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ORIGINAL ARTICLE

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Seasonal change in sexual size dimorphism of the major cheliped in the hermit crab *Pagurus minutus*

Abstract Sexual size dimorphism is a common phenomenon in the animal kingdom, and its seasonal change has been reported in some species that possess traits dimorphic only in males and specialized for male mating success. However, few studies have examined seasonal change in sexual dimorphism of traits possessed by both sexes. Here, we examined the reproductive biology of the hermit crab *Pagurus minutus*, at a sandflat in the Waka River estuary, Japan, with special reference to seasonal changes in sexual dimorphism of the large claw (major cheliped) size by conducting population and precopulatory guarding-pair sampling. Previous investigation demonstrated that the major cheliped is used as a weapon, and its size, more than body size, determines the winner in male–male contests of this species. We found ovigerous females from November to April, peaking in January, when 80% of females were ovigerous. Sexual size dimorphism of the major cheliped was observed; the degree of dimorphism increased in the reproductive season, when only males possessed an enlarged major cheliped. In addition, in the reproductive season, precopulatory guarding males had a larger body and larger relative size of the major cheliped than did solitary males, although the major cheliped size in guarding males seemed to reach an upper limit. These results suggest that seasonal change in sexual dimorphism of the major cheliped size in *P. minutus* strongly reflects sexual selection favoring the development of this natural weaponry, and that the degree of the dimorphism might be limited through natural selection.

Keywords Decapoda · Morphology · Reproductive success · Seasonal variation · Secondary sexual traits

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Introduction

Sexual dimorphism in morphological traits evolves in the context of sexual selection and is well documented in various taxa, as these traits are often more evident in males than in females (Andersson 1994). Body size is one of the most studied traits showing sexual dimorphism, but other features, including natural weaponry and ornamentation, also exhibit obvious differences between the sexes (Berglund et al. 1996; Hunt et al. 2009). For example, marine mammals as a group present the greatest body size dimorphism, with males of the most dimorphic taxa (seals) being on average three times larger in body mass than females (Weckerly 1998). Red coloration in the three-spined stickleback *Gasterosteus aculeatus* (Candolin 1999) and the elongated eyestalks of *Cyrtodiopsis* stalk-eyed flies (Panhuis and Wilkinson 1999) are examples of conspicuous male traits that function in both male–male contests and female mate choice.

In animals with a distinct reproductive season, the degree of sexual dimorphism often changes seasonally, depending on the type of contribution of the trait to its possessors. One type are traits that contribute only to reproduction: in this case, seasonal changes in sexual dimorphism occur because males display the traits only during the reproductive season. For example, just before mating, salmonid males develop an enlarged jaw and increased hump height for use in competition for females or spawning sites (Fleming and Gross 1994; Hendry and Berg 1999). Other types of traits function not only in sexual contexts but also in general behaviors and are thus often observed in both sexes. These shared traits can nevertheless develop further in males in the reproductive season, than otherwise exhibited in the non-reproductive season, because of their reproductive advantages. For example, both sexes of some decapod crustaceans possess an enlarged cheliped, which they generally use for foraging, predator avoidance, and contest competition (Juanes and Smith 1995; Duffy and Thiel 2007). However, sexual dimorphism of cheliped size in decapods is

also well known (Crane 1975; Lee and Seed 1992; Lee 1995; Mariappan et al. 2000; Emlen 2008), and in some species the major cheliped of males increases in size in the reproductive season (cf. Bueno and Shimizu 2009). Although many studies have focused on the type of sexual dimorphism specialized for male mating success (Andersson 1994), relatively few studies (such as Koga et al. 2010) have examined seasonal changes in the sexual dimorphism of traits possessed by both sexes.

In *Pagurus* hermit crabs, both the male and female have an enlarged right claw (the major cheliped). Because the major cheliped is used as a weapon and helps the animal win contests—in which both sexes participate (Yoshino and Goshima 2002; Briffa and Dallaway 2007)—over gastropod shells (Neil 1985; Imafuku 1989), this trait is important regardless of sex. However, sexual size dimorphism of the major cheliped is common in this genus (e.g., *P. nigrofascia*, Yasuda et al. 2011; *P. filholi*, Matsuo et al. 2015; *P. bernhardus*, Briffa and Dallaway 2007; Doake et al. 2010) as well as in other decapods. Recent empirical studies have demonstrated that males of *Pagurus* use the major cheliped as a physical weapon, and that a larger major cheliped increases the probability of winning in male–male contests during the reproductive season (Yasuda et al. 2011, 2012, 2014). Hermit crabs also show flexible allocation of energy to body growth and major cheliped size (Blackstone 1985); changes in this allocation are associated with the reproductive cycle (Yasuda et al. 2014). Together, these findings suggest that major cheliped size in *Pagurus* species differs between the reproductive and non-reproductive seasons, but especially in males because of its advantage for mating success.

Here, we examine the relationship between major cheliped size and season in *Pagurus minutus*. Male–male contests in this species are resolved according to the relative size of the major cheliped rather than body size (Yasuda and Koga 2016), but no studies have focused on the status of the major cheliped in a natural setting. First we describe (1) the reproductive biology of *P. minutus*, including sex ratio, the frequency of ovigerous females, and body size distributions. We then examine whether major cheliped size shows (2) sexual dimorphism or (3) seasonal change in either sex. *Pagurus* males grasp the aperture of the gastropod shell occupied by a sexually mature female for several days during the reproductive season (Imafuku 1986; Goshima et al. 1998). Because this precopulatory mate guarding is typically necessary to achieve copulation (but see Minouchi and Goshima 1998), we also compared (4) body size and major cheliped size between solitary males collected in the reproductive season and guarding males.

Methods

Field collection

Each month from May 2014 to April 2015, we collected solitary *Pagurus minutus* from a sandflat at Nunohiki, in the Waka River estuary, Wakayama, Japan (34°10′N, 135°10′E), as samples of the population. Field sampling was also conducted in November 2015 because no samples had been available for collection in November 2014. Each month, we randomly cast a sieve (33 cm in diameter) several times on the sandy ground. After each cast, we went to where it had landed, turned it upside down, and collected all solitary *P. minutus* found underneath.

In the laboratory, we recorded whether a crab possessed or had autotomized (shed) the major cheliped, and identified the sex of each individual under a dissecting microscope on the basis of the position of the gonopores (i.e., at the coxae of the third pereiopods in females and at the coxae of the fifth pereiopods in males). Approximately 90% of the crabs possessed a major cheliped, regardless of sex (Table 1), and there was no significant difference in the frequency of possession of a major cheliped between the sexes (Fisher's exact test, $P = 0.97$). We then measured the shield length (SL: calcified anterior portion of the cephalothorax) of all crabs, as an index of body size, to the nearest 0.1 mm using a digital caliper. In individuals possessing a major cheliped we also measured the propodus length of the major cheliped (PL: total length of the propodus) to the nearest 0.1 mm using a digital caliper. Females were also checked for eggs attached to the pleopods (i.e., whether a female was ovigerous).

From December 2014 to April 2015, we also randomly collected precopulatory guarding pairs of *P. minutus* from the same sampling site; the reproductive season of this species at this site is from November to April (see 'Results'). Because, unlike solitary crabs, guarding pairs are often found on vertical structural objects, such as algae, boulders or sea banks, we did not use a sieve to collect guarding pairs. The sampling area for guarding pairs was the same as that for solitary crabs throughout the study, but the main difference between guarding pairs and solitary crabs was deemed to be their use of vertical space. In the laboratory, we checked to see whether the male in each guarding pair possessed or had autotomized the major cheliped. Because precopulatory guarding in this genus is performed by the left (i.e., minor) cheliped, males can guard a female as usual even if they have autotomized their major cheliped. We then measured SL and PL in guarding males in the same way as for samples from the general population. There was no significant difference in the frequency of males with a major cheliped between solitary males collected from December to April and guarding males ($P = 0.61$; Table 1). We did not use data on the females in the guarding pairs.

Analyses

We first used data on all crabs in the population sampling, regardless of the presence or absence of a major cheliped (males, $N = 747$; females, $N = 1537$), to de-

Table 1 Sample sizes of field collections of solitary and guarding pairs of the hermit crab *Pagurus minutus*

Sampling date	N	Males			Females				
		N	Cheliped possession		N	Ovigerous		Cheliped possession	
			N	(%)		N	(%)	N	(%)
Solitary crabs (general population)									
11 May 2014	208	93	81	(87.1)	115	1	(0.9)	105	(91.3)
28 Jun 2014	213	111	99	(89.2)	102	0	(0)	89	(87.3)
12 Jul 2014	194	93	84	(90.3)	101	0	(0)	85	(84.2)
8 Aug 2014	147	112	99	(88.4)	35	0	(0)	29	(82.9)
27 Sep 2014	195	81	68	(84.0)	114	0	(0)	95	(83.3)
23 Oct 2014	196	56	54	(96.4)	140	0	(0)	118	(84.3)
24 Nov 2015*	214	27	27	(100)	187	58	(31.0)	182	(97.3)
22 Dec 2014	165	27	23	(85.2)	138	64	(46.4)	105	(76.1)
24 Jan 2015	171	16	14	(87.5)	155	124	(80.0)	146	(94.2)
21 Feb 2015	203	17	16	(94.1)	186	138	(74.2)	181	(97.3)
20 Mar 2015	178	51	47	(92.2)	127	74	(58.3)	117	(92.1)
2 Apr 2015	200	63	59	(93.7)	137	48	(35.0)	133	(97.1)
Total	2284	747	671	(89.8)	1537	507	–	1385	(90.1)
Guarding pairs									
24 Dec 2014	42		30	(71.4)	–	–	–	–	–
24 Jan 2015	39		33	(84.6)	–	–	–	–	–
17 Feb 2015	40		37	(92.5)	–	–	–	–	–
20 Mar 2015	40		36	(90.0)	–	–	–	–	–
2 Apr 2015	43		39	(90.7)	–	–	–	–	–
Total	204		175	(85.8)	–	–	–	–	–

* Data from November 2014 were unavailable

scribe basic population parameters, namely sex ratio, frequency of ovigerous females, and body size (SL) distribution in each month. For the monthly data, we used a binomial test to examine sex ratio, and we used the Kolmogorov–Smirnov test to compare the size distributions of SL between the sexes. Both analyses were also applied to pooled data from the non-reproductive season (May to October: males, $N = 546$; females, $N = 607$; see Results), the reproductive season (November to April: males, $N = 201$; females, $N = 930$; see Results), and all months (May to April) to examine overall trends.

Next, we used a subset of data on crabs in the population sampling, namely those crabs that had a major cheliped (males, $N = 671$; females, $N = 1385$; Table 1), to examine sexual dimorphism in major cheliped size. Assuming relative growth (allometry), PL and SL were $\log_{10}$-transformed, and we compared $\log_{10}(PL)$ between sexes using analysis of covariance (ANCOVA). To investigate whether there were seasonal changes in major cheliped size between the reproductive and non-reproductive seasons, and whether the trends differed between sexes, the data for males and females were then separately analyzed. To compare the $\log_{10}$-transformed SL–PL relationship between the seasons, we used ANCOVA for both sexes. We considered the interaction between SL and season in the analysis of males, but not in the analysis of females for which we found no significant interaction between SL and season ($P = 0.44$).

To compare the body sizes of solitary and guarding males, we used all solitary males collected from December to April, and all guarding males regardless of the presence or absence of a major cheliped (solitary males, $N = 201$; guarding males, $N = 204$), and applied the Kolmogorov–Smirnov test to the data for each month and to the pooled data for all months. We then used a subset of data on males with a major cheliped (solitary males, $N = 186$; guarding males, $N = 175$) to compare $\log_{10}$-transformed major cheliped size between the two male groups (solitary or guarding) by using ANCOVA. Because the interaction between SL and group was not significant ($P = 0.43$), we excluded this variable from the model. All statistical analyses were performed with R version 3.2.3 (R Core Team 2015).

Results

Population characteristics

Table 1 summarizes the numbers of *Pagurus minutus* collected. We examined the sex ratio of solitary crabs recovered from May 2014 to April 2015 (excluding November 2014) and in November 2015 (Fig. 1a). Whereas the sex ratio was significantly male-biased in August (binomial test, $P < 0.001$), the ratios were female-biased for September to April ($P < 0.02$; Fig. 1a); there were no significant sex-ratio biases for May to July ($P > 0.14$; Fig. 1a). In the three sets of pooled data, although there was no significant bias in the non-reproductive season ($P = 0.08$), there was a significant
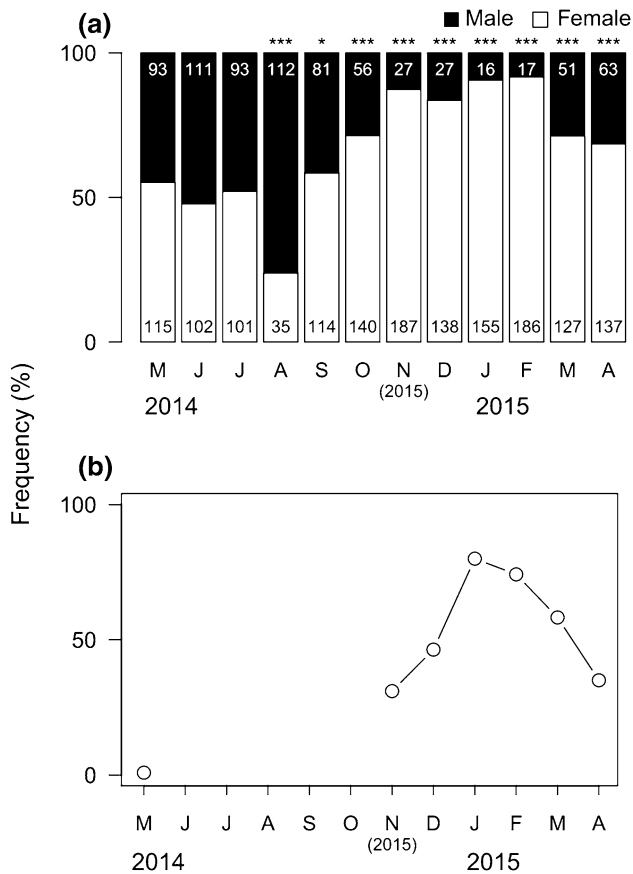
Fig. 1 Results of monthly field collections of solitary hermit crabs *Pagurus minutus*. Percentages of **a** males and females, and of **b** ovigerous females. Field collections were conducted from May 2014 to April 2015 (excluding November 2014) and in November 2015. In **a**, *numbers in each bar* indicate sample size of each sex, and *asterisks* indicate significant biases in the sex ratio (binomial test: *P < 0.05; ***P < 0.001), for each month

female bias in both the reproductive season ($P < 0.001$) and in all the months combined ($P < 0.001$).

We then examined monthly changes in the frequency of ovigerous females (Fig. 1b). Ovigerous females were found in May and from November to April, with their frequency peaking at 80% in January and dropping to a minimum of about 1% in May (Fig. 1b; Table 1). Few females were ovigerous in May, whereas more than 30% of females were ovigerous from November to April, suggesting that the main reproductive season of *P. minutus* at the study site is from November to April.

We analyzed the monthly body-size patterns of both sexes (Fig. 2). The pattern of SL distribution differed significantly between the sexes in May, October, December, February, and March (Kolmogorov–Smirnov test, all values of $D > 0.23$, $P < 0.04$), but not in the other months (all values of $D < 0.17$, $P > 0.18$). In May and March, the modes of female size (3.4–3.6 mm SL in May, 2.4–2.8 mm SL in March) were larger than those of males (2.2 mm SL in May, 2.4 mm SL in March; Fig. 2). But in October the mode of female size

(2.4, 2.8–3.0 mm SL) was smaller than that of males (3.4–3.6 mm SL; Fig. 2). In December and February, but possibly because of the small sample sizes of males ($N = 27$ and 17, respectively), the shapes of the size distributions in both sexes seemed to be different from each other, and only females showed clear modes of SL, at 2.8 mm in December and 2.4–2.6 mm in February (Fig. 2). Minimum size of ovigerous females was 2.0 mm SL, in November, December, and March (Fig. 2).

Finally, we examined the three overall trends in SL distribution in solitary *P. minutus* (Fig. 3). No significant difference was found between the sexes in the non-reproductive season (Kolmogorov–Smirnov test, $D = 0.06$, $P = 0.21$; Fig. 3a), but significant differences were found in the reproductive season ($D = 0.11$, $P = 0.04$; Fig. 3b) as well as across all months combined ($D = 0.10$, $P < 0.001$; Fig. 3c). In the reproductive season, although the size modes of both sexes were 2.6–2.8 mm SL, the number of females was approximately six times that of males (Fig. 3b). Ovigerous females were observed in all size classes of solitary females except for the smallest class (Fig. 3b). Across all months combined, the mode of female size (2.6–3.0 mm SL) was larger than that of males (2.4–2.6 mm SL; Fig. 3c).

Comparison of major cheliped size between sexes and seasons

We investigated the relationship between body size and major cheliped size in solitary *P. minutus* (Fig. 4). In all solitary crabs possessing a major cheliped, PL increased with SL in both sexes (Fig. 4a; Table 2), and there was a significant interaction between SL and sex (Table 3), indicating sexual dimorphism in the PL of the major cheliped as the PL of males increased at a higher allometric rate than occurred in females (Fig. 4a; Table 2). Comparison between the reproductive and non-reproductive seasons revealed a significant interaction between SL and season in males (Table 3). Males possessed a clearly larger major cheliped in the reproductive season than in the non-reproductive season (Table 2), especially when males were larger than 3.0 mm SL (Fig. 4b), whereas relative size of the female major cheliped was approximately the same between the seasons (Fig. 4c; Tables 2, 3).

Comparisons between solitary and guarding males

We plotted the body size distributions (using SL data) of solitary and guarding males for December 2014 to April 2015 (Fig. 5). The patterns of size distribution differed significantly between the two male groups in all months (Kolmogorov–Smirnov test, all vales of $D > 0.35$, $P < 0.04$); the modes of size of the guarding males were larger than those of the solitary males in all months
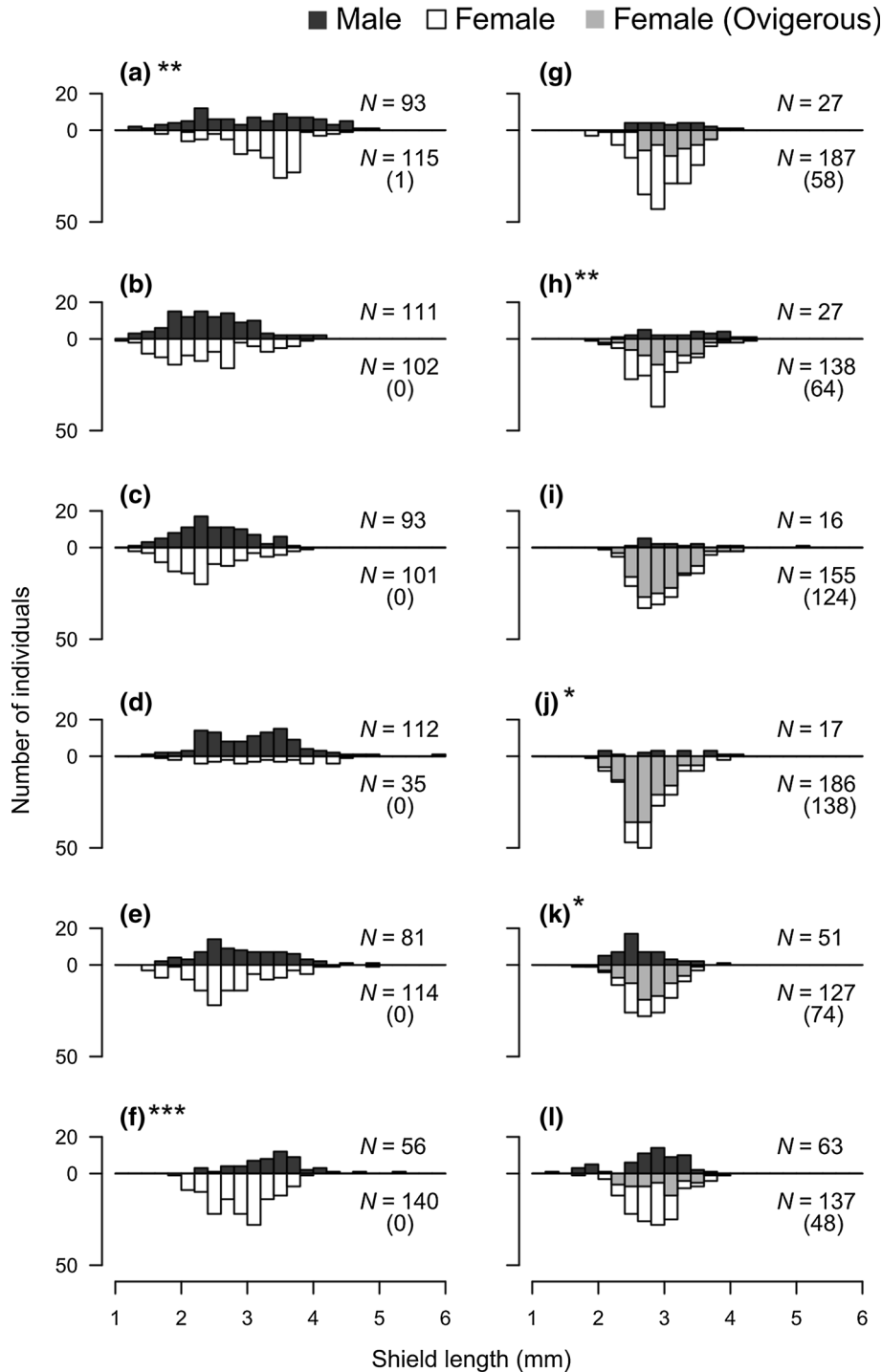
Fig. 2 Body size distributions of solitary male and female hermit crabs *Pagurus minutus* from May 2014 to April 2015 (excluding November 2014) and in November 2015: **a** May 2014, **b** June 2014, **c** July 2014, **d** August 2014, **e** September 2014, **f** October 2014, **g** November 2015, **h** December 2014, **i** January 2015, **j** February 2015, **k** March 2015, and **l** April 2015. Shield length is an index of body size. *Numbers in parentheses* indicate numbers of ovigerous females. Significant differences between sexes are shown by *asterisks* (Kolmogorov–Smirnov test: *P < 0.05; **P < 0.01; ***P < 0.001)

(Fig. 5). Minimum size of guarding males was 2.2 mm SL, found in January (Fig. 5).

We then compared overall SL and PL in solitary males and guarding males (Fig. 6). The overall trends of SL distribution also differed significantly between

guarding and solitary males (Kolmogorov–Smirnov test, $D = 0.65$, $P < 0.001$), with a larger body size in guarding males (mode, 3.6–3.8 mm SL) than in solitary males (2.4–2.8 mm SL; Fig. 6a). Given that the modes of SL in solitary males and females were similar in the
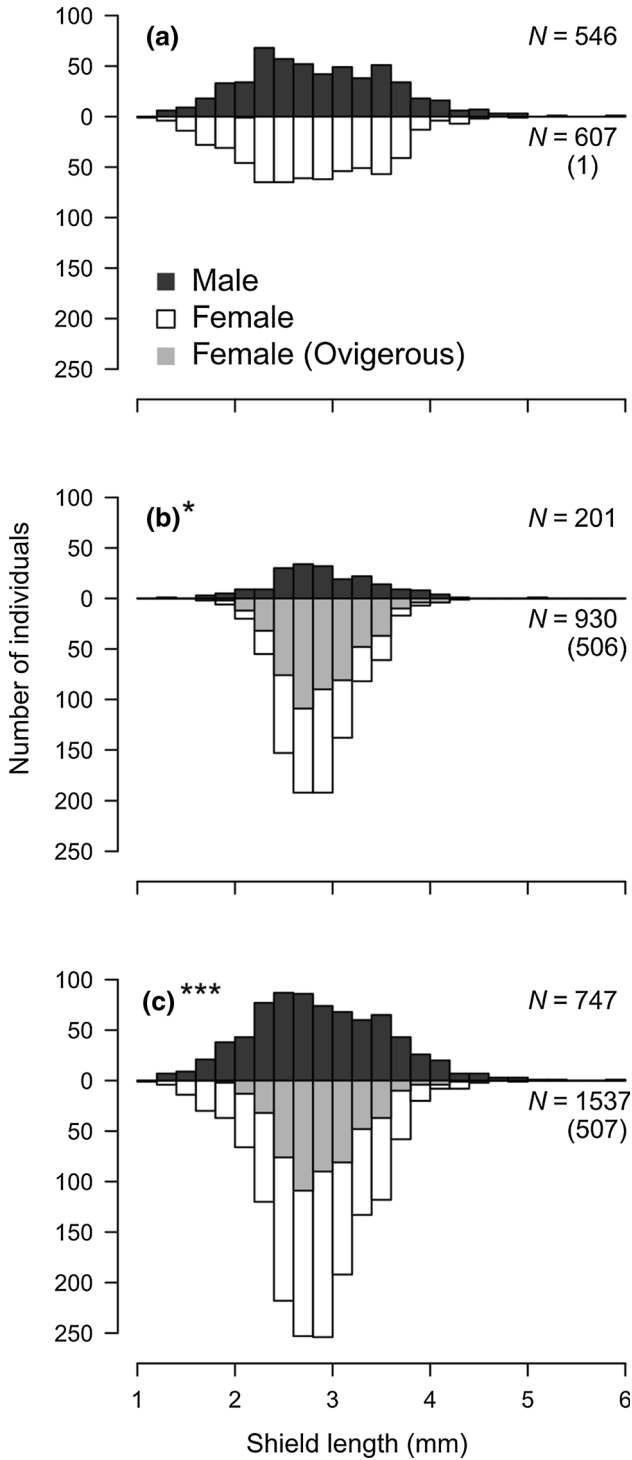
Fig. 3 Body size distributions of solitary male and female hermit crabs *Pagurus minutus* in **a** the non-reproductive season (May to October), **b** the reproductive season (November to April), and **c** all seasons combined. Shield length is an index of body size. *Numbers in parentheses* indicate numbers of ovigerous females. Significant differences between sexes are shown by *asterisks* (Kolmogorov–Smirnov test: *$P < 0.05$; ***$P < 0.001$)

Fig. 4 Relationships between major cheliped size and body size in solitary hermit crabs *Pagurus minutus*. Shown are comparisons between **a** sexes, **b** males during reproductive and non-reproductive seasons, and **c** females during reproductive and non-reproductive seasons. Note that *x*- and *y*-axes have $\log_{10}$ scales. In both **a** and **b**, the slopes of the regression lines differed significantly from each other (ANCOVA: ***$P < 0.001$)

reproductive season (Fig. 3b; see above), the body sizes of guarding males were also larger than those of solitary female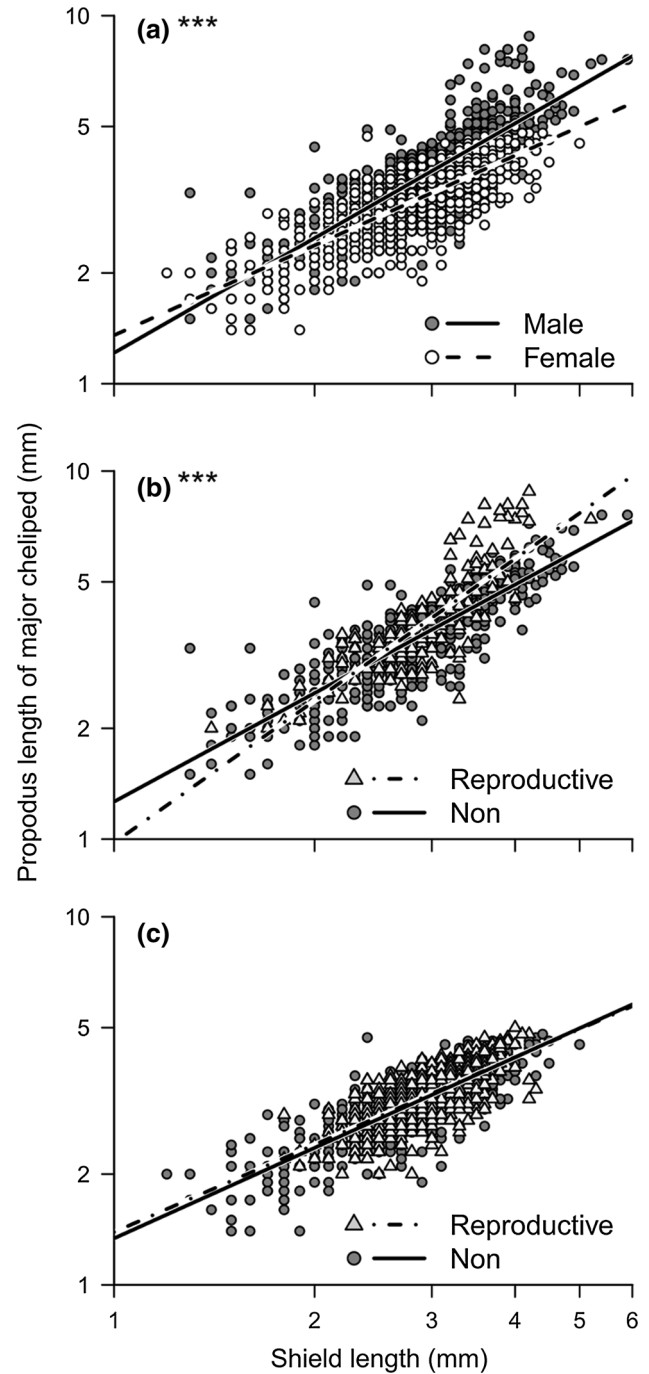s in the reproductive season. Almost all guarding males (195/204, 95.5%) were larger than 3.0 mm SL, whereas fewer than half the solitary males attained this size (78/201, 38.8%; Fig. 6a). Among those males with a major cheliped, guarding males had a relatively larger major cheliped than did solitary males, and the differ-

Table 2 Allometric relationships between body size and major cheliped size in *Pagurus minutus*

Category	N	Allometric relationship			*Statistical test for slope	
		Equation		R^2	t	P
Solitary crabs						
All crabs						
Males	671	$\mathrm{Log_{10}(PL)} = 0.08 + 1.04 \times \mathrm{log_{10}(SL)}$		0.705	39.97	<0.001
Females	1385	$\mathrm{Log_{10}(PL)} = 0.13 + 0.81 \times \mathrm{log_{10}(SL)}$		0.634	48.95	<0.001
Males						
Non-reproductive season	485	$\mathrm{Log_{10}(PL)} = 0.10 + 0.98 \times \mathrm{log_{10}(SL)}$		0.730	36.14	<0.001
Reproductive season	186	$\mathrm{Log_{10}(PL)} = -0.01 + 1.28 \times \mathrm{log_{10}(SL)}$		0.685	19.99	<0.001
Females						
Non-reproductive season	521	$\mathrm{Log_{10}(PL)} = 0.13 + 0.82 \times \mathrm{log_{10}(SL)}$		0.701	34.89	<0.001
Reproductive season	864	$\mathrm{Log_{10}(PL)} = 0.14 + 0.79 \times \mathrm{log_{10}(SL)}$		0.513	30.12	<0.001
Guarding males	175	$\mathrm{Log_{10}(PL)} = 0.16 + 1.19 \times \mathrm{log_{10}(SL)}$		0.534	14.07	<0.001

PL propodus length of major cheliped, *SL* shield length of cephalothorax (an index of body size)
* Each slope was examined if it was significantly different from zero

Table 3 Results of ANCOVA for the comparison of major cheliped size (i.e., $\mathrm{log_{10}}$-transformed propodus length of major cheliped) between sexes and seasons in solitary crabs

	df	MS	F	P
Comparison between sexes (N = 2056)				
$\mathrm{Log_{10}(SL)}$	1	13.90	4188.64	<0.001
Sex	1	1.33	403.02	<0.001
$\mathrm{Log_{10}(SL)} \times$ sex	1	0.21	62.51	<0.001
Residuals	2052	0.003		
Comparison between reproductive and non-reproductive seasons in males (N = 671)				
$\mathrm{Log_{10}(SL)}$	1	8.23	1690.53	<0.001
Season	1	0.1	20.61	<0.001
$\mathrm{Log_{10}(SL)} \times$ season	1	0.10	20.35	<0.001
Residuals	667	0.005		
*Comparison between reproductive and non-reproductive seasons in females (N = 1385)				
$\mathrm{Log_{10}(SL)}$	1	5.82	2398.39	<0.001
Season	1	0.006	2.621	0.106
Residuals	1382	0.002		

MS mean square, *SL* shield length (an index of body size)
* Interaction between $\mathrm{log_{10}(SL)}$ and season was excluded because it was not significant (P = 0.44)

ence was significant (Fig. 6b; Tables 2, 4), although there was some overlap in major cheliped size between males in the two groups. The size of the major cheliped in guarding males seemed to reach an upper limit at about 10 mm PL (Fig. 6b).

Discussion

We found distinct sexual dimorphism in major cheliped size in the hermit crab *Pagurus minutus*. Sexual dimorphism in cheliped size has been reported in other decapods (reviewed by Mariappan et al. 2000; Emlen 2008), including several hermit crab species (Asakura 1987; Yasuda et al. 2011; Doake et al. 2010). Although both male–male contests and female mate choice are common mechanisms leading to sexual dimorphism (Andersson 1994; Hunt et al. 2009), Contreras-Garduño and Córdoba-Aguilar (2006) suggest that male–male contests alone, not female choice, directly explains sexual size dimorphism in *Pagurus* hermit crabs. This is because,

although a larger body or larger major cheliped increases fighting success during male–male contests (Wada et al. 1999; Okamura and Goshima 2010; Suzuki et al. 2012; Yasuda et al. 2014), females might have less opportunity to directly choose a male because precopulatory mate guarding is initiated by the approach of a male, not by the female (Suzuki et al. 2012). Given that the outcomes of male–male contests are determined primarily by the relative size of the major cheliped in *P. minutus* (Yasuda and Koga 2016), the sexual dimorphism of major cheliped size in this species thus follows the general pattern of this genus.

The advantage for male mating success of having a larger major cheliped would also explain why only male *P. minutus* showed a seasonal change in major cheliped size. Solitary males in the reproductive season possessed a major cheliped that was clearly larger, especially when they were at a body size comparable to the guarding males. This suggests that at least some solitary males have the potential for mate guarding and mating success. As we found here, male decapods often increase
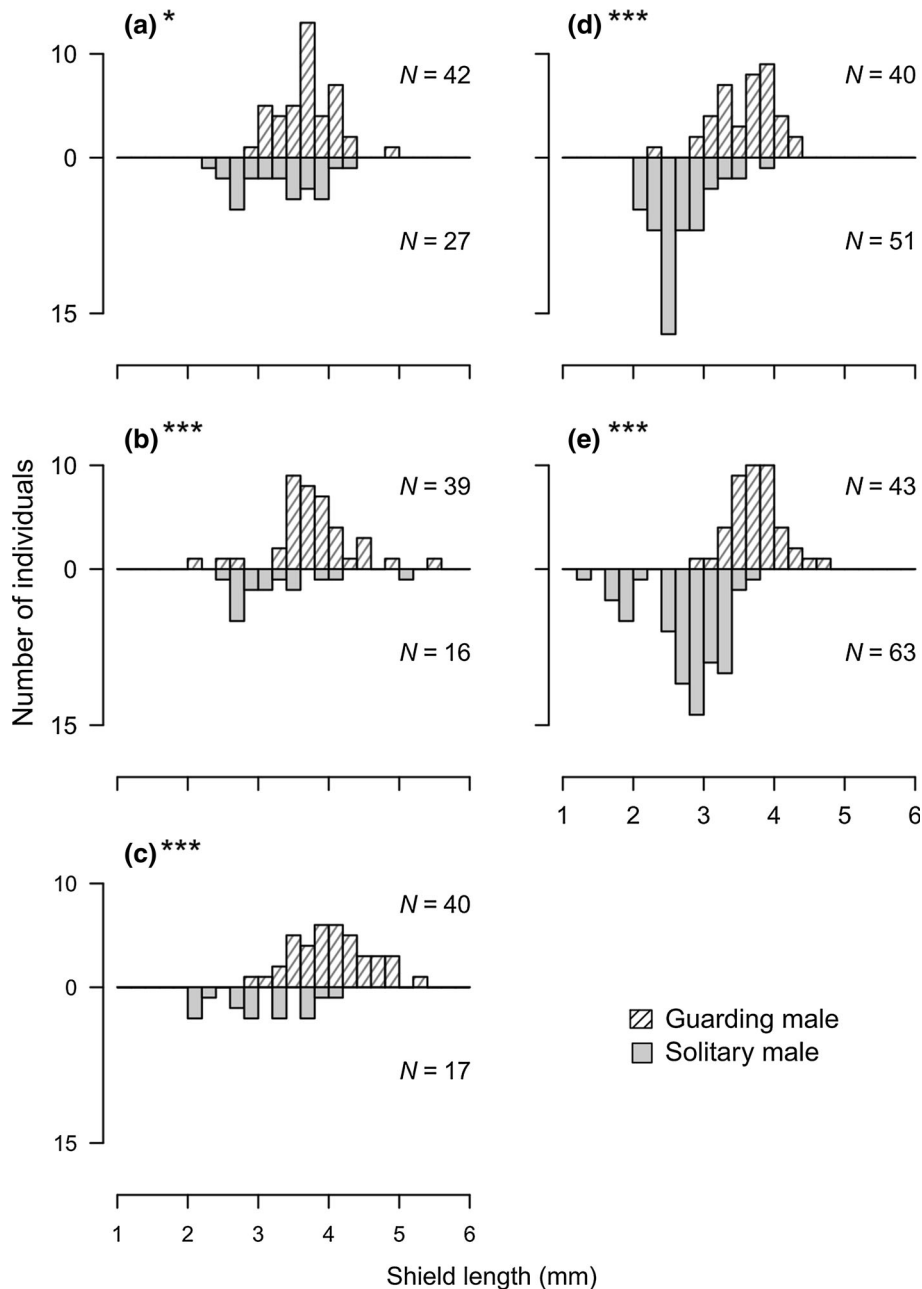
Fig. 5 Body size distributions of solitary male and guarding male *Pagurus minutus* collected from December 2014 to April 2015: **a** December 2014, **b** January 2015, **c** February 2015, **d** March 2015, and **e** April 2015. Shield length is an index of body size. Significant differences in shield length between the two types of males were found in all months (Kolmogorov–Smirnov test: *P < 0.05; ***P < 0.001)

their investment in cheliped growth in the reproductive season. For example, in Cambaridae crayfishes, adult males molt from a non-reproductive form (form II) to a reproductive form (form I) (Stein et al. 1977; Butler and Stein 1985). Male crayfish of form I are more likely to be dominant than those of form II because they possess a relatively larger chela (Guiasu and Dunham 1998). Matsuo et al. (2015) examined the regeneration pattern of the major cheliped in both sexes of the hermit crab *P. filholi* during the reproductive season; males showed greater regeneration of the major cheliped than did fe-

males, but males had reduced body size growth. Moreover, the male's investment in the major cheliped size can change, even over a single reproductive season. Koga et al. (2010) reported that, in the hermit crab *Diogenes nitidimanus*, larger males possess a relatively larger major cheliped than smaller males, especially around the reproductive peak, but this trend disappears later in the same season. Because the sample sizes of males possessing a major cheliped were relatively small from November to April ($N = 14$–59), we did not examine whether the major cheliped size of males
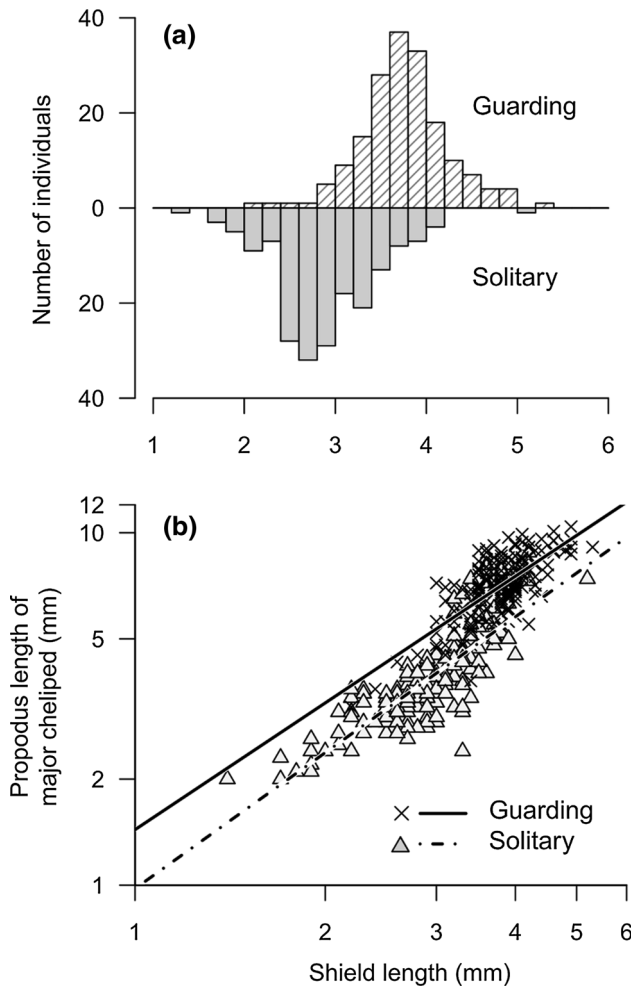
Fig. 6 Comparisons of data for *Pagurus minutus* solitary males and guarding males collected from December 2014 to April 2015: **a** Body size distribution, and **b** relationship between major cheliped size and body size. In **b**, x- and y-axes have $\log_{10}$ scales. Shield length is an index of body size. Guarding males had significantly larger bodies (Kolmogorov–Smirnov test: $P < 0.001$) and larger major chelipeds (ANCOVA: $P < 0.001$) than solitary males

Table 4 Results of ANCOVA for the comparison of major cheliped size (i.e., propodus length of major cheliped) between guarding and solitary males in the reproductive season; the total number of males was 361

	df	MS	F	P
$\log_{10}$(SL)	1	8.48	1806.7	< 0.001
Guarding	1	0.79	169.1	< 0.001
Residuals	358	0.005		

MS mean square, *SL* shield length (an index of body size)
Interaction between $\log_{10}$(SL) and guarding was excluded because it was not significant ($P = 0.43$)

changed within a single reproductive season, thus more research will be needed to test this possibility.

Although a larger major cheliped is likely favored in the sexual selection of male *P. minutus*, the size of the major cheliped in the guarding males seemed to reach an upper limit at about 10 mm PL. The simplest

explanation for this finding may be the constraint imposed by the size of the shells occupied by the crabs, as this is one of the most important determinants of overall body size in hermit crabs (Fotheringham 1976). However, Blackstone (1985) demonstrated that *P. longicarpus* males can produce a longer major cheliped even when they use small shells, suggesting that there are other factors operating to determine the size limit of the male's major cheliped. These factors could include the costs of having a larger major cheliped. As is the case in other taxa (e.g., the now extinct Irish elk *Megaloceros giganteus*, Moen et al. 1999; the swordtail *Xiphophorus montezumae*, Basolo and Alcaraz 2003), in decapod crustaceans, increasing the size of a sexually selected trait (e.g., the major cheliped) imposes a higher energetic or locomotor cost on the male possessor (Allen and Levinton 2007; Wilson et al. 2009; Doake et al. 2010). Moreover, given the possibility that some conspicuous male morphologies, such as coloration in guppies (Endler 1980) and a large claw in the fiddler crab (Koga et al. 2001), may attract predators (Magnhagen 1991; Zuk and Kolluru 1998), a larger major cheliped in *P. minutus* might increase the frequency of attacks by predators. These substantial potential costs relating to a larger major cheliped might limit the degree of sexual size dimorphism in this trait through natural selection (Allen and Levinton 2007; Doake et al. 2010).

Unlike major cheliped size, the body size of solitary crabs in the reproductive season was larger in females as compared to males. This size sexual dimorphism is inconsistent with the findings of many studies of various taxa (e.g., marine mammals, Weckerly 1998; the wandering albatross *Diomedea exulans*, Shaffer et al. 2001; review by Shine 1989; Andersson 1994), including hermit crabs (Wada et al. 1996; Contreras-Garduño and Córdoba-Aguilar 2006; Koga et al. 2010). One possible explanation is unintended sampling bias due to male–female differences in habitat use, especially among larger crabs. In *P. minutus*, solitary crabs and precopulatory guarding pairs were typically distributed in different areas of the sampling site, and the larger males (> 3.0 mm SL) were typically the guarding males in all months. This suggests that the frequency of larger males in the area where we collected solitary crabs (i.e., the general collection area) may have been lower than expected given the true size distribution of the population. Even if larger males tended to return to the general collection area after copulation, they might subsequently move back to the guarding area rather than stay in the general area, since larger males are more likely to initiate the guarding of a new female. Alternately, many of the solitary females collected were ovigerous across almost all size classes, suggesting that females tend to achieve copulation regardless of their size. Because males stop guarding the female just after copulation, and since the ovigerous period in *P. minutus* at our study site has been estimated at more than a month (Nakano et al. 2016), ovigerous females are possibly not guarded by a male

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and might not relocate to the guarding area as part of a guarding pair, at least during this period. Females, more than males, were therefore expected to remain in the general collection area after copulation, regardless of female size. This difference in habitat use between the sexes might have caused the apparent sexual dimorphism of body size in the solitary crabs that were collected during the reproductive season.

Although we have no relevant quantitative data to show, solitary *P. minutus* were generally found on sandy ground, whereas guarding pairs mostly occupied vertical objects (e.g., sea banks and algae). Because guarding pairs are formed by a male grasping the shell in which a female shelter, this crab distribution is easily associated with male habitat use related to guarding status. A similar pattern of crab distribution has been observed in *P. filholi* (Kawaminami and Goshima 2015) and *P. nigrofascia* (CI Yasuda, personal observation). Kawaminami and Goshima (2015) suggest that climb-up behavior by a guarding male helps to sequester a guarded female from rival males and hence to avoid male–male contests. Yasuda and Koga (2016) reported that most solitary *P. minutus* males (>80%) initiate a contest against the guarding males they encounter, and more than half the intruders may succeed in takeover of a guarded female from her original 'owner' during escalated male–male contests. This indicates that prior guarding position (owner advantage) is less effective in this species than in other *Pagurus* species (e.g., *P. middendorffii*, Wada et al. 1999). Therefore, different habitat use by guarding and solitary *P. minutus* males may also help to decrease the rate of encounters between potential rivals and so improve mating success.

In summary, we examined the reproductive biology of *P. minutus* at our study site and compared the size of the major cheliped between sexes, seasons, and male guarding status. Although this appendage functions as a weapon in both sexes of species of *Pagurus*, we found sexual size dimorphism of the trait in *P. minutus*, and this dimorphism appeared enhanced in the reproductive season because solitary males possessed a relatively large major cheliped in this season but females did not. Given that a larger major cheliped increases male guarding and fighting success in this species, this seasonal change in sexual size dimorphism of the major cheliped may strongly reflect sexual selection acting on males via male–male competition. *P. minutus* has a long reproductive season (about 6 months) at the site studied; thus, if the relative size of the major cheliped of males changes during this period, then the degree of sexual dimorphism might change even within a single reproductive season, but further investigation is needed to determine this possibility.

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