ASSESSMENT OF *CALANUS HELGOLANDICUS* EMBRYO AND NAUPLIAR MORTALITY USING FLUORESCENT PROBES

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Abstract

A diatom diet of *Thalassiosira rotula* and *Skeletonema costatum* induced egg mortality and abnormal development of hatched copepod *Calanus helgolandicus* nauplii, depending on the number of ingested cells and the duration of feeding. Here we use fluorescent techniques to evaluate copepod embryo mortality following spawning, using the vital probe SYTOX Green, and to assess apoptosis in abnormal nauplii using the TUNEL enzymatic assay.

Key-words: Copepod; vital fluorescent probes; apoptosis

In recent years it has been shown that several species of diatoms produce α–β unsaturated aldehydes that block embryonic divisions in various species of invertebrates, including copepods (1-3). The cellular target of these molecules is still unknown but previous studies have demonstrated that decadienal, one of the main unsaturated aldehydes isolated from diatoms, depolymerises microtubules and actin filaments in sea urchin and tunicate embryos (1;4) and induces apoptosis in copepod and sea urchin embryos (5). When copepods feed a diatom diet, egg viability is reduced in a time and concentration dependent manner (5;6). At lower diatom concentrations, some embryos develop to hatching but the embryos thus generated are strongly malformed(6). Here we apply two different fluorescent techniques to study the effect of diatom diets on the reproductive physiology of the copepod *Calanus helgolandicus*: a protocol to rapidly calculate embryo mortality soon after spawning, using the vital fluorescent probe SYTOX Green (Molecular probes), and an enzymatic *in-situ* labeled nucleotide assay (dUTP nick-end labelling, TUNEL) to evaluate the induction of apoptosis in abnormal nauplii.

SYTOX Green is a non-permeant nucleic acid stain that enters only into cells with damaged plasma membranes such as in dead cells that then appear with green fluorescent nuclei. *Calanus helgolandicus* embryos, produced by females fed the diatom *Thalassiosira rotula,* were incubated in chitinase solution (1U/ml for 50 min at 20°C) to permeabilize the chitinous wall soon after egg spawning. Embryos were then incubated in SYTOX Green 20 µM for 50 min, and observed with the epifluorescent or confocal laser scanning microscope. Egg mortality was determined as the number of fluorescent as opposed to non-fluorescent embryos. DNA fragmentation due to apoptosis was detected with the TUNEL kit (Boheringher GmbH) on abnormal *C. helgolandicus* nauplii produced by females fed the diatom *Skeletonema costatum*.

Eight days after feeding, percentage egg mortality increased to 75% when embryos were allowed to develop naturally to hatching (Fig. 1 control). SYTOX Green-treated embryos gave similar results for embryo mortality (Fig. 1 Sytox + and insert). Figure 2 shows a deformed nauplius positively stained with TUNEL (arrow) indicating activation of apoptotic processes in abnormal tissues. Such nauplii die soon after hatching.

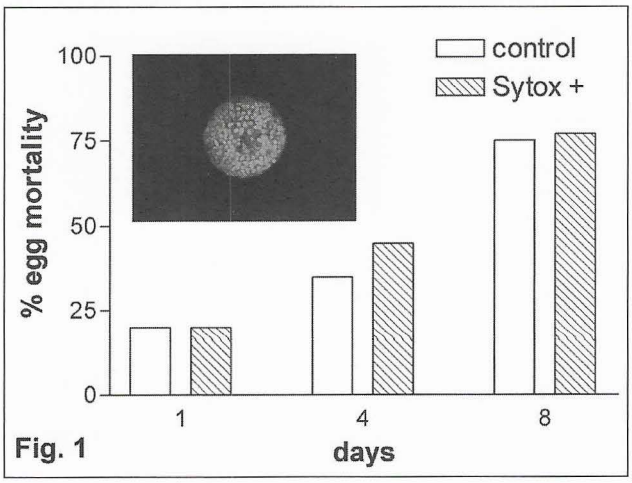

Fig. 1

The possibility of applying immuno-fluorescence techniques to stain embryos and nauplii opens new perspectives in studies on the reproductive physiology of copepods and zooplankton, allowing for the rapid assessment (2h) of hatching success and abnormal embryonic and post-embryonic development compared to other conventional techniques.

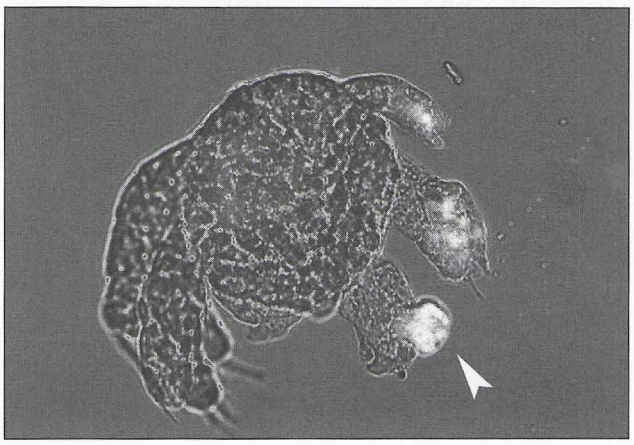

Fig. 2.

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