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SEASONAL AND INTER-SPECIES COMPARISON OF ASYMMETRY IN THE GENITAL SYSTEM OF SOME SPECIES OF THE OCEANIC COPEPOD GENUS *METRIDIA*

BY

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Short title: ASYMMETRY IN THE GENITAL SYSTEM OF *METRIDIA* SPP.

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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.

30 is connected to the oviduct on the same side, either left or right, this suggests that half of
31 the eggs produced by unilaterally inseminated females remain unfertilized. The
32 morphology of the genital structures and literature data of the egg hatching rates of
33 *Metridia* spp. indicate that almost half of the eggs produced by females are not viable
34 and are, thus, wasted.

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 & July, 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

52 (95% of total) and that the morphotype of leg 5 of the males was predominantly
53 left-sided (98%). Concerning the genital system of adult females, *Metridia* spp. have
54 paired lateral spermathecae (left and right) in the genital double-somite, and
55 spermatozoa placed in the spermathecae by males during copulation are released with
56 eggs after the synchronized opening of the seminal and egg-laying ducts (Cuoc et al.,
57 1997). Because insemination, spawning, and fertilization occur independently of the
58 other side, it is assumed that half of the eggs produced by females in which only one
59 spermatheca bears sperm remain unfertilized; thus, almost half of the eggs produced by
60 females may be not viable and are wasted (Ershova & Kosobokova, 2012). Because
61 previous observations of adult female insemination of *Metridia* spp. based on field
62 samples were limited to *M. longa* in the White Sea, it is not clear whether asymmetrical
63 fertilization is a common phenomenon for other *Metridia* species from oceans
64 throughout the world. Seasonal or inter-annual changes in female insemination within
65 the species also require evaluation.

66 In the present study, we observed seasonal and inter-annual changes in the
67 insemination of adult females and in morphotypes of the adult male leg 5 of *M.*
68 *okhotensis* Brodsky, 1950 and *M. pacifica* Brodsky, 1950 in the Okhotsk Sea over
69 two-and-a-half years. The same parameters were observed among seven *Metridia*

70 species distributed in the epipelagic to abyssopelagic (5000 m) layers of oceans
71 throughout the world. An inter-species comparison was also performed for both
72 parameters.

73 MATERIAL AND METHODS

74 Field sampling

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

84 For inter-species comparison, we used formalin-preserved samples collected by
85 various research vessels and institutions from the epipelagic to abyssopelagic zones
86 (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.

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(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

120 correlation between left-insemination in adult females and the lefty morphotype of the
121 adult males (fig. 4). For all *Metridia* species, only one spermatheca was inseminated in
122 most samples (table II). The ratio of asymmetric insemination of adult females varied by
123 species. Right-insemination was dominant for *M. curticauda*, *M. gerlachei* and *M.*
124 *asymmetrica* (as the ventral spermatheca) whereas left insemination was common for *M.*
125 *longa*, *M. okhotensis*, *M. pacifica* and *M. similis.* It should be noted that the asymmetric
126 skewness was more moderate for *M. gerlachei*, *M. longa* and *M. pacifica*. Thus, these
127 three epipelagic species showed a minor-sided insemination of 21-34% of the total adult
128 female population (table II).

129 DISCUSSION

130 Adult males and some females of metridinid copepods are characterized by
131 asymmetrical morphology. For *Pleuromamma*, or "looking-glass copepods", also
132 belonging to the Metridinidae, the composition of asymmetrical morphology and their
133 vertical and seasonal changes were observed by Ferrari (1985) and Ferrari & Hayek
134 (1990). However, the ultimate causes of the asymmetry and their consequences, as well
135 as their function for population maintenance, remained unsolved problems (Ferrari &
136 Hayek, 1990). Concerning the mating behaviour of *Metridia*, males clasp the caudal

137 rami or urosome of females (cf. Ohtsuka & Huys, 2001). Because the spermatophores
138 of *Metridia* are located at either the left, or the right side of the fixation site around the
139 copulatory pore of the females (Cuoc et al., 1997), males use the inner process of leg 5
140 to attach the spermatophore. The attachment site (left or right) varies with lefty or righty
141 males (Ershova & Kosobokova, 2012). Thus bilateral insemination of females may be
142 the result of multiple copulations with both lefty and righty males, as mentioned by
143 Cuoc et al. (1997) and Ershova & Kosobokova (2012).

144 The special features of asymmetry in female insemination and the male
145 morphology of *Metridia* species evaluated in this study can be summarized in the
146 following four points: (1) The ratios of asymmetric female insemination and male
147 morphotype were found to be correlated (fig. 4). (2) Within a species, both female and
148 male ratios of asymmetry showed little seasonality throughout the year (fig. 3). (3) The
149 ratio of asymmetry of female insemination was common for seven *Metridia* species
150 from oceans throughout the world, but the ratios varied among species (table II). (4) For
151 the epipelagic species in each ocean, for which insemination occurred on both sides, the
152 lower percentage did show appreciable variation (21-34%) (table II). Among these four
153 features, the former two are also expressed in *M. longa* in the White Sea (Ershova &
154 Kosobokova, 2012). For the latter two features, regional variations were recognized for

155 epipelagic species: right-inseminated females were dominant among *M. pacifica* in the
156 Okhotsk Sea (62%, fig. 3C), whereas left-inseminated females were more abundant in
157 the North Pacific and Bering Sea (54-64%, table II). The variation in asymmetry within
158 populations of the epipelagic species in each region (e.g., *M. gerlachei*, *M. longa* and *M.*
159 *pacifica*) could be interpreted as a reflection of the presence of regional variability in the
160 asymmetry of the epipelagic species.

161 Concerning the adult female genital system of calanoid copepods, there is a
162 pair of lateral gonads connected by oviducts to the lateral, paired gonopores
163 (Bradford-Grieve et al., 2010). To increase the effectiveness of the fertilization of eggs,
164 adult females of calanoid copepods have seminal receptacles to deposit spermatozoa,
165 and/or a genital operculum, the large board covering the gonopores (Bradford-Grieve et
166 al., 2010). Within calanoid copepods, the genital systems of adult females are
167 morphologically divided into three types: those containing both genital operculum and
168 spermathecae (e.g., Calanoidea); those with no genital operculum, but with
169 spermathecae (e.g., Arietelloidea); and those with genital operculum, but without
170 spermathecae (e.g., Diaptomoidea, except Acartiidae) (Bradford-Grieve et al., 2010).
171 The Metridinidae treated in this study are classified as Arietelloidea (i.e., no genital
172 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).

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%

209 and that of *M. okhotensis* varied greatly (Yamaguchi et al., 2010). The cause of the low
210 egg-hatching rate of *Metridia* species has often been discussed; however, it has long
211 remained an unsolved problem. Egg cannibalism by adult females may be a possible
212 cause. Plourde & Joly (2008) compared egg-hatching rates under the presence or
213 absence of an egg-separating mesh (which has the function of preventing egg
214 cannibalism by adult females) for *Calanus finmarchicus* (Gunnerus, 1760) and *M. longa*.
215 The resulting egg-hatching rate of *C. finmarchicus* was reported to be 74-81%, whereas
216 that of *M. longa* remained low (29-51%), even in the presence of an egg-separating
217 mesh, which suggests that cannibalism by adult females is not the (main) cause of the
218 low hatching rate of species of *Metridia* (Plourde & Joly, 2008).

219 As an alternative hypothesis for *Metridia*, mate choice during copulation (a
220 female produces eggs in only her left or right ovary/oviduct, not both) may be
221 considered. At the time of mating, a female with left ovary/oviduct egg production will
222 select only a lefty male who can inseminate her left spermatheca. A female with right
223 ovary/oviduct egg production will select only a righty male who can inseminate her
224 right spermatheca. However, this hypothesis may not be the case. For all *Metridia*
225 females treated in this study, we confirmed that both of the examined left and right
226 ovaries/oviducts of females were filled with eggs. Gonad maturation diagrams are

227 reported for several *Metridia* species (Tande & Grønvik, 1983; Batchelder, 1986). From
228 these diagrams it becomes clear, that both the left and right ovary and oviduct were
229 functional and filled with eggs. Thus, this alternative hypothesis may not be a correct
230 description of the actual situation in *Metridia*.

231 ## CONCLUSION

232 In this study, unilateral insemination (e.g., left or right) was common for adult
233 females of various *Metridia* species from oceans throughout the world. Females of
234 species of *Metridia* have each spermatheca connected to only one of two lateral
235 oviducts. Thus, both fertilization and spawning on one side may occur independently of
236 the other side (Cuoc et al., 1997). This suggests that only half of the eggs produced by
237 females of *Metridia* spp. that are inseminated on only one side remain unfertilized
238 (Ershova & Kosobokova, 2012). Thus, the low egg hatching rate of *Metridia* species
239 (29-68%) found in laboratory experiments (e.g., the paradox of *Metridia*, Plourde &
240 Joly, 2008) might be a reflection of this morphology of the female genital system. To
241 confirm this hypothesis, egg hatching experiments with the separation of adult female
242 insemination (i.e., non-, left-, right-, or bilateral-insemination) are required for future
243 study.

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322 **Figure legends**

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

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

331 fig. 3. A, B, *Metridia okhotensis* Brodsky, 1950; and, C, D, *Metridia pacifica* Brodsky,
332 1950: A, C, seasonal/inter-annual changes in the insemination of
333 spermathecae of adult females (C6F); B, D, morphotype of leg 5 of adult
334 males (C6M); all of these samples were from Rausu in the Okhotsk Sea, and
335 taken from June 2007 to November 2009.

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

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	Locatiom	Station	Lattitude	Longitude	Date	Ship	Sampling gear	Depth (m)
1	*Metridia asymmetrica* Brodsky, 1950	NW Pacific	Site H	41°30'N	145°47'E	7-8 Sep. '99	“Tansei-Maru”	Gamaguchi-net	0-5000
2	*M. asymmetrica*		KNOT	44°00'N	155°00'E	19-21 Aug. '98	“Hakurei-Maru No. 2”	VMPS	0-4000
3	*M. asymmetrica*		KNOT	44°00'N	155°00'E	19-21 Aug. '98	“Hakurei-Maru No. 2”	VMPS	0-4000
4	*M. asymmetrica*	NE Pacific	OS12106	51°15'N	150°00'W	15 July '12	“Oshoro-Maru”	VMPS	0-3000
5	*M. asymmetrica*	Bering Sea	AB	53°28'N	177°00'W	14 June '06	“Oshoro-Maru”	VMPS	0-3000
6	*Metridia curticauda* Giesbrecht, 1889	NW Pacific	Site H	41°30'N	145°47'E	07-08 Sep. '99	“Tansei-Maru”	Gamaguchi-net	0-5000
7	*M. curticauda*	NE Pacific	OS12106	51°15'N	150°00'W	15 July '12	“Oshoro-Maru”	VMPS	0-3000
8	*M. curticauda*	Bering Sea	AB	53°28'N	177°00'W	14 June '06	“Oshoro-Maru”	VMPS	0-3000
9	*Metridia gerlachei* Giesbrecht, 1902	Southern Ocean	138	66°00'S	65°00'E	19 Jan. '84	“Kaiyo-Maru”	WP-2	0-500
10	*M. gerlachei*		142	65°00'S	65°00'E	20 Jan. '84	“Kaiyo-Maru”	WP-2	0-500
11	*M. gerlachei*		159	65°00'S	67°30'E	23 Jan. '84	“Kaiyo-Maru”	WP-2	0-500
12	*M. gerlachei*		51	63°43'S	72°30'E	13 Dec. '83	“Kaiyo-Maru”	MTD	500
13	*M. gerlachei*		167	66°46'S	69°53'E	25 Jan. '84	“Kaiyo-Maru”	MTD	500
14	*M. gerlachei*		171	65°00'S	70°00'E	26 Jan. '84	“Kaiyo-Maru”	MTD	500
15	*Metridia longa* (Lubbock, 1854)	Barents Sea	862	72°30'N	28°00'E	13 Sep. '01	“G.O. Sars”	MOCNESS	0-283
16	*M. longa*		868	72°30'N	40°30'E	14 Sep. '01	“G.O. Sars”	MOCNESS	0-340

TABLE I

(Continued)

Code	Species	Locatiom	Station	Lattitude	Longitude	Date	Ship	Sampling gear	Depth (m)
17	*M. longa*		905	75°04'N	32°01'E	20 Sep. '01	"G.O. Sars"	MOCNESS	0-233
18	*M. longa*	Arctic Ocean	32	74°32'N	161°58'W	8 Sep. 2013	"Mirai"	VMPS	0-1000
19	*M. longa*		56	73°48'N	159°59'W	27 Sep. 2013	"Mirai"	VMPS	0-1000
20	*Metridia okhotensis* Brodsky, 1950	NW Pacific	Site H	41°30'N	145°47'E	07-08 Sep. '99	"Tansei-Maru"	Gamaguchi-net	0-5000
21	*M. okhotensis*	Bering Sea	AB	53°28'N	177°00'W	14 June '06	"Oshoro-Maru"	VMPS	0-3000
22	*Metridia pacifica* Brodsky, 1950	NW Pacific	Site H	41°30'N	145°47'E	07-08 Sep. '99	"Tansei-Maru"	Gamaguchi-net	0-5000
23	*M. pacifica*	NE Pacific	OS12106	51°15'N	150°00'W	15 July '12	"Oshoro-Maru"	VMPS	0-3000
24	*M. pacifica*	Bering Sea	AB	53°28'N	177°00'W	14 June '06	"Oshoro-Maru"	VMPS	0-3000
25	*Metridia similis* Brodsky, 1950	NW Pacific	Site H	40°36'N	147°30'E	28 Aug. '04	"Tansei-Maru"	Gamaguchi-net	3000-5000
26	*M. similis*		Site H	42°29'N	147°31'E	29 Aug. '04	"Tansei-Maru"	Gamaguchi-net	3000-5000
27	*M. similis*		Site H	42°28'N	147°33'E	29 Aug. '04	"Tansei-Maru"	Gamaguchi-net	3000-5000
28	*M. similis*		Site H	42°26'N	147°35'E	29 Aug. '04	"Tansei-Maru"	Gamaguchi-net	3000-5000
29	*M. similis*		Site H	41°30'N	145°47'E	07-08 Sep. '99	"Tansei-Maru"	Gamaguchi-net	0-5000
30	*M. similis*		KNOT	44°00'N	155°00'E	19-21 Aug. '98	"Hakurei-Maru No. 2"	VMPS	0-4000
31	*M. similis*	NE Pacific	OS12106	51°15'N	150°00'W	15 July '12	"Oshoro-Maru"	VMPS	0-3000
32	*M. similis*	Bering Sea	AB	53°28'N	177°00'W	14 June '06	"Oshoro-Maru"	VMPS	0-3000

TABLE II

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

Species (habitat depth)	Station code (*n*)	Composition to total adult females (%)			
		Non-ins.	Left-ins.	Right-ins.	Bilateral-ins.
Metridia asymmetrica Brodsky, 1950 (D)	1 (25)	20.0	4.0	64	12.0
	2 (5)	10.3	0.8	81.7	7.1
	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
Metridia curticauda 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
Metridia gerlachei 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
Metridia longa (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
Metridia okhotensis 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
Metridia pacifica 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
Metridia similis 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
	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.

TABLE III

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

Species	Location	Hatching rate (%) range (mean)	References
Metridia longa (Lubbock, 1854)	Lower St. Lawrence Estuary	29-51	Plourde & Joly (2008)
Metridia pacifica 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)
Acartia spp.	South Coast of England	86-99	Castro-Longoria (2003)
Centropages hamatus (Lilljeborg, 1853)	Long Island Sound	65-95	Tang et al. (1998)
Calanus spp.	Western Greenland	75-93	Jung-Madsen & Nielsen (2015)
Neocalanus spp.	Oyashio region	81-93	Saito & Tsuda (2000)
Temora longicornis (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.

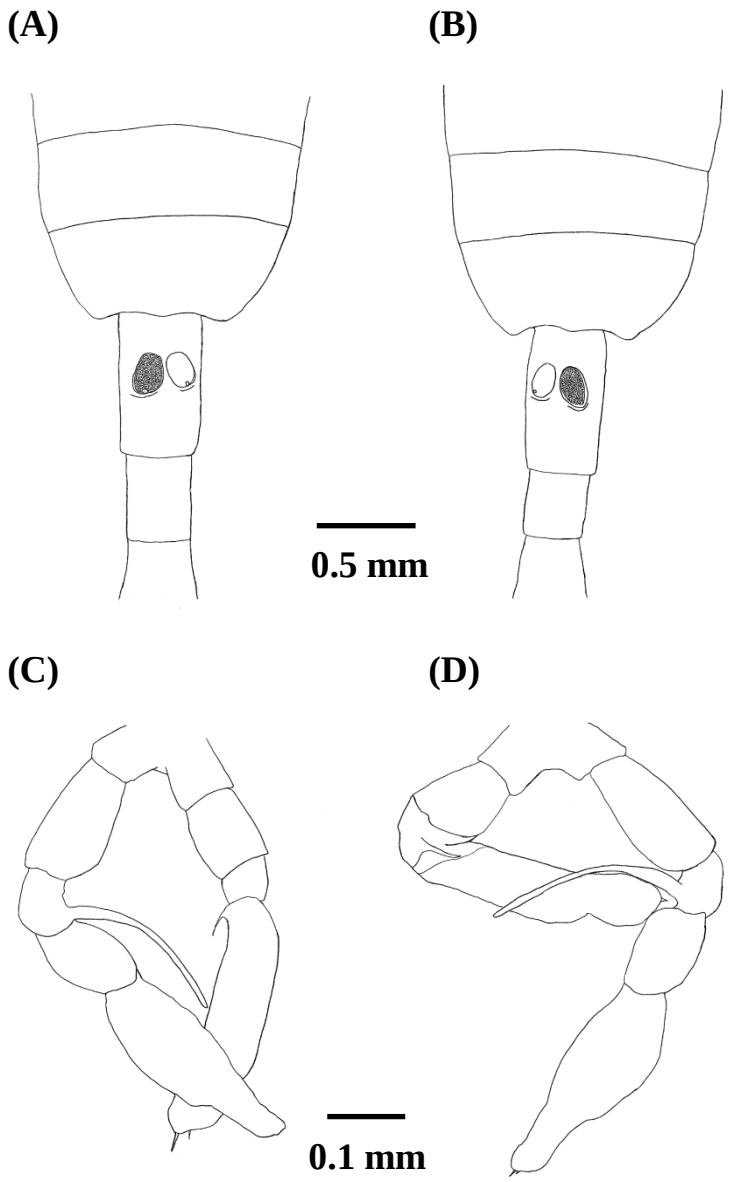


fig. 2. Arima et al.

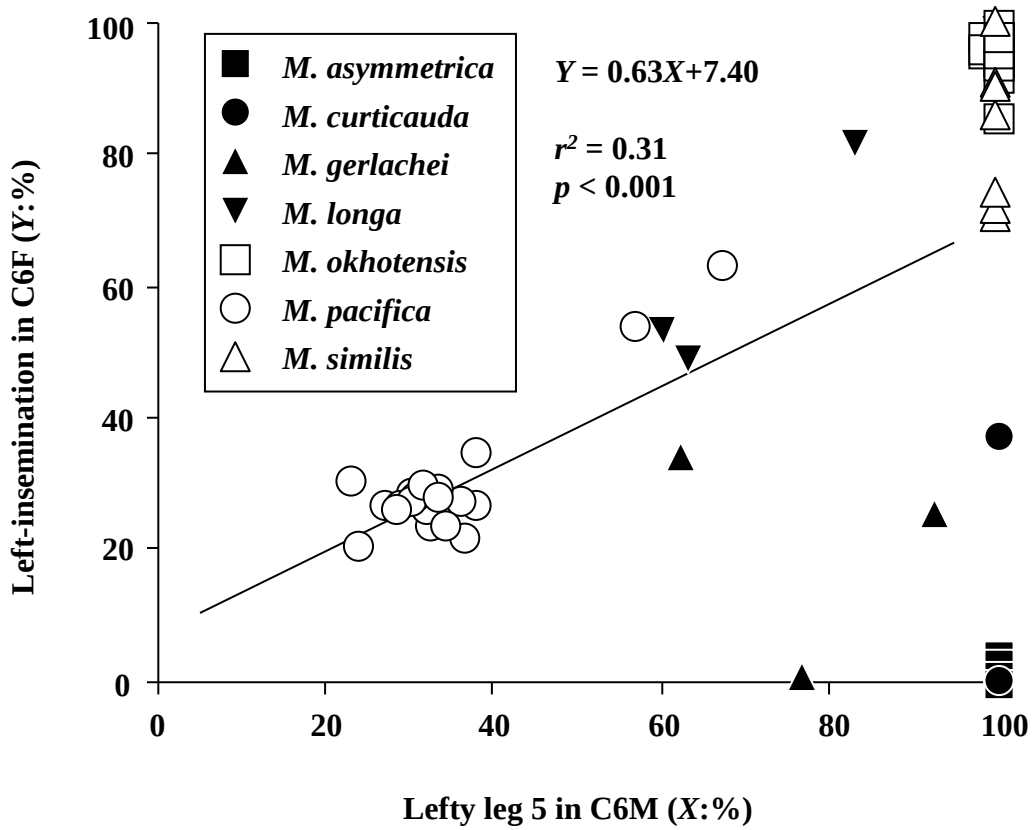


fig. 4. Arima et al.

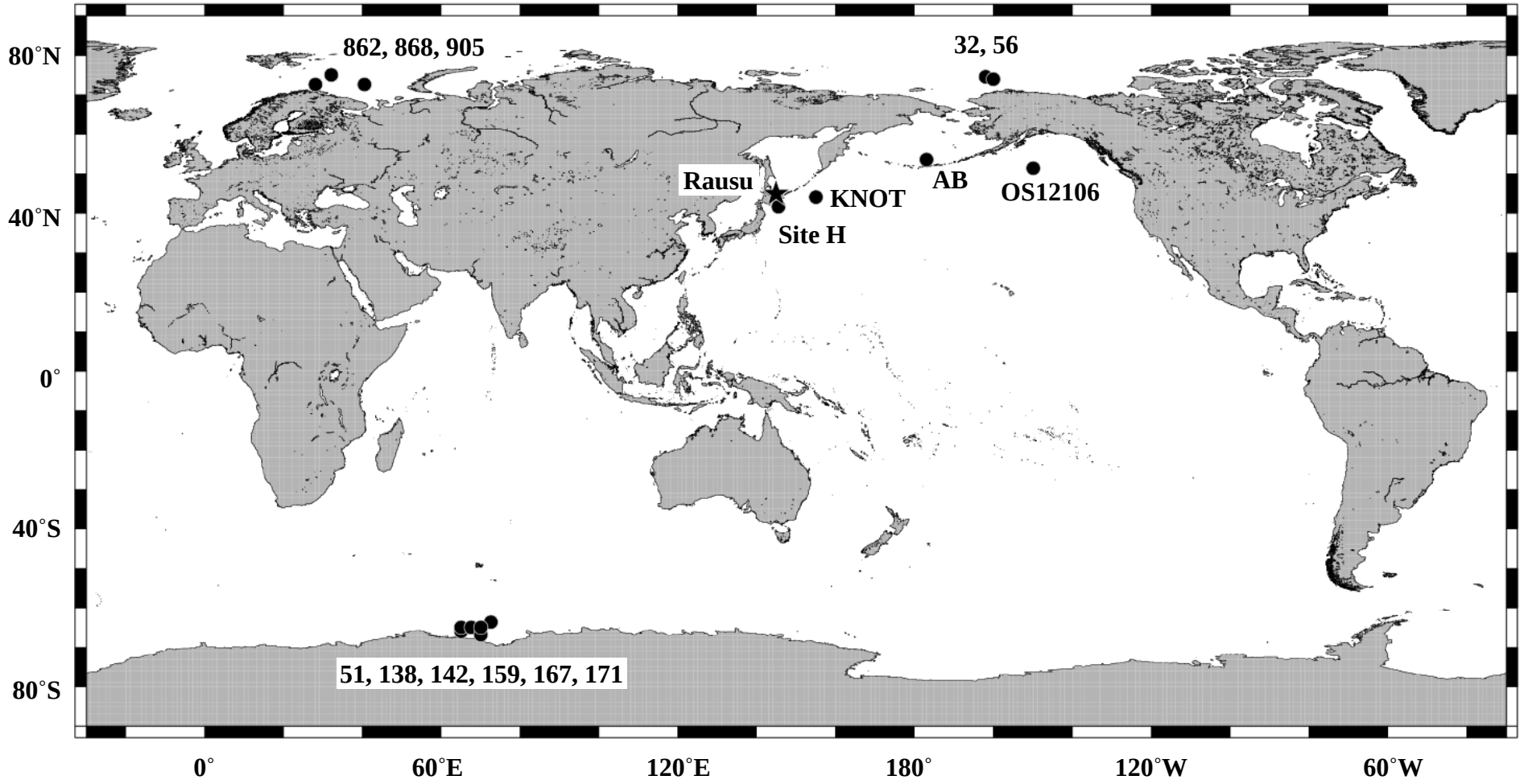


fig. 1. Arima et al.

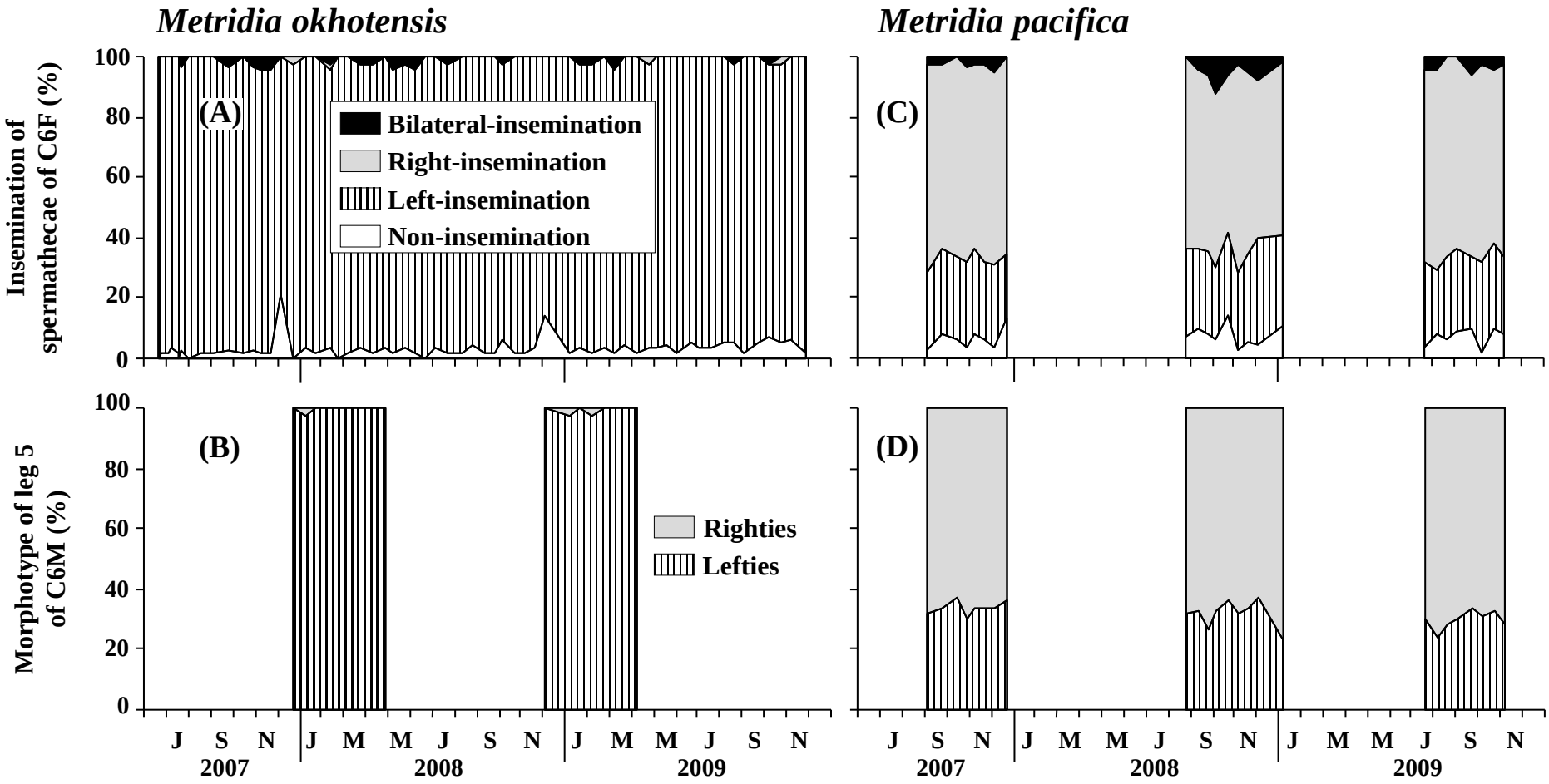


fig. 3. Arima et al.