



SEASONAL AND INTER-SPECIES COMPARISON OF ASYMMETRY IN
THE GENITAL SYSTEM OF SOME SPECIES OF THE OCEANIC
COPEPOD GENUS *METRIDIA* (COPEPODA, CALANOIDA)

BY

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ABSTRACT

The seasonal and inter-annual changes in the asymmetry of female insemination and the male leg 5 of the planktonic calanoid copepods *Metridia okhotensis* and *M. pacifica* were investigated in the Okhotsk Sea. An inter-species comparison of both parameters was also carried out on seven *Metridia* species collected from oceans throughout the world. For *M. okhotensis* from the Okhotsk Sea, most of the females showed left-side insemination (annual average: 95.7%) and most of the males showed left-side asymmetry (99.7%) of the long inner process of the second exopodal segment in the fifth leg throughout the year. However, sympatric *M. pacifica* showed different ratios of asymmetry for female insemination and male morphotypes with a left : right = 1 : 2 ratio throughout the year. For the seven *Metridia* species from the global oceans treated in this study, ratios of asymmetry for female insemination and male morphotypes were correlated with each other. One-sided insemination (i.e., only left or only right insemination) was a common pattern for various *Metridia* species from global oceans, but their ratios varied by species. Previously, low hatching rates (29-68%) of eggs were reported for various *Metridia* spp. in laboratory experiments, but the reasons for these low rates were unclear. Because each spermatheca of *Metridia* spp. is connected to the oviduct on the same side, either left or right, this suggests that half of the eggs produced by unilaterally inseminated females remain unfertilized. The morphology of the genital structures and literature data of the egg hatching rates of *Metridia* spp. indicate that almost half of the eggs produced by females are not viable and are, thus, wasted.

RÉSUMÉ

Les changements saisonniers et interannuels de l'asymétrie de l'insémination de la femelle et de la patte 5 du mâle des copépodes calanoïdes planctoniques *Metridia okhotensis* et *M. pacifica*

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ont été étudiés dans la mer d'Okhotsk. Une comparaison des deux paramètres entre espèces a été menée également chez sept espèces de *Metridia* collectées dans les océans du monde entier. Chez *M. okhotensis* de la mer d'Okhotsk, la plupart des femelles ont montré une insémination du côté gauche (moyenne annuelle : 95,7%) et la plupart des mâles ont montré une asymétrie gauche (99,7%) du long processus interne du second segment de l'exopodite de la cinquième patte tout au long de l'année. Cependant, les *M. pacifica* sympatriques montraient des rapports différents sur l'année de l'asymétrie d'insémination des femelles et des morphotypes mâles avec un rapport gauche : droite = 1 : 2. Chez les sept espèces de *Metridia* des océans mondiaux traitées dans cette étude, les rapports d'asymétrie d'insémination des femelles et des morphotypes mâles était corrélés entre eux. L'insémination d'un seul côté (uniquement à gauche ou uniquement à droite) est un modèle commun pour diverses espèces de *Metridia* des océans, mais leurs rapports variaient suivant les espèces. Précédemment, des taux faibles d'éclosion des oeufs (29-68%) avaient été observés chez diverses espèces de *Metridia* spp. lors d'expériences en laboratoire, mais les raisons invoquées pour expliquer ces taux faibles étaient peu claires. Chaque spermatheque de *Metridia* spp. est reliée à l'oviducte du même côté, soit gauche, soit droit, et cela suggère que la moitié des œufs produits chez les femelles inséminées unilatéralement (d'un seul côté) restent non fécondés. La morphologie des structures génitales et les données de la littérature concernant les taux d'éclosion des oeufs de *Metridia* spp. indiquent que presque la moitié des œufs produits par les femelles ne sont pas viables et sont ainsi perdus.

INTRODUCTION

Calanoid copepods belonging to the genus *Metridia* are distributed from the epipelagic to abyssopelagic layers of oceans throughout the world, and are often listed as the locally dominant planktonic species (Mauchline, 1998). Because of their ecological importance, the reproduction and egg-hatching rate of *Metridia* spp. from various oceans were studied in laboratory conditions (Halsband-Lenk, 2005; Hopcroft et al., 2005; Plourde & Joly, 2008; Yamaguchi et al., 2010). The egg-hatching rate of *Metridia* spp. (29-68%) is commonly reported to be lower than that of other calanoid copepods. Possible explanations for this lower rate include cannibalism by the adult females (Hopcroft et al., 2005; Plourde & Joly, 2008) or the negative effect of diatoms on egg development (Halsband-Lenk, 2005). However, factors responsible for the low egg-hatching rate of *Metridia* spp. are not fully understood; thus a satisfactory explanation cannot be given, and this discrepancy between high copepod-abundance and the low egg-hatching rate is expressed as the "paradox of the *Metridia* species" (Plourde & Joly, 2008).

Recently, another possible reason for the low egg-hatching rate of *Metridia* spp. was suggested. Ershova & Kosobokova (2012) reported that most of the adult females of *M. longa* (Lubbock, 1854) in the White Sea showed only left-sided insemination (95% of total) and that the morphotype of leg 5 of the males was predominantly left-sided (98%). Concerning the genital system of adult females, *Metridia* spp. have paired lateral spermathecae (left and right) in the genital double-somite, and spermatozoa placed in the spermathecae by males during

copulation are released with eggs after the synchronized opening of the seminal and egg-laying ducts (Cuoc et al., 1997). Because insemination, spawning, and fertilization occur independently of the other side, it is assumed that half of the eggs produced by females in which only one spermatheca bears sperm remain unfertilized; thus, almost half of the eggs produced by females may be not viable and are wasted (Ershova & Kosobokova, 2012). Because previous observations of adult female insemination of *Metridia* spp. based on field samples were limited to *M. longa* in the White Sea, it is not clear whether asymmetrical fertilization is a common phenomenon for other *Metridia* species from oceans throughout the world. Seasonal or inter-annual changes in female insemination within the species also require evaluation.

In the present study, we observed seasonal and inter-annual changes in the insemination of adult females and in morphotypes of the adult male leg 5 of *M. okhotensis* Brodsky, 1950 and *M. pacifica* Brodsky, 1950 in the Okhotsk Sea over two-and-a-half years. The same parameters were observed among seven *Metridia* species distributed in the epipelagic to abyssopelagic (5000 m) layers of oceans throughout the world. An inter-species comparison was also performed for both parameters.

MATERIAL AND METHODS

Field sampling

The seasonal and inter-annual samples were collected at the Rausu deep-ocean water-sampling site in Rausu Harbor in the Okhotsk Sea. This facility can pump-up deep-ocean water from a depth of ca. 350 m, 2.8 km from shore, with a speed of 106 m³/h. This deep-ocean water was filtered through a strainer (mesh size 420 μm), and after 3-135 h of pumping (average 20.1 h), with water filtered between 315 and 14 175 m³, the resulting zooplankton samples were collected and preserved with 5-10% (v/v) formalin. From 20 June 2007 to 28 November 2009 a total of 62 zooplankton samples were collected, and the adult females and males of *M. okhotensis* and *M. pacifica* were sorted from the samples.

For inter-species comparison, we used formalin-preserved samples collected by various research vessels and institutions from the epipelagic to abyssopelagic zones (down to 5000 m) of oceans throughout the world (i.e., the eastern and western North Pacific, the Bering Sea, the Arctic Ocean, the Barents Sea and the Southern Ocean) (fig. 1, table I). From these samples, we sorted the adult females and males of *M. asymmetrica* Brodsky, 1950, *M. curticauda* Giesbrecht, 1889, *M. gerlachei* Giesbrecht, 1902, *M. longa*, *M. okhotensis*, *M. pacifica*, and *M. similis* Brodsky, 1950. For details about sampling date, location, and depth of each sample, see table I.

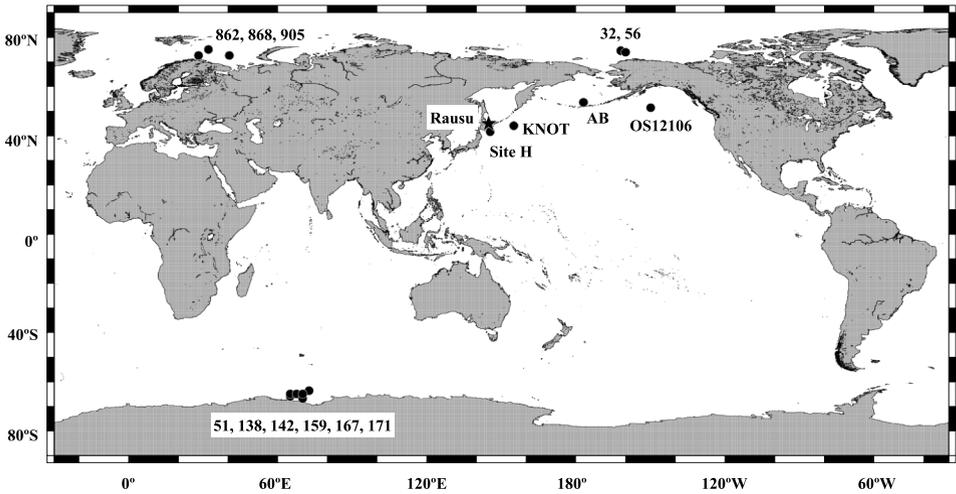


Fig. 1. Location of the sampling stations for various *Metridia* spp. Seasonal/inter-annual samples were collected at Rausu in the Okhotsk Sea from June 2007 to November 2009 (star). Samples for inter-species comparison were collected at 15 stations from oceans throughout the world (circles). See table I for the details of the sampling data.

Morphological observation

According to Ershova & Kosobokova (2012), adult female insemination of *Metridia* species was observed and counted for four categories: non-, left-, right- or bilateral-insemination. The spermathecae were always full of sperm or empty on either or both sides (fig. 2A, B). Unlike other *Metridia* species, both spermathecae of *M. asymmetrica* are located on the left side of the genital double-somite but dorsal and ventral to one another (cf. Brodsky, 1967). For *M. asymmetrica*, the dorsal spermatheca was treated as the left and the ventral spermatheca as the right. For adult males, we observed the position of the long inner process on the second exopodal segment of the fifth leg (i.e., either left, or right) and termed left and right specimens as lefty or righty, respectively (fig. 2C, D) (cf. Ershova & Kosobokova, 2012).

RESULTS

Seasonal and inter-annual changes

Throughout the study period, the location of the spermathecae bearing sperm in adult females and the morphology of the adult male fifth leg of *Metridia okhotensis* and *M. pacifica* in the Okhotsk Sea were constant and showed species-specific values (fig. 3). *M. okhotensis* adult females predominantly showed left-insemination throughout the year (average 95.7%), followed by non-insemination

TABLE I
 Sampling data of various *Metridia* species that were used to observe the insemination of adult females and morphotypes of adult males from oceans throughout the world

Code	Species	Location	Station	Latitude	Longitude	Date	Ship	Sampling gear	Depth (m)
1	<i>Metridia asymmetrica</i> Brodsky, 1950	NW Pacific	Site H	41°30'N	145°47'E	7-8 September 1999	"Tansei-Maru"	Gamaguchi net	0-5000
2	<i>M. asymmetrica</i>		KNOT	44°00'N	155°00'E	19-21 August 1998	"Hakurei-Maru No. 2"	VMPS	0-4000
3	<i>M. asymmetrica</i>		KNOT	44°00'N	155°00'E	19-21 August 1998	"Hakurei-Maru No. 2"	VMPS	0-4000
4	<i>M. asymmetrica</i>	NE Pacific	OS12106	51°15'N	150°00'W	15 July 2012	"Oshoru-Maru"	VMPS	0-3000
5	<i>M. asymmetrica</i>	Bering Sea	AB	53°28'N	177°00'W	14 June 2006	"Oshoru-Maru"	VMPS	0-3000
6	<i>Metridia curticauda</i> Giesbrecht, 1889	NW Pacific	Site H	41°30'N	145°47'E	7-8 September 1999	"Tansei-Maru"	Gamaguchi net	0-5000
7	<i>M. curticauda</i>	NE Pacific	OS12106	51°15'N	150°00'W	15 July 2012	"Oshoru-Maru"	VMPS	0-3000
8	<i>M. curticauda</i>	Bering Sea	AB	53°28'N	177°00'W	14 June 2006	"Oshoru-Maru"	VMPS	0-3000
9	<i>Metridia gertachei</i> Giesbrecht, 1902	Southern Ocean	138	66°00'S	65°00'E	19 January 1984	"Kaiyo-Maru"	WP-2	0-500
10	<i>M. gertachei</i>		142	65°00'S	65°00'E	20 January 1984	"Kaiyo-Maru"	WP-2	0-500
11	<i>M. gertachei</i>		159	65°00'S	67°00'E	23 January 1984	"Kaiyo-Maru"	WP-2	0-500
12	<i>M. gertachei</i>		51	63°43'S	72°30'E	13 December 1983	"Kaiyo-Maru"	MTD	500
13	<i>M. gertachei</i>		167	66°46'S	69°53'E	25 January 1984	"Kaiyo-Maru"	MTD	500
14	<i>M. gertachei</i>		171	65°00'S	70°00'E	26 January 1984	"Kaiyo-Maru"	MTD	500
15	<i>Metridia longa</i> (Lubbock, 1854)	Barents Sea	862	72°30'N	28°00'E	13 September 2001	"G. O. Sars"	MOCNESS	0-283
16	<i>M. longa</i>		868	72°30'N	40°30'E	14 September 2001	"G. O. Sars"	MOCNESS	0-340

TABLE I
(Continued)

Code	Species	Location	Station	Latitude	Longitude	Date	Ship	Sampling gear	Depth (m)
17	<i>M. longa</i>		905	75°04'N	32°01'E	20 September 2001	"G. O. Sars"	MOCNESS	0-233
18	<i>M. longa</i>	Arctic Ocean	32	74°32'N	161°58'W	8 September 2013	"Mirai"	VMPS	0-1000
19	<i>M. longa</i>		56	73°48'N	159°59'W	27 September 2013	"Mirai"	VMPS	0-1000
20	<i>Metridia okhotensis</i> Brodsky, 1950	NW Pacific	Site H	41°30'N	145°47'E	7-8 September 1999	"Tansei-Maru"	Gamaguchi net	0-5000
21	<i>M. okhotensis</i>	Bering Sea	AB	53°28'N	177°00'W	14 June 2006	"Oshoru-Maru"	VMPS	0-3000
22	<i>Metridia pacifica</i> Brodsky, 1950	NW Pacific	Site H	41°30'N	145°47'E	7-8 September 1999	"Tansei-Maru"	Gamaguchi net	0-5000
23	<i>M. pacifica</i>	NE Pacific	OS12106	51°15'N	150°00'W	15 July 2012	"Oshoru-Maru"	VMPS	0-3000
24	<i>M. pacifica</i>	Bering Sea	AB	53°28'N	177°00'W	14 June 2006	"Oshoru-Maru"	VMPS	0-3000
25	<i>Metridia similis</i> Brodsky, 1950	NW Pacific	Site H	40°36'N	147°30'E	28 August 2004	"Tansei-Maru"	Gamaguchi net	3000-5000
26	<i>M. similis</i>		Site H	42°29'N	147°31'E	29 August 2004	"Tansei-Maru"	Gamaguchi net	3000-5000
27	<i>M. similis</i>		Site H	42°28'N	147°33'E	29 August 2004	"Tansei-Maru"	Gamaguchi net	3000-5000
28	<i>M. similis</i>		Site H	42°26'N	147°35'E	29 August 2004	"Tansei-Maru"	Gamaguchi net	3000-5000
29	<i>M. similis</i>		Site H	41°30'N	145°47'E	7-8 September 1999	"Tansei-Maru"	Gamaguchi net	0-5000
30	<i>M. similis</i>		KNOT	44°00'N	155°00'E	19-21 August 1998	"Hakurei-Maru No. 2"	VMPS	0-4000
31	<i>M. similis</i>	NE Pacific	OS12106	51°15'N	150°00'W	15 July 2012	"Oshoru-Maru"	VMPS	0-3000
32	<i>M. similis</i>	Bering Sea	AB	53°28'N	177°00'W	14 June 2006	"Oshoru-Maru"	VMPS	0-3000

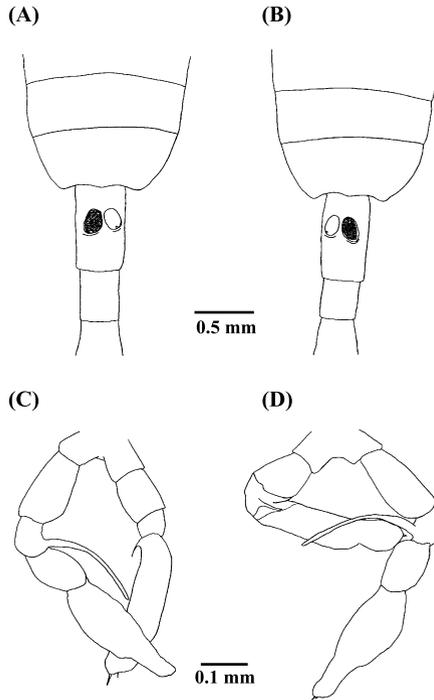


Fig. 2. *Metridia okhotensis* Brodsky, 1950; A, C6F spermathecae with left insemination; B, spermathecae with right insemination; C, C6M fifth leg of lefty male; D, fifth leg of righty male.

(3.4%), bilateral-insemination (0.8%) and right-insemination (0.1%) (fig. 3A). Most adult males of *M. okhotensis* were classified as left-insemination (99.7%), with very few classified as right-insemination (0.3%; fig. 3B). For the sympatric *M. pacifica*, adult females showed a different asymmetry ratio; the annual average of left-insemination and right-insemination were 27.2 and 62.3%, respectively, followed by non-insemination (7.1%) and bilateral-insemination (3.3%) (fig. 3C). The percentages of lefty and righty adult males were 31.7 and 68.3%, respectively, which is comparable to the left vs. right percentages found in the adult females (fig. 3D).

Inter-species comparison

Seven *Metridia* species from oceans throughout the world showed a positive correlation between left-insemination in adult females and the lefty morphotype of the adult males (fig. 4). For all *Metridia* species, only one spermatheca was inseminated in most samples (table II). The ratio of asymmetric insemination of adult females varied by species. Right-insemination was dominant for *M. curticauda*, *M. gerlachei* and *M. asymmetrica* (as the ventral spermatheca) whereas left insemination was common for *M. longa*, *M. okhotensis*, *M. pacifica* and *M.*

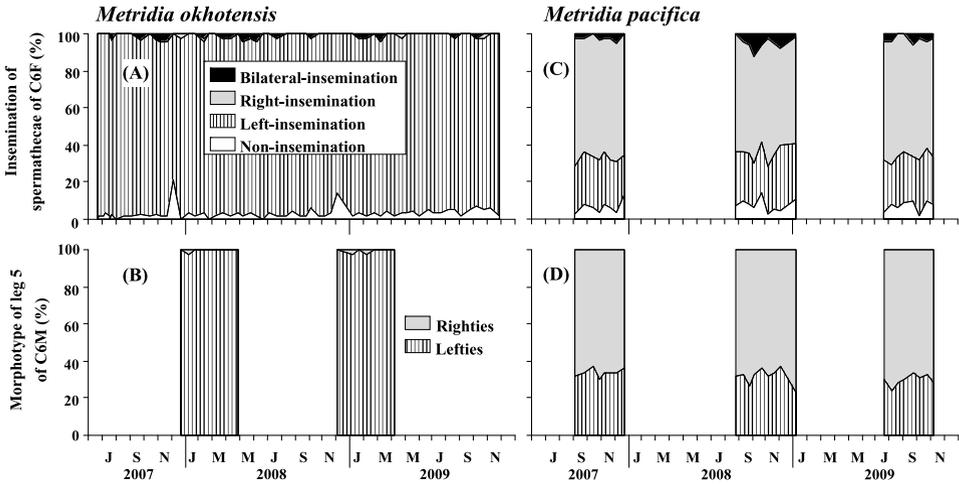


Fig. 3. A, B, *Metridia okhotensis* Brodsky, 1950; and, C, D, *Metridia pacifica* Brodsky, 1950; A, C, seasonal/inter-annual changes in the insemination of spermathecae of adult females (C6F); B, D, morphotype of leg 5 of adult males (C6M); all of these samples were from Rausu in the Okhotsk Sea, and taken from June 2007 to November 2009.

similis. It should be noted that the asymmetric skewness was more moderate for *M. gerlachei*, *M. longa* and *M. pacifica*. Thus, these three epipelagic species showed a minor-sided insemination of 21-34% of the total adult female population (table II).

DISCUSSION

Adult males and some females of metridinid copepods are characterized by asymmetrical morphology. For *Pleuromamma*, or “looking-glass copepods”, also

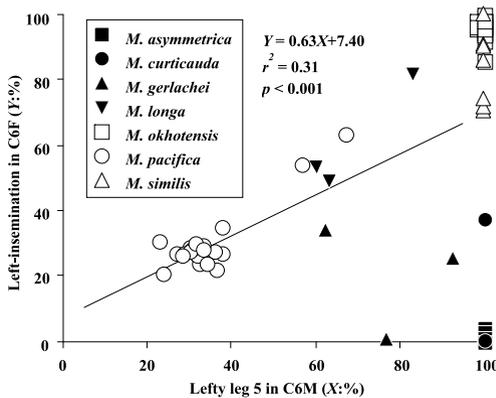


Fig. 4. Relationship between the composition of left-insemination in adult females (C6F) and composition of lefty leg 5 in adult males (C6M) for various *Metridia* species from oceans throughout the world.

TABLE II

Inter-species comparison of the insemination (ins.) of adult females of various *Metridia* species from oceans around the world

Species (habitat depth)	Code (<i>n</i>)	Percentage of total adult females			
		Non-ins.	Left-ins.	Right-ins.	Bilateral-ins.
<i>Metridia asymmetrica</i> Brodsky, 1950 (D)	1 (25)	20.0	4.0	64.0	12.0
	2 (5)	10.3	0.8	81.7	7.4
	3 (33)	12.5	0	87.5	0
	4 (126)	0	0	100	0
	5 (8)	6.1	3.0	87.9	3.0
	Mean	9.8	1.6	84.2	4.4
<i>Metridia curticauda</i> Giesbrecht, 1889 (D)	6 (8)	50.0	37.5	12.5	0
	7 (17)	0	0	94.1	5.9
	8 (8)	12.5	0	87.5	0
	Mean	20.8	12.5	64.7	2.0
<i>Metridia gerlachei</i> Giesbrecht, 1902 (E)	9 (31)	16.1	35.5	48.4	0
	10 (105)	16.2	45.7	38.1	0
	11 (226)	10.2	45.6	44.2	0
	12 (24)	0	33.3	62.5	4.2
	13 (4)	25.0	25.0	50.0	0
	14 (3)	0	0	100	0
	Mean	11.2	30.9	57.2	0.7
<i>Metridia longa</i> (Lubbock, 1854) (E)	15 (9)	0	88.9	11.1	0
	16 (56)	0	50.0	46.4	3.6
	17 (24)	0	54.2	41.7	4.2
	18 (3)	0	100	0	0
	19 (17)	11.8	82.4	5.9	0
	Mean	2.4	75.1	21.0	1.5
<i>Metridia okhotensis</i> Brodsky, 1950 (E)	20 (40)	15.0	80.0	0	5.0
	21 (1)	0	100	0	0
	Mean	7.5	90.0	0	2.5
<i>Metridia pacifica</i> Brodsky, 1950 (E)	22 (64)	12.5	54.7	32.8	0
	23 (23)	4.2	54.2	41.7	0
	24 (74)	5.4	63.5	28.4	2.7
	Mean	7.4	57.5	34.3	0.9
<i>Metridia similis</i> Brodsky, 1950 (D)	25 (12)	0	100	0	0
	26 (35)	11.4	85.7	0	2.9
	27 (31)	9.7	90.3	0	0
	28 (40)	2.5	90.0	5.0	2.5
	29 (10)	30.0	70.0	0	0
	30 (5)	26.3	73.7	0	0

TABLE II
(Continued)

Species (habitat depth)	Code (<i>n</i>)	Percentage of total adult females			
		Non-ins.	Left-ins.	Right-ins.	Bilateral-ins.
	31 (7)	40.0	60.0	0	0
	32 (19)	0	71.4	14.3	14.3
	Mean	15.0	80.1	2.4	2.5

For codes, see table I. Abbreviations: *n*, number of observed specimens, the grand mean was calculated for each species; D, deep-sea zone; E, epipelagic zone.

belonging to the Metridinidae, the composition of asymmetrical morphology and their vertical and seasonal changes were observed by Ferrari (1985) and Ferrari & Hayek (1990). However, the ultimate causes of the asymmetry and their consequences, as well as their function for population maintenance, remained unsolved problems (Ferrari & Hayek, 1990). Concerning the mating behaviour of *Metridia*, males clasp the caudal rami or urosome of females (cf. Ohtsuka & Huys, 2001). Because the spermatophores of *Metridia* are located at either the left, or the right side of the fixation site around the copulatory pore of the females (Cuoc et al., 1997), males use the inner process of leg 5 to attach the spermatophore. The attachment site (left or right) varies with lefty or righty males (Ershova & Kosobokova, 2012). Thus bilateral insemination of females may be the result of multiple copulations with both lefty and righty males, as mentioned by Cuoc et al. (1997) and Ershova & Kosobokova (2012).

The special features of asymmetry in female insemination and the male morphology of *Metridia* species evaluated in this study can be summarized in the following four points: (1) The ratios of asymmetric female insemination and male morphotype were found to be correlated (fig. 4). (2) Within a species, both female and male ratios of asymmetry showed little seasonality throughout the year (fig. 3). (3) The ratio of asymmetry of female insemination was common for seven *Metridia* species from oceans throughout the world, but the ratios varied among species (table II). (4) For the epipelagic species in each ocean, for which insemination occurred on both sides, the lower percentage did show appreciable variation (21-34%) (table II). Among these four features, the former two are also expressed in *M. longa* in the White Sea (Ershova & Kosobokova, 2012). For the latter two features, regional variations were recognized for epipelagic species: right-inseminated females were dominant among *M. pacifica* in the Okhotsk Sea (62%, fig. 3C), whereas left-inseminated females were more abundant in the North Pacific and Bering Sea (54-64%, table II). The variation in asymmetry within populations of the epipelagic species in each region (e.g., *M. gerlachei*, *M. longa* and *M. pacifica*)

could be interpreted as a reflection of the presence of regional variability in the asymmetry of the epipelagic species.

Concerning the adult female genital system of calanoid copepods, there is a pair of lateral gonads connected by oviducts to the lateral, paired gonopores (Bradford-Grieve et al., 2010). To increase the effectiveness of the fertilization of eggs, adult females of calanoid copepods have seminal receptacles to deposit spermatozoa, and/or a genital operculum, the large board covering the gonopores (Bradford-Grieve et al., 2010). Within calanoid copepods, the genital systems of adult females are morphologically divided into three types: those containing both genital operculum and spermathecae (e.g., Calanoidea); those with no genital operculum, but with spermathecae (e.g., Arietelloidea); and those with genital operculum, but without spermathecae (e.g., Diaptomoidea, except Acartiidae) (Bradford-Grieve et al., 2010). The Metridinidae treated in this study are classified as Arietelloidea (i.e., no genital operculum, while having spermathecae).

For the species with a genital operculum, the fertilization of eggs occurs in the genital atrium, which is the space between the genital operculum and the gonopores (Barthélémy et al., 1998; Bradford-Grieve et al., 2010). Metridinidae accumulate spermatozoa in spermathecae through the copulatory pores, and fertilization occurs outside the body after the synchronized opening of gonopores (eggs) and the seminal ducts (spermatozoa) (Cuoc et al., 1997). Because the spermathecae and gonopores on both sides are laterally separated, insemination, spawning, and fertilization of *Metridia* species are thought to occur on each side independently of the other side (Cuoc et al., 1997). This suggests that half of the female's eggs produced on the side that has not been inseminated remain unfertilized, and thus, almost half of the eggs produced by females may not be viable and are hence wasted (Ershova & Kosobokova, 2012).

The genital system of *Acartia* is superficially similar to that of *Metridia* (i.e., no genital operculum and the presence of right and left seminal receptacles (= spermathecae); Barthélémy, 1999). However, the egg-hatching rate of *Acartia* is reported to be normal (86-99%, Castro-Longoria, 2003; table III), which may be related to the copulatory behaviour of *Acartia*. Thus, *Acartia* males place a spermatophore at the fixation site located midway between the left and right copulatory pores of females, and this configuration is associated with a simultaneous insemination of the two receptacles due to the bifid structure of the spermatophore (Steuer, 1923; Barthélémy, 1999). This is why a normal egg-hatching rate is observed for *Acartia*, even though the genital system is superficially similar to that of *Metridia*. These modes thus differ from those observed in *Metridia*, in which the insemination of the two receptacles implies the occurrence of two separate matings (Cuoc et al., 1997).

TABLE III
Summary of egg-hatching rates of *Metridia* spp. from oceans around the world

Species	Location	Mean hatching rate (%)	Reference
<i>Metridia longa</i> (Lubbock, 1854)	Lower St Lawrence Estuary	29-51	Plourde & Joly (1998)
<i>Metridia pacifica</i> Brodsky, 1950	Dabob Bay, Washington	40-68	Halsband-Lenk (2005)
	Gulf of Alaska	(51)	Hopcroft et al. (2005)
	Oyashio region	(67)	Yamaguchi et al. (2010)
<i>Acartia</i> spp.	South coast of England	86-99	Castro-Longoria (2003)
<i>Centropages hamatus</i> (Lilljeborg, 1853)	Long Island Sound	65-95	Tang et al. (1998)
<i>Calanus</i> spp.	Western Greenland	75-93	Jung-Madsen & Nielsen (2015)
<i>Neocalanus</i> spp.	Oyashio region	81-93	Saito & Tsuda (2000)
<i>Temora longicornis</i> (Müller, O. F., 1785)	Long Island Sound	70-90	Tang et al. (1998)

For comparison common egg-hatching rates of other copepod species are also summarised.

In this study, it was revealed that the insemination of only one of two spermathecae on an adult female was a common phenomenon for various *Metridia* species from oceans throughout the world (table II). Because *Metridia* is a dominant zooplankton species found in various oceans, many laboratory experiments on the egg-hatching rate have been performed (table III). Commonly in these studies, an extremely low egg-hatching rate has been reported. Thus, 49% of *M. pacifica* eggs did not develop normally, and abnormal eggs without membranes were also observed (Hopcroft et al., 2005). For *M. pacifica* in Dabob Bay, Washington, experiments were conducted with specimens collected from the surface and deep layers by applying two types of incubation methods (incubations by multiple wells and chambers); however, egg-hatching rate remained low (40-68%) throughout the experiments and abnormal eggs with broken membranes were observed (Halsband-Lenk, 2005). In the Oyashio region, during spring, the mean egg hatching rate of *M. pacifica* was reported as 67% and that of *M. okhotensis* varied greatly (Yamaguchi et al., 2010). The cause of the low egg-hatching rate of *Metridia* species has often been discussed; however, it has long remained an unsolved problem. Egg cannibalism by adult females may be a possible cause. Plourde & Joly (2008) compared egg-hatching rates under the presence or absence of an egg-separating mesh (which has the function of preventing egg cannibalism by adult females) for *Calanus finmarchicus* (Gunnerus, 1760) and *M. longa*. The resulting egg-hatching rate of *C. finmarchicus* was reported to be 74-81%, whereas that of *M. longa* remained low

(29-51%), even in the presence of an egg-separating mesh, which suggests that cannibalism by adult females is not the (main) cause of the low hatching rate of species of *Metridia* (Plourde & Joly, 2008).

As an alternative hypothesis for *Metridia*, mate choice during copulation (a female produces eggs in only her left or right ovary/oviduct, not both) may be considered. At the time of mating, a female with left ovary/oviduct egg production will select only a lefty male who can inseminate her left spermatheca. A female with right ovary/oviduct egg production will select only a righty male who can inseminate her right spermatheca. However, this hypothesis may not be the case. For all *Metridia* females treated in this study, we confirmed that both of the examined left and right ovaries/oviducts of females were filled with eggs. Gonad maturation diagrams are reported for several *Metridia* species (Tande & Grønvik, 1983; Batchelder, 1986). From these diagrams it becomes clear, that both the left and right ovary and oviduct were functional and filled with eggs. Thus, this alternative hypothesis may not be a correct description of the actual situation in *Metridia*.

CONCLUSION

In this study, unilateral insemination (e.g., left or right) was common for adult females of various *Metridia* species from oceans throughout the world. Females of species of *Metridia* have each spermatheca connected to only one of two lateral oviducts. Thus, both fertilization and spawning on one side may occur independently of the other side (Cuoc et al., 1997). This suggests that only half of the eggs produced by females of *Metridia* spp. that are inseminated on only one side remain unfertilized (Ershova & Kosobokova, 2012). Thus, the low egg-hatching rate of *Metridia* species (29-68%) found in laboratory experiments (e.g., the paradox of *Metridia*, Plourde & Joly, 2008) might be a reflection of this morphology of the female genital system. To confirm this hypothesis, egg hatching experiments with the separation of adult female insemination (i.e., non-, left-, right-, or bilateral-insemination) are required for future study.

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