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Distribution of *Holtodrilus truncatus*, a Branchiobdellidan Ectosymbiotic on Atyid Shrimps in the Kii Peninsula, Western Japan, with Reference to Salinity Tolerance and Host Preference

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The distribution of the branchiobdellidan *Holtodrilus truncatus* (Liang, 1963), which is symbiotic on atyid shrimps, was surveyed in 26 river catchments, covering almost all of the Kii Peninsula, western Japan. *Holtodrilus truncatus* has been recorded in 10 rivers located from the southwest to north-east regions of the peninsula. *Paratya compressa* was the dominant host species in all rivers, with a lower prevalence on *Caridina leucosticta*, *C. multidentata*, *C. typus*, and *Neocaridina denticulata*. The longitudinal distribution of *H. truncatus* was limited to the middle and upper reaches, while its main host *P. compressa* occurred widely from the lower to the upper reaches. The numbers of *H. truncatus* and their cocoons per host increased in summer and decreased in winter, in accordance with the abundance of the host *P. compressa*. The body size of *P. compressa* was larger in individuals with *H. truncatus* than those without *H. truncatus* in some rivers, while in another river, sizes of the two groups were not different. A salinity tolerance experiment revealed that the threshold of salinity tolerance of *H. truncatus* occurred between 0–0.5%. A choice experiment between two host species showed that *H. truncatus* preferred *C. leucosticta* to *C. multidentata*, *P. compressa* to *C. multidentata*, and *P. compressa* to *C. leucosticta*.

Key words: Branchiobdellida, atyid shrimp, symbiont, geographical distribution, host preference, longitudinal distribution

INTRODUCTION

The order Branchiobdellida (Annelida: Clitellata) contains 150 species worldwide (Gelder, 1999). They mostly cohabit with crayfish, and sometimes with freshwater crabs (Gelder et al., 2001), shrimps (Gelder, 1999; Ohtaka et al., 2012) and isopods (Gelder, 1999). In northern Japan, 12 native species of branchiobdellidans have been recorded to cohabit with Japanese native crayfish, *Cambaroides japonicus* (Ohtaka, 2010; Ohtaka and Gelder, 2015), and three exotic species have been recorded from alien crayfish (Ohtaka et al., 2005; Ohtaka, 2007). Recently, *Holtodrilus truncatus* (Liang, 1963), which is known to occur on the atyid shrimps in China (Ohtaka et al., 2012) and Taiwan (Ohtaka and Chen, 2010), has been recorded cohabiting with the atyid shrimps in Hyogo Prefecture (Niwa et al., 2005; Niwa and Ohtaka, 2006), and in Okinawa Prefecture (Fujita et al., 2010). Furthermore, Yamato (personal communication) has found a branchiobdellidan, which was identified as *Holtodrilus truncatus* by Ohtaka (Ohtaka, personal communication)

occurring on the atyid shrimps in the river system of the Kii Peninsula (Wakayama Prefecture). However, these reports are fragmentary and the distribution of branchiobdellidans and their hosts in Japan has not been quantitatively investigated, although there have been a few studies dealing with the distribution of branchiobdellidans in relation to environmental conditions (Berry and Holt, 1959; DeWitt et al., 2013). Although branchiobdellidans are considered to be intolerant to salinity (Gelder, 2006), there have been no salinity tolerance experiments reported for *H. truncatus*. As with the host preference of branchiobdellidans, there has been one study examining the host preference of *Cambarincola ingens* for two crayfish species (Brown et al., 2004). The influence of branchiobdellidans on host crayfish has been studied in terms of growth or survival of the host (Brown et al., 2002, 2012; Lee et al., 2009; Ames et al., 2015). However their influence on host shrimps has not been explored.

This study aimed to clarify the geographical distribution of the branchiobdellidan *Holtodrilus truncatus* in the river systems of the Kii Peninsula, and the seasonal pattern of the longitudinal distribution of the symbiont and its host shrimps. In order to know whether or not the symbiont influences the host shrimps, the body size and egg number of the host shrimp were also compared between shrimps with symbionts and those without symbionts. Laboratory experi-

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ments were undertaken to determine the salinity tolerance and host preference of *H. truncatus*, with both factors then related to the distribution of the symbionts.

MATERIALS AND METHODS

Field survey

To determine the distribution of *H. truncatus* in the Kii Peninsula, atyid shrimps were collected at one to eight stations from each of 26 rivers from May 2012 to August 2013 (Fig. 1, Table 1). The lowermost station in each river was situated in the tidally influenced area. In the Koza River, the survey was conducted in August, September, and October 2012, December 2013, and March and May 2014 to capture the seasonal distribution patterns of atyid shrimps and *H. truncatus*.

Sampling stations in each river were selected at waterside locations covered with vegetation. Sampling of the shrimps was standardized in such that a dip-net (mesh size: 2 mm, diameter: 36 cm) was used at 10 waterside areas (ca. 100-m length, 20–100-cm depth) in each site. All shrimps collected were fixed by 100% ethanol on capturing. The probability that *H. truncatus* was detached from the host shrimp in 100% ethanol was 5.81% (9/155) and the probability that the host shrimp became a non-carrier by detaching of *H. truncatus* by ethanol fixing was 1.6% (1/63), which was obtained from 99 shrimps (*Paratya compressa*) collected in the Koza River on 23 June 2013, and fixed individually by 100% ethanol. These probabilities, however, were not incorporated to represent the abundance of *H. truncatus*. The fixed shrimps were then sorted and their species identified according to Hamano et al. (2000). The carapace length (CL) of each shrimp was measured from the anterior margin of the carapace behind the insertion of the eyestalk to the most distal posterior margin using the micrometer of a stereoscopic microscope (SZ-ST: Olympus, Tokyo, Japan). When *H. truncatus* was found on the shrimp, the attached body part of the shrimp was recorded, and the length of straightened body of *H. truncatus* was measured from the anterior end to the posterior end under a stereoscopic microscope. When the branchiobdellidan cocoons were found on the shrimp, the attached body part of the shrimp was recorded, and their sizes (major axis and minor axis) were measured under a stereoscopic microscope.

Egg number of atyid shrimps

The number of eggs in ovigerous shrimps (*Paratya compressa*) with non-eyed eggs were counted under a stereoscopic microscope, with separate counts for shrimps with and without *H. truncatus* in samples from the Ohta, Iseji, Oroshi, and Tahara rivers, where more than 19 ovigerous shrimps were collected.

Salinity tolerance

The salinity tolerance of *H. truncatus* was examined from 17 June to 10 July 2014. The water temperatures ranged from 22 to 27°C. We poured fresh water (Chlorine - neutralized water, "Tetra Contra Chlorine": Tetra Aquatic Asia Pacific Ltd, Luzerne, Singapore) to a depth of 10 cm into a plastic tank (8 × 15 × 10-cm height), where we placed either two or five *H. truncatus* that were removed from atyid shrimps from the Iseji River. The experiment was conducted for five salinity levels: 0, 0.5, 1, 1.5, and 3‰, which were prepared using "SEA water (dry type)" (Gex. Co., Ltd., Osaka, Japan). *Holtodrilus truncatus* was removed from the host as follows. The thorax of the shrimp with *H. truncatus* was placed into a dish that was filled with water. The carapace was detached from the shrimp's body with a pair of tweezers, and the *H. truncatus* was extracted

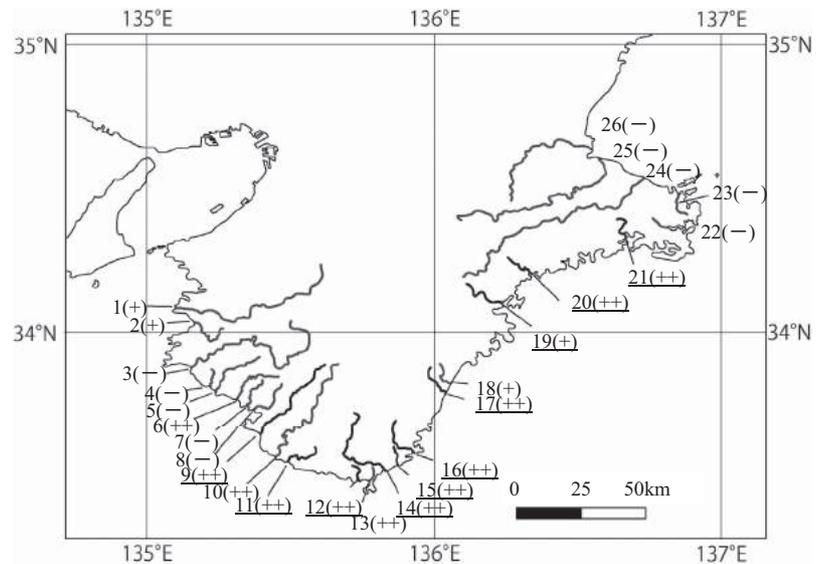


Fig. 1. Occurrence of branchiobdellidans (underline) and *P. compressa* (parentheses) in the 26 rivers surveyed. The abundance of *P. compressa* is shown by the number of individuals collected (+: less than 50, ++: more than 50). River numbers (1–26) correspond to those in Table 1.

Table 1. Sampling periods and number of sampling sites for each of 26 rivers surveyed. River numbers correspond to those of Fig. 1.

	River	Date	No. sampling sites
1	Arida	Aug 7–10, 2012	3
2	Hiro	Aug 1–2, 2012	3
3	Hidaka	Jun 14–15, 2012	3
4	Inami	Jul 4–5, 2012	3
5	Kirime	Jul 5–6, 2012	3
6	Minabe	Jul 10–11, 2012	3
7	Hidariaidu	Jun 11–12, 2012	3
8	Haya	Sep 26–27, 2012	5
9	Tonda	Jul 24–26, 2012	6
10	Hiki	Jul 26–28, 2012	3
11	Susami	Jun 6–7, 2012	3
12	Arita	Jun 26–27, 2012	3
13	Kujino	Sep 11–12, 2012	3
14	Koza	Aug 21–24, 2012	7
		Sep 20–22, 2012	7
		Oct 3–5, 2012	7
		Dec 20–21, 2013	7
		Mar 22–23, 2014	7
		May 17–18, 2014	7
15	Tahara	Jun 1–2, 2012	3
16	Ohta	May 29–30, 2012	3
17	Oroshi	Aug 9, 2013	2
18	Ichiki	Aug 9, 2013	2
19	Choshi	Aug 9, 2013	2
20	Akaba	Aug 8, 2013	1
21	Iseji	Aug 8, 2013	2
22	Isobe	Sep 6, 2013	1
23	Kamo	Sep 5, 2013	2
24	Miya	Sep 4–7, 2012	8
25	Kushida	Sep 5, 2013	2
26	Kumozu	Sep 6, 2013	2

Table 2. The prevalence of branchiobdellidans on host shrimps of *P. compressa* in each of 10 rivers.

	Tonda R.	Susami R.	Arita R.	Koza R.	Tahara R.	Ohta R.	Oroshi R.	Choshi R.	Akaba R.	Iseji R.
<i>P. compressa</i>	2.9% (50/1799)	40.4% (34/84)	17.2% (14/81)	11.0% (327/2963)	53.5% (106/196)	19.6% (49/249)	17.1% (70/408)	15.9% (7/44)	14.8% (11/74)	19.0% (42/220)
<i>C. leucosticta</i>	0.0% (0/1629)	0.2% (1/414)	13.8% (10/72)	0.0% (0/3409)	3.2% (1/31)	0.7% (2/291)	0.0% (0/7)	0.0% (0/12)	3.5% (10/287)	1.2% (13/1122)
<i>C. multidentata</i>	0.0% (0/44)	0.0% (0/143)	1.8% (1/57)	0.0% (0/55)	0.0% (0/9)	0.0% (0/8)				0.0% (0/16)
<i>C. typus</i>	0.0% (0/5)		1.0% (2/206)	0.0% (0/32)	0.0% (0/1)	0.0% (0/2)			0.0% (0/7)	0.0% (0/9)
<i>N. denticulata</i>	100% (1/1)			100% (1/1)						
<i>C. serratiostris</i>	0.0% (0/41)	0.0% (0/80)	0.0% (0/77)	0.0% (0/106)	0.0% (0/9)	0.0% (0/57)			0.0% (0/36)	0.0% (0/38)

from the carapace using a piece of cardboard. Similar-sized large individuals (ca. 5 mm in body length) were used for the experiment. A check was made to confirm if they were alive or not once per day in the 0 and 0.5% salinities, every hour in 1% salinity, every 15 minutes in 1.5% salinity, and continuously in 3% salinity. The number of test *H. truncatus* specimens at each concentration was 24 in 0%, 20 in 0.5%, 30 in 1%, 25 in 1.5%, and 12 in 3%. During the experiment no food was provided for test individuals. When test individuals floated or did not attach onto any part of the tank, they were considered dead.

Host preference

A host preference experiment of *H. truncatus* from the Iseji River was conducted from 6 September to 6 October 2013, and 17 June to 5 July 2014. The water temperature ranged from 21 to 27°C. We poured fresh water to a depth of 10 cm into a plastic tank (8 × 15 × 10-cm height). Two atyids of different species were placed in the tank, and then one individual of *H. truncatus* was placed into the same tank. The method used to extract *H. truncatus* from the host (*Paratya compressa*) was the same as that used in the salinity tolerance experiment. The test animals of *H. truncatus* were similar-sized large ones (ca. 5 mm in body length). The experiment ended when *H. truncatus* was found located under the carapace of either shrimp or when it had disappeared following a daily check. Three combinations of two host species were performed: *Paratya compressa* – *Caridina leucosticta* (40 replicates), *P. compressa* – *Caridina multidentata* (36 replicates), and *C. leucosticta* – *C. multidentata* (39 replicates), where each test individual of *H. truncatus* and shrimps was used once. Two shrimps used for each experiment was similar-sized males or females (non-ovigerous), with the difference of carapace lengths being less than 10% of the larger shrimp. Collected locality of the shrimps was the Iseji River for *P. compressa* and *P. leucosticta*, and the Koza River for *C. multidentata*.

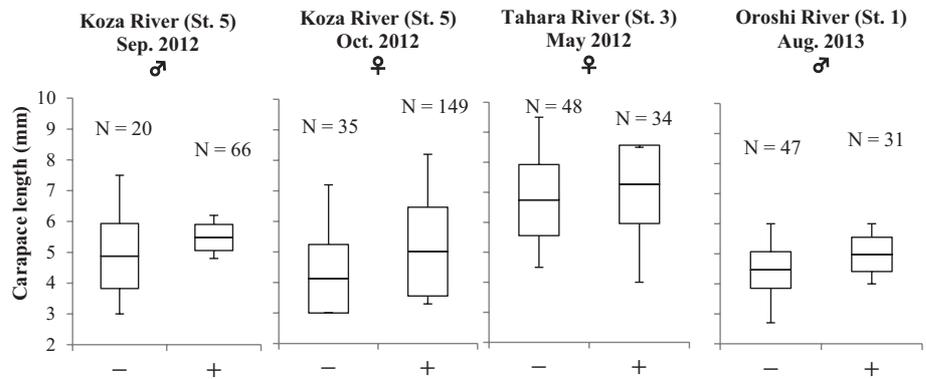


Fig. 2. The carapace lengths (mean with standard deviation and range) of male or female *P. compressa* with (+) and without (-) branchiobdellidans at sampling sites where more than 20 *P. compressa* specimens were collected.

Table 3. Carapace length (mm) of *P. compressa* carrying cocoons, small worms, and large worms of branchiobdellidans and their comparison among the three shrimp groups by ANOVA.

	Cocoon		Small		Large		one-way ANOVA
	N	mean ± SD	N	mean ± SD	N	mean ± SD	
Koza River							
Aug. 2012	28	5.5 ± 1.13	32	5.6 ± 1.05	110	5.7 ± 1.02	F = 2.14 P = 0.12
Sep.	17	5.6 ± 0.88	22	5.8 ± 0.87	72	5.6 ± 1.13	F = 0.89 P = 0.50
Oct.	6	6.3 ± 1.46	25	4.8 ± 1.62	93	4.8 ± 1.51	F = 2.78 P = 0.07
Dec. 2013	0		0		0		
Mar. 2014	0		0		3	5.4 ± 0.51	
May	2	7.4 ± 1.13	5	6.8 ± 0.57	7	4.9 ± 1.27	F = 6.76 P = 0.01
Tahara River	7	5.8 ± 1.90	36	6.0 ± 1.22	92	6.0 ± 1.06	F = 0.16 P = 0.85
Ohta River	10	4.3 ± 0.36	7	4.9 ± 1.00	58	4.7 ± 1.03	F = 0.89 P = 0.43
Oroshi River	12	4.9 ± 0.76	3	4.0 ± 1.40	66	4.9 ± 0.80	F = 2.13 P = 0.13

Data analysis

To compare the host size between shrimps with and without *H. truncatus*, the carapace lengths were compared by a t-test between *P. compressa* with and without *H. truncatus* from the sampling sites where more than 20 *P. compressa* specimens were collected.

A general linear model was used to examine whether the cohabiting of branchiobdellidans, the carapace length of *P. compressa*, the river, and their interaction affected the egg number of shrimps (fixed effect: cohabiting of branchiobdellidans, carapace length, and river). Because the interactions between the cohabiting of branchiobdellidans and the carapace length of *P. compressa*, or

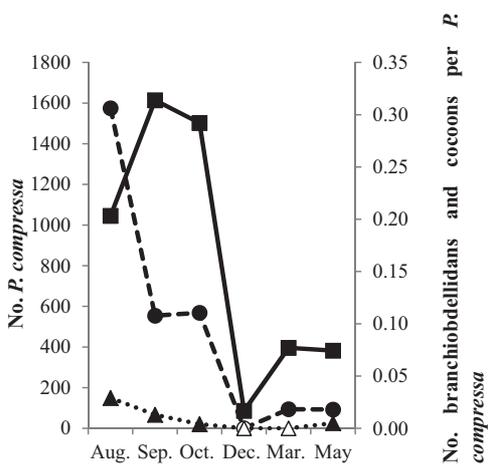


Fig. 3. Seasonal changes in the abundance of *P. compressa* (square), branchiobdellidans (circle), and cocoons (triangle) in the Koza River. The total number of shrimps collected, the number of branchiobdellidans per shrimp, and the number of cocoons per shrimp are shown. Open triangles and squares indicate a zero value.

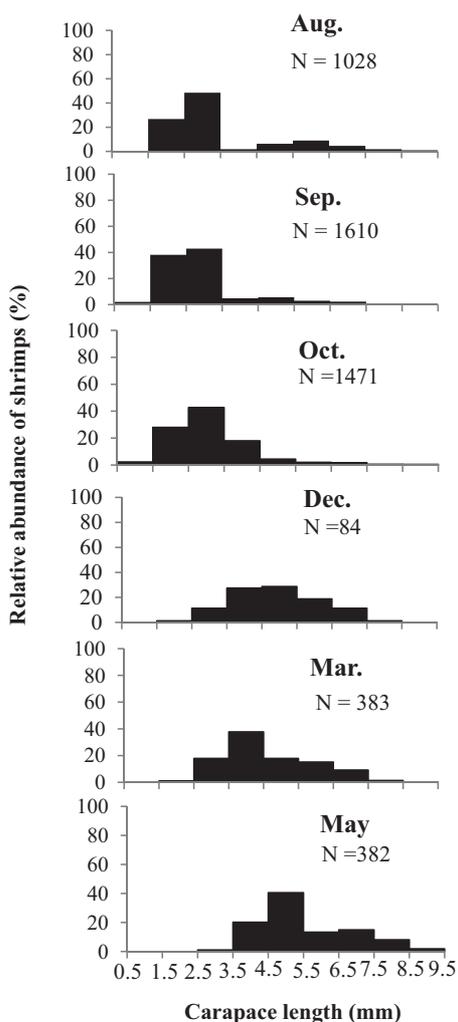


Fig. 4. Seasonal changes in size frequency distributions of *P. compressa* in the Koza River. N: total number of individuals collected in each month.

between the river and carapace length of *P. compressa* were not significant, the analysis was performed excluding these interactions. The model was regarded to be optimal through the use of Akaike information criterion with a correction for sample size (AICc).

A one-way ANOVA was used to compare the carapace lengths of *P. compressa* carrying *H. truncatus* among the shrimps with cocoons, those with small worms (CL < 2.5 mm), and those with large worms (CL ≥ 2.5 mm).

Table 4. Result of general linear model for the effect of cohabiting of brachiobdellidans, carapace length, and the river on the egg number carried by ovigerous *P. compressa*.

	d.f.	F	P
Cohabiting of branchiobdellidans	1	0.08	0.780
Log ₁₀ (Carapace length of <i>P. compressa</i>)	1	33.28	<.0001
River	3	5.64	0.001
River × Cohabiting of branchiobdellidans	3	2.64	0.053

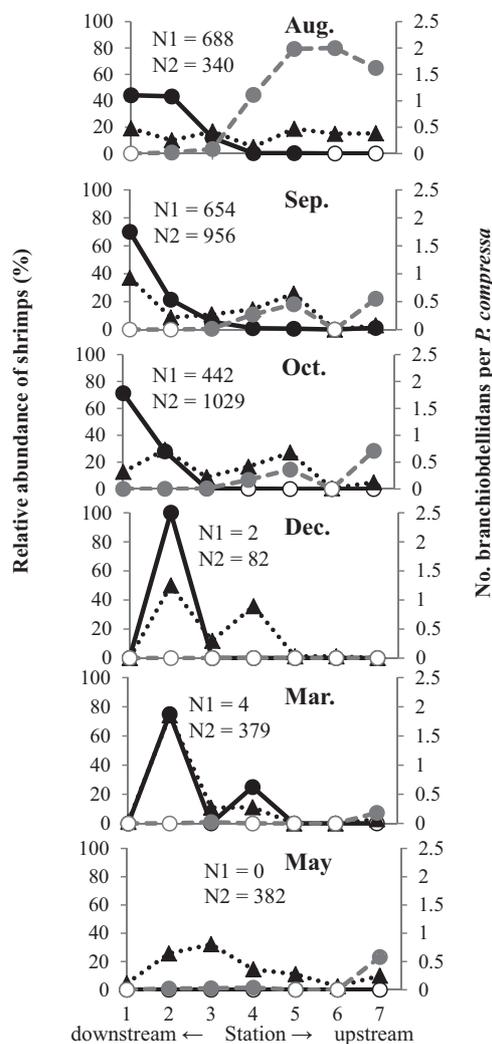


Fig. 5. Seasonal changes in the longitudinal distribution of small *P. compressa* (carapace length < 2.5 mm) (solid line), large *P. compressa* (carapace length ≥ 2.5 mm) (dotted line) and the number of branchiobdellidans per *P. compressa* (broken line) in the Koza River. Open circles and triangles indicate a zero value. N1: total number of small *P. compressa* collected in each month, N2: total number of large *P. compressa* collected in each month.

A comparison of the five groups of shrimps placed in different salinity conditions was performed using the log-rank test (Ishimura et al., 2003).

The host preference of *H. truncatus* was compared by a binomial test between *P. compressa*, *C. leucosticta* and *C. multidentata* to determine whether *H. truncatus* preferred a specific host species.

All statistical analyses were performed using JMP (SAS Institute, 2013).

RESULTS

Field survey

Holtodrilus truncatus was found in 10 of the 26 rivers surveyed, which were located from the southwest to northeast of the Kii Peninsula (Fig. 1). Their host shrimps were *P. compressa*, *C. leucosticta*, *C. multidentata*, *C. typus*, and *N. denticulata*, of which *P. compressa* was the most dominant (Table 2). Most of the *H. truncatus* were found inside the carapace of the host, with some on the external surface of the antennule, the rostrum, the carapace and the abdomen. Body length of *H. truncatus* ranged from 1.0 mm to 5.0 mm (mean ± SD = 2.4 ± 0.8 mm, n = 1153). Some rivers contained many *P. compressa*, but no *H. truncatus* (Fig. 1).

The prevalence (the number of individuals of a host species inhabited by branchiobdellidians divided by the number of hosts examined) of *H. truncatus* on *P. compressa* was highest in the Tahara River (Table 2). The intensity (the mean number of individuals of branchiobdellidians in a single infected host in a sample) was highest in the Iseji River (mean ± SD = 4.1 ± 6.4, max: 29) and lowest in the Ohta River (1.1 ± 0.3, max: 2).

The carapace lengths of *P. compressa* larger than the smallest shrimp carrying *H. truncatus* (2.5 mm CL), were compared between those with and without the branchiobdellidan (Fig. 2). The carapace lengths of *P. compressa* with *H. truncatus* were significantly larger than those without *H. truncatus* in the Koza and Oroshi rivers (*t*-test, Koza River males: *t* = -3.75, *P* = 0.0003, Koza River females: *t* = -3.39, *P* = 0.0015, Oroshi River males: *t* = -3.79, *P* = 0.0003), while in the Tahara River the carapace length was not significantly different between the two groups (*t*-test: *t* = -1.87, *P* = 0.06).

The branchiobdellidan cocoons (major axis: mean ± SD = 0.92 ± 0.18 mm, minor axis: 0.71 ± 0.15 mm, n = 78) were found mostly inside the host carapace, deposited on the inner surface of the carapace, with some on the abdomen of ovigerous female shrimps.

The carapace lengths of *P. compressa* carrying *H. truncatus* were not significantly different among the shrimps with cocoons, those with small worms (body length < 0.2 mm), and those with large worms (body length ≥ 0.2 mm), except for in May 2014 in the Koza River (Table 3).

The analysis by the general linear model showed that the egg number was significantly influenced by carapace length and the river, but not by the cohabiting of branchiobdellidians (Table 4, *R*² = 0.49). The interaction between the cohabiting of branchiobdel-

lidians and the river was not significant.

The abundance of worms and cocoons of *H. truncatus* displayed a similar seasonal pattern to the abundance of their main host *P. compressa* (Fig. 3). Both the worms and cocoons of *H. truncatus* were abundant in summer, then decreased and were absent or were present only in small numbers in winter to spring. Similarly, *P. compressa* was abundant in summer to autumn, but decreased to low levels in winter to spring. The size structure of *P. compressa* (Fig. 4) displayed a seasonal change: in summer small shrimp of 2.5–3.5-mm CL dominated, then the peak size class shifted to 3.5–5.5-mm CL in the following winter and spring. Small shrimps that did not bear *H. truncatus* were common in summer to autumn, but in winter to spring they were found only rarely.

The longitudinal distribution of *P. compressa* in the Koza River (Fig. 5) showed that *P. compressa* smaller than the smallest shrimp carrying *H. truncatus* occurred in the lower reach from August to October, while *P. compressa* larger than 2.5 mm occurred widely from the lower to upper reach. In contrast, *H. truncatus* occurred in the middle to the upper reaches, with few specimens collected at the lowermost station (tidally-influenced area) (Fig. 5).

Laboratory experiments

The salinity tolerance experiment revealed that the survival rate of *H. truncatus* was significantly greater at 0 than 0.5% (Fig. 6. $\chi^2 = 14.36$, *P* = 0.0003). At 1, 1.5, and 3%, the survival rate was significantly lower under the higher condi-

Table 5. The comparison of survival rate among three groups of *H. truncatus* under three different salinity conditions by log-rank test. Probability for comparison was corrected with sequential Bonferroni method for multiple tests.

	χ^2	<i>P</i>
0%–0.5%	14.36	0.0003
1.0%–1.5%	13.00	0.0003
1.5%–3.0%	39.65	<0.0001
1.0%–3.0%	45.00	<0.0001

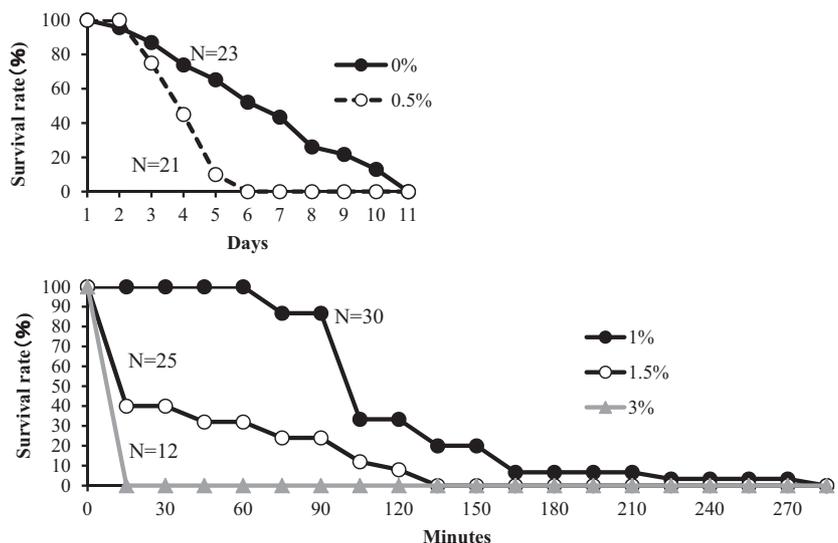


Fig. 6. Survival rate of branchiobdellidians under the indicated salinity conditions.

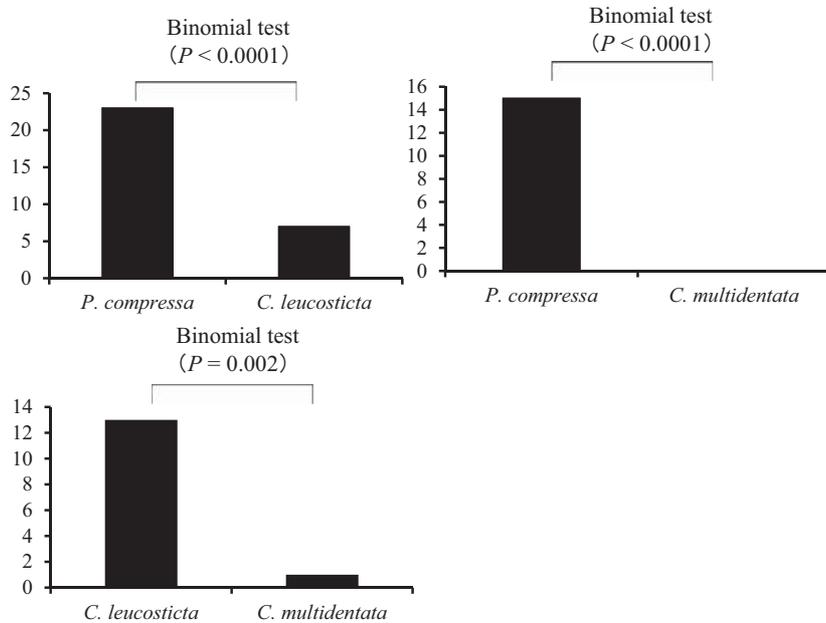


Fig. 7. Number of replicates where each of two shrimp species was inhabited by a branchiobdellidan in the host selection experiment, and a comparison of the two shrimp species using a binomial test.

tion than under the lower condition for all combinations (Fig. 6, Table 5).

The host preference experiment showed that *H. truncatus* significantly preferred *C. leucosticta* to *C. multidentata*, *P. compressa* to *C. multidentata*, and *P. compressa* to *C. leucosticta* (Fig. 7).

DISCUSSION

Holtodrilus truncatus was collected from 10 of 26 rivers located from southwest to northeast in the Kii Peninsula, indicating a limited distribution. Niwa and Ohtaka (2006) recorded *H. truncatus* from only 7 of 17 rivers surveyed within a 60-km range of Hyogo Prefecture. The branchiobdellidans in Hyogo Prefecture are reported to have been introduced from China (Niwa, 2011). The other known record of *H. truncatus* from Japan is from Miyakojima Island, Ryukyu Islands (Fujita et al., 2010). These records show that a limited distribution is a common feature of *H. truncatus* in Japan.

In this study, five species of atyid shrimp (*P. compressa*, *C. leucosticta*, *C. multidentata*, *C. typus*, and *N. denticulata*) bore *H. truncatus*, but no *H. truncatus* were found on *C. serratiostris*. *Caridina typus* (Fujita et al., 2010) and *N. denticulata* (Niwa et al., 2005) are known to be hosts for branchiobdellidans, but this is the first report that branchiobdellidans were found from *P. compressa*, *C. leucosticta* and *C. multidentata*. All the findings suggest that *H. truncatus* has weak host specificity for the shrimps of the family Atyidae. The non-occurrence of *H. truncatus* on *C. serratiostris* may be due to the shrimp species being distributed mainly in brackish waters (Shokita, 1975; Suzuki, 1993; Hamano et al., 2000; Usami et al., 2008; Tanaka et al., 2013) where *H. truncatus* rarely occurs, as shown from the longitudinal distribution in this study.

Holtodrilus truncatus in the Kii Peninsula were found

mostly inside the carapace of host shrimps, with some being on the external surface of the carapace. *Holtodrilus truncatus* from Miyakojima Island in Okinawa Prefecture (Fujita et al., 2010) and Hyogo Prefecture (Niwa et al., 2005) have been found mainly on the external body surface of the shrimps but *H. truncatus* on *Neocaridina* spp. in China has been reported to be attached to the same body parts as in *H. truncatus* in the Kii Peninsula (Ohtaka et al., 2012). *Holtodrilus truncatus* appears to have inter-populational difference in preference for attachment site of the host body.

The maximum size of *H. truncatus* from the Kii Peninsula was about 5 mm, which was similar to specimens from Okinawa (Fujita et al., 2010). In contrast, *H. truncatus* from Hyogo Prefecture was recorded as 1.5–2.5 mm (Ohtaka, 2007). Consequently, there would be inter-populational variation in their body size of *H. truncatus*.

Paratya compressa individuals with *H. truncatus* were larger than those without *H. truncatus* in two rivers. This phenomenon can be explained by the increased surface

area for attaching, the decreased frequency of molting (Young, 1966; Bishop, 1968; Koeppe, 1975) and decreased surface area for grooming in larger hosts (Farrell et al., 2014; Skelton et al., 2014). It is also probable that the growth of the host is promoted by branchiobdellidans, as known for some crayfishes (Brown et al., 2002, 2012; Lee et al., 2009; Ames et al., 2015). Although the reason for the body size difference of the host shrimps is indeterminable, the influence of *H. truncatus* on the growth of the host shrimp, if any, could not be negative, because there is no data showing that the host shrimps with *H. truncatus* was smaller than those without *H. truncatus*.

The size of *P. compressa* did not differ among shrimps with large *H. truncatus*, those with small *H. truncatus*, and those with cocoons. This indicates that the host size did not differ among the life stages of *H. truncatus*. However, Mori et al. (2001) reported that immature and mature branchiobdellidans (*Branchiobdella italica*) were found mainly on juvenile and mature crayfish, respectively.

The number of eggs in *P. compressa* specimens was influenced by the body size of ovigerous females and the river, but not by the cohabiting of *H. truncatus*. Different egg numbers among rivers may be caused by differences in nutritional conditions among rivers. The interaction between the river and the cohabiting of branchiobdellidans was insignificant for the effect on egg number in *P. compressa*. This finding indicates that the cohabiting of *H. truncatus* does not influence the number of eggs in the host shrimps.

The abundance of *H. truncatus* and its cocoons displayed similar seasonal dynamics to the host shrimp *P. compressa*, with an increase in summer and a decrease in winter. Such a seasonal pattern has also been observed in another atyid species, *C. multidentata* (Hamano et al., 1992). The period when cocoons occur (May, August, September, and October) suggests that the reproductive

season of *H. truncatus* is from spring through autumn.

The carapace length of *P. compressa* carrying *H. truncatus* was greater than 2.5 mm. The monthly size structure of *P. compressa* indicated that shrimps larger than 2.5 mm were more abundant in winter to spring than in summer to autumn. The abundance of *P. compressa* available for branchiobdellidans was therefore higher in winter to spring than in the warm season. However, the abundance of branchiobdellidans in winter to spring was very low. This suggests that the abundance of *H. truncatus* per *P. compressa* was extremely high in the warm season. The seasonal trend that symbiont abundance was reduced in the cold season has been shown in other branchiobdellidan species on crayfishes (Young, 1966; Bishop, 1968; DeWitt et al., 2013).

The longitudinal distribution of *P. compressa* was wide, from the lower to the upper reach, which has also been reported in Tokushima (Hamano et al., 2000) and Chiba (Usami et al., 2008). *Paratya compressa* larger than 2.5-mm CL, which can carry *H. truncatus*, also occurred widely from the lower reach with tidal influence to the upper reach. However, *H. truncatus* was distributed from the middle to upper reaches, which disagreed with the distribution between host shrimps and *H. truncatus*. Non-occurrence of *H. truncatus* in the lower reach with tidal influence could be due to the weak salinity tolerance of *H. truncatus*, as shown by the salinity tolerance experiment in the present study. The susceptibility of branchiobdellidans to salinity stress was also reported by Overstreet (1998) and Gelder et al. (2006).

The preference experiment showed that *H. truncatus* prefers *C. leucosticta* to *C. multidentata*, *P. compressa* to *C. multidentata*, and *P. compressa* to *C. leucosticta*. The overwhelming preference for *P. compressa* was reflected in the higher proportion of *P. compressa* bearing *H. truncatus* compared to other atyid species in the rivers surveyed. Similarly, the branchiobdellidan *Cambarincola ingens* preferred the crayfish *C. chasmodactylus* to another crayfish, *O. cristavarius*, and the prevalence of the branchiobdellidan on *C. chasmodactylus* was higher than on *O. cristavarius* in the field (Brown et al., 2004).

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