Description of the first haemocytic rod-shaped virus from a penaeid prawn

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ABSTRACT: Epizootic losses of hybrid *Penaeus esculentus* by *P. monodon* crosses initiated an electron microscopical investigation. A haemocytic rod-shaped virus was found in the gill tissues as well as infectious hypodermal and haematopoietic necrosis virus in other tissues (reported elsewhere). Within the cytoplasm of small-granule haemocytes and the nucleus of other unrecognised cells, rod-shaped virions were present. The virions were 588 × 119 nm with extraordinarily long virions being 888 nm. Some were encapsulated by a smaller envelope which forced the long virions to become either V- or U-shaped. Empty capsids 496 × 50 nm were also seen in the nucleoplasm. Capsid originators were 50 to 60 nm long. The virus is named penaeid haemocytic rod-shaped virus (PHRV) until its relationship to other haemocytic viruses can be ascertained. However, PHRV showed affinities to the genus *Bracovirus* within the Polydnaviridae and the size and morphology were somewhat similar to rod-shaped virus of *Carcinus maenus* (RV-CM).

INTRODUCTION

Baculoviruses are the most commonly found viruses in crustaceans, there being at least 12 named types. Furthermore, they are often associated with disease in both penaeid prawns and portunid crabs (Johnson 1988, Johnson & Lightner 1988). Records of the 3 haemocyte-infecting, rod-shaped viruses similar to baculoviruses are restricted at present to the portunid crabs of the Atlantic Ocean. Developmentally, they are less typically like a baculovirus and share some characteristics with polydnaviruses (Johnson 1988). During an investigation into epizootic mortalities of *Penaeus esculentus* hybridised with *P. monodon*, a number of viruses were visualised including infectious hypodermal and haematopoietic necrosis virus (IHHNV) and lymphoidal parvovirus (Owens et al. 1992). This paper describes the detection of a rod-shaped virus in haemocytes, which represents the first haemocytic rod-shaped virus seen in penaeid prawns.

MATERIALS AND METHODS

Hybrid prawns (*Penaeus esculentus* crossed with *P. monodon*) were produced and raised as described in Owens et al. (1992). Moribund prawns were collected from holding tanks just after the major epizootic on Day 100 and fixed in Davidson's fixative. Histological processing and staining followed the methods outlined by Culling et al. (1985). For electron microscopy, diced gill tissue was fixed in 2.5 % glutaldehyde/2 % paraformaldehyde in cacodylate buffer or 10 % formalin and postfixed in 1 % osmium tetroxide. The tissue was dehydrated and mounted in Spurr's (Tm) resin. Sections were cut on an LKB Ultratome at 500 Å (50 nm), stained with uranyl acetate/70 % methanol and lead citrate and viewed at 80 kV on a Jeol 2000FX transmission electron microscope. Terminology of virion components follows Federici (1986) and Johnson (1988). Measurements of virion components were taken directly from photographs or by using a cartographer's wheel for bent virions. Only intact virions which were identified by envelopes at both polar ends were used in measurements.

RESULTS

The histological findings are reported in Owens et al. (1992). Briefly, Cowdrey type A intranuclear inclusion bodies were widespread throughout all mesodermal
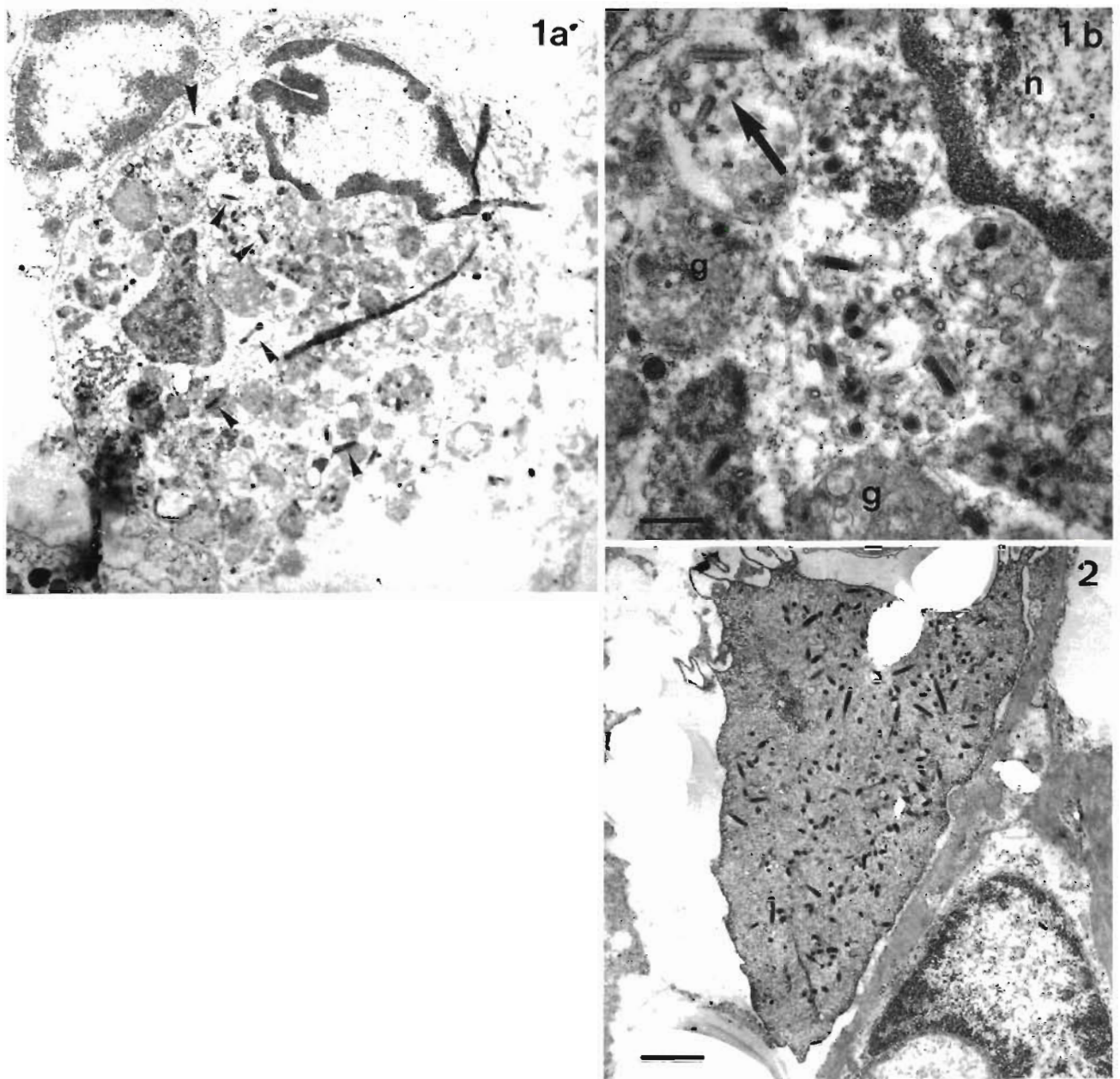
Figs. 1 to 8. Rod-shaped virus in hybrid prawns (*Penaeus esculentus* × *P. monodon*). Fig. 1a. A small-granule haemocyte show-
ing rod-shaped virions (small arrowheads) and empty capsids (large arrowhead) in the cytoplasm ×6237. Note the rounded gran-
ules throughout the cytoplasm of the haemocyte. Scale bar = 1.6 µm. Fig. 1b. Higher magnification (×25 000) of Fig. 1a at the
large arrowhead: small-granule haemocyte (g) with rod-shaped virions in thin-walled, cytoplasmic vacuoles. Empty capsids
(arrow) and nucleocapsids are present. Note the slightly hyperchromatic nucleus (n) with some nucleoplasm changes. Scale bar
= 400 nm. Fig. 2. Nucleus of an infected cell displaying dense virogenic stroma and numerous rod-shaped virions, ×6800. Scale
bar = 1.5 µm. Fig. 3. V-shaped virion within a small envelope ×50 000. Two enveloped, bent empty capsids are visible in cross
section (★). Scale bar =200 nm. Fig. 4. U-shaped virion (×50 000). Also present, a virion attached via a membrane to a capsid orig-
inator at right angles (solid arrowheads). Empty, truncated enveloped capsids are also visible (open arrowheads). Scale bar =
200 nm. Fig. 5. Cigar-shaped virions with the specialised apex visible (solid arrowheads), ×100 000. Some marginated chroma-
tin is also present (c). Scale bar = 100 nm. Fig. 6. Empty capsids (e) ×50 000. Two possible capsid originators (arrowheads) with
1 end still open, the one on the left clearly showing the internal originator. One possible originator attached by a membrane to a
fully formed nucleocapsid. Note some cross sections of bent virions. Scale bar = 200 nm. Fig. 7. Two capsid originators (arrow-
heads) ×25 000. Marginated chromatin is present (c) and a cytoplasmic mitochondrion (m). Scale bar = 400 nm. Fig. 8. Cross sec-
tion of a mature virion showing the 2 protein layers of envelope (arrowheads) separated by the electron-lucent lipid layer, a
slightly less electron-dense inner ring (intermediate layer, arrow) and then the nucleoprotein core, ×170 000. Terminology from
Federici (1986). Scale bar = 53 nm
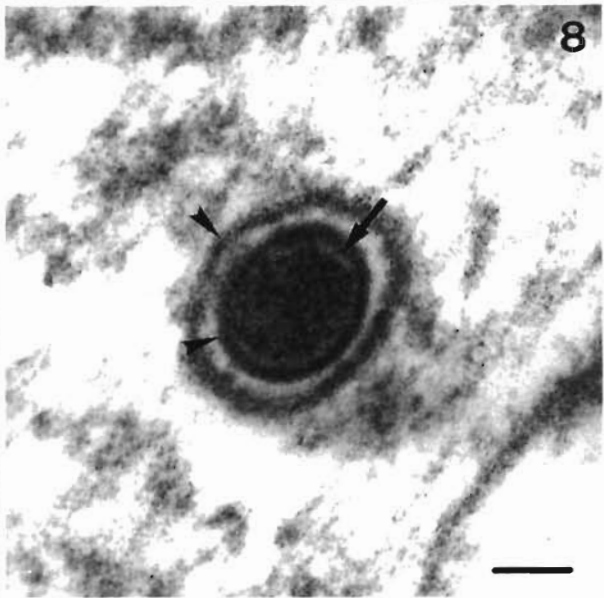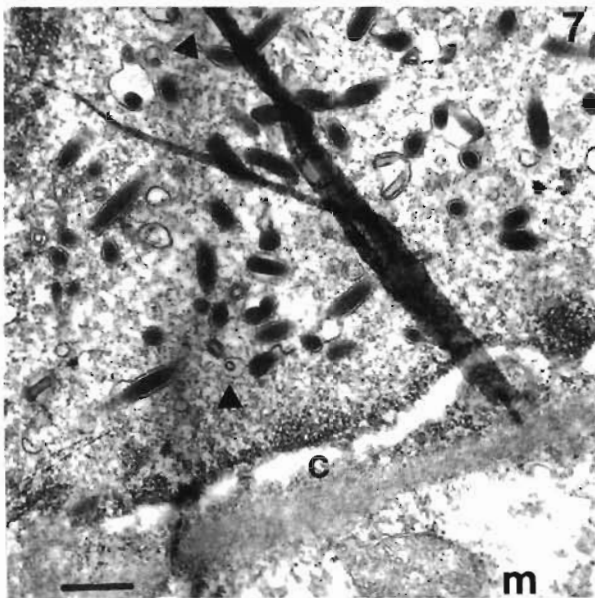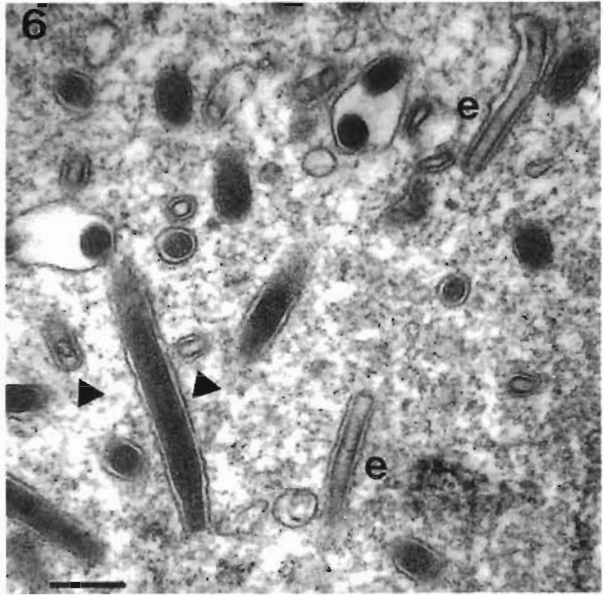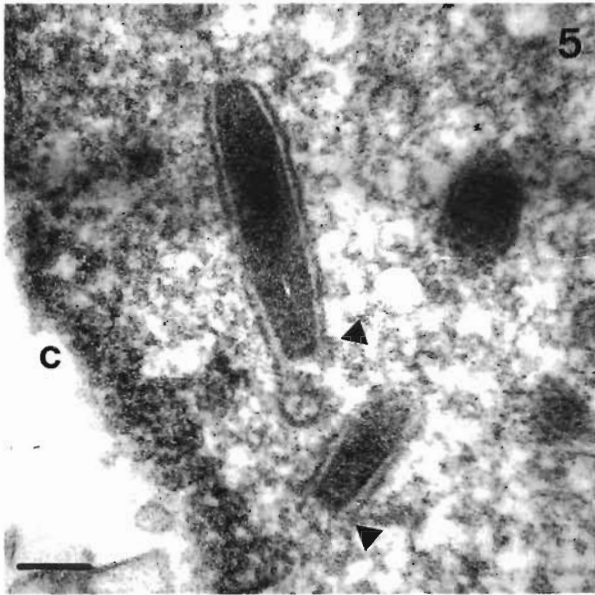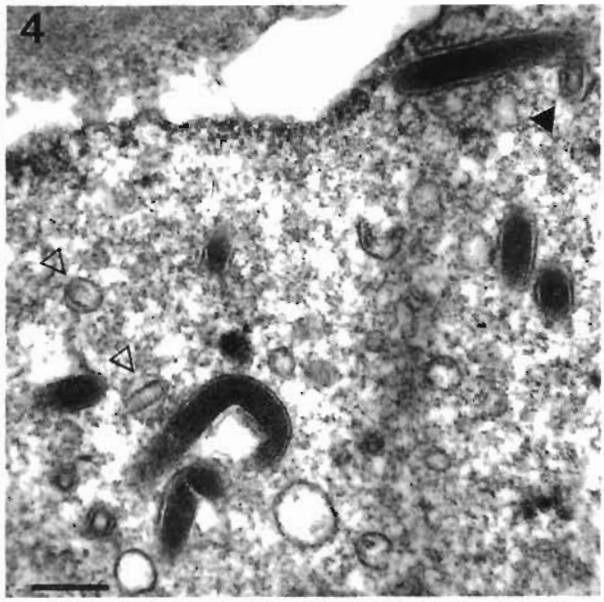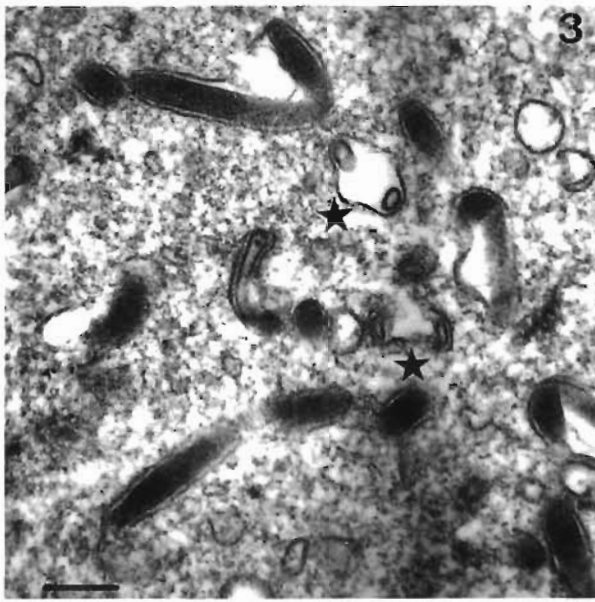
and ectodermally derived tissues. These inclusions are pathognomonic for IHHNV which is believed to be responsible for the epizootic.

In gill preparations, a number of cells were seen to harbour rod-shaped virions. The cell types included small-granule haemocytes (Fig. 1a, b) and unrecognisable cell types (Fig. 2). Bar-shaped virions, viral cross-sections and empty capsids were observed within thin-walled, membrane-bound vacuoles and free in the cytoplasm of the haemocytes. The nuclei of these haemocytes displayed slight margination and darkening of the chromatin, and nucleoplasm changes but lacked virions (Fig. 1a, b). This was assumed to represent recent phagocytosis without viral replication but possible nuclear infection. Some patently infected cells showed the remnants of nuclear chromatin which identified the main area of virogenesis as the nucleus (Fig. 2). In some infected cells, the nucleus could not be distinguished nor could cytoplasmic organelles. It was assumed that the virogenic stroma had obscured organelles and that the cytoplasmic-nuclear boundaries had merged.

Many of the virions were contained singly in a envelope that caused them to bend in either a V-shape (Fig. 3) or a U-shape (Fig. 4). The extra-long, mostly bent nucleocapsids measured 888 × 86 nm mean size (Table 1) whilst a second group of straight nucleocapsids were somewhat smaller at 542 × 86 nm mean size. Some virions had a cigar or baseball bat shape (Fig. 5). No evidence of the nucleocapsids acquiring either a primary or secondary envelope by budding through the nuclear or cell membranes was seen. The single envelope appeared to be totally formed de novo.

Infected cells often had partially enveloped, empty capsids throughout their contents (Fig. 6). The empty capsids (presumed to be fully formed) measured 496 × 83 nm mean size including the envelope. These empty capsids were smaller than filled nucleocapsids. Many fully enveloped but empty capsids were seen and they were sometimes bent within the envelope (Fig. 3).

A number of capsid originators were seen, some in association with almost complete virions but still shar-ing a membrane (Fig. 6). The membranes did not fully enclose the capsid originators which measured 50 to 60 nm (Figs. 6 & 7). The specialised apex thought to develop from the capsid originator (Johnson 1988) was not easily recognised in these preparations (Fig. 5).

Cross sections of virions showed the envelope to have 2 electron-dense protein rings separated by an electron-lucent lipid ring (Fig. 8). Inside the envelope, just preceding the capsid and nucleoprotein core, the intermediate layer (slightly electron-lucent) was visible.

DISCUSSION

The presence of empty capsids and capsid originators as well as complete virions suggests that viral replication is taking place and that the prawn is not acting only as a carrier host. As haemocytes are the only identifiable cell type that had virions present, and considering the great similarity of this virus to the crab haemocyte-infecting rod-shaped viruses, it is believed that haemocytes are a tissue that supports virogenesis of this virus. The present case represents the first record of a rod-shaped viral infection of the haemocytes of a penaeid or, indeed, any non-portunid marine crustacean. It increases the known range of haemocytic rod-shaped viruses to the Pacific Ocean and Australia.

The virions, nucleocapsids and capsids are considerably longer than any other haemocytic rod-shaped virus (Table 1). Similarly, virion and nucleocapsid widths are also greater than for Rod-B. However, empty capsid width is less than either RV-CM or Rod-B whilst nucleocapsid width is intermediate between them (Table 1). This phenomenon of the capsid expanding when filled with nucleoprotein is common in haemocytic rod-shaped viruses (Johnson 1988). Based on morphometrics, the current haemocytic rod-shaped virus is dissimilar to known portunid haemocytic rod-shaped viruses. Therefore, the virus herein is named penaeid haemocytic rod-shaped virus (PHRV) until more is known about its relationship to other haemocytic rod-shaped viruses.

Table 1. Morphometrics (nm) of penaeid haemocytic baculovirus and comparisons with other haemocytic baculoviruses

	PHRV	RV-CM	Rod-B	Bracovirus	Ichnovirus
Extraordinary virion length	840–920	95–760	–	30–150	–
Ordinary virion length	430–640	370–390	–	–	–
Virion width	112–125	85–100	–	–	–
Nucleocapsid length	410–600	190–540	220–260	30–150	330
Nucleocapsid width	80–90	95–110	75–85	40	85
Empty capsid length	442–525	210–280	220–280	–	–
Empty capsid width	45–55	65–70	53–60	–	–
Capsid originators	50–60	53–64	53–64	–	–

Of interest was the total lack of budding of the PHRV nucleocapsid through either the nuclear membrane or the cellular membrane to acquire the envelope. In the gut-infecting baculoviruses, non-occluded virions have been shown to acquire their envelope in this manner, especially those that are going to infect other cells in the same host (Federici 1986). Similarly, non-occluded baculoviruses of marine animals such as *Scylla serrata* receive their second envelope via budding (Anderson & Prior 1992).

PHRV shows affinities to the *Bracovirus* within the Polydnaviridae (Francki et al. 1991). In particular, the great variable length of the nucleocapsids and the presence of only 1 de novo-produced envelope are indicative of *Bracovirus*. The variable length of the virions is also suggestive of segmented DNA. PHRV is also somewhat similar to the *Ichnovirus* within the same family in that a few smaller virions tend to be prolate ellipsoids (cigar shaped) and the size of the virions is closer to that reported for *Ichnovirus* than that reported for *Bracovirus*. However, the *Ichnovirus* have 2 envelopes, 1 of which is acquired by budding, not 1 de novo envelope as seen here. Johnson (1988) suggested that the rod-shaped haemocytic infecting viruses of Crustacea showed several similarities to the Polydnaviridae and this virus supports those suggestions.

The concurrent infection with IHHNV may have allowed expression of an otherwise latent virus. Alternatively, the host may possibly have been genetically immuno-compromised (Owens et al. 1992), and the penaeid may not be the normal host for this virus. Indeed, the fact that all previous cases have been in

portunid crabs suggests that local portunids should be examined for further cases of PHRV. Further investigations for PHRV are being undertaken so a clearer picture of its epidemiology can be obtained.

LITERATURE CITED

Anderson, I. G., Prior, H. C. (1992). Baculovirus infections in the mud crab, *Scylla serrata*, and a freshwater crayfish, *Cherax quadricarinatus*, from Australia. J. Invertebr. Pathol. 60: 265–273

Culling, C. F. A., Allison, R. T., Barr, W. T. (1985). Cellular pathology techniques,. 4th edn. Butterworths, London

Federici, B. A. (1986). Ultrastructure of baculoviruses. In: Granados, R. R., Federici, B. A. (eds.) The biology of baculoviruses. Vol. I. Biological properties and molecular biology. CRC Press, Boca Raton, p. 61–88

Francki, R. I. B., Fauquet, C. M., Knudson, D. L., Brown, F. (1991). Classification and nomenclature of viruses. Arch. Virol. Suppl. 2: 1–450

Johnson, P. T (1988). Rod-shaped nuclear viruses of crustaceans: hemocyte-infecting species. Dis. aquat. Org. 5: 111–122

Johnson, P. T., Lightner, D. V. (1988). Rod-shaped nuclear viruses of crustaceans: gut-infecting species. Dis. aquat. Org. 5: 123–141

Owens, L., Anderson, I. G., Kenway, M., Trott, L., Benzie, J. A. H. (1992). Infectious hypodermal and haematopoietic necrosis virus (IHHNV) in a hybrid penaeid prawn from tropical Australia. Dis. aquat. Org. 14: 219–228

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