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Embryonic, early larval development time, hatching mechanism and interbrood period of the sac-spawning euphausiid *Nyctiphanes simplex* Hansen

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Females of the sac-spawning euphausiid Nyctiphanes simplex Hansen were incubated under shipboard laboratory conditions to observe the embryonic and larval development time and hatching mechanism. Females ready to spawn have a pale pink ovary that extends from the back of the stomach to the first abdominal segment, filling most of the haemocoel. This species usually behaves as a total spawner (produces one batch of oöcytes per cycle of the ovary) leaving an 'empty' space in the cephalothorax where the spent ovary is located. After spawning, the young oöcytes mature and turn pale pink. The eggs do not have a measurable perivitelline space (PVS) in any of the embryonic stages (6.6 × magnification). The embryos hatch as nauplius (80–91 h after spawning, 16°C ± 1°C). They further develop into pseudometanauplii (PMN, 90–105 h after spawning) and metanauplii (MN, 92–140 h after spawning) inside the ovigerous sac. The nauplius breaks the thin and fragile chorion by increasing the volume of the body and by using the first and second antennae. We call this an 'expansion' hatching mechanism, the fifth distinct hatching mechanism observed so far among euphausiids. N. simplex larvae escape from the ovigerous sac late in the MN stage (5 days after spawning), just a few hours before molting into calyptopis 1 (C1) (0.5–4 h). This delayed release extends protection by the female, likely decreasing the risk of predation or early cannibalism. Additionally, this may save energy by not swimming independently increasing the time of not return if the calyptopis does not find favorable feeding conditions. Females are not ready to spawn again until at least two days after the previous batch of embryos leaves the ovigerous sac. The interbrood period (IBP) observed ranged between 7 and 15 days at 16–18°C. This IBP is about one-fourth to half than was previously assumed for this species suggesting a significant underestimation of the fecundity of this species. N. simplex hatching success usually was 100%, except for a few females with all of their embryos dying during embryonic development. Other females either molted before releasing the embryos, or the oöcytes were spawned unfertilized (0% hatching success), particularly during winter conditions. Efficient hatching and late free-swimming strategy may partially explain why this species is the most abundant neritic euphausiid in the southern part of the California Current System (CCS) and in the Gulf of California.

INTRODUCTION

Euphausiids have two reproductive strategies: (i) the broadcast-spawners shed their eggs freely into the ocean and (ii) the sac-spawners protect their embryos in a sac attached to the posterior pairs of thoracic legs. In several marine ecosystems the broadcast-spawning species dominate the euphausiid assemblage in biomass and abundance and form immense standing stocks, as in

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the northern Pacific (*Euphausia pacifica* Hansen, *Thysanoessa spinifera* Holmes and *Thysanoessa inermis* Krøyer), the northern Atlantic and Mediterranean Sea (*Meganyctiphanes norvegica* M. Sars), and the Antarctic region (*Euphausia superba* Dana and *Euphausia crystallorophias* Holt and Tattersall). The eggs of broadcast-spawning species sink and the larvae drift early in the life cycle; thus, their early-life stages [eggs, nauplius (N), and metanauplius (MN)] are commonly collected with zooplankton nets (Feinberg and Peterson, 2003; Gómez-Gutiérrez, 2003b). The MN must develop into calyptopis 1 (C1) to feed for the first time on exogenous food. In other marine ecosystems like the subtropical neritic regions of the eutrophic Eastern Boundary Current Systems (California, Humboldt, Canary, and Benguela), sac-spawning euphausiid species of the genus *Nyctiphanes* can dominate the secondary productivity at levels similar to those of the broadcast-spawners in other regions (Mauchline and Fisher, 1969). The water column in the regions which *Nyctiphanes* spp. dominate secondary productivity is virtually devoid of euphausiid eggs, nauplii, pseudometanauplii (PMN) and metanauplii, and usually only stages older than calyptopis are collected with nets. Why do *Nyctiphanes* species dominate in those specific neritic regions of the ocean?

In the neritic zone of the southern part of the California Current System (CCS) and in the Humboldt Current System, *N. simplex* Hansen accounts for more than 95% of the euphausiid biomass and abundance (Brinton, 1962; Lavaniegos, 1994; Gómez-Gutiérrez, 1995; Gómez-Gutiérrez et al., 1995). High secondary productivity of this species has been partly attributed to its short life span of about 6–7 months, continuous gonad maturation and indirectly associated with high egg-production rates (Lavaniegos, 1992, 1995; Gómez-Gutiérrez, 1995), large larval recruitment (Lavaniegos, 1994, 1995; Gómez-Gutiérrez, 1995; De Silva-Dávila and Palomares-García, 1998, 2002; De Silva-Dávila *et al.*, 2002), and flexibility in the development pathways of the early furcilia stages (Lavaniegos, 1992, 1994; Gómez-Gutiérrez, 1996). Temperate species usually have longer life span >1 year and several of them have limited or intermitent reproductive season (Ross and Quetin, 2000). Evidently, *N. simplex* attains large standing stocks in these upwelling regions by high fecundity, high survival and the advantages of swarming behavior as a strategy to reduce predation (Hamner, 1984), but little is known about its recruitment and early larval life, because few experimental studies have been done. Traditionally it has been considered that the sac-spawning euphausiid species has a significant lower fecundity than broadcast euphausiid spawning species. The relatively small brood size and relatively long female brooding time that the females must invest at each brood are, perhaps, two of the main arguments to assume that sac-spawning euphausiids may have relatively long IBPs and reduced fecundity throughout their life cycle (Hosie and Ritz, 1983; Gendron, 1992; Lavaniegos, 1995). This perspective has been prevailing principally due to the lack of experimental and observational studies to study the development time, hatching time, hatching mechanism, brood size and IBP of the sac-spawning euphausiid species (Ross and Quetin, 2000); all them are significant variables to estimate the secondary productivity due reproduction. This study is the first estimation of the embryonic development time of *N. simplex* and, so far, the first direct observation of the IBP in any sac-spawning euphausiid species under laboratory conditions. These measurements may have significant consequences in terms of previous secondary productivity estimations based on preserved specimens that assumed an IBP of 30 days for *Nyctiphanes australis* G.O. Sars (Hosie and Ritz, 1983) and *N. simplex* (Lavaniegos, 1995).

Additionally, we report measurements of early larval development times [(nauplius 1 (N1) to calyptopis 3 (C3)] of this species, which extends earlier work by Lavaniegos (Lavaniegos, 1992) who studied larval development times using field collected calyptopis 2 (C2) and C3 (with unknown ages) to follow subsequent development from furcilia to juvenile. It is useful to understand how long the female keeps the embryos in the ovigerous sac before she can produce a new batch. Also it is relevant to understand how *N. simplex* larvae hatches and to estimate its hatching success under laboratory conditions, because these processes affect larval recruitment. Hatching is a critical period, involving substantial physiological and behavioral changes (Davis, 1968, 1981; Anderson, 1982; Saigusa, 1996). Sometimes a large proportion of euphausiid embryos die during hatching (Gómez-Gutiérrez, 2002).

The hatching mechanism of euphausiids, the method by which an embryo breaks the chorion, has been considered historically as simple and straightforward (Sars, 1898; Mauchline and Fisher, 1969). In a recent review of the Order Euphausiacea the prevailing concept that broadcast-spawning euphausiid embryos hatch exclusively as N and that sac-spawning euphausiid embryos hatch as PMN or MN was still reported (Casanova, 2003). However, recent studies demonstrated in the laboratory that euphausiid embryos have the potential for considerable flexibility in the hatching schedule and hatching mechanism among species, for the same species and even for sibling embryos within the same brood (Gómez-Gutiérrez, 2002, 2003a,b). He described four different hatching mechanisms, termed: backward, forward, flipping and push-off, observed in three broadcast-spawning species *E. pacifica*, *T. spinifera*, and *Thysa-*

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noessa inspinata Nemoto, and one sac-spawning species *Nematoscelis difficilis* Hansen from the northern CCS. The push-off hatching mechanism was exclusively observed in *N. difficilis*. However, this species also can hatch occasionally (<2%) prematurely as nauplius 2 (N2) by using the backward hatching mechanism commonly used by broadcast-spawning euphausiid species (Gómez-Gutiérrez, 2002, 2003a) (Table I). Hatching of nauplii for sac-spawning euphausiids has been briefly described for *Stylocheiron carinatum* G.O. Sars, (Ponomareva, 1969) and in more detail for *N. difficilis* (Gómez-Gutiérrez, 2003a,b). These two species are usually more abundant in oceanic than nearshore conditions. For example, *N. difficilis* is endemic in the transition zone of the northern Pacific, and although it is the most abundant sac-spawning euphausiid in the North Pacific Drift (ca. 37–45° N), *N. difficilis* is not numerous nearshore in the northern Pacific and west coast of Baja California peninsula, two zones dominated by broadcast-spawning species like *E. pacifica* and *T. spinifera* (Gómez-Gutiérrez, 2003a,b; Gómez-Gutiérrez *et al.*, in press) and the sac-spawning species *N. simplex* (Gómez-Gutiérrez *et al.*, 1995). However, in the Gulf of California throughout the year and offshore in the west coast of Baja California during anomalous cold years, *N. difficilis* is the second-most abundant euphausiid after *N. simplex* (Brinton and Townsend, 2003,1980; Gómez-Gutierrez *et al.*, 1995; De Silva-Davila and Palomares-García, 2002).

Studying the brood size, development time, hatching time, hatching mechanism, hatching success and reproductive biology of a highly abundant sac-spawning euphausiid species like *N. simplex* may provide clues

Table I: Hatching mechanisms of the broadcast-spawning (B) or the sac-spawning (S) euphausiid species observed under laboratory conditions from mature females collected in the field along the northeastern Pacific. The euphausiid species in which the hatching mechanism has been inferred from a brief description of this process, drawings and/or photographs are indicated with asterisks

Hatching mechanism	Species	Reference
Backward: The nauplius 1 (N1) pushes against the chorion with the posterior part of the abdomen producing a protuberance. The pressure breaks the chorion, and the N1 pushes itself backward with the first and second antennae and mandible to slide out from the chorion	*Euphausia superba* (B)* *Euphausia pacifica* (B) *Thysanoessa spinifera* (B) *Thysanoessa inspinata* (B) *Nematoscelis difficilis* (S)	Ross and Quetin (1982)*; George (1984)*; Gómez-Gutiérrez (2002, 2003a,b)
Forward: The nauplius 2 (N2) and metanauplius (MN) break the chorion with the first and second antennae, hatching forward	*Thysanoessa inermis* (B)** *Stylocheiron carinatum* (S)*** *Euphausia pacifica* (B) *Thysanoessa spinifera* (B) *Thysanoessa inspinata* (B) *Euphausia eximia* (B)****	Zelikman (1961)**; Ponomareva (1969)***; Gómez-Gutiérrez (2002); This study****
Flipping: The calyptopis (C1) slit the chorion using their telson spines extending and flipping the abdomen outside the egg	*Euphausia pacifica* (B)	Gómez-Gutiérrez (2002)
Push-off: The pseudometanauplius (PMN) or MN embryos extend and contract their first and second antennae in a swimming movement, breaking the chorion in almost equal halves joined by one small section in the anterior part of the chorion. The PMN or MN hatch and escapes from the ovigerous sac almost simultaneously	*Nematoscelis difficilis* (S)	Gómez-Gutiérrez (2003a,b)
Expansion: The nauplius hatch breaking the thin chorion with the body growth, but is the MN stage that escapes from the ovigerous sac about 2 days after hatching	*Nyctiphanes simplex* (S)	This study

Backward hatching is the usual hatching mechanism for broadcast-spawning species and unusually early hatching mechanism for sac-spawning species. The forward and flipping are delayed hatching mechanisms for broadcast-spawning euphausiids and the forward is early hatching for sac-spawning species, both are relatively unusual. The push-off and the expansion hatching mechanisms have been observed exclusively in sac-spawning euphausiids.

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about why such species are particularly successful in this highly productive, upwelling ecosystem. The goal of this study was to measure the embryonic and early larval development time, to observe the hatching mechanism, to understand the hatching success process of *N. simplex*, and to estimate how long it would take a female to produce a new brood. These observations have significant consequences in terms of the estimation of the secondary productivity. Although the brood size have been reported for several sac-spawning euphausiid species (Ponomareva, 1969; Nemoto *et al.*, 1972; Gendron, 1992; Lavaniegos, 1995; Wilson *et al.*, 2003), as far the author's knowledge, this study represents the first experimental work to estimate the IBP of any sac-spawning euphausiid species around the world providing a new insight about the potential fecundity of the euphausiids with this reproductive strategy.

METHOD

Field sampling and shipboard incubation of the mature female euphausiids

Euphausiids were collected during three oceanographic cruises from 16 March to 2 April, from 29 June to 16 July, and from November 29 to December 18, 2004 over the continental shelf and in the middle part of Bahía Magdalena, Baja California Sur, México (25°40 N to 24°20 N, 113°00 W to 111°8 W) on board the R/V El Puma (Universidad Nacional Autónoma de México). From 86 plankton tows, we found euphausiids at only 45 tows. Mature females were commonly collected from dense near-bottom aggregations detected with a split-beam, 120-kHz echo sounder (Simrad EY-60, with a nominal beam width of 7 degrees. The ping rate was 3 pings s^{-1} and noise threshold was set at –80 dB), usually between 1 and 10 m above the seafloor (in locations with depths from 40 to 100 m) in the mouth of the bay both day and night. In several locations, the euphausiids were virtually in contact with the seafloor.

Gravid females of *N. simplex*, identified because they are pale pink of the ovary when the oöcytes are in meiosis, were collected with a 1-m diameter 'live' net, 5-m long and constructed of black 300-μm mesh. The cod end was PVC, 0.22-m diameter and 0.70-m long. The net was equipped with an underwater lamp (Ikelite Inc., provideo–lite II system of 50 Watts) to attract euphausiids, a Sea Bird Microcat CTD attached to the towing line above, and a high-resolution submarine video camera Multi SeaCam (Deep Sea Power & Light, San Diego, CA, USA, with lens of f = 2.8 mm, and the depth of field of 10 cm to infinity) attached to the ring of the net. Live zooplankton samples were obtained by lowering the net to the depth where the echosounder and the video camera showed large euphausiid densities for less than 10 min and sampling while the ship was drifting. This was to avoid damage of the euphausiids. In some locations during March, densities of euphausiids were very high, collecting about 90 kg of *N. simplex* adults just in one tow of 30 minutes, so another shorter net tow was made to collect additional healthy individuals for incubation. Animals were incubated in a shipboard cold room at 16°C (±1°C) during the oceanographic cruises of March and July and 18°C (±1°C) during December, approximating that of their natural environment.

In practice it was difficult to distinguish and sort out the living gravid females with pinky coloration for incubation while on the ship, in contrast with easily detected blue-purple gonad of *E. pacifica* and gray-green colored gonads of *T. spinifera* (Gómez-Gutiérrez, 2003b). Thus, at each station we incubated individually, for at least 48 h (observing them every 12 h), more than 30 healthy looking adults in 1-L bottles and about 200 adults in groups of ten animals per 1-L bottle filled with sieved (20-μm mesh) seawater from 4-m depth. If a female with a pink gonad or with a new formed ovigerous sac was detected in the bottles with ten animals, she was isolated in a 1-L bottle for observation.

Biometry and development time of the embryos

Several of the *N. simplex* ovigerous females (n = 58 females from March and July oceanographic cruises) were used to observe embryonic development, they were incubated on board (16°C) until all the eggs hatched and the larvae escaped from the ovigerous sac. Hatching success was evaluated as the percentage of embryos that hatched of the total spawned. The ovigerous sac of each female was monitored as frequently as possible (usually every 4–6 h). Development of the eggs was monitored using a digital camera (Olympus, Camedia 3040, 3.3×10^6 pixel resolution). We classified development of the embryos after spawning as proposed for several broadcast and sac-spawning euphausiid species (Ponomareva, 1963; Quetin and Ross, 1984, 1989; George and Strömberg, 1985; Gómez-Gutiérrez, 2002, 2003a,b) distinguishing the following stages: single cells (SC), multiple cell (MC), blastula (B), gastrula (G), early limb-bud (eLB), late limb-bud (lLB), and twitching (TW). The diameter of the chorion and the embryo were measured with a micrometer. The just-spawned (JS) and unfertilized (U) eggs of *N. simplex* were elliptical, they were measured from its longest axis and its perpendicular axis of the previous measurement. We determined the larval development time after hatching and after the larval escape from the ovigerous sac until

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C3. Each larval stage was identified according to the following criteria. The nauplius phase (N) is characterized by three pairs of functional appendages: uniramous first antennae, biramous second antennae and mandibles, the body is oval and unsegmented, for simplicity in this study was not distinguished between the N1 and N2 stages in the naupliar phase (Boden, 1951). The PMN has two pairs of functional appendages (first and second antennae), the mandible is reduced and no longer serves as swimming appendages. Buds of the first and second maxillae and maxillipeds are present. The abdomen is short and from lateral view has barely a posterior hump. The MN is similar than the pseudometanauplius but the abdomen is longer and bears posterior spines and from lateral view it has a pronounced posterior hump. The calyptopis phase is characterized by compound eyes beneath the carapace. There are three stages in this phase. The C1 has non-segmented abdomen and this becomes segmented in C2, the uropods develop in the C3, along with the separation of the telson from the 6th abdominal segment (Brinton *et al.*, 2000). The first feeding larval stage of *N. simplex* is the C1. When the larvae reached this stage, we changed the seawater every day and an unknown amount of food was added. This was a mix of phytoplankton-microzooplankton obtained from a pump with an intake at 4-m depth in the hull of the ship. The food was concentrated with a sieve of 20-μm mesh net.

Interbrood period

All the females incubated that spawned and released their larvae were measured the total lengths (mm), and then they were incubated for several additional days with abundant field-caught phytoplankton and microzooplankton concentrated with a sieve of 20-μm mesh net to observe whether they spawned again, the time between two consecutive spawns is the interbrood period (IBP). During July cruise, we added a male with spermatophore to each female that already released her embryos from the ovigerous sac with the goal to observe mating or that the female produce fertilized eggs.

Hatching mechanism, embryos escape from the ovigerous sac and molt from MN to C1

Several processes like hatching, embryo escape from the ovigerous sac and early larval molting are very brief. Thus, to observe and measure them we monitored more frequently the incubated females according to the following criteria:

Hatching mechanism: When the embryos reached the TW stage inside the ovigerous sac, that is when the nauplius had taken shape inside the chorion, the appendages were freely suspended from the body moving them actively and the heart pulsates (Quetin and Ross, 1984, 1989; Gómez-Gutiérrez, 2002), we observed them every 30 minutes to detect when the embryos begin to hatch. The female was always manipulated with a plastic spoon. We compared the *N. simplex* hatching mechanism with the four previous hatching mechanisms known for euphausiids (Table I) (Gómez-Gutiérrez, 2002, 2003a,b).

Embryo escape: When the larvae in the ovigerous sac were older than 12 h after they reached the MN stage, they were monitored every ten minutes. Once the first MN escaped, we observed the female's ovigerous sac almost continuously turning on the stereoscope light only to take the digital photograph to avoid the light and heat of the lamp damage the larvae.

Early larval molting process: When we observed that a female released her embryos, we sorted out the female and observed the free-swimming MN stage as frequently as possible, usually <30 minutes to observe when they molted to the first feeding stage C1 taking photographs of this hitherto undescribed process.

RESULTS

Embryonic development and morphological shapes of the ovigerous sac

A sequence of *N. simplex* ovigerous females carrying selected successive embryonic developmental stages is shown in Fig. 1(A–E). Females carry eggs in two unsymmetrical, membranous sacs attached along their broad, anterior sides to the outer edges of the endopodites of the sixth and seventh periopods, and by their narrow posterior ends to the exopodites of the eighth thoracic limbs (Fig. 1B). The function of the eighth thoracic limbs is to support the posterior part of the ovigerous sac. The eighth thoracic limb also helps to move the ovigerous sac up and down, presumably helping the oxygenation of the brood. As in *N. difficilis*, both ovigerous sacs are usually in contact with the ventral part of the female, but she can move them down and up while swimming. We observed the formation of new ovigerous masses (oviposition). The females spawned all of their eggs in no more than 4 h (n = 6 females broods). The ovigerous sac may have different shapes (pear-shaped, elliptical, or irregular) depending on the number of eggs produced and their developmental stage of the embryos (Fig. 1A–E). After all the eggs were spawned and accommodated in the ovigerous sac, the female usually has a characteristic 'empty' space in the cephalothorax in the position of the spent ovary (Fig. 1B), indicating that this species behaves as a total spawner, that is a female produces one batch of

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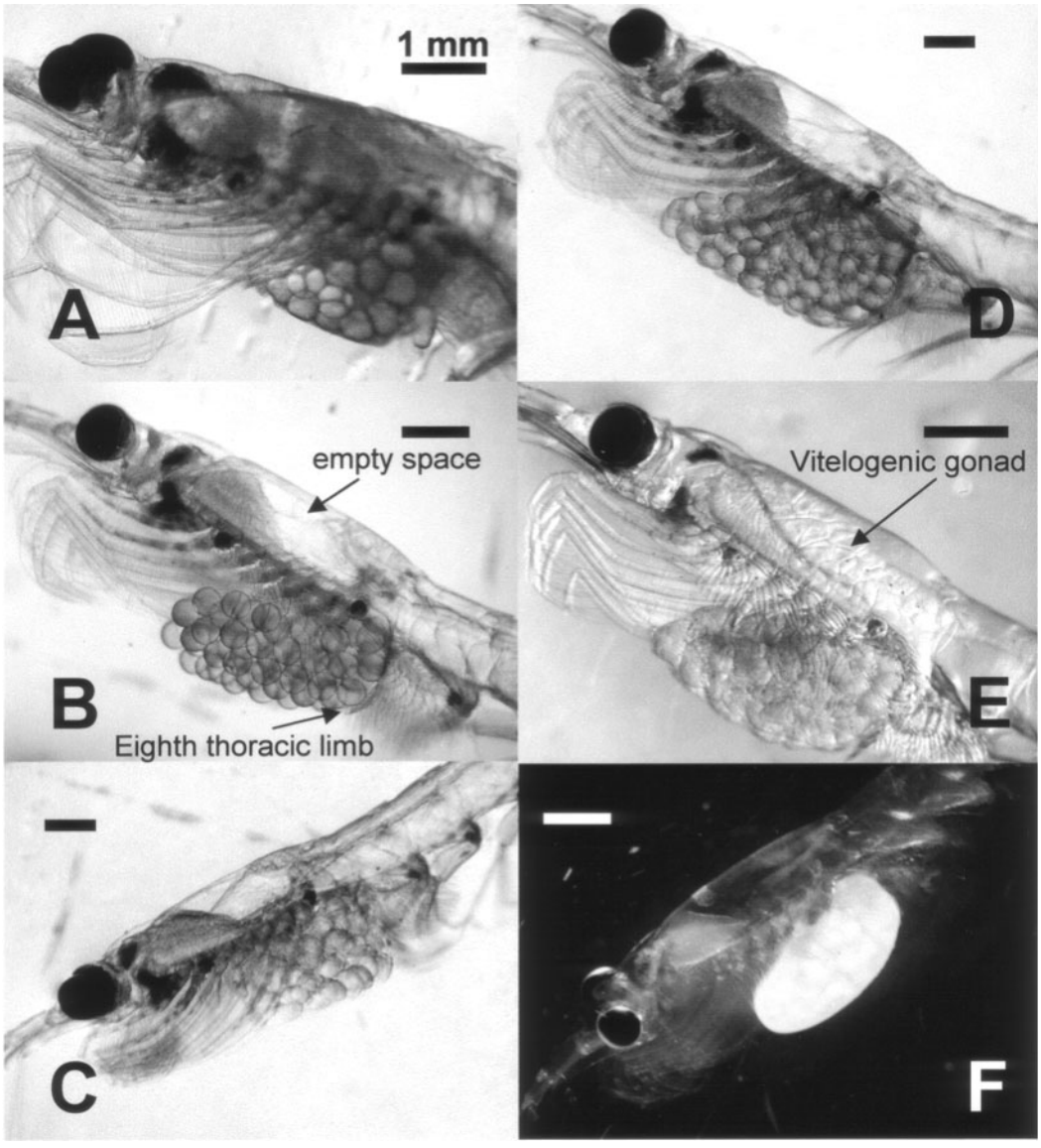

Fig. 1. *Nyctiphanes simplex*. Shape and appearance of the ovigerous sac at different selected embryonic developmental stages (1× magnification). (**A**) Female bearing just-spawned elliptical eggs, the gonad still filled with oöcytes in meiosis phase (stage IV). (**B**) Female with a complete ovigerous sac filled with spherical single-cell (SC) and multiple-cell (MC) embryos. The carapace shows the typical empty space after completing the spawning event. The same external female appearance with the ovigerous sac is for blastula gastrula and early limb-bud stage embryonic stages (not showed). (**C**) Female with nauplii hatching inside the ovigerous sac larvae are not organized in rows anymore. Females bear ovigerous sac with (**D**) pseudometanauplius and (**E**) metanauplius stages. This last female shows an ovary in vitelogenesis with lipid yolk accumulation (thick ovary and with semitransparent oöcytes in oc2 and oc3 type) while having metanauplius (MN) in the ovigerous sac. (**E**) The ovigerous sac becomes visibly darker as the larvae develops and significantly increases their size. (**F**) Female with white ovigerous sac with all the embryos dead during embryonic development. The ovigerous sac is completely detached several days after the embryos died. Scale bar = 1 mm.

oöcytes per cycle of the ovary. Sometimes females still have an empty appearance even after the embryos were released from the ovigerous sac, but sometimes the ovaries develop again during the external embryonic development in the ovigerous sac, thus advanced ovigerous females may be also in vitelogenic (stage III) or mature (stage IV) ovarian development (Fig. 1E). Additionally, the females that have lost her ovigerous sac (the embryos already were released) have a typical empty space between the seventh and the eighth pair of limbs. This is a valuable indicator that a female previously produced an ovigerous sac when collected directly from the sea (Fig. 2D).

Healthy JS eggs have different shapes because the chorion is soft. They are opaque, highly compacted in the sac, and surrounded by a thin, transparent oviger-

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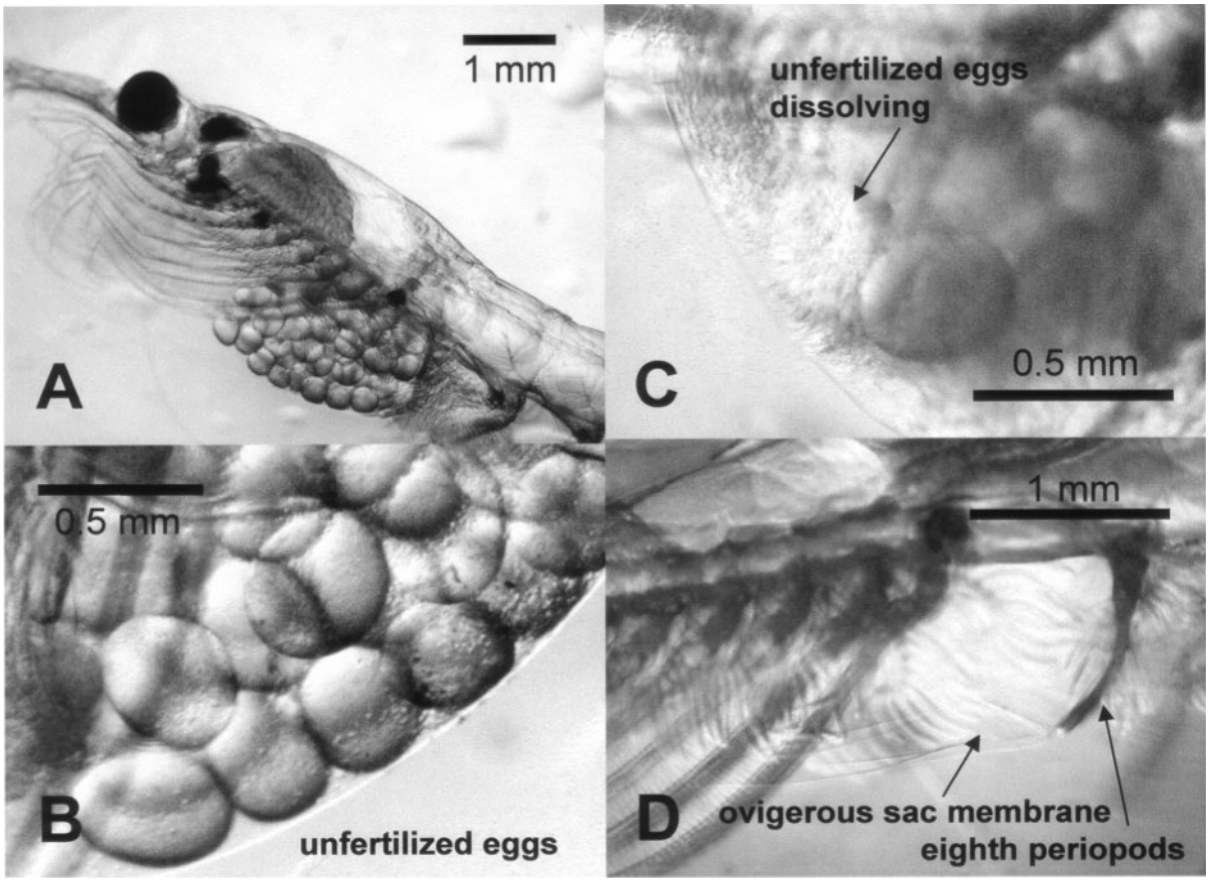

Fig. 2. (**A**) Several *Nyctiphanes simplex* females spawned (unfertilized eggs) again in two days after their metanauplius were released from the ovigerous sac or during the first spawning after collection. (**B**) The unfertilized eggs were almost identical in appearance to fertilized elliptical just-spawned egg, excepting the former are more transparent that the latter. (**C**) The female filled its ovigerous sac with about 30 eggs per sac, the picture shows the disintegration of unfertilized eggs several hours after spawning and (**D**) transparent and empty ovigerous sac membrane because of the complete disintegration of the unfertilized eggs. A and D scale bars = 1 mm, B and C scale bars = 0.5 mm.

ous-sac membrane (Fig. 1A). During early embryo development from the SC to eLB stage, the egg membrane becomes more rigid and spherical with some empty spaces among the eggs. During those stages, the eggs within the ovigerous sac occupy neat rows and are semitransparent (Fig. 1B–E). As with other species of the genus *Nyctiphanes*, the naupliar stage of *N. simplex* is passed within the ovigerous sac. The eggs become elliptical during the lLB and the TW stages (Fig. 1C). The TW stage is relatively brief pre-hatching stage and the embryos always hatch as nauplius. After hatching, the nauplii are no longer arranged in rows. When the larvae reach the PMN stage, the volume of the ovigerous sac increases considerably (Fig. 1D), and when they molt into the MN stage, the ovigerous sac reaches its maximum size and the larvae become darker and more robust (Fig. 1E). During March and July cruises at least fifty-five females had 100% hatching success. The ovigerous sac of three females was white and the embryos died from unknown causes during their development detaching from the female several hours after the death of the embryos (Fig. 1F).

Biometry of the embryonic developmental stage

Table II summarizes the biometry of *N. simplex* for embryonic developmental stages (diameter), the early larval stages (total length) after hatching but before leaving the ovigerous sac, and the free-swimming calyptopis stage. The JS eggs are elliptical, averaging 0.384-mm long and 0.333-mm wide (1.165 length/width ratio), the unfertilized eggs (U) have an average length of 0.325 mm. When the eggs become spherical (in the SC stage), the multiple-cell stage (MC) have an average diameter of 0.339 mm, the blastula (B) 0.336 mm, the gastrula stage (G) 0.348 mm, the eLB 0.381 mm and lLB stage 0.381 mm. The TW and just-hatched nauplius (measurements combined) are significantly larger than the previous embryonic

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Table II: Minimum, maximum, and average chorion diameter and early larval total length of Nyctiphanes simplex measured in mm per developmental stage in the ovigerous sac of females incubated under shipboard laboratory conditions (16°C ± 1°C)

Embryo stage	JS length	JS width	JS length/width	U	MC	B	G	eLB	TW	PMN	MN	C1
N	20	20	20	7	70	30	60	8	19	10	57	10
Minimum	0.345	0.300	1.021	0.225	0.300	0.260	0.315	0.371	0.340	0.360	0.450	0.630
Maximum	0.465	0.375	1.500	0.425	0.390	0.375	0.390	0.399	0.418	0.460	0.555	1.037
Average	0.384	0.333	1.165	0.325	0.339	0.336	0.348	0.381	0.405	0.408	0.513	0.878

The eggs and larvae of 35 females were measured. JS, just-spawned eggs (elliptical, fertile); U, unfertilized eggs; MC, multiple cell; B, blastula;-G, gastrula; eLB, early limb-bud; TW, twitching nauplius (combined the nauplius inside the chorion and the nauplius after hatching); PMN, pseudometanauplius; and MN, metanauplius. Free-swimming C1, Calyptopis 1 stage (first feeding stage). *N*, total number of embryos and early larvae measured from at least two different females.

stages, with an average length of 0.405 mm. After-hatching, the PM and MN grow in average to 0.408 and 0.513-mm total length respectively. This increase in dimensions from SC eggs to MN is about 34% (Table II), visually evidenced in the sequential pictures of the Fig. 1(A–E). Clearly, nutrient rich yolk is converted to larger structures. With the extension of the abdomen, the C1 have an average total length of 0.878 mm.

Interbrood period

After all of the larvae had escaped from the ovigerous sacs under laboratory conditions, we fed the females (n = 55, during March and July oceanographic cruises) with sieved concentrated local phytoplankton–zooplankton mixture for several days to observe the interbrood period, the time between two consecutive spawns. The amount of food was unknown but certainly the concentration was low enough to avoid artificial high concentrations. Several females produced unfertilized eggs in a second spawning under shipboard laboratory conditions. In July 2004, from the 55 females incubated, twelve of them produced eggs (always unfertile, they resembled the JS stage, but they are transparent, they never became spherical nor divide during more than 24 h of observation) two days after all the larvae of the previous spawning escaped from the ovigerous sac (IBP of 7 days) and one more female in approximately 15 days (Fig. 2A). The IBP, however, may be longer than the observed 15 days IBP because the duration of sampling period of each of the research cruises was 16 days. The rest of the females died or they never spawned under shipboard conditions. The spawning of unfertilized eggs probably means that *N. simplex* females must mate to fertilize the oöcytes each time after they produce an ovigerous sac. During July cruise, we added a male to the containers of several individual females with an ovigerous sac, but they did not produce fertilized eggs in the second spawning in the laboratory conditions. During December 2004 oceanographic cruise, relatively high proportion of just-collected females from the field produced unfertilized eggs in the first spawn aboard the ship (<24 h after collection). Particularly in three stations located in the mouth of Bahía Magdalena where all the euphausiid were females and all of them produced unfertilized eggs (n = 64 females). This proportion was reduced over the continental shelf (21% females produced unfertilized eggs) and the rest produced healthy ovigerous sacs (n = 90 females, two stations). In those continental shelf stations males with spermatophores were observed. This means that production of unfertilized eggs is not necessarily a shipboard laboratory artifact but still unclear if this happens because euphausiid swarms are biased to females or the environmental conditions were oligotrophic (comparing the Winter with Spring and Summer environmental conditions).

When females that produced unfertilized eggs in the field (12 females during July and 68 females during December, 2004) were incubated under shipboard laboratory conditions, the unfertilized oöcytes were also elliptical, but more transparent than the JS fertilized eggs, which look like oil transparent globules (Figs 2A and B, and Fig. 3A) They always disintegrated into smaller oily droplets several hours after spawning (Fig. 2C). The empty ovigerous-sac membrane remains attached to the female for several days before she discards it (Fig. 2D). We observed at least 12 females that molted between 1 and 48 h (average 23 h) after the last MN left the ovigerous sac. Females did not detach their ovigerous sacs during hatching of the embryos, as observed for *N. difficilis* (Gómez-Gutiérrez, 2003a) in the northern CCS. One female lost her ovigerous sac with live MN larvae when she molted four days after spawning before releasing her larvae. All those larvae died, probably from the lack of oxygenation that the female provides by swimming and constantly moving her ovigerous sac.

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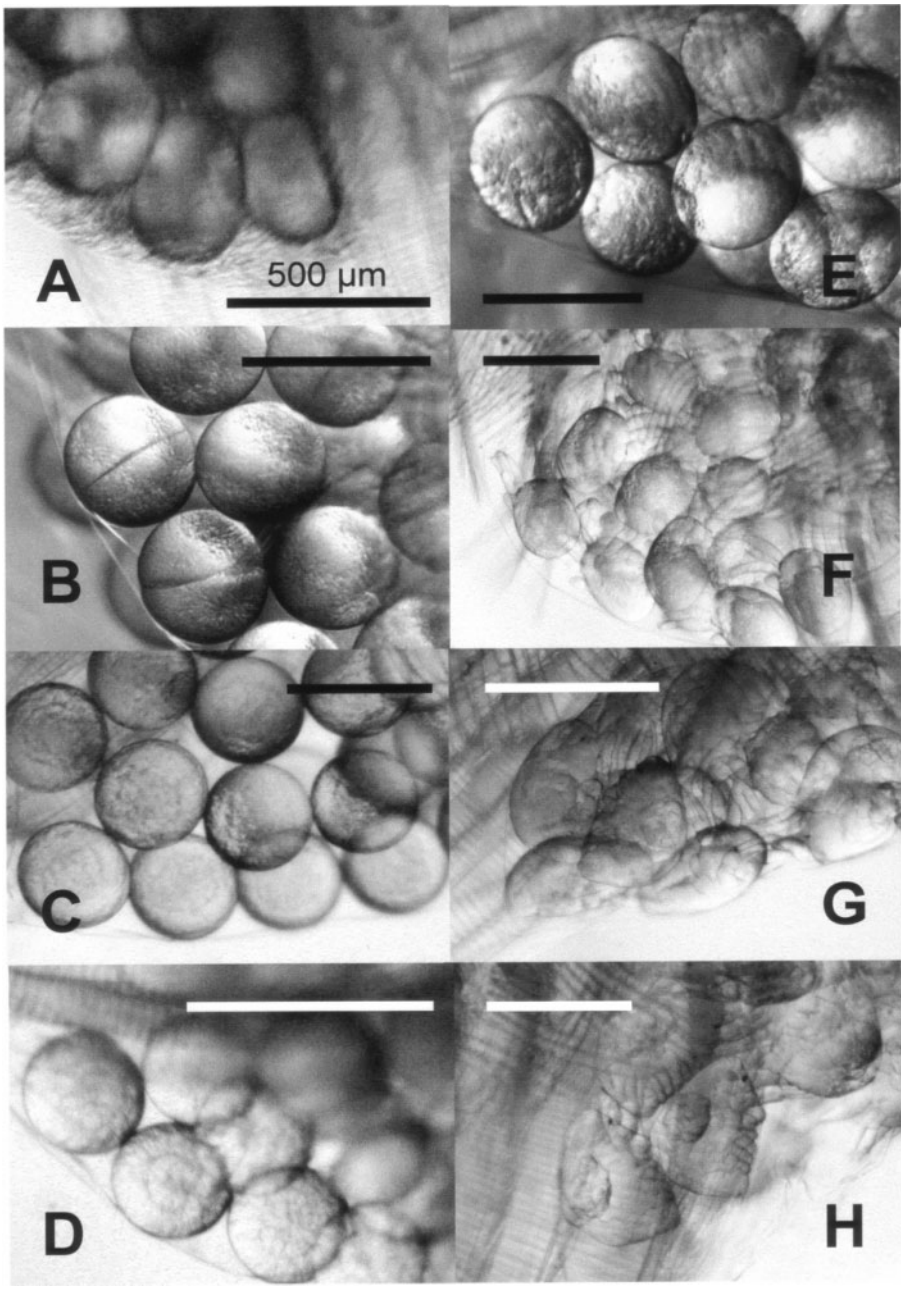

Fig. 3. *Nyctiphanes simplex*. Embryo development in the ovigerous sac and hatching mechanism (4× magnification). (**A**) Just-spawned elliptical eggs. (**B**) Spherical single-cell embryo stage and multiple-cell embryo stage. (**C**) Blastula stage with two layers of cells. (**D**) Gastrula stage showing three layers of cells. (**E**) Late limb-bud stage with elliptical shape (the early limb-bud stage is similar but still having spherical shape, picture not showed). (**F**) Nauplius breaking the chorion and hatching while the embryo grows. (**G**) Pseudometanauplius with rudimentary mandible in the ovigerous sac. (**H**) Metanauplius with black nauplius eye. Scale bar = 500 µm.

Hatching mechanism and larval escape from the ovigerous sac

A sequence of the embryonic development stages of *N. simplex* is shown in Fig. 3(A–H). This figure shows that JS elliptical eggs have a soft and flexible chorion and assume variable elliptical shapes (Fig. 3A), spherical SC embryo stage and MC embryo stage have a stronger chorion (Fig. 3B), blastula stage (B) with two layers of cells (Fig. 3C), gastrula stage (G) showing three layers of cells (Fig. 3D), lLB stage with elliptical shape (Fig. 3E) [eLB stage is similar to lLB but with less elliptical shape and appendages are just forming (picture not showed)], Nauplius breaking

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the chorion and hatching while the embryo grows (Fig. 3F), pseudometanauplius with rudimentary mandible in the ovigerous sac (Fig. 3G), and MN with black nauplius eye (Fig. 3H). The embryos between JS and TW stages do not have a visible perivitelline space (PVS) and from SC to eLB are spherical (Fig. 3A–D), they become elliptical again when the embryos reach the lLB stage (Fig. 3E).

The embryonic and early larval developmental times are summarized in the Table III. There was considerable female-to-female variability that sometimes makes the final and initial development times of consecutive embryonic stages overlap. This variability was considerably larger than developmental time variability within the same ovigerous sac. The eggs and larvae remain in the ovigerous sac as embryos for about 82 h and as postembryonic larval stages for 116 h after spawning, then they leave the female protection. The nauplius breaks the chorion as the embryo grows, weakly helped by movements of the first and second antennae, between 80 and 91 h after spawning (Fig. 3F and Table III). The embryos hatch without an apparent anterior to posterior order in the ovigerous sac. It is difficult to observe the empty chorions inside the ovigerous sac because they are thin and transparent. The larvae develop in the ovigerous sac to the PMN stage, between 90 and 105 h after spawning (Fig. 3G) and the MN stage, between 92 and 140 h after spawning (Fig. 3H). The PMN develops a nauplius eye in the ventral posterior part of the larva, easily observed when they are alive that become darker in the MN stage (Figs 3G and H) but much less evident when the larvae are preserved. The larvae escape from the ovigerous sac late in the MN stage, between 96 and 144 h after spawning, and just a few hours (between 0.5 and 10 hors after the larvae escape) before they molt to the first feeding C1 stage (Table III). The three calyptopis stages have asynchronous development times, the C1 develops between 101 and 167 h, C2 between 119 and 351 h, and C3 between 215 and 508 h after spawning (Table III).

Molt from free-swimming metanauplius to calyptopis 1 stage

We observed the molt from the MN to the C1 several times (Fig. 4A–F). Molting is quite rapid, less than 3 minutes. In a lateral view, the late MN stage has a big dorsal hump, where the primordial abdomen develops to become the abdomen of the C1. The telson looks like a small bump in the posterior part of the body (Fig. 4A). Suddenly, this bump begins to extend from the carapace as the MN expands its abdomen (Fig. 4B). Eventually the MN extends

Table III: Observed initial, final, and median (the time when about the 50% of the embryos reach a specific development stage) development time (h) of Nyctiphanes simplex per embryonic or larval stage under shipboard laboratory conditions (16°C ± 1°C, 50 females with broods were observed during March and July, 2004)

Embryo development stage	Initial (h)	Final (h)	Median (h)	Median (days)
Just-spawned eggs (JS)	0.0	0.5	0.3	0.01
Single Cell (SC) spherical shape	0.3	3.6	1.8	0.08
Multiple Cell (MC)	3.0	8.0	5.5	0.23
Blastula (B)	8.0	34.0	21.0	0.88
Gastrula (G)	32.0	44.0	39.0	1.63
Early limb-bud (eLB)	54.0	65.0	59.5	2.48
Late limb-bud (lLB)	67.0	81.0	68.0	2.83
Twitching nauplius (TW) & hatching time	80.0	91.0	82.0	3.42
Pseudometanauplius (PMN)	90.0	115.0	97.5	4.06
Metanauplius (MN)	92.0	140.0	116.0	4.83
Molts from MN-C1 after release	96.0	144.0	117.0	4.87
Calyptopis 1 (C1)	101.0	167.0	130.0	5.42
Calyptopis 2 (C2)	119.0	351.0	189.0	7.86
Calyptopis 3 (C3)	215.0	508.0	325.0	13.54

The time of the twitching stage, when the egg has a nauplius completely developed inside the chorion and its appendages are freely moving, strongly overlaps with the nauplius hatching time inside the ovigerous sac. The embryos escape from the ovigerous sac late in the metanauplius stage having a short free-swimming period (average 1 h, but it can be extended to 10 h) before molting into the first feeding calyptopis 1 stage (C1). The nomenclature of the development stages of the eggs after spawning and early larval stages was adapted from previous studies (Ponomareva, 1963; George and Strömberg, 1985; Brinton et al., 2000; Gómez-Gutiérrez, 2002, 2003a).

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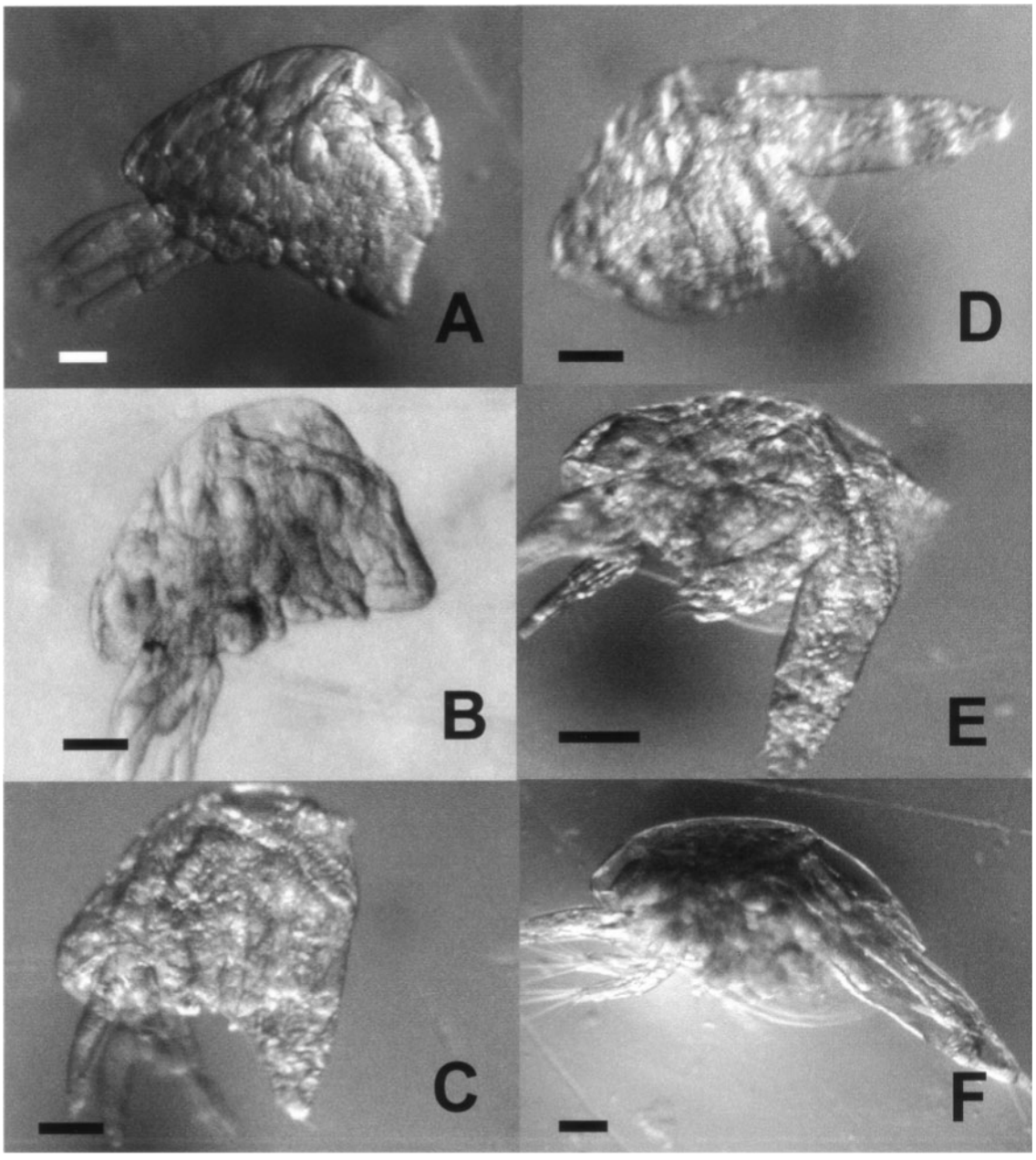

Fig. 4. *Nyctiphanes simplex*. Sequence of the molting process of the just released metanauplius (MN) stage from the female's ovigerous sac to the first feeding larval stage Calyptopis 1 (C1). Most of the MN molt in less than a few hours after they leave the female ovigerous sac. (**A**) The MN has typically a dorsal hump where the abdomen is folded. (**B**) The posterior part of the MN is extruded slowly. (**C**) The larvae breaks the posterior part of the exoskeleton and extends most of the abdomen. (**D**) The just-molted C1 extends the abdomen taking the typical shape of the calyptopis. This process usually takes <3 minutes. (**E**) This C1 has rudimentary mouth parts, which develop completely several hours later; the eyes begin to be evident. (**F**) C1 completely developed with red colored pigmentation in the posterior part of the abdomen. Scale bar = 100 μm.

the abdomen still flexed forward breaking the exoskeleton, the mandible, maxillula and maxila extend and are ready for feeding (Fig. 4C). Then the C1 flips its abdomen backward, assuming the characteristic calyptopis shape. The newly molted C1 stage has rudimentary eyes and feeding appendages, and the organism usually swims quickly (Fig. 4D). The C1 completes the development of the feeding appendages in less than 12 h, the eye becomes more complex, setae are added to the thoracic appendages, and the overall size increases (Fig. 4E). The C1 can feed and

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have colored stomach and a red spot in the base of the telson a day after they molt from MN (Fig. 4F).

DISCUSSION

Embryonic and early larval development time

The time from spawning to hatching as nauplii was between 80 and 91 h and the duration from spawning to larval release as MN was approximately 116 h (~5 days at 16°C). This is remarkably longer hatching time than the observed in *N. difficilis* hatching as PMN between 55 and 60 h or as MN about 84 h after spawning (2.5 and 3.5 days respectively at 10°C). This difference was unexpected because it is well-known that egg development times are shorter at higher temperatures (Herring, 1974; Gillooly *et al.*, 2002). The advantages of prolonged maternal protection and swimming energy saving strategy promoted by a late embryo release may be important adaptations not solely regulated by temperature.

Lavaniegos (Lavaniegos, 1992) reported the larval development time of *N. simplex* under laboratory conditions (14°C ± 0.5°C) from field collected C2 and C3 of unknown age to juveniles. She deduced the average age from hatching to the midpoints of C2 was 8.5 days and to C3 was 11.5 days, assuming that the larvae had spent one day becoming MN after leaving the ovigerous sac as PMN, and each successive instar of the calyptopis phase lasted 3 days (synchronous development time) (Table IV). We measured the *N. simplex* development times at 16°C from hatching to C3 as 8.7 days. Because we observed that the stage that escapes the ovigerous sac is always the MN stage, our estimated time from sac departure to C3 does not include the observed average development time from PMN to MN (0.75 days). We observed progressively increasing stage durations of calyptopis stages (asynchronous development time) with the C1 stage duration (0.6 days) shorter than C2 (2.5 days), which was shorter than C3 (5.6 days) (n = 55 females with ovigerous sacs) (Table IV). These development times were faster than those assumed by Lavaniegos (Lavaniegos, 1992) even after subtracting the PMN-MN duration (C2 = 7.5 days and C3 = 10.5 days). The calyptopis can reach the first furcilia stage (F1) in just half the time previously thought.

In the past it was assumed for simplicity that the calyptopis stages of *N. simplex* had isochronal developmental time, the time spent in each stage is the same for all stages (Lavaniegos, 1992). This was partially because they have not been directly measured from egg to C3 in any of the four *Nyctiphanes* species, but indirectly estimated from field collected C2 or C3 with unknown age (Le Roux, 1973; Ritz and Hosie, 1982; Hosie and Ritz, 1983; Pillar, 1984; Lavaniegos, 1992). However, the assumption of isochronal larval development in euphausiids can result in substantial errors in estimates of instar-specific mortality rates and, consequently, can seriously bias the interpretation of mortality patterns in nature and their possible causes. Asynchronous calyptopis developmental times have been reported in the broadcast-spawning euphausiids *E. pacifica* (Ross, 1981), *T. spinifera* (Summers, 1993) and the sac-spawning euphausiids *N. difficilis* (Gopalakrishnan, 1972, 1973) and *N. simplex* (this study). Asynchronous development times may be the usual pattern for euphausiids since high individual variability of development time has been observed, even

Table IV: Median larval development time of Nyctiphanes simplex in days (in parentheses in hours) deduced by Lavaniegos (Lavaniegos, 1992) and observed during this study from several ovigerous females

Stage	Lavaniegos (1992)		This study	
	Median age in days (h)	Cumulative time in days (h)	Median age in days (h)	Cumulative time in days (h)
PMN	Assumed hatching stage	Unknown	5 (120)	
MN	1 (24)	1 (24)	Observed hatching staged	0.6 (14)
C1	3 (72)	4 (96)	0.6 (14)	0.6 (14)
C2	3 (72)	7 (168)	2.5 (59)	3.1 (74)
C3	3 (72)	10 (72)	5.6 (134)	8.7 (209)

In the past it was assumed that the hatching stage of *N. simplex* was the pseudometanauplius and that the early larval developmental stages have isochronal development time (Boden, 1951; Lavaniegos, 1992).

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for the individuals of the same larval stage (Le Roux, 1973; Pillar, 1984). Even in copepods where supposedly it is relatively common to observe isochronal developmental times, no species follows the isochronal rule strictly (Peterson, 2001). It is well know that neritic species like *Nyctiphanes* have large variability in furcilia development pathways (pleopod development) associated with the temperature and feeding conditions (Le Roux, 1973; Pillar, 1984; Lavaniegos, 1994; Gómez-Gutiérrez, 1996) not observed in oceanic species with non-variable development of the pleopods (Pillar, 1984). It is likely that calyptopis stage duration is also dependent of the environmental conditions; however, this has not been specifically tested for *Nyctiphanes* species.

The ecological success of *N. simplex* in this ecosystem has not been well understood perhaps because of the lack of experimental studies. In the past it was assumed that the development time of the eggs of *N. simplex* was 30 days (thus, the IBP should be longer than 1 month), and egg production was estimated at 0.09 mg m^{-2} d^{-1} or 33 mg m^{-2} y^{-1}, representing 1.9% of the total *N. simplex* secondary production (1736 mg m^{-2} y^{-1}; egg production 1.9% + exuviae 23.4% + body growth 74.7%) (Lavaniegos, 1995). We observed that embryonic development time is 5 days at 16°C and that most of the females that spawned twice under laboratory conditions had an IBP of 7 days. This means that the egg production of this species has been underestimated and is at least four times greater (0.36 mg m^{-2} d^{-1} or 132 mg m^{-2} y^{-1}) than Lavaniegos's estimates assuming an IBP of 30 days. Thus, egg production of *N. simplex* may represent about 7% of the total secondary production (1736 mg m^{-2} y^{-1}) estimated by Lavaniegos (Lavaniegos, 1995). The minimum possible IBP of 7 days of the sac-spawning *N. simplex* is relatively longer than the average IBP of 3 days observed in *E. pacifica*, a broadcast-spawning species (between 2 and 21 days) (Gómez-Gutiérrez, 2003b). Other favorable traits to explain the great success of *N. simplex* in comparison with other euphausiid species may be rapid body-growth rates (Lavaniegos, 1995; Gómez-Gutiérrez *et al.*, 1996; De Silva-Dávila and Palomares-García, 1998), which is high when compared even with the congener *N. australis* G. O. Sars (Ritz and Hosie, 1982; Hosie and Ritz, 1983) and high morphogenetic flexibility of the furcilia stages (Lavaniegos, 1992, 1994; Gómez-Gutiérrez, 1996).

Boden (Boden, 1951) described the eggs of *N. simplex,* from preserved samples, to have an extremely small PVS. We did not observe PVS in any of the embryo developmental stages of *N. simplex*. *Nematoscelis difficilis* has a narrow PVS (<20 μm) (Nemoto *et al.*, 1972; Gómez-Gutiérrez, 2003a). Sac-spawning species tend to have a smaller PVS than broadcast-spawning species, perhaps as an adaptation to package the greatest number and larger embryos in the least amount of space in the ovigerous sac (Gómez-Gutiérrez, 2003b). This decrease in the PVS is apparently extreme in *N. simplex* and perhaps generalized for species of this genus (Ritz and Hosie, 1982; Hosie and Ritz, 1983; Stuart and Nicol, 1986; Gómez-Gutierrez, 2003b).

Hatching success

Gómez-Gutiérrez (Gómez-Gutiérrez, 2002) proposed that 'backward' hatching of the N1 stage is the normal hatching mechanism for several broadcast-spawning euphausiids (*E. pacifica*, *T. spinifera* and *T. inspinata*) (Table I). The backward hatching as nauplius was associated with a significantly greater hatching success than the extended-hatching schedules with forward or flipping hatching. Gómez-Gutiérrez (Gómez-Gutiérrez, 2003a) demonstrated that PMN and MN stages of *N. difficilis*, a sac-spawner, use a fourth hatching mechanism called 'push-off' hatching, also associated with great hatching success (Table I). Other studies also indicate very high hatching success for *N. difficilis* (Komaki, 1967; Gopalakrishnan, 1972, 1973; Nemoto *et al.*, 1972). *Nyctiphanes simplex* also showed remarkably high hatching success (usually 100%), excepting for three females whose eggs all died, changing the color of their ovigerous sac to bright white (Fig. 1F). We did not observe dead or deformed embryos inside the ovigerous sac. High hatching success could be an inherent characteristic of the sac-spawning euphausiids in comparison with more variable and temperature-dependent hatching success observed in broadcast-spawning euphausiids (Iguchi and Ikeda, 1994; Gómez-Gutiérrez, 2002, 2003b). The N1 and N2 of the broadcast-spawning species (*E. pacifica*, *T. spinifera* and *T. inspinata*), the N2 of the sac-spawning species *N. difficilis* have a red coloration in the posterior part of the body (where they hatch using the backward hatching mechanism). This was interpreted as a region where enzymes could help to dissolve the chorion (Gómez-Gutiérrez, 2002, 2003a). *Nyctiphanes simplex* N1 never had a red coloration in any part of the body during hatching (using the expansion hatching mechanism). This represents a significant physiological and behavioral modification in the hatching mode in comparison with other euphausiid species.

Nyctiphanes simplex interbrood period and reproductive strategy

It have been suggested that euphausiids of the genus *Nyctiphanes* (Hosie and Ritz, 1983; Gómez-Gutiérrez, 1995; Lavaniegos, 1995) and other tropical sac-spawning euphausiid species like *Pseudeuphausia latifrons* (Wilson

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et al., 2003) have continuous reproduction (continuous oögenesis); defined by Ross and Quetin (Ross and Quetin, 2000) as the reproduction that is no regression to a spent stage once maturity is reached. We consider that *N. simplex* behaves as a total spawner because the females produce one batch of oöcytes per cycle of the ovary (the carapace looks 'empty' after spawning) and we observed the development of a new batch of oöcytes after spawning (Fig. 1E). This is different to the broadcast *E. superba* and *M. norvegica* that produce three batches of oöcytes per cycle of the ovary, that is after spawning of the mature egg batch, the ovary is not empty (Cuzin-Roudy, 2000). Our experimental observations indicate that *N. simplex*, like *N. difficilis*, cannot produce a new batch of eggs until the previous produced embryos have hatched and escaped from the ovigerous sac. The IBP of *N. simplex* was usually of 7 days although one female had an IBP of 15 days. Because the females were incubated under laboratory conditions, the estimates of IBP may not be necessarily representative of the environment. Thus, the IPB should be interpreted as the potential time between two consecutive spawns of an individual female. However, we believe that the IBP of 7 days really happens in nature because it is the shortest possible IBP that this sac-spawning euphausiid can produce a new batch of eggs considering that the embryos in the ovigerous sac develop in 5 days and the female usually molt at least one day after spawning.

The females that spawned a second time under laboratory conditions always produced unfertilized eggs and that females usually molted 1 or 2 days after they released their embryos. This suggests that they need to remate earlier or after they release their previous embryo batch and require an efficient synchronization between egg production and molting processes. A broadcast-spawning euphausiid, *E. pacifica*, also produces unfertilized eggs from the second spawn after collection under laboratory conditions (Gómez-Gutiérrez, 2003b). The need to mate with different males every time after the female produced an ovigerous sac could be a strategy to enhance the genetic variability of individual female's broods throughout her life span. Otherwise, if sperms of a single male were stored in a spermatheca to fertilize several female's broods, she could decrease significantly its odds to increase the embryo's fitness. This last strategy could be adaptive whether the species has low densities and the female have little odds to find a male, but *N. simplex* mating process should be enhanced because dense krill swarms usually have a nearly 1:1 sex ratio (Gendron, 1992; Gómez-Gutiérrez, 1995).

Brinton *et al.* (Brinton *et al.*, 2000), based on Einarsson (Einarsson, 1945) observation reported that spermatophores have not been observed attached to the female of any *Nyctiphanes* species, suggesting that fertilization may occur within the external egg sac. Even we have not seen the spermatophore attached to *N. simplex* females, we believe that fertilization should be internal because gravid females collected from the field and insolated into incubation bottles produced fertilized eggs within the next 48 h. Otherwise, in case that the fertilization would be external, all the females that spawned under laboratory condition after collection in the field would be unfertilized due to the absence of males to immediately fertilize the JS eggs into the ovigerous sac. We observed females collected directly from the field that produced unfertilized eggs (in relatively low proportion during July and high proportion during December; 2004). In such stations only females were collected and/or occurred in stations with oligotrophic conditions in comparison with more productive seasons of spring (March) and summer (July) the same year.

Nyctiphanes simplex females molted at least two days after all the MN embryos leave the ovigerous sac, thus spawning does not occur close to the actual molt (ecdysis). *Euphausia superba* can release one or up to three spawns independently of the molt process (Nicol, 1989), but *M. norvegica* showed that two successive molt cycles are necessary for a complete cycle of egg production, a vitellogenic molt cycle preceding the spawning molt cycle (Cuzin-Roudy and Buchholz, 1999; Cuzin-Roudy, 2000).

Hatching mechanism

Boden (Boden, 1951) suggested that *N. simplex* larvae emerge from the ovigerous sac in the PMN stage, molting almost immediately to the MN stage in the water column. He based this on the observation of rearing experiments with *Nyctiphanes couchi* Bell reported by Lebour (Lebour, 1924, 1925) and substantiated because only ovigerous females were present and PMN were seldom encountered in the plankton. This agrees with the old notion that sac-spawning species hatch exclusively as PNM or MN (Casanova, 2003). Only recently has it become recognized that five species of euphausiids have the potential for flexibility in the hatching mechanism, being able to hatch in the nauplius, PMN, MN or even the C1 stages in the laboratory (Gómez-Gutiérrez, 2002, 2003a,b).

The subtropical, neritic, sac-spawning euphausiid *N. simplex* has a distinctive hatching mechanism ('expansion' hatching mechanism, the nauplii break the chorion as they grow) than the observed under laboratory conditions for *E. pacifica*, *T. spinifera*, *T. inspinata* and *N. difficilis* along the Oregon coast (Gómez-Gutiérrez, 2002, 2003a,b). This is the fifth distinct hatching mechanism discovered in the family Euphausiidae and differs in

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the hatching stage and hatching schedule from the two hatching mechanisms observed in the sac-spawning euphausiid *N. difficilis* under similar shipboard laboratory conditions (Gómez-Gutiérrez, 2003a,b). *Nyctiphanes simplex* embryos always hatch as nauplius, and then remain inside the ovigerous sac, developing into PMN and MN, increasing the volume of the sac and squeezing the embryos until they are virtually motionless. The larvae leave the ovigerous sac exclusively as MN just 0.5–10 h before they molt into C1. This short free-swimming duration explains why this stage was not commonly collected in standard zooplankton samples in other studies (Lavaniegos, 1994, 1995; Gómez-Gutiérrez *et al.*, 1995, 1996; Gómez-Gutiérrez, 1995; De Silva-Dávila and Palomares-García, 2002). *Nematoscelis difficilis* embryos hatch rarely as N2 (<2.7%), and usually as PMN or MN, escaping immediately from the ovigerous sac after hatching either as PMN or as MN. Because *N. difficilis* larvae do not wait inside the sac the PMN and MN may be exposed to larger predation risk in the water column without the female's protection. Another difference is that the larvae of *N. simplex* do not hatch progressively from the distal end to the proximal end of the sac (toward the female's thelycum) as observed for *N. difficilis* (Gómez-Gutiérrez, 2003a,b), but by breaking the chorion as the nauplii grow. Thus, the *N. simplex* hatching schedule resembles those observed in mysids (Wortham-Neal and Wayne-Price, 2002) in which the larvae hatch inside the ovigerous sac long before they emerge from it.

Nyctiphanes simplex embryos also hatch differently than the embryos of the sac-spawning *S. carinatum* (Ponomareva, 1969), which apparently use the forward hatching mechanism (Gómez-Gutiérrez, 2002, 2003a,b). Ponomareva (Ponomareva, 1969) reported *S. carinatum* embryos hatching as nauplii by extending and contracting the anterior pair of appendages, pinning the chorion with short, dense spines, parting their appendages to rupture the nearest part of the egg membrane, and then emerging into the surrounding water. Hatching and escaping from the ovigerous sac are almost simultaneous, like in *N. difficilis*. She also observed that the nauplii swam near the ruptured bag, but she did not follow the larval development after the MN stage.

It is interesting that *N. simplex*, which is a numerically dominant euphausiid species in this habitat (Lavaniegos, 1992, 1994, 1995; Gómez-Gutiérrez, 1995; Gómez-Gutiérrez, 1995, 1996; De Silva-Dávila and Palomares-García, 2002), uses a different hatching mechanism than two comparatively less abundant sac-spawning euphausiid species. Perhaps, the late schedule of embryo release with enhanced maternal protection and the energy saved by not swimming extend the trophic point-of-no-return in the field (Ross and Quetin, 1989), significantly promoting survival in this susceptible phase of the life cycle. The point-of-no return is the time at which the first exogenous feeding stage (C1) remains starving but cannot survive even if enough food is suddenly provided (Ross and Quetin, 1989).

We observed that *N. simplex* and *N. difficilis* females do no eat their healthy eggs and post-hatching stages while develop in their ovigerous sac, excepting when they are dead (Gómez-Gutiérrez, 2003a). This is a significant difference in comparison with broadcast-spawning species in which cannibalism of eggs or early larvae stages have been sometimes observed (Gómez-Gutiérrez, 2003b). During the December 2004 oceanographic cruise we observed the spawning (brood size of 70 eggs fem^{-1}), hatching time and hatching mechanism of a blue gonad stage IV female (18.6 mm total length) of the tropical euphausiid *Euphausia eximia* Hansen incubated at 18°C. All the embryos hatched as MN stage using the forward hatching mechanism (Table I), with variable hatching time among the sibling embryos (90 to 102 h after spawning) (unpublished data).

The hatching mechanisms of the euphausiids have been experimentally observed in only nine of the 86 species currently described (Table I). Thus an extensive research effort is needed to close the immense gap of knowledge about early embryology and larval behavior of the species of the order Euphausiacea. The relevance to study hatching mechanisms in other euphausiid species is that they have shown high flexibility in the hatching schedule and hatching mechanisms; each one associated with differential hatching success and sometimes significantly decreasing the fitness of the individuals. The hatching mechanism and mortality during this process are critical factors to estimate the early larval recruitment of the population. Additionally, it is expected that related euphausiid species will share similar hatching mechanisms and developmental strategies, reflecting their common phylogenies and ecological adaptations. An interpretation of the phylogenetic trends should be deduced by combining genetic (Jarman *et al.*, 2000a,b; Jarman, 2001), embryological (Alwes and Scholtz, 2004), morphological (Casanova, 1984, 2003; Maas and Waloszek, 2001) and early-life behavioral information (Gómez-Gutiérrez, 2002, 2003a,b; this study) to obtain a general perspective about the phylogenetic relationships among the euphausiid species. The identification of novel hatching mechanisms in other euphausiid species, the identification of the most common hatching mechanisms in different euphausiid genera or species or the comparison of these variables between broadcast and sac-spawning euphausiids, might provide evidence about the adaptive role of hatching mechanics in survival and general euphausiid species fitness.

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