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Experimental Study on Brood Size, Egg Hatchability and Early Development of a Euphausiid *Euphausia pacifica* from Toyama Bay, Southern Japan Sea*

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Abstract

Brood size, egg hatchability and larval development of *Euphausia pacifica* were studied using specimens from Toyama Bay, southern Japan Sea. Brood size ranged from 12 to 296 eggs and was correlated loosely with the size of females. This brood size range is equivalent to 0.4-6.9% of the female's dry weight. Egg hatchability and larval development were examined at 7 graded temperatures (1, 5, 10, 15, 20, 22 and 25℃). Egg hatchability was high (96.3-99.5%) in the temperature range of 1-20℃, but was 70.3% at 22℃, and only 1.1% at 25℃. Development time (D, days) from the Nauplius I stage to Calyptopis I stage was temperature (T, °C) dependent within the range of 5-20℃, and the relationship is expressed as $\log_{10} D = -0.0405\ T + 1.246$. Nauplius I died without further development at 1, 22 and 25℃. Thus, post-embryonic development is more temperature sensitive than embryonic development in *E. pacifica*. The present results are compared with those reported by other workers on the same species and other euphausiid species.

Key words : *Euphausia pacifica*, brood size, egg hatchability, larval development, Toyama Bay

Introduction

A euphausiid *Euphausia pacifica* HANSEN is distributed in the northern North Pacific Ocean, Bering Sea, Okhotsk Sea and Japan Sea (BRINTON 1962), and is an integral prey of many pelagic and demersal fishes, squids and sea birds (OKIYAMA 1965, TAKEUCHI 1972, ODATE 1991, HUNT *et al*. 1993).

Among the 10 euphausiid species known in the Japan Sea, *E. pacifica* is considered to be the most predominant species in the central and southern waters (ENDO and KOMAKI 1979). According to the data of IGUCHI *et al*. (1993) in Toyama Bay, *E. pacifica* spawn in February-June, and newly-hatched larvae reach maturity by the end of the first year. Estimated life span of *E. pacifica* is <21 months. Active growth of *E. pacifica* in Toyama Bay is limited only in February-June during the year. The long period of growth stagnation

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from summer to autumn is due to the fact that they live in food-limited deep waters to avoid the high, lethal temperatures (>20℃) of the euphotic zone during these seasons. *E. pacifica* is a typical herbivore, so their access to the euphotic zone is essential to achieve "normal" growth. Both the spawning season and growth pattern of *E. pacifica* is known to differ considerably from one habitat to another, as reflected in regional variations in oceanographycal conditions and the production cycle (IGUCHI *et al.* 1993).

As an extension of the study of IGUCHI *et al.* (1993), we investigated brood size, egg hatchability and larval development of *E. pacifica* in this study. Special attention was paid to the effects of temperature on egg hatchability and larval development.

Materials and methods

1 Animals

Live specimens of female *E. pacifica* were collected aboard the R. V. *Mizuho-Maru* from an offshore station in Toyama Bay (37°00′ N, 137°14′ E), southern Japan Sea, during March and April 1992. Oblique hauls with a 6-ft Issacs-Kidd Midwater Trawl (IKMT) and a fish-larva net (130cm diameter, 0.5mm mesh) were made from approximately 200m depth to the surface at night. Seawater collected from 250m with Niskin bottles was used throughout the following experiments.

2 Brood size

On board the ship, undamaged females with spermatophores were selected and transferred individually into 900 ml glass bottles filled with natural seawater with a wide bore pipette. To prevent spawned eggs from predation by the females, 1mm mesh netting was fixed 2-3cm above the bottom of each bottle (cf. ROSS and QUETIN 1983, HARRINGTON and IKEDA 1986). The bottles were immersed in a dark tank on the deck of the ship, and kept at a constant temperature (ca. 11℃) by a continuous overflow of surface seawater. The bottles were inspected every morning for eggs. When eggs were found, both the females and eggs were removed from the bottle and preserved in 10% buffered formalin-seawater. The number of spawned eggs was counted and the body length of the females (*BL*: from the anterior tip of the rostrum to the posterior end of the telson) was measured later in the land laboratory. To convert *BL* into dry weight (*DW*, mg) the following equation was established: $\log_{10} DW - 3.156 \log_{10} BL - 3.002$ ($r = 0.991$, $N = 167$). The diameter of the eggs was measured under a microscope to the nearest 0.005mm. Some of the preserved eggs were rinsed briefly with distilled water, blotted on filter paper and dried at 60℃ for a day. *DW* of the eggs was determined using an electrical balance (Mettler M3) to a precision of 1 μg.

3 Egg hatchability

Gravid *E. pacifica* females were brought back to the land laboratory to obtain eggs. A batch of spawned eggs (ca. 10 eggs) was transferred to each 100 ml glass bottle filled with filtered seawater (Whatman GF/F) and incubated at 7 graded temperatures (1, 5, 10, 15, 20, 22 and 25℃). These bottles were checked every morning for nauplii hatched-out. The incubation period extended up to 14 days.

4 Early development

A batch of 10 nauplii obtained from the "egg hatchability" experiment was placed into 100 ml glass bottles filled with filtered seawater and incubated at graded temperatures (1, 5, 10, 15 and 20℃). Preliminary tests indicated the immediate death of nauplii without development at temperatures of 22 and 25℃.

The larval stage of *E. pacifica* includes Nauplius I-II (2 stages), Metanauplius (1 stage), Calyptopis I-III (3 stages) and Furcilia I-VI (6 stages) (SUH *et al*. 1993). The development of newly hatched nauplii (Nauplius I stage) was traced up to the Calyptopis I stage by either collecting casted moult or sacrificing few live specimens at regular intervals. No food was provided as feeding is known to start from Calyptopis I stage in *E. pacifica* (cf. MAUCHLINE and FISHER 1969).

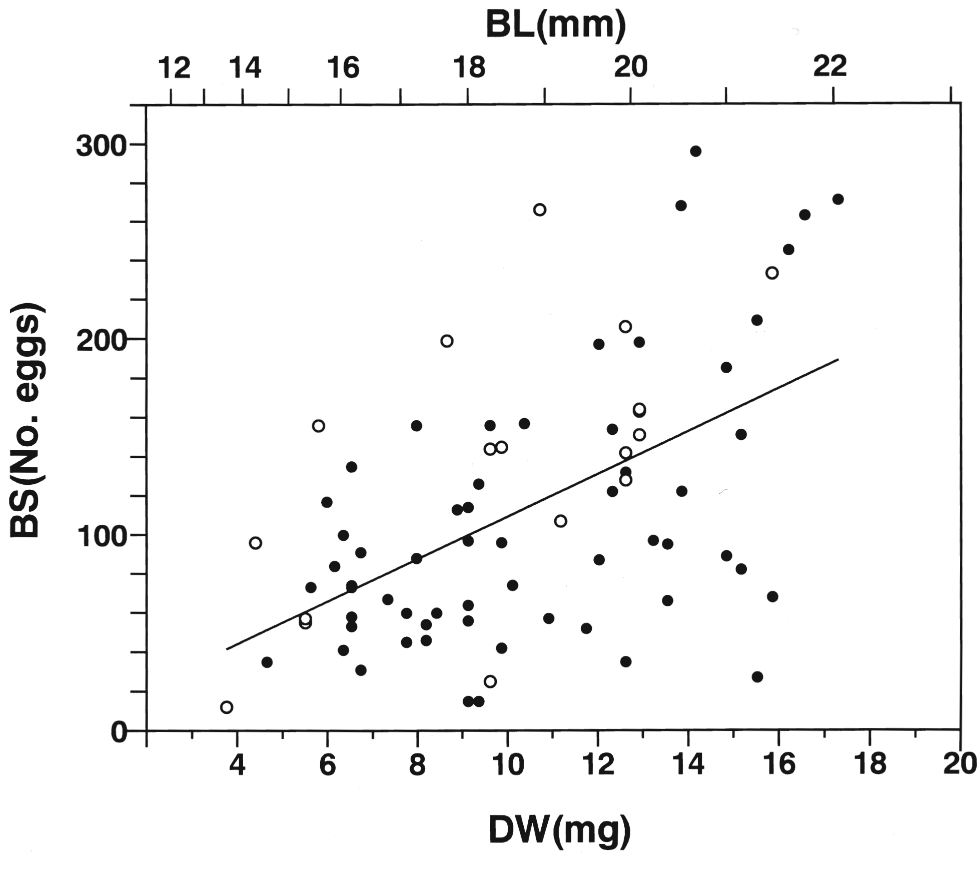

Fig. 1. Relationship between brood size (*BS*: the number of eggs) and dry weight (*DW*: mg) (bottom abscissa) and between brood size and body length (*BL*: mm) (top abscissa) of female *Euphausia pacifica* in Toyama Bay. Open circle: March 1992 data, closed circle: April 1992 data. Regression line is $BS=10.88DW+0.53$.

Table 1. Egg hatchability and its hatching time of *Euphausia pacifica* determined at 7 temperatures. N: number of replicates.

Temperature (°C)	N	Hatchability, mean±SD (%)	Hatching time (days)
1	5	99.5±1.5	7
5	5	96.3±4.6	4
10	5	97.0±3.0	2
15	5	98.2±3.6	1
20	4	98.7±2.3	<1
22	6	70.3±35.6	<1
25	5	1.1±2.3	<1

Results

1 Brood size

The size of spawning females ranged from 13.6 to 22.1mm in BL, or from 3.8 to 17.3mg in DW. Brood size (BS) varied from 12 to 296 eggs. A large scatter of the data was seen in the relationship between BS and DW (or BL) (Fig. 1). The regression equation was computed as $BS=10.88DW+0.53$ ($r=0.539$, $N=77$, $p<0.01$). The correlation coefficient (r) did not improve when BS regressed on BL in stead of DW, i.e. $BS=17.73\ BL-214.76$ ($r=0.525$, $N=77$, $p<0.01$).

Replicated measurements ($N=9$) showed that the DW of the single egg was 3.3(±0.2, SD)μg. Based on this data, the observed brood size is re-expressed as 0.4-6.9% of the female on DW basis. Egg diameter varied from 430 to 505μm, with a mean of 460(±20) μm. Correlation between BL and egg diameter was found to be insignificant ($r=0.037$, $N=25$, $p \gg 0.05$).

2 Egg hatchability

The results of the egg hatchability experiment are summarized in Table 1. Within the temperature range of 1 to 20℃, egg hatchability was consistently high (mean: 96.3-99.5%). The results at 22℃ were highly variable but its mean (70.3%) was appreciably lower than those at 1 to 20℃. The lowest hatchability (1.1%) was recorded for 25℃. Nauplii hatched out almost synchronously. Hatching time decreased with the increase of temperature (7 days at 1℃ to <1 day at ≧20℃).

3 Early development

Newly hatched nauplii lived for few days but all died without developing to the Nauplius II stage at 1℃. Assuming that all specimens sacrificed in the course of experiment were alive at the end of experiment, mean mortality was calculated as 100% at 1℃, 43.2% at 5℃, 35.8% at 10℃, 34.4% at 15℃ and 44.6% at 20℃. The time required to reach Calyptopis I stage was 12.3(±0.5) days at 5℃, 6.2(±0.4) days at 10℃, 4.0(±0.0) days at 15℃ and 3.0(±0.0) days at 20℃ experiments. The relationship between these mean development

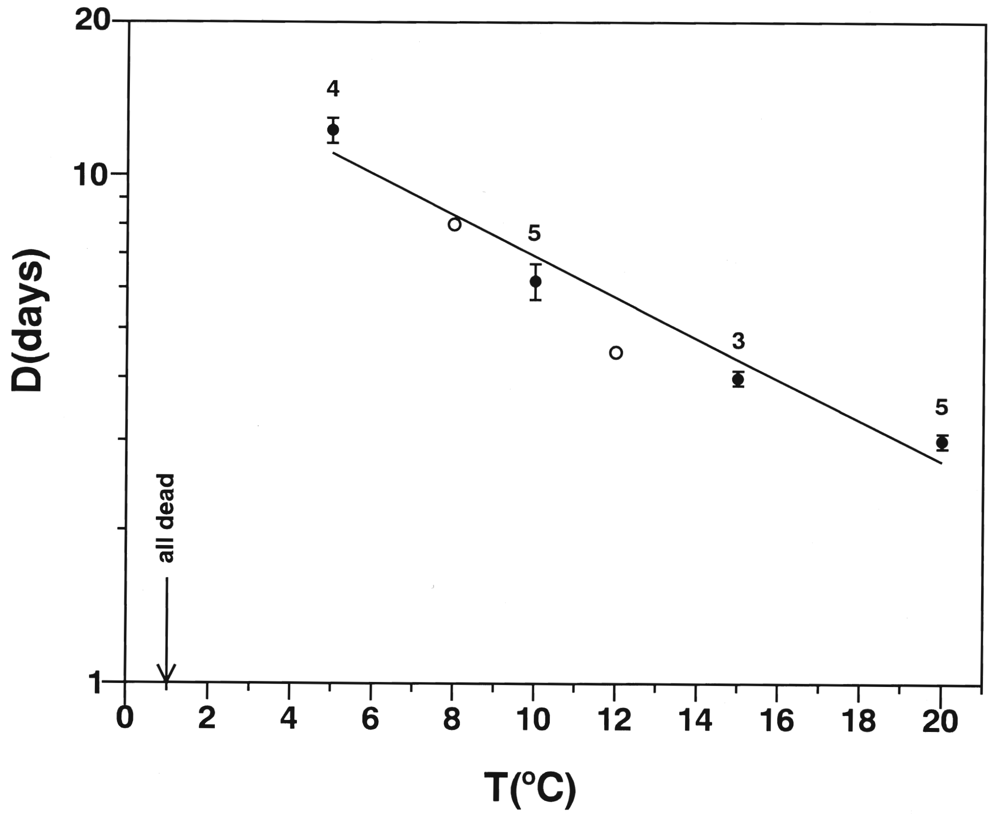

Fig. 2. Relationship between developmental time (D: days) from hatching to Calyptopis I and temperature (T: ℃) of *E. pacifica*. Vertical bar denotes the 95% confidence interval, and numerals numbers, the number of replicates. Regression line is $\log_{10} D = -0.0405T + 1.246$. Two open circles are the data of ROSS (1981) obtained at 8 and 12℃.

times (D, days) and temperature (T, ℃) is linear on a semi-log graph (Fig. 2), and expressed as $\log_{10} D = -0.0405T + 1.246$ ($r = 0.982$, $N = 4$).

The development rate is the reciprocal of the development time ($= 1/D$), hence $\log_{10} (1/D) = 0.0405T - 1.246$. From equation (3) in IKEDA (1985) a Q_{10} is calculated as 2.54 for the growth of early larvae of *E. pacifica.*

Discussion

Brood size of *E. pacifica* has been estimated as 100-1400 eggs, based on ovary volume and the counting of ovarian eggs (PONOMAREVA 1966a, MAUCHLINE and FISHER 1969). Brood size calculated from ovarian volume is less accurate, and counting ovarian eggs often leads to an overestimation as all ovarian eggs are not necessarily released. As a more reliable measure of brood size, ROSS *et al.* (1982) counted the number of eggs actually released in the laboratory by female *E. pacifica* from Puget Sound, Washington, USA. ROSS *et al.*'s (1982) data indicated that the larger the females the greater the brood size, which is in agreement with the present results (Table 2). The greatest brood size recorded by ROSS *et al.* (1982) is 266 eggs, as compared with 296 eggs in this study. From the comparison in Table 2, it is apparent that the brood size of *E. pacifica* in Toyama Bay is comparable to that of the same species in Puget Sound, USA.

The correlation between brood size and female size was found to be rather loose in this study ($r=0.539$), as was also the case in ROSS *et al.* (1982) ($r=0.357$). Clearly, female size alone is not a determining factor of brood size of *E. pacifica*. The breeding history of females and other unknown factors may also be important to determine the brood size of this euphausiid.

LASKER (1966) measured DW of individual eggs for *E. pacifica* from off California. His results (5 μg per egg) were greater than the 3.3 μg determined with preserved eggs in this study. Preservation of eggs in formalin-seawater may be a possible cause for these different results, but our single measurement on fresh eggs yielded 3.8μg per egg, which is still less than that of LASKER (1966). Considerable variations in the size of eggs collected at different times and localities within the same species are well known in euphausiids (MAUCHLINE and FISHER 1969).

Fecundity is defined as the product of brood size and brood number. Assuming no synchrony in the spawning of individual females, ROSS *et al.* (1982) estimated that female *E. pacifica* in Puget Sound release brood every 2-3 days in the early spawning season and every 10 days during the late spawning season. As a result, large individual female may release 25-31 broods over a 2 months' spawning season. BRINTON (1976) observed pulses of egg abundance of *E. pacifica* every 2 months during the upwelling season off southern California and suggested that individual females spawn three times during their lives. However, repeated spawnings of *E. pacifica* have not been verified experimentally. In this study, we maintained post-spawn females in the laboratory to examine this multiple spawning mode of *E. pacifica*. Despite providing sufficient food and continued regular moulting, the re-maturation of the females was never observed. While our results may be argued as laboratory artifacts, the lack of direct evidence makes it difficult to justify this multiple spawning hypothesis for *E. pacifica* in Toyama Bay. It is relevant to note that

Table 2. Comparison of brood size (BS) of three body length (BL) classes of female *Euphausia pacifica*. (A): Present study, (B): ROSS *et al.* (1982). N: number of females.

	BL (mm)					
	Small <17		Medium 17 to <19		Large 19-23	
	(A)	(B)	(A)	(B)	(A)	(B)
N	18	27	26	19	33	13
BS (No. eggs)						
min	12	3	15	3	27	71
max	156	265	266	204	296	234
mean	75	60	95	147	147	132

multiple spawnings of females in a single spawning season has also been speculated in other euphausiids: *Euphausia superba* in the waters around the Antarctic Peninsula (ROSS and QUETIN 1983), *Nyctiphanes australis* off Tasmania (HOSIE and RITZ 1983), and *Euphausia lucens* in the southern Benguela Current (PILLAR and STUART 1988).

This study demonstrated for the first time that the eggs of *E. pacifica* could hatch successfully at a wide temperature range of 1 to 20℃. This temperature range is much wider than 10-16℃ of the spawning season (February through May) in Toyama Bay, but overlaps that to which post-larvae of *E. pacifica* encounter in their lifetime in the same area (cf. IGUCHI *et al*. 1993). ROSS (1981) reported that *E. pacifica* eggs are highly sensitive to handling, and once sorted (handled) eggs show low hatchability (24%) compared with those undisturbed (61%). Although eggs used for the hatchability test of this study are all sorted once, hatchabilities exhibited by those incubated in 1 to 20℃ are even higher than that of undisturbed eggs in ROSS *et al*. (1982), suggesting that the general experimental procedures of this study are superior to those used by ROSS *et al*. (1982), or possible geographical variation in the sensitivity of egg hatching in this species. Egg hatchability of *E. pacifica* recorded in this study is also higher than the 51% (KIKUNO 1981) and 70% (HARRINGTON and IKEDA 1986) observed on *E. superba*.

Successful development from Nauplius I to Calyptopis I stage via Nauplius II and Metanauplius was achieved in a temperature range of 5 to 20℃ without appreciable differences in their mortalities. Development time was temperature dependent, with the shortest (3.0 days) at 20℃ and the longest (12.3 days) at 5℃. In a previous experiment by ROSS (1981), development time to Calyptopis I was 8.0 days at 8℃ and 4.5 days at 12℃. Both data of ROSS (1981) are close to our results (Fig. 2). MAUCHLINE (1977) established a growth model for *E. pacifica* based on moulting interval and moult increment. According to his model, in which the temperature is not specified, the development time from hatching to Calyptopis I is 6.7 days, which corresponds to the time at 10.4℃, as estimated from our regression equation $\log_{10} D = -0.0405T + 1.246$. Temperature dependence of the growth rate as accessed by Q_{10} was 2.54, which falls in the range of Q_{10} (2.1-2.6) reported for the oxygen consumption rate-temperature relationship of this species (SMALL *et al*. 1966, PARANJAPE 1967).

The present data indicate that *E. pacifica* eggs can hatch at 1℃, but its nauplii can not develop further at this temperature. Water as cold as ≦1℃ is widespread at ca. 300-350m depth througout the entire Japan Sea in all season. Therefore, spawned eggs that sank below 300-350m depth are not expected to contribute to the recruitment of *E. pacifica* in the Japan Sea. From this view it is interesting to note that the spawning season for *E. pacifica* distributed in cold waters such as the Okhotsk Sea and off Kamchatcha is spring-early summer (NEMOTO 1957, PONOMAREVA 1966b), thus avoiding sub-zero water temperatures in winter season. Based on field data analysis, BRINTON (1976) considers that a combination of the temperature of 9-16℃ and abundant food phytoplankton is essential to induce spawning of *E. pacifica*. Abundant phytoplankton is needed largely for growth and gonad maturation in adults. As far as embryonic development and early post-embryonic development (Nauplius I to Calyptopis I) are concerned, the present data suggests that *E. pacifica* is capable of tolerating much wider temperatures of 5 to 20℃ without appreciable difference in mortality. While this wide thermal range of early development of *E. pacifica* demonstrated in the present study requires further confirmation on *E. pacifica* in other waters, this may be one of the mechanisms which allow the wide geographical distribution of this euphausiid in the North Pacific.

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富山湾におけるツノナシオキアミの抱卵数，ふ化率と初期幼生発育について

井口　直樹・池田　　勉

富山湾から採集されたツノナシオキアミ（*Euphausia pacifica*)を用いて，産卵数，ふ化率と幼生発育について調べた結果，産卵数は12-296であり，雌の体重と緩やかな相関があった．ふ化率は1-20℃では高く(95%以上)，これ以上の高水温では低かった．ノープリウスＩからカリプトピスＩまでの発育時間（D，日）と水温（T，℃）との関係は5-20℃の範囲で $\log_{10} D=-0.0405\,T+1.246$ であった．これらの結果を他海域の同種やその他のオキアミ類と比較した．