Marine Biology (1997) 127: 587–597

F. Chen · N. H. Marcus

Subitaneous, diapause, and delayed-hatching eggs of planktonic copepods from the northern Gulf of Mexico: morphology and hatching success

Received: 9 September 1996 / Accepted: 1 October 1996

Abstract Experiments were conducted to examine the morphology and hatching success of eggs, either spawned by freshly caught planktonic copepods or recovered from bottom sediments in the northern Gulf of Mexico. Collections were made between August 1992 and September 1995. Eggs of nine species were described and these differed in their diameter, color and surface attributes. Three types of eggs were distinguished: subitaneous, diapause, and delayed-hatching. Three species, *Labidocera aestiva* Wheeler, *Acartia tonsa* Dana, and *Centropages velificatus* (Oliveira) produced only subitaneous eggs. Hatching success varied greatly among these species. Two species, *Labidocera mirabilis* Fleminger and *Centropages hamatus* (Lilljeborg) produced diapause eggs and subitaneous eggs. The length of the refractory phase of the diapause eggs differed greatly both within and between these two species. A third type of dormant egg, delayed-hatching, was recognized in *Labidocera scotti* Giesbrecht and *Pontella meadi* Wheeler. The existence of delayed-hatching eggs may be an adaptive response of subtropical species to less seasonal fluctuation. Based upon morphological characteristics of the eggs and rearing of nauplii to an identifiable stage, benthic dormant eggs of eight species of calanoid copepods were also identified.

Introduction

Two distinct types of eggs, subitaneous and diapause eggs, are well recognized in marine planktonic copepods (reviewed by Grice and Marcus 1981; Uye 1985). Subitaneous eggs are able to hatch immediately (within hours to days, Kiørboe and Sabatini 1994) in situ, which leads to growth of the current population. They may, however, become quiescent (development-retarded) when they are exposed to adverse environmental conditions (e.g., low oxygen; Uye and Fleminger 1976). Such conditions frequently occur in marine bottom sediments (e.g., Revsbech et al. 1980a, b). In contrast, diapause eggs are in a state of arrested development and do not hatch, even under favorable conditions, until they complete a refractory phase. The duration of the refractory phase varies within and among species and can be modified by external factors, e.g., temperature (Marcus 1987).

Grice and Marcus (1981) suggested that both quiescent subitaneous and diapause eggs might occur in sediments, especially in shallow waters. They referred to quiescent subitaneous and diapause eggs occurring in the sediments as dormant. Since the duration of development and hatching differ for these two egg types, these two sources of eggs should affect naupliar recruitment and thus the population dynamics of copepods differently. Moreover, results showed that diapause eggs survive longer in sediment than quiescent subitaneous eggs (e.g., Hairston and Olds 1984). Thus, if viable dormant eggs accumulate in the sea bed over an extended period of time (e.g., greater than a reproductive season), their hatching may slow the rate of evolutionary change of species (Hairston and De Stasio 1988; Marcus et al. 1994). Presently, 38 marine copepod species are known to have a dormant egg phase, of which only 14 have been shown to produce diapause eggs (Marcus 1996). With increasing interest in the ecological and/or evolutionary significance of benthic egg banks, studies are needed to determine the types and hatching characteristics of eggs produced by copepods.

Two procedures are currently used to identify subitaneous and diapause eggs of planktonic copepods. In the first, eggs spawned in the laboratory are incubated at ambient field temperatures for a few days and hatching success is determined. The eggs that hatch are classified as subitaneous, and those that do not are classified as diapause and/or nonviable. To distinguish diapause

Communicated by J.P. Grassle, New Brunswick

F. Chen (✉) · N.H. Marcus
Department of Oceanography, Florida State University, Tallahassee, Florida 32306, USA

from nonviable eggs, unhatched eggs are incubated for an additional period of time ranging from weeks to months, during which the incubation temperature may be modified. Diapause eggs hatch at substantial levels after either warming or cooling for a period of time (i.e., after completion of the refractory phase; Grice and Gibson 1977, 1981; Kasahara and Uye 1979; Uye 1985; Marcus 1987; Chen and Li 1991). Nonviable eggs disintegrate. With the second procedure, eggs recovered from the sea bed can be identified to species based upon their morphology, or by rearing nauplii that hatch from the eggs to identifiable stages (Marcus 1990). Classification of these is more problematic because in most species subitaneous and diapause eggs do not differ morphologically.

Extensive studies on dormant eggs have been carried out for marine planktonic copepods from northern temperate areas, which typically undergo marked seasonal fluctuations in environmental factors (review by Grice and Marcus 1981). The production of diapause eggs is considered to be a genetic, adaptive response of species to predictable environmental fluctuation (Marcus 1979), and critical to the perpetuation of species year-after-year in these regions (Grice and Marcus 1981). Studies of copepod population dynamics in some subtropical and tropical waters led investigators to suggest that dormant eggs occur in these areas as well (Tranter and Abraham 1971; Fleminger 1979), but the actual existence of such eggs was not documented until recently (Li et al. 1989; Marcus 1989; Chen and Li 1991; Marcus 1991). Marcus (1989) demonstrated that *Centropages hamatus*, a planktonic copepod that occurs during the winter and spring in the northern Gulf of Mexico, produced diapause eggs, and she reported the occurrence of large numbers of dormant eggs in the sea bed. She also noted the occurrence of benthic eggs of other planktonic copepods including *Acartia tonsa, Labidocera aestiva* and *L. scotti* in this region, but these eggs were not distinguished as diapause or quiescent subitaneous eggs. Further observations on the hatching success of eggs produced by field-caught females are required to classify these eggs. In order to understand naupliar recruitment patterns in subtropical and tropical regions it is necessary to distinguish the types of eggs produced by copepods in these areas, as is the case in temperate regions. Since subtropical and tropical regions are generally believed to undergo less seasonal fluctuation than temperate regions it may be that diapause eggs are not as common at these latitudes.

Materials and methods

Collection of zooplankton and description of the study areas

Zooplankton were collected using 0.75 m plankton nets, either 243- or 333-μm mesh with nonfiltering cod ends. Sampling was conducted weekly from 2 June 1993 to 28 September 1995 in shallow (2 to 3 m) inshore waters near Turkey Point, Florida, USA (29°51′N; 84°31′W). Sea surface temperature and salinity were recorded at the time of collection. Temperature was determined with a thermometer placed in a bucket of freshly collected surface water. Salinity of the same sample of water was determined with a hand-held refractometer. The water column is generally well mixed though some stratification occurs in summer. The plankton sample was diluted with ambient seawater, stored in insulated buckets and transported to the laboratory within 2 h.

Collection, isolation, and identification of eggs

Eggs spawned by field-collected females under laboratory conditions

Immediately upon arrival at the laboratory, adult copepods were sorted into 100-ml glass dishes or 1000-ml beakers containing glass-fiber filtered (GFF) seawater. Males and females were added to achieve a sex ratio (female:male) of 5:1 to 10:1 when the number of females was sufficient. Each 1000-ml beaker contained approximately 100 copepods, and each 100-ml glass dish, 10 to 15 copepods. A mixture of *Scrippsiella trochoidea* (Clone Peri), *Gymnodinium nelsoni* (Clone GSBL) and *Prorocentrum micans* (Clone Proro) was added to the containers to achieve a food concentration of 150 cells ml^{-1} of each species. Salinity in the vessels was kept at values (± 3 to 4‰) close to those in the field at the time of collection. The dishes and/or beakers were placed in incubators at temperatures and photoperiods comparable to those in the field at the time of sampling.

Eggs spawned by females after 16 to 20 h of incubation were collected by filtering the contents of the dishes and/or beakers through 243-, or 153-, and then 48-μm screens. Eggs retained on the latter were rinsed into a clean glass dish with GFF seawater. Some of these eggs were transferred with a micropipette to microscope slides and observed with a Zeiss compound microscope to determine morphological attributes and egg size. Measurement of egg diameter excluded the surface attributes of eggs when such attributes were present. Egg diameter was expressed as mean (μm) ± 1 SD based on the measurement of 20 to 30 eggs. Aliquots of the remaining eggs spawned by the females were incubated to determine hatching success.

Eggs from the sea bed

Bottom sediments were collected from Louisiana coastal waters (28°51′N; 91°05′W) on 2 to 5 August 1992, and from nearshore regions of Turkey Point, Florida in August and September 1993. In Louisiana, sediment samples were obtained using a cylindrical core tube (4.8 cm diam × 30 cm length) that was pressed into the sea bed by SCUBA divers. On deck the cores were extruded to a depth of 5.0 cm, using a modified version of the Fuller and Butman (1988) extruder (see Marcus 1990). The core material was preserved in plastic screw-cap test tubes with 5% (v/v) formalin. At Turkey Point, sediment samples were collected on 17 August 1993 using a Peterson grab. Three subsamples were scooped from the top 1 to 2 cm of sediment in the grab. Two of these subsamples were placed in screw-cap test tubes and preserved with 5% (v/v) formalin. Material from the third subsample was placed in a screw-cap test tube, resuspended in GFF seawater and kept in an insulated chest for transfer to the laboratory, where it was used to determine the hatching success of eggs isolated from the sediments. Three additional core samples were taken by a diver at Turkey Point on 19 September 1993, and the eggs from the top 3 cm of these samples were also used to determine hatching success.

To separate eggs from the sediments, each core sample was suspended in 5-μm filtered seawater, sonicated for 30 s (No. 4 setting, Branson Sonifier Cell Disruptor 200), filtered over a 48 μm Nitex screen, suspended in a concentrated solution of sucrose (1:1 sucrose:distilled water), and centrifuged for 3 min. The material remaining in suspension was filtered over a 48 μm screen, washed thoroughly with seawater and transferred to a dish containing GFF seawater.

Identification of eggs from the sea bed was based on their morphological similarity to eggs spawned in the laboratory by known species. Egg diameter was expressed as mean (± 1 SD) based on the measurement of 20 to 30 eggs. In addition, hatched nauplii from dormant eggs that were recovered from sediments at Turkey Point were placed in 1000-ml beakers containing GFF seawater and fed either *Gymnodinium nelsoni* (Clone GSBL) or a mixture of *Scrippsiella trochoidea* (Clone Peri), *Gymnodinium nelsoni* (Clone GSBL) and *Prorocentrum micans* (Clone Proro) at a concentration of 150 cells ml^{-1} of each species. Temperature and photoperiod were set at 20 °C and 14 h light:10 h dark, respectively. The adults and/or juveniles from these rearings were preserved in 5% (v/v) formalin for species identification.

Incubation of eggs to determine hatching success

Eggs spawned under laboratory conditions

Eggs were transferred to polystyrene tissue culture plates containing GFF seawater, and incubated at the same temperature and salinity used to incubate the spawning females. The eggs of *Labidocera mirabilis, L. scotti* and *Pontella meadi* were also incubated at additional temperatures ranging from 11 to 30 °C. Eggs of *Centropages hamatus* were incubated at 20 °C for 7 d. Unhatched eggs were then transferred to 25 °C and kept at this temperature for up to 6 months. A batch of eggs was transferred monthly to 15 °C. Unless otherwise indicated in the presentation of the results, at least three replicates of 30 or more eggs each were used for each species. For some species, fewer eggs were used because an insufficient number of eggs were spawned. Egg hatching rates were monitored with a Wild dissecting microscope every 1 to 2 d until no viable eggs remained. When the duration of incubation exceeded 3 weeks, the monitoring interval was increased to 5–7 d. The hatch values of the replicates were averaged to obtain mean hatching success ± 1 SD.

Eggs from the sea bed

The live benthic samples collected at Turkey Point on 17 August and 19 September 1993 were stored at 5 to 7 °C in a refrigerator for 2 weeks and 2 d, respectively. Eggs from these cores were isolated using the procedures as for the preserved samples; they were then sorted into polystyrene tissue culture plates containing GFF seawater on the basis of size, color, and surface attributes. These eggs were incubated at different temperatures (20, 25 and 30 °C), and hatching success was monitored every 2 to 3 d until all viable eggs hatched.

Results

Morphology of eggs

Eggs spawned under laboratory conditions

The seasonal occurrence of females of nine species of free-spawning calanoid copepods in weekly plankton samples from Turkey Point, Florida are shown together with the size, color and surface characteristics of their eggs (Table 1). Eggs differed in diameter, color and surface attributes. *Centropages hamatus* eggs were smallest with a diameter of 68.2 to 69.4 μm, and the largest eggs were from *Pontella meadi*, with a diameter of 141.0 μm. Two distinct types of surface attributes were obvious among the eggs: those without any ornamentation (smooth surface, e.g., *Calanopia americana, Labidocera aestiva* and *L. scotti*) and those with spines (e.g., *Pontella meadi*, *Centropages velificatus*, *C. hamatus* and *Acartia tonsa*) or cases (e.g., *Tortanus setacaudatus*).

Some species produced eggs with variable surface attributes. *Labidocera mirabilis* and *Acartia tonsa* produced both smooth and spiny eggs. In *L. mirabilis*, green-colored eggs were either spiny or smooth. Black eggs, however, were always spiny. Green eggs were subitaneous and black eggs were diapause, according to their hatching success. *Centropages hamatus* also produced smooth and spiny eggs, and spine length varied seasonally. Eggs with longer spines (15 to 32 μm, = Type II; Table 1) occurred only from January to April when the population declined and finally disappeared from the plankton. These eggs did not hatch at ambient temperatures. In contrast, smooth eggs or eggs with short spines (<13 μm, = Type I; Table 1) on their surfaces were produced by females from November to April and these hatched immediately at ambient field temperatures. For all copepods, with the exception of *L. mirabilis*, egg color did not vary within the species.

Table 1 Characteristics of eggs produced in the laboratory by field-collected planktonic copepods from Turkey Point, Florida. Occurrence refers to period when adult females were present in weekly plankton samples between June 1993 and September 1995

Species	Surface attributes	Diameter (μm)[a]	Color	Occurrence
Calanopia americana Dahl	Smooth	129.5 (1.7)	Green	Aug
Labidocera aestiva Wheeler	Smooth	96.8 (1.5)	Green	Year-round
Labidocera mirabilis Fleminger	Smooth or spiny	99.1 (1.4)	Black or green	Apr–Sep
Labidocera scotti Giesbrecht	Smooth	104.5 (1.8)	Green	May–Dec
Pontella meadi Wheeler	Spiny	141.0 (3.9)	Black	Apr–Oct
Centropages velificatus (Oliveira)	Spiny	76.6 (1.9)	Yellow	Apr–Dec
Centropages hamatus (Lilljeborg)	Type I[b]	68.2 (1.8)	Dark gray	Nov–Apr
	Type II[c]	69.4 (2.9)	Dark gray	Jan–Apr
Acartia tonsa Dana	Smooth or spiny	77.8 (1.5)	Gray	Year-round
Tortanus setacaudatus Williams	Cases	100.0 (1.4)	Brown-yellow	Apr–Jul

[a]Excluding ornamentation on surface of eggs; means with SD in *parentheses*
[b]Smooth or spiny, spines < 13 μm long
[c]Spiny, spines > 15–32 μm long

Eggs from the sea bed

Dormant eggs of seven species of planktonic copepods: *Calanopia americana, Labidocera aestiva, L. scotti, Pontella meadi, Centropages velificatus, C. hamatus* and *Acartia tonsa* were identified from the northern Gulf of Mexico (Table 2) based on their morphological similarity to eggs spawned by females in the laboratory (Table 1). Of these species, hatched nauplii from dormant eggs of four copepods (*L. aestiva, P. meadi*, *A. tonsa and C. hamatus*) were also reared to identifiable stages ranging from the third copepodite stage (CIII; *A. tonsa* and *P. meadi*) to adulthood (*L. aestiva* and *C. hamatus*). In *C. hamatus*, two distinct types of eggs were obvious in terms of their surface spine length, Type I (short spines) and II (long spines). The identification of dormant eggs of an eighth species, *Anomalocera ornata*, was based on the morphological similarity to eggs of *Anomalocera patersoni* (Ianora and Santella 1991) and the morphology of copepodite stages that were reared from nauplii that hatched from eggs recovered from the sea bed.

Classification of eggs according to hatching success

Eggs spawned under laboratory conditions

Based on the response of eggs to incubation temperatures, three types of eggs were distinguished: (1) subitaneous, (2) diapause and (3) delayed-hatching eggs. All species studied produced subitaneous eggs. Two also produced diapause eggs, and two species produced eggs that hatched over an extended period of time. We refer to these as delayed-hatching eggs.

The eggs of four species, *Labidocera aestiva, Acartia tonsa*, *Centropages velificatus* and *Calanopia americana* hatched within 2 to 7 d at ambient temperatures. Since the unhatched eggs were considered as nonviable because they degraded within the same short period of time, the eggs were classified as subitaneous. *L. aestiva* and *A. tonsa* produced these eggs year-round, but hatching success was variable (Fig. 1). In *L. aestiva*, fluctuation in hatching success occurred between October 1993 and February 1994 (40 to 100%), as well as between May and August 1994 (48 to 96%), respectively. During the rest of the year, hatching success was generally high (>80%). In *A. tonsa*, a high hatching success (>85%) was found throughout the summer and winter of 1993, during which the ambient field salinity was consistently high. Low hatching success (5 to 52%) occurred between early December 1993 and late March 1994 (Fig. 1). During the summer of 1994, hatching success varied greatly (25 to 99%). Both the decline and fluctuations in hatching success in eggs of *A. tonsa* occurred during periods of reduced and fluctuating salinities (Fig. 1). Hatching success was not correlated with the surface attributes of eggs of *A. tonsa* (Table 1). In *C. velificatus*, hatching success was consistently high (79

Table 2 Characteristics of copepod eggs recovered in bottom sediments from Louisiana coastal waters (*LA*; 2 to 5 August 1992) and Turkey Point, Florida (*FL*; 17 August 1993)

Species	Location	Surface attributes	Diameter (μm)[a]	Color	Identifiable stages
Calanopia americana	FL	Smooth	130.0 (8.3)	Green	–
Labidocera aestiva	LA	Smooth	97.5 (1.4)	Green	–
	FL	Smooth	95.9 (3.3)	Green	Adult
L. scotti	LA	Smooth	103.5 (2.5)	Green	–
	FL	Smooth	103.3 (2.7)	Green	–
Pontella meadi	LA	Spiny	147.5 (–)	Black	–
	FL	Spiny	142.3 (2.4)	Black	CIII
Centropages velificatus	LA	Spiny	73.0 (–)	Yellow	–
	FL	Spiny	79.0 (1.7)	Yellow	–
C. hamatus					
Type I	LA	Short spines	71.0 (–)	Dark gray	–
	FL	Short spines	73.3 (1.4)	Dark gray	Adult
Type II	LA	Long spines	71.0 (1.2)	Dark gray	–
	FL	Long spines	71.1 (0.8)	Dark gray	Adult
Acartia tonsa	LA	Smooth or	73.0 (1.2)	Gray	–
	FL	spiny	76.9 (1.9)		CIII
Anomalocera ornata Sutcliffe	LA	Cases	144.9 (4.3)	Brown	–
	FL	Cases	151.8 (3.5)	Brown	Late copepodites

[a]Means with SD in *parentheses*

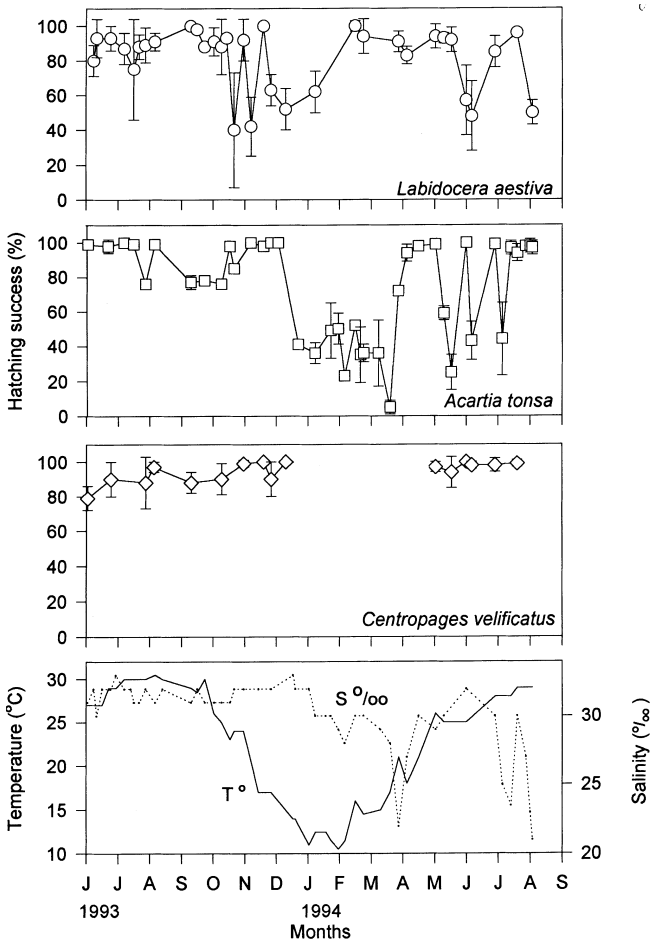

Fig. 1 *Labidocera aestiva*, *Acartia tonsa*, *Centropages velificatus*. Seasonal variation in hatching success of eggs of three species of planktonic copepods from Turkey Point, Florida. *Bottom panel* sea surface water temperature and salinity at the time of collection

to 100%) when adults were present, i.e., during the summer/fall (Fig. 1). A single collection on 9 August 1993 showed a high hatching success (97 ± 5%) in subitaneous eggs of *Calanopia americana*.

Two species, *Labidocera mirabilis* and *Centropages hamatus*, produced diapause eggs as well as subitaneous eggs. The immediate hatching success of eggs of *L. mirabilis* was generally high (80 to 100%) except on 23 June 1993 and 16 August 1995 (Table 3). For these two collections, hatching did not occur until 29 and 24 d of incubation, respectively. Maximum hatching success (62 and 29%) was achieved by Day 31 and 56, respectively. Although these eggs hatched without a modification of incubation temperature, they were classified as diapause eggs because hatching did not occur for several weeks, and, when hatching finally commenced, it increased rapidly. Two morphologically distinct types of eggs, one with a smooth surface and/or short spines, and the other with long spines were observed for *Centropages hamatus* (Table 1). The eggs with short spines hatched immediately at ambient temperatures and were thus classified as subitaneous eggs. Those with long spines did not hatch immediately at ambient temperatures at the time of collection. When these unhatched eggs were kept at 25 °C for different periods for up to 6 months, and were then transferred monthly to 15 °C, they hatched, although the time to hatch varied (Fig. 2). Incubation time at 25 °C and hatching time at 15 °C were inversely related. No eggs hatched at 25 °C for 7 months. The eggs with long spines were classified as diapause eggs. They maintained dormancy at 25 °C and required a minimum of 5 to 6 months at this temperature to achieve a 50% hatch within 2 to 3 d when placed at 15 °C. This contrasts with eggs incubated at 25 °C for less time, e.g., 1 month. Such eggs required 2 to 3 months at 15 °C before a 50% hatch level was attained.

Table 3 *Labidocera mirabilis*. Mean hatching success (± 1 SD) and embryonic duration of eggs spawned in the laboratory by field-collected copepods from Turkey Point, Florida

Collection date	Field temp. (°C)	Incubat. temp. (°C)	No. of eggs	Cumulative hatching success (%)	Days to hatch at	
					50%[a]	100%[b]
1993						
23 Jun	–	25	21	62 (11)	30	31
1994						
5 May	25	25	101	95 (5)	1	2
31 May	–	25	89	87 (3)	2	6
22 Jun	28	25	29	97 (0)	2	4
1995						
6 Apr	18	19	86	99 (2)	2	4
10 Apr	22	20	40	80 (28)	2	5
17 Apr	23	23	117	84 (5)	1	2
3 May	25	25	102	92 (5)	1	3
15 May	28	28	22	100 (0)	1	2
16 Aug	31	25	17	29 (23)	35	56

[a]Estimated in terms of hatching of viable eggs
[b]Time to hatching of all viable eggs incubated

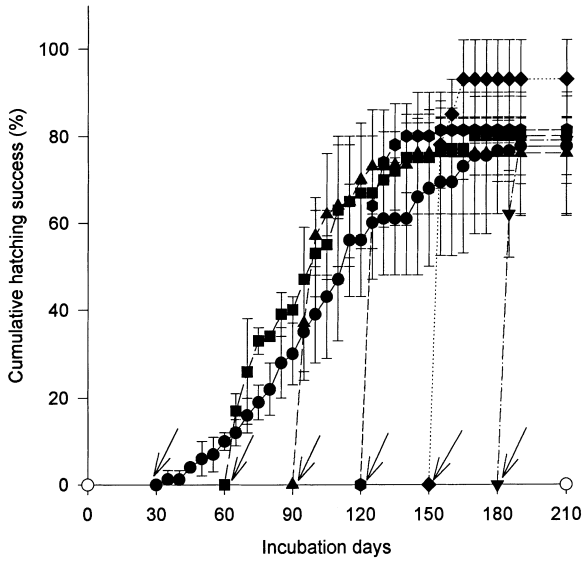

Fig. 2 *Centropages hamatus.* Cumulative hatching success (mean ± 1 SD) of diapause eggs transferred (indicated as *arrows*) to 15 °C after incubation at 25 °C for periods up to 6 months. *Open circle* shows control in which eggs were incubated at 25 °C

A third type of egg, delayed-hatching, was found in *Labidocera scotti* (Table 4) and *Pontella meadi* (Table 5). Hatching success in eggs of *L. scotti* was generally high (86 ± 12%) at 25 °C with little variation (range: 58 to 92%); intermediate at 28 to 30 °C (63 ± 47%) and at 20 °C (66 ± 25%) and more variable (range: 30 to 97% and 20 to 92% at 28 to 30 and 20 °C, respectively); and considerably lower (5 ± 7%) at 11 °C and less variable (0 to 25%). A single observation at 15 °C revealed a hatching success of 61 ± 12%. The time for all viable eggs to hatch varied with the date of collection. In most cases, it took 1 to 8 d for 50% of the viable eggs to hatch. However, delayed-hatching was apparent for the remaining unhatched eggs. The greatest time delays were 13, 48 and 81 d for eggs incubated at 28 to 30, 25 and 20 °C, respectively. A high hatching success (77 to 100%) was also found in eggs of *Pontella meadi* (Table 5), regardless of incubation temperature. The eggs from 15 of 17 collections had hatching typical of subitaneous eggs: high hatching success in 2 to 3 d. Some eggs from two collection dates (2 June and 17 August 1993) only hatched after extended incubation (Fig. 3). When unhatched eggs that had been incubated at 25 °C for 7 d after

Table 4 *Labidocera scotti.* Mean hatching success (± 1 SD) and embryonic duration of eggs spawned in the laboratory by field-collected copepods from Turkey Point, Florida

Collection date	Field temp. (°C)	Incubat. temp. (°C)	No. of eggs	Cumulative hatching success (%)	Days to hatch at	
					50%[a]	100%[b]
1993						
29 Sep	26	30	40	30 (7)	2	13
		25	93	71 (6)	0–1	18
		20	40	20 (0)	11	29
		11	40	0		
10 Oct	25	25	60	85 (16)	1–2	37–48
11 Oct	24	25	37	84	0–1	12
		20	40	81 (13)	10	41
		11	60	1 (1)		
14 Oct	23	25	54	86 (10)	8–9	25
		20	58	47 (17)	2–3	81
		11	60	5 (2)		
18 Oct	24	25	62	89 (3)	3–4	18
27 Oct	21	25	55	84 (13)	9–10	34
		20	60	38 (2)	17	69
		11	60	3 (4)	115	134
3 Nov	18	25	59	98 (2)	0–1	27
		20	79	77 (10)	2–3	61
		11	81	25 (10)	3	159
15 Nov	20	20	40	85 (7)	1–2	54
		11	64	16 (1)	3–4	8
22 Nov	17	20	62	68 (8)	1–2	23
1994						
14 May	26	25	82	98 (4)	0–1	21
26 May	25	25	43	98 (3)	0–1	28
5 Dec	20	20	77	87 (12)	0–1	3
1995						
11 Aug	28	25	37	58 (14)	8	14
23 Aug	30	25	93	93 (7)	0–1	8
5 Sep	28	28	90	97 (3)	0–1	2
		25	90	98 (4)	0–1	11
		20	90	92 (11)	0–1	24
		15	90	61 (12)	0–1	26

[a]Estimated in terms of hatching of viable eggs
[b]Time to hatching of all viable eggs incubated

Table 5 *Pontella meadi.* Mean hatching success (± 1 SD) and embryonic duration of the eggs spawned in the laboratory by field-collected copepods from Turkey Point, Florida

Collection date	Field temp. (°C)	Incubat. temp. (°C)	No. of eggs	Cumulative hatching success (%)	Days to hatch at	
					50%[a]	100%[b]
1993						
2 Jun	–	25	144	98 (4)	1–4	34
5 Aug	–	28	59	97 (5)	0–1	2
		20	62	98 (2)	1	3
9 Aug	–	30	45	98 (3)	0–1	2
		25	93	94 (3)	0–1	2
17 Aug	–	30	59	90 (5)	0–1	20
		25	59	97 (5)	0–1	41
		20	63	97 (0)	1–2	55
29 Sep	26	30	28	96 (5)	0–1	2
		25	29	93 (0)	0–1	2
		20	28	93 (10)	1	3
		11	28	86 (0)	8	12
6 Oct	26	25	33	100 (0)	0–1	3
27 Oct	23	25	70	99 (2)	1–2	5
		20	72	100 (0)	3–4	6
		11	72	97 (4)	7	9
1994						
11 Apr	21	20	75	100 (0)	3–4	4
27 Apr	26	25	28	96 (5)	1–2	2
22 Jun	28	28	40	98 (4)	1–2	4
8 Aug	28	28	32	84 (6)	0–1	8
1995						
19 Apr	25	25	90	99 (2)	0–1	3
24 Apr	25	25	90	100 (0)	0–1	3
5 May	25	25	89	100 (0)	0–1	2
18 Aug	31	30	71	93 (4)	0–1	3
5 Sep	28	28	61	79 (10)	0–1	11
		25	39	82 (19)	0–1	3
		20	60	77 (6)	0–1	11
		15	60	78 (8)	0–1	11
28 Sep	25	25	136	82 (5)	0–2	5

[a]Estimated in terms of hatching of viable eggs
[b]Time to hatching of all viable eggs incubated

spawning were kept at the same temperature, they hatched sporadically over the next 40 d (Fig. 4). These delayed-hatching eggs from *L. scotti* and *P. meadi* are distinguished from diapause eggs because hatching was gradual over an extended incubation period.

Eggs from the sea bed

The hatching success of eggs recovered from the sea bed is shown in Table 6. Both diapause and subitaneous eggs of *Centropages hamatus* occurred in the sea bed. A high hatching success (80 to 100%) was found for Type II eggs (diapause eggs) of *C. hamatus* at 20 °C. No Type II eggs hatched at 25 and 30 °C. This is comparable to the result that diapause eggs of this species do not hatch, i.e., maintain dormancy at high temperature (e.g. 25 °C). A relatively low hatching success (21 to 22%) was found for Type I eggs (quiescent subitaneous eggs). Other species, including *Labidocera aestiva* and *Acartia tonsa*, showed low hatching success. No eggs of *A. tonsa* hatched at 25 and 30 °C. These eggs were considered to be quiescent subitaneous eggs.

Discussion

This study was initiated: (1) to determine the types and hatching success of eggs of calanoid copepods from the northern Gulf of Mexico and (2) to identify the types of eggs of planktonic copepods in the sea bed of the northern Gulf of Mexico. The results indicated: (1) seasonal differences in the hatching success of subitaneous eggs among different species; (2) diverse egg types, including quiescent subitaneous, diapause and delayed-hatching eggs; and (3) distinct types of dormant eggs in the sea bed.

The results showed seasonal fluctuations in the hatching success of two species which occur throughout the year, *Labidocera aestiva* and *Acartia tonsa*; this contrasts with *Centropages velificatus*, which maintained high hatching success whenever it was present in the

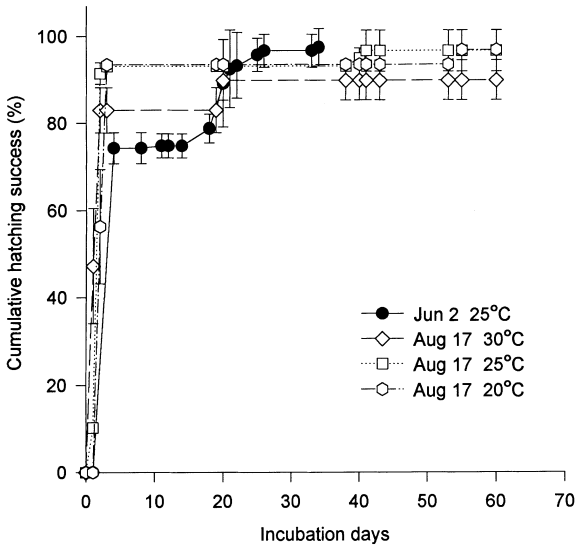

Fig. 3 *Pontella meadi*. Cumulative hatching success (mean ± 1 SD) of eggs from females collected 2 June and 17 August 1993 incubated at temperatures shown

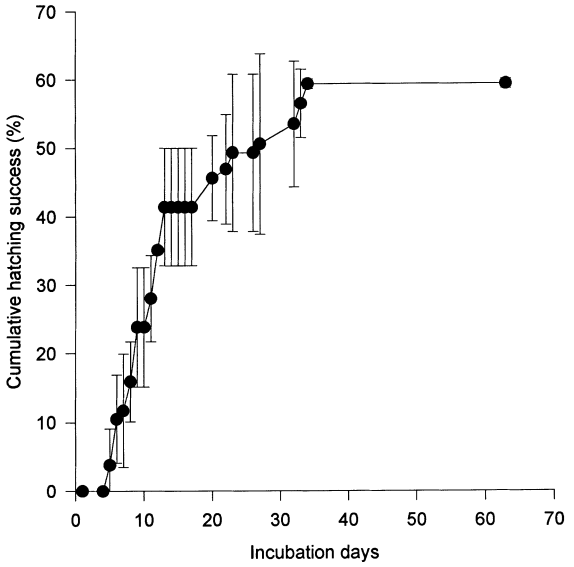

Fig. 4 *Pontella meadi*. Cumulative hatching success (mean ± 1 SD) of delayed-hatching eggs at 25 °C. Eggs had been preincubated at 25 °C for 7 d and this period is excluded from "Incubation days" axis

plankton. Several studies have examined seasonal trends in the hatching success of marine planktonic copepod eggs. Ambler (1985) reported that egg viability of *A. tonsa* from East Lagoon, Texas varied with season, from nearly 100% in the spring to <70% in the fall. A similar trend was found for *Centropages typicus* from the Gulf of Naples, Italy (Ianora et al. 1992), whereas an opposite trend with higher hatching success during fall (and/or summer) and lower during spring (and/or winter) was apparent in *Tortanus dextrilobatus* and *Labidocera euchaeta* from Xiamen waters, China (Li et al. 1989), as well as in *Temora stylifera* from the Bay of Naples, Italy (Ianora and Poulet 1993).

The causes for reduced hatching success in these studies were not determined. Factor(s) both acting prior to and after spawning of eggs could be responsible for fluctuation in hatching success of subitaneous eggs. In this study, declines and fluctuations in hatching success in subitaneous eggs of *Acartia tonsa* coincided with reduced and fluctuating salinities. Uye and Fleminger (1976) found that the range of salinity permitting >50% hatching varied among *Acartia* species, the lower limit being 17 to 20‰. We found that lowest hatching success (5%) of *A. tonsa* eggs occurred in March 1994 when the salinity dropped to 22‰, the lowest value for the year. But change in salinity did not appear to affect hatching success in subitaneous eggs of *Labidocera aestiva* or *Centropages velificatus*. This suggests that there are differences among species in tolerance to low salinity. Although we did not determine the quality or quantity of food available in the field, diet is another factor which can affect egg viability. Poulet et al. (1994) found that the hatching success of *Calanus helgolandicus* eggs was significantly lower when adult females were fed the

Table 6 Hatching success (%, ± 1 SD) at different temperatures of benthic dormant eggs collected 17 August and 19 September 1993 from Turkey Point, Florida. Number of eggs incubated shown in *parentheses*. Freshly collected sediments were stored at 7 °C for 14 d (17 August 1993) and 2 d (19 September 1993), respectively, before eggs were isolated and then incubated at different temperatures

Species	Temperature (°C)		
	20	25	30
Labidocera aestiva			
17 Aug	19 ± 3 (58)	26 ± 13 (62)	19 ± 2 (77)
19 Sep	25 ± 0 (24)	59 ± 23 (24)	35 ± 2 (26)
Centropages hamatus			
Type I			
17 Aug	21 (14)	–	–
19 Sep	22 (9)	–	–
TypeII			
17 Aug	90 ± 14 (20)	0 ± 0 (20)	0 ± 0 (20)
19 Sep	85 (13)	–	–
Acartia tonsa			
17 Aug	10 ± 14 (20)	0 ± 0 (20)	0 ± 0 (16)
19 Sep	50 (12)	–	–

diatom *Thalassiosira rotula* than with the dinoflagellate *Prorocentrum minimum*. Similar results were found for another planktonic copepod, *Centropages typicus* (Miralto et al. 1995).

Two species of calanoid copepods, *Labidocera mirabilis* and *Centropages hamatus*, produced diapause eggs. The length of the refractory phase differed both within and between these two species: up to 2 months for *L. mirabilis* and up to 6 months for *C. hamatus*. Diapause eggs of *L. mirabilis* did not require a modification of temperature to induce hatching. Sporadic hatching occurred after the eggs were incubated at constant temperature, i.e., 25 °C. This is the first report of diapause in this species. A similar hatching response was obtained for diapause eggs of the marine planktonic copepod *Tortanus dejuginii* from Xiamen waters, China (Chen and Li 1991) and the freshwater species *Eurytemora affinis* from Lake Ohnuma Hokkaido, Japan (Ban and Minoda 1991). In contrast, the hatching of diapause eggs of *C. hamatus* was inhibited at 25 °C. A period of prewarming (5 to 6 months) at 25 °C was required to induce substantial hatching of these eggs within 2 to 3 d at a low (favorable) temperature (15 °C). The requirement of prewarming of diapause eggs and the inhibition of hatching at 25 °C suggests that diapause eggs produced during a winter/spring period will not hatch until the following fall. This is essential because the planktonic phase cannot survive high summer temperatures (e.g., >29 °C, Chen unpublished).

Eggs of two pontellid copepods, *Labidocera scotti* and *Pontella meadi*, from Turkey Point, Florida hatched over a broad time span (up to 81 d in *L. scotti*) when incubated at temperatures within a few degrees of in situ values, and these were classified as delayed-hatching. This is the first evidence of delayed embryonic development in a marine planktonic copepod, although delayed naupliar development (up to 50 d) has been reported for a meiobenthic copepod (Coull and Dudley 1976). It is interesting to note that *Pontella meadi* from Buzzards Bay, Massachusetts produced diapause eggs which required 4 to 8 weeks of preincubation at 2 to 3 or 5 to 6 °C for substantial hatching to occur (Grice and Gibson 1977); whereas *P. meadi* from Turkey Point, Florida produced delayed-hatching eggs. This may reflect genetic differences between populations from these two widely separated sites. Marcus (1984) demonstrated genetic variation in the diapause response of *Labidocera aestiva* along the Atlantic coast of the United States.

Eggs classified as delayed-hatching, hatch gradually over an extended period of time regardless of incubation temperature, and they do not require a change in temperature to hatch. In contrast, diapause eggs hatch within a few days after completion of the refractory phase. The hatching of some diapause eggs requires chilling or warming followed by an increase or decrease in temperature. However, some diapause eggs (e.g., *Labidocera mirabilis* in this study) undergo a refractory period but do not require a change in temperature to induce hatching. Sullivan and McManus (1986) found that resting eggs of *Acartia hudsonica* required only a few days of exposure to warm temperature (20 °C) before they would hatch at a cold temperature. Eggs did not hatch when they were kept at 20 °C (Sullivan and McManus 1986). An analogous hatching response was also observed in *Acartia californiensis* eggs: hatching was inhibited at low temperature (9 to 13 °C) and resumed at high temperature (i.e., 21 °C, Johnson 1980). According to the present definition, we classify these eggs as diapause.

The cause for delayed-hatching of eggs is unknown. Kahan et al. (1988) demonstrated that maternal inhibition of hatching of an harpacticoid copepod, *Tigriopus japonicus*, occurred at high population densities. Further studies are needed to elucidate whether such a mechanism also exists in marine planktonic copepods, such as *Labidocera scotti* and *Pontella meadi.*

Differences in the time to hatching may be a response to environmental conditions. Kiørboe and Sabatini (1994) suggested that the hatching time of eggs of pelagic copepods should vary due to differences in selection pressure, e.g., predation. The hatching time of freely spawned calanoid eggs is about one-third that of carried cyclopoid eggs, whereas egg-carrying calanoids seem to be intermediate. Free-spawning calanoids "pay a price" for the short hatching time; their nauplii do not possess a fully developed gut at hatching, and they are unable to feed until the second and/or third naupliar stages (Landry 1983), while some Oithonidae nauplii start feeding immediately upon hatching (Uchima and Hirano 1986). Thus early hatching in free-spawning calanoids may be a response to predators, because suspended eggs experience very high mortality rates due to predation (Kiørboe and Sabatini 1994). Delayed-hatching in copepod eggs could be a bet-hedging strategy to promote the survival of some offspring. By spreading out the period over which eggs hatch, a female might extend her offspring's abilities to utilize resources, e.g., temporal differences in food quality and quantity, and reduce mortality of the offspring by avoiding heavy predation over some limited period of the year. Sih and Moore (1993) demonstrated delayed hatching of salamander eggs in response to enhanced larval predation risk.

This study found benthic eggs of eight species of copepods in sediments from the northern Gulf of Mexico. Of these, the benthic eggs of *Labidocera aestiva*, *Centropages velificatus* (= *furcatus*), *C. hamatus,* and *Acartia tonsa* in this region had been reported previously (Marcus 1989), although the egg types were not determined. This study demonstrated that the benthic eggs of *L. aestiva*, *C. velificatus*, and *A. tonsa* in sediments from the northern Gulf of Mexico were quiescent subitaneous eggs. Although there was no evidence of diapause egg production by field-collected *Calanopia americana*, we obtained eggs on only one occasion (9 August 1993). Thus, our data are insufficient to determine whether the eggs of *C. americana* that were isolated from the sediments were quiescent subitaneous

or diapause eggs. Classification of the eggs of *Pontella meadi* and *Anomalocera ornata* obtained from the sediments was not possible. For *P. meadi*, both egg types are produced, but they cannot be distinguished morphologically. For *A. ornata,* some benthic eggs isolated from sediments hatched sporadically over a relatively long time up to 45 d (Chen personal observation), suggesting that these eggs were either delayed-hatching or diapause eggs which gradually completed their refractory phases.

A much higher hatching success was found for benthic diapause eggs (80 to 100%) than for subitaneous eggs (21 to 22%) in *Centropages hamatus*. The diapause eggs had probably completed their refractory phase and were ready to hatch when exposed to suitable conditions. Differences in hatching probably reflect differences in the capacity of these eggs to survive conditions in the sea bed. Marcus and Lutz (1994) found that the hatching success of subitaneous eggs of *C. hamatus* decreased to 5–40% when eggs were exposed to anoxia for 32 d. On the other hand, the survival rate of diapause eggs of *C. hamatus* under anoxia remains extremely high (>80%) when incubated at 25 °C for 8 months (Chen and Marcus unpublished). The higher hatching success of benthic diapause eggs in comparison to benthic quiescent subitaneous eggs suggests that diapause eggs play a more important role in the seasonal occurrence of *C. hamatus* in this region.

Compared with higher latitudes (e.g., northern temperate regions), where most of the work on diapause eggs of marine copepods has been conducted, subtropical areas such as the northern Gulf of Mexico undergo less seasonal fluctuation in environmental conditions (e.g., temperature and photoperiod). These conditions may favor the existence of intermediate types of dormancy in copepods. Indeed, this study has shown that two marine planktonic copepods are capable of producing a third type of egg: delayed-hatching eggs (i.e., *Labidocera scotti* and *Pontella meadi*) and two other species (*L. mirabilis* and *Centropages hamatus*) produce diapause eggs with great variation in the length of their refractory phase. How common delayed-hatching eggs are in marine copepods remains to be determined.

Acknowledgements We thank L. Stadler, C.E. Wiese, and R. Lutz for the assistance they provided with sample collection. We also thank N. Rabalais for assistance in obtaining the samples from Louisiana. Work was supported by the National Science Foundation under Grant No. OCE-9202322 awarded to NHM. Publication No. 1088 of the Florida State University Marine Laboratory.

References

Ambler JW (1985) Seasonal factors affecting egg production and viability of eggs of *Acartia tonsa* Dana from East Lagoon, Galveston, Texas. Estuar cstl Shelf Sci 20: 743–746

Ban S, Minoda T (1991) The effect of temperature on the development and hatching of diapause and subitaneous eggs in *Eurytemora affinis* (Copepoda: Calanoida) in Lake Ohnuma, Hokkaido, Japan. Bull Plankton Soc Jap (Spec Vol): 299–308

Chen F, Li SJ (1991) On the seasonal distribution and the hatching rate of the diapause eggs in *Tortanus* from Xiamen waters. Acta oceanol sin 13: 721–727 (in Chinese)

Coull BC, Dudley BW (1976) Delayed naupliar development of meiobenthic copepods. Biol Bull mar biol Lab, Woods Hole 150: 38–46

Fleminger A (1979) *Labidocera* (Copepoda: Calanoida): new and poorly known Caribbean species with a key to species in the western Atlantic. Bull mar Sci 29: 170–190

Fuller CM, Butman CA (1988) A simple technique for fine-scale, vertical sectioning of fresh sediment cores. J sedim Petrol 58: 763–768

Grice GD, Gibson VR (1977) Resting eggs in *Pontella meadi* (Copepoda: Calanoida). J Fish Res Bd Can 34: 410–412

Grice GD, Gibson VR (1981) Hatching of eggs of *Pontella mediterranea* Claus (Copepoda: Calanoida). Vie Milieu 31: 49–51

Grice GD, Marcus NH (1981) Dormant eggs of marine copepods. Oceanogr mar Biol A Rev 19: 125–140

Hairston NG, De Stasio BT (1988) Rate of evolution slowed by a dormant propagule pool. Nature 336: 239–242

Hairston NG, Olds EJ (1984) Population differences in the timing of diapause adaptations in a spatially heterogeneous environment. Oecologia 61: 42–48

Ianora A, Mazzocchi MG, Grottoli R (1992) Seasonal fluctuations in fecundity and hatching success in the planktonic copepod *Centropages typicus.* J Plankton Res 14: 1483–1494

Ianora A, Poulet SA (1993) Egg viability in the copepod *Temora stylifera.* Limnol Oceanogr 38: 1615–1626

Ianora A, Santella L (1991) Diapause embryos in the neustonic copepod *Anomalocera patersoni.* Mar Biol 108: 387–395

Johnson JK (1980) Effects of temperature and salinity on production and hatching of dormant eggs of *Acartia californiensis* (Copepoda) in an Oregon estuary. Fish Bull US 77: 567–584

Kahan D, Berman Y, Bar-El T (1988) Maternal inhibition of hatching at high population densities in *Tigriopus japonicus.* Biol Bull mar biol Lab, Woods Hole 174: 139–144

Kasahara S, Uye S (1979) Calanoid copepod eggs in sea-bottom muds. V. Seasonal changes in hatching of subitaneous and diapause eggs of *Tortanus forcipatus.* Mar Biol 55: 63–68

Kiørboe T, Sabatini M (1994) Reproductive and life cycle strategies in egg-carrying cyclopoid and free-spawning calanoid copepods. J Plankton Res 16: 1353–1366

Landry MR (1983) The development of marine calanoids with comment on the isochronal rule. Limnol Oceanogr 28: 614–624

Li SJ, Chen F, Wang GZ (1989) Studies on the feature of eggs and its hatching rates of some planktonic copepods in Xiamen waters. J Xiamen Univ (nat Sci) 28: 538–543 (in Chinese)

Marcus NH (1979) On the population biology and nature of diapause of *Labidocera aestiva* (Copepoda: Calanoida). Biol Bull mar biol Lab, Woods Hole 157: 297–305

Marcus NH (1984) Variation in the diapause response of *Labidocera aestiva* (Copepoda: Calanoida) from different latitudes and its importance in the evolutionary process. Biol Bull mar biol Lab, Woods Hole 166: 127–139

Marcus NH (1987) Differences in the duration of egg diapause of *Labidocera aestiva* (Copepoda: Calanoida) from the Woods Hole, Massachusetts, region. Biol Bull mar biol Lab, Woods Hole 173: 169–177

Marcus NH (1989) Abundance in bottom sediments and hatching requirements of eggs of *Centropages hamatus* (Copepoda: Calanoida) from the Alligator Harbor region, Florida. Biol Bull mar biol Lab, Woods Hole 176: 142–146

Marcus NH (1990) Calanoid copepod, cladoceran, and rotifer eggs in sea-bottom sediments of northern California coastal waters: identification, occurrence, and hatching. Mar Biol 105: 413–418

Marcus NH (1991) Planktonic copepods in a sub-tropical estuary: seasonal patterns in the abundance of adults, copepodites, nauplii, and eggs in the sea bed. Biol Bull mar biol Lab, Woods Hole 181: 269–274

Marcus NH (1996) Ecological and evolutionary significance of resting eggs in marine copepods: past, present, and future studies. Hydrobiologia 320: 141–152

Marcus NH, Lutz RV (1994) Effects of anoxia on the viability of subitaneous eggs of planktonic copepods. Mar Biol 121: 83–87

Marcus NH, Lutz RV, Burnett W, Cable P (1994) Age, viability, and vertical distribution of zooplankton resting eggs from an anoxic basin: evidence of an egg bank. Limnol Oceanogr 39: 154–158

Miralto A, Ianora A, Poulet SA (1995) Food type induces different reproductive response in the copepod *Centropages typicus*. J Plankton Res 17: 1521–1534

Poulet SA, Ianora A, Miralto A, Meijer L (1994) Do diatoms arrest embryonic development in copepods? Mar Ecol Prog Ser 111: 79–86

Revsbech NP, Jorgensen BB, Blackburn TH (1980a) Oxygen in the sea bottom measured with a microelectrode. Science, NY 207: 1355–1356

Revsbech NP, Sorensen J, Jorgensen BB, Blackburn TH, Lomholt JP (1980b) Distribution of oxygen in marine sediments measured with microelectrodes. Limnol Oceanogr 25: 403–411

Sih A, Moore RD (1993) Delayed hatching of salamander eggs in response to enhanced larval predation risk. Am Nat 142: 947–960

Sullivan BK, McManus LT (1986) Factors controlling seasonal succession of the copepods *Acartia hudsonica* and *A. tonsa* in Narragansett Bay, Rhode Island: temperature and resting egg production. Mar Ecol Prog Ser 28: 121–128

Tranter D, Abraham S (1971) Coexistence of species of Acartiidae (Copepoda) in the Cochin Backwater, a monsoonal estuarine lagoon. Mar Biol 11: 222–241

Uchima M, Hirano R (1986) Food of *Oithona davisae* (Copepoda: Cyclopoida) and the effect of food concentration at first feeding on the larval growth. Bull Plankton Soc Jap 33: 21–28

Uye S (1985) Resting egg production as a life history strategy of marine planktonic copepods. Bull mar Sci 37: 440–449

Uye S, Fleminger A (1976) Effect of various environmental factors on egg development of several species of *Acartia* in Southern California. Mar Biol 38: 253–262