

## The Occurrence of Dual-gender Imposex in Japanese Freshwater Crab

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**Tributyltin (TBT)-induced imposex phenomena have been reported only in mollusks. Our findings reveal that imposex is not a phenomenon exclusive to mollusks. The imposex phenomenon has been found to occur in a common Japanese freshwater crab, *Geothelphusa dehaani*, which inhabits TBT-uncontaminated mountain streams in Japan. In addition, the imposex of *G. dehaani* occurred not only in females but also in males (dual-gender imposex): either female genital opening (gonopore) or a single ovary occurred in male crabs. However, the definitive causal factors and mechanics relating to the occurrence of imposex are still unclear.**

**Key words** — endocrine disrupters, imposex, freshwater crab

### INTRODUCTION

There are a number of reports of wild animals with secondary sexual characteristics and/or reproductive abilities impacted by environmental endocrine disrupters (EEDs).<sup>1)</sup> By contrast, to this date, there have been relatively few EED invertebrate studies, although more than 99% of the known animal species on our planet are invertebrates.<sup>2)</sup> The superimposition of male characters in female gastropods has been given the name ‘imposex’ by Smith.<sup>3)</sup> This condition has been reported in many kinds of gastropods and some bivalves,<sup>2)</sup> but no examples have been observed in other phyla.

It may be that a number of invertebrates are sensitive to EEDs. Araki *et al.* (1995) reported that

during a one-year survey, beginning in September 1990, six abnormal crabs were found out of 263 individuals collected in the Taniyama River, a small stream in north Kyusyu, Fukuoka, Japan.<sup>4)</sup> This crab species populates mainland Japan, excepting Hokkaido. The objective of this study is to reveal by reinvestigation the possibility of EEDs affecting invertebrates in the environment.

### MATERIALS AND METHODS

**Animals** — The crabs were found from among 207 individuals collected in small streams flowing into the Koga Dam, Fukuoka, Japan (33°41′N, 130°31′E) on October 6 and December 11, 1999. As a control site, 159 crabs were collected from Shiratani River, about 1 km outside of the World Heritage Forest, Yakushima Island, Japan (70 km south from the Japan mainland), on June 25, 1999.

**Analysis of the River Water Sample** — Trace organic contaminants in water were extracted by liquid–liquid extraction using dichloromethane. The extracts were concentrated and subjected to simultaneous determination using a GC/MS system comprising a Varian 3400GC and a Finnigan MAT ITS40MS (an ion trap mass spectrometer). Inductively Coupled Plasma Mass Spectrometry (Seiko Instruments, Inc. SPQ9000) was utilized for qualitative and quantitative analysis of inorganic elements in water.

### RESULTS AND DISCUSSION

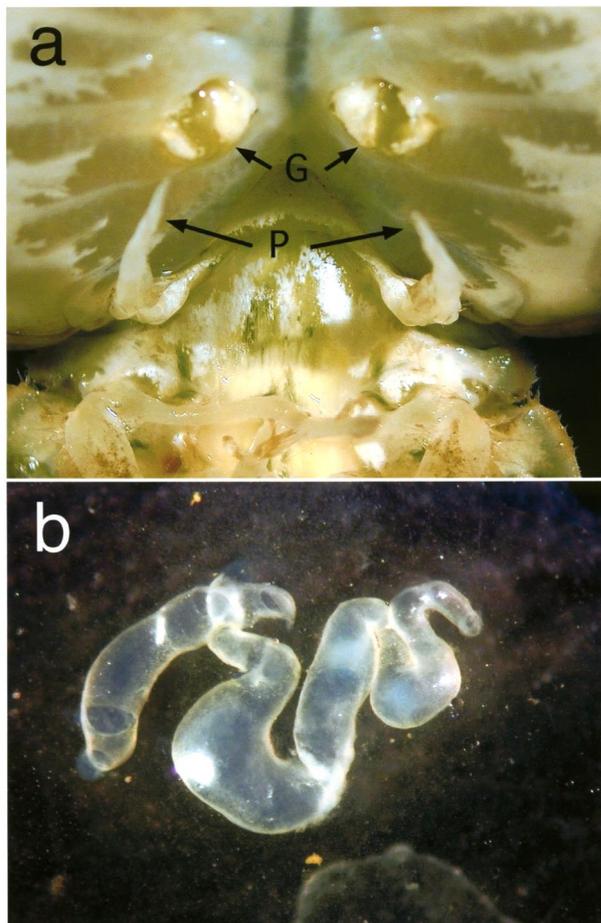
It was found that malformations occurred at a high frequency (Table 1). Consequently, the following types of imposex were observed at Taniyama River: (a) Twelve (12.4%) out of 97 female crabs

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**Table 1.** Occurrence of Imposex in *Geothelphusa dehaani*

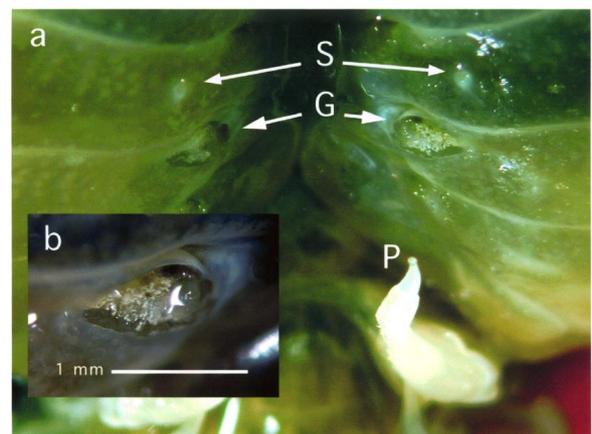
	Number of individuals (percentage)			
	Shiratani River [control]	Taniyama River	Unnamed tributary	Yakuoji River
Male				
N	62 (100%)	31 (66.0%)	4 (80.0%)	3 (37.5%)
G	0	16 (34.0%)	1 (20.0%)	5 (62.5%)
Female				
N	97 (100%)	85 (87.6%)	29 (90.6%)	13 (72.2%)
P	0	12 (12.4%)	3 ( 9.4%)	5 (27.8%)
Concentration of arsenic ( $\mu\text{g}\cdot\text{l}^{-1}$ )	< 0.2	7.5	2.2	10.8

N: normal, G: gonopore-like openings occurred, P: penis-like appendage occurred.

**Fig. 1.** Imposex of a Female *Geothelphusa dehaani*

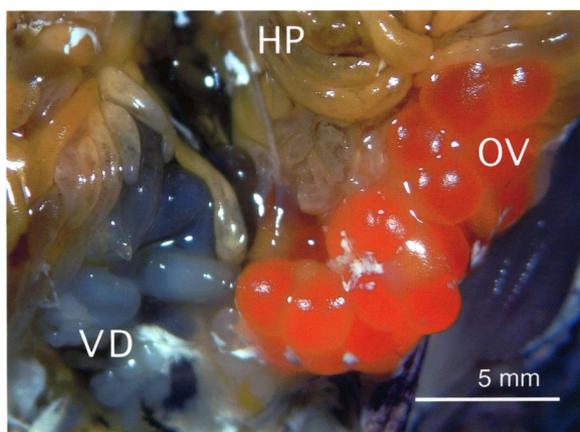
a, Penis-like appendages (P) occurring on the 1st abdominal segment of a female crab. (G) gonopore. b, Vas deferens removed from inside of the penis-like appendage.

had a pair of penis-like appendages that were analogous to the true penis (1st pleopods), and one individual had a single appendage (Fig. 1a, Table 1) on the 1st abdominal segment. Vasa deferentia passed through the inside of the appendages (Fig. 1b). On the 2nd abdominal segment, there was no append-

**Fig. 2.** Gonopore-like Openings

(G, b) occurred on the 6th abdominal segments of male *Geothelphusa dehaani*. (P) penis. (S) sternal knobs. Sternal knobs exist in normal crabs of both sexes.

age analogous to the posterior pleopod of the male. Seminal receptacles in all the imposex-females were filled with sperm. One individual was ovigerous, with 11 juveniles hatched out. Therefore, the imposex-females were not necessarily sterile. (b) Sixteen (34.0%) out of 47 males crabs had a pair of gonopore-like openings on the 6th thoracic sternite (Fig. 2) and one male had two pairs of openings on the 5th and 6th thoracic sternites. These openings were covered with membrane operculums analogous with those of true gonopores. (c) Ovaries were found in two males. One individual had gonopore-like openings; the other individual was externally normal. The average egg diameter was about 2.5 mm at the maturation stage.<sup>5)</sup> The ovaries developed on the right side of the hemocoel and were connected to the vas deferens (Fig. 3). (d) A malformation of the ovary was found in one of the 14 females dissected. Some of the ovarian cells had become atrophied and



**Fig. 3.** Occurrence of an Ovary in the Hemocoel of Male *Geothelphusa dehaani* (OV) ovary; (VD) vas deferens; (HP) hepatopancreas. The ovary is connected to the vas deferens.



**Fig. 4.** Abnormally Developed Ovary of Female *Geothelphusa dehaani*

turned white; other cells had adhered or become fused to each other (Fig. 4).

We collected 159 crabs from the Shiratani River as a control group, about 1 km outside of the World Heritage Forest, Yakushima Island, Japan (70 km south from the Japan mainland), on June 25, 1999. We found no occurrence of abnormalities among them (Table 1).

We are currently expanding our research area, and have found *G. dehaani* imposex in two rivers, an unnamed tributary of the Taniyama River, and also in the Yakuoji River, which is approximately 2 km north and separated by one ridge from the Taniyama River collection point (Table 1).

Although many reproductive disorders of wild animals have been reported, there have been no

imposex disorders reported as occurring in the both sexes in the same population. Rhizocephalan or epicaridean parasites sometimes feminize the male brachyuran hosts; *i.e.*, the male chelipeds become smaller, and abdominal width becomes larger (parasitic castration).<sup>6)</sup> However, no parasites were detected in the present population, and the types of malformation in the males were quite different from those of parasitic castration. It seems likely that the causal factor for these conditions is a chemical substance or substances.

We have examined the water samples for 266 chemical substances, which have been detected from the environmental waters;<sup>7)</sup> *i.e.*, 56 pesticides, 88 aliphatic and aromatic hydrocarbons, 49 oxygenated compounds including phenols, 60 amines and related compounds, and 13 others. Twenty of these chemical substances are listed in the US-EPA as possible EEDs.<sup>8)</sup> The results show no prominent concentration of any of these chemical substances ( $< 0.05 \mu\text{g}\cdot\text{l}^{-1}$ ). Nevertheless, imposex of gastropods has been demonstrated to be induced by the anti-fouling-paint ingredient tributyltin (TBT).<sup>2,9,10)</sup> The result of our elemental analysis revealed no high concentration of tin, nor that of any other heavy metals ( $< 0.01 \mu\text{g}\cdot\text{l}^{-1}$ ), except arsenic (data not shown). At present, the causal relationship between arsenic and imposex occurrence is still unclear and other causative environmental factors may exist.

EEDs are assumed to affect the sexual development of animals if exposed at an early stage of embryo development, even if the EED concentration is low. Unlike common brachyurans, *G. dehaani* progress through their larval stage in a large capsule of the egg and hatch out as a juvenile crab. As the growth of *G. dehaani* is rather slow (it takes 3 years to reach maturation size<sup>11)</sup>), some EEDs may accumulate in the yolk of the large egg ( $> 3$  mm in diameter), which is over 1000 times as large as that of a similar size shore crab, *Hemigrapsus sanguineus*. This may be why this phenomenon is observed only in this species. Further research will be necessary to determine whether these species-specific features limit the occurrence of imposex to *G. dehaani*.

If EEDs negatively impact the reproductive ability of invertebrates, imposex and other unusual phenomena may be highly transitory indications of a progressive probability for extinction. Thus, the study of abnormalities such as imposex is contingent upon the availability of the species under study, while some measure of reproductive ability is maintained within the population. Once extinct, the ex-

istence of an imposex/EED relationship in an invertebrate population passes from our view.

Research findings demonstrate that carnivorous mammals or birds at a high trophic level are susceptible to pollutants, but the present results suggest that attention should also be paid to small invertebrates at a lower trophic level.

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