Use of fluorescent probes in studies on copepod reproduction and development

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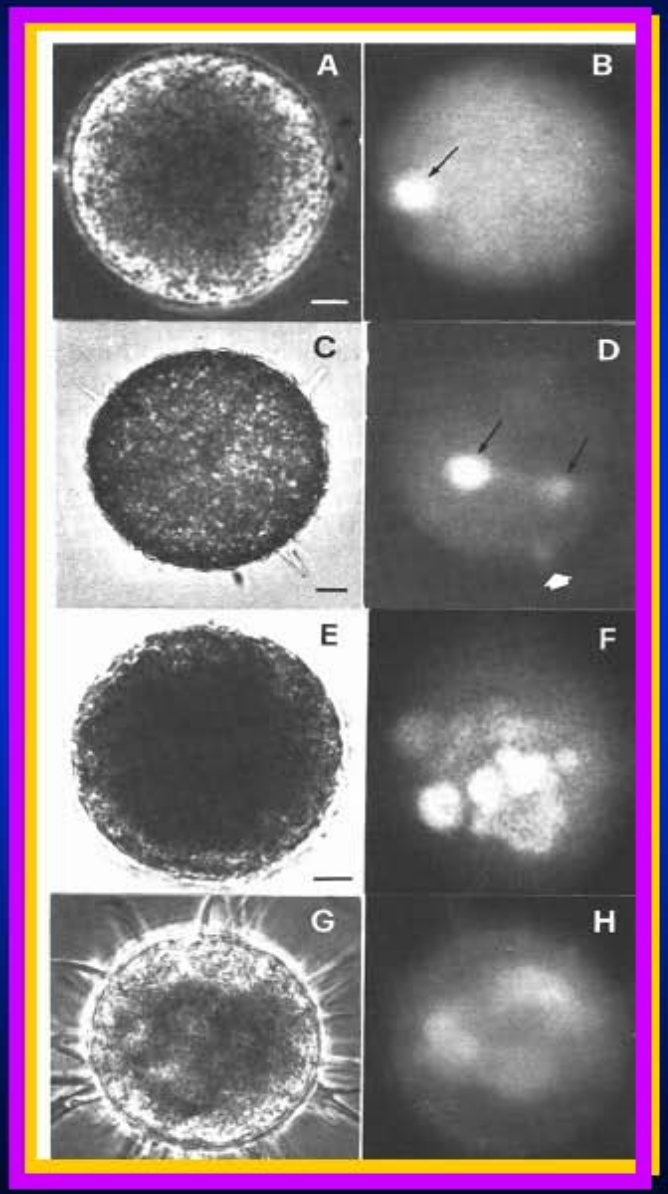
What are fluorescent molecular probes?

A fluorescent probe is a fluorophore designed to localize specific cellular structures within a defined region of a biological specimen or to respond to a specific stimulus.

Fluorescent probes are common analytical tools in the life sciences and biotechnology commonly used to label nucleic acids, proteins and other bio-molecules.

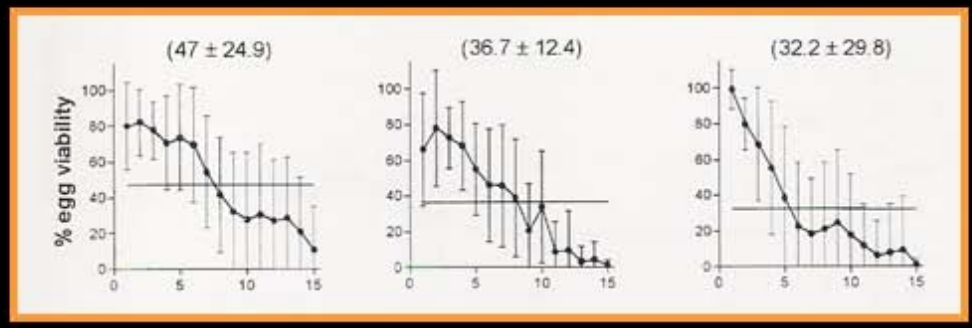
Centropages typicus viable and nonviable eggs



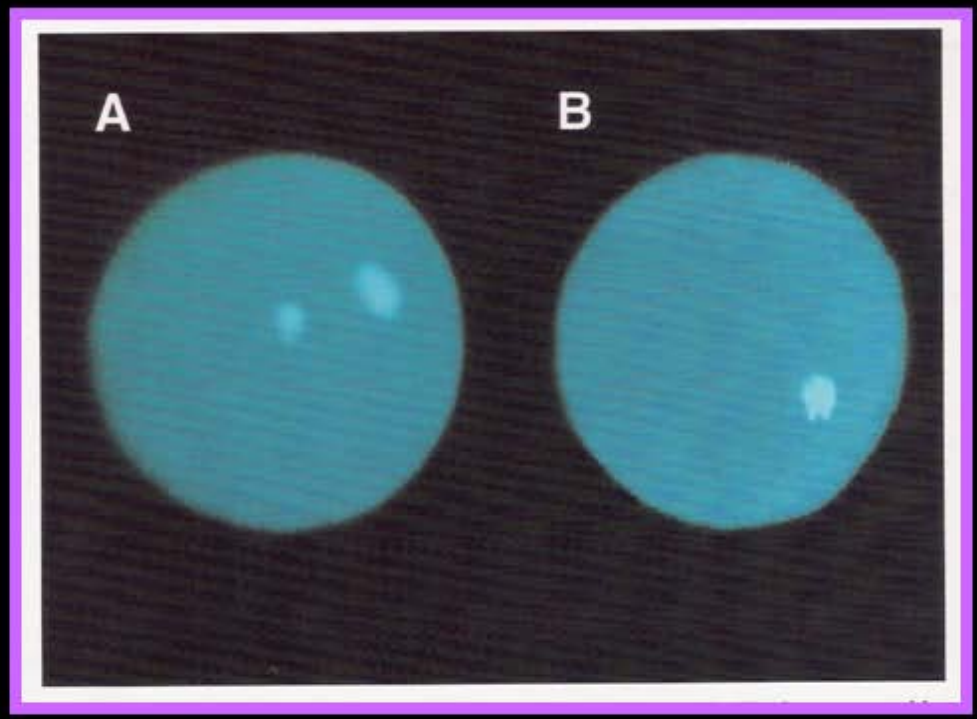
Eggs stained with the vital dye Hoechst 33342 which Stains intracellular nuclei

from Ianora et al. 1992

Hatching failure in *Temora stylifera* due to dinoflagellate diets (*Prorocentrum micans*, *Gymnodinium sanguinium* and *Gonyaulax polyedra*) compared to control (*P. minimum*)

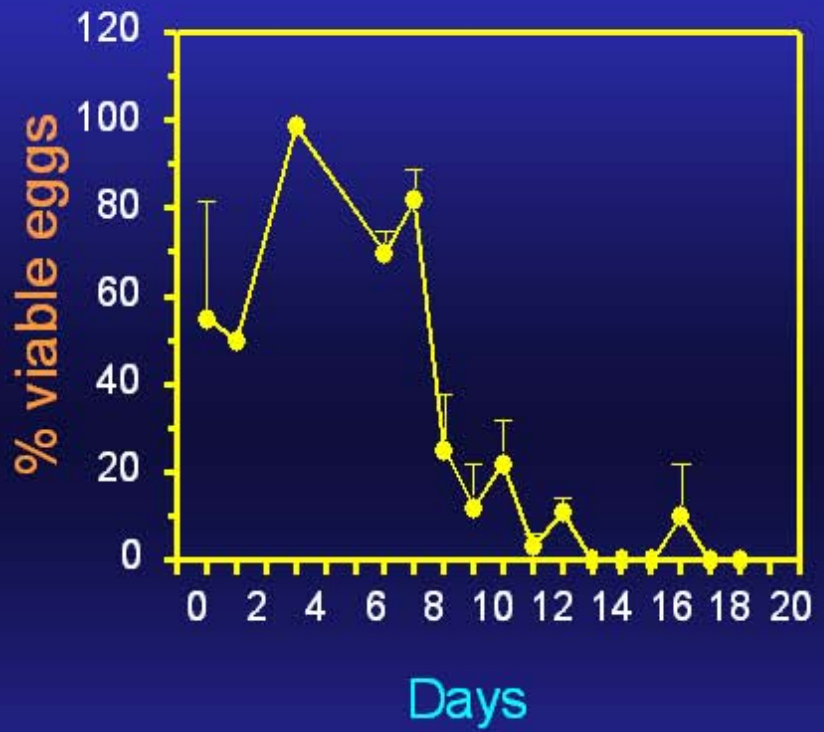


Eggs stained with Hoechst 33342

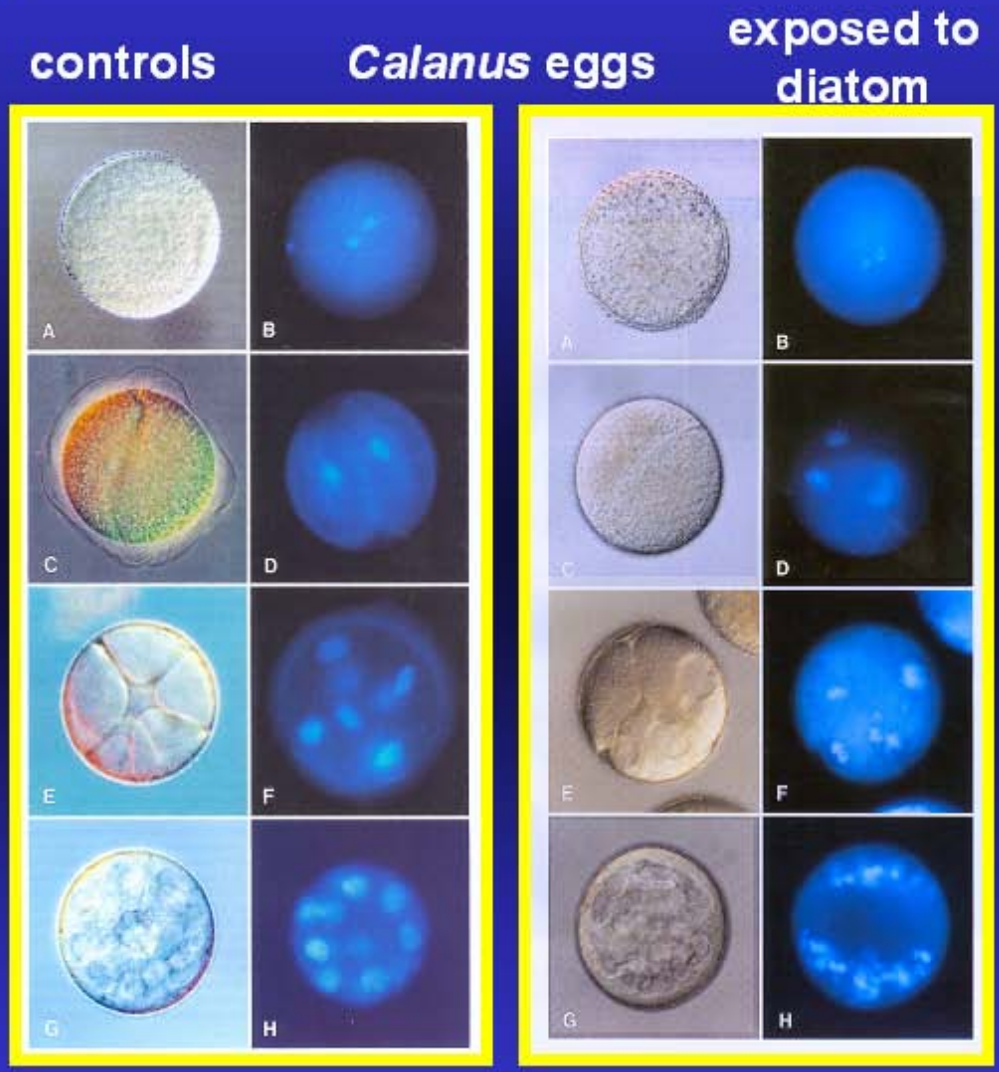


Ianora et al. Limnol. Oceanogr. 1999

Calanus helgolandicus fed *Phaeodactylum tricornutum*



Eggs stained with Hoechst 33342



from Poulet et al. 1995

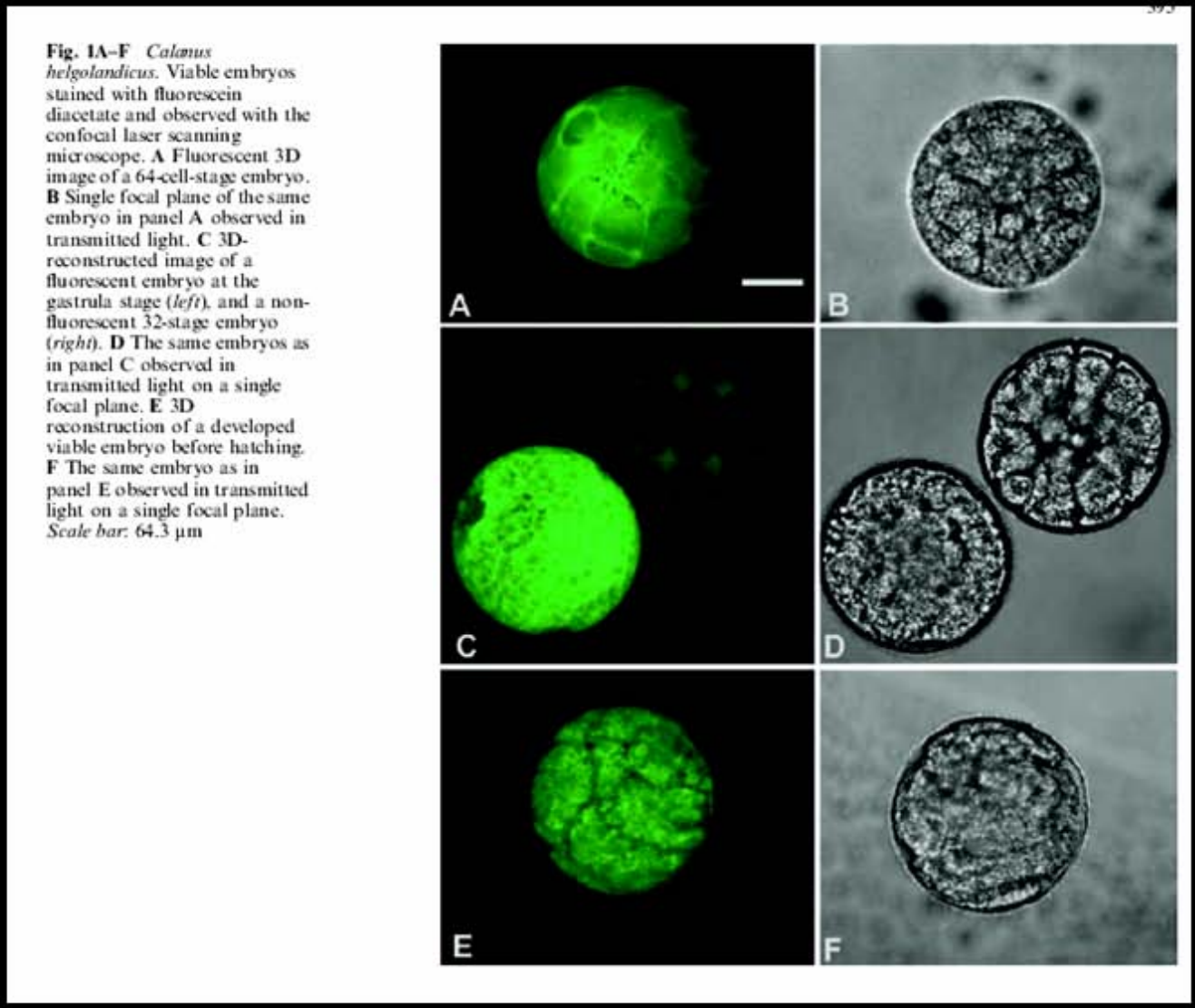
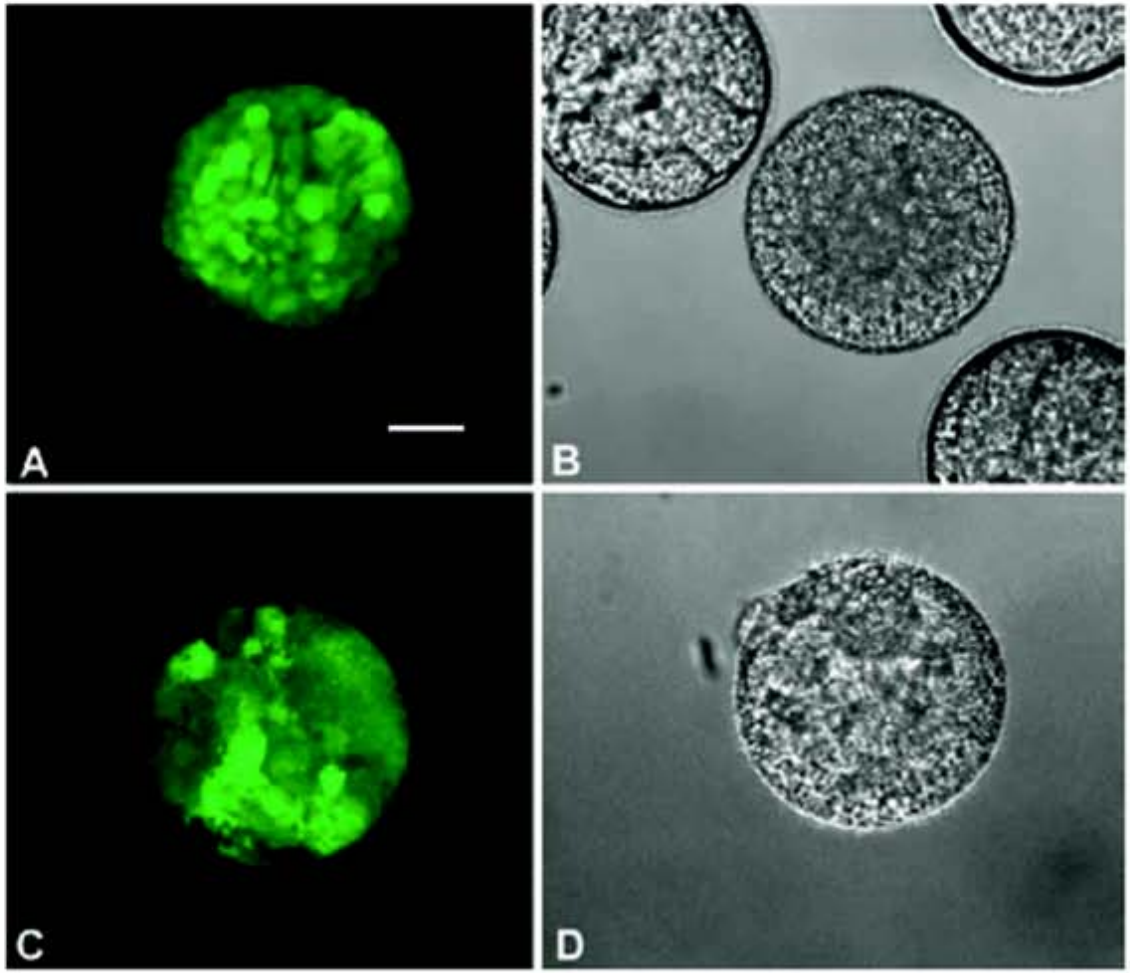


Fig. 1A–F *Calanus helgolandicus*. Viable embryos stained with fluorescein diacetate and observed with the confocal laser scanning microscope. **A** Fluorescent 3D image of a 64-cell-stage embryo. **B** Single focal plane of the same embryo in panel A observed in transmitted light. **C** 3D-reconstructed image of a fluorescent embryo at the gastrula stage (*left*), and a non-fluorescent 32-stage embryo (*right*). **D** The same embryos as in panel C observed in transmitted light on a single focal plane. **E** 3D reconstruction of a developed viable embryo before hatching. **F** The same embryo as in panel E observed in transmitted light on a single focal plane. *Scale bar*: 64.3 µm

When FDA penetrates into viable cells, esterases produce free fluorescent fluorescein and cells appear fluorescent in green, whereas cells with an inactive metabolism are not fluorescent.

Buttino et al. Mar. Biol. 2004

Fig. 2A–D *Calanus helgolandicus.* Non-viable embryos stained with SYTOX green and observed with the confocal laser scanning microscope. **A** Fluorescent 3D image of non-viable morula embryo. **B** Transmitted light of the same field as in panel A; the darker embryo at the center of the field is positively stained with SYTOX green. **C** Fluorescent 3D image of an abnormal embryo with dispersed chromatin. **D** The same embryo as in panel C observed in transmitted light on a single focal plane. *Scale bar*: 58.5 µm



SYTOX green is a nucleic acid stain that enters only into cells with damaged plasma membranes, such as in dead cells, which then appear with green fluorescent nuclei.

Buttino et al. Mar. Biol. 2004

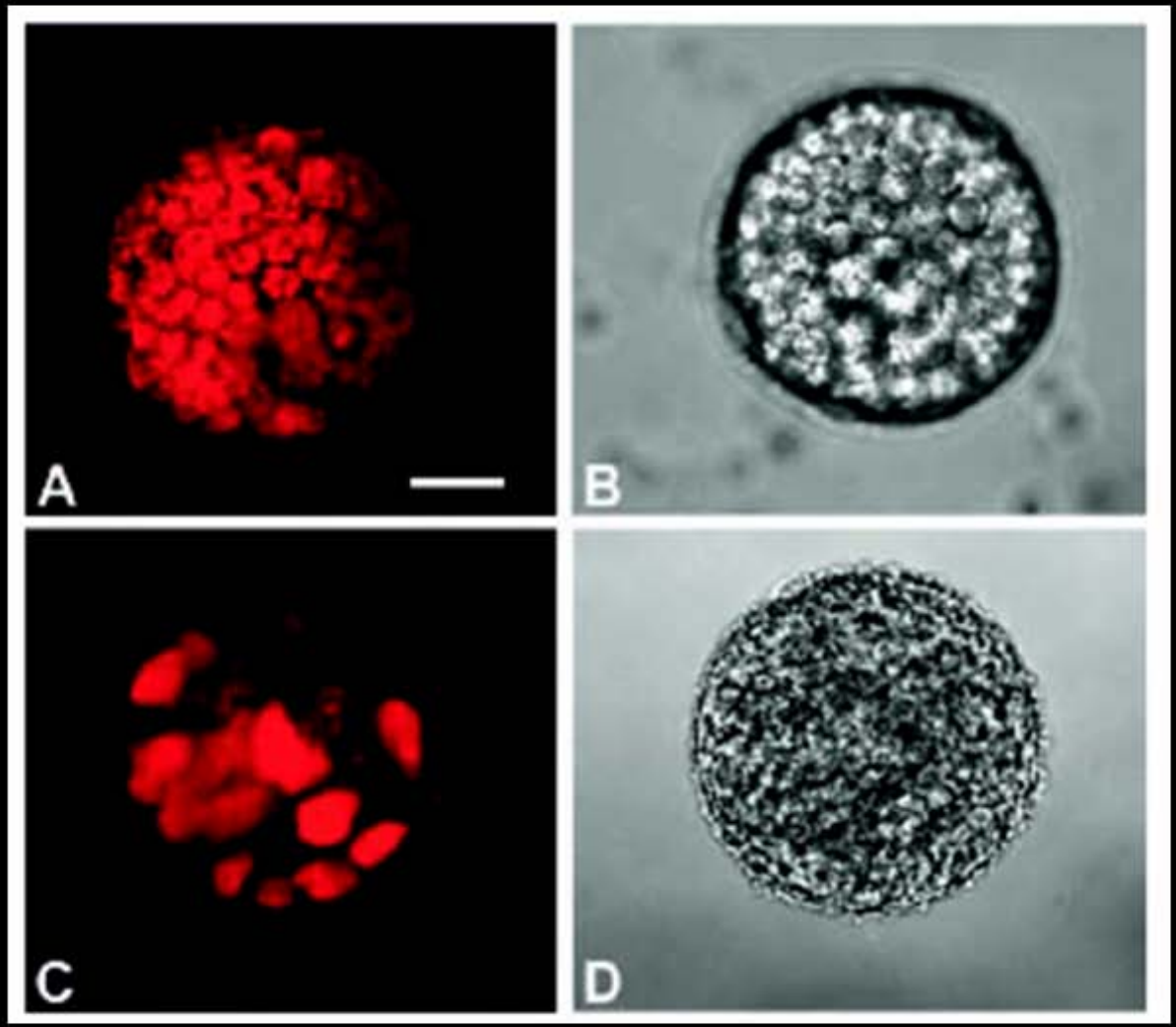


Fig. 3A–H *Calanus helgolandicus*. Non-viable embryos stained with 7-aminoactinomicycin D (**A–D**) and auto-fluorescent unstained embryos (**E–H**), observed with the confocal laser scanning microscope. **A** 3D reconstruction of a fluorescent embryo at the morula stage. **B** The same embryo as in panel A observed in transmitted light on a single focal plane. **C** 3D reconstruction of a fluorescent embryo; nuclei are asymmetrically distributed in the cytoplasm. **D** The same embryo as in panel C observed in

Buttino et al. Mar. Biol. 2004

7-AAD is a fluorescent DNA probe that is excluded from live cells; dead cells appear with red fluorescent nuclei.

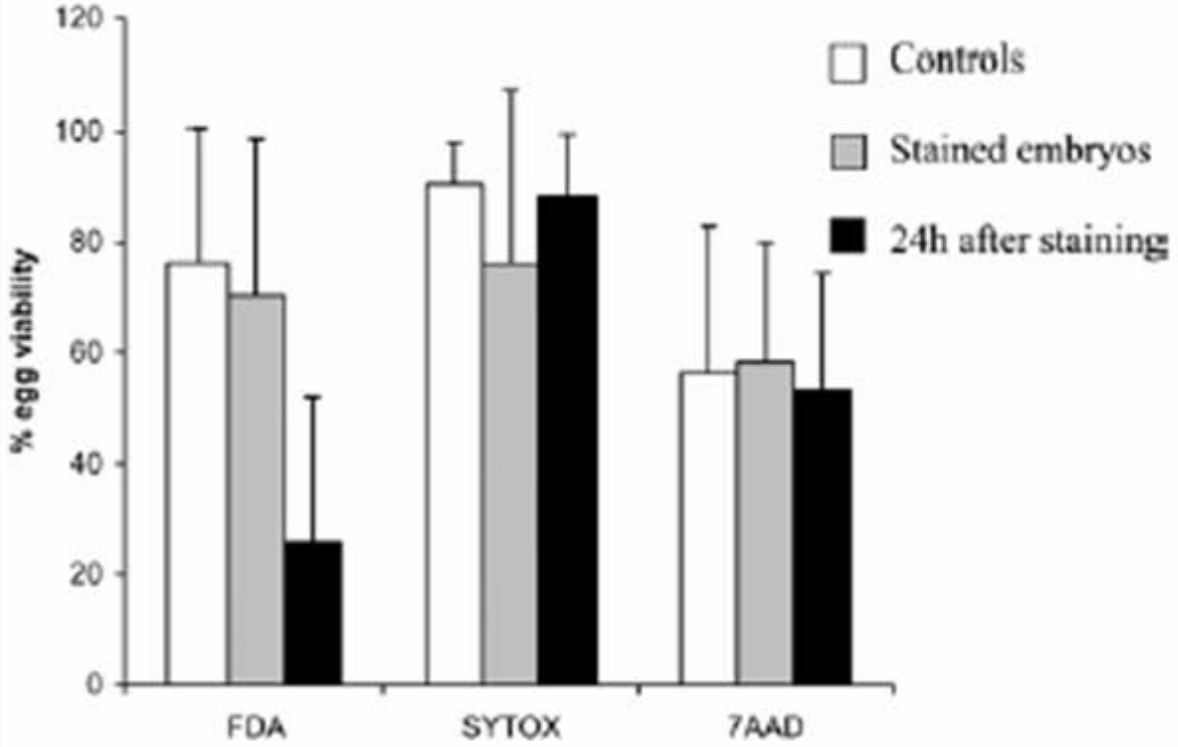
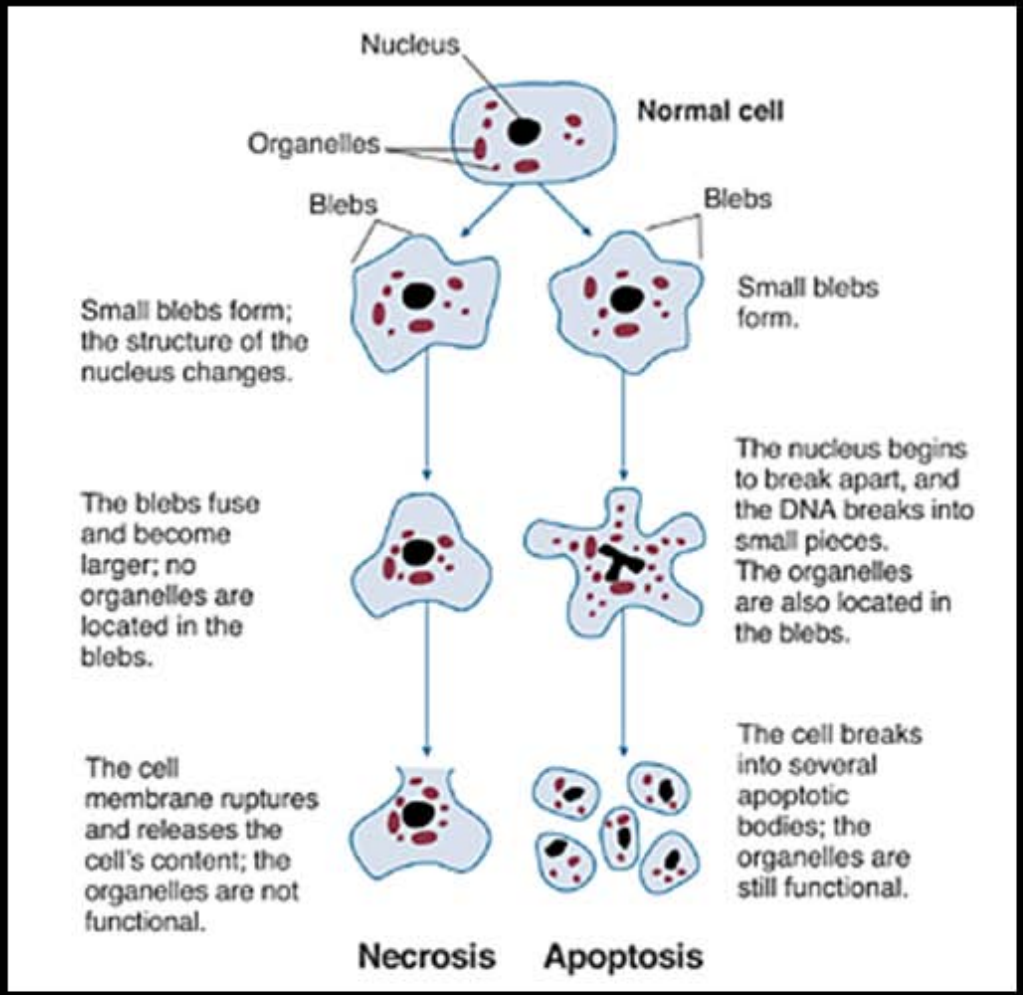


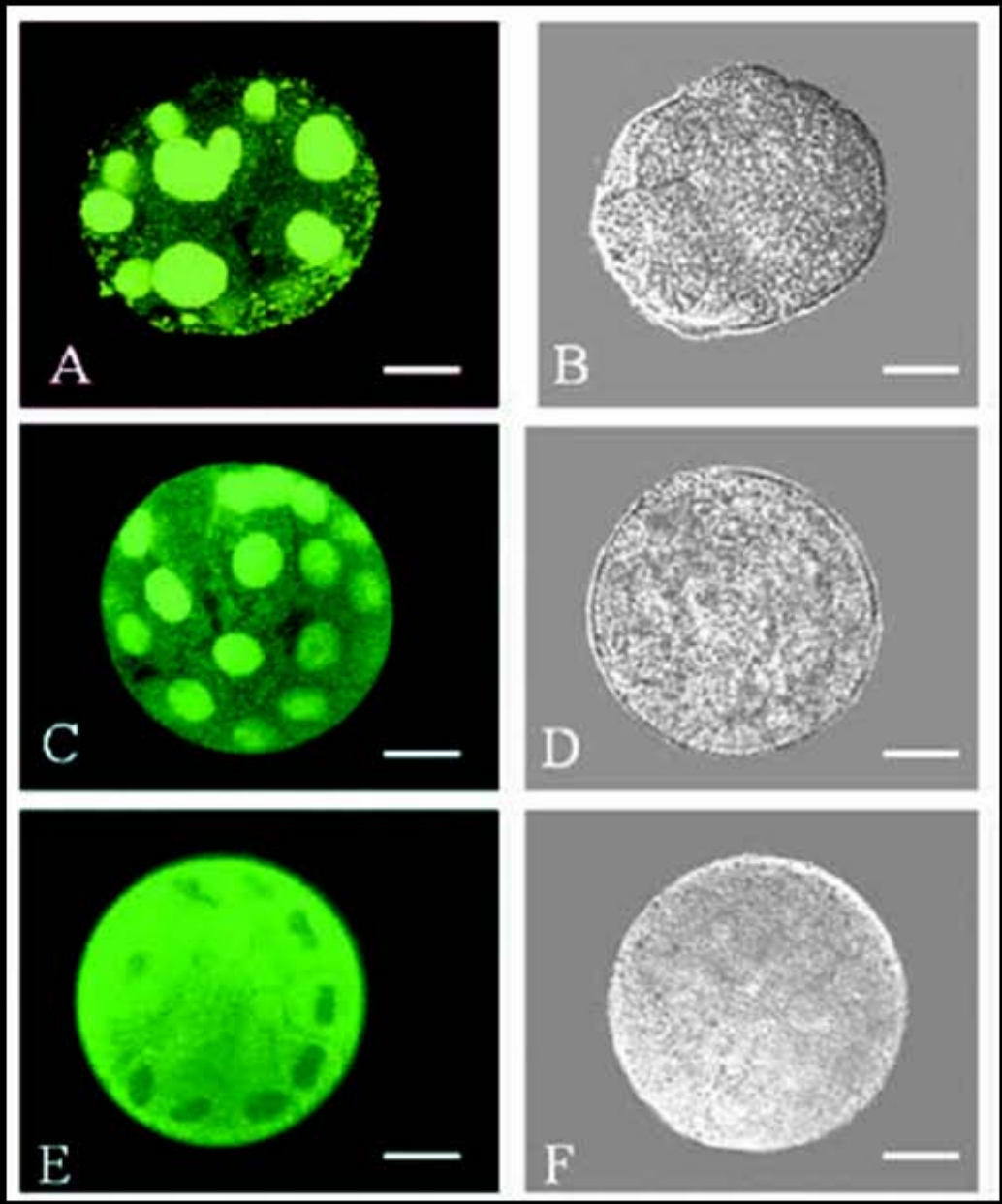
Fig. 4 *Calanus helgolandicus*. Comparison between the percentage of egg viability for unstained embryos (*controls*), FDA-fluorescent embryos, for SYTOX green– and 7-AAD–non-fluorescent embryos (*stained embryos*), and for embryos stained with the three probes and then allowed to hatch (*24 h after staining*) (mean ± SD)

Buttino et al. 2004

How do cells die?



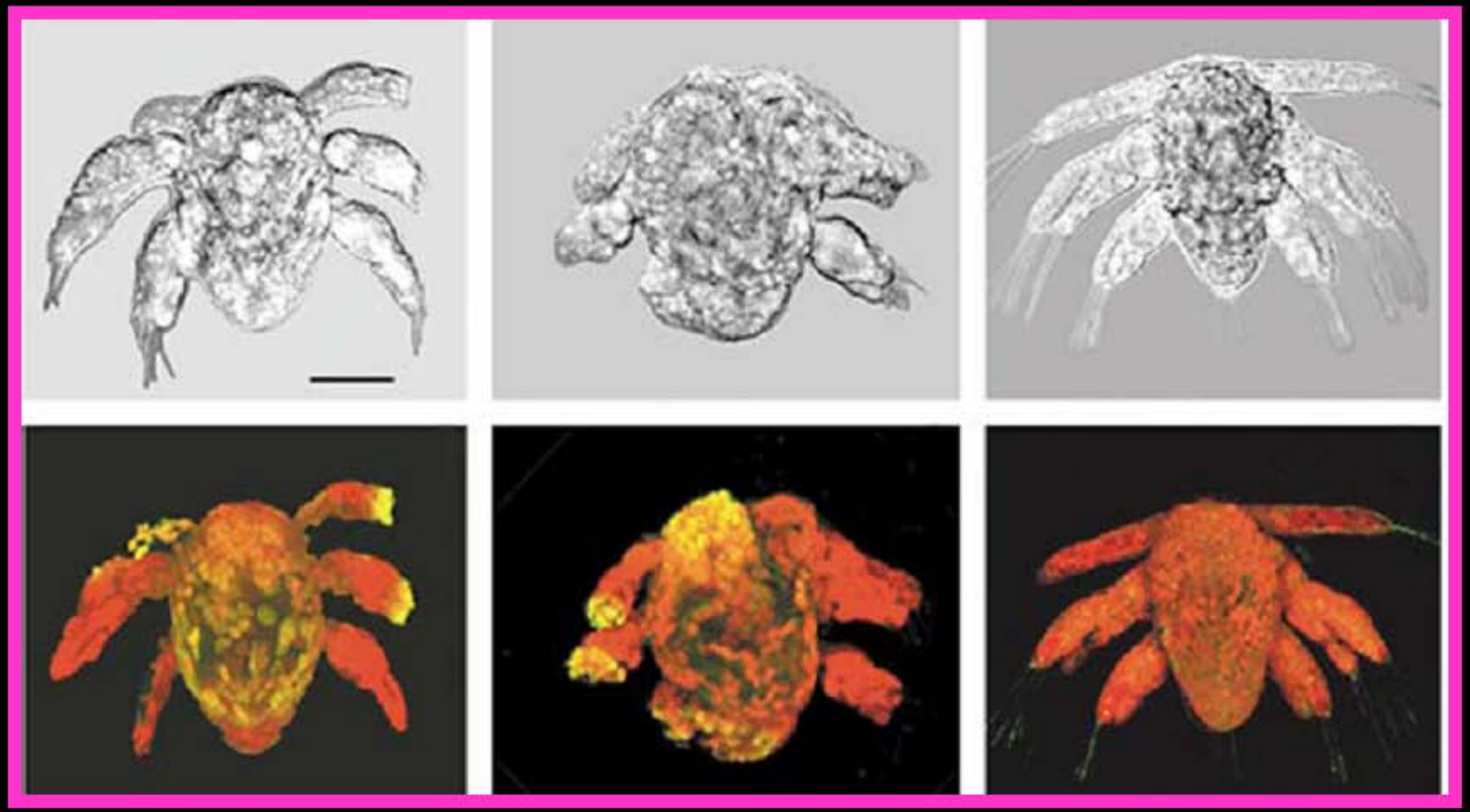
Necrosis (passive cell death) and apoptosis (active self-destruction).
In necrosis the cellular metabolism breaks down, the cell swells and cellular membranes decompose.
In apoptosis the cell rounds and shrinks; the DNA is systematically fragmented.



Calanus helgolandicus **embryos stained with TUNEL dye to detect apoptosis(programmed cell death)**

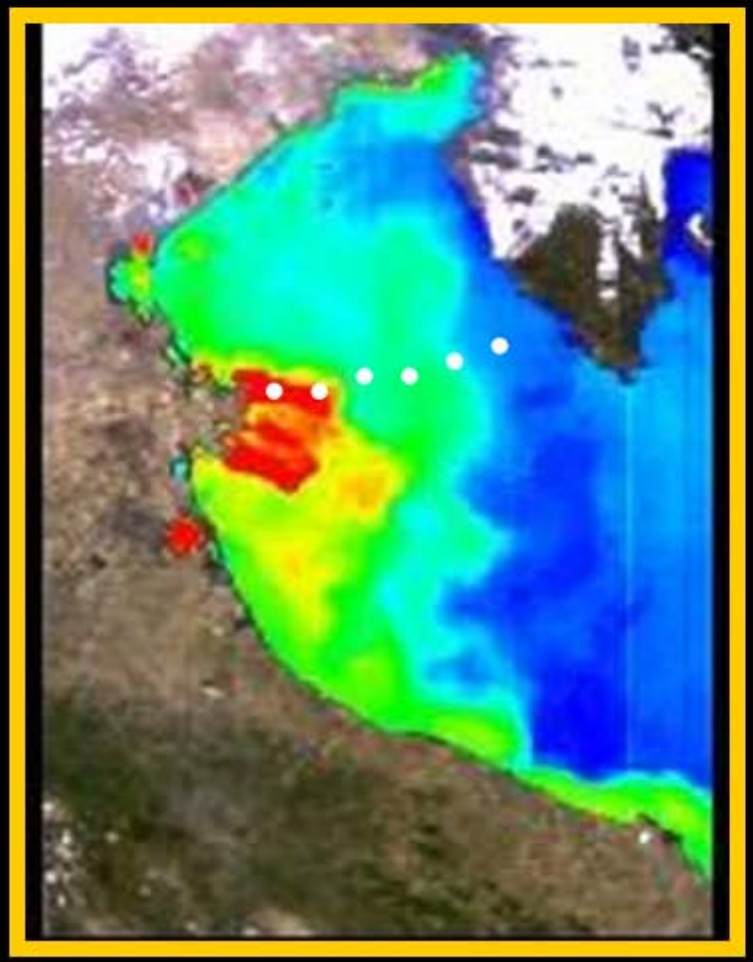
Romano et al. J Exp Biol 2003

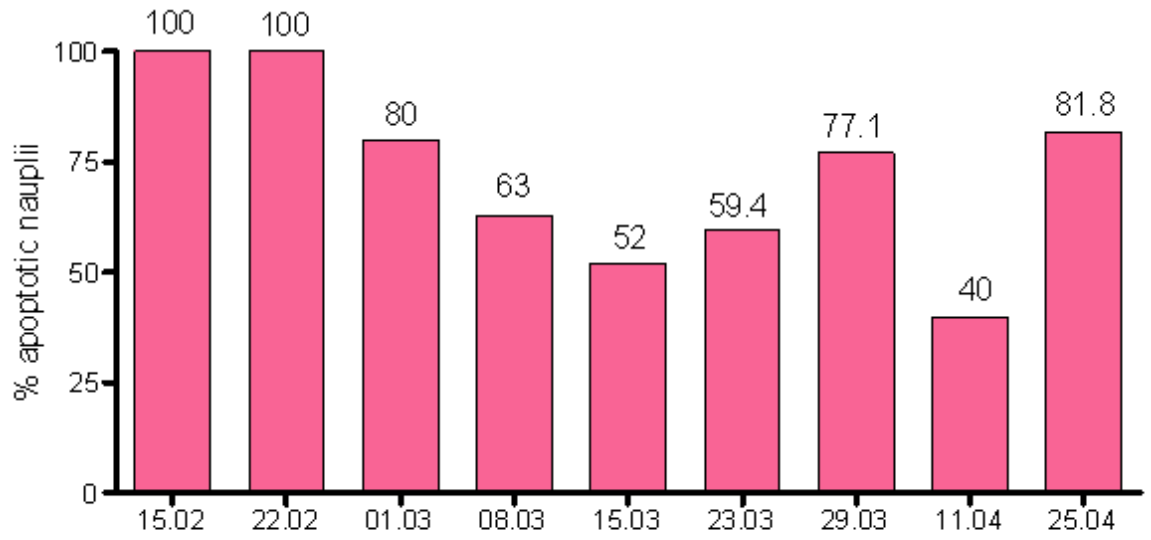
TUNEL dye used to detect cell death in newly spawned embyros (yellow) and propidium iodide (red) to color all of the nuclei



Ianora et al. Nature 2004

North Adriatic Sea




% apoptotic naupli
100
75
50
25
0
100
100
80
63
52
59.4
77.1
40
81.8
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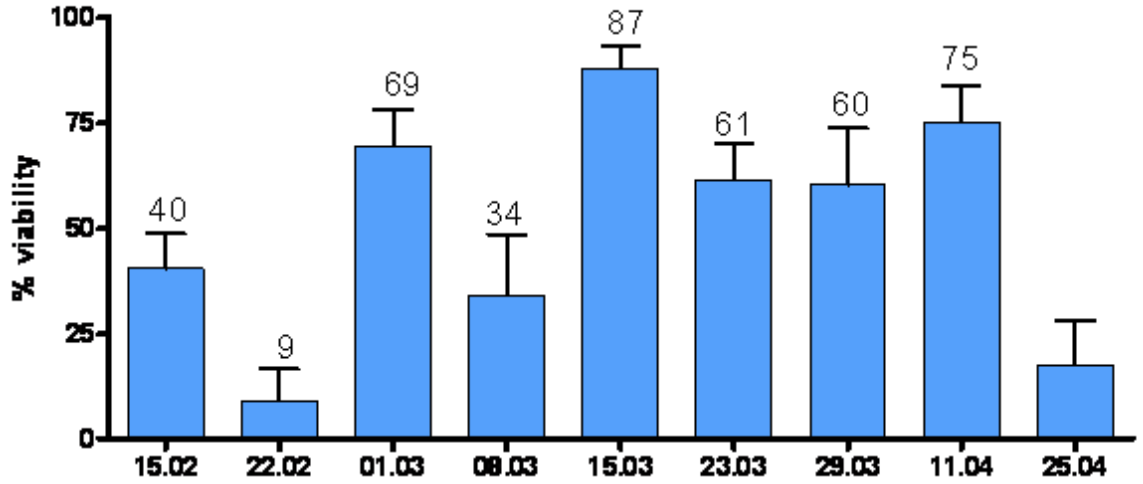
15.03
23.03
29.03
11.04
25.04


% viability
100
75
50
25
0
40
9
69
34
87
61
60
75
15.02
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08.03
15.03
23.03
29.03
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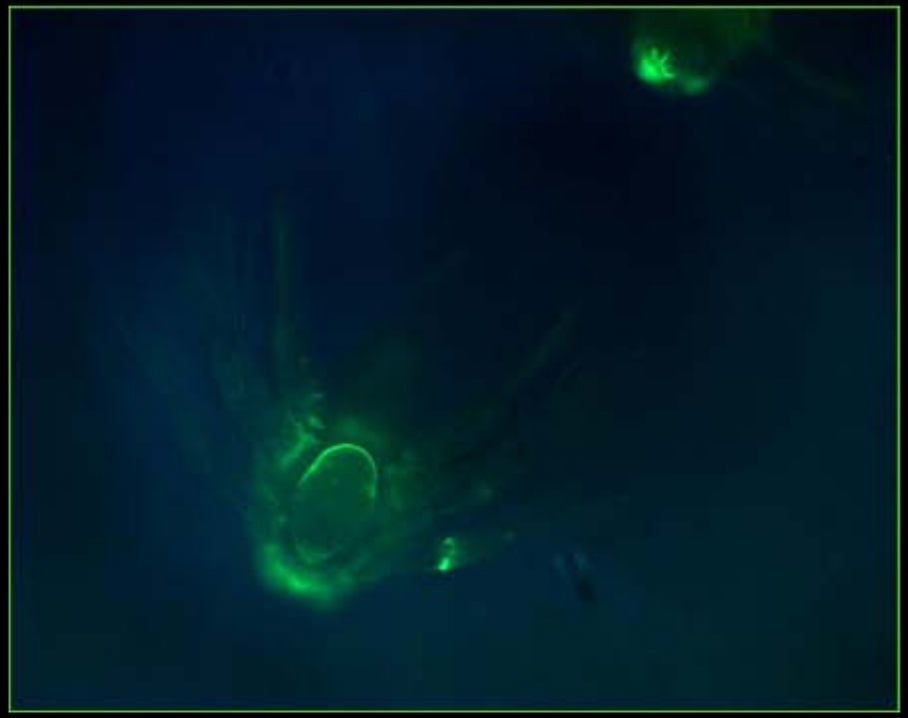
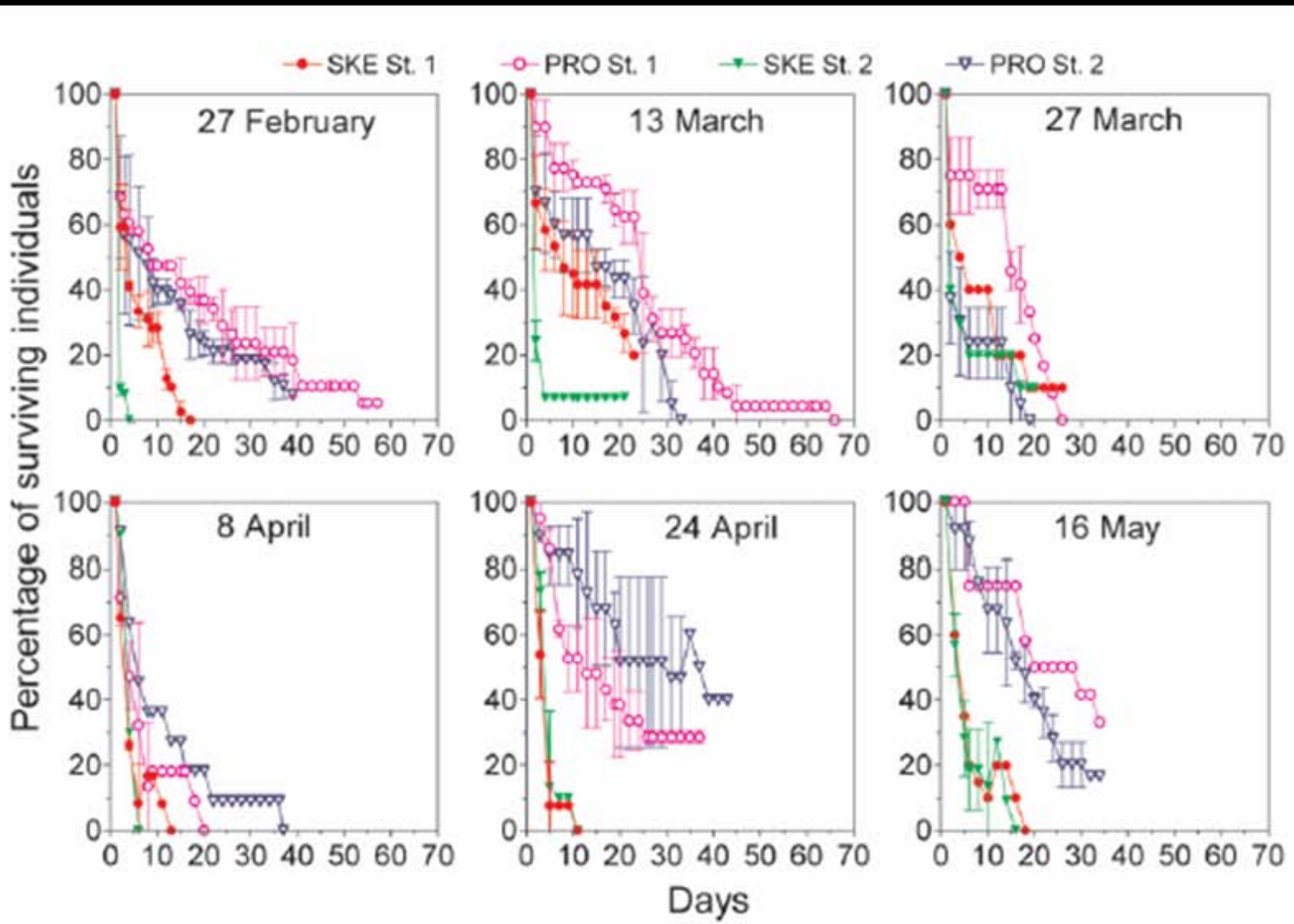
25.04





Ianora et al. Nature 2004

Summary

-Vital fluorochromes to detect egg fertilization success and normal/abnormal embryogenesis (Hoechst 33342, DAPI, Acridine orange)
-Vital fluorochromes to discriminate between viable/non-viable eggs (FDA, SYTOX Green, 7-AAD)
-Fluorochromes to detect apoptosis and cell death (TUNEL, MitoLight)