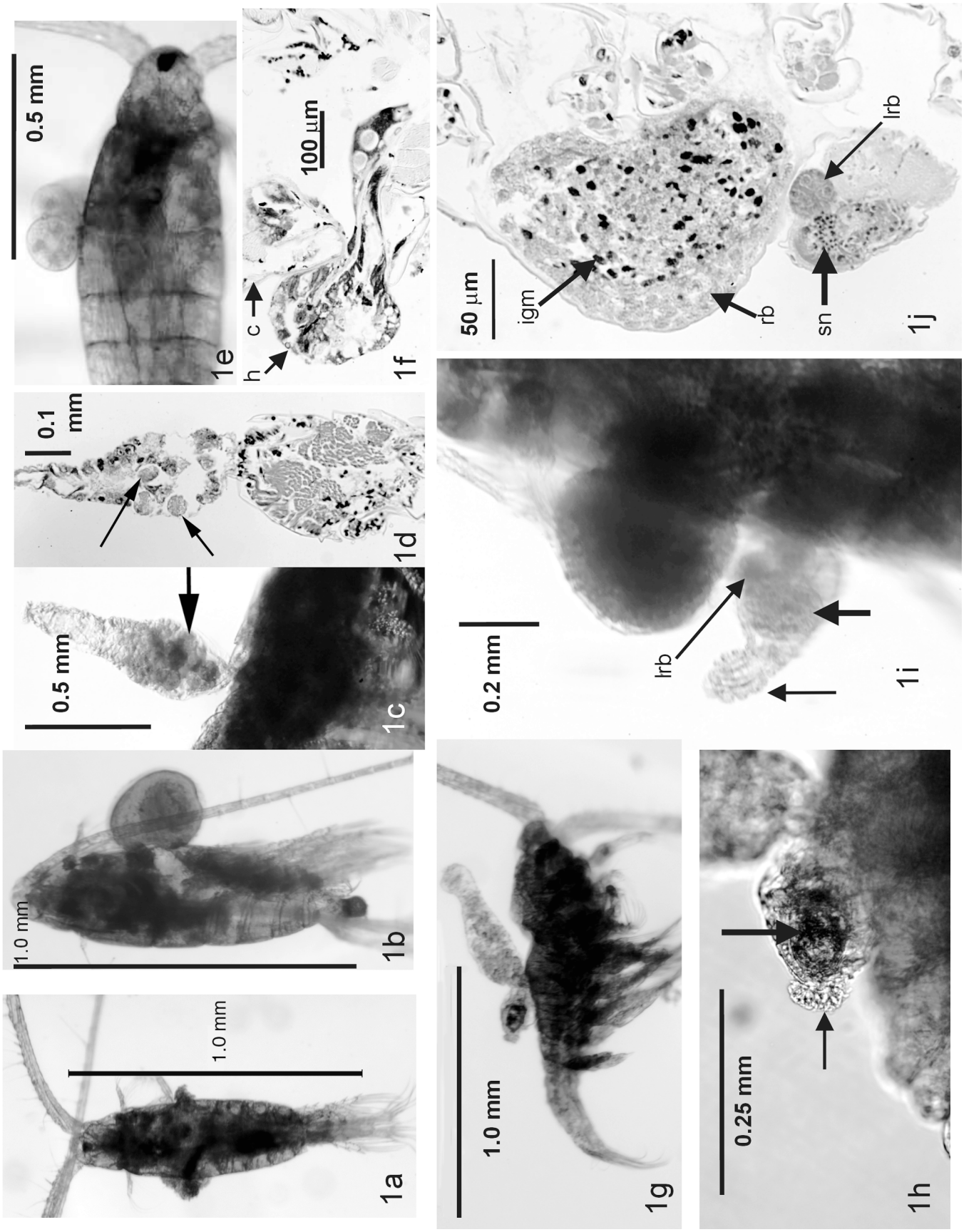

Fig. 1. Gross morphology and histological sections of copepods with cysts and hernias from Patterson Lake, Michigan. (*a*) Adult *Skistodiaptomus oregonensis* with hernias at the articulation of 1st and 2nd prosomal segments. (*b*) *S. oregonensis* with large cyst similar to tumor-like anomalies reported from Lake Michigan (Omair et al. 1999). (*c*) *S. oregonensis* bearing elongated, transparent cyst of the type thought to be an ellobiopsid parasite. Note round sporulation bodies (arrow). (*d*) Histological section of specimen in Fig. 1*c*; round bodies are indicated by arrows. (*e*) Live *S. oregonensis* with hernia induced by piercing the carapace with a hypodermic needle. (*f*) Histological section of a hernia (h) protruding from a break in the carapace (c). (*g*) External view of pyriform and elongated cysts thought to be ellobiopsid parasites. (*h*) Enlarged view of pyriform cyst. Note knurled distal end (small arrow) and ovoid interior body (larger arrow) characteristic of ellobiopsid parasite. (*i*) Another example of a pyriform cyst with knurled end (small arrow), ovoid interior body (larger arrow), and large round body (lrb). Large, dark cyst above may be a different stage of the parasite. (*j*) Histological section of cysts shown in Fig. 1*i*. Note various stages of spore development: round bodies (rb) with no nuclear material along outer periphery, nuclei of various sizes among inner granular material (igm), abundance of small nuclei (sn) within the larger ovoid body. Large round body (lrb) is undergoing cleavage and may become the next ovoid body.

caused by parasites. Although the mature parasite was found only on adult and nearly adult calanoid copepods, the co-occurrence in copepod populations of parasites and herniated individuals in Patterson Lake, Lake Michigan (J. Cavaletto, GLERL, Ann Arbor, Mich., personal communication), and the Baltic Sea (T. Shchuka, Institute of Global Climate and Ecology of the Russian Academy of Science and Rosgidromet, Moscow, personal communication) suggests a connection between parasites and hernias. Recognizable ellobiopsid parasites were rare compared with other types of cysts in Patterson Lake, which may explain why they have been overlooked in previous reports of cysts and TLAs.

The sporadic nature of ellobiopsid infestations has been reported by others (Rayner and King 1986, Al-Yamani and Fahmi 1995). In Patterson Lake, cysts on copepods appeared with high frequency in mid-October only, suggesting that the cysts developed within a few weeks. Although cysts were rare in late October, it is unlikely that they were shed by molting because most cysts are deeply anchored through a small hole in the carapace (Hoffman and Yancey 1966). It is more likely that increased mortality resulting from infection, starvation, inability to molt, or increased susceptibility to predation may explain the absence of animals bearing cysts in late October.

Nearly opposite patterns of infections were observed between calanoid and cyclopoid species. In calanoids, cysts were most frequent in adults. Conversely, cysts were absent among adult cyclopoids but occurred frequently on nauplii. Cyclopoid nauplii were also highly affected in several Russian lakes where cyst frequencies as high as 69% were observed (Silina and Khudolei 1994). The absence of cysts on adult cyclopoid copepods in all reports of cysts, TLAs, or ellobiopsid parasites suggests that although cyclopoid juveniles may be susceptible, adult cyclopoids are not suitable hosts for the parasites.

The effect of cysts on individuals or populations is difficult to determine without more frequent sampling intervals or observations of infected live animals. Some species of ellobiopsid parasites are thought to sterilize the host or reduce gonad development (Ramirez and Dato 1989). In Patterson Lake, egg-bearing females were uncommon, but some *S. Oregonensis* carried a few eggs as well as ellobiopsid cysts. *Ellobiopsis* was also thought to have played a role in the replacement of *Tropodiaptomus spectabilis* by a competitor, *Metadiaptomus transvaalensis*, which was immune to the parasites (Rayner and King 1986).

Although this is the first record of cysts on copepods from a small lake in North America, and only the second record of ellobiopsid parasites occurring in fresh water, it is difficult to know whether this phenomenon is new. At low frequencies, cysts would be easily missed in a standard analysis of plankton samples. Even when cysts are common, those having the characteristics of ellobiopsid parasites are rare. If, as in Patterson Lake, high frequencies of cysts are present for only a few days or weeks, even weekly collections could miss the period in which cysts would be frequent enough to become obvious.

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