Aquatic Animals

水生動物 第2024巻
令和6年2月

Sex ratios in five amphidromous atyid shrimp species collected in the Banda River, Boso Peninsula, Japan

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Abstract

We investigated the sex ratio in five amphidromous atyid shrimp species of the genera *Caridina* and *Paratya*; namely, *C. leucosticta*, *C. multidentata*, *C. serratirostris*, *C. typus* and *P. compressa*. Our surveys revealed slight or significant male-biased sex ratios for *Caridina* and *Paratya* species, particularly during the breeding season, except for *C. serratirostris*, which had a significant female-biased sex ratio. Size-specific sex ratios were observed as male-biased and female-biased for the middle and large size classes, respectively. We discuss the possible causes of biased sex ratios in atyid shrimps.

Key words: *Caridina*; *Paratya*; reproduction; biased sex ratio; size-specific sex ratio; mating system

Introduction

Five amphidromous atyid shrimp species of the genera *Caridina* and *Paratya* (Decapoda; Caridea; Atyidae) are widely distributed in western and southern Japan (~24–35°N); these species are *C. leucosticta* Stimpson 1860, *C. multidentata* Stimpson 1860, *C. serratirostris* De Man 1892, *C. typus* H. Milne-Edwards 1837 and *P. compressa* (De Haan 1844) (Shokita 1979; Hamano and Hayashi 1992; Suzuki et al. 1993; Usami et al. 2008; Watanabe and Kano 2009; Saito et al. 2012; Yatsuya et al. 2012; Soomro et al. 2016; Maruyama and Okamoto 2022). The occurrence and distributional characteristics of amphidromous atyids have been intensively studied in rivers and streams (e.g. Shokita 1979; Suzuki et al. 1993; Usami et al. 2008). The population structure and dynamics, including recruitment, growth and reproduction, have also been investigated for *C. leucosticta* (Shokita 1979; Yamahira et al. 2007; Yatsuya et al. 2013), *C. multidentata* (Shokita 1979; Hamano and Hayashi 1992), *C. serratirostris* (Shokita 1979) and *C. typus* (Shokita 1979; Imai and Oonuki 2022). However, the sex ratio, which is an important life history trait, has not been documented for these atyid shrimps. Here, we report the sex ratios for five amphidromous atyid shrimp species based on the collection records from our previous study (Yamada et al. 2024).

Materials and Methods

Shrimp collection and treatment

We previously conducted monthly field surveys from May 2017 to August 2019 to collect atyid shrimps in the Banda River (34°58´N, 139°46´E), Boso Peninsula, Japan, to elucidate the seasonal occurrence patterns of atyids by analysing the relationship between stream temperature and the number of shrimps collected (Yamada et al. 2024). The carapace length of each collected specimen was measured to the nearest 0.01 mm from the posterior margin of the orbit to the dorsal posterior end of the carapace using a stereomicroscope equipped with a digital camera and an image analysis system (Nikon Digital Sight and NIS-Elements software, Nikon Corp., Tokyo, Japan). The second pleopod was then removed from the specimen with forceps and examined under a microscope to determine sex based on the presence (male) or absence (female) of an appendix masculina on the endopod. Individuals smaller than the minimum size of males were considered juveniles of each species (see Tables S2–S6 in Yamada et al. (2024) for the minimum carapace lengths of males). Females were recorded as ovigerous or non-ovigerous. The records of shrimps collected during the entire survey period are summarised in Table 1.

Table 1. Collection records of five amphidromous atyid shrimp species of the genera *Caridina* and *Paratya* during the monthly field surveys conducted from May 2017 to August 2019 in the Banda River, Boso Peninsula, Japan. Data are derived from Yamada et al. (2024).

Species	Number of shrimps			
	Juveniles	Males	Females	Total
C. leucosticta	3	88	67	158
C. multidentata	4	333	147	484
C. serratirostris	0	275	454	729
C. typus	3	397	271	671
P. compressa	24	3574	2053	5651

Data analysis

The sex ratio was calculated as (number of males)/(number of males and females) for all survey months and for survey months when ovigerous females did not occur (i.e. non-breeding months) or occurred (i.e. breeding months). Ovigerous females of our target atyid shrimps occurred during the warm seasons from spring to autumn (see Tables S2–S6 in Yamada et al. (2024) for the non-breeding and breeding months). To assess whether the sex ratios differed between the non-breeding and breeding months, the independence of the occurrence of males and females between these months was assessed using the chi-squared test. Sexual dimorphism of body size was evident for all five atyid shrimp species (Yamada et al. 2024), as the females were larger than males (Fig. 1). Therefore, the sex ratio was also calculated for the carapace length classes at 0.5 mm intervals for each species. This was done for non-breeding and breeding months, because the sex ratios

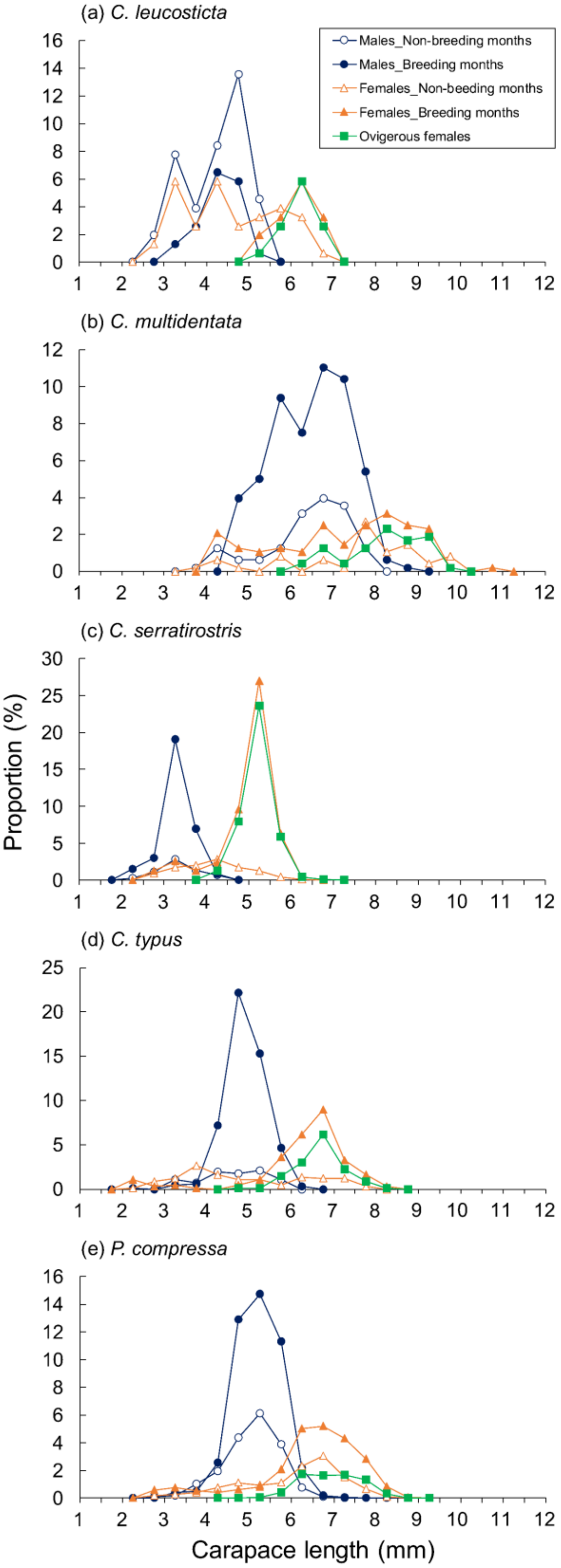

Fig. 1. Carapace length frequency distributions of males, females and ovigerous females for five amphidromous atyid shrimp species of the genera *Caridina* and *Paratya* collected in the Banda River: *C. leucosticta* (a), *C. multidentata* (b), *C. serratirostris* (c), *C. typus* (d) and *P. compressa* (e).

Table 2. Sex ratios (number of males/number of males and females) of five atyid shrimp species of the genera *Caridina* and *Paratya* during all survey months and during survey months when ovigerous females did not occur (non-breeding months, NB) or occurred (breeding months, B). A binomial test was used to test the null hypothesis for the sex ratio (H_0; sex ratio = 0.5). A chi-squared test was employed to evaluate the independence of the occurrence of males and females during non-breeding and breeding months.

Species	Survey month	Number of males (1)	Number of females (2)	Sex ratio [1/(1+2)]	Binomial test	Chi-squared test		
					p	χ^2 value	df	p
C. leucosticta	All	88	67	0.568	0.1079	0.1926	1	0.6607
	NB	62	45	0.579	0.1215			
	B	26	22	0.542	0.6655			
C. multidentata	All	333	147	0.694	< 0.0001	2.749	1	0.0973
	NB	76	44	0.633	0.0045			
	B	257	103	0.714	< 0.0001			
C. serratirostris	All	275	454	0.377	< 0.0001	0.2107	1	0.6462
	NB	46	82	0.359	0.0019			
	B	229	372	0.381	< 0.0001			
C. typus	All	397	271	0.594	< 0.0001	30.09	1	< 0.0001
	NB	58	88	0.397	0.0161			
	B	339	183	0.649	< 0.0001			
P. compressa	All	3574	2053	0.635	< 0.0001	13.88	1	0.0002
	NB	1048	700	0.600	< 0.0001			
	B	2526	1353	0.651	< 0.0001			

significantly differed between these months in some species. A binomial test was used to test the null hypothesis for the sex ratio (H_0; sex ratio = 0.5) because Fisher's theory indicates that natural selection favours a 1:1 sex ratio (Fisher 1930).

Results

Sex ratios of five atyid shrimp species are presented in Table 2. The sex ratio during the study period was balanced in *C. leucosticta* (0.568, $p = 0.1079$). It was male-biased in *C. multidentata* (0.694, $p < 0.0001$), *C. typus* (0.594, $p < 0.0001$), and *P. compressa* (0.635, $p < 0.0001$) and female-biased in *C. serratirostris* (0.377, $p < 0.0001$). The sex ratio did not vary between the non-breeding and breeding months for *C. leucosticta* ($\chi^2 = 0.1926$, df = 1, $p = 0.6606$) and *C. serratirostris* ($\chi^2 = 0.2107$, df = 1, $p = 0.6462$). In *C. typus*, the sex ratio was female-biased in the non-breeding months (0.397, $p = 0.0161$) and male-biased in the breeding months (0.649, $p < 0.0001$) ($\chi^2 = 30.09$, df = 1, $p < 0.0001$). In *P. compressa*, although the sex ratio was male-biased in both the non-breeding (0.600, $p < 0.0001$) and breeding months (0.651, $p < 0.0001$), the proportion of males was significantly higher in the breeding moths than in the non-breeding months ($\chi^2 = 13.88$, df = 1, $p = 0.0002$). This feature was also observed for *C. multidentata* (non-breeding months, 0.633, $p = 0.0045$; breeding months, 0.714, $p < 0.0001$), although the occurrence of males and females was not significantly different between the non-breeding and breeding months ($\chi^2 = 2.749$, df = 1, $p = 0.0973$).

The sex ratios were plotted against the carapace length class midpoints in Fig. 2 (see Table S1 for corresponding test statistics for the sex ratios within the size classes). Overall, the sex ratios exhibited an asymmetrical bell shape: they were highly male-biased and female-biased in the middle and large size classes, respectively.

Discussion

While Fisher's theory indicates that natural selection

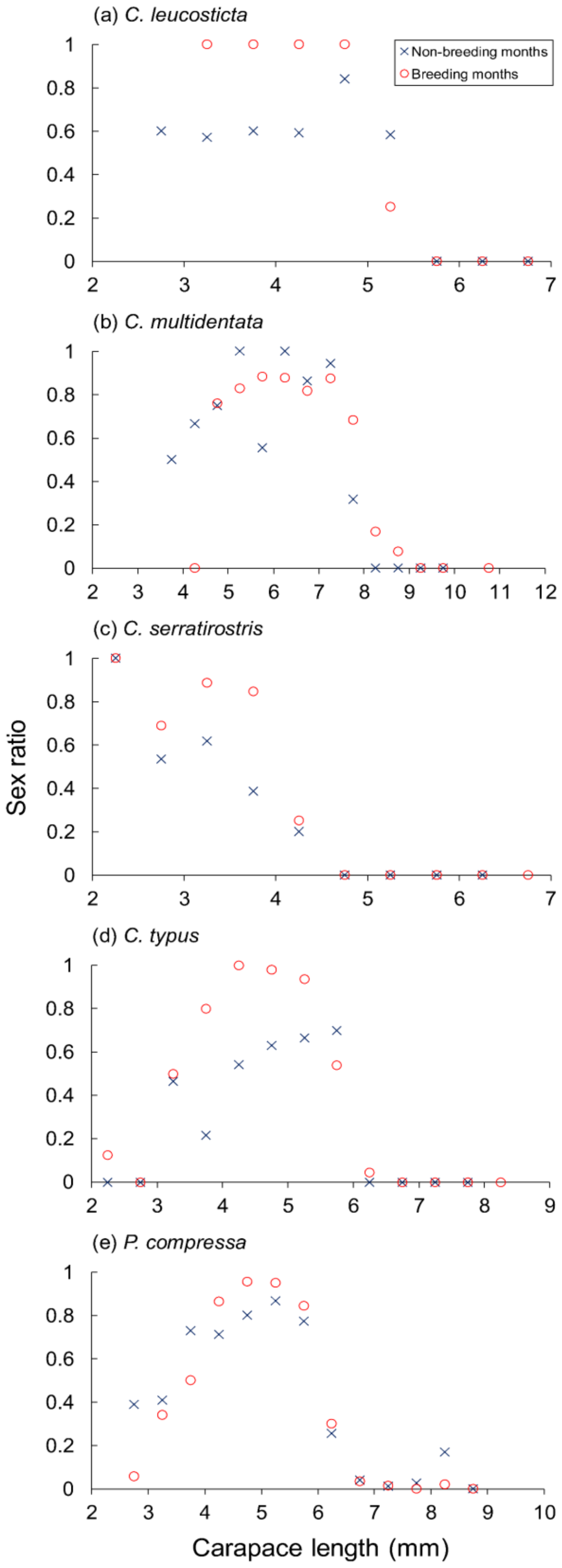

Fig. 2. Sex ratio in relation to the carapace length class mid-point for five amphidromous atyid shrimp species of the genera *Caridina* and *Paratya* collected in the Banda River: *C. leucosticta* (a), *C. multidentata* (b), *C. serratirostris* (c), *C. typus* (d) and *P. compressa* (e). The sex ratio was calculated as (number of males)/(number of males and females).

favours a 1:1 sex ratio (Fisher 1930), unbiased sex ratios are frequently observed in decapod crustacean species (Wenner 1972; Ewers-Saucedo 2019), including atyid shrimps. For instance, sex ratios have been reported as 0.38–0.63 for eight *Caridina* species (De Silva 1988a, 1988b; Choy and Ng 1991; Yam and Dudgeon 2005; Leuven et al. 2008; Han et al. 2011; Zare et al. 2011).

Our surveys revealed either slight or significant male-biased sex ratios for *Caridina* and *Paratya* species (0.57–0.69), particularly during the breeding months. The exception was *C. serratirostris*, which had a significant female-biased sex ratio (0.38). Hamano and Hayashi (1992) collected 598 males and 381 females of *C. multidentata* during a one-year field study conducted in a small stream (33°47´N, 134°36´E) in Tokushima Prefecture, Japan; thus, their sex ratio can be calculated as 0.61, which was similar to that of our *C. multidentata* population (0.69).

Sex-specific behaviour has been proposed as one of the causes of sex ratio bias (Ewers-Saucedo 2019), and mating behaviour may influence the observed sex ratio of atyid shrimps during the breeding season. A pure-searching mating system is thought to be the most common type among free-living caridean shrimps (Bauer and Abdalla 2001; Correa and Thiel 2003), and a small size is advantageous for males in this mating system due to increased agility, lower energetic costs and low conspicuousness to predators (Correa and Thiel 2003). When a searching male finds a receptive female, it transfers spermatophores during a brief mating without exhibiting complex behaviour, such as the courtship of females or aggression against other males. This mating behaviour has been reported for the atyid shrimp *Caridina laevis* Heller 1862 (Pillai 1960).

The body size (carapace length) of our target atyid shrimps showed a distinct intersexual dimorphism, with males being significantly smaller than females (Fig. 1) (also see Yamada et al. 2024), as previously reported for the same atyid species (Shokita 1979;

Hamano and Hayashi 1992; Yamahira et al. 2007; Yatsuya et al. 2013; Imai and Oonuki 2022). *Caridina* and *Paratya* species with small male sizes may exhibit a pure-searching mating system. The pure-searching behaviour of males against females may explain the male-biased sex ratio of atyid shrimps observed at our sampling sites during the breeding season. However, a biased sex ratio during the non-breeding season or all seasons would not be explained by male reproductive behaviour. The mating system, behaviour and microhabitats of males and females should be investigated further to reveal the mechanisms underlying the biased sex ratios in atyid shrimps.

Reflecting the sexual size dimorphism observed in our target atyid shrimps (i.e. females larger than males; Fig. 1), the sex ratios were highly skewed to males and females in the middle and large size classes, respectively (Fig. 2). A similar phenomenon has been reported for several *Caridina* and *Paratya* species, such as *C. fossarum* Heller 1862 (Zare et al. 2011), *C. pristis* Roux 1931 (De Silva 1988b), *C. simoni* Bouvier 1904 (De Silva 1988a), and *P. curvirostris* (Heller 1862) (Carpenter 1978). Sexual size dimorphism depends on the sex-specific growth patterns in *C. leucosticta* and *P. compressa* (Yamahira et al. 2007; Yamada et al. 2024), as new summer recruits grow linearly until the end of autumn in both sexes, reaching a similar body size to that of adult males. The females then resume growth in the next spring and reach a larger body size. The larger female body size may be advantageous for increased fitness of males and females because clutch size is positively correlated with female body size in the *Caridina* and *Paratya* species (Shokita 1979).

Apart from the sexual size dimorphism, which depends on the sex-specific growth patterns, sex changes from male to female—i.e. protandric hermaphroditism—could not be ruled out for small size overlaps between males and large adult females (Fig. 1) (Wenner 1972). Indeed, *P. curvirostris* is thought to be protandrous, based on histological and morphological investigations (Carpenter 1978). Reproductive systems should be further examined to clarify the causes of the size-specific sex ratios observed in the *Caridina* and *Paratya* species.

Acknowledgments

We thank the members of the laboratory for helping with laboratory work. We are grateful to two anonymous reviewers for their constructive comments and suggestions. This study was supported by JSPS KAKENHI grant number JP17K00578 to KH.

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Received: 5 February 2024 | Accepted: 21 February 2024 | Published: 24 February 2024