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Histology of Herniations through the Body Wall and Cuticle of Zooplankton from the Laurentian Great Lakes

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Zooplankton of the Laurentian Great Lakes developed hernial protrusions whose gross appearance matches those on zooplankton described elsewhere in the world. We have carried out a histologic and cytologic analysis of the protrusions and found that they are composed of apparently degenerating or necrotic tissue(s) that has been expressed from the organism through the process of herniation. At their base the protrusions are continuous with viable tissue(s) within the organism through a fissure in the exoskeleton. Our observations lead us to suspect that these hernial protrusions are lethal. The development of such protrusions in zooplankton may be a worldwide phenomenon, but the cause of the herniation remains a mystery. © 2001 Academic Press

Key Words: **Great Lakes; zooplankton; surface protrusions; herniation.**

INTRODUCTION

This article deals with the composition of protrusions on the surface of various species of zooplankton in the Laurentian Great Lakes; it also includes information on the species affected. Such protrusions have also been recorded on zooplankton found in various "polluted seas" (Cristafi and Crescenti, 1977) in a Michigan inland lake (Bridgeman *et al.*, 2000) and elsewhere (Silina and Khudolei, 1994). Recently, calanoid copepods in Lago Maggiore in Italy were reported to bear protrusions referred to as "cysts" (Manca *et al.*, 1996), although no proof was offered that the lesions were cystic.

All of the protrusions that we have observed on zooplankton and those described elsewhere appeared on various sites of the body. Despite the striking appearance (Omair *et al.*, 1999) and widespread distribution of these protrusions, no attempt has been made to determine their composition and origin. As might be expected, speculation has been made as to whether these unusual lesions are neoplastic (Vanderploeg, 1999). However, the presence of these protrusions on immature as well as adult zooplankton and their simultaneous presence on many species (reminiscent of an infectious disease) are out of character for the development of neoplasms. We have, therefore, undertaken a study to determine the composition and origin of these protrusions found on several species of zooplankton from Lake Michigan, Lake Huron, the River Raisin (a tributary of Lake Erie), and the St. Clair River (the channel connecting Lake Huron with Lake St. Clair).

METHODS

Field Methods

Zooplankton were collected with a 63-μm mesh, 0.5-m-diameter plankton net towed vertically in water columns from several sites in Lakes Michigan and Huron and some tributaries. Samples were usually preserved in a 10% solution of formaldehyde; sometimes they were examined fresh and then preserved. A fresh sample was collected from Lake Michigan at 3 m on 2 June 1999, placed on ice, and transported to Ann Arbor, Michigan. The next day, nine freshly caught *Epischura lacustris* spp. with protrusions were fixed in glutaraldehyde for electron microscopy. Aceto-orcein squash preparations of the dissected protrusions of *E. lacustris* as well as protrusions dissected from *Bythotrephes cederstroemi*, *Diaptomus* spp. and *Eurytemora* spp. were performed. Similarly, on 11 June 1999, a sample was collected at 6 m and 114 zooplankters (70% *E. lacustris*, 30% a mixture of *Eurytemora* and *Diaptomus* spp.) were fixed in glutaraldehyde for routine histological examination.

Data on the prevalence of protrusions and the classification of the zooplankton were based on a qualita-

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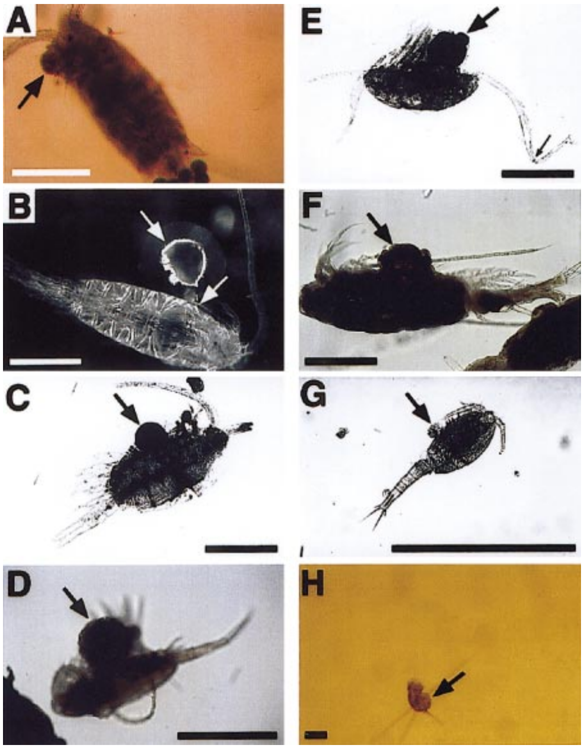

FIG. 1. Composite photograph illustrating protrusions on Great Lakes zooplankton. Arrows point to the protrusions (bar, 0.5 mm unless otherwise stated). Most protrusions appear to arise from the ventral surface of the organisms. (A) *Eurytemora affinis;* female bearing eggs and an anteriorly situated protrusion. (B) *Limnocalanus macrurus* cleared in xylene showing a large protrusion connected to the surface of the zooplankton by a pedicle (arrow). The nature of the bright rim in the protrusion is unknown. (C) *E. affinis* with a large protrusion on its ventral surface. (D) Female *Diaptomus* sp. with a protrusion on its ventral surface. This omnivorous organism inhabits inshore and offshore waters. (E) A male *Diaptomus* sp. with a geniculated right antenna (arrow). It bears an irregularly shaped protrusion. (F) *Epischura lacustris,* an offshore and inshore inhabitant and a predator of microzooplankton. It has a protrusion on its ventral surface. (G) *Cyclops* spp., an inshore and offshore predator, with a small protrusion on its ventral surface. (H) *Keratella* spp., the most common limnetic rotifer of the Great Lakes, with a protrusion on its posterior aspect (bar, 0.1 mm).

tive examination of 50 preserved samples from 1995 through 1999, primarily from Muskegon, Michigan, along the eastern Lake Michigan shoreline; however, some came from the Raisin River, the St. Clair River, and the Straits of Mackinaw in northern Lake Michigan. We also examined 12 unpreserved samples, 10 of which were brought back to the laboratory about 20 h after collection and 2 of which were examined within 2 h of collection. These samples were examined for the presence of live or dead zooplankters bearing protrusions.

Laboratory Methods

We employed several techniques to investigate the composition and origin of the protrusions: (1)

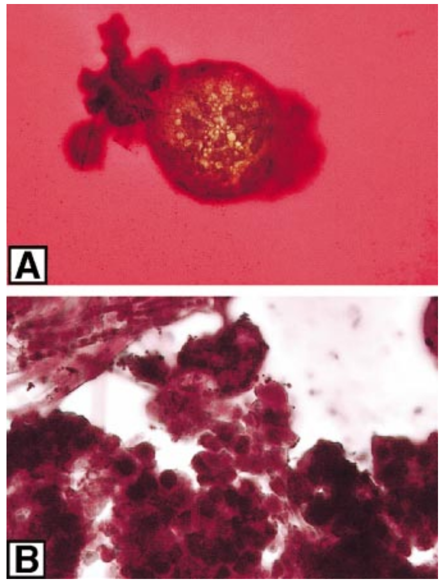

FIG. 2. *Epischura lacustris* with a protrusion. (A) A protrusion dissected from the organism. The internal structure, consisting of tiny round bodies, suggests the presence of cells (scale bar, 0.5 mm). (B) Squash preparation of the protrusion reveals its contents to be cells; their lack of clarity may be due to necrosis or degeneration (original magnification, ×400).

dissection of protrusions from organisms for aceto-orcein squash preparations, (2) scanning and transmission electron microscopy (SEM and TEM), and (3) routine histologic sectioning of entire organisms with subsequent staining with hematoxylin and eosin (H&E).

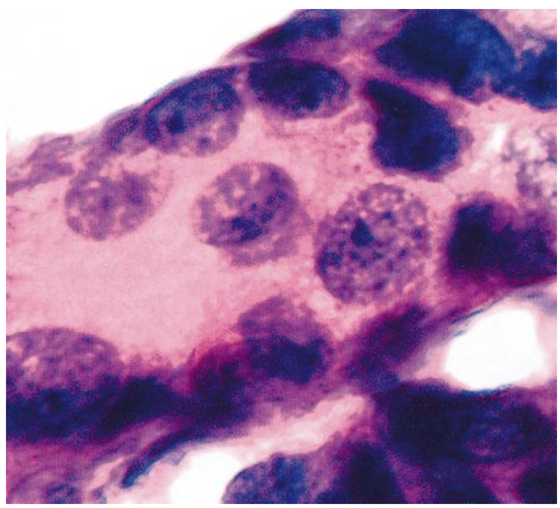

FIG. 3. Squash preparation of a dissected protrusion from *Bythotrephes cederstroemi,* a nonindigenous species. It is composed of small cells of fairly uniform size and shape, with high nucleocytoplasmic ratios and granular chromatin. Many cells are degenerated, as manifested by smudgy chromatin, cracks in the nuclear membranes, and tiny nuclear vacuoles (original magnification, ×1000).

TABLE 1
Zooplankton Taxa ($n = 33$) Found in the Laurentian Great Lakes

Taxa (developmental stage/sex)
Native crustaceans
Alona spp.
Alonopsis spp.
Bosmina spp.
Ceriodaphnia sp.
**Chydorus* sp.
*Copepod nauplii
**Cyclops* spp. C1–C5
**C. bicuspidatus* M, F
C. vernalis M, F
Daphnia galeata
D. longiremis
D. pulicaria
D. retrocurva
**Diaptomus* spp. C1–C5
**D. ashlandi* M, F
D. minutus M, F
D. oregonensis M, F
**D. sicilis* M, F
**Diaphanosoma* sp.
**Epischura* sp. C1–C5, M, F
Eubosmina sp.
Eurycercus sp.
**Eurytemora* spp. C1–C5, M, F
Holopedium sp.
Leptodora sp.
**Limnocalanus* spp. C1–C5, M, F
**Mesocyclops* spp. C1–C5
**M. edax* M, F
**Polyphemus* sp.
Tropocyclops spp. C1–C5, M, F
Exotic crustaceans
**Bythotrephes cederstroemi*
Rotifers
**Keratella* spp.
**Stephanoceros* spp. (uncommon)

Note. Taxa found with protrusions ($n = 17$) are marked with an asterisk. Of the 28 zooplankton species (26 common and 2 uncommon), 11 common species and 2 uncommon species (*B. cederstroemi* and *Stephanoceros*) bore protrusions. Two uncommon species bearing protrusions are also included. M, male; F, female; C1–C5 refers to the successive stages of development.

Squash Preparations

A dissected protrusion was put in a drop of aceto-orcein solution on a slide for 3 min. It was then washed with dilute acetic acid to remove excess stain, a coverslip was placed on it, and the protrusion was then squashed onto the slide and examined under a microscope.

Electron Microscopy

Specimens for electron microscopy were fixed in a solution containing 3% glutaraldehyde, 3% formaldehyde, and 3% cacodylate buffer diluted 1:2 with distilled water. Specimens for TEM were dehydrated and embedded in Poly 812 resin. Sections 1 μm thick were cut on a LKB Pyramitome, thin sectioned with a Reichart Supernova ultratome, and then examined on a LEO EM 900 electron microscope. Specimens for SEM were fixed, dehydrated, and critical point-dried. The specimens were individually mounted on aluminum stubs, then gold coated, and examined with an ISI Super IIIA scanning electron microscope.

Histologic Sectioning for H&E Staining

Specimens for H&E staining were deposited in a 15-mL centrifuge tube containing physiologic saline which was then centrifuged at 2500 rpm for 5 min. The supernatant was decanted and three drops of human plasma followed by three drops of thrombin solution were added to the sediment. On agitating the material at the apex of the centrifuge tube a clot developed within seconds. The clot was fixed in 10% buffered formaldehyde for at least 30 min, after which it was dehydrated and embedded in paraffin. Sections 5 μm thick were cut from the block and stained with H&E.

RESULTS

Distribution of Protrusions

The protrusions we observed, like those described elsewhere, occurred at various sites on the body; most were large, solitary, and smoothly round, whereas others were small or multiple or irregularly contoured (Fig. 1).

We observed protrusions on 46% of the 28 common species of zooplankton and on 53% of the 32 taxa collected from the Great Lakes and contiguous waters (Table 1). Immature stages and both sexes of copepods were affected as well as two species of rotifers. Even *B. cederstroemi*, a newly introduced exotic species and a predator on other zooplankton (Evans, 1988), was affected.

Squash Preparations

Squash preparations of the dissected protrusions appear to be composed of darkly staining, monomorphic, small round cells with high nucleocytoplasmic ratios and generally smudgy chromatin (Fig. 2), although some contain obvious chromocenters (Fig. 3). On the whole, morphologic details of any cells identified in these dissected protrusions were obscure, presumably due to degeneration or necrosis.

Electron Microscopy

Nine protrusion-bearing zooplankters (*E. lacustris*) were studied by scanning electron microscopy. The SEM plates show that the protrusions of all nine arise from the area between hard plates (somites) of the exoskeleton and that they appear to issue from a fis-

FIG. 4. Scanning electron micrograph of a protrusion from *E. lacustris*. All protrusions examined by this technique were found to issue from a fissure between somites of the exoskeleton. About 40% of protrusions studied had the rough surface illustrated here (original magnification, ×50).

sure in the membrane that connects the hard plates with each other (Fig. 4). Many of the protrusions displayed by SEM, including three of the nine examined by SEM, have a rough surface, which, under higher magnification, is seen to be caused by heavy growth of bacilli (Fig. 5). Protrusions not covered by bacilli are seen to be bounded by a limiting membrane. None of the somites show bacterial growth on their surface.

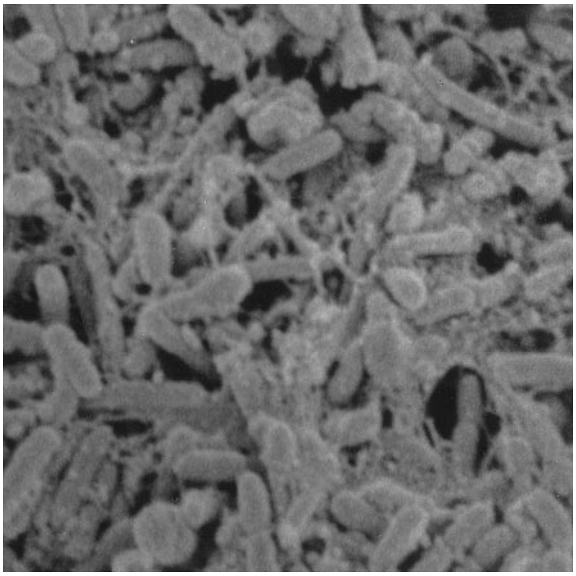

FIG. 5. Scanning electron micrograph of the surface of the protrusion from *E. lacustris* illustrated in Fig. 4. The rough surface is caused by a heavy growth of bacilli (original magnification, ×9000).

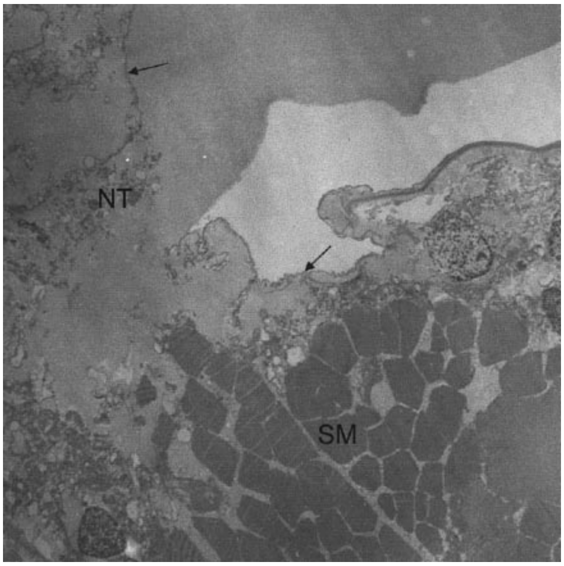

FIG. 6. Transmission electron micrograph of a protrusion and contiguous body of the zooplankter *E. lacustris*. The protrusion consists of necrotic tissue (NT). Viable skeletal muscle (SM) is shown within the zooplankter extending toward the protrusion. A limiting membrane (arrow) is present.

The contents of protrusions examined by TEM consist of necrotic tissue. Limiting membrane is present. The necrotic tissue in the protrusions is in continuity with viable tissue within the organisms through a fissure between the thoracic somites (Fig. 6).

Histologic Sections Stained with H&E

Histologic sectioning of the deposit of organisms obtained by centrifugation reveals the protrusions to be composed of apparently necrotic or degenerating tissue(s), often unidentifiable as to type. A fortuitous sagittal section of one of the organisms (*E. lacustris*) reveals a large protrusion that has been expressed through a fissure between the thoracic somites (Fig. 7). The protrusion is composed of the same tissue as the viable tissue within the organism at the point from which it is expressed; however, the herniated tissue does not have the same crisp clarity as the viable tissue, illustrating its degenerated or necrotic state. The contents of the protrusion are bounded by a membrane which appears to be in continuity with an internal membrane over the surface of the ovary. Furthermore, the intersomite membrane appears to have ruptured.

DISCUSSION

We found that 45% of the common zooplankton species and 52% of the taxa bore protrusions; individuals of both sexes and at all stages of development were affected, as well as one nonindigeneous species.

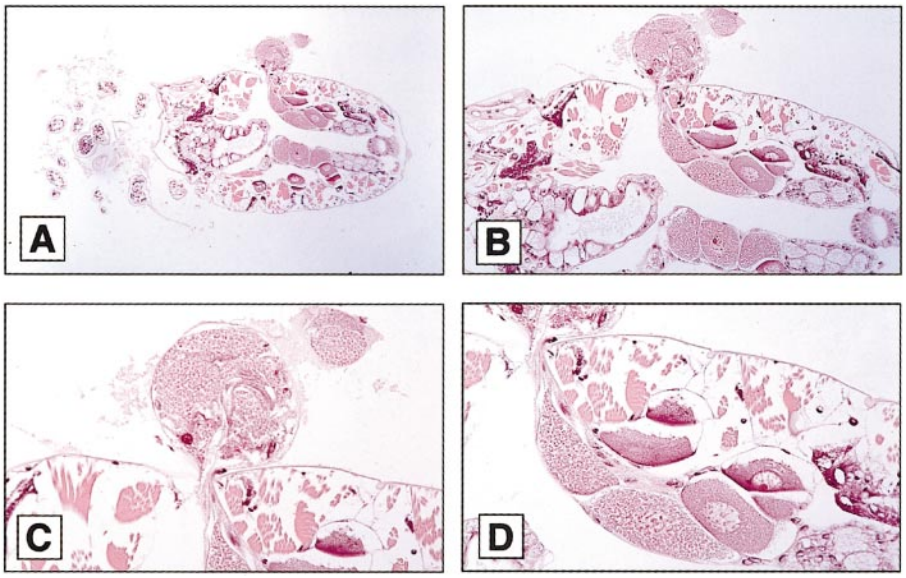

FIG. 7. Sagittal section of a zooplankter (*E. lacustris*, female) stained with H&E. (A) Low-power view illustrating the size and position of a protrusion and the presence of an external membrane on the metasomal ventral surface of the zooplankter (original magnification, ×100). (B) Higher power view demonstrating seeming continuity between the protrusion and tissue within the organism (original magnification, ×200). (C) Higher power view confirming the continuity between the protrusion and tissue within the organism. The protrusion, which is bounded by a thin membrane, issues from the organism through a fissure between hard plates of the exoskeleton and is in continuity with membrane covering the ovary. The contents of the protrusion consist mainly of degenerated or necrotic eosinophilic granular material, which contains one distinct nucleus and some tiny cyanophilic particles, presumably nuclear remnants. In addition, nonviable eosinophilic strands, presumably skeletal muscle, are present amid the granular material. The base of the protrusion is in clear continuity with identical ovarian tissue within the organism (original magnification, ×400). (D) This image illustrates the herniated protrusion issuing from the organism; continuity between the external protrusion and internal tissues of the organism is obvious. In this section the internal tissue consists of skeletal muscle, intestinal tract, and large ovarian cells toward the point of extrusion. The incarcerated ovarian tissue is not as morphologically well preserved as the ovarian tissue within the organism, evidence of degeneration or necrosis (original magnification, ×400).

Our observations of these protrusions on the surface of zooplankton demonstrate that they are neither cystic nor neoplastic; instead, they are composed of tissue(s) expressed from within the organism and are associated with limiting membrane. They appear to have herniated between the somites.

In some of the Great Lakes, zooplankton populations are being negatively affected by phosphorous decline, competition from zebra mussels, which filter algal food resources (Bridgeman *et al.*, 1995), and predation from the exotic zooplankter *B. cederstroemi*. Therefore, zooplankton-bearing protrusions could be subjected to an additional mortality risk on a population already stressed. It is worthy of note that one species of protrusion-bearing zooplankton (*Mesocyclops*) has disappeared from Lago Maggiore in Italy (Manca *et al.*, 1996). Further investigation is being planned to attempt to elucidate the etiologic circumstances associated with their development.

In these tiny organisms it is not possible to analyze precisely the pathogenesis of the protrusions. It appears that they develop by a process of herniation in the membranous intersomite region. This raises the question of whether the membrane itself had initially split, thus allowing a hernial protrusion to develop, or whether the intersomite membrane itself bulges along with the hernial sac and its contents. Our observations on Fig. 7 are consistent with the former situation. The possibility that herniation is initiated by puncture wounds caused by external predators or parasites has some plausibility. However, none of our observations allow us to be certain as to the exact pathogenesis of these herniations.

All zooplankton with protrusions that we examined from fresh unpreserved samples were dead, whereas those in the same sample without such lesions were alive. We suspect, therefore, that these protrusions are, in some manner, lethal to the organisms by impairing movement and feeding, thereby rendering them vulnerable to predation by other invertebrates and vertebrates, such as fish. On the other hand, just the toxic effect of herniated, incarcerated, and necrotic tissue on an organism may cause or contribute to its death.

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