TRANSMISSION ELECTRON MICROSCOPY OF MARINE CRUSTACEAN EGGS

BY

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ABSTRACT

Ultrastructural investigations of eggs can be important in helping to understand embryonic development. There are few transmission electron microscope studies of marine arthropod eggs, however, as they have proved difficult to fix and infiltrate with resin. Here, we describe a modification of a standard method that allows the preparation of the quite different eggs of the marine copepod, Acartia tonsa and the lobster, Homarus gammarus, for transmission electron microscopy. By using double fixation and an extended resin infiltration time we obtained good preparations for electron microscopy. We anticipate that these modifications to the standard protocol will be widely applicable and useful for the study of the eggs and early developmental stages of many marine arthropod taxa.

RÉSUMÉ

Les recherches sur l’ultrastructure des œufs peuvent être importantes en aidant à comprendre le développement embryonnaire. Il existe cependant peu d’études en microscopie électronique à transmission sur les œufs d’arthropodes marins, car il est difficile de les fixer et d’y infiltrer de la résine. Dans ce travail, nous décrivons une modification de la méthode standard, qui permet la préparation pour la microscopie électronique à transmission d’œufs aussi différents que ceux du copépode marin Acartia tonsa et du homard Homarus gammarus. En utilisant une double fixation et un temps plus long d’infiltration de la résine, nous avons obtenu de bonnes préparations pour la microscopie électronique. Nous prévoyons que ces modifications du protocole standard seront largement applicables et utiles pour l’étude des œufs et des premiers stades de développement de nombreux taxons d’arthropodes marins.

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Crustaceana 80 (6): 739-745

Also available online: www.brill.nl/cr
INTRODUCTION

Transmission electron microscopy (TEM) remains a valuable tool for studying organisms at the ultrastructural level. In marine organisms these studies have helped address questions ranging from immunology (Wootton & Pipe, 2003) to population biology (Norbín, 2001). There are few successful ultrastructural investigations of arthropod eggs, however, despite this being an important developmental stage. The egg envelope and the high internal lipid content of marine arthropod eggs both present particular problems for fixation and resin infiltration (Blades-Eckelbarger & Marcus, 1992; Santella & Ianora, 1992), which are key stages in preparing good TEM specimens.

Methods of fixation and resin embedding for TEM are often species and tissue specific in some way. Primary fixation normally uses a mixture of paraformaldehyde-glutaraldehyde often for varying periods of time, e.g., 90 minutes for soft tissues (Kikuchi et al., 2002) or up to 1 month in specimens with an exoskeleton (Santella & Ianora, 1992). Secondary treatment with osmium tetroxide over a period of no more than 2.5 hours is standard for fixation of lipids (Høeg et al., 2004) and this stage may be combined with the primary fixative (Hayat, 1981). There are several embedding media available for TEM (“TAAB”: Luxford & Murphy, 1992; “Epon-Araldite”: Sloane et al., 1994; “Spurrs”: Menzies & Kourteva, 1998; and “LR White”: Radford & White, 1998) that differ in their ability to penetrate the specimen and in their hardness; infiltration times of 24 hours are usual for these resins (Paffenhöfer & Loyd, 2000).

During a TEM investigation of the presence of endosymbiotic bacteria in the ovaries and eggs of the marine copepod, *Acartia tonsa* (Dana, 1848) and the European lobster, *Homarus gammarus* (Linnaeus, 1758), we have developed a successful method for the fixation and resin infiltration of these quite different marine arthropod eggs that involves extended fixation and infiltration times.

MATERIALS AND METHODS

Specimen collection

Eggs of *Acartia tonsa* were collected from adult females reared in the laboratory. Stage 1 or stage 2 (Helluy & Beltz, 1991) eggs of *Homarus gammarus* were collected from gravid females caught off the Cornish coast of England.

Fixation

Upon collection, *A. tonsa* and *H. gammarus* eggs were fixed at 4°C for 5 weeks in 0.1 M sodium cacodylate buffer containing 2% paraformaldehyde and 2.5%