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Reproductive Biology of *Pseudodiaptomus marinus* (Copepoda : Calanoida) in the Inland Sea of Japan[1), 2)]

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Abstract

The rates of daily egg production were estimated for the egg sac-carrying calanoid copepod *Pseudodiaptomus marinus* SATO by an integration of data from field and laboratory investigations. The brood size was positively related to the body size of adult females which was in turn negatively related to the environmental temperature. The development time of embryos (D in days) was a function of temperature (T in °C) as expressed by the equation: $D=448\ (T-1.0)^{-1.80}$. Under excess food conditions, the duration from the hatching of brood to the production of the successive one was generally short and constant (0.14 d) at different temperatures. Assuming sufficient food supply and continuous breeding, the daily reproductive rates of the natural population of *P. marinus* were high from May to October but much lower from January to March. The ratios of daily egg production: female body weight increased linearly with temperature.

The egg sac-carrying calanoid copepod *Pseudodiaptomus marinus* SATO is a perennial in the Inland Sea of Japan, but its population abundance is small compared with the total copepod abundance (HIROTA 1962, 1964). Meanwhile, it has been reported that *P. marinus* is easy to rear under laboratory conditions when compared with other calanoid species (UYE & ONBÉ 1975), and it is therefore listed as one of the copepod species recommended for mass cultivation (OMORI 1974). Later an attempt was made to cultivate this species in the laboratory by examining the effects of physical factors and food conditions on breeding (IWASAKI & KAMIYA 1977). In spite of these researches, our knowledge of the biology of *P. marinus* is still inadequate.

The present paper describes the reproductive rates of *P. marinus*, which are parameters of both population birth rate and population production, by an integration of field and laboratory data. Generally, it is difficult to estimate accurate rates of egg production and total fecundity of copepods in the field. However, the advantages of direct observation of egg sac deposition in the laboratory enabled us to measure the rates of fecundity of *P. marinus* much easier than copepods which shed eggs freely into the water.

Materials and Methods

Regular zooplankton sampling was carried out for a year between 28 April, 1980 and 28 April, 1981, generally at one week intervals. From November, 1980 to March, 1981, the

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2) 瀬戸内海における *Pseudodiaptomus marinus* (橈脚亜綱: カラヌス目) の繁殖生態

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sampling was infrequent (ca. 2 week intervals). *P. marinus* were collected by vertical hauls from the bottom to the surface using a 0.45 m net (94 μm mesh size) fitted with a flowmeter. Samples were taken from the deck of a ferryboat whilst at its berth at Tomo, Fukuyama (Fig. 1), during 20:00–21:00 local time. Since this species is not truely planktonic (IWASAKI & KAMIYA 1977), i.e. it is epibenthic during the daytime and disperses through water column during the nighttime, only the nighttime collections were used in the quantitative survey of abundance. After collection, the zooplankton was immediately preserved in 5 % formalin-seawater solution. Seawater samples were taken by water bottles at the surface, 1-m deep and just above the bottom, and temperature readings were made with a hand-held thermometer.

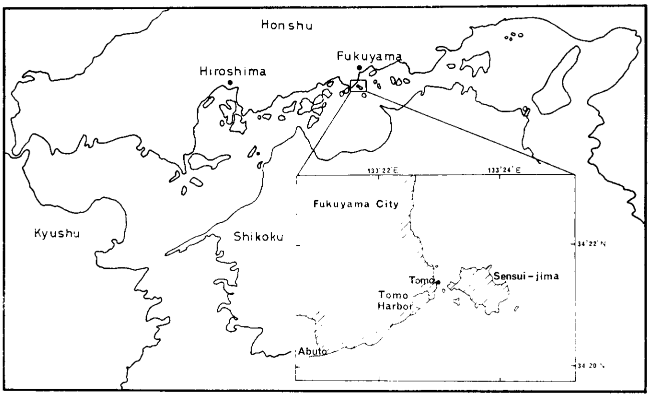

Fig. 1. Location of the sampling station at Tomo, Fukuyama

Following the descriptions of various developmental stages of *P. marinus* by GRINDLEY & GRICE (1969) and UYE & ONBÉ (1975), the individuals belonging to each stage were counted from whole samples or split sub-samples under the dissecting microscope. Their body length, from the front border of the head to the tip of the furca, was measured using an eyepiece micrometer. The number of eggs contained in an egg sac was counted by careful dissection of sacs either carried by the females or after detachment from the female body.

To investigate the egg production of *P. marinus* under laboratory conditions, ovigerous females were isolated from the live zooplankton samples collected at Tomo, and were reared at 20°C under constant illumination (ca. 200 lx). Preliminary experiments demonstrated that the diatom *Thalassiosira decipiens* supported higher egg production than the flagellate *Monochrysis lutheri* and *Dunaliella tertiolecta*. It was also found that the daily egg production, i.e. the number of eggs produced per female per day, increased with the increase of *T. decipiens* concentration and was satiated at concentrations higher than 3,130 cells$\cdot$ml^{-1}. This was equivalent to 272 μg C$\cdot$l^{-1} from the relationship between carbon content and cell volume (STRATHMANN 1967). To investigate the effect of temperature on egg production, ten ovigerous females were individually reared at each of 4 different temperatures (10.3, 14.6, 19.7 and 23.8°C) maintained in a water bath which established a temperature gradient. The animals were contained in glass bottles of 7.5 cm in depth and 4.2 cm in diameter containing 50 ml of glassfiber (Whatman GF/C) filtered seawater and *T. decipiens* at a concentration of ca. 1×10^4 cells•ml^{-1}. Frequent observations (1–2 h intervals) were made from 9:00 to 24:00 on their egg sac production and egg hatching rate during 9 to 14 days of experiment; during the first 2 full days, the monitoring was conducted hourly. After the third day, events that occurred from 0:00 to 9:00 were excluded from the estimate of development time. Animals were transferred to new media every 2 days.

The carbon content of a female with or without an egg sac was measured by Yanagimoto CHN analyzer (MT-3) following the procedures as described by UYE (in press). The carbon weight of a female was subtracted from that of an egg sac-carrying female, and this result was divided by the mean number of eggs in the sac to give the carbon weight of an egg.

Results

Field Observations

1. Seasonal Change in Water Temperature (Fig. 2)

The water temperature rose after the start of investigation to a maximum of 25.3°C at the surface twice on 21 July and 26 August, 1980, and then fell to a minimum of 7.0°C on 2 March, 1981. Thermal stratification was slight in the spring and summer, but in other seasons the temperature was vertically homogeneous. The maximum temperature recorded was lower than the usual annual highest temperature which was 28–29°C (KASAHARA et al. 1975, UYE unpublished), reflecting the unusually lower air temperature in the summer of 1980.

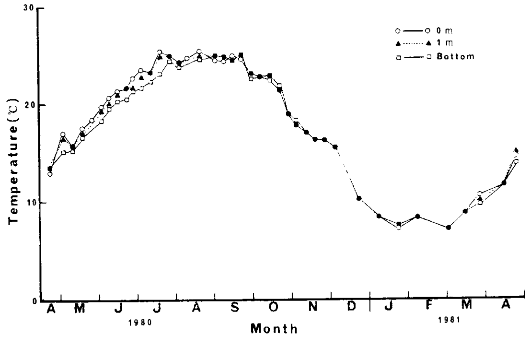

Fig. 2. Seasonal change in temperature at the surface, 1 m deep and just above the bottom (ca. 7 m deep).

2. Seasonal Change in Abundance (Fig. 3)

The total abundance of *P. marinus* fluctuated very much during the study period. Three distinct peaks were found in June–July, August and October, among which the June–July peak was most prominent (9.79×10^2 indiv • m^{-3}). At the time of peak abundance, the copepodites plus adults were much more numerous than the nauplii which were generally less variable in abundance. The copepods were least abundant during the winter, when no nauplii were collected although small numbers of adults and later copepodites were present.

3. Seasonal Change in Sex Ratio (Fig. 4)

Although the sexual differences first appear in CIV stage (UYE & ONBÉ 1975), the seasonal change in sex ratio was investigated in only CV and adult stages. The ratios varied markedly in both stages. General pattern showed that the ratios were nearly equal in both stages when the copepods were in peak abundance, but adult females were more abundant during the winter. Wilcoxon's signed-rank test demonstrated that the female : male ratios were significantly higher for adults, but not for CVs ($p=0.05$). This fact may indicate that adult females live longer than adult males.

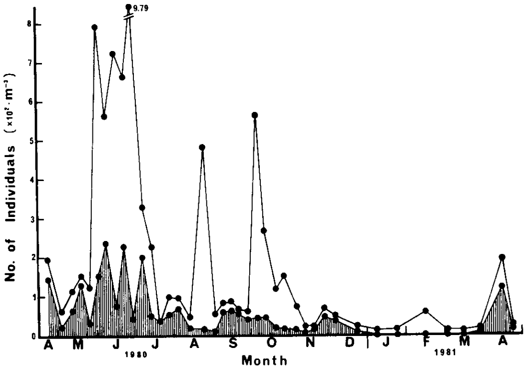

Fig. 3. Seasonal change in abundance of *Pseudodiaptomus marinus*. Hatched area denotes the abundance of nauplii.

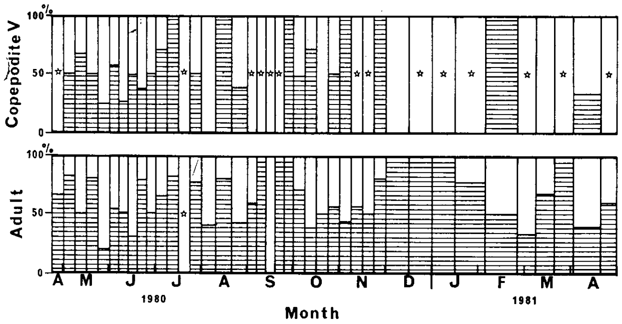

Fig. 4. Seasonal change in sex ratio in adult and copepodite V stages of *Pseudodiaptomus marinus*. Hatched columns show the ratios of female. Asterisks denote absence of copepods.

4. Seasonal Change in Body Length of Adult Females (Fig. 5)

The seasonal variation in body size was observed for most of the developmental stages, among which it was most remarkable for adult females. At the beginning of the investigation, females larger than 1,500 μm in body length were found, and the length decreased from June to September; the minimum size was ca. 1,250 μm. Then length increased to ca. 1,500 μm in December, and was constantly larger during the winter and spring. These patterns were essentially similar to those of many neritic copepod species (DEEVEY 1960, MCLAREN 1963), showing inverse relationship between the size of adult females (L in μm) and the environmental temperature (T in °C), which was described by the equation:

$$L=1{,}630-12.0\,T \quad (r=-0.812)$$

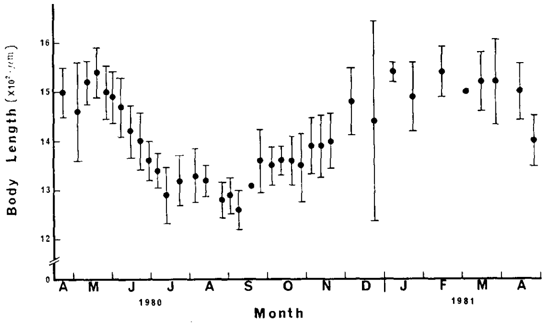

Fig. 5. Seasonal change in the mean body length of adult females of *Pseudodiaptomus marinus*. Verical lines denote 2 standard deviation of the mean.

5. Seasonal Change in Brood Size (Fig. 6)

The pattern of seasonal variation in the brood size was similar to that of the body length of adult females, i.e. the size was largest (ca. 35 eggs per egg sac) in May and smallest in September (ca. 20 eggs). Smaller brood size was also found in the winter in spite of the larger body size of maternal copepods. The lower temperature might hinder attainment of normal egg production in this season. Excepting for the data from January to March, when the water temperature ranged between 7.0 and 10.4°C, a significant negative relationship between the brood size (En) and the surface water temperature (T) was found. The regression equation was:

$$En=47.6-0.998\,T \quad (r=-0.640).$$

When the number of eggs per egg sac (En) was plotted against the body length (L) of adult females (Fig. 7), the following equation was given:

$$En=-57.6+0.0611L \quad (r=0.889).$$

In this case, the data from January to March were again excluded. A similar positive relationship between the number of eggs in a sac and female size has also been known for several species of copepods (MCLAREN 1965, CORKETT & MCLAREN 1969, HOPKINS 1977).

The carbon content of an egg was calculated to be 0.10 μg, and a brood carbon weight was determined by multiplying this by the mean number of eggs per sac. The brood carbon (Ec in μg) was plotted against the respective female body carbon (C in μg) (Fig. 8), and gave the following regression equation:

$$Ec=-0.356+0.477C \quad (r=0.902).$$

From this relationship, an egg sac corresponds to 40-44% of body carbon of the maternal copepods.

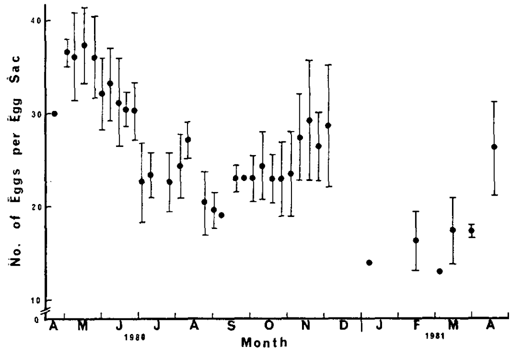

Fig. 6. Seasonal change in the mean number of eggs per egg sac of *Pseudodiaptomus marinus*. Vertical lines denote 2 standard deviation of the mean.

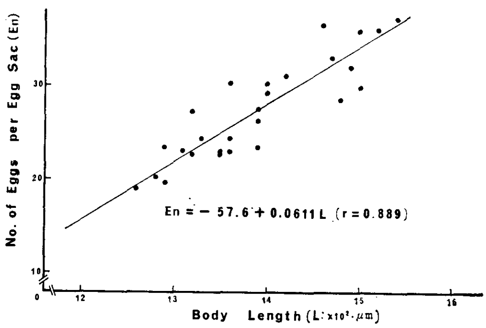

Fig. 7. Relationship between the mean egg number per egg sac and mean body length of adult females of *Pseudodiaptomus marinus*.

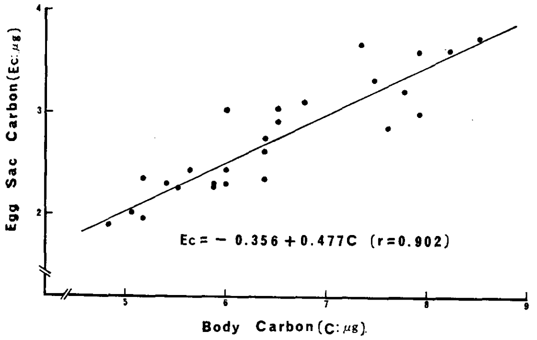

Fig. 8. Relationship between the mean egg sac carbon and mean body carbon of adult females of *Pseudodiaptomus marinus*.

Egg Production and Hatching under Laboratory Conditions

1. General Pattern of Egg Laying and Development

Ripe females of *P. marinus* contained ash-colored eggs both in the ovary and oviduct. The eggs were extruded through the genital pore, presumably being fertilized on the way, and swelled into spheres forming an egg mass which was enveloped within an egg sac membrane. The egg sac was attached to the genital segment until hatching occurred. Freshly laid eggs were an opaque green, but their color became pinkish as they developed. As hatching approached, the nauplius stage I made jerky movements inside the egg membrane. The actual process of hatching of *P. marinus* was not observed in detail, but it might be similar to that described for *Pseudocalanus minutus*, *Oithona similis* and *Tigriopus fulvus* (MARSHALL & ORR 1954). At the time of hatching, it was observed that the females swung their abdomen up and down and also created a backward water current with their swimming legs to assist in the escape of nauplii from the egg sac. In most cases, the hatching was completed within several minutes, and then the empty egg sac was discarded. In some cases, however, the process continued for several hours before all the eggs hatched out. Successive broods were produced usually within a few hours after the hatching of the previous one.

2. Effect of Temperature on Egg Production

Table 1 summarizes the results of egg production of *P. marinus* at 4 different temperatures. The mean brood size for 10 individuals varied from 20.8 at 19.7°C to 26.8 at 14.6°C, but there were no significant differences in the mean brood size at each temperature (Kruskal-Wallis' test, $p=0.05$). The interbrood period increased from 1.8 d at 23.8°C to 8.3 d at 10.3°C as the incubation temperature decreased. The interbrood period was largely determined by the development time of embryos, since there was no significant effect of temperature on the duration between the hatching of an old sac and the production of a new one ($p=0.05$). The duration (D in days) from laying to hatching increased exponentially with the decrease of temperature (T), and was expressed by the following Bělehrådek equation:

$$D=448\ (T-1.0)^{-1.80}\quad (r=0.999).$$

The mean number of eggs produced per female per day was determined by dividing the brood size by the interbrood period, and increased from 3.0 at 10.3°C to 11.8 at 23.8°C.

TABLE 1. EGG PRODUCTION OF *Pseudodiaptomus marinus* FEMALES MAINTAINED AT VARIOUS TEMPERATURES. STANDARD DEVIATIONS ARE SHOWN IN PARENTHESES.

Temperature (°C)	10.3 (1.0)	14.6 (1.0)	19.7 (0.5)	23.8 (0.4)
No. of eggs per sac	23.9 (5.5)	26.8 (4.3)	20.8 (0.5)	21.2 (5.3)
Interbrood period (days)	8.3 (0.33)	4.4 (0.57)	2.4 (0.24)	1.8 (0.23)
Interval between egg sacs (days)	0.15(0.14)	0.15(0.17)	0.13(0.05)	0.13(0.08)
Development time (days)	8.1	4.2	2.3	1.7
No. of eggs produced per female per day	3.0 (0.66)	6.2 (1.1)	8.2 (3.2)	11.8 (3.6)

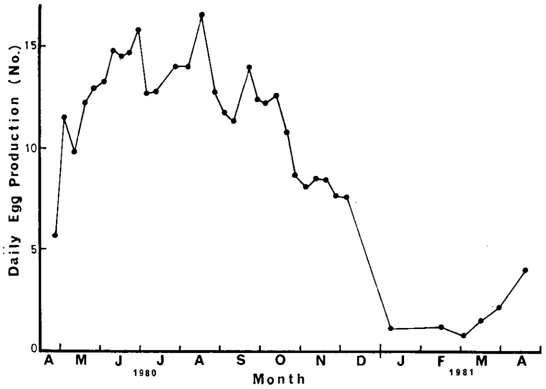

Fig. 9. Seasonal change in daily egg production of *Pseudodiaptomus marinus*.

Daily Egg Production of the Population in Nature

In order to estimate daily egg production in nature, field and laboratory data were integrated. Assuming the population of *P. marinus* was continuously breeding, the daily fecundity was calculated by dividing the mean brood size by the interbrood period. The interbrood period was determined from the development time calculated from the equation, $D=448\,(T-1.0)^{-1.80}$, by substituting the surface water temperature on each sampling occasion, to which was added the constant interval without egg sacs (0.14 d). The seasonal variation in daily egg production is presented in Fig. 9.

The daily egg production was constantly higher from May to October showing 3 apparent peaks in June, August and September. From October to January, the rate was sharply reduced and it was extremely low between January and March. The daily fecundity (*Fn*) was plotted against the water temperature (*T*) at the respective sampling time (Fig. 10). There was a significant linear relationship between them, giving the following equation:

$$Fn=-4.17+0.756T \quad (r=0.919).$$

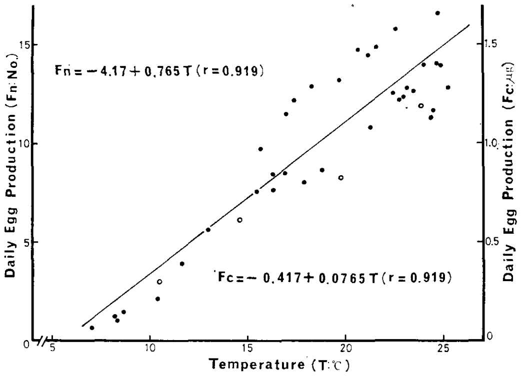

Fig. 10. Relationship between daily egg produciton of *Pseudodiaptomus marinus* and water temperature. The results for laboratory maintained animals (see Table 1) were also shown by open circles.

Discussion

Population abundance is a result of the balance between birth rate and death rate, although the actual process of population dynamics of *P. marinus* has not been studied in the present investigation. However, an indication of population birth rate can be seen from the daily egg production as presented in Fig. 9, which clearly showed that the birth rate was continuously high from May to October. Three peaks of the daily egg production observed in June, August and September, respectively (Fig. 9), were shortly followed by the peak of total abundance (Fig. 3). At the time of peak egg production, the water temperature ranged from 20 to 25°C, which coincided with the temperature range for the highest population growth for *P. marinus* in the laboratory (IWASAKI & KAMIYA 1977). The daily egg production was very low in the winter but was never zero (Fig. 9), although no nauplii were collected by the net at this time (Fig. 3). Probably the hatched larvae did not survive well or the nauplii, if present, were supposed to inactively stay on the sea-bottom. The fecundity of *P. marinus* was probably not limited by food deficiency in the winter because chlorophyll *a* level was not much decreased compared to other seasons in this study site (unpublished).

It has been reported that the reproductive rates of copepods are strongly influenced by food quantity besides temperature (COMITA & COMITA 1966, HARRIS 1977, CHECKLEY 1980, SEKIGUCHI et al. 1980, UYE 1981). These authors have demonstrated that females of copepods lay eggs at a rate in proportion to food abundance below a critical concentration, above this concentration the rate is independent of the food supply. In the present study, the food satiated condition for egg production of *P. marinus* was obtained by feeding with *T. decipiens* at concentrations higher than 3,130 cells$\cdot$ml^{-1}. If the food supply was inadequate, the inter-brood period would be expected to increase since the duration from the hatching of brood to the production of the successive one would presumably be affected by food supply as observed for *Pseudocalanus minutus* (CORKETT & McLAREN 1969). Under excess food conditions, the reproductive rates are largely affected by the temperature (UYE 1981). The daily egg production determined from laboratory maintained females was also plotted against the water temperature (Fig. 10). The plotts were slightly lower than the regression line obtained from the natural population (Fig. 10), which in turn indicates that the food abundance may be in excess in the field and the wild population can always reproduce at maximum rate at a given temperature. In fact, *P. marinus* is epibenthic during the daytime and thus can ingest plentiful food deposited on the sea-bottom.

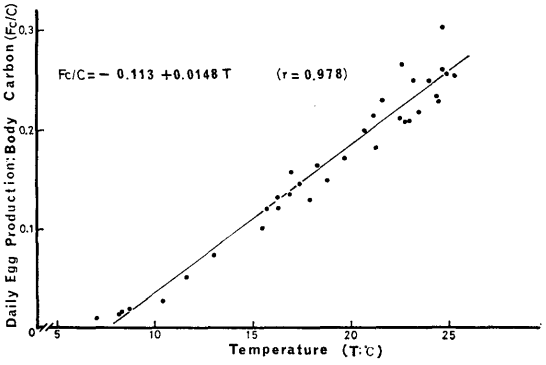

Fig. 11. Relationship between the ratio of daily egg production: female body carbon weight of *Pseudodiaptomus marinus* and water temperature.

Production of a copepod population is attained by somatic production and egg production. Assuming no somatic production for matured females of *P. marinus*, the ratios of daily egg production (*Fc*) to female body weight (*C*), which is equivalent to empirically often used production : biomass (P : B) ratios in the production study, were determined from each sampling date. Carbon based ratios increased linearly with temperature from 0.035 at 10°C to 0.26 at 25°C (Fig. 11). The regression equation was:

$$Fc/C = -0.113 + 0.0148T \quad (r = 0.978).$$

Because of the larger size of adult females, the egg production of *P. marinus* constitutes a large portion of the population production compared with the somatic production attained by juveniles during growth (in preparation).

CORKETT & MCLAREN (1978) calculated the ratios of daily egg production : female body weight in dry weight for *Pseudocalanus elongatus*; they were 0.10, 0.12, 0.14 and 0.16 at 8, 10, 12.5 and 15°C, respectively. The same ratios were calculated to be 0.031, 0.082, 0.19, 0.28 and 0.55 at 5, 10, 15, 20 and 25°C, respectively, on nitrogen basis for *Tigriopus brevicornis* which also lays eggs in an egg sac (HARRIS 1973). The potential of egg production of *P. marinus* may be higher than the former, but apparently lower than the latter. The rate of egg production for sac-carrying copepods is in general lower than that for copepods which lay single eggs, since the fecundity of the former is limited by the number of eggs in the sac and the rate of production of sacs (CORKETT & ZILLIOUX 1975). Such difference of reproductive rate may be closely related to the life history trait of respective species of copepod since the reproduction is balanced by the mortality.

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Literature Cited

CHECKLEY, D. M., JR., 1980. The egg production of a marine planktonic copepod in relation to its food supply: laboratory studies. *Limnol. Oceanogr.*, **25**: 430-446.

COMITA, G. W. & J. J. COMITA, 1966. Egg production in *Tigriopus brevicornis*, pp. 171-185. In *Some Contemporary Studies in Marine Science* (ed. H. BARNES). George Allen & Unwin, London.

CORKETT, C. J. & I. A. MCLAREN, 1969. Egg production and oil storage by the copepod *Pseudocalanus* in the laboratory. *J. exp. mar. Biol. Ecol.*, **3**: 90-105.

CORKETT, C. J. & I. A. MCLAREN, 1978. The biology of *Pseudocalanus*. *Adv. mar. Biol.* **15**: 1-231.

CORKETT, C. J. & E. J. ZILLIOUX, 1975. Studies on the effect of temperature on the egg laying of three species of calanoid copepods in the laboratory (*Acartia tonsa*, *Temora longicornis* and *Pseudocalanus elongatus*). *Bull. Plankton Soc. Japan*, **21**: 77-85.

DEEVEY, G. E., 1960. Relative effects of temperature and food on seasonal variations in length of marine copepods in some eastern American and western European waters. *Bull. Bingham oceanogr. Coll.*, **17**: 54-86.

GRINDLEY, J. R. & G. D. GRICE, 1969. A redescription of *Pseudodiaptomus marinus* SATO (Copepoda: Calanoida) and its occurrence at the island of Mauritius. *Crustaceana*, **16**: 125-134.

HARRIS, R. P., 1973. Feeding, growth, reproduction and nitrogen utilization by the harpacticoid, copepod, *Tigriopus brevicornis*. *J. mar. biol. Ass. U. K.*, **53**: 785-800.

HARRIS, R. P., 1977. Some aspects of the biology of the harpacticoid copepod, *Scottolana canadensis* (WILLEY), maintained in laboratory culture. *Chesapeake Sci.*, **18**: 245-252.

HIROTA, R., 1962. Species composition and seasonal changes of copepod fauna in the vicinity of Mukaishima. *J. oceanogr. Soc. Japan*, **18**: 35-40.

HIROTA, R., 1964. Zooplankton investigations in Hiuchi-nada in the Setonaikai (Inland Sea of

Japan). I. The seasonal occurrence of copepods at the three stations in Hiuchi-nada. *J. oceanogr. Soc. Japan,* **20**: 24-31.

HOPKINS, C. C. E., 1977. The relationship between maternal body size and clutch size, development time and egg mortality in *Euchaeta norvegica* (Copepoda: Calanoida) from Loch Etive, Scotland. *J. mar. biol. Ass. U.K.,* **57**: 723-733.

IWASAKI, H. & S. KAMIYA, 1977. Cultivation of marine copepod, *Pseudodiaptomus marinus* SATO. *Bull. Plankton Soc. Japan,* **24**: 44-54 (In Japanese with English abstract).

KASAHARA, S., S. UYE & T. ONBÉ, 1975. Calanoid copepod eggs in sea-bottom mud. II. Seasonal cycles of abundance in the populations of several species of copepods and their eggs in the Inland Sea of Japan. *Mar. Biol.,* **31**: 25-29.

MARSHALL, S. M. & A. P. ORR, 1954. Hatching in *Calanus finmarchicus* and some other copepods. *J. mar. biol. Ass. U.K.,* **33**: 393-401.

McLAREN, I. A., 1963. Effect of temperature on growth of zooplankton, and the adaptive value of vertical migration. *J. Fish. Res. Bd Can.,* **20**: 685-727.

McLAREN, I. A., 1965. Some relationships between temperature and egg size, development rate, and fecundity of the copepod *Pseudocalanus. Limnol. Oceanogr.,* **10**: 528-538.

OMORI, M., 1973. Cultivation of marine copepods. *Bull. Plankton Soc. Japan,* **20**: 3-11.

SEKIGUCHI, H., I. A. McLAREN & C. J. CORKETT, 1980. Relationship between growth rate and egg production in the copepod *Acartia clausi hudsonica. Mar. Biol.,* **58**: 133-138.

STRATHMANN, R. R., 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. *Limnol. Oceanogr.,* **12**: 411-418.

UYE, S., 1981. Fecundity studies of neritic calanoid copepods *Acartia clausi* GIESBRECHT and *A. steueri* SMIRNOV: a simple empirical model of daily egg production. *J. exp. mar. Biol. Ecol.,* **50**: 255-271.

UYE, S., 1982. Length-weight relationships of important zooplankton from the Inland Sea of Japan. *J. oceanogr. Soc. Japan,* (in press)

UYE, S. & T. ONBÉ, 1975. The developmental stages of *Pseudodiaptomus marinus* SATO (Copepoda: Calanoida) reared in the laboratory. *Bull. Plankton Soc. Japan,* **21**: 65-76.