

Intersexuality in *Acanthomysis mitsukurii* (Mysidacea) in Sendai Bay, northeastern Japan

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Received 20 December 2000; accepted 13 May 2001

Abstract: Specimens of the mysid, *Acanthomysis mitsukurii*, were collected monthly using a bottom plankton sampler at 6 stations with sandy bottom sediments in Sendai Bay between July 1993 and June 1994 and once in March 2000. Seven intersex individuals were found from a total of 9282 specimens. All intersex animals bear a pair of elongated fourth pleopods extending to the posterior edge of the fifth abdominal somite and a marsupium. Six of the 7 individuals have a smaller marsupium than that of a normal mature female. Genital papillae were observable neither under a binocular microscope nor from any histological section. The intersex animals subjected to histological analysis contained testicular tissue, indicating that these animals were feminized males. Intersexuality occurred only in the overwintering generation collected in January and March. Also intersex animals were collected at only one station, which is located off a pulp mill effluent outfall. The percentage occurrence of intersex individuals in adults collected at this station in January and March 1994 and in March 2000 was 2.5%. Nonylphenol and bisphenol-A, which are known to be xeno-estrogens in vertebrates, were detected only at this station, indicating that this station was more polluted than the other stations. However, the causality of intersexuality by the pollutants remains uncertain. Further studies are required to elucidate the mechanism of induction of intersexuality in *A. mitsukurii*.

Key words: mysid, *Acanthomysis mitsukurii*, intersex, endocrine disrupter

Introduction

The mysid, *Acanthomysis mitsukurii*, is one of the dominant species in the mysid communities in the Pacific coastal waters of Japan (Yamada et al. 1994; Yamada 2000) and has been known to form the main dietary item for juvenile Japanese flounder *Paralichthys olivaceus* (Yamada et al. 1998), which is one of the most important coastal commercial fish in Japan. We found intersex individuals in the specimens of *A. mitsukurii* collected during monthly samplings in Sendai Bay. These samplings were conducted to research the distribution, population dynamics and productivity of this species (Yamada 2000).

Intersexuality is a well-known phenomenon in crustaceans such as the Isopoda (Smith 1977; Korczynski 1985, 1988), Amphipoda (Bulnheim 1978; Buikema et al. 1980; Hastings 1981) and Copepoda (Moore & Stevenson 1994).

However, this phenomenon in Mysidacea has so far only been reported in *Neomysis integer* (Kinne 1955; Hough et al. 1992).

We examined the occurrence of intersexual individuals of *A. mitsukurii* and their external anatomical and internal gonadal characteristics and discuss relationships to pollution in the bay.

Materials and Methods

Mysids

Mysids were collected monthly using a bottom plankton sampler (0.6 m in mouth width, 0.4 m in height, 0.5 or 0.76 mm mesh size) at 4 stations (Stns A, B, C, D) over sandy sediments in Sendai Bay, 12 times between July 1993 and June 1994 (Fig. 1). Samplings were conducted at 8-m depth at Stns A, B, D and 4, 8, 12, 16-m depths at Stn C. On 15 March 2000, mysids were also caught at 4 stations

(Stns C, D, E, F) at 8–10-m bottom depth. Samples were fixed in 10% neutralized formalin-seawater.

More than 100 specimens (up to 200 specimens) from each net sample were examined with respect to sexual characteristics under a binocular microscope. For samples where less than 100 specimens were caught, all specimens were examined. *Acanthomysis mitsukurii* collected on 15 March 2000 and all the intersex specimens were examined for the presence of parasites at $\times 64$ under a binocular microscope (SZH-141, Olympus Optical Co. Ltd., Tokyo, Japan).

Four intersex individuals of the five caught on 21 January 1994 and some normal males and females caught in March 2000 were embedded in paraffin and transversely sectioned at a width of $4\ \mu\text{m}$. Sections were stained with Carazzi's hematoxylin-eosin (Watanabe 1981) for histological observations.

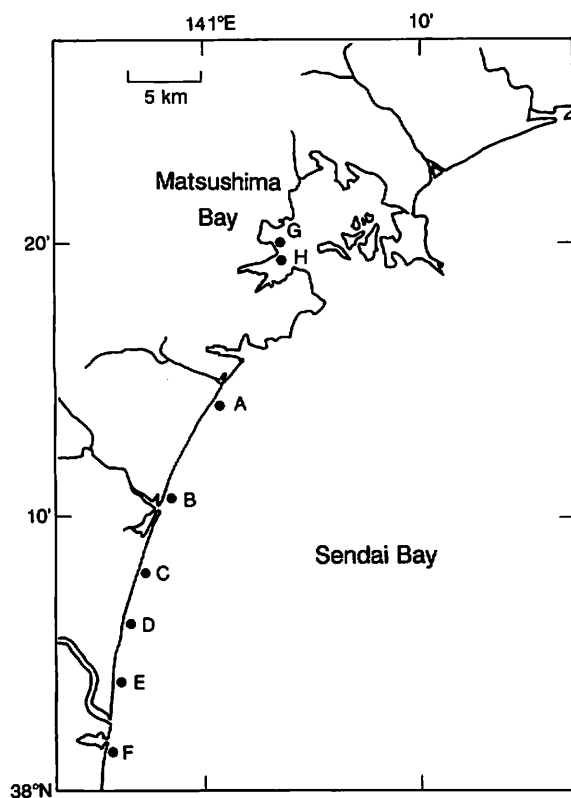


Fig. 1. Sampling stations for mysids and seawater.

Analysis of estrogenic compounds

Surface seawater samples were taken in two 2-liter glass bottles from water collected with a stainless steel bucket at Stns D and G on 29 November 1999 and at Stns C, D, E, F, G, H on 15 March 2000 for the analysis of bisphenol-A and nonylphenol concentrations. To prevent chemical decomposition of the compounds, 2 g of ascorbic acid was added to each bottle soon after sampling and the samples were kept in a refrigerator at 3°C until analysis, which was carried out within three weeks of sampling. The vertical profiles of temperature and salinity showed that the seawater was well mixed vertically at all stations during the March sampling period. Concentrations of bisphenol-A and nonylphenol were determined according to the modified official analytical methods developed by the Environment Agency, Japan (Environment Agency 1998). The lowest detection levels of bisphenol-A and nonylphenol are $10\ \text{ng l}^{-1}$ and $100\ \text{ng l}^{-1}$, respectively.

Results

A total of 9282 specimens of *Acanthomysis mitsukurii* were examined. At Stn D, 5 intersex individuals were found of the 92 adults collected in January and one of the 29 adults in March 1994 (Table 1). In addition, one intersex individual was collected from the same station in March 2000 (Table 2). The percentage occurrence of intersexuality in adults collected at this station in January and March 1994 and in March 2000 was 2.5%. There were no intersexual adult specimens from the other stations in 1993, 1994 or in 2000.

Seven specimens identified as intersexual individuals bore a marsupium and a pair of elongated fourth pleopods which extended to the posterior edge of the fifth abdominal somite (Fig. 2). The marsupium was made up of 2 pairs of brood plates and covered the area in which the genital papillae should develop. The marsupium of intersexual individuals did not contain embryos. Six of the 7 intersex animals had a visibly smaller marsupium than that of normal mature females (Fig. 2). Genital papillae were not observable under a binocular microscope. Body lengths (distance from the base of the eye stalk to the end of the telson) of these animals ranged from 7.1 to 8.3 mm and were in the normal body length range for adults occurring in winter–

Table 1. Occurrence of intersex individuals in *Acanthomysis mitsukurii* caught at Stn D. There were no intersex individuals in the mysid specimens caught at Stns A, B, C (1993–1994).

Date	Jul 22	Aug 18	Sep 1	Sep 28	Oct 28	Nov 16	Dec 16	Jan 21	Mar 19	Apr 18	May 10	Jun 23
No. of mysids examined	198	195	154	196	170	128	124	102	29	16	44	187
No. of adults (A)	16	152	133	121	106	26	51	92	29	16	13	77
No. of intersex individuals (B)	0	0	0	0	0	0	0	5	1	0	0	0
% occurrence in adults (B/A $\times 100$)	0	0	0	0	0	0	0	5.4	3.4	0	0	0

Table 2. Occurrence of an intersex individual of *Acanthomysis mitsukurii* collected in Sendai Bay on 15 March 2000. Values in parentheses indicate the number of individuals with trematode parasites.

Stn	Immature	Female	Male	Intersex
C	18	9 (3)	3 (1)	0
D	74	80 (16)	76 (4)	1 (1)
E	2	0	0	0
F	7	35 (4)	29 (7)	0

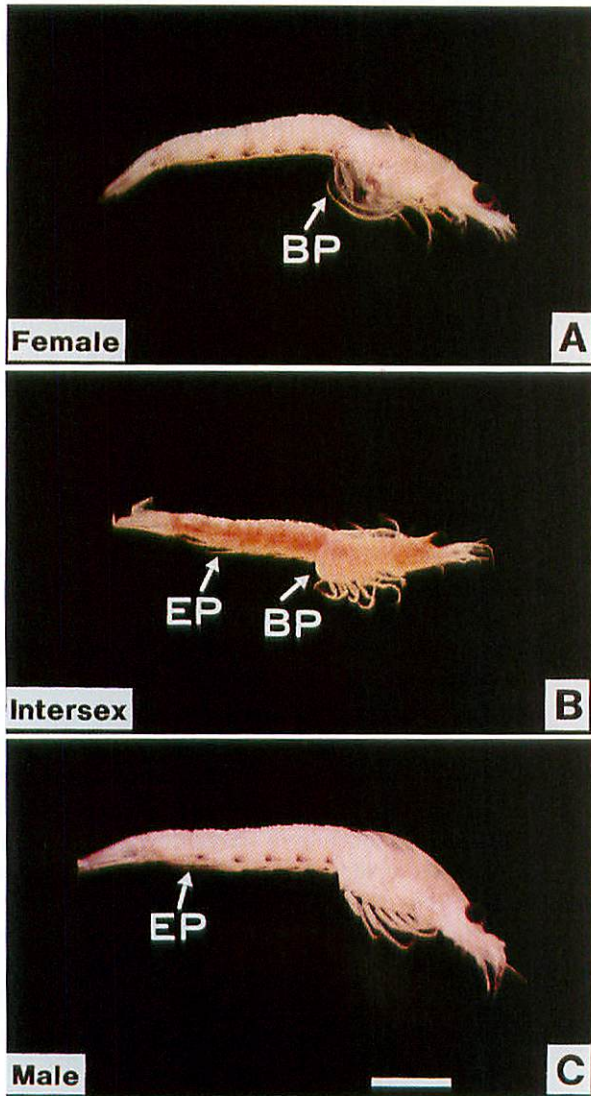


Fig. 2. Lateral view of a normal female (A), intersex specimen (B) and normal male (C) of *Acanthomysis mitsukurii*. BP; brood plates (marsupium), EP; elongated fourth pleopod. Bar indicates 2 mm.

spring (Yamada et al. 1998; Yamada 2000).

All of the 4 intersex animals from 1993–1994 which were subjected to histological analysis contained testicular tissue with spermatocytes and spermatozoa (Fig. 3A).

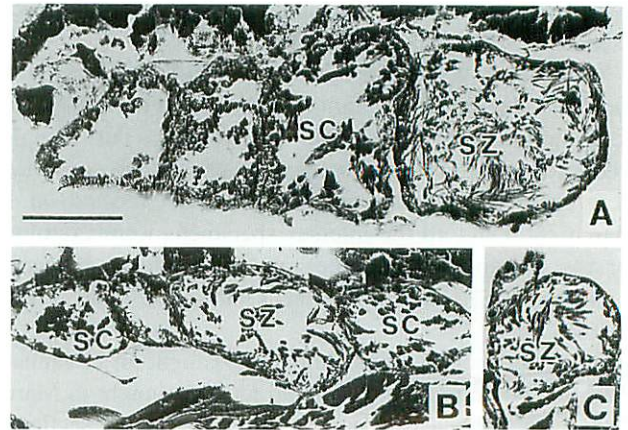


Fig. 3. Transverse sections of the gonad of *Acanthomysis mitsukurii*. A. Intersex (body length: 7.5 mm). B. Male (body length: 9.5 mm). C. Male (body length: 6.8 mm). SC, spermatocyte; SZ, spermatozoa. Bar indicates 0.1 mm.

Table 3. Concentrations (ng l^{-1}) of bisphenol-A and nonylphenol in seawater collected from Sendai Bay. ND indicates not detected.

Stn	29 Dec. 1999				15 March 2000			
	D	G	C	D	E	F	G	H
bisphenol-A	280	ND	ND	10	ND	ND	ND	ND
nonylphenol	200	ND	ND	400	ND	ND	ND	ND

Transverse sections of normal males also revealed spermatocytes and spermatozoa in the testis (Fig. 3B, C). The gonads in the intersex specimens looked like functional testes, but distinct genital papillae were not observable in any of the histological sections. Trematode parasites occurred in one of the intersex specimens collected in January 1994 and in one individual in March 2000. However, these parasites were also commonly associated with normal males and females collected at other stations (Table 2).

Bisphenol-A and nonylphenol were detected only at Stn D (Table 3). Concentrations of these compounds were less than the lowest detectable level at the other stations.

Discussion

In spite of monthly sampling over a one year period at 4–6 stations, intersex individuals of *Acanthomysis mitsukurii* were caught only at Stn D in January and March (no sampling in February). Intersexuality in mysids has only been reported for *Neomysis integer* (Kinne 1955; Hough et al. 1992), but in these reports there was no description of a specific season or site of occurrence. All four intersex specimens examined in the histological analysis were identified as males. However, light microscopy and the histological observations revealed an absence of genital papillae, indicating that these animals were feminized and castrated

males. This feminization in *A. mitsukurii* is the opposite phenomenon to intersex in *Neomysis integer* which is due to the masculinization of females with fully functional ovaries and developing embryos (Kinne 1955; Hough et al. 1992).

In some species, parasites can induce intersexuality (Charniaux-Cotton et al. 1992). The parasite species which induce intersexuality in crustaceans belong to very different systematic groups: microsporidians in the amphipod *Gammarus duebeni* (Bulnheim 1978), degeneans and unidentified cysts in the amphipod *Ampelisca brevicornis* (Hastings 1981), rhizocephalans in the crab *Portunus pelagicus* (Shields & Wood 1993). Trematodean parasites were found infecting two intersex mysids in the present study. A wide range of parasites have been reported in mysids (Mauchline 1980), but there are no records of parasite-induced intersexuality. Because 5 of the 7 intersex animals did not have parasites and these parasites are found commonly in both normal males and females of *A. mitsukurii*, parasites as the cause of intersexuality appears unlikely.

Environmental factors such as photoperiod (Bulnheim 1978) and temperature (Ginsburger-Vogel & Magniette-Mergault 1981), and genetic abnormality (Ginsburger-Vogel & Charniaux-Cotton 1982) have been reported to be linked to the occurrence of intersexuality. However, as all the intersex animals in the present study were collected at one specific station in Sendai Bay, it is unlikely that natural factors were responsible for inducing the intersexuality but rather that it was a causative agent specific to Stn D.

Water pollution may also induce intersexuality in crustaceans (Moore & Stevenson 1994). Stn D where the intersex animals were sampled is located about 100 m from a pulp mill effluent outfall. Both bisphenol-A and nonylphenol, which have estrogenic effects on vertebrate animals (Krishnan et al. 1993; Jobling et al. 1996), were detected only at Stn D. Although the relationship between these compounds and the pulp mill effluent is unknown, pulp mills use bisphenol-A as one of the materials in resins and nonylphenol as a surface-active agent used in paper production. The concentration of nonylphenol at Stn D ranged from 200 to 400 ng l⁻¹. This was lower than the lowest values, approximately 1–50 µg l⁻¹, which have been reported to exert endocrine responses in crustaceans, (Baldwin et al. 1997; Brown et al. 1999; Billinghamurst et al. 2000). However, there are no reports in which nonylphenol has been shown to cause intersexuality in invertebrates. Phytoestrogens derived from wood, such as sitosterol, are present in pulp mill effluents and have been reported to suppress testicular steroid production and to impair the reproductive capacity of male fish (MacLatchy & van der Kraak 1995). Butyltin compounds such as tributyltin (TBT) have also been reported to induce imposex in some neogastropod species (Horiguchi et al. 1995) and to cause alternations in testosterone metabolism in daphnids (LeBlanc & McLachlan 2000). However, in addition to these compounds, a number of possible endocrine disrupting chemicals are known

(Depledge & Billinghamurst 1999) and the effect of these pollutants on intersexuality in *A. mitsukurii* remains unclear. Detection of bisphenol-A and nonylphenol at Stn D may indicate that this station has been polluted more than the other stations.

It is also notable that the intersex animals were collected only during the low temperature season. The productivity and biomass of *A. mitsukurii* become maximal at around 20°C, in summer and autumn in northeastern Japan (Yamada et al. 1998; Yamada 2000). In rearing experiments at 20°C, *A. mitsukurii* reaches the stage at which secondary sex characteristics occur at about 16 d after release from the marsupium (Yamada & Yamashita 2000) and these individuals have an average life span of about 40 d (Yamada et al. 1995). In contrast, the bottom temperature during the period between January and March ranges from 7 to 10°C at 10-m depth in Sendai Bay (Yamada 2000). The age to occurrence of secondary sex characteristics was estimated to be 109 d at 9°C in rearing experiments (Yamada & Yamashita 2000), indicating a life span of several months. The specimens caught between January and March are thought to be the overwintering generation that is characterized by a long life span, low reproduction rate and large body size (Yamada 2000). This overwintering generation may be exposed to endocrine disrupters for a much longer period before maturation compared with the warm season generation, resulting in a higher possibility of the manifestation of intersexuality. Further, the overwintering individuals might have different physiological traits from the highly reproductive generation appearing during the warm season. If these speculations are true, it would indicate that the horizontal migration of the mysids is restricted within a small area affected by discharged water from the pulp mill effluent outfall. However, there is no information on the migration of this species. Further studies are required to elucidate the mechanisms of induction of intersexuality in *A. mitsukurii*.

Acknowledgments

We are grateful to Dr K. Nagasawa of the National Fisheries Institute of Aquaculture for identification of the parasites. We also thank Dr Y. Oshima of Kyushu University and Dr H. Takada of the Tokyo University of Agriculture and Technology for helpful comments on our research.

This is contribution ED-01-II-2-1 of the Agriculture, Forestry and Fisheries Research Council Secretariat, Ministry of Agriculture, Forestry and Fisheries, Japan.

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