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Reproductive biology, embryo and early larval morphology, and development rates of krill (*Euphausia lamelligera* and *Euphausia distinguenda*), endemic to the Eastern Tropical Pacific

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ABSTRACT: The reproductive biology and early life phases of tropical broadcast spawning krill are largely unknown worldwide. This investigation provides the first published data on the reproductive period, brood size, embryo and nauplius-to-metanauplius morphology, biometry, development, and hatching success rates of two of the smallest krill species known (*Euphausia lamelligera*, <11 mm and *E. distinguenda*, <14.5 mm total length), endemic to the Eastern Tropical Pacific. Embryos were obtained from gravid females collected on the Jalisco continental shelf (Mexico) every 2 wk from July 2011 to June 2012, and incubated under laboratory conditions. Both species spawned throughout the year (with higher brood sizes between Jan and Jun), showing similar mean interspecific brood sizes: *E. lamelligera*, 34 eggs female^{-1} (range: 4–95) and *E. distinguenda*, 36 eggs female^{-1} (range: 14–72). *E. distinguenda* spawned larger eggs (chorion 0.700, embryo 0.329 mean diameters, perivitelline space [PVS] 0.185 mm) than *E. lamelligera* (chorion 0.405, embryo 0.291, PVS 0.057 mm). Both species had high hatching success (>66%) with the shortest hatching times (9 to 14 h) known so far for any species of the Order Euphausiacea. *E. distinguenda* was significantly larger than *E. lamelligera* at each early larval stage. A pseudometanauplius stage (molting between nauplius and metanauplius stages), previously thought to be an exclusive stage of sac-spawning species, was observed for both broadcast spawning species. Our results support the hypothesis that both species exhibit a continuous but seasonally variant spawning reproductive strategy associated with female body size and seasonal coastal upwelling dynamics, and show brood sizes within the low range of variability known for temperate krill species.

KEY WORDS: *Euphausia lamelligera* · *Euphausia distinguenda* · Embryogenesis · Nauplius · Development times · Reproductive biology · Eastern Tropical Pacific

INTRODUCTION

Very little is known about the reproductive biology/ecology of tropical euphausiids (Order Euphausiacea, commonly known as krill), except for their zoogeographic distribution, vertical distribution, seasonal abundance patterns, population structure, and sex ratio. This is because relatively little research has been done in low latitude marine ecosystems, as tropical krill are small (not suitable for a fishery), and because it has been long assumed that they occur in low abundance, have low secondary production rates, and do not form dense aggregations like subtropical, temperate, and polar krill species. There-

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fore, our understanding of their ecological function and contribution to the productivity rates of zooplankton in the tropical food web is still incipient and deserves scientific attention (Wang 1965, Wilson et al. 2003, Ambriz-Arreola et al. 2012). Knowledge is particularly deficient in reproductive strategies, embryology and early larval stage ontogeny and taxonomy for endemic krill species of the Eastern Tropical Pacific (ETP), but their vertical and horizontal distribution and abundance are relatively well known (Valentine & Ayala 1976, Brinton 1979, Färber-Lorda et al. 1994, 2004, 2010, Fernández-Álamo & Färber-Lorda 2006, Ambriz-Arreola et al. 2012).

Fifteen krill species have been recorded in the northern region of the ETP off Mexico (Brinton 1962, 1979, Mundhenke 1969). Five of them are endemic and include two of the smallest and numerically dominant krill species in the ETP region: *Euphausia lamelligera* (<11 mm total length) and *E. distinguenda* (<14.5 mm total length) (Baker et al. 1990, Brinton et al. 2000, Ambriz-Arreola et al. 2012). *E. distinguenda* has larger heterozygosity (as a proxy of variability in large outbreeding) than temperate and polar species (Valentine & Ayala 1976), suggesting a trend of low genetic variability in high latitude species and high genetic variability in low latitude species. High endemism of zooplankton species in the ETP is likely due to long-term evolutionary adaptation and speciation in this region, which features year-round high temperatures, pronounced water column stratification, and a shallow upper boundary of the oxygen minimum zone (caused by regional relatively low horizontal speed current circulation and relatively high surface primary production/respiration rates that deplete oxygen concentrations in deeper waters). It has been proposed that these environmental conditions promote low seasonal variability of zooplankton standing stock and relatively low zooplankton biomass (Fernández-Álamo & Färber-Lorda 2006). However, so far there have been no estimations of brood size or studies of the reproductive season for any tropical krill species in the ETP.

Recently, it was demonstrated that *E. lamelligera* and *E. distinguenda* show strong seasonal and interannual variability of larval (calyptopis and furcilia phases) and postlarval (juvenile and adult phases) abundances associated with upwelling and downwelling coastal variability, suggesting that both species reproduce throughout the year (Ambriz-Arreola et al. 2012). Reproductive biology (gonad development and brood sizes) and spawning periods of both species (as with most broadcast tropical species) have not been studied (Ross & Quetin 2000). There are relatively few studies than have counted the oocytes inside the carapace or the eggs of sac-spawning tropical species (Mathew 1980, Wilson et al. 2003). The lack of information is partially because the morphology of embryonic and early larval stages nauplii and pseudometanauplii (indicative of recent spawning) have not yet been described in tropical krill species (Gómez-Gutiérrez et al. 2010a). Previous studies suggested a pattern of continuous reproduction for krill species inhabiting the mid-latitudes and equatorial regions, strongly linked to seasonal upwelling cycles and high food availability (Pillar & Stuart 1988, Ross & Quetin 2000, Ambriz-Arreola et al. 2012). In the present study, we evaluate the hypothesis that *E. lamelligera* and *E. distinguenda* exhibit continuous reproduction, albeit with some variability in spawning intensity associated with seasonal upwelling dynamics from the northern region of the ETP, measuring their monthly mean brood size under laboratory conditions from gravid females collected in the field.

Margaret Knight from the Scripps Institution Oceanography (San Diego, CA, USA) described the morphology of the metanauplius-to-furciliae stages of *E. lamelligera* and *E. distinguenda* from specimens collected in the field (Brinton et al. 2000). These descriptions require confirmation through identification of eggs spawned by gravid females incubated under laboratory conditions and following their embryonic and early larval development (nauplius-to-metanauplius stages). Mathew (1971, 1975) described *E. distinguenda* calyptopis and furcilia stages from field samples collected in the Indian Sea. However, it is currently well known that previous reports of *E. distinguenda* in the Red and Indian Seas were actually describing the endemic species *E. sibogae* (Brinton 1975, Brinton et al. 2000). Because the morphology of embryos (commonly known as 'eggs') and nauplii stages of *E. distinguenda* and *E. lamelligera* are unknown, it is not possible to directly infer the reproductive activity in time and space from field zooplankton samples. So far, no attempts have been made to incubate gravid tropical krill females to record brood size, hatching success, and describe biometry and morphology of embryos and early larval stages (Ross & Quetin 2000). This investigation is a first step toward monitoring those stages from field samples in order to investigate the distribution and seasonal reproductive periods of each species, similar to studies of krill species in other regions of the world (Gómez-Gutiérrez et al. 2005, 2010a, Plourde et al. 2011).

Embryonic development of euphausiids was first described for the North Atlantic krill *Meganyc-*

tiphanes norvegica (Sars 1898, Taube 1909, 1915). Cell division from single cell to gastrulation stages of *M. norvegica* was described in unprecedented detail using cell lineage maps with fluorescence staining (Alwes & Scholtz 2004). As far as we know, the only previous illustrations and brief descriptions from shipboard gravid female incubations are of embryos and early larval stages of the tropical sac-spawning *Stylocheiron carinatum* (Ponomareva 1969) and the broadcast spawning *E. eximia* (Knight 1980). Observations of transparent live embryos of *E. superba* (George 1984, George & Strömberg 1985, Jia et al. 2014), *E. pacifica, Thysanoessa spinifera, T. inspinata, Nematoscelis difficilis* (Gómez-Gutiérrez 2002, 2003, 2006, Gómez-Gutiérrez et al. 2010a) and *N. simplex* (Gómez-Gutiérrez & Robinson 2005, Gómez-Gutiérrez et al. 2010b) have also improved our understanding of embryonic development and hatching success of euphausiids for species with broadcast and sac-spawning reproductive strategies. Embryogenesis and internal morphology of early larval stages (metanauplius and calyptopis 1) of the sac-spawning *N. simplex* were recently studied using histological techniques (Montuy-Gómez et al. 2012).

The biometry of eggs was recently revised and updated for all available krill species worldwide by Gómez-Gutiérrez et al. (2010a). These authors reported mean egg diameter measurements of *E. distinguenda* (0.320 mm chorion diameter and 0.218 mm embryo diameter), but they did not report the biometry for each embryonic stage. The biometry of embryonic and early larval stages of *E. lamelligera* is currently unknown. Study of the temporal and geographical distributions of their eggs may elucidate species preferences for spawning season and location, centers of larval dispersion, and clues about how embryos survive in the ocean (Gómez-Gutiérrez et al. 2010a). Thus, the goals of the present study were to (1) determine the reproductive biology (brood size and spawning season) and (2) define the morphometric differences of *E. lamelligera* and *E. distinguenda* embryos to be able to identify them from field samples, and measure their hatching success, (3) describe the early larval stage nauplius (1 and 2) and re-describe the metanauplius stage (for confirmation purposes) to complete the taxonomic description previously known only from metanauplius, calyptopis, and furcilia stages (Brinton et al. 2000), and (4) estimate embryonic and early larval stage development rates of these 2 tropical krill species for comparison with species from other latitudinal and zoogeographic distribution patterns.

MATERIALS AND METHODS

Collection of euphausiids and spawning experiments

Gravid females of *Euphausia lamelligera* and *E. distinguenda* were collected every 2 wk during a time series of plankton sampling at Bahía de Navidad station, Mexico (19° 09′ 03″ N, 104° 44′ 50″ W), located on the continental shelf of the northeastern tropical Pacific Ocean. Ambriz-Arreola et al. (2012) proposed, based on the vertical structure of the water column, that 3 seasonal climatic periods prevail in the Cabo Corrientes region: a mixed period from February to May, typified by well-mixed water column, relatively low temperatures (<24°C), high salinities (>34), shallow mixed layer depth (<30 m), and high plankton biomass driven by intense coastal upwelling events (coastal upwelling index, CUI > 100 m^3 s^{-1} per 100 m coastline); a stratified period from July to November characterized by strong vertical stratification of the water column, high temperatures (>25°C), low salinities (<34) (due to the regional rainy season), relatively deep mixed layer depth (>40 m depth), and low plankton biomass (oligotrophic conditions); and 2 transitional semi-mixed periods; one from mixed-to-stratified conditions (June) and another from stratified-to-mixed conditions (December/January).

Twenty zooplankton samples were collected from July 2011 through June 2012 at night (~05:00 h) from a 6 m length fiberglass boat. Zooplankton trawls (<30 m depth) were carried out using a net (1 m diameter, 3 m long, 300 µm mesh, with a closed cod end 0.22 m in diameter and 0.70 m long) towed at a constant speed (~4 km h^{-1}) for 10 min. The zooplankton sample was gently diluted into a 20 l insulated container filled with *in situ* surface seawater and transported (usually within 1 h) to the land-based laboratory.

Gravid females of both krill species were visually identified by the presence of intense blue-colored ovaries (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/s001p143_supp.pdf). Active and healthy gravid females were placed individually into 250 ml glass bottles (8.5 cm height × 6.6 cm diameter) containing filtered sea surface water (64 µm) collected at the sampling station. The specimens were incubated under continuous dark conditions inside a plastic cooler. Incubation temperature was set at 25 ± 1°C, which represents the average temperature recorded in the upper water column (<30 m depth) at the study area. Additional incubations were conducted at temperatures from 22 to 29°C to estimate development time ranges in their seasonally chang-

ing habitat (Ambriz-Arreola et al. 2012). The females usually spawned between 07:00 and 08:00 h (<2 h after they were incubated). After each female spawned, the embryos were monitored every 10 min during the first hour to detect the rapid early embryonic development stages (1 cell to 64 cell multiple stages) and every hour for further embryonic development stages (blastula stage to twitching stage until hatching process). After hatching, the early larval stages were observed every 6 h (nauplius-to-metanauplius stages). Each spawned female was removed from the incubation bottle to avoid cannibalization of the eggs and to measure its total length (from the forward carapace rim to the tip of the telson, expressed in mm), carapace length (CL, from the forward carapace rim to the posterior notch of the carapace), and preserved in 5% formalin with saturated sodium borate. The spawned eggs were left untouched to avoid altering their embryonic and further larval development rates.

Brood size, biometry, and embryonic development time

We used the nomenclature of the development stages of embryos after spawning according to previous studies of several broadcast krill species (Ponomareva 1963, Quetin & Ross 1984, 1989, George & Strömberg 1985, Gómez-Gutiérrez 2002), distinguishing the following embryonic stages: single cell (SC), multiple cell (MC), early blastula (eB), late blastula (lB), early gastrula (eG), late gastrula (lG), post gastrula (pG), early limb-bud (eLB), late limb-bud (lLB), and twitching (TW) stages. Eggs of each spawning event were counted to measure the females' brood size (number of eggs produced per female per spawning event) and at least 15 live eggs from each brood were photographed (recording the stereoscope magnification zoom for each digital photograph) under an optical microscope (Carl Zeiss, Axiostar Plus) equipped with a calibrated micrometer and a digital AxioCam lCc1 camera (Carl Zeiss, with 5× and 10× magnification zoom) connected to a desktop computer. The chorion and embryo diameters at each embryonic development stage were measured from these scaled color photographs. The embryo is suspended in fluid within the chorion, moving freely and sinking to the bottom side of the shell. Therefore, the perivitelline space (PVS) is not always symmetrical for measurement purposes. Thus, we calculated the PVS as follows (Gómez-Gutiérrez et al. 2010a):

$$PVS = CD - ED/2 \quad (1)$$

where CD is the chorion diameter and ED is the embryo diameter. We used Axiovision v.4.0 software to obtain digital images of each embryo and early larval stage and measure their morphological features. Acquired digital images were post-processed for brightness and contrast using Adobe Photoshop CS5, and Adobe Illustrator CS5 for subsequent image assembling (see Figs. 3–6, Figs. S1 & S2 in the Supplement). The eggs spawned from each female were transferred into multi-well trays and observed regularly until they developed from the nauplius to metanauplius stage. Embryonic hatching success was estimated as the percentage of embryos that hatched from the brood size of each incubated female.

The average biometric measurements of the embryos (chorion, embryo, and PVS diameters) of *E. lamelligera* and *E. distinguenda* for each embryonic stage were statistically compared using a Mann-Whitney *U*-test. The intraspecific and interspecific multiple average comparison of brood size (eggs female^{-1}) and female total length (mm) among climate periods were tested using Kruskal-Wallis test followed by Tukey's test (Zar 1996). Linear regression analysis was used to determine the relationship between brood size versus female total length and carapace length of the 2 species. All statistical analyses were performed using Statistica v.7.0 (Stat Soft). Embryonic development and hatching time were compared with those observed in other krill species from polar, temperate, and subtropical latitudes to explore interspecific development rate patterns among krill species from different ecosystems, incubated at approximately the average temperature recorded in their natural habitats (Quetin & Ross 1984, George & Strömberg 1985, 1989, Gómez-Gutiérrez 2002, 2003, Gómez-Gutiérrez & Robinson 2005, Montuy-Gómez et al. 2012, Jia et al. 2014).

Early larval stages morphology and development rates

Digital photographs of live embryos and early larval stages (nauplius to metanauplius) were taken to measure their biometry and development morphology. Embryos and early larvae were observed at least every 30 min during the first day after spawning, and typically every 4 h the next day while recording time elapsed between spawning and hatching events. Additional pictures of formalin-preserved nauplii-to-

metanauplii appendages were taken with an inverse microscope (Olympus model CKX41, 10 to 40× magnification) equipped with a digital camera (Evolution model MP, using QCapture-Pro v.6.0 software) and a calibrated micrometer to observe the morphology and biometry of each pair of first and second antennae and the mandible. Appendages were drawn with thin ink pens, copying them from enlarged photographic images using transparent wax paper.

RESULTS

Brood size of *E. lamelligera* and *E. distinguenda*

A total of 173 gravid female *Euphausia lamelligera* (n = 115) and *E. distinguenda* (n = 58) were collected on 20 of 24 sampling days during the bi-weekly time series (July 2011 to June 2012) (Fig. 1a). Brood size ranged from 4 to 95 eggs female^{-1} for *E. lamelligera* (average ± SD = 34 ± 20) and from 14 to 72 eggs female^{-1} for *E. distinguenda* (36 ± 13). The average brood size of both species was significantly different among the mixed, semi-mixed, and stratified climatic periods (Kruskal-Wallis post hoc test, $p < 0.001$). The brood sizes of *E. lamelligera* were significantly higher during the mixed period than during the stratified and semi-mixed periods ($p = 0.001$), whereas *E. distinguenda* brood sizes were higher during the semi-mixed period than during the mixed and stratified periods ($p = 0.001$; Fig. 1b). These temporal differences suggest a distinct phenology of maximum reproductive activity for each species. Gravid female total length ranged from 6.5 to 10.8 mm (average 8.4 mm) for *E. lamelligera* and 9.5 to 14.4 mm (average 11.9 mm) for *E. distinguenda*. Therefore, *E. lamelligera* can attain first reproduction at about 60% and *E. distinguenda* at 66% of their respective maximum sizes recorded in this study. In concordance with brood sizes, *E. lamelligera* females were significantly larger during the mixed period than during the semi-mixed and stratified periods (Kruskal-Wallis test, $p = 0.001$) (Fig. 1a,c). However, there were no significant differences in average total length of *E. distinguenda* among months or climatic periods (Mann-Whitney *U*-test, $p > 0.05$) (Fig. 1a,c). Fecundity presented seasonal variability, with brood size increasing with total length, and a significant positive linear relationship was observed in both species, explaining about 45% of total brood size variability (Fig. 2a, Table 1). Similarly, a significant regression of brood size against carapace length of *E. lamelligera* and *E. distinguenda* was found (Fig. 2e, Table 1).

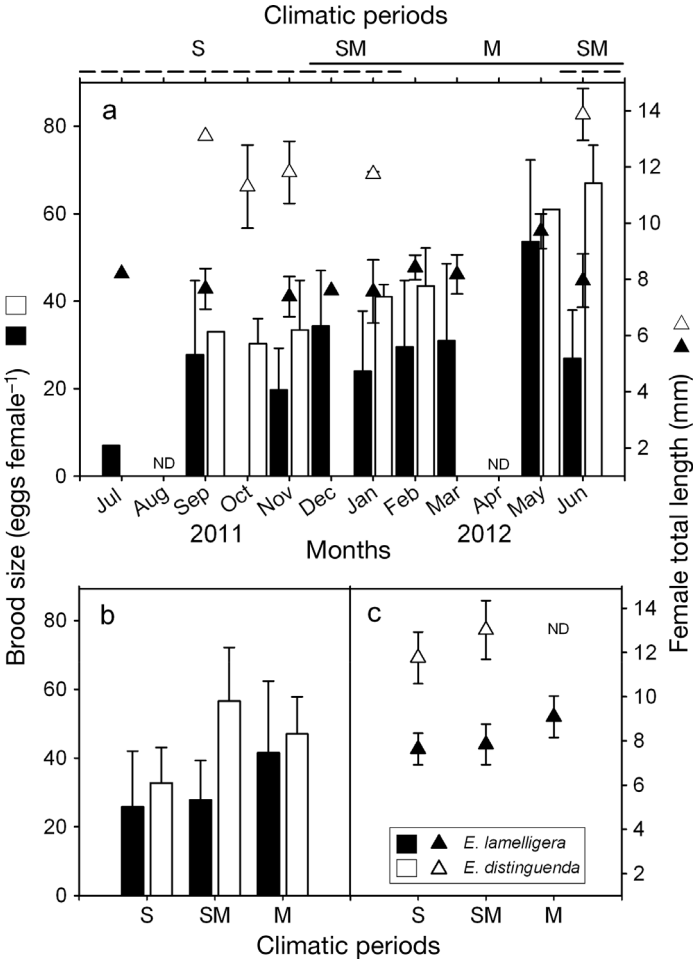

Fig. 1. Brood size (bars) and female total length (triangles) of *Euphausia lamelligera* (N = 115) and *E. distinguenda* (N = 58), showing (a) monthly mean and standard deviation (SD) of brood size spawned under laboratory conditions (July 2011 to June 2012), (b) brood size and (c) female total length per climatic period as defined in the coastal region off Cabo Corrientes (Ambriz-Arreola et al. 2012). SM = semi-mixed, M = mixed, S = stratified, ND = no data

Linear regression indicated that chorion diameter, embryo diameter, and PVS had a significant negative correlation with total length (Fig. 2b–d, Table 1) and carapace size (Fig. 2f–h, Table 1) of *E. distinguenda*. However, none of these 3 embryonic biometric correlations were significant for *E. lamelligera*.

Embryo morphology of *E. lamelligera* and *E. distinguenda*

The photographs of all embryonic development stages of *E. lamelligera* (Fig. 3) and *E. distinguenda* (Fig. 4) showed similar morphology for both krill species, and also similar to that observed in other broad-

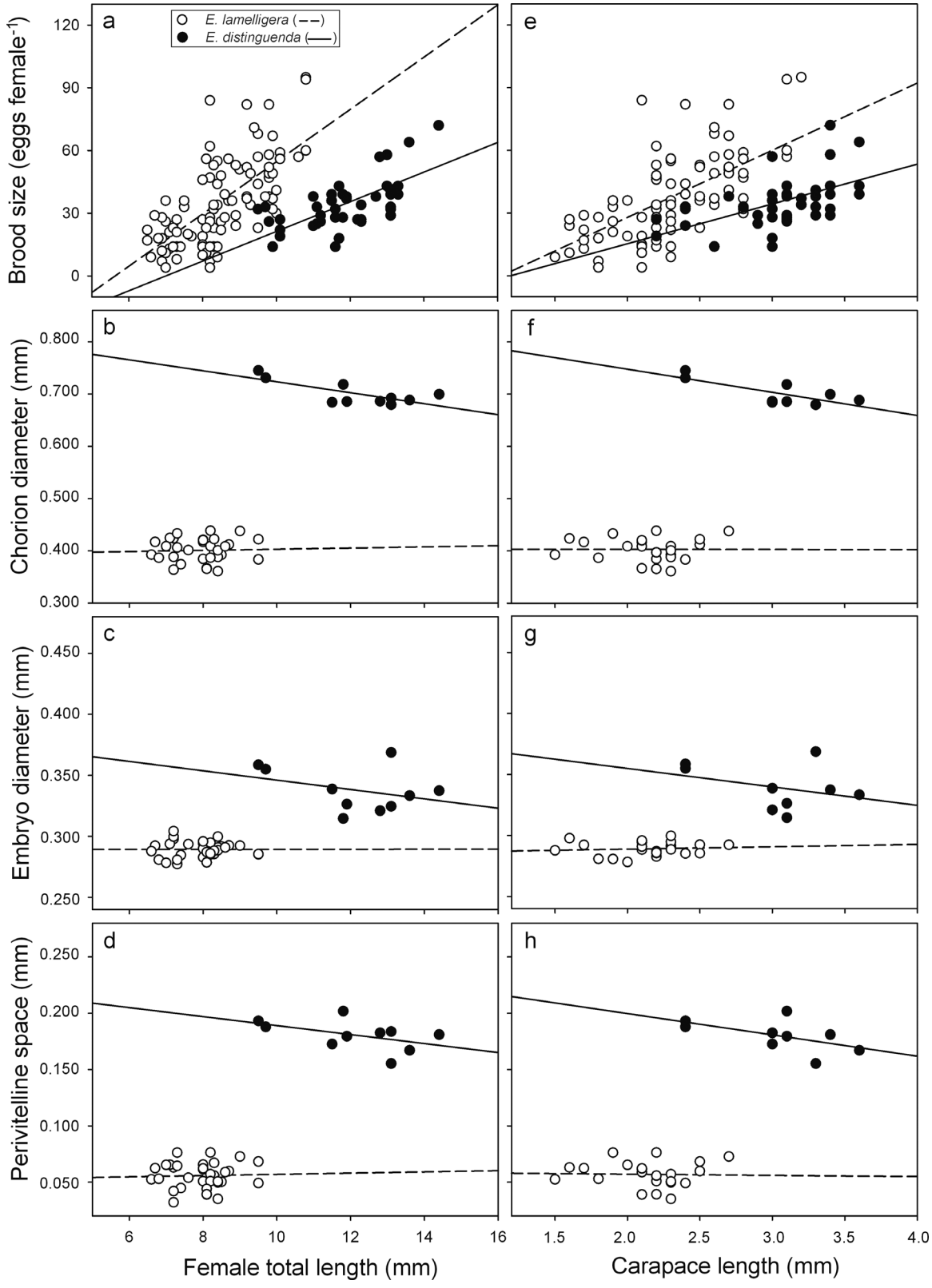

Fig. 2. (a,e) Brood size, (b,f) average chorion, (c,g) embryo diameters, and (d,h) perivitelline space as a function of female total length (a–d), and carapace length (e–h) of *Euphausia lamelligera* and *E. distinguenda* collected in the Cabo Corrientes region from July 2011 to June 2012. The equations of the linear regression model are given in Table 1

Table 1. Linear regression equations of the brood size (BS), chorion diameter (CD), embryo diameter (ED), and perivitelline space (PVS) as a function of total length (TL) and carapace length (CL) of the eggs of *Euphausia lamelligera* and *E. distinguenda* females spawned under laboratory conditions

Relationship	*Euphausia lamelligera*				*Euphausia distinguenda*			
	a	*b*	Adj r^2	p	*a*	*b*	Adj r^2	p
BS vs. TL	−70.345	12.498	0.464	<0.001	−49.626	7.093	0.434	<0.001
CD vs. TL	0.392	0.112	0.001	0.838	0.828	−0.011	0.497	0.014
ED vs. TL	0.289	<0.001	<0.001	0.988	0.384	−0.004	0.010	0.328
PVS vs. TL	0.514	<0.001	<0.001	0.843	0.229	−0.003	0.139	0.156
BS vs. CL	−36.297	32.112	0.423	<0.001	−22.706	19.028	0.247	<0.001
CD vs. CL	0.403	−0.001	<0.001	0.989	0.836	−0.044	0.522	0.017
ED vs. CL	0.285	0.002	<0.001	0.652	0.385	−0.015	<0.001	0.369
PVS vs. CL	0.059	−0.001	<0.001	0.898	0.238	−0.019	0.211	0.12

cast spawning species (Taube 1909, 1915, George & Strömberg 1985, Gómez-Gutiérrez 2002, Alwes & Scholtz 2004, Gómez-Gutiérrez et al. 2010a, Jia et al. 2014). Therefore, here we only briefly re-describe the stages following the nomenclature of George & Strömberg (1985) and Jia et al. (2014).

Single cell stage: recently spawned eggs still had the blue coloration of the oocytes from the mature gonad. The embryo developed quickly, taking a spherical shape and homogeneous opaque beige coloration, showing evenly distributed yolk granules. The cytoplasm was accumulated around the nucleus. Two exceedingly thin membranes surrounded the spherical, fertilized, and uncleft zygote; the outer membrane was the fertilization jelly (sensu Jia et al. 2014, also known as jelly coat; Tarling et al. 2009) and the inner membrane was the chorion (also known as embryo membrane; Jia et al. 2014). A third membrane, termed the vitelline membrane, surrounded the dividing zygote (Figs. 3a & 4a). Both species had a considerably large and transparent PVS that immediately expanded after spawning, and that remained throughout the development to hatching.

Multiple cell stage: the fates of the various cells and cell groups were followed for only the first 5 cleavages because all the early cleavages were holoblastic, leading to easily definable 2, 4, 8, 16, and 32-cell stages. The first cleavage was meridional (with a slight furrow) and resulted in a 2-cell stage with apparently equally sized cells (Figs. 3b & 4b). The second cleavage was also meridional, but slightly unequal leading to a 4-cell stage with unequally sized cells. The third cleavage (8-cell stage) was equatorial and unequal resulting in 4 smaller and 4 larger blastomeres. At the fourth cleavage an asynchrony began, with the 4 smaller cells at the animal pole cleaving a little faster than the 4 cells in the vegetative half (16-cell stage). The fifth cleavage leading to the 32-cell stage showed increased asynchrony (Fig. 3d). After the 32-cell stage it was difficult to follow the precise pattern of cleavage in living embryos of both species.

Blastula stage: there was a delay in cleavage in 1 or 2 cells at the vegetative pole. The cleavages continued and a clear blastula stage developed with larger cells at the vegetative pole. On the basis of the general cell size, this stage can be divided into an early blastula with big cells (Fig. 3e) and a late blastula with smaller cells (Figs. 3f & 5c).

Gastrulation: over the subsequent cleavages, gastrulation took place (Figs. 3g–i & 4d,e). During the early phase of gastrulation (Figs. 3g–h & 4d) a few large cells were observed migrating into the embryo as well as a blastopore that was only occasionally visible, presumably because of its short duration. During this stage, the mesendoderm was evident. In contrast, the late gastrula stage was characterized by a large number of bigger ectodermal cells, but still with an undifferentiated interior with relatively few cells (Figs. 3i & 4e).

Early limb-bud stage: the embryos of both species overall still had a spherical shape. In the early limb-bud stage the limb primordia were seen; in lateral view they appeared as incipient ridges or, in horizontal view, outlined with lateral invaginations between the 3 pairs of limbs (Figs. 3j & 4f). In this stage, the presence of a vitelline membrane surrounding the embryo was still evident. In live embryos, the posterior region of the body showed a reddish coloration (always observed in the same position independent of the nauplius orientation, discarding a possible light artifact of the stereoscope).

Late limb-bud stage: the distal ends of the limbs became free, tubelike structures developing setae on the tip of antenna 1 (A_1), antenna 2 (A_2), and the biramous mandible (Figs. 3k & 4g). The posterior quarter of the body still showed a reddish coloration. At

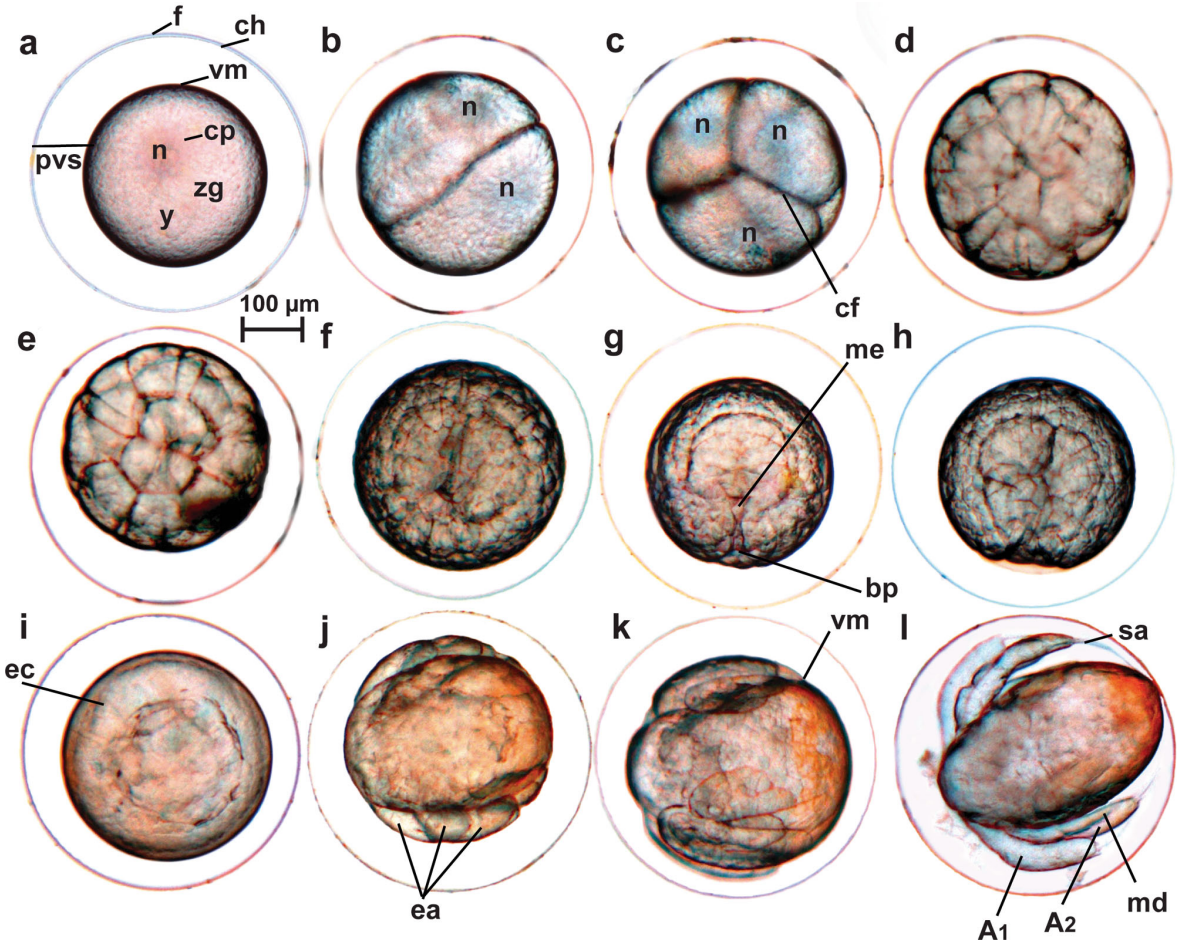

Fig. 3. Embryonic development stages of *Euphausia lamelligera*: (a) single cell, (b) 2-cells, (c) 4-cells, (d) 32-cells, (e) early blastula, (f) late blastula, (g) early gastrula, (h) late gastrula, (i) post gastrula, (j) early limb-bud, (k) late limb-bud, and (l) twitching stage. Embryo morphology: f = fertilization jelly, ch = chorion (also known as embryo membrane), pvs = perivitelline space, vm = vitelline membrane, n = nucleus, cp = cytoplasm, zg = zygote, y = yolk, cf = cross-furrows, me = mesendoderm, bp = blastopore, ec = ectodermal cells, ea = early appendages (buds), sa = setae, A_1 = antenna 1, A_2 = antenna 2, md = mandible

the late limb-bud stage the mouth was discernible as an invagination, which represented the beginning of the formation of a stomodeum. *E. lamelligera* embryos were still spherical in shape, but *E. distinguenda* late limb-bud stage embryos were thinner and oval shaped.

Twitching stage: the last stage before hatching; no vitelline membrane was evident, the muscles of the limbs were differentiated, and the nervous system was already functional (Figs. 3l & 4h). The posterior half of the body had a reddish coloration, as seen in previous stages. This stage could be recognized by the pulsating heart, as well as the limbs separating from the body prior to the hatching process. Lastly, both species showed an oval shaped nauplius inside the chorion.

Embryo biometry of *E. lamelligera* and *E. distinguenda*

We report the biometry of each embryonic and larval development stages because they have relevant taxonomic characteristics for species identifications of specimens collected in the field (Table 2). A Mann-Whitney *U*-test showed interspecific significant differences in morphological biometry of embryos and chorion diameters, and PVS measurements between these 2 krill species throughout the entire embryonic ontogeny ($p < 0.0001$, Table 3). *E. distinguenda* embryos had a significantly larger average diameter (0.700 ± 0.063 mm), embryo size (0.329 ± 0.027 mm), and PVS (0.185 ± 0.031 mm) than *E. lamelligera* diameter (0.405 ± 0.026 mm),

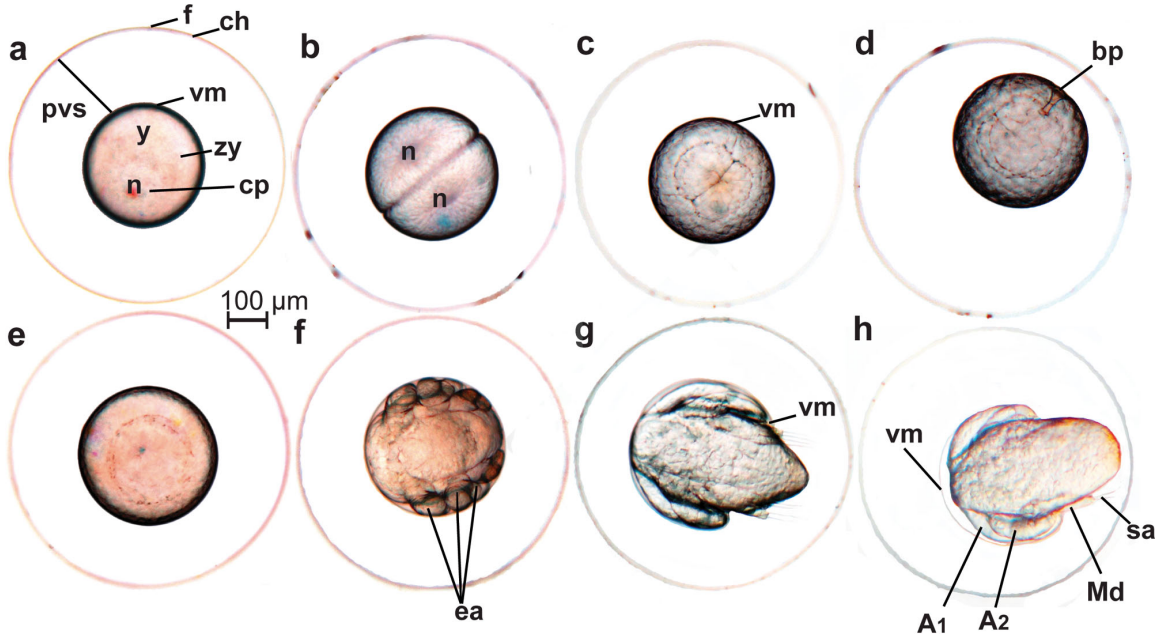

Fig. 4. Embryonic development stages of *Euphausia distinguenda*: (a) single cell, (b) 2-cells, (c) blastula, (d) gastrula, (e) post gastrula, (f) early limb-bud, (g) late limb-bud, and (h) twitching stage; see Fig. 3 for embryo morphology abbreviations

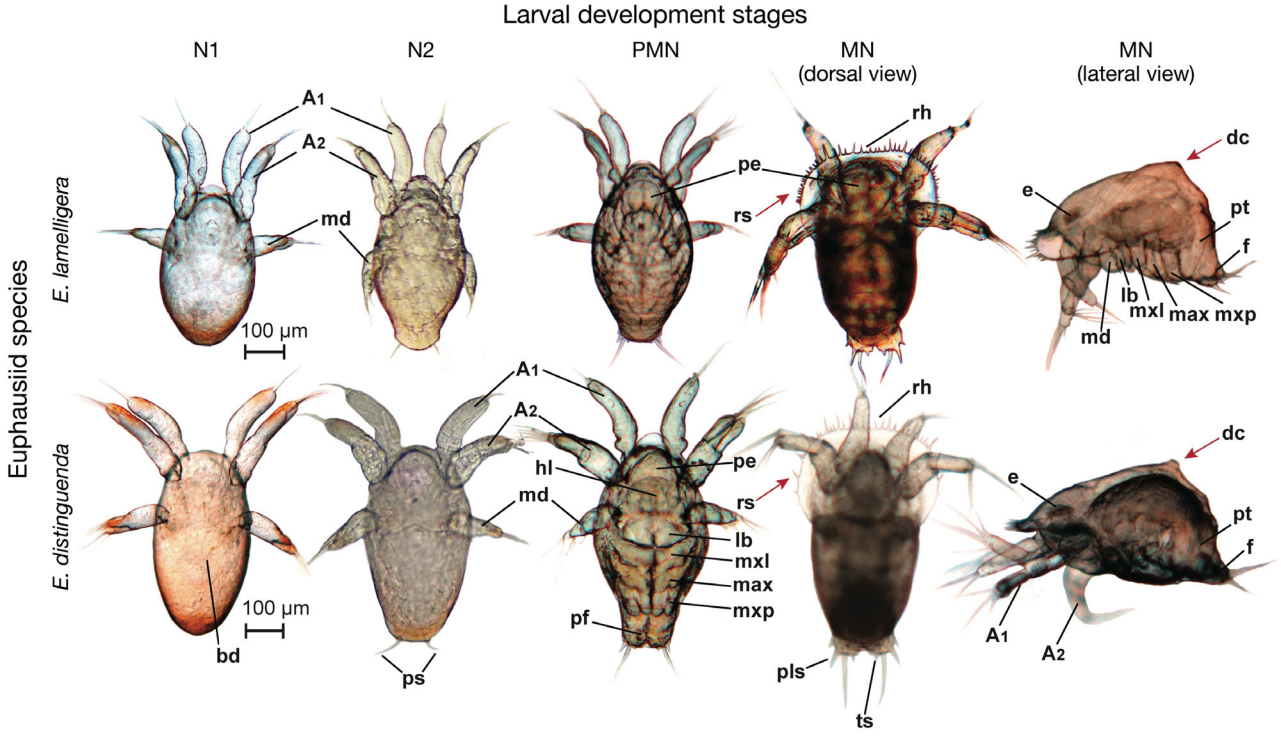

Fig. 5. *Euphausia lamelligera* and *E. distinguenda* early larval development stages: N1 = nauplius 1, N2 = nauplius 2, PMN = pseudometanauplius, MN = metanauplius. Early larval morphology: bd = body, A_1 = antenna 1, A_2 = antenna 2, md = mandible, hl = head lobe, pe = primordial eye, rs = rostral spines, lb = labrum, max = maxilla, mxl = maxillule, mxp = maxilliped, pf = primordial furca, ps = posterior spines, pls = posterolateral spines, ts = terminal spines, rh = rostral hood, e = eye, dc = dorsal crest, pt = primordial trunk, f = furca

embryo size (0.291 ± 0.011 mm), and PVS (0.057 ± 0.013 mm) in all embryonic stages ($p < 0.0001$, Table 3). Additionally, *E. lamelligera* spawned soft, sticky eggs (frequently observed with particles attached, but not attached to each other), with smaller chorion, embryo diameter and PVS than the larger *E. distinguenda* eggs (hard chorion, non-sticky). These morphological and biometric features of the eggs were useful for ootaxanomic purposes to distinguish the eggs of both species.

Table 2. Biometry of the chorion and embryo diameter, and perivitelline space of the eggs of *Euphausia lamelligera* and *E. distinguenda* females spawned under laboratory conditions and reared through early embryonic stages. SC = single cell, MC = multiple cells, eLB = early limb-bud, lLB = late limb-bud, TW = twitching stage, n = number of measured eggs

Embryo development stage	n	Chorion diameter (mm) Average	Range	SD	Embryo diameter (mm) Average	Range	SD	Perivitelline space (mm) Average	Range	SD
E. lamelligera										
SC	47	0.409	0.345–0.515	0.038	0.297	0.274–0.328	0.015	0.056	0.034–0.096	0.014
MC	68	0.411	0.355–0.447	0.020	0.293	0.277–0.310	0.007	0.059	0.034–0.076	0.009
Blastula	205	0.398	0.335–0.453	0.027	0.291	0.264–0.313	0.008	0.053	0.025–0.085	0.012
Gastrula	215	0.397	0.283–0.440	0.022	0.289	0.258–0.355	0.010	0.054	0.021–0-078	0.012
eLB	120	0.413	0.339–0.467	0.024	0.290	0.268–0.320	0.010	0.061	0.024–0.083	0.012
lLB	228	0.415	0.316–0.494	0.027	0.290	0.257–0316	0.010	0.062	0.026–0.090	0.013
TW	84	0.399	0.339–0.440	0.020	0.298	0.249–0.338	0.015	0.051	0.024–0.068	0.009
All	967	0.405	0.283–0.515	0.026	0.291	0.249–0.355	0.011	0.057	0.021–0.096	0.013
E. distinguenda										
SC	82	0.706	0.513–1.002	0.065	0.318	0.237–0.337	0.013	0.194	0.095–0.342	0.030
MC	49	0.727	0.581–0.787	0.046	0.320	0.235–0.338	0.018	0.203	0.162–0.233	0.018
Blastula	36	0.723	0.602–0.769	0.032	0.323	0.291–0.321	0.009	0.200	0.155–0.222	0.015
Gastrula	40	0.680	0.534–0.885	0.056	0.311	0.228–0.366	0.028	0.184	0.136–0.267	0.024
eLB	5	0.639	0.613–0.653	0.017	0.328	0.317–0.332	0.006	0.156	0.142–0.161	0.008
lLB	138	0.691	0.502–0.992	0.064	0.337	0.232–0.402	0.022	0.177	0.070–0.310	0.028
TW	38	0.692	0.588–1.015	0.082	0.364	0.302–0.494	0.040	0.164	0.082–0.322	0.044
All	338	0.700	0.502–1.015	0.063	0.329	0.228–0.494	0.027	0.185	0.070–0.342	0.031

Table 3. Mann-Whitney comparison of the interspecific biometry of embryos and early larval stages of *Euphausia lamelligera* and *E. distinguenda* incubated under laboratory conditions and reared through early embryonic and larval stages. SC = single cell, MC = multiple cells, eLB = early limb-bud, lLB = late limb-bud, TW = twitching stage, N = nauplius, PMN = pseudometa-nauplius, MN = metanauplius

Development stage	Chorion diameter *Z*-value	p-value	Embryo diameter *Z*-value	p-value	Perivitelline space *Z*-value	p-value	No. of specimens measured *E. lamelligera*	*E. distinguenda*
Embryos								
SC	−9.426	<0.0001	−6.338	<0.0001	−9.425	<0.0001	47	82
MC	−9.204	<0.0001	−8.425	<0.0001	−9.205	<0.0001	68	49
Blastula	−9.565	<0.0001	−9.246	<0.0001	−9.564	<0.0001	205	36
Gastrula	−10.039	<0.0001	−5.601	<0.0001	−10.038	<0.0001	215	40
eLB	−3.780	0.0001	−3.742	0.0001	−3.779	0.0001	120	5
lLB	−16.037	<0.0001	−15.327	<0.0001	−15.956	<0.0001	228	138
TW	−8.823	<0.0001	−8.503	<0.0001	−8.824	<0.0001	84	38
	Total length *Z*-value	p-value	Width *Z*-value	p-value	Total length/width *Z*-value	p-value	No. of specimens measured *E. lamelligera*	*E. distinguenda*
Early larvae								
N1	−12.661	<0.0001	−0.836	−0.4032	−7.111	<0.0001	223	75
N2	−11.858	<0.0001	–	–	–	–	135	159
PMN	−8.814	<0.0001	−6.814	<0.0001	−0.678	<0.0001	35	154
MN	−11.691	<0.0001	−5.790	<0.0001	−0.615	0.539	196	66

Description of early larval stages of *E. lamelligera* and *E. distinguenda*

The most notable discovery of the present study was the observation of an intermediate larval stage between the nauplius 2 (N2) and metanauplius (MN) stages, here interpreted as a pseudometanauplius (PMN) stage. This is a relevant life cycle observation for these 2 broadcasting species because until now, PMN has been thought to be an exclusive larvae stage for krill species with a sac-spawning reproductive strategy. There is strong evidence that this is a distinct larval stage because the PMN-to-MN molting process was directly observed, and it had significant morphological differences that distinguished it from the N2 and MN stages (see Fig. S2 in the Supplement at www.int-res.com/articles/suppl/s001p143_supp.pdf). Statistical comparison of the interspecific morphological and biometric features of early larval stages (nauplius 1 [N1], N2, PMN, and MN) showed significant stage-dependent differences between both species ($p < 0.0001$). *E. lamelligera* were smaller and slightly thinner than *E. distinguenda* in all early larval stages (Fig. 5, Tables 3 & 4), except the N1 stage which did not show significant differences in width between both krill species ($p > 0.05$). The most prominent morphological characters distinguishing the early larval stages of both species are described below.

Nauplius 1. *E. lamelligera* N1 had an oval body shape in dorsal view, with an average total length of 0.352 mm and width of 0.241 mm (Table 4), with 3 pairs of appendages: A_1 unsegmented, with 2 terminal long seta and 1 small terminal spine; A_2, endopod with 2 setae, exopod with 4 setae and 1 terminal small seta; mandible biramous, unsegmented, endopod and exopod each with 3 setae (Figs. 5 & 6). *E. distinguenda* N1 was also ovoid in shape, with average total length of 0.422 mm and average width of 0.243 mm (Table 4). N1 had 3 pairs of appendages: A_1 unseg-

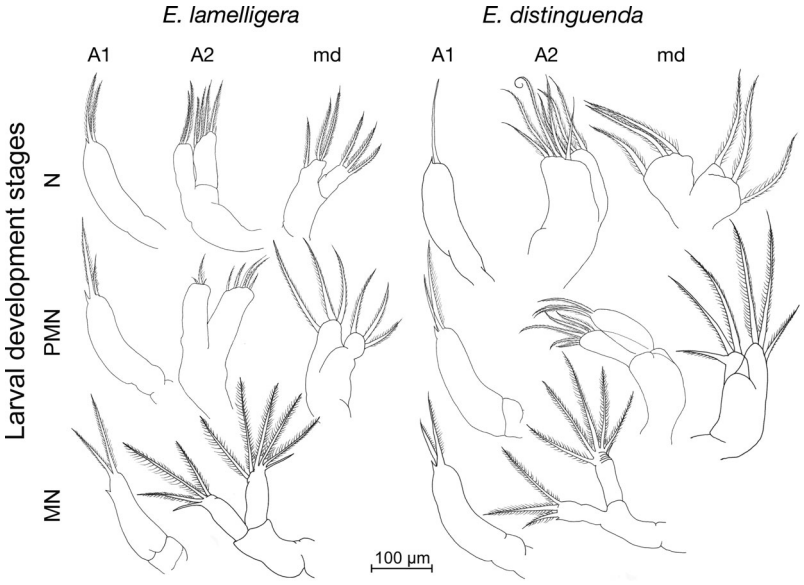

Fig. 6. Swimming appendages of early larval development stages: (N) nauplius, (PMN) pseudometanauplius, and (MN) metanauplius of the tropical krill *Euphausia lamelligera* and *E. distinguenda*. A_1 = antenna 1, A_2 = antenna 2, md = mandible

Table 4. Biometry of body length, width and length/width ratio of the early larval development stages of *Euphausia lamelligera* and *E. distinguenda* reared under laboratory conditions. N = nauplius, PMN = pseudometanauplius, MN = metanauplius, n = number of measured larvae

Larval development stage	n	Length (mm)			Width (mm)			Length/Width (mm)		
		Average	Range	SD	Average	Range	SD	Average	Range	SD
E. lamelligera										
N1	223	0.352	0.296–0.409	0.021	0.241	0.207–0.301	0.014	1.464	1.011–1.729	0.110
N2	135	0.403	0.343–0.447	0.020	0.254	0.228–0.276	0.009	1.589	1.351–1.725	0.085
PMN	35	0.401	0.376–0.428	0.012	0.237	0.225–0.251	0.006	1.691	1.490–1.854	0.064
MN	196	0.439	0.380–0.497	0.020	0.239	0.200–0.291	0.020	1.830	1.418–2.242	0.149
E. distinguenda										
N1	75	0.422	0.363–0.467	0.020	0.243	0.229–0.259	0.008	1.788	1.679–1.990	0.078
N2	159	0.451	0.365–0.542	0.031	–	–	–	–	–	–
PMN	154	0.469	0.378–0.520	0.032	0.261	0.228–0.296	0.017	1.831	1.640–2.051	0.010
MN	66	0.499	0.476–0.566	0.022	0.266	0.239–0.290	0.015	1.861	1.686–2.083	0.112

mented, with 1 terminal long seta and 1 small terminal spine; A_2, endopod with 5 setae, exopod with 3 setae; mandible biramous, unsegmented, endopod and exopod each with 3 setae (Figs. 5 & 6). Overall, *E. distinguenda* had a longer body length N1 than *E. lamelligera*. Both species were distinguishable in the N1 stage because *E. lamelligera* had 2 setae in the antenna and *E. distinguenda* had only one; and because *E. lamelligera* had a total length:width ratio of 1.46 versus 1.79 for *E. distinguenda*.

Nauplius 2. *E. lamelligera* N2 had a slightly longer body length in comparison with N1, armed with 1 pair of spines located at the posterior margin of the body. Average total length was 0.401 mm and width was 0.254 mm (Table 4), with 3 pairs of appendages (Fig. 5).

E. distinguenda had an ovoid shape and was longer than N1, with an average total length of 0.451 mm, but similar in width (Table 4). It presented 3 pairs of appendages (Fig. 5). As in N1, both species were distinguished in N2 by *E. lamelligera* having 2 setae in the antenna whereas *E. distinguenda* had only one.

Pseudometanauplius. *E. lamelligera* PMN had an elongated oval shape in dorsal view, partially due to the formation of a primordial furca, approximately 2 times longer than its width (Table 4). The posterior margin was armed with 4 pairs of spines: one pair of small posterolateral (outer) spines, one pair of relatively large third spines, and 2 pairs of small rudimentary medial terminal spines. Three pairs of appendages were present: A_1 and mandible as in N1 and N2, and A_2; the endopod of A_2 was armed with 3 setae and the exopod with 5 setae (Figs. 5 & 6).

E. distinguenda PMN had a pear shaped body, with an oval-shaped head region and elongated posterior extension of the body with 2 posterolateral pairs of spines and one pair of small to rudimentary medial terminal spines. Average total length was 0.469 mm and width was 0.261 mm (Table 4). PMN had 3 pairs of appendages: A_1 was unsegmented, with 2 terminal long setas and 1 small terminal spine, and A_2 and mandible morphology were similar to N1 and N2 (Figs. 5 & 6). *E. distinguenda* had considerably larger PMN than *E. lamelligera* but no difference in the number of setae in their appendages.

In the PMN stage, both species were already developing a primordial eye, a prominent head lobe, and rudimentary labrum, maxillule, maxilla, and biramous maxilliped that distinguished them from the N2 stage. They lacked the rostral carapace hood fringed with spines typical of the MN stage (Fig. 6).

Metanauplius. *E. lamelligera* MN had an average total length of 0.439 mm and width of 0.239 mm (Table 4). A_1 was unsegmented, with 2 long terminal setae and 1 small subterminal spine. A_2 had an endopod with 4 setae; an exopod with 5 long setae and one terminal seta, and reduced mandible (Figs. 5 & 6). The rostral hood of the carapace was fringed with small marginal rostral spines with larger rostral spines interspersed on the frontal margin and one small pointed dorsal crest without spines. The telson had 2 small pairs of posterolateral spines and 2 pairs of telson spines; the outer pair of telson spines were considerably longer and articulated with the telson (Fig. 5).

E. distinguenda MN had an average total length of 0.499 mm and width of 0.266 mm (Table 4). A_1 was similar to PMN stage; A_2 with endopod bearing 3 setae and 1 subterminal seta; the exopod had 5 long setae and one terminal seta, and the mandible was reduced (Fig. 6). The rostral carapace hood was fringed with strong spines interspersed with smaller spines, and had a very distinctive dorsal crest without spines. The telson was short, as described in *E. lamelligera* (Fig. 6).

The MN stage of both species showed prominent development of a pigmented eye, and the labrum, maxilla, maxillule, and a biramous maxilliped were longer than in the PMN stage. Inside the MN hump a primordial trunk was developing, and in the posterior tip a furcal development was already present (Fig. 5).

E. distinguenda MN was larger, wider and had a higher length/width ratio than *E. lamelligera*. They also had a distinct number of setae in the antenna, and different spinal arrangements on the carapace, which were morphological features of taxonomic value: *E. lamelligera* had a carapace fringed with small marginal spines with larger spines interspersed on the frontal margin; in contrast, *E. distinguenda* had a carapace fringed with prominent spines spaced around the margin of the frontal hood and with smaller spines interspersed. Finally, they had distinct dorsal keels; *E. lamelligera* had a small pointed crest while *E. distinguenda* had a prominent dorsal keel.

Development times of *E. lamelligera* and *E. distinguenda*

These 2 small tropical krill species presented a rapid embryonic development with an average hatching time of 12 h (25°C) after spawning (range 9 to 14 h at 29 to 22°C) (Table 5). The duration of the development time among embryonic and early larval stages was similar in both species. For example, both species had similar average development times to reach the blastula (~2 h), eLB (5 h), and TW stages (11 h)

Table 5. Mean development time (at 25°C) and range (at 22 to 29°C) of the embryos and early larval stages after spawning of *Euphausia lamelligera* and *E. distinguenda* incubated under laboratory conditions. SC = single cell, MC = multiple cells, eLB = early limb-bud, lLB = late limb-bud, TW = twitching stages; N = nauplius, PMN = pseudometanauplius, MN = metanauplius. N = total number of gravid females, n = number of specimens measured

Development stage	*Euphausia lamelligera* (N = 110)				*Euphausia distinguenda* (N = 58)			
	n	Average time (h)	SD	Range	n	Average time (h)	SD	Range
Embryos								
SC	4	0.00	–	–	5	0.00	–	–
MC	8	0.45	0.33	0.30–1.44	3	1.45	0.05	0.39–1.49
Blastula	13	1.77	0.45	1.21–2.30	3	2.23	0.42	1.47–2.44
Gastrula	19	2.91	1.10	1.32–5.51	5	4.02	0.64	2.27–3.12
Early larvae								
eLB	13	5.32	0.94	3.33–6.21	2	5.26	0.43	5.32–6.10
lLB	15	8.84	1.22	5.53–9.40	9	8.13	0.91	7.32–10.34
TW	8	10.85	0.53	9.07–11.02	5	10.22	1.02	9.45–12.47
N1	15	11.93	1.63	9.37–14.26	7	12.29	0.70	10.10–13.18
N2	8	16.60	4.31	14.38–25.41	9	18.09	3.39	16.08–27.31
PMN	2	20.14	1.13	19.53–27.19	6	26.68	2.70	23.05–29.11
MN	5	29.30	2.32	22.40–32.48	4	29.51	1.69	28.07–34.28

after spawning; and ~12, 18, 24, and 30 h for N1, N2, PMN, and MN, respectively (Table 5). In both species the diameter of the embryo increased throughout embryonic development until the twitching stage (Figs. 3a–l & 4a–h, Table 2), when the embryo typically hatched. N1 was observed hatching exclusively using the backward hatching mechanism (Gómez-Gutiérrez 2002), with hatching process duration shorter for *E. lamelligera* (<2 min) than for *E. distinguenda* (<30 min). Hatching success was consistently high throughout the year for both tropical species: eggs spawned by 12 female *E. lamelligera* had an average hatching success of 90% (range: 77 to 100%), whereas eggs spawned from 25 *E. distinguenda* females had an average of 91%, ranging from 66 to 100%.

DISCUSSION

To the best of our knowledge, this is the first study to report brood size and give a complete description and biometry of embryonic and early larval nauplius-to-metanauplius development stages, ontogenetic stage duration, and hatching success of any broadcast tropical krill species in the Order Euphausiacea. We successfully incubated two of the smallest broadcast spawning krill species in the world (*Euphausia lamelligera*, <11 mm and *E. distinguenda*, <14.5 mm total length) and explored patterns in the seasonal variability of their brood sizes, concluding that both species reproduce throughout the year although with distinct seasonal peaks in mean brood sizes.

Reproductive seasonal pattern and embryo biometry of tropical broadcast spawning krill species

Ambriz-Arreola et al. (2012) suggested that *E. lamelligera* and *E. distinguenda* reproduce year-round because calyptopis larval abundances numerically dominated the krill populations off the Cabo Corrientes region based on monthly sampling during 1996 to 1998. The continuous presence of gravid females and brood size observations of both tropical species in this northern region of the ETP confirm the continuous reproduction strategy of both species, although with distinct interspecific maximum reproduction rates among climatic periods (Fig. 1a). We detected the maximum brood size of *E. lamelligera* during the mixed period (February to May), and of *E. distinguenda* during the semi-mixed periods (June and December/January), both having a statistically significant linear association of brood size with female total length and carapace length (Figs. 1a–c & 2a,e), as has been commonly reported for subtropical sac-spawning species (Wilson et al. 2003, Gómez-Gutiérrez & Robinson 2005, Gómez-Gutiérrez et al. 2010b, 2012). Interestingly, in polar (*E. superba*) and temperate (*E. pacifica* and *Thysanoessa spinifera*) broadcast spawning krill species, a typical dome shape association of brood size versus total length was recorded (Nicol et al. 1995, Gómez-Gutiérrez et al. 2006, Feinberg et al. 2007, 2013), with older females decreasing brood size after reaching their maximum reproductive activity, even with a larger body size (and carapace volume). In these relatively large krill species with lifespans ranging from 2 to

7 yr, oogenesis should metabolically decrease with female age. Because *E. distinguenda* and *E. lamelligera* are among the smallest broadcast spawning krill species in the world, we presume they have shorter life span (likely <1 yr) than subtropical, temperate, and polar species, due to the relatively high temperatures that prevail in the ecosystem they inhabit. As a result of this shorter life span, tropical krill do not appear to reach a body size and age where females decrease their reproductive effort. However, brood size is not only influenced by female size; food availability seems to affect spawning patterns as well, which is related to the occurrence of intense upwelling events that promote relatively high chlorophyll *a* (chl *a*) concentrations and primary productivity during the mixed and semi-mixed climatic periods at Cabo Corrientes (Cepeda-Morales et al. 2009, López-Sandoval et al. 2009a,b, Ambriz-Arreola et al. 2012). Both species spawned smaller brood sizes during the stratified season. In Cabo Corrientes, Ambriz-Arreola et al. (2012) demonstrated that healthy *E. lamelligera* and *E. distinguenda* had a high hepatosomatic index (i.e. the proportion of the size of the hepatopancreas and the cephalothorax; this proxy helps to infer body conditions and recent trophic conditions) during the most productive mixed and semi-mixed periods. High chl *a* concentrations would provide energy to produce eggs with higher lipid content for embryonic development, and relatively high temperatures would increase growth and development rates of early larval stages compared with those species inhabiting higher latitudes. This evidence confirms that although both species can spawn in any season of the year, their maximum brood sizes overall occurred during periods of high chl *a* concentrations (mixed and semi-mixed periods), suggesting that seasonality plays a primary role in the magnitude of the female reproductive life history (size, brood size, embryonic and larval development) of *E. lamelligera* and *E. distinguenda* in the northern region of the ETP.

Even though both species have among the smallest mean brood sizes currently known for broadcast species of the Order Euphausiacea, continuous reproduction, fast ontogenetic development, and a presumed lifespan of <1 yr (as seen in the subtropical species *Nyctiphanes simplex*; Lavaniegos 1992) compensate for their relatively low brood sizes per spawning event. The interbrood period of these tropical species is still unknown, but it is probably shorter than in temperate broadcast spawning species. In *E. lucens* from the Benguela Current System and *E. pacifica* from the California Current System, spawning occurs every 3 to 7 d for several months (Stuart 1992, Feinberg et al. 2007), and as it is well known that high temperatures increase metabolic rates, we can reasonably assume that the mean interbrood periods of both tropical species is 3 d (range 2 to 4 d). Precise estimations of egg production rates require further direct observations of interbrood periods and the number of consecutive spawns a female can produce in its lifespan in order to estimate total fecundity. The mean brood size of *E. lamelligera* (33 eggs female^{-1}, range 3 to 96, n = 115) and *E. distinguenda* (36 eggs female^{-1}, range 15 to 72, n = 58) can be considered relatively low in comparison with krill species of higher latitudes (Nicol et al. 1995, Feinberg et al. 2007, Gómez-Gutiérrez et al. 2007).

An interspecific comparison of mean chorion and embryo diameter as a function of female total length showed that *E. lamelligera* and *E. distinguenda* are among the smallest gravid females in the world (Fig. 7), having some of the smallest eggs and mean brood sizes compared to the other 24 broadcast spawning species (Brinton et al. 2000, Gómez-Gutiérrez et al. 2010a). The chorion and embryo diameters measured for *E. lamelligera* (0.405 and 0.291 mm, respectively) were a size near the predictive linear regression model (see Fig. 7). In contrast, *E. distinguenda* (chorion diameter = 0.700, embryo diameter = 0.329 mm) spawned eggs with a chorion diameter in proportion to its total length, well above the average predicted for the rest of the broadcast spawning species (Fig. 7a). The ecological or adaptive value of having a very large PVS is unknown. However, Timofeev et al. (2004) pointed out that a large PVS is a protective feature for embryos developing under unfavorable environmental conditions. Because our krill collections came from a relatively small coastal region within the distribution range of both krill species, the PVS could vary more spatiotemporally than that which was observed in the present study, considering that these species seasonally invade northern latitudes along the west coast of the Baja California peninsula and Gulf of California (Brinton & Townsend 1980, Lavaniegos et al. 1989).

Description of embryonic and early larval stages of tropical krill species

The taxonomic description of the metanauplius, calyptopis, and larval furcilia stages of *E. lamelligera* and *E. distinguenda* was previously reported from specimens directly collected in the field (Brinton et al. 2000). We confirmed that the morphological descrip-

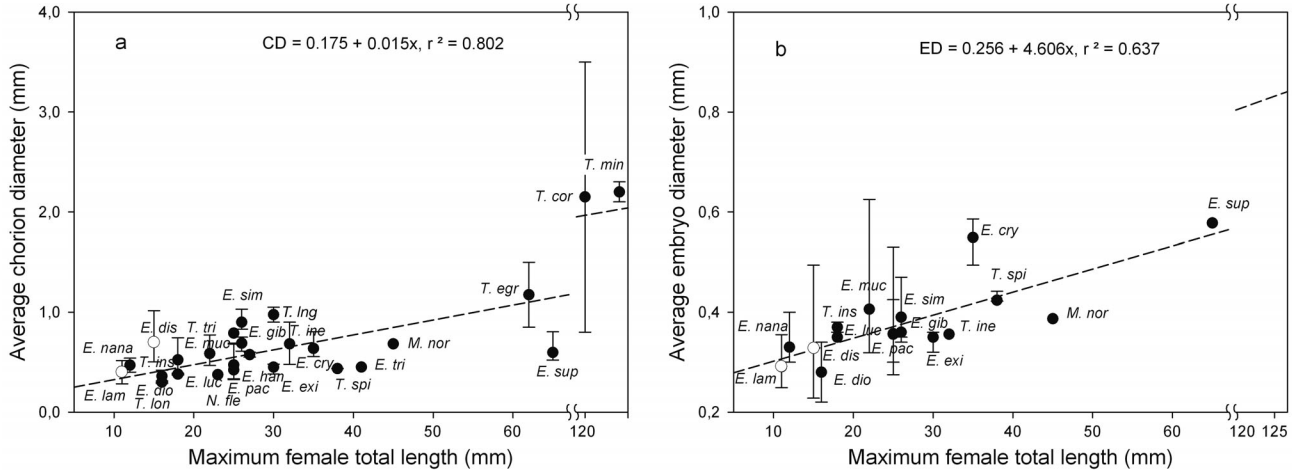

Fig. 7. Interspecific comparison of average female length (Baker et al. 1990) and average (a) chorion and (b) embryo diameter of *Euphausia lamelligera* and *E. distinguenda* (open circles) and 24 other broadcast spawning species (filled circles). Bars = min. and max. values recorded based on Mauchline (1988), Brinton et al. (2000), and Gómez-Gutiérrez et al. (2010a). Dashed line = adjusted linear regression with equation shown. *E. cry* = *Euphausia crystallorophias*, *E. dio* = *E. diomideae*, *E. dis* = *E. distinguenda*, *E. exi* = *E. eximia*, *E. gib* = *E. gibboides*, *E. han* = *E. hanseni*, *E. luc* = *E. lucens*, *E. lam* = *E. lamelligera*, *E. muc* = *E. mucronata*, *E. nana* = *E. nana*, *E. sim* = *E. similis*, *E. sup* = *E. superba*, *E. tri* = *E. tricantha*, *M. nor* = *Meganyctiphanes norvegica*, *N. fle* = *Nematobrachion flexipes*, *T. ine* = *Thysanoessa inermis*, *T. ins* = *Thysanoessa inspinata*, *T. lon* = *Thysanoessa longicaudata*, *T. lng* = *Thysanoessa longipes*, *T. ras* = *Thysanoessa raschi*, *T. spi* = *Thysanoessa spinifera*, *T. cor* = *Thysanopoda cornuta*, *T. egr* = *Thysanopoda egregia*, *T. min* = *Thysanopoda minyops*, *T. tri* = *Thysanopoda tricuspidata*

tions of the MN stage are correct based on evidence from gravid females that spawned under laboratory conditions. Additionally, we provided morphological data on the previously unknown embryonic and early larval development (N1, N2, and PMN) stages. The embryology of both tropical species exhibited the same ontogeny as previously detailed morphological descriptions and cell maps known for several species with broadcast and sac-spawning reproductive strategies (Sars 1898, Taube 1909, 1915, George 1984, Gómez-Gutiérrez 2002, 2003, 2006, Alwes & Scholtz 2004, Gómez-Gutiérrez et al. 2010a, Montuy-Gómez et al. 2012, Jia et al. 2014). However, *E. distinguenda* eggs had distinctive morphology, with the chorion diameter well above the theoretically expected size considering the female total length of this species (<14.5 mm), whereas the embryo diameter was near the predicted average (this means anomalously large PVS) (Gómez-Gutiérrez et al. 2010a, our Fig. 7). This morphological feature makes the identification of species from eggs collected directly from the water column in the northern ETP relatively easier.

A monthly time series (1996 to 1998) from the Mexican central Pacific (19° N, 105° W) reported that of the total abundance of 8 krill species (larval and postlarval phases), *E. distinguenda* contributed between 88 and 90% (oceanic affinity), and *E. lamelligera* contributed ~7% (neritic affinity) (Ambriz-Arreola et al. 2012). Thus, we expect that most of the eggs collected in this region should belong to these 2 numerically dominant species. The biometric measurements obtained in the present study will assist in the identification of *E. lamelligera* and *E. distinguenda* eggs obtained from field zooplankton collections in order to infer spatio-temporal distribution patterns — as in previous studies off the Oregon coast where spawning periods and cross-shelf distribution was defined for the 2 numerically dominant krill *E. pacifica* (oceanic affinity) and *T. spinifera* (neritic affinity) (Feinberg & Peterson 2003, Gómez Gutiérrez et al. 2005, 2010a), and in the lower St. Lawrence Estuary with the spatio-temporal spawning patterns of *Meganyctiphanes norvegica* (Plourde et al. 2011).

Our study area is inhabited by 13 other tropical krill species that have low relative abundance (<5%). From this 5%, the most frequently sampled include 3 broadcast spawning species (*E. eximia, E. diomedeae*, and *E. tenera*) and 3 sac-spawning krill species (*Nematoscelis gracilis, Stylocheiron affine*, and *S. carinatum*) (Brinton 1979, Gómez-Gutiérrez & Hernández-Trujillo 1994, Färber-Lorda et al. 2004, 2010, Ambriz-Arreola et al. 2012). The mean chorion and PVS measurements of eggs of *E. eximia* are known (but not for each development stage) and the nauplii were not drawn because they are similar to

those observed to *E. gibboides* (Knight 1980). The early larval stages (N1 and N2) of both *E. tenera* and *E. diomedeae* remain undescribed, and intraspecific morphology should be compared in a future study.

A previous study reported that broadcast spawning krill species *E. pacifica*, *T. spinifera*, and *T. inspinata* can potentially employ 3 different hatching mechanisms: backward, forward, and flipping (Gómez-Gutiérrez 2002). This author stated that the backward hatching of the N1 stage is the main hatching mechanism for these temperate species, and also demonstrated that it is strongly associated with higher hatching success in comparison to forward and flipping mechanisms. Our observations showed that *E. lamelligera* and *E. distinguenda* embryos hatched exclusively as N1 using the backward hatching mechanism. Delayed hatching is typically associated with low temperatures and lack of stimulus to break the chorion. This temperature dependent reproductive adaptation explains the high hatching success (average 90%) observed in both tropical species, and their consecutive high numerical dominance with respect to the other 13 krill species that inhabit the northern region of the ETP (Brinton 1979, Gómez-Gutiérrez & Hernández-Trujillo 1994, Färber-Lorda et al. 2004, 2010, Ambriz-Arreola et al. 2012).

The PMN larval development stage was previously considered exclusive to the life cycle of sac-spawning krill species (Brinton et al. 2000). We clearly demonstrate that the PMN stage occurs in the life cycle of both *E. lamelligera* and *E. distinguenda*, albeit of relatively short duration, with evidence of PMN–MN exuvia release (see Fig. S2 in the Supplement). The morphology is similar to the PMN known for other sac-spawning krill species. This observational evidence indicates that species of sac-spawning and occasionally broadcast reproductive strategies develop through a PMN stage, contrary to the conceptualization that this stage is exclusive to sac-spawning euphausiid species. Knight (1975, 1980) did not find a PMN in the tropical broadcast spawning species *E. eximia* or *E. gibboides* during rearing experiments.

With respect to the MN stage, an intraspecific comparison evidenced notable differences in morphology among *E. lamelligera* and *E. distinguenda* and those of congeners of the genus *Euphausia*. *E. eximia* and *E. diomedeae* MN have a high dorsal crest with short and long spines, respectively, and the *E. tenera* MN possess 2 pairs of spines on the anterior margin of the carapace (Knight 1980, Brinton et al. 2000). In contrast, the MN of *E. distinguenda* we described has a slightly more marked dorsal keel than previously illustrated in Brinton et al. (2000) (Fig. 5). The MN stage of *E. lamelligera* and *E. distinguenda* has dorsal keels without spines and distinct spinal arrangements on the carapace (Fig. 5). The unique morphological characteristics in the MN stage of *E. lamelligera* and *E. distinguenda* observed in our study help to distinguish them from other *Euphausia* species that inhabit the ETP.

Embryonic development rates of tropical krill species

Our measurements of embryonic and early larval development times confirmed the expectation that higher temperatures in tropical habitats promote faster metabolic rates, resulting in shorter development times. We argue that *E. lamelligera* and *E. distinguenda* are not only among the smallest krill in the world, with the lowest brood sizes for broadcast spawning species, but also the species with the fastest development rates recorded so far in the Order Euphausiacea. Comparing embryonic development, hatching time, and early larval development time from spawning to MN stage for other species (Fig. 8), it is clear that polar and temperate broadcast spawning species have embryos and larvae with development times several times longer than species from tropical ecosystems like *E. lamelligera* and *E. distinguenda*. Both tropical species reached the N1 stage (12 h after hatching; Table 5) 8 and 12 times faster than the subtropical sac-spawning species *N. simplex* (91 h after hatching; Gómez-Gutiérrez & Robinson 2005), and Antarctic krill *E. superba* (144 h after hatching; Quetin & Ross 1984). It is well known that embryonic and early larval development rates of Antarctic species *E. superba* (Ross et al. 1988, Yoshida et al. 2004), and temperate species *E. pacifica* and *Thysanoessa inermis* (Iguchi & Ikeda 1994, Gómez-Gutiérrez 2002, Pinchuk & Hopcroft 2006) are closely associated with temperature and food quality and quantity for females (that fuel oogenesis) and for larvae since exogenous feeding begins from calyptopis 1. In fact, we observed that females spawned their eggs relatively quickly, mostly because of their relatively low brood sizes, completing their release of eggs 1 or 2 h after we started their incubations (with an approximate maximum spawning rate of 96 eggs in less than 1 h). Overall, this total release of eggs was faster than the Antarctic krill *E. superba,* which spawns between 1400 and 4000 eggs over a period of up to 10 h (5 to 30 eggs min^{-1}), suggesting that spawning in these 2 tropical species does not

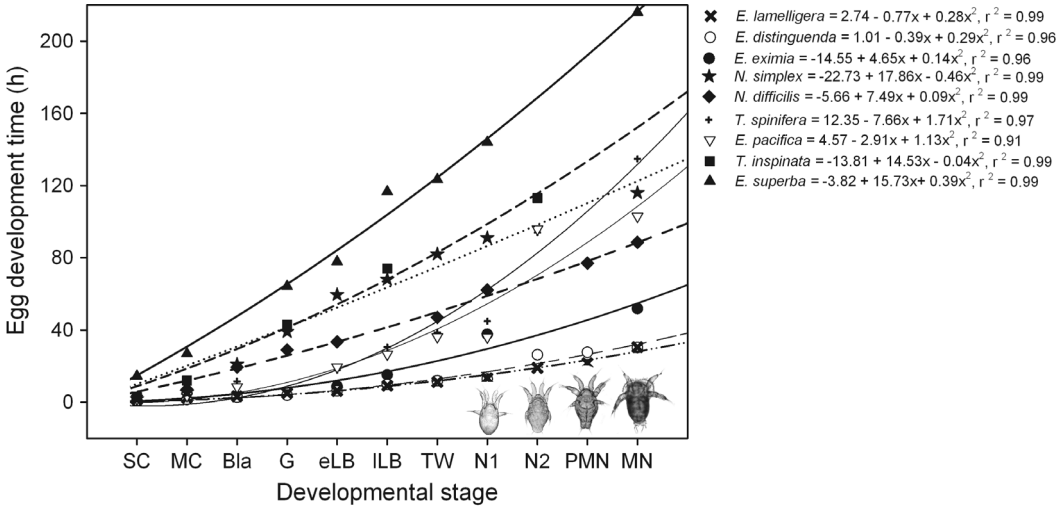

Fig. 8. Interspecific comparison of the embryonic and early larval development time of the tropical krill *Euphausia lamelligera*, *E. distinguenda* (25 ± 1°C; this study), and *E. eximia* (18 ± 1°C; Gómez-Gutiérrez unpubl. data), the subtropical species *Nyctiphanes simplex* (16 ± 1°C; Gómez-Gutiérrez & Robinson 2005), the temperate species *Nematoscelis difficilis*, *Thysanoessa spinifera*, *T. inspinata,* and *E. pacifica* (10 ± 1°C; Gómez-Gutiérrez 2002, 2003), and the Antarctic krill *E. superba* (1 ± 1°C; Quetin & Ross 1984, 0.5°C; Jia et al. 2014). These incubation temperatures represent the mean temperature of the water column each krill species inhabits. Adjusted linear regression (second order polynomial fit) was calculated for each krill species. All regression lines are significant ($p < 0.05$)

modify female swimming behavior for very long, and therefore reduces vulnerability to predators (Tarling et al. 2009).

In conclusion, our study demonstrated that (1) the *E. lamelligera* and *E. distinguenda* populations exhibit continuous spawning patterns, but with a spawning maximum strongly associated with seasonal upwelling dynamics from the northern region of the ETP, as predicted by species inhabiting other tropical and temperate ecosystems (Pillar & Stuart 1988, Ross & Quetin 2000). (2) We established morphological and biometric features of diagnostic and taxonomic value to identify the eggs and early larval stages (N1, N2, PMN, and MN) of these 2 tropical species; this will allow identification of species collected from the field in order to infer preferences for spawning season and location, centers of larval dispersion, and clues about how embryos survive in the ocean. (3) The PMN stage is no longer thought to be exclusive for sac-spawning species, giving the new perspective that some broadcast spawning species can develop through this larval stage. (4) The previous description of the MN stage of both species (Brinton et al. 2000) was morphologically and biometrically almost identical to that obtained under laboratory conditions. (5) Both *E. lamelligera* and *E. distinguenda* exhibit the fastest embryonic and early larval development rates (and likely female egg release due to their relatively small brood sizes) reported to date for any species of the Order Euphausiacea. Finally, the scientific knowledge gained in the present study will help advance our rudimentary understanding of the reproductive biology/ecology of tropical krill, and represents a first step towards estimating krill secondary productivity in tropical regions that appear to be within the lower limit of temperate krill productivity from the Northern Eastern Pacific (Gómez-Gutiérrez et al. 2007).

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