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SEASONAL ABUNDANCE, POPULATION STRUCTURE, SEX RATIO AND GONAD MATURATION OF *METRIDIA OKHOTENSIS* BRODSKY, 1950 IN THE OKHOTSK SEA: ANALYSIS OF SAMPLES COLLECTED BY PUMPING UP FROM DEEP WATER

BY

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ABSTRACT

In the Okhotsk Sea, the calanoid copepod *Metridia okhotensis* Brodsky, 1950 is the dominant component of zooplankton, accounting for 61% of the annual mean total pelagic copepods. Although this organism is important, little ecological information is available for *M. okhotensis* in the Okhotsk Sea because of the ice cover during winter, which prevents the collection of seasonal samples in this region. Here, we report the seasonal changes in the population structure, sex ratio and female gonad maturation of *M. okhotensis*. The data are from samples collected using water pumped from a depth of 350 m off Rausu Harbour in the Okhotsk Sea at 2-week intervals over a 2.5-year period. Due to the mesh size of the strainer (420 μm), *M. okhotensis* was collected from C3 to adults. The sex ratio of C5 (female : male) was approximately 1 : 1 throughout the year. In contrast, the sex ratio of C6 (adult) showed a clear seasonality, with males (C6M) occurring only from December to May and females (C6F) dominating during the other seasons. The gonad maturation of C6F was scored using five categories, and their composition also showed clear seasonality. From January to April, gonads developed rapidly from stage I (immature) to V (spawning). During the other seasons, the majority of C6F had immature gonads. Based on these data, we conclude that this species likely has a diapause phase for C6F, with immature gonads, and C5M from June to November. Moulting from C5M to C6M began in December. Accompanying the occurrence of C6M, C6F were fertilized from December to January. C6F underwent gonad maturation from January to April and performed primary reproduction from April to May. Thereafter, *M. okhotensis* entered diapause from June to November.

Key words. — Diapause, gonad maturation, *Metridia okhotensis*, Okhotsk Sea, sex ratio

RÉSUMÉ

Dans la mer d'Okhotsk, le copépode calanoïde *Metridia okhotensis* est le composant dominant du zooplancton et représente 61% de la moyenne annuelle des copépodes pélagiques. Bien que

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cet organisme soit important, peu d'informations écologiques sont disponibles sur *M. okhotensis* dans la mer d'Okhotsk en raison de la couverture de glace durant l'hiver, qui empêche la collecte d'échantillons en toutes saisons dans cette région. Nous rapportons ici les changements saisonniers de la structure de la population, du sex-ratio, et de la maturation des gonades femelles de *M. okhotensis*. Les données ont été obtenues à partir d'échantillons collectés par pompage à une profondeur de 350 m au large du port de Rausu dans la mer d'Okhotsk, à des intervalles de deux semaines au cours d'une période de deux ans et demi. En raison du vide de maille utilisé (420 μm) pour la filtration, *M. okhotensis* a été récolté du stade C3 au stade adulte. Le sex-ratio des C5 (femelle : mâle) était approximativement de 1 : 1 tout au long de l'année. En revanche, le sex ratio des C6 (adultes) a montré une saisonnalité évidente, avec des mâles (C6M) présents seulement de décembre à mai et des femelles (C6F) dominantes aux autres saisons. La maturation des gonades des C6F a été classée en cinq catégories, et leur composition a montré aussi une saisonnalité nette. De janvier à avril, les gonades se développaient rapidement du stade I (immature) à V (reproduction). Au cours des autres saisons, la majorité des C6F présentaient des gonades immatures. Selon ces données, cette espèce avait probablement une phase de diapause chez les C6F avec des gonades immatures, et chez les C5M de juin à novembre. La mue de C5M à C6M a commencé en décembre. En présence des C6M, les C6F étaient fécondées de décembre à janvier. Les C6F réalisaient la maturation de leurs gonades de janvier à avril, puis la première reproduction d'avril à mai. Ensuite, *M. okhotensis* entrait en diapause de juin à novembre.

Mots clés. — Mer d'Okhotsk, *Metridia okhotensis*, diapause, maturation des gonades, sex-ratio

INTRODUCTION

The calanoid copepod *Metridia* spp. is distributed worldwide throughout the oceans and is listed as one of the dominant species, particularly among oceanic zooplankton fauna (Mauchline, 1998). In the North Pacific, *Metridia okhotensis* Brodsky, 1950 is listed as a secondary dominant metridinid species, following *M. pacifica* Brodsky, 1950 (Yamaguchi et al., 2002, 2010). The life cycle of *M. okhotensis* was only studied in the Oyashio region (Padmavati et al., 2004).

In the Oyashio region, *M. okhotensis* is consistently found at depth (250-1000 m) throughout the year, except for excursions to the subsurface layer for spawning during the phytoplankton bloom (Padmavati et al., 2004). A two-year life cycle with diapause and C1 and C5 was proposed for *M. okhotensis* (cf. Padmavati et al., 2004). However, there was a low abundance of middle copepodid stages (C2-C4) throughout the year. Padmavati et al. (2004) suggested that the population in the Oyashio region was actually the population originating from the adjacent Okhotsk Sea.

In the Okhotsk Sea, *M. okhotensis* accounts more than half of the numerical abundance in the zooplankton fauna (Asami et al., 2009; Yamaguchi, 2009; Shimada et al., 2012). Thus, their relative importance in the marine ecosystem varies with that in the Oyashio region. While *M. okhotensis* is important in the Okhotsk Sea, little information is available regarding its life cycle there, partially because of the ice cover during winter. Because of this temporal ice cover, the collection of seasonal samples on a fine time-scale is difficult when using ordinary ship observations.

In the town of Rausu, located on the southern coast of the Okhotsk Sea, deep-ocean water was collected by pumping up every day from a depth of 350 m for refrigerating fishery products, food processing and other industries (Takahashi & Yamashita, 2005; Takahashi et al., 2014). Zooplankton contained in the deep-ocean water was removed through a strainer (mesh size 420 μm; Arima et al., 2015, 2016). These zooplankton samples can be found year-round with a fine time resolution; therefore, the analysis of the zooplankton samples collected from the deep-ocean water sampling site provides valuable information on the life cycle of *M. okhotensis* in the Okhotsk Sea.

In this study, we analysed zooplankton samples collected at the Rausu deep-ocean water-sampling site over a 2.5-year period, from June 2007 to December 2009. For *M. okhotensis*, the seasonal changes in the population structure, sex ratio and female gonad maturation were examined to evaluate its life cycle in the Okhotsk Sea. The life-cycle pattern of *M. okhotensis* was compared with that of other *Metridia* species, especially *M. okhotensis* from the Oyashio region (Padmavati et al., 2004).

METHODS

Field sampling

Seasonal zooplankton samples were collected at the Rausu deep-ocean water-sampling site in Rausu Harbour in the Okhotsk Sea (fig. 1). This facility can pump up deep-ocean water through a 268-mm diameter water inlet at a depth of approximately 350 m, 2.8 km from the shore with a water volume flow rate of 106 m^3 h^{-1} (Takahashi & Yamashita, 2005; Takahashi et al., 2014). The deep-ocean water was filtered through a strainer (mesh size 420 μm), and the remaining zooplankton was collected after 3-135 h of pumping of the deep-ocean water (mean: 20.1 h). The volume of filtered water was 315-14 175 m^3. From 20 June 2007 to 18 December 2009, a total of 352 zooplankton samples were collected and preserved with 5-10% borax-buffered formalin. The temperature and volume of the filtered water (m^3) were recorded at the same time.

Analysis of *Metridia okhotensis*

Sixty-two samples were selected in 2-week intervals throughout the sampling period, and subsamples (1-3% of the total) were examined under a microscope with the aid of a wide-bore pipette for each sample. Zooplankton was enumerated by taxon, and *Metridia okhotensis*, the most dominant copepod, were sorted and counted by stage. For adult females, gonad maturation was observed and scored

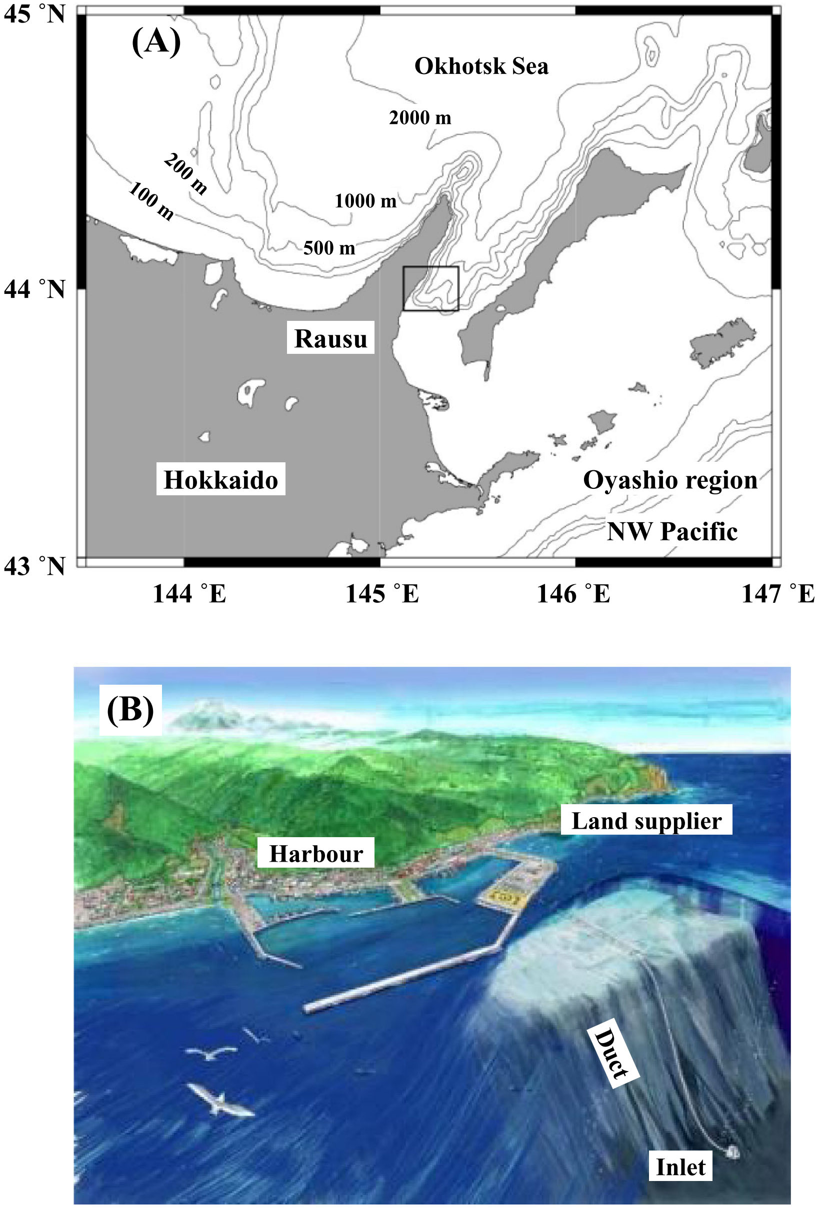


Fig. 1. A, Location of Rausu, eastern Hokkaido; and B, schema of Rausu Deep-Ocean Water sampling site. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/15685403.

into five categories: Immature, Developing-I and II, Mature and Spawning (Tande & Grønvik, 1983).

RESULTS

The temperature of the Rausu deep-ocean water at 350 m depth ranged from 0.5 to 5.1°C, with a maximum during November to early January, and reached a minimum from March to May (fig. 2A). Zooplankton abundance ranged from

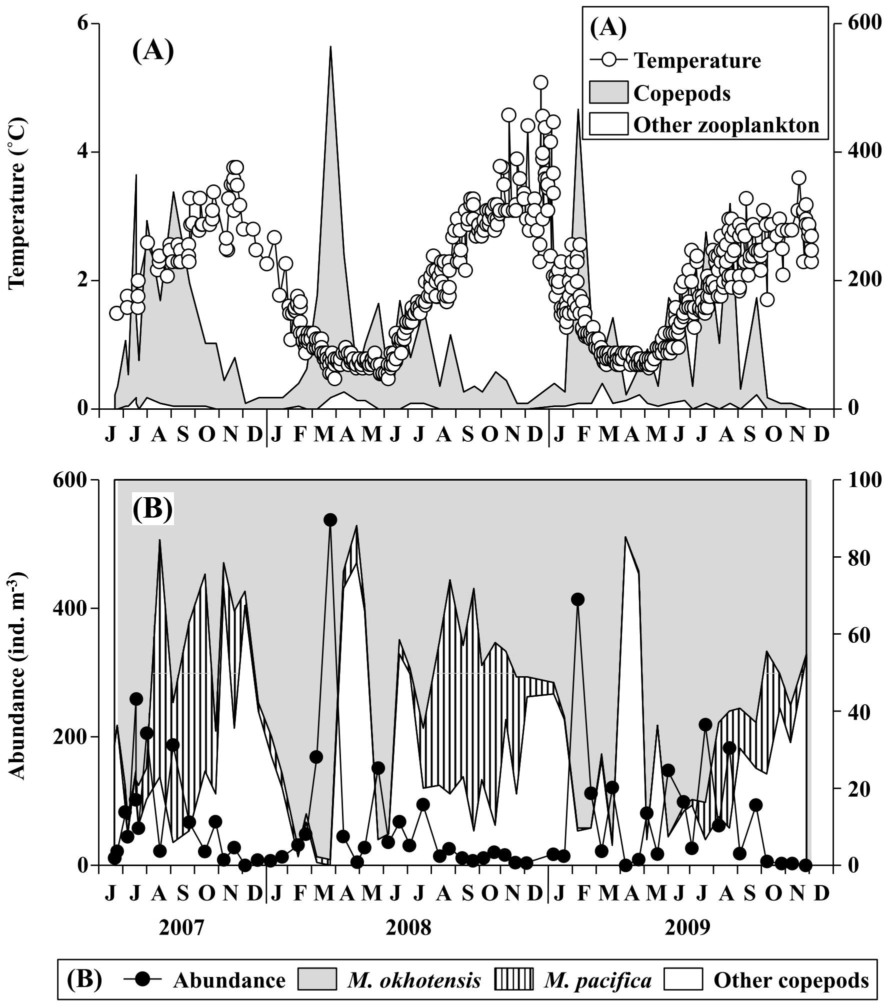


Fig. 2. A, Seasonal changes in temperature and zooplankton abundance (copepods and others) at 350 m and B, copepod abundance and species composition (*Metridia okhotensis* Brodsky, 1950, *Metridia pacifica* Brodsky, 1950 and other copepods) observed in the Rausu deep-ocean water from June 2007 to November 2009.

20 to 550 ind. m^{-3}. Copepods were the dominant taxon throughout the year and accounted for 90% of the annual mean total zooplankton abundance (fig. 2A). Within the copepod community, *Metridia okhotensis* was the most dominant species (annual mean: 61%), followed by the congener *M. pacifica* (12%). The abundance of *M. okhotensis* ranged between 1 and 539 ind. m^{-3} and was high from December to July, with sudden peaks in March 2008 and February 2009 (figs. 2B, 3A), whereas *M. pacifica* was abundant primarily from August to November (fig. 2B). A sudden decrease of the *M. okhotensis* abundance at 350 m was observed in April in 2008 and 2009 (fig. 2B).

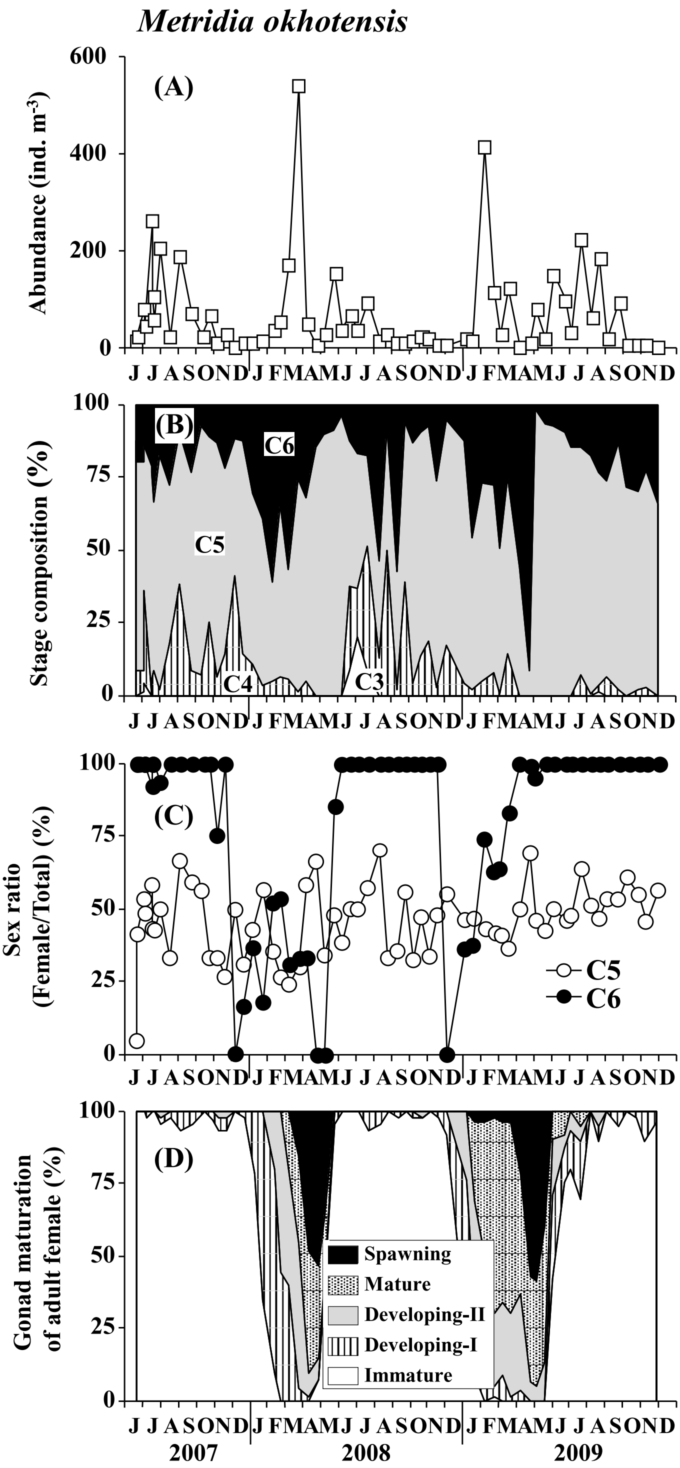
Metridia okhotensis was sampled only from C3 onward, due to the mesh size of the strainer. Throughout the year, C5 dominated at 350 m, whereas C6 was abundant from January to April at this depth. C4 was abundant from June to December, and C3 was observed only from June to July (fig. 3B). The sex ratio of C5 (C5F : C5M) at 350 m was nearly 1 : 1 throughout the year. However, the sex ratio of C6 (adult) showed clear seasonal changes, and C6F predominated from June to November. C6M was present from December to May and was sometimes more abundant than C6F during that period (fig. 3C). The gonad maturation of C6F showed clear seasonality, and the majority of C6F had immature gonads from July to November. Gonad development began rapidly after December and reached a peak of spawning females from April to May (fig. 3D).

DISCUSSION

The most comprehensive schema of the life cycle of *Metridia okhotensis* was obtained in the Oyashio region (Padmavati et al., 2004). *M. okhotensis* reside between 250 m and 1000 m in the Oyashio region, both day and night, throughout the year (Padmavati et al., 2004), and they ascend vertically (50-150 m during the daytime and 0-50 m at night) during April (Takahashi et al., 2009). In the present study, because the sampling inlet of the deep-ocean water was at a depth of 350 m, the peak abundance of *M. okhotensis* from February to March was likely the result of their seasonal upward migration from 250 m to 1000 m to shallower depths ($\leqslant$150 m depth) during the spring.

The shallowest vertical distribution ($\leqslant$150 m) of *M. okhotensis* (in April) probably induced a sudden decrease in their abundance within the deep-ocean (350 m) copepod community (fig. 2B). April corresponded to the period when

Fig. 3. A, Seasonal changes in abundance; B, stage composition; C, sex ratio of C5 and C6; and D, gonad maturation of C6F of *Metridia okhotensis* Brodsky, 1950 in the Rausu deep-ocean water from June 2007 to November 2009.


Metridia okhotensis
(A)
Abundance (ind. m-3)
600
400
200
0
J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
(B)
Stage composition (%)
C6
C5
C4
C3
100
75
50
25
0
(C)
Sex ratio (Female/Total) (%)
C5
C6
(D)
Gonad maturation of adult female (%)
Spawning
Mature
Developing-II
Developing-I
Immature
2007
2008
2009

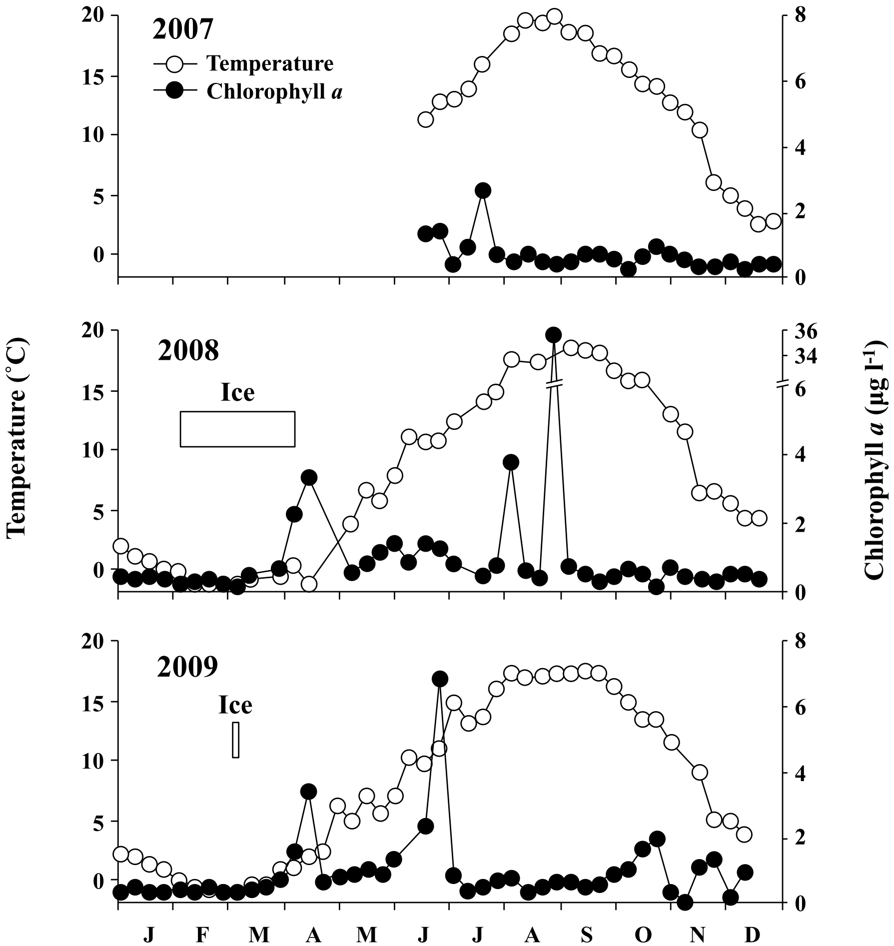


Fig. 4. Seasonal changes in sea surface temperature, chlorophyll *a* and ice coverage (shown as white bars) around the Rausu Harbour from 1 January 2007 to 26 December 2009 determined using satellite images (MODIS-Aqua).

phytoplankton bloom was observed at the sea surface layer (fig. 4). The peak of chlorophyll *a* was initially observed in April in 2008 and 2009. Thus, in April, the upward migration of *M. okhotensis* C6F with matured gonads (fig. 3D) during the spring phytoplankton bloom (fig. 4) suggested that active feeding on phytoplankton and reproduction occurred during this period.

Based on the population structure, sex ratio and gonad maturation data, the seasonal reproduction cycle of *M. okhotensis* was summarized as follows. From summer to autumn (June to November), *M. okhotensis* has a diapause phase for C6F with immature gonads and C5M. These diapause stages (C6F with immature gonads and C5M) are similar to those of *M. pacifica* in the Japan Sea (Hirakawa &

Imamura, 1993). Moulting from C5M to C6M begins in December, and males in C6 outnumber females at this time (fig. 3C). The general patterns of the sex ratio in this study (C5 was nearly equal in female and male abundance, while C6 was skewed for females) correspond well with those of *M. okhotensis* in the Oyashio region (Padmavati et al., 2004). The moulting timing of males, which is much earlier than the primary time for reproduction, corresponds to that of *M. longa* in a Norwegian fjord (Tande & Grønvik, 1983) and *M. pacifica* in the Japan Sea (Hirakawa & Imamura, 1993). Thereafter, C6F develops gonads, matures and spawns from April to May.

Most C6F in the Oyashio region had immature gonads throughout the year (cf. Padmavati et al., 2004). This is in contrast to the seasonal patterns in C6F gonad maturation of the two adjacent regions (Oyashio region and Okhotsk Sea) and strongly suggests that the population of *M. okhotensis* maintains the repetition of their life cycle in the Okhotsk Sea where their nursery grounds are located. However, the immature gonads of C6F throughout the year, and the anomalous population structure (lack of middle copepodid stages C2-C4) in the Oyashio region, suggests that their population in the Oyashio region originated from the Okhotsk Sea (Padmavati et al., 2004).

In the Okhotsk Sea in the present study, because phytoplankton food was scarce at the surface layer from December to March (fig. 4), the gonad maturation of C6F was likely achieved by using stored lipids in their body as energy. For the congener *Metridia longa* (Lubbock, 1854), gonad maturation using stored lipids as energy without feeding was well documented (Tande & Grønvik, 1983).

For the life cycle of *M. okhotensis*, Padmavati et al. (2004) reported a 2-year generation length in the Oyashio region, with a newly recruited population in the spring, an overwinter period at the C1 stage, development to C5 the next spring, a phytoplankton bloom and overwinter again, and moulting to C6F/M and reproduction during the next spring phytoplankton bloom. Two shortcomings were noted in this study. First, the large mesh size of the strainer (420 μm) prevented the collection of early copepodid stages (C1 and C2), and second, the fixed sampling depth (350 m) may have missed events occurring at the surface layer. Because of these shortcomings, we could not modify or add to the 2-year life history hypothesis. Despite these shortcomings, the analysis of the samples collected by pumped-up deep-water clearly revealed key aspects of the life cycle of *M. okhotensis*: diapause with C5M and C6F (June-November), arousal (December), gonad maturation (January-March) and reproduction (April-May) in the southern Okhotsk Sea.

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