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Occurrence of *Eurytemora americana* (Copepoda, Calanoida) in a small inlet of Japan: temporary domination and disappearance of the invasive alien species

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Abstract: The estuarine planktonic calanoid copepod *Eurytemora americana* is described for the first time from Japan based on specimens from Dokai Bay, a small inlet of northern Kyushu in 1975, when the species was exclusively dominant in the innermost bay. This is the southernmost record of the species in the Northern Hemisphere. Morphological descriptions are provided, especially on characters different from or overlooked in previous descriptions. Compared with the recent description of the species from Korea, a distinct morphology is found in the spinule number on the male fifth leg, suggesting inter-population variation and therefore no gene flow between the Japanese and Korean populations. *Eurytemora americana* in Dokai Bay is regarded as an invasive alien species introduced via ship ballast water. Despite the dominance in 1975, the copepod was not collected from brackish waters in the innermost part of the bay in 2008. Global warming in spring and/or winter periods after the 1970s is a possible reason for the disappearance of this cold water species.

Key words: ballast water, calanoid copepod, *Eurytemora americana*, global warming, invasive alien species

Introduction

Tsuruta & Yamada (1978) surveyed the spatio-temporal distribution of net plankton in Dokai Bay, a small inlet of northern Kyushu, Japan, from 1974 to 1976, and reported the abundant occurrence of the calanoid copepod *Eurytemora thompsoni* Willey, 1923 in the central and innermost parts of the bay from February to May. At their request, one (HU) of us identified this copepod following Brodsky's (1950) key more than 40 years ago. This species, however, had been synonymized with *Eurytemora americana* Williams, 1906 by Gurney (1933).

Eurytemora americana is a brackish-water species originally described from the Atlantic coast of North America (Williams 1906) and is widespread in relatively high latitudes of the Northern Hemisphere (Moon et al. 2016). In the Northwest Pacific, *E. americana* has been recorded from the coasts of the Japan Sea (Sakhalin), the Sea of Okhotsk (Amur Liman), and the Bering Sea (Kamchatka, Tkachen Bay) (Brodsky 1950 [as *E. thompsoni*], Kos 1977). More recently, Moon et al. (2016) described *E. americana* from the northeastern coast of South Korea. There are no records of the species from Japan other than that of Tsuruta & Yamada (1978 [as *E. thompsoni*]). The present study is the southernmost record of the species in the Northern Hemisphere.

Because Moon et al. (2016) described *Eurytemora americana* from Korea in detail with fine illustrations, we herein briefly describe the specimens collected by Tsuruta & Yamada (1978) in April 1975. Attention is paid to the characteristics that differ from or have been overlooked in previous descriptions (Williams 1906, Brodsky 1950, Heron 1964, Kos 1977, Brylinski 2009, Moon et al. 2016) and on those distinguishing from the three congeners in Japan, i.e. *E. affinis* (Poppe, 1880), *E. pacifica* Sato, 1913, and *E. herdmani* Thompson & Scott, 1897 (Tanaka 1965,

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Itoh 1997).

We also describe the copepod communities in the estuaries of the innermost part of Dokai Bay in 2008 to follow the fate of the *E. americana* population. The ratios of the dominant copepods to the total copepods in these samples were reported by Sakaguchi et al. (2011) but the detailed sampling locations and the numbers of each species were not presented. Discussion is made on possible mechanisms for occurrence of the population in Dokai Bay and subsequent changes in the community.

Materials and Methods

The formalin-preserved plankton sample from which *Eurytemora americana* was described was collected on 23 April 1975 at the innermost station of Dokai Bay (Tsuruta & Yamada 1978, Stn 5) by a surface tow of a 0.1 mm-mesh plankton net. Specimens were observed on depression slides with 70% lactic acid under a differential interference contrast microscope (Nikon E600). Body length was measured with an ocular micrometer on specimens having a relatively straight urosome. Since the urosome generally inclined dorsalward, the prosome length (from the tip of the cephalosome to the suture with the urosome) and the urosome length (from the suture with prosome to the end of the caudal ramus) were measured separately. The ratio of caudal ramus length to width was calculated by the length along the medial margin and the width at the segment base.

Line figures in Fig. 2 were prepared using the computer software package Adobe Illustrator® by tracing drawings made with a camera lucida for the figure of the male P5, and microscope photographs taken with a digital photomicroscopic camera system (Nikon DS-5M) for the other figures. Photo figures in Fig. 3 were made by combining photographs at various focal planes using the image processing software CombineZP (http://combinezp.software.informer.com/). Four kinds of armature elements, i.e. spine, seta, setule, and spinule, were distinguished according to the glossary defined by Huys & Boxshall (1991). Abbreviations used are P5 for the fifth leg and Exp1 (Exp2) for the first (second) exopodal segment.

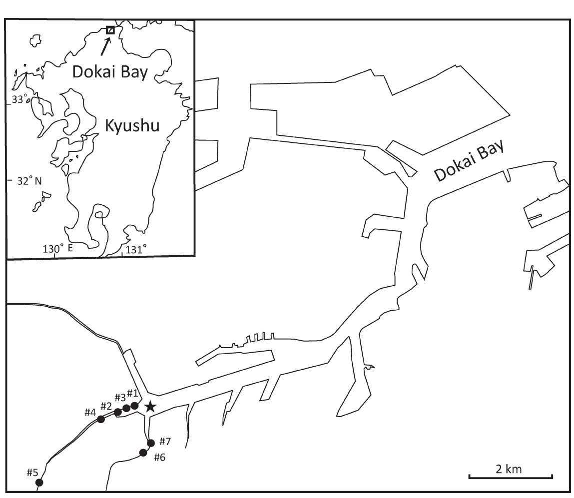

Fig. 1. Map of Dokai Bay with the sampling sites (#1–#7) in the present study and the innermost sampling station (★) in Tsuruta & Yamada (1978).

Zooplankton sampling in the brackish waters of the innermost part of the bay in 2008 was done with a 0.1 mm-mesh plankton net at a total of seven sites in or near the three river mouths on either 3 January, 20 February, or 11 March. Water temperature and salinity in the surface water were measured with a portable T-S meter (CM-21P, DJJ-TOA Corp., Japan). All copepods were counted in the whole samples and adult calanoid and cyclopoid copepods were identified to species.

Results

Systematic accounts

Family Temoridae G. O. Sars, 1903

Genus *Eurytemora* Giesbrecht, 1881

Eurytemora americana Williams, 1906 (Figs 1 and 2)

Material examined: Ten adult females and nine adult males collect from the innermost part (33°52′35″N, 130°45′01″E) of Dokai Bay on 23 April 1975. More than a half volume of the sample has been deposited in the National Museum of Nature and Science, Tokyo (NSMT Cr-25469).

Female: Body (Fig. 2A, B) length 1.29–1.51 mm (mean 1.40 mm, n=8); prosome length 1.4–1.5 times urosome. Cephalosome generally with small dorsal hump at center of posterior margin (Fig. 2B, indicated by arrow). Left posterior wings of prosome larger than right one. Genital double somite laterally swelling at midlength (Figs. 2A and 3A) and constricted posteriorly to swelling; genital aperture vase-shaped in ventral view with broad base and constricted neck (Fig. 3A) and, in lateral view, with rectangularly curved frontal end (Fig. 3B, indicated by arrow). Caudal ramus length 6.3–7.4 times width; rami nearly parallel but distal ends slightly apart from each other, with long setules along lateral and medial margins and conspicuous spinules on almost entire dorsal surface (Fig. 3C) or, in some specimens, lateral half of dorsal surface (Fig. 3D); 4 terminal caudal setae distally curved ventralward.

P5 (Figs. 2C, 3E) with 2-segmented exopod; Exp1 length 2–2.5 times width (excluding attenuation), with weak medial depression at midlength and gently curved pointed attenuation at distomedial corner; Exp2 short, about one-third of Exp1, with 2 posteriorly curved terminal setae; one specimen abnormally bearing spiniform seta on medial margin of second segment of left leg in addition to normal 2 setae (Fig. 3E, indicated by arrow).

Male: Body (Fig. 2D, E) length 1.17–1.38 mm (mean 1.26 mm, n=7); prosome length 1.2–1.4 times urosome; cephalosome usually with small dorsal hump (Fig. 2E, indicated by arrow). Caudal ramus (Fig. 3F) length 7.8–8.6

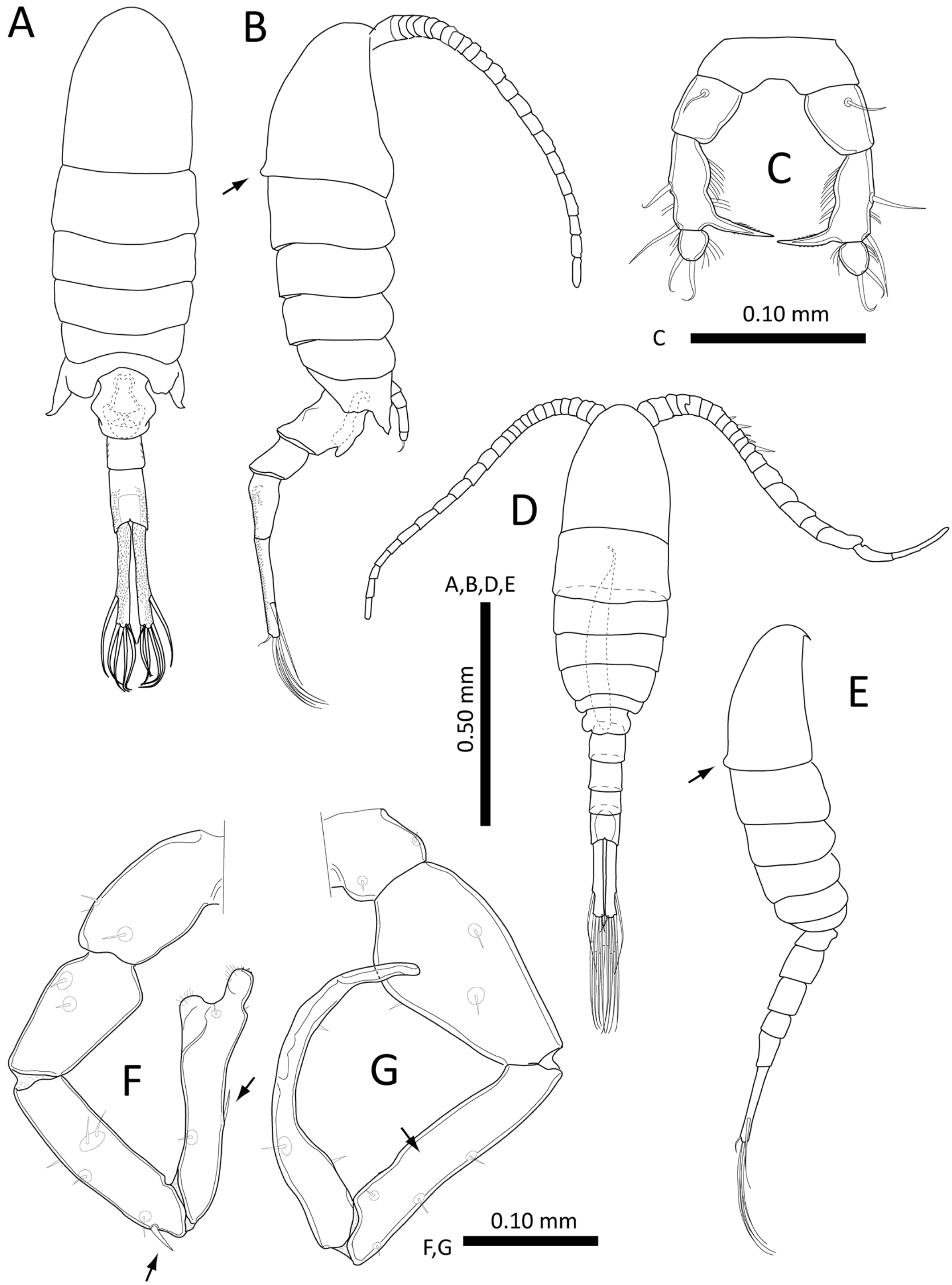

Fig. 2. *Eurytemora americana* from Dokai Bay. A, female habitus, dorsal view; B, female habitus (the same specimen as A), lateral view; C, female fifth legs, posterior; D, male habitus, dorsal view; E, male habitus (the same specimen as D), lateral view F, male left fifth leg, arrows indicating spines; G, male right fifth leg (the same specimen as F).

times width; rami parallel and distal ends generally not apart from each other, without setules along both margins; caudal setae as in female.

Left P5 (Fig. 2F) with 2 small spines each on Exp1 and Exp2 (indicated by arrow in Fig. 2F); basis broadest near base; Exp2 distally with 2 rounded bulbous processes with fine setules, subterminal one producing medialward and terminal one apically with 1–3 very tiny spinules; spinule numbers from coxa to Exp2: 3, 2, 5, 3, respectively. Right P5 (Fig. 2G) with basis swelling at proximal one-third; spinule numbers from coxa to Exp2: 2, 3, 4, 3; 3 of 4 spinules on Exp1 located near lateral margin and 1 near distomedial

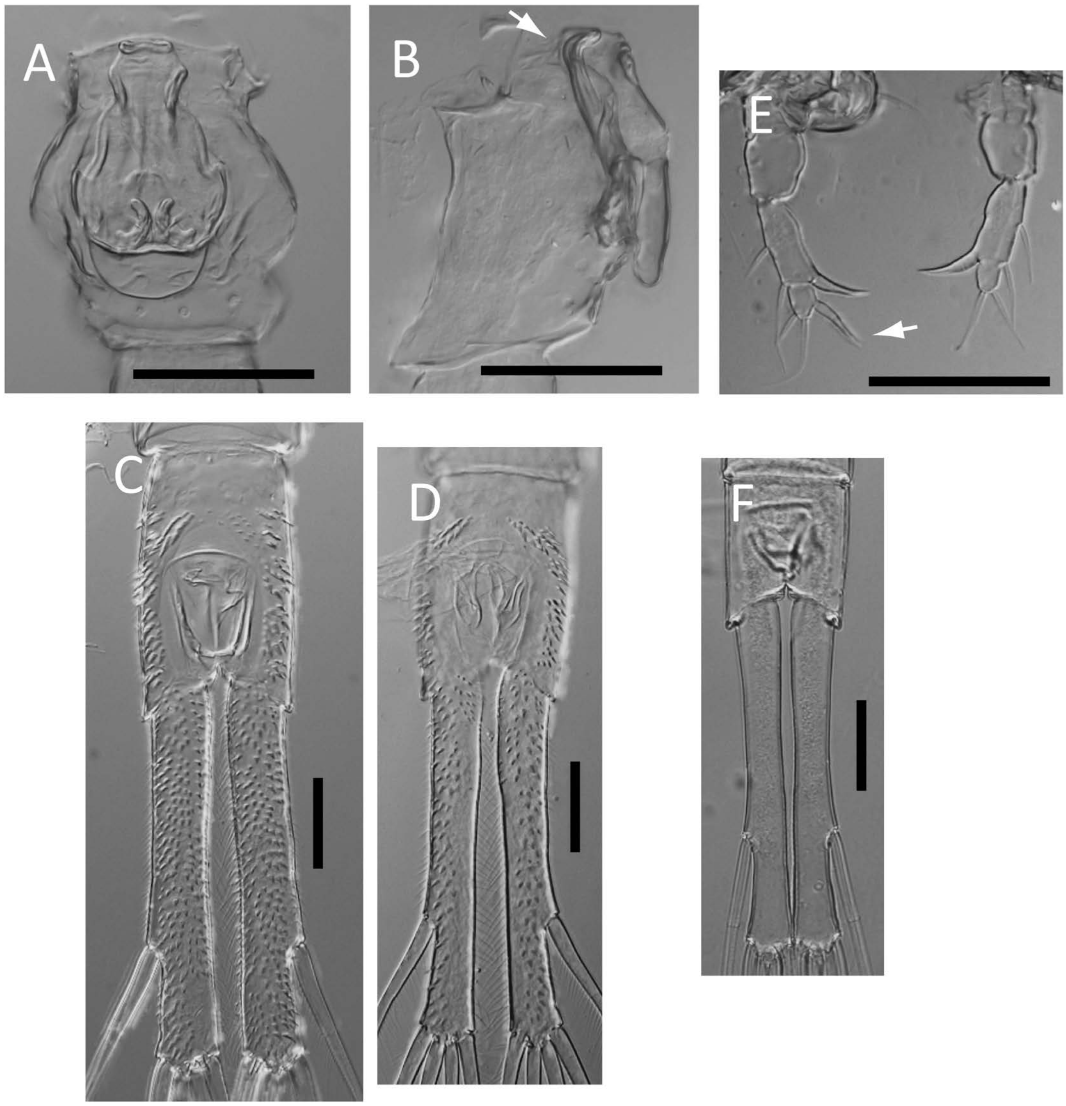

Fig. 3. *Eurytemora americana* from Dokai Bay. A, female genital aperture, ventral view; B, female genital aperture (the same specimen as A), lateral view, arrow indicating anterior end of seminal receptacle; C, D, female anal somite and caudal rami, dorsal view, different specimens; E, female fifth legs abnormally having a spine (arrowed) on the left second exopodal segment; F, male anal somite and caudal rami, dorsal view. Each photograph was made by combining those taken at different focal planes. Scale bars represent 0.10 mm.

corner.

Remarks

Although the original description of *Eurytemora americana* by Williams (1906) did not present enough information on the species-specific characters, Gurney (1933) and later authors, especially Heron (1964), Brylinski (2009) and Moon et al. (2016), have provided more distinct species-diagnostic characters. Those of the female are: slightly asymmetric prosomal wings with larger left one; genital double somite swelling at midlength and constricted posteriorly; anal somite and long caudal ramus dorsally with conspicuous spinules; P5 Exp1 twice as long as wide, with weak medial depression; and Exp2 shorter than a half of Exp1. The male diagnostic characters are: long caudal rami without dorsal spinules; left P5 Exp2 with two rounded bulbous processes on distal part; and right P5 basis swelling proximally.

Compared with the recent description of *E. americana* from South Korea (Moon et al. 2016), our specimens have minor differences in the following characters (those of Korean specimens are given in brackets):

(1) cephalosome with a small dorsal hump in both sexes [present only in male];
(2) female genital aperture vase-shaped with constricted neck in ventral view [without constricted neck in their fig. 2];
(3) female caudal ramus is covered with spinules generally on almost entire dorsal surface [without spinules on medial half];
(4) terminal caudal setae distally curved ventralward in both sexes [almost straight];

(5) male left P5 with two spines each on Exp1 and Exp2 [these spines were described as setae];
(6) and male right P5 Exp1 with four spinules (three laterally and one distomedially) [in addition to these four spinules, one medial spinule present at the location indicated by arrow in Fig. 2G].

Some of these perceived may be due to individual variability in the specimens examined or could be due to the methodology of the respective observations. However, at least the difference in the spinule number on the male right P5 Exp1 is apparently an inter-population variation, because all the male P5 Exp1s of the present specimens examined had four spinules.

Three Japanese congeners are easily distinguishable from *Eurytemora americana*. *Eurytemora affinis* is closest to *E. americana* among the three species, but the former differs by having the genital double somite with more prominent lateral swelling, the female P5 Exp2 longer than a half of Exp1 (excluding attenuation), the male P5 basis without swelling proximally, and the male P5 Exp2 terminally with acute tip (Itoh 1997, Mizuno 2000). The other two Japanese congeners, *E. herdmani* and *E. pacifica*, have caudal rami without dorsal spinules and a quite different P5 in the females. The distal part of their male left P5 Exp2 is somewhat acute instead of having two rounded bulbous processes and the right P5 base does not swell proximally (Sato 1913).

According to Tsuruta & Yamada (1979, Fig. 2a), the surface water temperature and salinity at the collection site (Stn 5) on the sampling day (23 April 1975) were 14°C and 22, respectively. Those from February to May, when *Eurytemora americana* abundantly occurred there from 1975 to 1976, were approximately 8–22°C and 20–33, respectively. Zooplankton in the present sample consisted exclusively of various stages of *E. americana*, with a much smaller number of *Acartia omorii* Bradford, 1976.

Copepod community in brackish waters of innermost part of Dokai Bay in 2008

The temperature and salinity ranges in the surface water at the various sampling sites were 9.6–14.1°C and 15.1–33.0, respectively (Table 1). The dominant copepod in the samples collected in 2008 was *Oithona davisae* Ferrari & Orsi, 1984, except for the sample from site #5, where the estuarine calanoids *Pseudodiaptomus inopinus* Burckhardt, 1913 and *Sinocalanus tenellus* (Kikuchi, 1928) were dominant. Three specimens of copepodites of *Eurytemora* were collected each from sites #1, #5, and #7, but these were probably *E. pacifica* Sato, 1913, because its adults occurred at sites #1 and #4. Thus *E. americana* was not found in the innermost estuaries of the bay in 2008.

Discussion

Moon et al. (2016) recently discovered *Eurytemora americana* from the northeast coast of South Korea. They suggested three possible explanations for the new occurrence in Korean waters, i.e. insufficient studies of the northern estuaries in Korea, misidentification in previous studies, and recent invasion to Korean waters. As for the

Table 1. The number of copepods collected from brackish waters in the innermost part of Dokai Bay in 2008. The site IDs correspond to those in Fig. 1.

Sampling date in 2008	3 Jan.		20 Feb.			11 Mar.	
Site ID	#1	#2	#3	#4	#5	#6	#7
Longitude (North 33°)	52′33.2″	52′30.1″	52′31.4″	52′31.4″	52′22.2″	51′55.8″	52′01.1″
Lattitude (East 130°)	44′46.3″	44′30.7″	44′42.1″	44′42.1″	44′15.6″	44′56.6″	45′05.6″
Water temperature (°C)	12.0	13.1	9.8	9.6	10.2	14.1	14.1
Salinity	33.0	22.0	22–20.2	25.6	24.3	15.1	18.9
Acartia hudsonica Pinhey, 1926	5		3	3	9	4	13
Acartia omorii Bradford, 1976					1		
Acartia spp. (copepodite)	1		7	38	22	8	5
Eurytemora pacifica Sato, 1913	1			1			
Eurytemora sp. (copepodite)	1				1		1
Paracalanus parvus s.l.	14						
Parvocalanus crassirostris (Dahl, 1894)	17		1	1			
Pseudodiaptomus inopinus Burckhardt, 1913					117		
Sinocalanus tenellus (Kikuchi, 1928)			4	3	125		
Oithona davisae Ferrari & Orsi, 1984	75	148	43	41	1	33	40
Unidentified cyclopoid						1	
Benthic harpacticoid						26	3
Total	114	148	58	87	276	72	62

population in Dokai Bay, introduction by ballast waters is the most probable explanation for its occurrence because of the following reasons:

(1) *Eurytemora americana* was abundant from February to May in the innermost part of Dokai Bay, where the surface salinity was 22–33. This species can co-occur with marine copepods (Moon et al. 2016, present study). These facts indicate that the species could occur from winter to spring in polyhaline brackish waters, such as the inner parts of enclosed bays. Copepod faunas in such bays along the Japan Sea coast of Japan have been studied during colder seasons by planktologists. For example, Yamazi (1956) investigated the distribution of the net plankton community in a brackish coastal lagoon, Yosanai-kai, Kyoto Prefecture in March and April. Ohtsuka et al. (1999) described the seasonal succession of the zooplankton fauna, in brackish Lake Naka-umi, Shimane Prefecture, in detail at several stations. One (HU) of us studied monthly copepod distribution throughout the year in Kumihama Bay, a strongly enclosed brackish (salinities$<$30) bay of Kyoto Prefecture (Ueda 1991). These studies suggest that the absence of *E. americana* from Japan, other than the records for Dokai Bay, is unlikely to be attributable to insufficient studies in Japan.

(2) If researchers other than copepod taxonomists collected *Eurytemora americana* in Japanese waters, they would have probably misidentified it to *Eurytemora affinis*, because the appearance of the two species is very similar and because *E. americana* has not been treated or described in any Japanese books or papers. Notwithstanding, records of *E. affinis* in Japan are all from fresh or oligohaline brackish waters (Mizuno 2000), which obviously differ from the polyhaline habitats of *E. americana*. Therefore, misidentification is unlikely, considering these previous records of *E. affinis*.

(3) Dokai Bay has been used as an industrial port, and is one of the three main ports of the City of Kitakyushu, the second largest city in Kyushu; these ports are collectively named the Port of Kitakyushu, the main trade port in western Japan (http://www.kitaqport.or.jp/index_e.html, accessed on 3 March 2017). Dokai Bay is surrounded by many manufactories, especially large steel works. Thus it is considered that export cargo ships would have discharged a large amount of ballast water into the bay water until and during the 1970s. In fact, although the data are not from the 1970s, the total amount of estimated take-in of ballast water at the Port of Kitakyushu in 2005 was about 30×10^4 tons, which was about 1.5 times greater than that at the Port of Osaka in the same year (Ohtsuka et al. 2008). Colonization of alien benthic invertebrates in Dokai Bay has already been reported by Kajiwara & Yamada (1997) and Kajiwara et al. (2015). As for copepods, intercontinental introduction of free-living copepods via ship ballast water is known especially for estuarine forms including *Eurytemora* (Reid & Pinto-Coelho 1994, Ohtsuka et al. 2004). Considering the attributes of Dokai Bay and estuarine copepods, as well as the sudden occurrence of the species during the 1970s, it can undoubtedly be concluded that *E. americana* in Dokai Bay was an invasive alien species introduced via ballast water.

Inter-population variation between Korean specimens (Moon et al. 2016) and the present specimens was observed in the spinule number on the male P5 and probably in ornamentation of the female caudal ramus. This suggests that the two populations are isolated from each other without gene flow, and that, given that both populations were introduced from elsewhere by ballast water, their sources were probably different. Genetic comparison between them is necessary to test these hypotheses.

The result of sampling in the innermost part of Dokai Bay from January to March 2008 was that *Eurytemora americana* was not collected in the brackish waters. There have been many papers reporting invasions of non-indigenous copepods (Reid & Pinto-Coelho l994, Bollens et al. 2002, Ohtsuka et al. 2004). Some of these alien copepods became dominant in the copepod community of their settled places and some spread from the range they settled first (Orsi & Walter 1991, Cordell et al. 1992, 2008, Orsi & Ohtsuka 1999, Bollens et al. 2002). However, so far it has been unreported that a planktonic copepod presumed to be invasive and that once dominated its new habitat, could have disappeared over the space of a few decades as is the case for *E. americana* in Dokai Bay.

Global warming is the most possible reason for the disappearance of *Eurytemora americana* from Dokai Bay. The bay is the southern limit of the species in the Northern Hemisphere. This means that the temperature in the bay is almost at the highest limit for the species to reproduce successfully. We do not have access to long-term data on the water temperature in Dokai Bay. However, the temperature in the brackish-waters of the innermost part of the bay is highly considered to be correlated to air temperature. The average air temperature in Fukuoka Prefecture, where Dokai Bay is located, during spring (March to May) has become higher by about 1°C over the last 40 years, with fluctuations of $<$2.5°C (Fig. 4A). In the springs of 1975 and 1976, when *E. americana* occurred abundantly (Tsuruta & Yamada 1978), the temperatures were lower by $>$0.5°C than the last 30-year average, whereas after the 1980s extremely high temperatures (higher by $>$1.0°C than the 30-year average) were recorded in 1998 and 2002. These high temperatures in spring might have been beyond the upper threshold for this cold-water species.

Because *Eurytemora americana* produces resting eggs (Marcus et al. 1994), it is considered that the population in Dokai Bay survived high temperatures in summer and autumn as resting eggs and reappeared in the next winter by the hatching of these eggs. It is well known that temperature is one of the main factors affecting hatching success of copepod resting eggs (e.g. Uye et al. 1979, Johnson 1980, Marcus 1996). Sullivan & McManus (1986) noted an interesting phenomenon about the cold-water co-

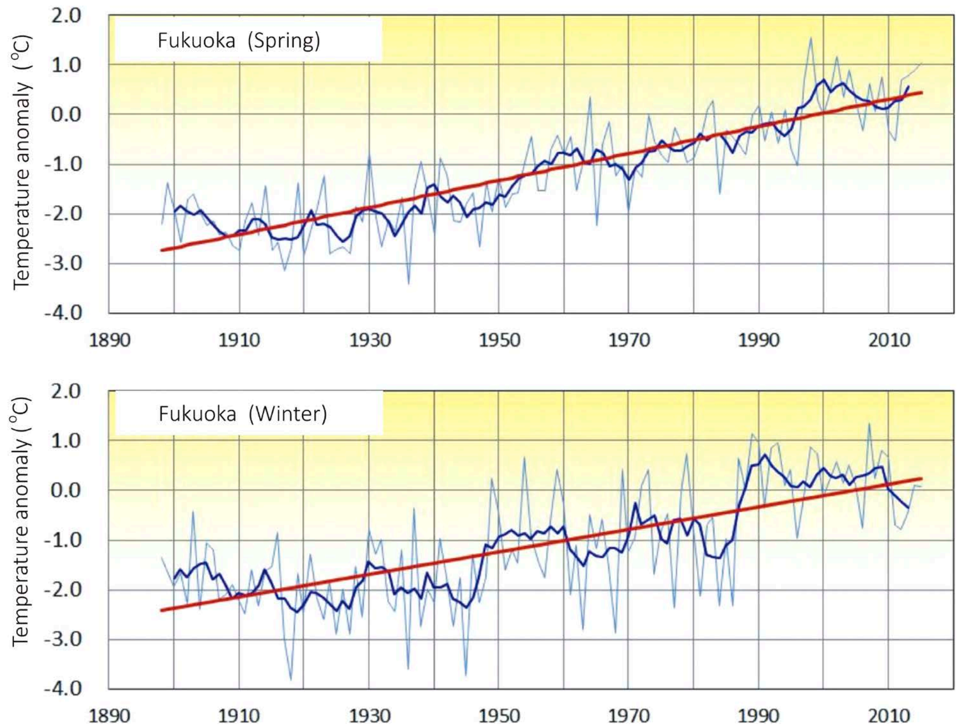

Fig. 4. Temperature deviation during spring (March–May) (A) and winter (December–February of next year) (B) in Fukuoka Prefecture from 1898 to 2015 (Fukuoka Regional Headquarters-Japan Meteorological Agency 2016; Japanese in the original figure was translated to English by the author.). Thin polygonal line, temperature anomaly (℃) from the mean temperature for 30 years from 1981 to 2010; thick polygonal line, 5-year running mean of temperature anomaly; thick straight line, long-term trend.

pepod *Acartia hudsonica* Pinhey, 1926. Most of the eggs that had not hatched by incubation at the ambient temperature (>16℃) hatched after exposure to low temperature (4–6℃); they regarded these eggs as resting ones. This implies that low temperature is necessary for the hatching of resting eggs in cold-water copepod. Accordingly, warming in winter, when the seasonal occurrence of *E. americana* in Dokai Bay would begin, is also a possible reason for its disappearance from the water column. The long-term change of the winter air temperature between the 1970s and the 1990s was more distinct than that of the spring temperature (Fig. 4B). That is, the 5-year running mean temperature became higher steeply during the late 1980s. The highest winter temperature after the 1970s was recorded in the 2007's winter (from December 2007 to February 2008), when the present sampling was made. If it is true that *E. americana* disappeared from Dokai Bay by failure in hatching of resting eggs due to warmer winters, they would occur again in colder winters. Zooplankton sampling especially in a spring after a colder winter, as well as sampling and incubation of resting eggs in bottom sediments of the innermost part of the bay, is necessary to test this hypothesis.

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