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Migration of Two Atyid Shrimps, *Caridina sakishimensis* (Fujino and Shokita, 1976) and *Caridina typus* (H. Millne Edwards, 1937) in Urabaru, Kikai-jima Island, Southern Japan

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Authors' Contribution
ANS designed the study and conducted the survey. This study is part of her thesis. HS supervised the research. STQ helped in statical analysis. WAB corrected the language of the manuscript.

Key words
Atyid shrimps, Amphidromy, Stream

ABSTRACT

The present study was conducted to study the presence of amphidromy in two atyid shrimps *Caridina sakishimensis* and *Caridina typus* along the continuum of Urabaru stream. Study was carried out to clearly outline the life cycle of an endangered atyid *C. sakishimensis* and its co-existing species *C. typus*. Entire continuum of stream was divided into five stations (St.1, St. 2, St. 3, St. 4 and St. 5). Monthly sampling was carried out using a scoop net (1 × 1 mm) via the sweep method, sweeping efforts were counted. The results revealed that *C. sakishiemnsis* was restricted at St. 1 only, a few juveniles of *C. sakishiemnsis* were recorded at St. 3, St. 4. The length frequency histograms showed the occurrence of large number of juveniles at St. 3, 4 and 5 in smaller length groups. Also *C. typus* ovigerous females exhibiting spawning migration to the downstream and its juvenile also migrate back to upstream after completing the larval development in Urbaru stream were also observed. Presence of *C. sakishsimensis* at only St. 1 indicates the species is not using the river continuum for migration. Due to cave dwelling habit of *C. sakishsimensis* and coral origin of Kikai-jima Island it can be concluded that the species might be using the underground river system for migration.

INTRODUCTION

Most of the aquatic animals migrate from one habitat to another for different purposes mainly for feeding, spawning etc (Dingle, 1996). In amphidromy, freshwater animals migrate from freshwater, involving spawning in freshwater and then larval drift to sea or estuarine environments. The development of these larvae complete in the marine environments then young return to freshwater for feeding where they grow and become mature (McDowall, 2007).

Some of the freshwater shrimps are diadromous but require marine environments to complete their life cycles (Bauer and Delahoussaye, 2008). In some type of diadromous species larvae drift downstream to estuaries for further development (Hamano, 1992; March *et al*., 1998; Benstead *et al*., 1999; Rome *et al*., 2009). After completion of the larval stages, the juvenile migrate back to the parental freshwater habitats. In some caridean species females migration is noted towards downstream for spawning in the estuarine environments (Ohtomi and Nakabayashi, 1999; Bauer and Delahoussaye, 2008; Ismael and New, 2000). Such type of migration of ovigerous females is to minimize larval mortality during the drift. This phenomenon is mainly reported in *Macrobrachium* species inhabiting larger river systems (Bauer and Delahoussaye, 2008; Rome *et al*., 2009; Ismael and New, 2000).

Caridina typus was observed as the most abundant species in the different streams of Kikaijima (Soomro *et al*., 2016). Hence this present study is conducted to clarify the life history characteristics and their amphidromy in the two atyid shrimps *C. sakishimensis* and *C. typus* occur in Urabaru streams, Kikai-jima Island, Southern Japan. The study involves the spawning grounds, occurrence of larval stages near estuarine areas and possible upstream migration of juveniles by both species.

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MATERIALS AND METHODS

Sampling and observation of specimens

The ecological distribution patterns of two atyid shrimps, monthly variation in developmental stages of eggs attached to ovigerous females including eyed and non-eyed stages, and the recruitment pattern of juveniles were investigated based on samples collected during May 2007 to December 2008. Sampling and observation of specimens was conducted in Urabaru stream of Kikai-jima Island, southern Japan.

In Kikai-jima, Urabaru was the only stream with accessibility for sampling on the entire stretch of the stream due to its short length (680 m from head to tail) and slow flows. Five sampling stations, Sts.1, to 5, were sampled along the river profile from the headwater to the river mouth and each station covered the area of about 20 m in length: St. 1 located at the headwater 680 m from the river mouth, and at 12 m altitude; St. 2 at 580 m and characterized by a framed culvert with roof, and bottom of concrete sand, and gravel deposits; St. 3 was located at 410 m, where the stream width ranged from 2 to 4 m, the stream bottom comprised of concrete and gravel deposits with green algae and aquatic plants dominating the flora at the station; St. 4 at 110 m with the upper part of the station located above a cascade with characteristics similar to station 3, while the lower part of the station (90 m from river mouth) below the cascade was a pool surrounded by a concrete wall with a sandy gravel bottom running approximately 10 cm deep; and St. 5 at river mouth but with finer sediments at the bottom and grown aquatic plants. There were small dams with approximately 1-m high cascades below Sts.1, 3 and 4. The depth of the Urabaru stream ranged from 20 to 60 cm.

Monthly samples were taken using a scoop net measuring 1×1 mm via the sweep method. Orbital total length (OTL) of sampled specimens was measured using a caliper to 0.05 mm accuracy. Identification and measurement of shrimps was carried out at site and specimen were not killed or preserved. The egg developmental stages of ovigerous females were categorized into eyed and non-eyed egg stage (Soomro *et al*., 2011).

Study on larval occurrence

Larval occurrence was studied over 32 h sampling where samples were taken at intervals of 2 h on the 4th and 5th of August 2009. Sampling was conducted at Sts.1, 3, 4 and 5. Station 2, which was very close to St. 1, was omitted for the purpose of this study. Samples were collected at each station using a 100 μm mesh size plankton net follows: approximately 200 liters water was filtered through the plankton net and all specimens collected and preserved immediately in 5 % formalin and later transferred to the laboratory for further analysis. All specimens were identified to family level by observing under a stereomicroscope (Nikon SMZ-U) and their total numbers were recorded. Larval identification to family level was conducted according to classifications adapted from Ito *et al*. (2003).

Measurements of physico-chemical parameters

Physico-chemical parameters temperature, conductivity, pH and salinity in the five stations in Urabaru stream were measured on the site using a digital meter (YSI model 63-10, YSI, USA).

Tidal data and sunset and sunrise timings Table I were obtained from the city office of Amami-Oshima during the larval sampling.

Table I. High and low tide timings and sunrise and sunset timings in Amami-Oshima.

Date	High tide time	Tidal level(cm)	Low tide time	Tidal level(cm)	Sunrise time	Sunset time
04-Aug	5:41	194	0:04	113	5:36	19:12
	19:03	195	12:26	44		
05-Aug	6:19	203	0:37	105	5:36	19:11
	19:29	201	12:57	39		

Data analysis

Histograms for length frequency analysis were plotted in MS Excel 2003 using 2-mm length classes. Individuals smaller than 8 mm OTL were difficult to sex due to undeveloped sexual appendages, hence all the individuals were categorized into: i) juveniles ii) ovigerous females and iii) others including non-ovigerous females and males. All ovigerous females were further categorized into two stages: a) eyed and b) non-eyed egg stage females.

For the analysis of variations between the OTL of juveniles at different stations, non-parametric Kruskal-Wallis test was conducted using SPSS® 13.0, followed by a post hoc Steel-Dwass test.

RESULTS

Physico-chemical parameters

Conductivity was highest at the headwater and decreased gradually towards the river mouth. On the contrary, pH was lowest at headwater, increasing towards the river mouth. High variations in temperature were recorded at Sts. 3, 4 and 5. In Sts. 1 and 2, monthly fluctuations in temperature were minimal, averaging 21.5 ±0.2 (mean ± SD). At Sts. 3 and 4 fluctuations were higher, with water temperatures averaging at 18.7 ± 8.2

and 19.4 ± 8.9 (mean ± SD) respectively. Similarly, at St. 5 the temperature also recorded high monthly fluctuations: mean ± SD at 22.8 ± 3.7 (Fig. 1).

During the sampling period for larvae, sunrise and sunset was recorded at 05:36 and 19:12, respectively. High tides timings were at 05: 41 and 19:03 and low tides at 00:04 and 12:26 on the 4th August, while on the 5th August, the timings were at 06:19 and 19:29, and at 00:37 and 12:57 for the high and low tides, respectively (Table I).

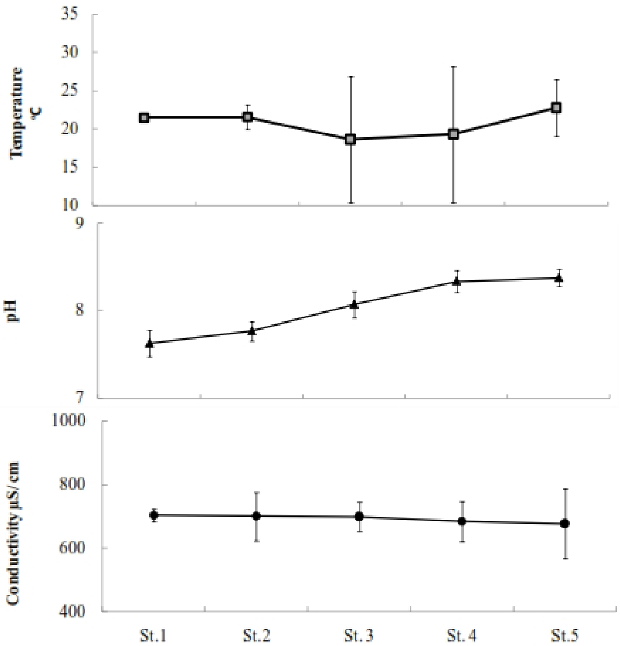

Fig. 1. Physico-chemical parameters recorded at the sampling stations. Whiskers represent the standard deviation of mean values at each station.

Abundance and occurrence of juveniles and ovigerous females

Caridina sakishimensis individuals were found at the St. 1 only. The size (OTL) of *C. sakishimensis* ranged from 6.6 to 25.6 mm. Ovigerous females were observed during June to September 2007 and May to October 2008 and their numbers were considerably high during this period. Juveniles in the 8 mm length class were recorded only during August and September 2007 and October and December 2008 (Fig. 2). Few juveniles in the length classes 6 and 8 mm were observed in the middle stations, at St. 3 during June, November and December 2007 and at St. 4 during June, October and December 2007 (Fig. 3). No specimens of *C. sakishimensis* were recorded in St. 2 and 5.

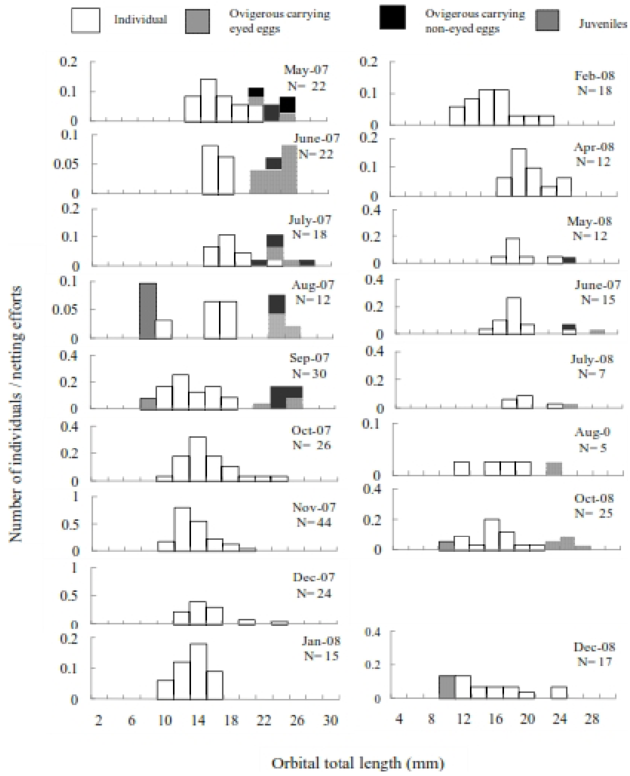

Fig. 2. Length-frequency analysis of orbital total length in *Caridina sakishimensis* showing monthly variations in OTL at St. 1 of Urabaru stream in Kikaijima, southern Japan.

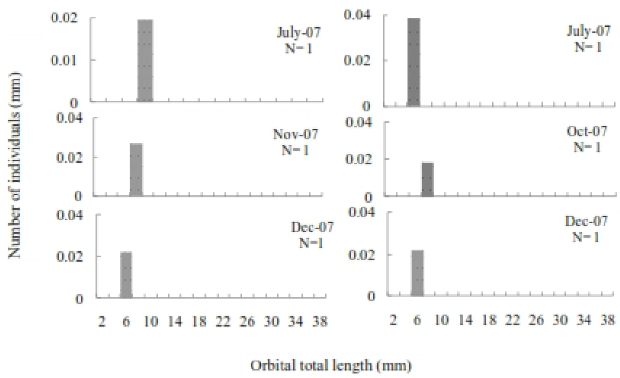

Fig. 3. Length-frequency analysis of orbital total length in *Caridina sakishimensis* showing monthly variations in the OTL at St. 3 and 4 of Urabaru stream in Kikaijima, southern Japan.

C. typus shrimp at St. 1 comprising of relatively smaller population with only a few ovigerous females. Such individuals were 5.5 to 23.7 mm OTL. Ovigerous females found in May and September 2007 and April to October 2008 in which non-eyed egg stage females were dominated. Juveniles of the 6 and 8 mm length classes were present from June 2007 to February 2008 and August to October 2008 as shown in (Fig. 4).

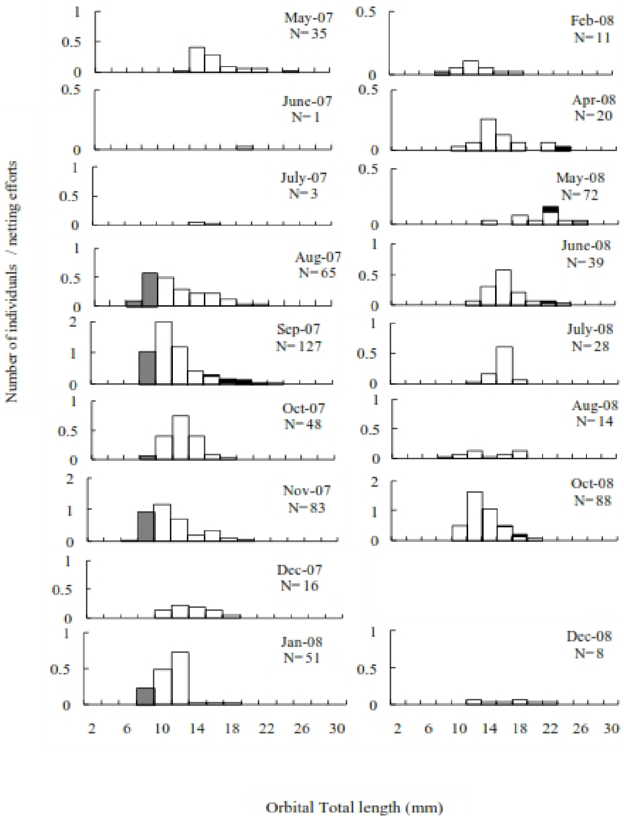

Fig. 4. Length-frequency analysis of orbital total length in *Caridina typus* showing monthly variations in the OTL at St. 1 of Urabaru stream in Kikaijima, southern Japan.

At St. 2, the population size of C. *typus* was among the individuals of 5.5 to 25.5 mm OTL. The 25.5 mm sized-specimen was an ovigerous female. Ovigerous females collected during June and September 2007 and April and December 2008 all were non-eyed eggs (Fig. 5).

At St. 3 the *C. typus* population was comprised of high numbers of specimens. The population size was increased at this station during August to December 2007, were 3.1 and 26.7 mm in OTL. Small sized individuals in the 4, 6 and 8 mm length classes are present from June to December 2007 and July and December 2008. Ovigerous females were recorded from May to October 2007, 2008 and were dominated by non-eyed stage females (Fig. 6).

At St. 4, ovigerous females of *C. typus* carrying eyed and non-eyed eggs were recorded in equal numbers during May to October 2007 and April to October 2008. Juveniles occurred during June through November 2007 and 2008, and the shrimps in 4 mm length class were the most abundant. The smallest and largest individuals at this station were 2.4 and 28.8 mm OTL, respectively (Fig. 7).

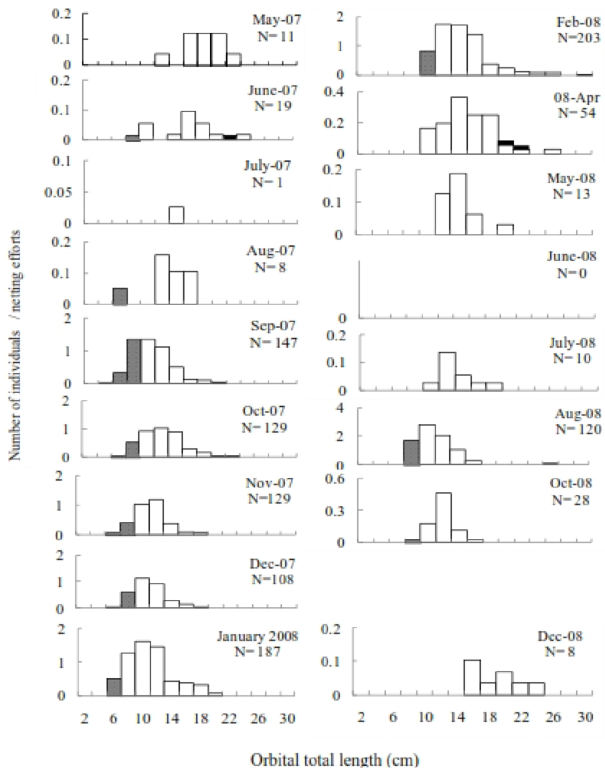

Fig. 5. Length-frequency analysis of orbital total length in *Caridina typus* showing monthly variations in the OTL at St. 2 of Urabaru stream in Kikaijima, southern Japan.

At St. 5, only a small number of individuals were recorded. Ovigerous females occurred from May to November 2007 and April to June 2008. During the study period, ovigerous females carrying eyed eggs generally appeared early (April 2008) at St. 5 compared to the appearance recorded at other stations. Further, juveniles were recorded early (May 2007) at this station compared to the other stations. The smallest and largest individuals were 3.4 and 27.2 mm OTL, respectively. Small sized individuals in the 4, 6, and 8 mm length class were observed during May to December 2007 and June to December 2008 (Fig. 8).

The average OTL of *C. typus* juveniles at the headwater stations, Sts.1 and 2 was larger, approximately 7.0 mm, compared to those of 6.3, 6 and 5.6 mm at Sts. 3, 4 and 5, respectively. The differences in the OTL sizes of *C. typus* juveniles were highly significant among the sampling stations (Kruskal-Wallis, $p<0.001$). Post hoc tests using Steel-Dwass showed highly significant differences ($p>0.01$) in OTL sizes of juveniles between Stations, St. 1 vs. 3; 1 vs. 4; 1 vs. 5; 2 vs. 3; 2 vs. 4 and St. 2 vs. 5 (Table II).

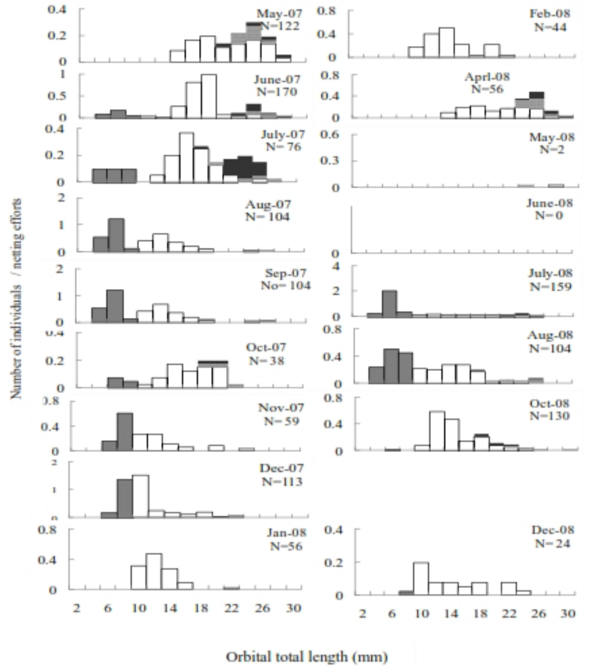

Fig. 6. Length-frequency analysis of orbital total length in *Caridina typus* showing monthly variations in the OTL at St. 3 of Urabaru stream in Kikaijima, southern Japan.

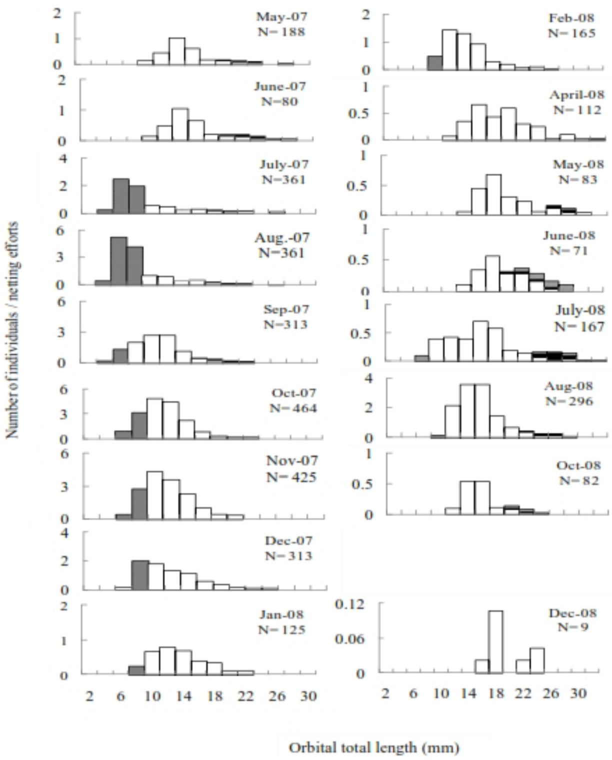

Fig. 7. Length-frequency analysis of orbital total length in *Caridina typus* showing monthly variations in the OTL at St. 4 of Urabaru stream in Kikaijima, southern Japan.

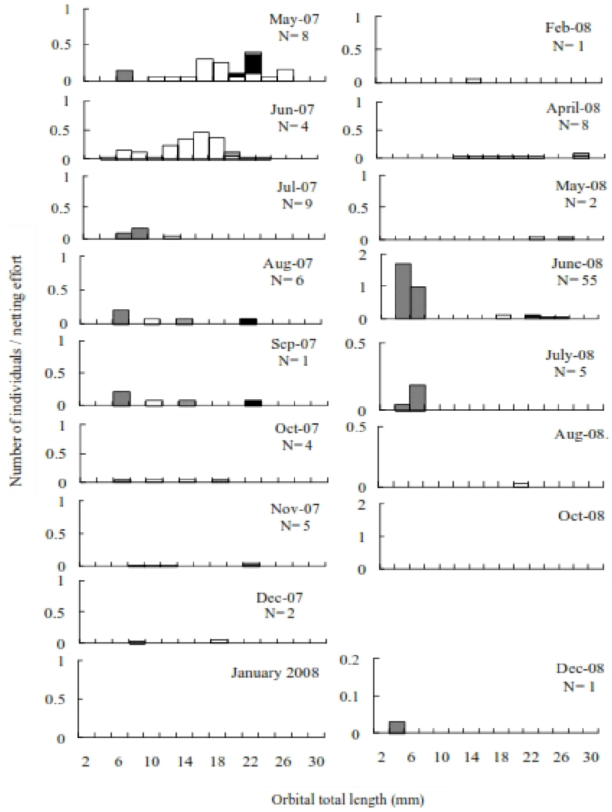

Fig. 8. Length-frequency analysis of orbital total length in *Caridina typus* showing monthly variations in the OTL at St. 5 of Urabaru stream in Kikaijima, southern Japan.

Table II. Mean values of orbital total length of *C. typus* with non-parametric *Kruskal-wallis* and Post hoc *Steel-Dewass* tests.

Stations	Mean length (OTL)	Kruskal-Wallis significance	Steel- Dewass significance
St-1	7		
St. 2	7		
St. 3	6.3	*P* > 0.001	
St. 4	6		
St. 5	5.6		
St. 1; Vs. 3, 4, 5			*P* > 0.01
St. 2; Vs. 3, 4, 5			*P* > 0.01

On the other hand, the number of *C. sakishimensis* juveniles was comparatively high at St. 1, with a few juveniles at Sts. 3 and 4 only, no juvenile was found at Sts. 2 and 5. Kruskal-Wallis test show no significant differences between the lengths of juveniles at Sts. 1, 3 and 4.

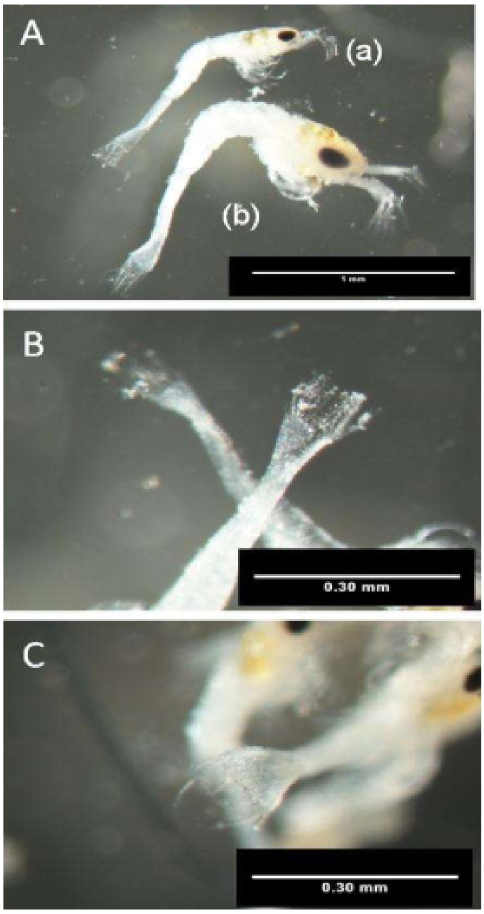

Fig. 9. Larval developmental stages of specimens sampled at the Urabaru headwater stream in Kikai-jima Island, southern Japan, showing (A) a) Stage I atyid larvae, b) Stage I palaemonid larvae, (B) the shape of telson of atyid larvae and (C) the shape of telson in palaemonid larvae.

Larval distribution and occurrence

Due to lack of information on the complete larval stages of *C. sakishimensis* and *C. typus*, larvae specimens collected were only identified to family level: larvae with bi-lobed telson and a small eye spot were identified as Atyid (Ito *et al*., 2003). Larvae with yolk globules stored in dorsal area of the carapace and possessing a telson without bifurcation (Fig. 9) were grouped under the family Palaemonidae. During the present study, a total of 613 larvae were captured, 609 belonged to family Atyidae and 4 in Palaemonidae. Most of the larvae occurred at St. 5, with over 91 % (556 specimens) of the total larvae, compared to <1 % at St. 1. (3 specimens) and St. 3 (2 specimens.), and 7.9 % (48 specimens) recorded at St. 4. Larval abundance was relatively higher at the river mouth and decreased toward upstream. Diurnal variations showed that at Sts. 4 and 5, larval abundances were relatively higher towards sunset at 19.00 hrs and the numbers increased within 2 h after sunset and then declining towards sunrise (Fig. 10).

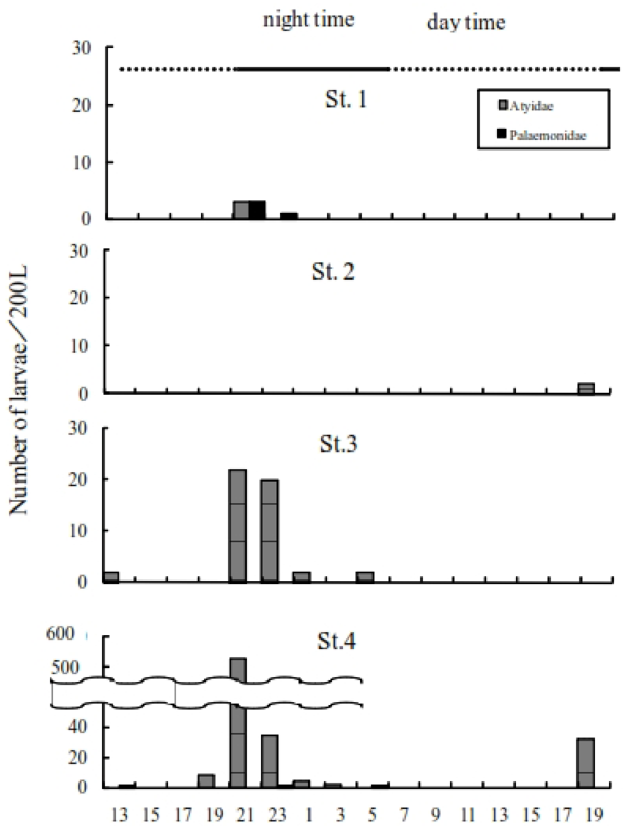

Fig. 10. Diurnal variations in the occurrence of planktonic larvae caught at the sampling stations (St. 1, 3, 4 and 5) of the headwater Urabaru stream in Kikaijima Island.

DISCUSSION

C. sakishimensis individuals occurred at St. 1 with large number of ovigerous females suggest that this species mainly inhabited the headwaters where breeding and spawning took place. Occurrence of a few juveniles at Sts. 3 and 4 was attributed to accidental drift due to heavy precipitation. Whereas, this species carries large numbers of small-sized eggs (Soomro *et al*., 2011), that is the characteristic of diadromous atyid shrimps with prolonged larval development completed in saline waters (Shokita, 1981; De Silva, 1988). The presence of larvae at the headwater St. 1 also confirms the spawning of *C. sakishimensis* in these headwater streams. However, restricted distribution of the juveniles at all stations except the headwaters also complicates the clarity of amphidromous life cycle in this species. In one of the other islands, Miyako-jima, the *C. sakishimensis* was reported as a cave dwelling species (Fujino and Shokita, 1975). Due to short formation history and coral origin, Ryukyu lime stone is highly porous and contain many caves and underground river systems. The present study postulates that *C. sakishimensis* may migrate through underground river systems as shown by its cave dwellings habits in Miyako-jima. To confirm the hypothesis of migration of *C. sakishimensis* through underground water reservoirs (man - made), a survey was conducted in one of the reservoirs in Kikai-jima with water depths ranging 50-75 cm. Planktonic sampling was conducted within the underground water storage system but no juveniles were found. The absence of juveniles may be attributed to the depth of this man-made reservoir which was also characterized by fast water flow.

The presence of ovigerous females with non-eyed stage at the headwater stations St. 1, and presence of a large number of ovigerous females carrying eyed-eggs at the downstream stations Sts. 3, 4 and 5 indicates that *C. typus* species exhibits downstream spawning migrations. Other atyid species, *C. japonica* spawned in headwaters of a small freshwater river in Tokushima, Japan (Hamano, 1992). Difference in the presence of various individuals can be attributed to the mechanism for the regulation of population (Jan *et al*., 2019). Presents report is the first detailed observation about downstream migration for hatching of an atyid in small stream systems.

The earlier occurrence of smaller size *C. typus* juveniles at the downstream stations and the larger sized juveniles at the upstream suggests that the juveniles of the species migrated upstream towards the headwater stations as growth progressed. The upstream migration of juveniles after metamorphosis has previously been reported for many caridean shrimps (Hamano, 1992).

The spawning seasons of *C. typus* run from March to December while juveniles start to recruit in August. Similarly, major spawning activities of *C. sakishimensis* were observed from June to September (Soomro *et al*., 2011). Based on the information of reproductive activities of both species, larval samples were collected during August. Larvae emerged soon after sunset and gradually number was increased as dark. At the river mouth St. 5 mostly *C. typus* were present. The higher abundance of atyid larvae after sun set was attributed to the nocturnal hatching of larvae and larval releasing behavior in the atyids. The larval release in *C. leucosticta* (Ideguchi, 2000), *C. typus*, *C. japonica serratirostris* and (Ideguchi *et al*., 2007) in Japanese rivers after sunset were also reported. A few larvae were found at St. 1. These larvae were possibly *C. sakishimensis,* since the ovigerous females of this species were restricted at the headwater St. 1 only. This speculation needs further confirmation since larvae were only identified up to family level and could not be differentiated to species level. On the other hand, comparatively high abundance of atyid larvae at the river mouth stations confirms a downstream spawning of *C. typus.* When downstream hatching occurs, significantly large numbers of larvae can be expected at the river mouth as compared to the upstream stations (Rome *et al*., 2009).

CONCLUSION

In this study, spawning migration of *C. typus* towards river mouth as well as upstream migration of juveniles was confirmed. However, further studies are necessary to clarify for the migration of *C. sakishimensis* ovigerous females and juveniles along the stream continuum that was not obvious in this study.

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Statement of conflict of interest

All authors declare no conflict of interest.

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