A NOVEL METHOD TO DETECT EMBRYO VIABILITY IN THE EGG-CARRYING COPEPOD *CLAUSOCALANUS FURCATUS*

Isabella Buttino *, Àurea Peralba and Maria Grazia Mazzocchi
Stazione Zoologica "Anton Dohrn", Napoli (I), Italy

Abstract

Copepods of the genus *Clausocalanus* are among the most abundant calanoids in Mediterranean zooplankton communities. However, the biology of this genus has been scarcely studied due to difficulties in species identification and rearing. Moreover, most *Clausocalanus* species carry the eggs, hindering an estimate of their egg production and viability. In fact, when the sac is separated from the female, eggs are unable to hatch. In this study we apply a novel method to *C. furcatus* to detect egg viability using vital fluorescent probe Fluorescein diacetate (FDA).

Key-words: vital fluorescent probe; confocal microscopy, fluorescein diacetate

Measurements of hatching success and naupliar viability in planktonic copepods provide important data for understanding recruitment rates and secondary production at sea (1). These parameters have also been used to test diet quality and toxic effects of marine natural products (2). In broadcast spawner copepods, hatching success can be easily calculated by allowing eggs to develop undisturbed to hatching (1). In egg-carrying copepods, however, this protocol is difficult to apply because embryos are not able to hatch once isolated from females, and total egg production rates can not be accurately defined.

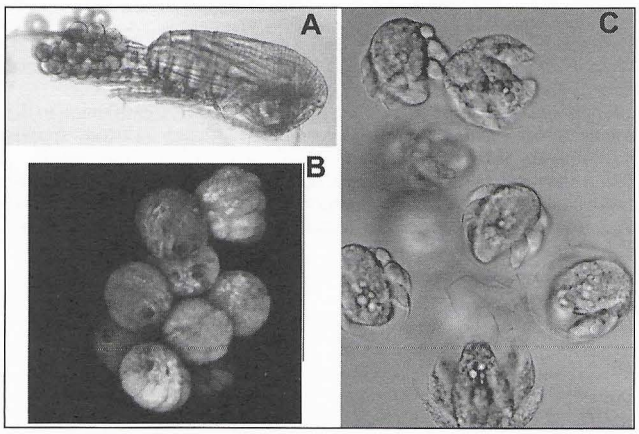
In plankton communities over a wide latitudinal range, very abundant copepods such as *Pseudocalanus* in the Atlantic Ocean, *Clausocalanus* in the Mediterranean Sea, *Oithona* and *Oncaea* in tropical environments, carry their embryos in sacs or egg masses.

In this study we analysed the egg viability of an egg-carrying calanoid copepod using a vital fluorescent probe Fluorescein diacetate (FDA) (Sigma-Aldrich). This dye penetrates in viable cells where esterases produce free fluorescent fluorescein and cells appear fluorescent in green.

Our target species was *Clausocalanus furcatus* (Brady, 1883), which is dominant in the epipelagic zone above the thermocline during summer in the Mediterranean Sea (3). *Clausocalanus furcatus* carry their eggs on the abdomen in a cylindrical mass that is discharged when the female is disturbed (4). Our method allowed analysing egg viability without disturbing females, and provided accurate measurement of egg production.

Clausocalanus furcatus females were sorted from zooplankton samples collected in the surface water of the Gulf of Naples (Southern Italy). They were incubated at 23°C in 2 l jars filled with natural particle assemblage collected at the sampling site with Niskin bottles. After 24h, the egg-carrying females were separated in two groups. One group were incubated in 7.5 µM FDA. After 15 min embryos were carefully separated from females and observed with the epifluorescent or confocal microscopy. The percentage of fluorescent embryos is a measure of their viability. Another group of females were incubated in 300 ml flask, left undisturbed until nauplii hatching and fixed in 4% paraformaldehyde. After settling, nauplii and eggs were counted under a stereomicroscope, as control.

Figure 1A shows a *C. furcatus* female carrying the egg mass. After FDA staining, viable embryos appeared fluorescent in green (Fig. 1B).



The same embryos hatched soon after observation confirming that they were viable (Fig. 1C). Some nauplii hatched after the embryos were removed from females probably because they were separated at the end of the embryonic development. The percentage of fluorescent embryos was 99± 1.5 sd similar to the controls. In free-spawning copepods this technique required previous permeabilisation of the chitinouse wall (5) while in egg-carrying copepods this treatment was not necessary, indicating that eggs lack hard chitin protection in egg-carrying species.

This simple and precise method allows to rapidly detect egg viability in egg-carrying copepods and could also be used to investigate other physiological aspects of egg production, such as remating frequency, or how long embryos must be carried for a successful hatching.

References

1 - Ianora A., 1998 Copepod life history traits in subtemperate regions. *J. Marine Syst.,* 15: 337-349.

2 - Miralto A., Guglielmo L., Zagami G., Buttino I., Granata A., Ianora A., 2003 Inhibition of population growth in the copepods *Acartia clausi* and *Calanus helgolandicus* during diatom blooms. *Mar. Ecol. Prog. Ser.,* 254: 253-268.

3 - Mazzocchi M.G. and Ribera d'Alcalà M. 1995 Recurrent patterns in zooplankton structure and succession in a variable coastal environment. *ICES Journal Marine Science,* 52: 679-691.

4 - Mazzocchi M.G. and Paffenhöffer G.A., 1998 First observations on the biology of Clausocalanus *furcatus* (Copepoda, Calanoida). *J. Plankton Res.,* 20: 331-342.

5 - Buttino I., Ianora A., Carotenuto Y., Zupo V. and Miralto A., 2003 Use of the Confocal Laser Scanning Microscope in studies on the developmental biology of marine invertebrates. *Microsc. Res. Tech.,* 60: 458-464.