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FULL PAPER

Monthly occurrence and feeding habits of larval and juvenile Ryukyu-ayu *Plecoglossus altivelis ryukyuensis* in an estuarine lake and coastal area of the Kawauchi River, Amami-oshima Island, southern Japan

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Abstract We examined monthly occurrence patterns and feeding habits of larvae and juveniles of the critically endangered Ryukyu-ayu *Plecoglossus altivelis ryukyuensis* in the lower reach of the Kawauchi River, Amami-oshima Island, southern Japan, for two successive years. The study area was classified into a brackish lake (Lake Uchiumi) and a coastal zone (Sumiyo Bay) based on monthly variations in water temperature and salinity. Larvae and juveniles occurred from December to April in the first year and from January to February in the second year, respectively. Smaller-sized and earlier developmental-staged *P. altivelis ryukyuensis* individuals tended to emerge in larger numbers in Lake Uchiumi than in Sumiyo Bay. The present data suggest that Lake Uchiumi, where the physical environment is characterized by lower temperature and salinity than in Sumiyo Bay, would be suitable as a nursery ground for this fish, especially for earlier developmental-staged larvae. No individuals ≥25.0 mm body length, which is the size at which they begin their upstream migration, occurred in Lake Uchiumi or Sumiyo Bay in April or May, when upstream migration is assumed to reach a peak based on previous studies, in either year. The peak of upstream migration, therefore, may have occurred several months earlier in the years covered in this study due to higher water temperature than in typical years. To assess the feeding habits of this fish, the gut contents were observed. In both areas, copepods were abundant in *P. altivelis ryukyuensis* diet with calanoids and *Oithona* spp. being the most abundant. The diet composition differed between Lake Uchiumi and Sumiyo Bay and clearer ontogenetic variation in the composition of the diet was observed in Lake Uchiumi. Small individuals fed on tintinnid ciliates, but the proportion of these organisms in the diet gradually decreased with growth. Large individuals fed exclusively on harpacticoid copepods and insects including demersal species. Overall, larval and juvenile *P. altivelis ryukyuensis* exhibited a generalist feeding habit that tended to increase with growth. It is indispensable to adequately manage the environmental conditions in the lower reach of the Kawauchi River, especially Lake Uchiumi, to conserve *P. altivelis ryukyuensis* population.

Keywords Larva · Juvenile · Amphidromous migration · Feeding habits · *Plecoglossus altivelis ryukyuensis*

Introduction

The Ryukyu-ayu *Plecoglossus altivelis ryukyuensis* (Salmoniformes, Plecoglossidae) is designated as critically endangered by the Japanese Ministry of the Environment and the Kagoshima Prefecture (Japanese Ministry of the Environment 2013; Kishino and Yonezawa 2013). *Plecoglossus altivelis ryukyuensis* is an amphidromous species (Yonezawa et al. 2016). They grow in the upper or middle reaches of rivers after upstream migration. Sexually mature fish then migrate downstream and spawn in sand- or gravel-bottomed glides in the lower reaches of rivers in winter. The life span for most *P. altivelis ryukyuensis* is one year because they die after spawning. Post-hatched larvae drift seaward, spending time in brackish or coastal waters growing and thereafter migrate upstream. Life

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history studies of the subspecies, specifically on the early life stages, have been conducted for the purpose of conservation of the subspecies (Oka et al. 1996; Kishino and Shinomiya 2003, 2004a, 2004b, 2005, 2006; Kishino et al. 2008; Kobari et al. 2012).

Plecoglossus altivelis ryukyuensis is closely related to the ayu *Plecoglossus altivelis altivelis*, which is widely distributed across the Japanese Archipelago and Korean Peninsula (Nishida 1988). It is thought that the ancestor of *P. altivelis ryukyuensis* was isolated from populations inhabiting mainland Japan in the Middle Pleistocene with the formation of the Ryukyu Archipelago and then evolved separately for a million years (Nishida 1985, 1986, 1988). The subspecies now inhabits only Okinawa-jima and Amami-oshima Islands (Nishida 1988), although the wild population of Okinawa-jima Island has been extinct since 1978 (Tachihara 2009). Fry produced by parent fish from the Yakugachi River on Amami-oshima Island were stocked in the rivers of Okinawa-jima in 1992, and some landlocked populations have now successfully established in reservoirs in northern Okinawa-jima Island (Tachihara 2009). Since then, fry have been continuously released into the rivers of Okinawa-jima. However, *P. altivelis ryukyuensis* still cannot reestablish their historical amphidromous migration on Okinawa-jima Island (Tachihara 2009).

Amami-oshima Island is the only area where living wild populations of *P. altivelis ryukyuensis* are found, mainly in four rivers: the Yakugachi River, the Sumiyo River, the Kawauchi River (in eastern Amami-oshima Island: our study site), and the Kawauchi River (in western Amami-oshima Island) (Yonezawa et al. 2016). The Yakugachi, Sumiyo, and Kawauchi Rivers flow into Sumiyo Bay on the eastern coast of the island while the western Kawauchi River flows into Yakeuchi Bay on the western coast. These four rivers are large rivers on Amami-oshima Island where spawning events of *P. altivelis ryukyuensis* occur every year (Yonezawa et al. 2016).

Plecoglossus altivelis ryukyuensis differs morphologically from *P. altivelis altivelis* (see Nishida 1986, 1988) with the maximum size of the former being smaller than the latter at 10–15 cm total length (Yonezawa et al. 2016). There are distinct differences between the early life history traits of the two subspecies. For example, *P. altivelis ryukyuensis* spawn from November to March and upstream-migrating individuals can be found from January to May, indicating that the duration of time that pelagic larval and juvenile stages can be found in estuarine and coastal waters before migrating upstream is shorter than for *P. altivelis altivelis* (Kishino and Shinomiya 2003, 2004a, 2004b). *Plecoglossus altivelis altivelis* migrate upstream at approximately 60–80 mm standard length just before or after the development of comb-like teeth (Takahashi et al. 2000; Kishino and Shinomiya 2003). Meanwhile, *P. altivelis ryukyuensis* individuals start to migrate upstream at approximately 25 mm standard length when the comb-like teeth are not completely formed and also when the number of fin rays is incomplete and pigmentation is insufficient (Kishino and Shinomiya 2005). Thus, upstream-migrating individuals of *P. altivelis ryukyuensis* are characterized by a smaller size, a younger age, and an earlier developmental stage compared to *P. altivelis altivelis* (Kishino and Shinomiya 2003). Larvae and juveniles of *Plecoglossus* species are vulnerable to high temperatures (Takahashi et al. 1999; Kishino and Shinomiya 2003). This is demonstrated by the deterioration of growth and development of *P. altivelis ryukyuensis* larvae in water temperatures $\geq$ 20°C in estuarine and coastal waters (Kishino and Shinomiya 2003). Therefore, it is speculated that shorter pelagic larval and juvenile stages evolved to adapt to the harsh conditions in Amami-oshima Island where the period of low water temperature (<20°C) is short in estuarine and coastal waters (Kishino and Shinomiya 2003).

Ecological information on *P. altivelis ryukyuensis* in the Kawauchi River and neighboring coastal areas is still limited, although some data have been presented on the feeding habits of larvae and juveniles in the surf zone in Sumiyo Bay and the appearance of upstream migrating individuals in the river (Oka et al. 1996; Kishino and Shinomiya 2004a). The ontogenetic habitat utilization pattern of larvae and juveniles in the area remains unknown, and their feeding habits are not completely understood throughout all of the early development stages. This area is one of the most important regions for the conservation of wild populations of this subspecies because it is one of its primary habitats. In this study, we aimed to describe the physical environment of Lake Uchiumi and the adjacent Sumiyo Bay located in the lower reach of the Kawauchi River, which is assumed to be an important nursery area for *P. altivelis ryukyuensis*, and to determine the monthly occurrence patterns and feeding habits of larvae and juveniles of this subspecies.

Materials and methods

After obtaining permission for fish collection following the prefectural rules and regulations, specimens were collected at fixed stations in the shallow area of Lake Uchiumi and the surf zone of Sumiyo Bay in the lower reach of the Kawauchi River from December 2012 to May 2013 (Fig. 1). Ten surveys were conducted. Surveys began at seven stations (nos. 1–4, 6, 7, and 10) on 27 December. On 9 January, two more stations (nos. 5 and 8) were added (Fig. 1), and surveys were conducted at all nine stations from that point on (surveys were conducted at station 9 in

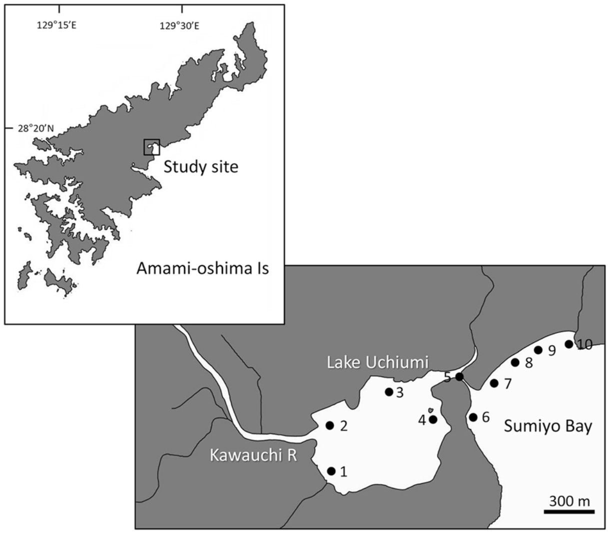

Fig. 1 Map of Amami-oshima Island and Lake Uchiumi and Sumiyo Bay at the mouth of the lower reach of the Kawauchi River in Amami-oshima Island. Numbers indicate survey stations

the following season; see the next paragraph). A larva net (opening: 50 × 110 cm; depth: 155 cm; mesh size: 0.7 mm) was towed at each station for 50 m along the coastline by two people who each held one side of the net. Water temperature and salinity of the surface layer were recorded using a stick thermometer and a handheld refractometer (MASTER-M/Millα, Atago Corporation), respectively. Surveys were performed at or around high tide during daytime. Specimens were fixed in 10 % neutral buffered formalin immediately after collection and then preserved in 70 % ethanol.

The following year, surveys were conducted as described above from December 2013 to April 2014. The number of specimens collected in the previous year exceeded 1,000 individuals and therefore monthly surveys were performed at three stations (3, 4, and 9; Fig. 1) to avoid serious effects on the reproduction of the subspecies. A supplementary collection was made with a scoop net (diameter: 30 cm; mesh size: 3.0 mm) at station 2 on 25 February 2014 to collect additional specimens for the analysis of feeding habits.

In the laboratory, we measured body length (BL: standard or notochord lengths) of all specimens to the nearest 0.1 mm using image-analyzing software (NIS-Elements D 3.1, Nikon Corporation) under a binocular stereomicroscope (Nikon SMZ1500, Nikon Corporation). For specimens over 25 mm, BL was measured using digital calipers (Mitutoyo Digimatic Caliper CD-20PMX, Mitutoyo Corporation). The developmental stage of each specimen was identified only for the specimens used in the analysis of feeding habits in the first year and for all specimens collected in the second year.

The CPUE (catch per unit effort: number of individuals per haul) of the larvae and juveniles was compared between Lake Uchiumi and Sumiyo Bay after samples were pooled each year due to small sample sizes. Because the assumption of homogeneity of variances for parametric analysis was not met, a nonparametric Mann–Whitney *U* test was employed to test the difference in CPUE between Lake Uchiumi and Sumiyo Bay using IBM SPSS Statistics 23 (IBM, Tokyo, Japan) statistical software. The CPUE of each size range (≤4.9, 5.0–9.9, 10.0–14.9, 15.0–19.9, 20.0–24.9, 25.0–29.9, and ≥30.0 mm BL) for the larvae and juveniles was compared between Lake Uchiumi and Sumiyo Bay to elucidate the monthly occurrence patterns. The specimens collected at station 2 by a scoop net for the analysis of feeding habits were excluded from this analysis.

The gut contents were analyzed and observed in a randomly selected subsample of specimens. Larvae and juveniles were mounted on microscope slides and their gut contents extracted using a fine needle. The gut contents were then covered with a drop of glycerin. The prey was identified and counted under a binocular stereomicroscope (Nikon SMZ1500, Nikon Corporation). We measured the body width (prey size) of any prey item that was intact and available for measurement to the nearest 0.1 μm using image analyzing software (NIS-Elements D 3.1, Nikon Corporation).

Feeding intensity (FI, %) was defined as the proportion of fish with prey in the gut (Kume et al. 2015a). This index was considered a measure of feeding success. Feeding ratio (FR) was defined as the number of prey items per gut (Kume et al. 2015a).

Diet composition was estimated using two indices for the prey items: percent number (N %) and frequency of occurrence (F %) (Kume et al. 2015a, 2015b). Percent number was calculated as the percentage of each item out of the total number of prey items examined, and frequency of occurrence was calculated as percent frequency occurrence of each prey item in all specimens. Larvae with empty guts were excluded from this analysis.

The Shannon–Wiener diversity index H' was calculated using mean N % of prey items (Krebs 1998):

$$H' = -\Sigma P_i \log P_i,$$

where Pi is the proportion of N % of the ith prey item in the diet of each individual. Index values at or near zero suggest a specialist tendency, whereas species with generalist habits tend to have relatively high values of feeding diversity (index values of >1) (Sano 1989; Horinouchi et al. 2008).

FI, FR, diet composition (N %), and H' were calculated for each size group (≤4.9, 5.0–9.9, 10.0–14.9, 15.0–19.9, 20.0–24.9, and ≥25.0 mm BL) and compared between groups to investigate ontogenetic variations. Because the assumption of homogeneity of variances for parametric analysis was not met, a nonparametric Mann–Whitney *U* test was employed using IBM SPSS Statistics 23 (IBM, Tokyo, Japan) to test if there was a difference in FR between larvae and juveniles at each size range among areas (bays) for the first and second years for Lake Uchiumi (samples collected in Sumiyo Bay in the second year were not used for analysis due to small sample size).

Prey size was plotted against the body length of larval and juvenile *Plecoglossus altivelis ryukyuensis* to determine if there was a relationship between them. The relationship was compared among years and areas.

Results

Physical environment of Lake Uchiumi and Sumiyo Bay. In the first year, **the** water temperature fluctuated around 20°C until early March at four stations in Sumiyo Bay (6–8, 10) and reached over 20°C after late March (Fig. 2). The temperature at the four stations in Lake Uchiumi (1–4) and at station 5 fluctuated more and exhibited generally lower values than stations in Sumiyo Bay, which reached approximately 25°C in late May. Additionally, in the second year water temperature was lower at two stations in Lake Uchiumi (3, 4) than the one in Sumiyo Bay (9) until late March and then increased to around 20°C in late April (Fig. 2).

In both years, the salinity fluctuated around 30 at the five stations in Sumiyo Bay (6–10) (Fig. 2). In contrast, the salinity varied more but was generally lower at the four stations in Lake Uchiumi (1–4) and at station 5 (Fig. 2).

Monthly occurrence pattern. A total of 1,203 and 266 specimens were collected in the first and second years, respectively. The mean CPUE of larvae and juveniles was higher in Lake Uchiumi than in Sumiyo Bay in both years (16.1 ind/haul in Lake Uchiumi and 10.6 ind/haul in Sumiyo Bay in the first year and 26.3 ind/haul in Lake Uchiumi and 0.6 ind/haul in Sumiyo Bay in the second year), although the CPUEs were not significantly different ($P = 0.648$ for the first year and $P = 0.382$ for the second year).

In both years, smaller-sized and earlier developmental-staged individuals tended to emerge in greater numbers in Lake Uchiumi than in Sumiyo Bay (Figs. 3 and 4). Station 5 was grouped with the other Uchiumi stations because of the physical similarity of the sites. Sizes ranged from 3.9 to 30.8 mm BL and from 4.5 to 26.9 mm BL in the first and second year, respectively.

In December of the first year, specimens were composed mainly of the smallest individuals (≤9.9 mm BL). In January and February, mid-sized individuals (5.0–19.9 mm BL) were more abundant in Lake Uchiumi than in Sumiyo Bay. In March, relatively large individuals (20.0–29.9 mm BL) emerged in large numbers in both regions. In April, only a few large individuals (20.0–24.9 mm BL) were found in Sumiyo Bay, and no larvae or juveniles were found in May.

In the second year, no larvae emerged in December. In January, specimens from newly hatched larvae with yolk sacs to juveniles were found, ranging from 4.5 to 26.9 mm BL. In Lake Uchiumi, small individuals (≤9.9 mm BL) comprised 61.6 % of the collected individuals. In Sumiyo Bay, only three specimens were collected, all of which were classified as post-flexion larvae and ranged from 19.8

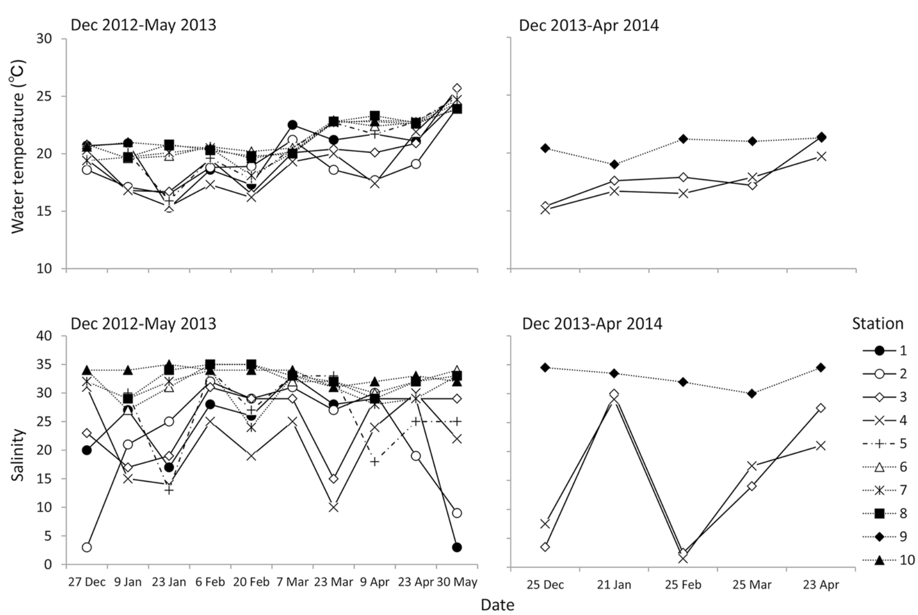

Fig. 2 Monthly changes in water temperature and salinity in the study area

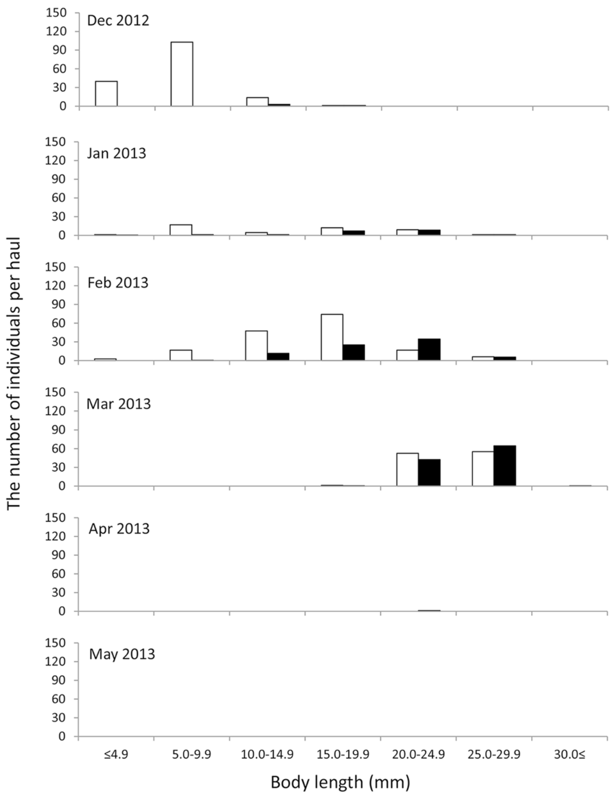

Fig. 3 Monthly changes in CPUE (number of individuals per haul) of each size range of larval and juvenile *Plecoglossus altivelis ryukyuensis* from December 2012 to May 2013. Open and closed bars indicate CPUE in Lake Uchiumi and Sumiyo Bay, respectively

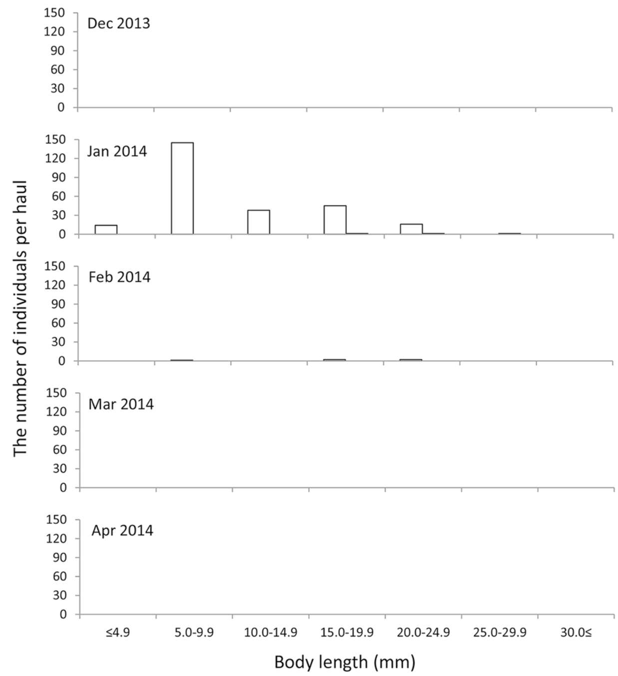

Fig. 4 Monthly changes in CPUE (number of individuals per haul) of each size range of larval and juvenile *Plecoglossus altivelis ryukyuensis* from December 2013 to April 2014. Open and closed bars indicate CPUE in Lake Uchiumi and Sumiyo Bay, respectively

to 26.9 mm BL. In February, no individuals occurred in Sumiyo Bay, and only a few individuals were observed in Lake Uchiumi, which were pre-flexion, flexion, and post-flexion larvae. These individuals ranged from 8.7 to 21.4 mm BL. In March and April, no specimens were collected.

Feeding habits. In total, 403 specimens (first year: $n = 283$; second year: $n = 120$), including additional samples ($n = 9$) collected at station 2 on 25 February 2014, were selected for gut content analysis. The sizes ranged from 3.9 to 30.8 mm BL and from 4.5 to 26.9 mm BL in the first and second year, respectively. For both years, specimens from newly hatched larvae to juveniles were used for the analysis.

Except for Sumiyo Bay in the second year in which there were not sufficient samples for analysis, the FI consistently increased during *Plecoglossus altivelis ryukyuensis* development in Lake Uchiumi and Sumiyo Bay (Table 1). FI was 0 % in larvae ≤4.9 mm, which were all yolk sac larvae, in both years, and it was 0 % in all yolk sac larvae including individuals ≥5.0 mm in both years. FI abruptly increased in the ≥10.0 mm and in the ≥5.0 mm groups in the first and second years, respectively. FI was higher in the ≥5.0 mm groups in Lake Uchiumi in the second year than in either bay in the first year. All individuals collected for diet composition analysis in the Sumiyo Bay in the second year ($n = 3$) fed on prey.

As with FI, there were not enough samples from Sumiyo Bay in the second year to analyze them for FR. For the other samples, the values of FR were 0 in individuals ≤4.9 mm and consistently increased throughout larval and juvenile development in Lake Uchiumi and Sumiyo Bay (Table 1). In the first year, there were no significant differences in FR amongst the size ranges ≥5.0 mm between Lake Uchiumi and Sumiyo Bay ($P = 0.351$ for 5.0-9.9 mm BL; $P = 0.450$ for 10.0–14.9 mm BL; $P = 0.270$ for 15.0–19.9 mm BL; $P = 0.249$ for 20.0–24.9 mm BL; $P = 0.898$ for ≥25.0 mm BL). Conversely, FR was significantly higher for all size ranges ≥5.0 mm in Lake Uchiumi in the second year than the first year ($P < 0.001$ for 5.0–9.9 mm BL; $P < 0.001$ for 10.0–14.9 mm BL; $P < 0.001$ for 15.0–19.9 mm BL; $P < 0.01$ for 20.0–24.9 mm BL; $P < 0.005$ for ≥25.0 mm BL).

For diet composition, 113 specimens with empty guts were excluded leaving 290 specimens for analysis. Larvae fed exclusively on copepods in both Lake Uchiumi and Sumiyo Bay throughout the study period (Table 2). Among the copepods identified, calanoids and *Oithona* spp. were the most abundant.

In Lake Uchiumi, ontogenetic variation in diet was observed in both years (Fig. 5). Individuals ≤24.9 mm fed

Table 1 Feeding intensity (FI) and feeding ratio (FR) of larval and juvenile *Plecoglossus altivelis ryukyuensis*

	December 2012–May 2013						December 2013–April 2014					
	Lake Uchiumi			Sumiyo Bay			Lake Uchiumi			Sumiyo Bay		
Size range (BL mm)	*n*	FI	FR	*n*	FI	FR	*n*	FI	FR	*n*	FI	FR
≤4.9	6	0	0	1	0	0	13	0	0	0	–	–
5.0-9.9	60	23.3	0.6	3	0	0	19	68.4	1.8	0	–	–
10.0-14.9	32	68.8	1.7	12	66.7	4.0	13	100	7.1	0	–	–
15.0-19.9	52	76.9	3.4	11	63.6	2.9	45	95.6	9.6	1	100	14.0
20.0-24.9	15	86.7	6.9	36	97.2	6.5	21	100	20.2	1	100	31.0
≥25.0	23	95.7	12.6	32	93.8	12.5	6	100	93.2	1	100	11.0

n number of specimens, *BL* standard or notochord lengths, *FI* proportion of fish with prey in the gut, *FR* number of prey items per gut

Table 2 Percent number (%N) and frequency of occurrence (%F) of prey items in the diet of larval and juvenile *Plecoglossus altivelis ryukyuensis*

		December 2012–May 2013				December 2013–April 2014			
		Lake Uchiumi (*n* = 111)		Sumiyo Bay (*n* = 80)		Lake Uchiumi (*n* = 96)		Sumiyo Bay (*n* = 3)	
Prey items		%F	%N	%F	%N	%F	%N	%F	%N
Tintinnina	Ptychocylididae spp.	9.0	6.0	0	0	4.2	1.8	0	0
Polychaeta	Nectochaeta sp.	0.9	0.2	0	0	0	0	0	0
Gastropoda	Veliger larvae	1.8	0.3	5.0	1.9	0	0	0	0
Copepoda	Nauplii	32.4	12.6	46.3	11.0	41.7	6.3	0	0
	Calanoida spp.	53.2	32.8	85.0	47.0	65.6	21.8	100	38.7
	Oithona spp.	44.1	24.5	50.0	14.6	84.4	49.4	100	55.3
	Microsetella spp.	7.2	1.6	8.8	0.8	15.6	1.4	0	0
	Euterpina spp.	5.4	0.7	1.3	0.1	15.6	1.4	0	0
	Benthic harpacticoida sp.	14.4	4.1	13.8	3.2	20.8	5.4	0	0
	Harpacticoida spp.	9.9	1.6	5.0	0.6	11.5	1.9	0	0
	Corycaeus spp.	4.5	0.5	25.0	4.8	14.6	1.1	33.3	3.0
	Oncaea spp.	8.1	1.6	22.5	3.4	24.0	5.3	33.3	3.0
	Poecilostomatoida spp.	1.8	0.1	0	0	4.2	0.5	0	0
	Others	5.4	1.6	3.8	1.9	6.3	1.1	0	0
Ostracoda	Myodocopa spp.	2.7	0.6	1.3	<0.1	0	0	0	0
Crustacea	Zoea larvae	4.5	0.9	3.8	1.0	1.0	<0.1	0	0
	Eggs	1.8	0.2	0	0	1.0	<0.1	0	0
	Others	0	0	12.5	1.8	3.1	0.2	0	0
Insecta	Isoptera spp.	0.9	0.8	0	0	0	0	0	0
	Acari spp.	0.9	0.2	0	0	0	0	0	0
	Ephemeroptera larvae	0	0	0	0	6.3	0.2	0	0
	Plecoptera larvae	0	0	0	0	1.0	<0.1	0	0
	Trichoptera larvae	0.9	<0.1	0	0	6.3	1.4	0	0
	Chironomidae larvae	0.9	<0.1	0	0	6.3	0.5	0	0
Appendicularia	spp.	0.9	0.1	0	0	0	0	0	0
Osteichthyes	Larvae	0.9	0.9	1.3	0.3	0	0	0	0
Others		19.8	8.1	22.5	7.6	7.3	0.3	0	0

%N percentage of each item out of the total number of prey items examined, *%F* percent frequency occurrence of each prey item in all specimens

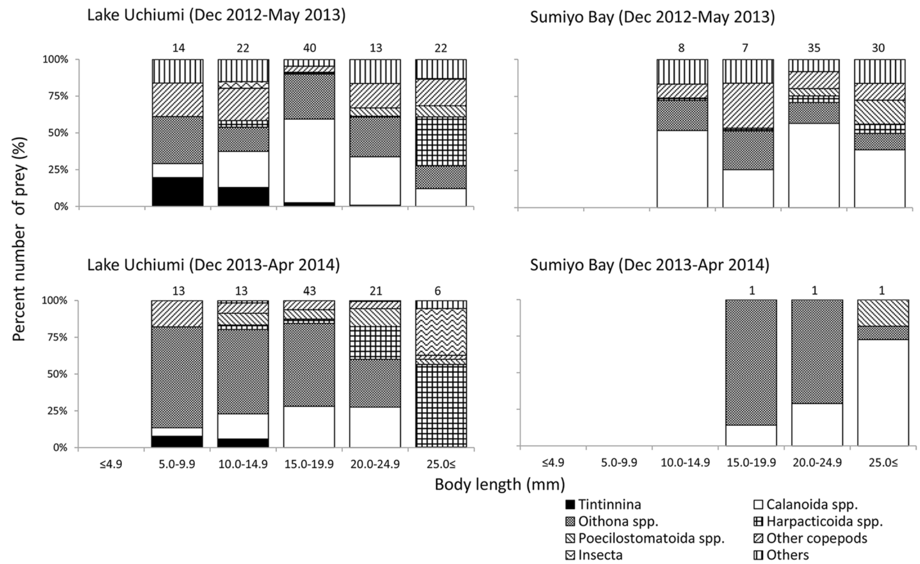

Fig. 5 Percent number (N %) of prey in the diet of each size range of larval and juvenile *Plecoglossus altivelis ryukyuensis*. Sample size is shown above each bar

Table 3 The Shannon–Wiener diversity index H' of larval and juvenile *Plecoglossus altivelis ryukyuensis*

	December 2012–May 2013				December 2013–April 2014			
Size range (BL mm)	*n*	Lake Uchiumi	*n*	Sumiyo Bay	*n*	Lake Uchiumi	*n*	Sumiyo Bay
≤4.9	0	–	0	–	0	–	0	–
5.0-9.9	14	2.2	0	–	13	1.3	0	–
10.0-14.9	22	2.7	8	1.7	13	2.0	0	–
15.0-19.9	40	1.6	7	2.1	43	1.7	1	0.6
20.0-24.9	13	2.0	35	2.3	21	2.8	1	0.9
≥25.0	22	3.5	30	3.0	6	2.8	1	1.3

n number of specimens, *BL* standard or notochord lengths

on tintinnid ciliates in Lake Uchiumi in the first year, and the proportion of the diet comprising these organisms gradually decreased with *P. altivelis ryukyuensis* growth. Copepods were abundant in all fish size ranges, and individuals ≤24.9 mm fed abundantly on calanoids and *Oithona* spp. while those ≥25.0 mm fed abundantly on harpacticoid copepods. Both terrestrial and aquatic insects were also observed in small numbers in the large-sized individuals ≥25.0 mm. In the second year, tintinnid ciliates were found in the diet of individuals ≤14.9 mm in Lake Uchiumi. Similar to the previous year, copepods were abundant in the diets of fish of all size ranges, and individuals ≤19.9 mm fed primarily on calanoids and *Oithona* spp. Harpacticoid copepods, calanoids, and *Oithona* spp. were abundant in the diet of the 20.0–24.9 mm group. Large-sized individuals (≥25.0 mm) fed exclusively on harpacticoid copepods and aquatic insects.

On the other hand, no clear ontogenetic differences were found in the diet of the individuals in Sumiyo Bay (Fig. 5). In Sumiyo Bay in the first year when a sufficient number of samples were available for analysis, calanoids and *Oithona* spp. were abundant in the diets of all size ranges. Tintinnid ciliates were not observed in small individuals in either year. The proportion of harpacticoid copepods did not increase with growth, and insects were not found in the diet of the large-sized individuals.

Except for specimens collected in Sumiyo Bay in the second year, the values of H' were over 1.00 in all size ranges in both Lake Uchiumi and Sumiyo Bay (Table 3). The values of H' increased generally with growth, although the values were low in the 15.0–19.9 and 20.0–24.9 mm groups in Lake Uchiumi in the first year and in the 15.0–19.9 mm in Lake Uchiumi in the second year (Table 3).

In the first year, prey size ranged from 0.0457 to 0.5424 mm in Lake Uchiumi and from 0.0878 to 0.8615 mm in Sumiyo Bay (Fig. 6), and in the second year, prey size ranged from 0.0410 to 0.5860 mm in Lake Uchiumi and from 0.0849 to 0.2977 mm in Sumiyo Bay (Fig. 6). There was no clear difference in prey size in Lake Uchiumi among years, and minimum and maximum prey sizes were larger in Sumiyo Bay in the first year than in Lake Uchiumi in both years. Except for the specimens in Sumiyo Bay in the second year, maximum prey size increased with growth while minimum size remained constant.

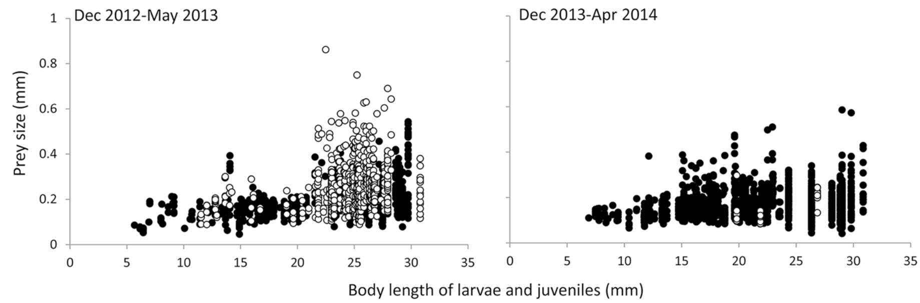

Fig. 6 Prey size against body size of larval and juvenile *Plecoglossus altivelis ryukyuensis*. *Closed* and *open circles* indicate data collected from the Lake Uchiumi and Sumiyo Bay, respectively

Discussion

Monthly occurrence and habitat shift. In the lower reaches of the Yakugachi River and western Kawauchi River, most of the larvae and juveniles of *Plecoglossus altivelis ryukyuensis* remain in the brackish waters and utilize a spatially restricted zone as their nursery area. (Kishino and Shinomiya 2005, 2006). Similarly in the present study, more larvae and juveniles, especially early developmental-staged individuals, occurred in Lake Uchiumi, which is classified as brackish, than in Sumiyo Bay in both years. It has been speculated that post-hatched larvae succeed in staying in the brackish waters by actively staying in the bottom layer during the daytime, thus decreasing the opportunity to drift offshore during ebb tides (Kishino and Shinomiya 2006).

Larval and juvenile *P. altivelis ryukyuensis* have been observed primarily during periods when water temperature is below 20°C in the lower reaches of Yakugachi and western Kawauchi Rivers (Kishino and Shinomiya 2005). *Plecoglossus altivelis altivelis* larvae have low survival rates in temperatures >20°C (Takahashi et al. 1999), and it has been suggested that high temperatures would detrimentally affect larval growth and development for *P. altivelis ryukyuensis* as well (Kishino and Shinomiya 2003). Kishino et al. (2008) compared the survival rates of *P. altivelis ryukyuensis* larvae under different temperatures and salinities and determined that survival rates were highest in temperatures of 15–18°C and salinities of 5–15. Such water temperature and salinity ranges parallel those of estuarine water during the season in which *P. altivelis ryukyuensis* larvae and juveniles occur. In addition to low water temperature, the physical condition of the estuarine area, where salinity is low, is suitable for larvae and juveniles of *P. altivelis ryukyuensis*, which have undeveloped osmoregulatory organs (Kishino et al. 2008). Compared with Sumiyo Bay, Lake Uchiumi, which is characterized by low temperatures and salinities, would be suitable for the growth and survival of larval and juvenile *P. altivelis ryukyuensis*, especially early developmental-staged individuals.

In Toyama and Tosa bays in mainland Japan, larvae of *P. altivelis altivelis* are distributed several kilometers offshore, which is influenced by river water, after their downstream migration, and they begin to migrate to the surf zones when they reach around 10 mm BL (Tago 2004; Yagi et al. 2006). In this study, no stations were fixed offshore in Sumiyo Bay, and therefore it was impossible to confirm the distribution patterns there. However, early developmental-staged larvae of ≤9.9 mm BL, including yolk-sac larvae, occurred abundantly in Lake Uchiumi, suggesting that their dispersion offshore would be limited in the study area. Their utilization pattern for the whole area of Lake Uchiumi also remains unknown, since stations were fixed only in the shallow area of the lake. Larvae and juveniles may possibly abundantly occur not only in the shallow area, but also in the deeper area of the lake.

The spawning season of *P. altivelis ryukyuensis* lasts from early November to early March, peaking from late November to early January in Yakugachi River (Kishino and Shinomiya 2004b). On December 28 of the first year, 40 specimens ≤4.9 mm, which are considered post-hatched larvae, and 103 specimens ≤ 9.9 mm were collected, comprising 90.5 % of the specimens collected in December. On the other hand, 14 specimens ≤4.9 mm and 145 specimens ≤9.9 mm were collected on January 21 of the second year, comprising 61.6 % of the specimens collected in January. *Plecoglossus altivelis ryukyuensis* take approximately 10 days to hatch after fertilization and reach 10 mm BL approximately 8 days after hatching (Kishino and Shinomiya 2003, 2006). Accordingly, it is thought that the peak of spawning occurred in mid-December of the first year and early January of the second year. No small or early developmental-staged individuals emerged after March of either year, suggesting that spawning ceased in late February or early March.

Upstream migration of *P. altivelis ryukyuensis* has been observed from late January to late May in the Yakugachi and Kawauchi Rivers with the peak being from April to May (Kishino and Shinomiya 2004a). Individuals ≥25.0 mm BL, which start to migrate upstream, were collected from January to March in the first year and in January of the second year. However, no individuals ≥25.0 mm BL occurred in either bay in April or May of either year. One potential reason for this is net avoidance, which increases with growth. Another reason could be that the peak of upstream migration may have shifted to several months earlier during the study period. In most of the stations in Lake Uchiumi and Sumiyo Bay, the water temperature was around or over 20°C in April of both years, which might be higher than that in the usual years. Moreover, in the lower reaches of the Yakugachi River, individuals gather and stay in the estuarine area just below the freshwater area for a certain amount of time to adapt to the freshwater environment before migrating upstream (Kishino and Shinomiya 2005). In the study area, many individuals may have moved upstream and stayed in the upper estuarine area beyond Lake Uchiumi to prepare for upstream migration.

Feeding habits. There has been only one study on feeding habits of this subspecies (Oka et al. 1996). In this study, the feeding habits of specimens ≥13.2 mm BL (mean value = 26.2 mm BL) collected in the surf zone of Sumiyo Bay (located adjacent to the present study stations) were investigated. In the present study, we elucidated the feeding habits for larvae and juveniles of all development stages of the subspecies from yolk-sac larvae to juveniles that were about to begin upstream migration.

The feeding intensity for yolk-sac larvae has been shown to be 23.0 % for land-locked *P. altivelis ryukyuensis* in the Fukuji Reservoir, Okinawa, and 40.8 %–47.9 % for *P. altivelis altivelis* in Tosa Bay (Kawakami and Tachihara 2005; Yagi et al. 2006). In contrast, yolk-sac larvae did not feed on prey in the present study. Rearing experiments have shown that larvae of *P. altivelis ryukyuensis* take five days to completely consume their yolk (Tachihara and Kawaguchi 2003), suggesting that in the present study area, they depend solely on their yolk as a source of nutrition for at least several days after hatching and therefore their growth rate might be lower.

In both years, both feeding intensity and feeding ratio increased with growth, indicating that foraging ability improves ontogenetically. Prey availability may have been more favorable in Lake Uchiumi in the second year than in Lake Uchiumi and Sumiyo Bay in the first year as indicated by both indices being higher in all size ranges in Lake Uchiumi in the second year.

Larvae and juveniles of species of *Plecoglossus* mainly feed on copepods (Hamada and Kinoshita 1988; Oka et al. 1996; Takahashi et al. 1999; Yagi et al. 2006). Oka et al. (1996) showed that relatively large larvae and juveniles (mean 26.2 mm BL) fed more selectively on calanoids than on *Oithona* spp. or nauplii, which were abundant in Sumiyo Bay. In our study, larvae and juveniles fed abundantly on *Oithona* spp. as well as calanoids, and the importance of copepod nauplii as prey was low.

The diet composition differed between Lake Uchiumi and Sumiyo Bay and clearer ontogenetic variation in the composition of the diet was observed in Lake Uchiumi. For example, insects were found in the diet of large-sized individuals only in Lake Uchiumi in both years, and they were more abundant in the second year than the first year. Although the composition of prey was not sampled directly from the water, it is speculated that the difference in the diet composition may reflect differences of prey availability in the ambient waters to some extent judging by the generalist habit of *P. altivelis ryukyuensis*. The absence of insects from large-sized individuals collected in Sumiyo Bay might be due to their absence in the coastal area.

Plecoglossus altivelis altivelis starts to adapt to benthic life before starting to migrate upstream (Hamada and Kinoshita 1988) at approximately 60–80 mm standard length. *Plecoglossus altivelis altivelis* started to feed on benthic prey such as Corophiidae and larval Chironomidae at 53.0 mm total length in the surf zone of Tosa Bay (Hamada and Kinoshita 1988). On the other hand, *P. altivelis ryukyuensis* starts to migrate upstream when it is much smaller at approximately 25 mm standard length (Kishino and Shinomiya 2005). In the present study, a substantial amount of benthic prey, such as benthic harpacticoids and larval insects, were found in the guts of individuals ≥25.0 mm BL in the first year and ≥20.0 mm BL in the second year, which were much smaller than *P. altivelis altivelis.* The data show that *P. altivelis ryukyuensis* also started to adapt to benthic life before starting to migrate upstream.

In larval and juvenile fish, the maximum prey size usually increases with body size, while the minimum size remains constant or increases slightly due to the increase in the capability to consume a wider range of prey sizes (Peters 1986; González-Quirós and Anadón 2001; Sassa and Kawaguchi 2005; Kume et al. 2015b). A similar trend was observed in our study. Interestingly, large individuals preyed on larger prey in Sumiyo Bay than in Lake Uchiumi, implying that large prey, which are preferred by large *P. altivelis ryukyuensis*, may have been more abundantly distributed in Sumiyo Bay than in Lake Uchiumi in the first year. Judging by the physical condition of the bay, Lake Uchiumi would appear to be more favorable to *P. altivelis ryukyuensis* than Sumiyo Bay. However, some large individuals, which had grown and developed in Lake Uchiumi and had adapted to higher temperatures and salinities, might have actively utilized the surf zone of Sumiyo Bay where prey availability for large individuals may have been better. In the second year, larvae and juveniles were rarely found in Sumiyo Bay. FI and FR were higher in Lake Uchiumi in the second year than in the first year, suggesting that prey availability may have been higher in the second year than in the first, and this higher prey availability in Lake Uchiumi could have impeded active feeding migration to Sumiyo Bay by larval and juvenile *P. altivelis ryukyuensis* in the second year. Further studies will be needed to understand the ways that larval and juvenile *P. altivelis ryukyuensis* utilize estuarine and coastal areas, namely Lake Uchiumi and Sumiyo Bay, in detail.

Conservation of *Plecoglossus altivelis ryukyuensis*. Our study showed that larvae and juveniles of *P. altivelis ryukyuensis* exclusively utilized estuarine lake and coastal areas in the lower reach of the Kawauchi River as their important nursery ground, feeding mainly on copepods such as calanoids and *Oithona* spp. It is essential to adequately manage the environmental conditions in the lower reach of the Kawauchi River, especially Lake Uchiumi, to conserve *P. altivelis ryukyuensis* population in the future.

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