Hatching mechanism and late hatching of the eggs of *Euphausia pacifica* Under laboratory conditions

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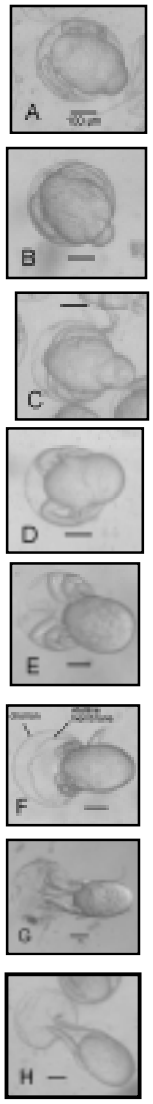
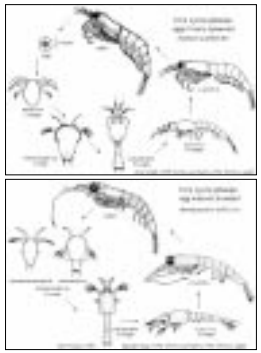
Hatching mechanism of *Euphausia pacifica*

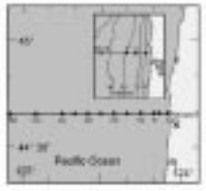


Life cycle for broadcast and brood sac spawner euphausiids



Field sampling.

Mature euphausiid females (purple females stage IV) were collected during the night along the Newport hydrographic line (NH). This line is located at 44° 40'N, Oregon, USA mainly in the NH5, NH15, NH20, and NH25 stations (the number indicates the distance from the coast in nautical miles). Females were transported to Hatfield Marine Science Center's cold room (10.5°C, ± 0.5°C) and incubated for at least 48 h.



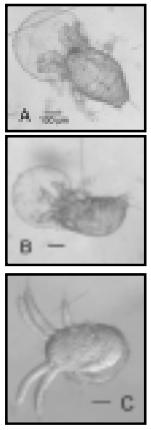
Life cycle and reproductive strategies

There are 86 species of euphausiids in the oceans. Euphausiids of the genera *Bentheuphausia, Euphausia, Thysanoessa, Meganyctiphanes*, and *Thysanopoda* spawn freely and hatch as nauplius 1 (N1), while the genera *Nematoscelis, Nyctiphanes, Pseudeuphausia*, and *Stylocheiron* brood their eggs and hatch in the early metanauplius phase as pseudometanauplii or as metanauplii. Diagrams from the life cycle of the euphausiids were taken from the CD's Euphausiids of the World Ocean (Brinton *et al.*, 2000).

Hatching mechanisms

There is very little information in the literature on the eclosion of euphausiid eggs, perhaps because hatching has been expected to be a simple process, because it that takes just few seconds and requires almost continuous observation under laboratory conditions, and because most researchers are most interested in the output of this process as hatching success (i.e. Ross *et al.* 1982, Iguchi and Ikeda, 1994).

Hatching of eggs of *Euphausia pacifica* Hansen was examined under laboratory conditions. Like all broadcast spawning euphausiids, *E. pacifica* usually hatches as nauplius 1 (N1). To the left it is shown a sequence of pictures of the hatching mechanism of this species (time to hatch ~35 h at 10.5°C, ± 0.5°C). Some hours before hatching the vitelline membrane breaks and the embryo is freely suspended within the chorion, later the embryo takes a slightly oval shape. When ready to hatch, the N1 pushes against the chorion with the posterior part of the abdomen producing a protuberance (A). The pressure breaks the chorion (B D), and the nauplius pushes itself backward with the first and second antennae and mandible to slide backward from the chorion (E F). After about ¾ of the body is outside, the nauplius bring all the appendages together to move backward without becoming stuck in the chorion (G H)). The vitelline membrane remains within the egg even after the nauplius leaves the chorion. Hatching takes 5 to 20 seconds, and most of the eggs in a clutch hatched during less than 2 hours.



Delayed hatching of *Euphausia pacifica*

Eggs from two of 26 clutches observed hatched as metanauplii (>200 h after spawning) or as calyptopis 1 (C1) stage (>232 h after spawning), rather than as nauplius 1 (N1). It is not known whether delayed hatching occurs in nature. Eggs with larvae in stages of development beyond N1 have not been observed from zooplankton samples. This is the first recorded report of a metanauplius or C1 inside the egg for any free spawning species of the order Euphausiacea. The broods of eggs of the late hatchers, showed different hatching mechanisms. Metanauplius emerged from the eggs after the chorion had completely disintegrated, perhaps by bacterial decomposition and it was easily broken with the help of the spines on the first and second antennae. The eggs with a C1 inside appear to have had a stronger chorion. The elasticity of the chorion allowed the development of the C1 inside the egg. The C1 usually broke the chorion with the mechanical force of the flipping abdomen, perhaps helped by the distal spines of the telson.

The hypothesis about the enzymatic hatching

Facts: In the live embryo just before hatching, the posterior part of the abdomen of the nauplius appears light red (see left color picture). No spine or egg tooth is present to break the chorion.

Hypothesis: Perhaps a chemical substance derived from the red spot like an enzyme helps to soften or break the chorion.

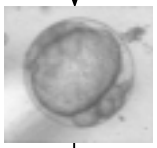
Hypothetical answer: An enzymatic mechanism has been observed in embryos of other crustaceans (De Vries and Forward, 1991; Saigusa and Terajima, 2000). Saigusa and Terajima (2000) reported that embryos of the estuarine crab *Sesarma haematocheir* have a pair of secretory glands of 30 µm diameter located in the dorsal thorax of the embryo, where the egg case is ruptured. They recognized two active substances released outside the egg case: one is a caseinolytic protease and the other an unidentified enzyme. The activity of the former was at a very low level compared with a standard casein enzyme. It might be digesting the thin, sticky internal layer enclosing the embryo but would not act on the thick, tough layer constituting the main component of the egg capsule (Saigusa 1996). The second substance, non identified so far, may cause the softening of the egg case.

Nauplius stuck in the empty chorion

Occasionally some of the N1 were unable to completely pass through the hole in the chorion, being held by their appendages (usually less than 1% of the nauplii hatched). Those animals tried to push themselves out for hours, working constantly, and some progressed to another developmental stage while still stuck in the chorion. Some larvae were observed to have developed to N2 (A) and, in a few cases, to metanauplius (B). Most of these animals died before freeing themselves from the chorion. An unusual way to hatch was observed when a nauplius broke the chorion at the anterior part of the egg with the appendages, then tried to push it self forward. However, this N1 remained stucked with the chorion around its abdomen until it died (C).

Late hatching of *Euphausia pacifica*

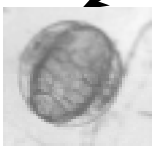
Nauplius 1 inside the egg
~35 h after spawning



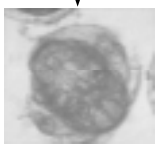
Nauplius 2 inside the egg
~85 h after spawning

Metanauplius inside the egg

The metanauplius inside the eggs showed lines in the thorax between blocks of tissue forming the cephalothoracic appendages (mandible, maxillae 1 and 2, and maxillipeds), and the presence of two pairs of functional appendages (first and second antennae)

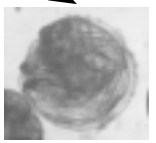


Metanauplius inside the egg
99 h after spawning



Metanauplius hatching
200 220 h after spawning

Metanauplius
225 h after spawning



Calyptopis 1 inside the egg
232 247 h after spawning

Calyptopis 1 hatching
232 247 h after spawning

Calyptopis 1
> 235 h after spawning