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Fluctuations in the sex ratio of the ostracod *Vargula hilgendorfii*

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Abstract

In the present study, the fluctuations in the sex ratio of the ostracod *Vargula hilgendorfii* (Müller, 1890) were studied. Specimens of stage-5 juveniles (last instar) were collected from Akita Port every 5 or 7 days and were reared until they reached maturity. The sex of the adults was identified under a stereomicroscope to determine the sex ratio. The sex ratio varied among collection dates, whereas at the beginning of the generation, the sex ratio was 1:1, a female-bias was apparent in the middle of the generation. These fluctuations were also observed in the second generation. This same sex ratio fluctuation was also observed in 2010 and 2011. These results revealed that the sex ratio varied among collection dates and suggested that sex determination is related to some specific environmental factors.

Keywords : environmental sex determination, sex ratio, Ostracoda, Myodocopida, *Vargula hilgendorfii*

INTRODUCTION

Vargula hilgendorfii (Müller, 1890) (Ostracoda: Myodocopida) is a luminescent marine crustacean with a double translucent carapace. This organism is native to Japanese coastal waters, and can be easily collected using a bait traps. There are some studies on its life cycle and ecology (Okada and Kato, 1949; Nakamura, 1954; Henmi and Okamoto, 2002; Mitome et al., 2007). Female *V. hilgendorfii* lay and brood approximately 50 eggs at a time under their carapace. The eggs are hatched in the carapace and immediately begin life in the benthic zone after being released by the mother. The juveniles (stage 1) released from the mother molt five times before becoming adults; therefore, they are referred to as A-5 (adult minus five). The stages of the juveniles as they molt until they reach maturation are referred to as A-4 (stage 2), A-3 (stage 3), A-2 (stage 4), and A-1 (stage 5) (Cohen and Morin, 1990; Ogo and Ohmiya, 2003). Mitome et al. (2007) reported that the ratio of males to females collected using bait traps is extremely unbalanced during some seasons. It has also been reported that the number of *V. hilgendorfii* chromosomes is 2n = 16, and the sex chromosomes display a XX/XO pattern (Moguilevsky, 1990). Based on this finding, it has been generally thought that the sex of *V. hilgendorfii* is genetically determined. Even though the sex ratio is 1:1, it is possible that the ratio of males and females collected by bait traps is biased to one sex. Males and females may not be equally attracted to the bait, and there also might be a difference in mortality between the two sexes. Meanwhile it has also been reported that environmental factors affect sex determination and cause fluctuations in the sex ratio for many animals, including crustaceans (Legrand et al., 1987; Naylor et al., 1988; Hobaek and Larsson, 1990; Korpelainen, 1990; Voordouw and Anholt, 2002). Based on these findings, it is possible that environmental factors might affect sex determination in *V. hilgendorfii*. Therefore, in this study, we aimed to clarify whether sex determination of *V. hilgendorfii* is the result of environmental factors or genetics (1:1).

In *V. hilgendorfii*, males and females can be easily identified by their external form after reaching adulthood (Nakamura, 1954; Ogo and Ohmiya, 2003). For males, in addition to being one size smaller than the female, the dorsal muscle bands (posterior part of body) are fewer, but much more swollen, than those in the

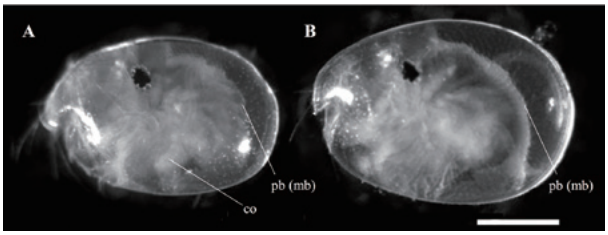

Fig. 1. Adult sexual dimorphism is expressed in carapace size and shape of posterior part of body. A, male; B, female; co = copulatory organ; pb = posterior part of body; mb = muscle bands. Scale bar = 1 mm.

female (Fig. 1; Vannier and Abe, 1993). Therefore, in the present study, we investigated sex ratio at adulthood. We collected A-1 specimens of *V. hilgendorfii* (those that will reach maturity after the next molt) and raised them in a laboratory until they reached maturity. After they became adults, we confirmed the female-male ratio. The advantage of this method is that it allowed us to reveal the sex ratio at one particular moment. The dynamics of the sex ratio could be elucidated by continuously collecting using this method.

MATERIALS AND METHODS

Specimens of *V. hilgendorfii* were collected from the north breakwater of Akita Port (39°45′16″N, 140°03′03″E) using bait traps. Raw chicken livers were placed in 500-mL thick walled glass containers with several 5 mm-diameter openings in the lid; the traps were sunk to the sea floor 2 h after sunset. The collection time ranged from 3 to 10 min in order to get an adequate number of A-1 specimens. Collections of *V. hilgendorfii* were performed 15 times from July

Table 1. Sex ratio and survival rate for A-1 *Vargula hilgendorfii* individuals reared to adulthood, and numbers of adult males and females collected in 2010 and 2011 using bait traps.

		Results of rearing				Number of collected individuals	
Date	Number of A-1	Number of males	Number of females	Female ratio (%)	Survival Rate (%)	Number of adult males	Number of adult females
Jul. 25, 2010	200	80	78	49.4	79.0	0	0
Jul. 30	200	56	66	54.1	61.0	411	297
Aug. 3	200	30	57	65.5 **	43.5	521	563
Aug. 7	200	39	87	69.0 **	63.0	1661	1950
Aug. 13	300	34	127	78.9 **	53.7	764	1391
Aug. 21	846	206	494	70.6 **	82.7	737	2003
Sep. 2	403	50	115	69.7 **	40.9	363	254
Sep. 9	400	63	66	51.2	32.3	26	30
Sep. 18	400	9	13	59.1	5.5	68	77
Sep. 24	400	31	45	59.2	19.0	391	311
Oct. 1	400	103	214	67.5 **	79.3	1704	2609
Oct. 8	400	146	194	57.1 **	85.0	1299	1064
Oct. 15	400	101	117	53.7	54.5	1016	884
Oct. 22	400	161	166	50.8	81.8	112	106
Oct. 29	400	137	227	62.4 **	91.0	1037	2598
Jul. 19, 2011	62	27	22	44.9	79.0	0	0
Jul. 25	264	119	107	47.3	85.6	10	124
Jul. 30	314	145	153	51.3	94.9	90	230
Aug. 4	250	121	106	46.7	90.8	489	318
Aug. 9	369	79	199	71.6 **	75.3	544	744
Aug. 14	165	48	97	66.9 **	87.9	518	873
Aug. 19	447	219	189	46.3	91.3	1959	3409
Aug. 24	687	201	184	47.8	56.0	582	973
Aug. 29	417	112	158	58.5 **	64.7	444	545
Sep. 2	802	199	340	63.1 **	67.2	1579	1026
Sep. 8	278	96	118	55.1	74.6	986	1495
Sep. 14	497	107	85	44.3	38.6	131	110
Sep. 22	865	378	432	53.3 *	93.5	801	318
Sep. 27	683	333	307	48.0	93.7	1060	616
Oct. 4	438	126	284	69.3 **	93.6	760	716
Oct. 9	385	113	267	70.3 **	98.7	328	392
Oct. 14	461	184	265	59.0 **	97.4	280	187
Oct. 19	502	224	248	52.5	94.4	612	278
Oct. 24	843	280	349	55.5 **	74.6	2187	1166
Oct. 30	648	262	347	57.0 **	94.0	3515	1024
Nov. 4	553	140	378	73.0 **	92.9	1125	950

*$p < 0.05$, **$p < 0.01$

25 to October 29, 2010 and 21times from July 19 to November 4, 2011. The collected specimens were brought to the laboratory and subsequently classified as A-1, adult female, adult male, or other with a stereomicroscope. The classification for each category was performed based on the size and the shape of the posterior part of the body. A-1 specimens were divided into groups of approximately 400 individuals and placed in plastic containers (15 cm diameter, 6 cm depth) until they reached adulthood. We confirmed the number of male and female specimens that had molted and matured, and removed them from the plastic containers. Sex ratio was expressed as ratio of females, it was to test the significant difference between the sex ratio in this study and the genetic sex ratio (1:1) by the binomial test. Each day, chicken livers were fed to the specimens, the remnants of the feed were removed, and the water was changed. Artificial seawater was used for seawater until August 13, 2010. After August 21, 2010, seawater obtained from the collection site was used to rear the specimens. Specimens were raised in an incubator at 24 °C in 2010 and at room temperature in 2011.

RESULTS

Table 1 shows the number of A-1 specimens and adults (male and female) collected on each day, and the number of male and female A-1 specimens that were reared to adulthood, the sex ratios (ratio of females), and the survival rates. At the beginning of each generation, the number of adult specimens collected was extremely small. Adults were not collected on July 25, 2010 or July 19, 2011. Furthermore, the number of adult specimens collected on September 9, 2010 and September 14, 2011, were only 56 and 241, respectively. The results of a binomial test revealed that on many of the collection days, the sex ratio was skewed toward females ($p < 0.05$ and $p < 0.01$). The sex ratios differed greatly between collection dates. The fluctuations in the sex ratios for 2010 and 2011 are shown in Fig. 2. The ratio of females gradually increased from 49.4 % on July 25, 2010, to 78.9% (first peak) on August 13. The sex ratio subsequently decreased to 51.2% on September 9, increased to the second peak of 67.5% on October 1, decreased to 50.8% on October 22, and increased to 62.4% on October 29. The fluctuations in 2011 were consistent with those observed in 2010. Fluctuations of the sex ratio during the first and second generations of 2010 and 2011 are superimposed together the peaks in Fig. 3. Similar fluctuations were consistently observed throughout the four generations.

DISCUSSION

Mitome et al. (2007) reported that two generations of *V. hilgendorfii* appear during the year in Akita Prefecture; adults emerge during late July (first generation) and early September (second generation), with almost no overlap between the two generations. Based on the number of adults collected in our study, we concluded that the first and second generations of adults emerge during late July and mid-September, respectively.

In this study, we revealed the dynamics of the sex ratio in *V. hilgendorfii*, which appears to fluctuate daily. The sex ratio in *V. hilgendorfii* biased to female, the measure of bias has changed. If the sex ratio is equal to 1:1, it indicates higher mortality in males before reaching A-1. It is possible that such as molting difficulties lead to a higher death rate in males. But then the measure of bias should have remained constant. Furthermore, the fluctuations were not due to the accidental death in males, because the fluctuations in the four generations were similar (Fig. 3). We concluded that the sex ratio fluctuation was caused by some unknown environmental factor.

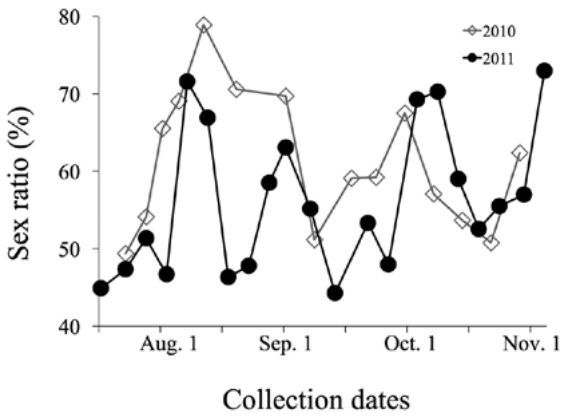

Fig. 2. Fluctuations in the sex ratio (proportion of females) across all A-1 collection dates. Similar fluctuations were observed in 2010 and 2011.

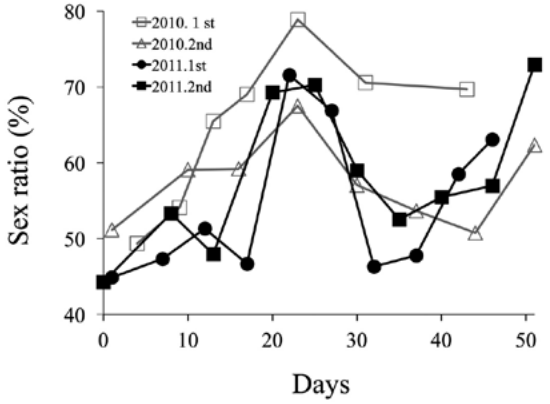

Fig. 3. Fluctuations in the sex ratio on the first and second generations of 2010 and 2011 superimposed on the center of the peak. Fluctuations were similar across all four generations.

It has been reported that *V. hilgendorfii* have XX and XO sex chromosomes. However, even in fish that possess XX and XY sex chromosomes, tendencies for female or male biases due to estrogen or water temperature have been reported (Yamamoto, 1953; Kitano et al., 1999;). At the beginning of the generation, *V. hilgendorfii* started with a 1:1 sex ratio, and therefore sex is determined genetically. However, we speculate that some unknown environmental factor causes the 1:1 sex ratio to skew toward more females.

Environmental sex determination has been reported in various animals (Legrand et. al., 1987; Korpelainen, 1990). The most well-known environmental factor affecting sex determination is temperature, but day length, nutrition, population density, chemical substances, and pH are also governing factors (Egami, 1951; Naylor et al., 1988; Hobaek and Larsson, 1990; Olmstead and Leblanc, 2002; Tatarazako et al., 2003; Oda et al., 2005). The sex ratios in this present study fluctuated greatly within a short period of time. The sex ratio on August 4, 2011 was 46.7 %, and 5 days later, it had increased to 71.6 %. Then on August 14, the sex ratio was 66.9 %, and 5 days later, it had decreased to 46.3 %. Altogether, these were fluctuations of more than 20 % during the 15 days period. In other generations, the range of fluctuations was smaller, but similar increases and decreases were noted during a short period time. It is unlikely that environmental factors, such as slow changes in seawater temperature and length of day, were the key signals involved in these fluctuations. Since similar fluctuations were observed in the first and second generations, there is a high possibility that, instead of external environmental changes (e.g., water temperature or day length), changes in *V. hilgendorfii* itself (population density, chemical substances released by *V. hilgendorfii*, etc.) are the key signals affecting these fluctuations. Currently, it is still unclear exactly when *V. hilgendorfii* sex determination occurs. Once this is elucidated, we should be able to focus on a narrower group of possible environmental factors responsible for this sex ratio fluctuation.

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