

Spiny but Subitaneous Eggs: Egg Morphology and Hatching in *Acartia* Copepods in the Tropics

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Egg surface ornamentation, egg production rates, and hatching success of two calanoid copepod species (*Acartia pacifica* and *Acartia* cf. *erythraea*) were examined in a tropical coral reef of Tioman Island, Malaysia, near the equator (2°N). All observed females of *A. pacifica* and *A. cf. erythraea* produced eggs with spines on the surface, either short or long, which are common in resting eggs in temperate ecosystems that hatch when environmental conditions are favorable or after a certain refractory period. However, all of these spiny eggs in the present study hatched within 48 hours when exposed to ambient seawater temperature, suggesting that they were subitaneous eggs. Such spine ornamentation has been thought to be an adaptive feature only for resting eggs to survive during egg dormancy, but the presence of spines on subitaneous eggs suggests further possible functions of these spines such as reducing the risks of ingestion by predators.

Key words: Copepods, Subitaneous egg, Spiny, Tropics, Malaysia.

BACKGROUND

Some copepods in temperate coastal waters are reported to produce two physiologically different types of eggs, subitaneous and resting eggs (Marcus 1996). Subitaneous eggs hatch within a few days after spawning. In contrast, resting eggs are produced to overcome adverse environmental conditions, and hatch when environmental conditions are favorable (quiescence eggs) or after a certain period of refractory phase (diapause eggs) (Marcus 1996). Temperature is the primary cue factor of dormancy in resting eggs

(e.g. Ban and Minoda 1991). Resting eggs tend to be produced during periods of lowering temperatures at the end of the growing season.

Subitaneous and resting eggs of copepods are often morphologically different to some extent, even if they are from the same species (Santella and Ianora 1990; Ianora and Santella 1991; Blades-Eckelbarger and Marcus 1992). In *Acartia* copepods, for example, many species show morphological differences between subitaneous and resting eggs, where subitaneous eggs are often smooth on the surface while resting eggs possess spines (Zillioux and Gonzalez 1972; Belmonte

1992; Belmonte and Puce 1994; Belmonte 1997; Belmonte 1998; Jiang et al. 2004; Onoue et al. 2004). Such spine ornamentation is thought to be an adaptive feature that lowers the risk of mortality and increases dispersal during egg dormancy (Belmonte et al. 1997).

Seasonal water temperatures and chlorophyll concentrations are almost constant in the tropical coastal waters (Yoshida et al. 2006; Zuraire et al. 2018), which suggests there is no compelling adaptive benefit to producing resting eggs (Marcus 1996; Yoshida et al. 2012a). As a result, reports of resting eggs in tropical copepods are not available, except in Hong Kong where resting eggs of *Tortanus forcipatus* were found in muddy bottom sediment (Dahms et al. 2006). Studies on egg morphology of copepods are limited to those conducted in temperate waters, and none are available from tropical waters. Recently, however, we found the eggs produced by *Acartia* copepods from tropical coral reef waters in Malaysia had spines on their surface that were morphologically similar to what previous studies have observed for resting eggs.

Here we examined whether the spiny eggs laid by *Acartia* copepods in tropical waters are subitaneous or resting eggs by investigating the egg production rate, hatching success and egg surface ornamentation. This is important because there has been extensive debate on the significance of spines on resting eggs, but if spines are found on eggs from copepods that are known to produce only subitaneous eggs, then this would contribute to our understanding of the evolutionary significance of egg surface ornamentation.

MATERIALS AND METHODS

Study site

All experiments and sample preparation were conducted at the Marine Park laboratory of Tioman Island (2°50'N 104°10'E), off the east coast of Peninsular Malaysia. The island forms a typical fringing coral reef dominated by *Acropora* corals. Live samples of *Acartia* copepods were collected at the jetty of the marine park by vertical tows of a plankton net (300 µm mesh opening) from the bottom to the surface during the night (22:00-24:00). Live samples to estimate the egg production rate and hatching success were collected in September-October 2007, while sampling devoted to the morphological study of eggs was carried out in October 2010. Water temperature and chlorophyll-*a* concentrations during the study periods were 29.3 ± 0.5°C and 0.24 ± 0.05 µg l⁻¹, and 31.0 ± 0.3°C and 0.26 ± 0.06 µg l⁻¹, respectively. Two *Acartia* species were found; *Acartia pacifica* and *Acartia cf. erythraea* with

the average abundance of 45 ± 58 inds. m⁻³ and 8 ± 7 inds. m⁻³, respectively, during the September-October 2007 sampling period.

Egg production

Adult females of *A. pacifica* and *A. cf. erythraea* collected in September-October 2007 to estimate the egg production rate (EPR) were transferred to the laboratory in an insulated container within 5 min. Each time, up to sixty adult females of each species were sorted under a dissecting microscope. Females were individually placed in an incubation chamber (diameter: 1.5 cm; height: 4.5 cm) with a 180 µm sieve 1 cm above the bottom to avoid cannibalism of eggs, immersed in a 50 ml beaker containing ca. 40 ml of ambient seawater pre-screened through a 35 µm mesh to avoid contamination from other eggs. Incubation of females were conducted under near ambient temperatures (29 ± 0.3°C) in the dark. After 24 h, the females were removed from their chambers and the number of eggs was counted.

To describe the physiological nature of the eggs, the hatching success was examined. All eggs spawned by *A. pacifica* females (*n* = 38) and *A. cf. erythraea* females (*n* = 8) were placed in multi-well dishes (NUNC) with ca. 5 ml of seawater pre-filtered through GF/F (Whatman) filters, and incubated at ambient temperature. Hatching success was monitored at intervals of ca. 12 h and subsequently up to 60 h. The eggs that hatched within the time determined by the Bělehrádek's temperature function of egg development time (Uye 1980) plus 24 h were defined as subitaneous eggs (Onoue et al. 2004).

Egg morphology

Adult females collected in October 2010 for the morphological study of eggs were individually maintained for 12 h in a 50 ml beaker filled with ambient seawater pre-screened through GF/F filters (Whatman) under dark and in situ temperature (29.8°C). Eggs spawned by the females were collected and fixed in 4% glutaraldehyde solution diluted with filtered seawater.

The fixed eggs were washed with distilled water to remove as much attached debris as possible. Eggs were then put on specimen stubs in a drop of distilled water. The stub was frozen through contact with a metal block cooled to about -100°C (Ohta et al. 2011), and then dried by vacuum sublimation using a freeze drying system (Aqua FD-6500, SUN Technologies). After the eggs dried, they were coated with an osmium coater (PMC-5000, APCO) to make the samples conductive,

and observed with a field-emission scanning electron microscope (FESEM) (JSM-6301F, JEOL).

RESULTS

In the laboratory, *Acartia pacifica* and *Acartia cf. erythraea* females laid eggs of morphologically different types as distinguished by light and SEM microscopy: one with fine short spines (*A. pacifica*) and the other with thick longer spines (*A. cf. erythraea*) (Figs. 1a-d). The spines grow densely packed on the egg surface for *A. pacifica*, while those for *A. cf. erythraea* are comparatively less dense, each spine possessing a thick base tapering toward a distinct forked tip. SEM analysis revealed the spines in *A. pacifica* eggs also forked at the end (Fig. 1e). The spines for both species resemble thin fibers twined together (Figs. 1e, f). No pores or hollowed spines were observed on the surface. Average (mean \pm SD) spine length was $4.0 \pm 1.7 \mu\text{m}$ for *A. pacifica* eggs and $29.7 \pm 5.3 \mu\text{m}$ for *A. cf. erythraea* eggs.

Females of *A. pacifica* and *A. cf. erythraea* spawned eggs at rates of 6.5-13.3 eggs female⁻¹ d⁻¹ (overall mean: 10.3 ± 2.4 eggs female⁻¹ d⁻¹) and 7.9-22.0 eggs female⁻¹ d⁻¹ (overall mean: 14.7 ± 6.3 eggs female⁻¹ d⁻¹), respectively (Table 1). No smooth eggs were spawned from the two species. Hatching patterns of eggs from each sampling date are shown in figure 2. Eggs spawned by both *A. pacifica* and *A. cf. erythraea* hatched within 24-48 hours, and were categorized as subitaneous eggs.

DISCUSSION

This paper presents the hatching success and surface ornamentation of eggs laid by *Acartia* copepods from a tropical coral reef water near the

equator (2°N). The mean egg production rates (EPR) of *Acartia pacifica* (10 eggs female⁻¹ d⁻¹) and *Acartia cf. erythraea* (15 eggs female⁻¹ d⁻¹) were within the range of previously reported EPR for *A. pacifica* (6-37 eggs female⁻¹ d⁻¹) and *A. erythraea* (7-16 eggs female⁻¹ d⁻¹) (Checkley et al. 1992; Kurihara et al. 2004; Sun et al. 2011; Yoshida et al. 2012a). The eggs laid by the two *Acartia* species were categorized as subitaneous eggs in this study with a hatching rate of 100% within 24-48 hours. Similar hatching rates were commonly observed in the coastal waters of Peninsular Malaysia (the Malacca Strait), reporting the hatching rates of *A. pacifica* and *A. erythraea* were 80% and 100%, respectively (Yoshida et al. 2012b).

The eggs from our study distinguished as subitaneous possessed spines, though morphology varied with species. Previous studies of egg dormancy of *A. pacifica* and *A. erythraea* from temperate waters reported two morphologically different eggs, smooth subitaneous eggs and spiny resting (diapause) eggs (Zillioux and Gonzalez 1972; Kasahara et al. 1974; Jiang et al. 2004). For example, a study from Xiamen Bay, China reported diapause eggs of *A. pacifica* in the sediments possessed spines (Jiang et al. 2004). A previous study from the Inland Sea of Japan also reported that *A. erythraea* produced resting eggs with spines as a strategy for overwintering (Kasahara et al. 1974).

Unlike previous reports, however, the subitaneous eggs laid by *A. pacifica* and *A. cf. erythraea* from our study in tropical coral reef water have spines on their surface. Similar results were observed from the Limfjord estuary in Denmark, where not all spiny eggs were resting but were instead subitaneous, though there was no mention of the adaptive function of spines on subitaneous eggs (Hansen et al. 2010). They reported up to 60% of subitaneous eggs spawned by *Centropages hamatus* possessed spines but some were smooth on the surface (Hansen et al. 2010). On the other hand, all eggs

Table 1. Abundance of adult female *Acartia pacifica* and *Acartia cf. erythraea*, number of females used to determine egg production rate (EPR) in September-October 2007. EPR is expressed as eggs female⁻¹ day⁻¹ \pm standard deviation (SD)

Date (dd/mm/yy)	<i>A. pacifica</i>		<i>Acartia cf. erythraea</i>	
	No. of females	EPR (eggs female ⁻¹ day ⁻¹)	No. of females	EPR (eggs female ⁻¹ day ⁻¹)
29/09/07	7	11.3 \pm 5.9	1	17.0
30/09/07	6	6.5 \pm 6.5	1	22.0
01/10/07	4	11.5 \pm 7.6		
03/10/07	31	8.4 \pm 4.4		
05/10/07	21	12.2 \pm 2.8		
06/10/07	60	11.1 \pm 6.0	3	18.3 \pm 14.0
07/10/07	6	7.8 \pm 4.7	21	7.9 \pm 5.6
09/10/07	31	13.3 \pm 10.5	30	8.4 \pm 5.9

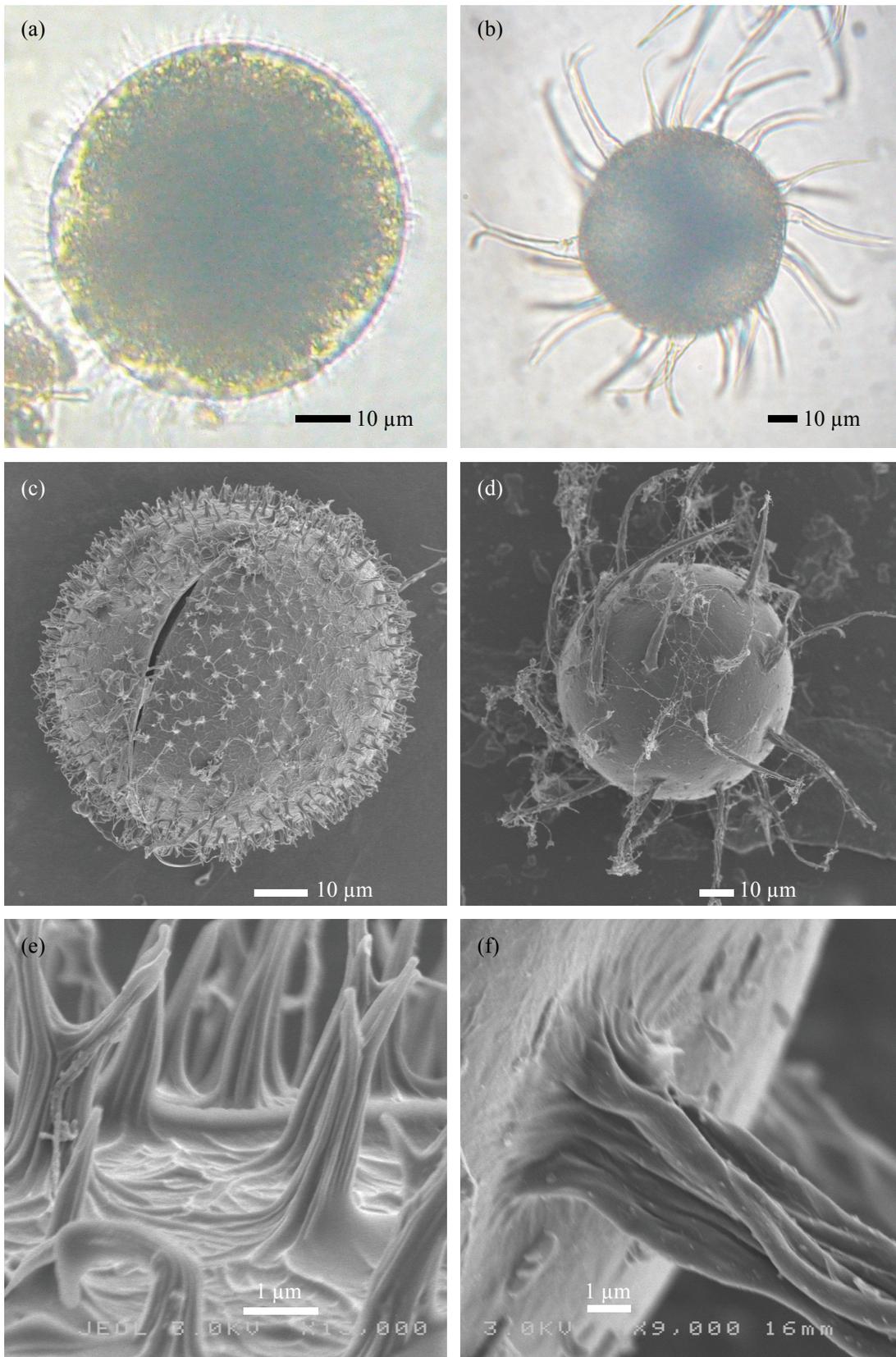


Fig. 1. Light microscope images of eggs spawned by (a) *Acartia pacifica* and (b) *Acartia* cf. *erythraea*, and scanning electron micrograph of (c, e) *A. pacifica* eggs and (d, f) *Acartia* cf. *erythraea* eggs.

we observed on Tioman Island, east coast of Peninsular Malaysia, were subitaneous and spiny. This result contrasts with the eggs produced by *A. pacifica* and *A. erythraea* (morphologically similar to *A. cf. erythraea* in this study) found in the Malacca Strait, west coast of Peninsular Malaysia that were smooth subitaneous eggs (Yoshida et al. 2006; Yoshida et al. 2012b). The morphological differences between eggs could be due to discrete distant populations or, alternatively, the *A. pacifica* and *A. cf. erythraea* from Tioman Island could be another species of *Acartia*. We were not able to genetically confirm if the species are conspecific

between the Malacca Straits and the study site; thus we cannot reject the possibility of different species, which needs to be examined in the near future. More importantly, the fact that all (100%) eggs produced in this study were spiny and subitaneous garnered our interest as it has not been reported previously.

Spine ornamentation in resting eggs are thought to be an adaptive strategy to reduce the risk being ingested by predators or buried in sediment during egg dormancy (Belmonte et al. 1997). The spines of copepod eggs have also been considered to serve as i) floating devices that help in passive dispersal and ii) as a means of chemical exchange through hollowed spines (Belmonte et al. 1997). The spines of *A. cf. erythraea* eggs in this study are conspicuously longer (> 30% of egg diameter) than those of *A. erythraea* reported from Japan, which are less than 10% of the eggs diameter (Kasahara et al. 1974). In contrast to silty or muddy bottoms found in temperate coastal regions where diapause eggs are often observed, the relatively coarser coral reef sediment in this study does not seem to support the hypothesis that spine ornamentation helps reduce the risk of getting buried in sediment. Furthermore, the idea that spines contribute to egg dispersal may not hold in subitaneous eggs, which hatch soon after spawning. No pores or hollowed spines often observed in resting eggs (Onoue et al. 2004) were found on the egg surface, excluding the likelihood of chemical exchange as an adaptive function. The function of the spines remains unclear. The spines' adaptive function could be reducing the risk of ingestion by predators prior to hatching in the water column or/on the bottom (Belmonte 1997).

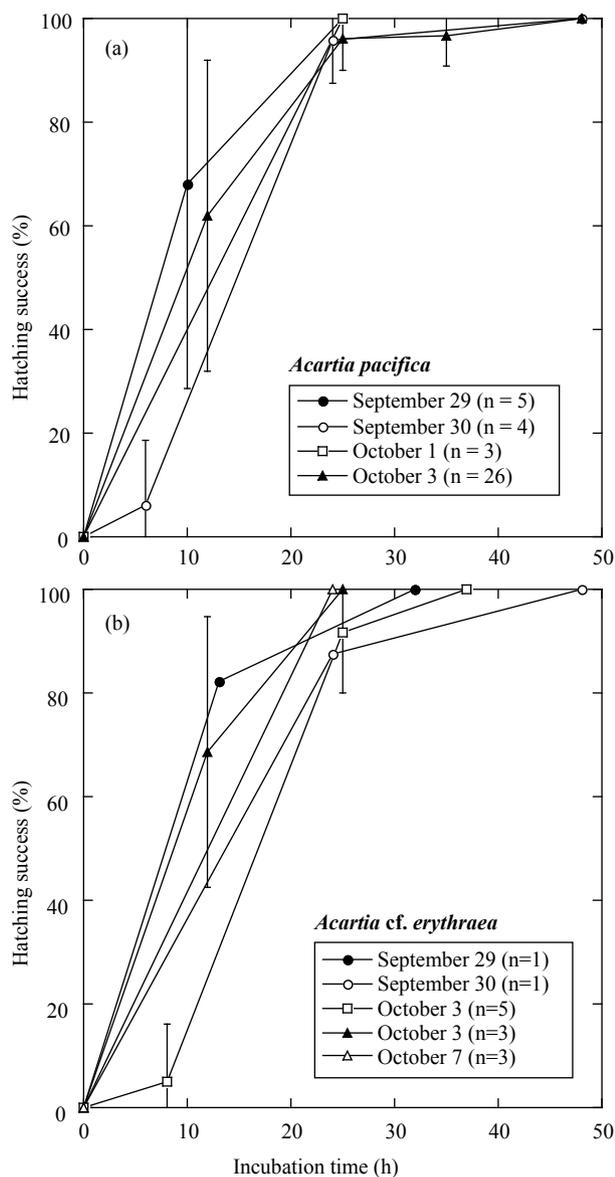


Fig. 2. Cumulative hatching success of eggs spawned by (a) *Acartia pacifica* and (b) *Acartia cf. erythraea* on Tioman Island in September-October 2007. The date of each symbol indicates sampling date of females. Error bars indicate standard deviation.

CONCLUSIONS

This study showed, for the first time, subitaneous eggs with spines on their surface from *Acartia* copepods in the tropical coral reef waters of Malaysia, which were previously reported to produce only resting eggs (quiescence eggs or diapause eggs). Such spine ornamentation has been thought to be an adaptive feature only for resting eggs to survive during egg dormancy, but the presence of spines on subitaneous eggs suggests further possible functions of these spines. We propose a future investigation on the ecological importance of spiny eggs in copepods inhabiting coral reef ecosystems in order to obtain a better understanding of the morphological function of spine ornamentation in copepod eggs.

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Availability of data and materials: All the key datasets are provided in this article.

Ethical approval consent to participate: Not applicable.

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