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FULL PAPER

Vertical migration in streams: seasonal use of the hyporheic zone by the spinous loach *Cobitis shikokuensis*

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Abstract Vertical hydrological connectivity between the surface stream and benthic and hyporheic zones plays a key ecological role in the biodiversity of lotic ecosystems because it allows surface and benthic organisms to use the hyporheic zone as a seasonal habitat and refuge. Use of the hyporheic zone by surface/benthic organisms has been well studied in invertebrates, but little is known about the importance of this connectivity for fishes. We investigated streambed surface and hyporheic densities (5–10, 15–20 and 20–25 cm below the streambed surface) of a stream fish, *Cobitis shikokuensis*, over a 20-month period in the Shigenobu River, southwestern Japan, to test the hypothesis that it uses the hyporheic zone for spawning and overwintering. In total, 1,804 individuals (13–58 mm total length) were captured from 33 streambed surface samplings and 102 individuals (10–46 mm total length) were present in 1,147 samples of 57 hyporheic samplings. Population densities in both zones peaked in late summer–early autumn due to the recruitment of age 0+ fish and a female with eggs was found in the hyporheic zone during the reproductive season. Both 0+ and older fish were absent from the streambed surface during winter, and fish densities were also lower in the hyporheic zone at this time. However, the vertical distribution of the fish tended to be skewed towards the deeper hyporheic layers from autumn to spring. These findings indicate that *C. shikokuensis* vertically migrates between the streambed surface and the hyporheic zone for spawning, rearing and overwintering, suggesting that the integrity of vertical hydrological connectivity in lotic systems is crucial for certain fish species.

Keywords Habitat use · Habitat connectivity · Migration · Interstices · Burrowing

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Introduction

Many fishes migrate across different habitats to complete their life history. For example, diadromous fishes such as salmon and eel migrate tens to hundreds of kilometres between rivers and seas for their reproduction (Tsukamoto et al. 2002; Eliason et al. 2011). Some non-diadromous species also migrate within freshwater bodies. In river–floodplain systems, various stream fishes move into inundated floodplains and oxbows from the main channels for spawning, rearing and/or feeding (Poizat and Corivelli 1997; Molls 1999; Saint-Paul et al. 2000). Although migration distances are diverse among species, their directions can be broadly categorised into two groups, namely, longitudinal (i.e., along the river channel) and lateral (i.e., across the floodplain). The common occurrence of migration among stream fishes emphasises the importance of hydrological connectivity among different habitats in maintaining fish populations and communities (Fullerton et al. 2010).

Vertical hydrological connectivity between surface and ground waters is well recognised as an important third dimension in lotic ecosystems because it plays a key role in biogeochemical processes in these ecosystems and in structuring aquatic communities (Ward 1989; Boulton 2007; Boulton et al. 2010). Previous studies have shown that the hyporheic zone, the saturated interstitial spaces below the streambed (Orghidan 1959, 2010; Boulton et al. 1998), provides habitats not only for the diverse invertebrates that complete their life cycles there (Malard et al. 2003; Datry et al. 2007), but also for benthic invertebrates, some of which migrate into this more stable part of the stream for rearing and/or feeding (Malard et al. 2003; Navel et al. 2010), as well as for seeking refuge from adverse conditions in the surface stream (the 'hyporheic refuge hypothesis'; Palmer et al. 1992; Dole-Olivier 2011). These findings suggest the importance of vertical hydrological connectivity for surface and benthic organisms. However, few empirical studies have investigated its importance in terms of fish migration.

Several studies have sporadically reported the occurrence of stream fishes in the hyporheic zone. For example, Stegman and Minckley (1959) found three fish species—slender madtom *Noturus exilis*, fantail darter *Etheostoma flabellare lineolatum*, and banded sculpin *Cottus carolinae*—lying in the interstices of the streambed in an Illinois creek, approximately 5–8 cm below the streambed surface. In addition, Jurajda and Rulík (2001) detected adult stone loach *Barbatula barbatula* in sediment traps placed at a depth of 15 cm in the hyporheic zone of the River Morava, Czech Republic. Furthermore, we recently found adult and juvenile spinous loach *Cobitis shikokuensis* using the hyporheic zone as a refuge from channel drying in an intermittent river in Japan (Kawanishi et al. 2013). These findings suggest that certain fish species use the hyporheic zone more frequently than has generally been recognised. However, fish surveys in the hyporheic zone are rare and span a short time period (a few days to several months). Because the hyporheic zone typically has more stable water temperature and lower flow velocity than the surface stream (Boulton et al. 1998; Poole and Berman 2001; Malard et al. 2003), stream fishes might use the hyporheic zone seasonally as an overwintering habitat and/or as a nursery for juveniles.

The main difficulty in investigating the use of the hyporheic zone by stream fishes is that the small interstices and compacted sediments of this zone render it difficult to use conventional sampling methods for stream fishes, such as snorkelling and electrofishing. Therefore, some studies have used artificial hyporheic sediments consisting of an experimental container or channel filled with substrates under semi-field or laboratory conditions (Phillips and Claire 1966; Davey et al. 2006; Heggenes et al. 2013). Heggenes et al. (2013) reported the use of interstitial spaces by young Atlantic salmon *Salmo salar* by using translucent polyvinyl chloride tubes filled with substrates (particle size, 16–60 mm) and indicated that this species easily moved into and through the interstitial spaces of the artificial hyporheic sediments. Such experimental studies have contributed to our knowledge of the potential role of the hyporheic zone for stream fishes. However, it should be noted that interstices in experimental settings may not represent natural hyporheic conditions (e.g., water quality and temperature, hydrological exchange and interstitial pore size), and habitat use by stream fishes in the 'real' hyporheic zone is still largely unknown.

Hyporheic invertebrate assemblages have often been investigated using pump sampling methods (Clinton et al. 1996; Stubbington et al. 2011; Datry 2012). In these methods, hyporheic water containing organisms is pumped from a sampling well that is installed in the streambed. Recently, we successfully applied a similar pump sampling method to sample a small stream fish *Cobitis shikokuensis* surviving in the hyporheic zone during streambed drying (Kawanishi et al. 2013). In this study, we further investigated the seasonal use of the hyporheic zone by *C. shikokuensis*. This species (formerly known as *Cobitis takatsuensis*; Suzawa 2006) has long been believed to burrow into the hyporheic zone for spawning and overwintering (Shimizu 2002). In an investigation of the densities of *C. shikokuensis* on the streambed surface over a 1-year period, Shimizu (2002) observed many mature females on the streambed from May to July (late spring to early summer in southwestern Japan), but never found their eggs on the streambed surface, suggesting that this species spawns in the hyporheic zone. Furthermore, *C. shikokuensis* was absent from the streambed surface during winter when the water temperature fell below 15 °C, suggesting that it burrows into the hyporheic zone for overwintering (Shimizu 2002). However, to date, there has been little direct evidence of these seasonal uses of the hyporheic zone. In this study, we thus examined the densities of *C. shikokuensis* on the streambed surface and in the hyporheic zone of the Shigenobu River, southwestern Japan, over a 20-month period to determine the seasonal use of the hyporheic zone. We hypothesised that (1) the hyporheic density increases during summer in association with reproduction and thus summer hyporheic populations would include a number of 0+ fish and (2) the hyporheic density also increases as the streambed surface density decreases in autumn–winter as a result of overwintering.

Materials and methods

Study area. The study was conducted in the Shigenobu River (watershed area, 445 km^2; main stem length, 36 km; 33°48′N, 132°42′E) on Shikoku Island, southwestern Japan. In this region, annual precipitation is approximately 1,300 mm, with the rainy season occurring in early summer (late June–July; Fig. 1a), and the average monthly air temperature ranges from 6 °C in January to 28 °C in

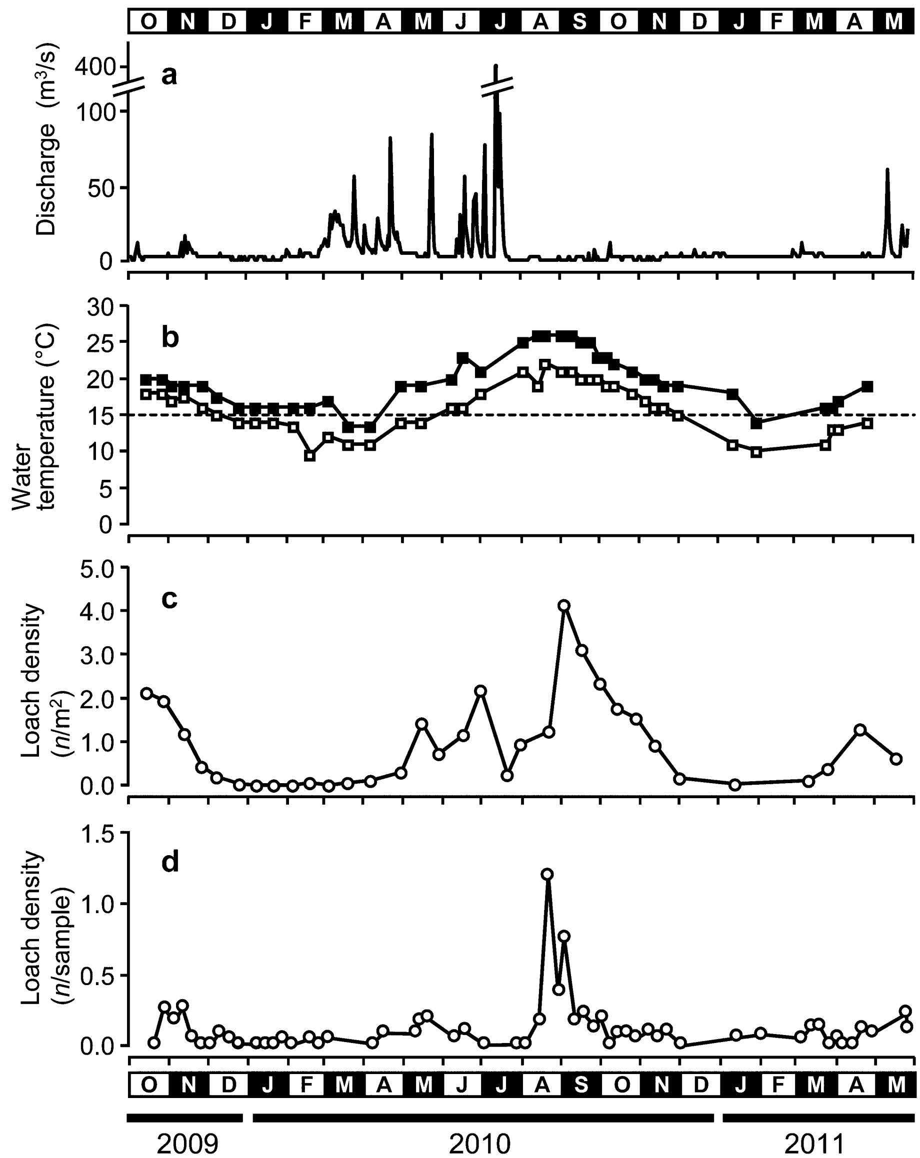


Fig. 1 River discharge (**a**), maximum (*solid square*) and minimum (*open square*) surface water temperatures (**b**), and densities of *Cobitis shikokuensis* on the streambed surface (**c**) and in the hyporheic zone (**d**) in the Shigenobu River, southwestern Japan, from October 2009 to May 2011. Discharge data were supplied by Deai gauging station located in the lower river, ca. 6 km downstream of the study reach. The *dashed line* in the figure on water temperature was drawn for reference

August. The Shigenobu River is perennial in its upper and lower reaches, but includes intermittent or ephemeral braided channels in its middle reaches ('mid-reach drying' type; Lake 2003) owing to thick alluvial deposits from the mountainous catchments with high sediment yields.

A study reach that included a pool-riffle-glide sequence (approximately 200 m in length, 3–10 m wetted width) was established in a braided channel (approximately 230 m bankfull width) dominated by pebbles and cobbles (dominant particle size 17–256 mm). The study reach was located near the downstream end of an intermittent reach (30 m elevation), where the flow permanence is restored by upwelling ground water. In the study reach, *Cobitis shikokuensis* was sampled from the streambed surface and the hyporheic zone during a 20-month period from October 2009 to May 2011: autumn (October–November) 2009, winter (December–February) 2009/2010, spring (March–May) 2010, summer (June–August) 2010, autumn (September–November) 2010, winter (December–February) 2010/2011 and spring (March–May) 2011.

Streambed sampling. To determine seasonal changes in the density of *C. shikokuensis* on the streambed surface, a quadrat (2 m × 20–40 m) was established in the study reach on each sampling occasion (Table 1). *C. shikokuensis* inside this quadrat was sampled using an electrofishing unit (Model LR24 Backpack Electrofisher; Smith-Root Inc.), and the number of individuals captured was counted and their total length measured, after which they were released alive. The density was expressed as the number of captured individuals per unit area (n/m^2). During the study period, this sampling was conducted 1–3 times a month (Table 1), with the exception of February 2011 when

Table 1 Summary of the streambed surface and hyporheic zone surveys for *Cobitis shikokuensis*. The total number of sampling occasions and hyporheic samples and the range of quadrat sizes are shown for each season of each year

Year	Season (months)	Streambed surface		Hyporheic zone				
		No. of occasions	Quadrat size (m^2)	No. of occasions	No. of samples			
					Total	Sediment depth		
						5–10 cm	15–20 cm	20–25 cm
2009	Autumn (Oct.–Nov.)	4	40–60	6	139	44	48	47
2009/10	Winter (Dec.–Feb.)	6	40	11	263	88	87	88
2010	Spring (Mar.–May)	6	40–80	6	135	41	47	47
	Summer (Jun.–Aug.)	5	60–70	8	127	36	44	47
	Autumn (Sep.–Nov.)	6	60–70	12	215	62	90	63
2010/11	Winter (Dec.–Feb.)	2	60	3	55	16	21	18
2011	Spring (Mar.–May)	4	60–70	11	213	59	78	76
Total		33		57	1,147	346	415	386

sampling could not be conducted. Streambed-surface sampling was typically conducted more than 1 day after hyporheic sampling to avoid potential sampling disturbance on each other. The maximum and minimum water temperatures of the surface water were recorded 40 times during the study period at 6–53-day intervals using a mercury thermometer placed in the study reach (Fig. 1b).

Hyporheic sampling. The seasonal use of the hyporheic zone by *C. shikokuensis* was investigated by directly capturing fish from this zone using a pump sampling method with a sampling well. The well consisted of a polyvinyl chloride pipe (length, 100 cm; inner diameter, 4.4 cm) that was plugged at one end with a cone-shaped tip made of reinforced plastic. The bottom 5–10 cm of this pipe was perforated with 24 evenly spaced holes of 1.5-cm diameter. The wells were installed at three hyporheic depths (intake zones at 5–10, 15–20 and 20–25 cm below the streambed surface) using a sledgehammer and they were left *in situ*. Several sets of the three wells were arranged <1 m from the edge of the water along the river channel (eight sets in October 2009–August 2010 and 10 sets in September 2010–May 2011), with a distance of 50–70 cm between wells within a set and >2 m between sets. The wells were sampled 1–5 times a month (Table 1).

On each sampling occasion, hyporheic water (15 L per sample) was extracted from the wells of each set in a random sequence by inserting a short length of plastic tube and applying a vacuum to this tube using a hand bilge pump (NRS Kayak Bilge Pump; NRS, Moscow, ID, USA). The extracted water was sieved through a 2-mm mesh net to retain the fish, and all captured individuals were then counted and measured (total length). Fish density was expressed as the number of individuals per sample. Wells were sometimes lost during the study period (e.g., by spates), in which case a new well was installed at the same location.

Statistical analyses. To test whether the densities of fish on the streambed surface and in the hyporheic zone changed seasonally, the abundance data were analysed using generalised linear mixed models (GLMMs) with a Poisson error structure and a log link function. The response variable was the number of individuals captured on each sampling occasion, offset by the sampling effort (i.e., the quadrat area for streambed surface density and the number of hyporheic samples for hyporheic density); the explanatory variables were the month in which each sampling occurred, which was treated as a categorical fixed factor, and the year (first or second year) as a categorical random factor. The significance of the models was tested using likelihood ratio tests against a null model (i.e., the intercept-only model). Post hoc comparisons among months were conducted with Tukey contrasts (Hothorn et al. 2008). For months when more than five individuals had been captured from both the streambed surface and the hyporheic zone, total length of the fish was compared between the streambed surface and the hyporheic zone using the Wilcoxon rank-sum test. Seasonal trends in the vertical distribution of *C. shikokuensis* in the hyporheic zone were analysed for each season of each year, with the exception of winter 2010/11, which was excluded due to the small number of individuals collected ($n = 2$). Because the numbers of hyporheic samples (i.e., sampling effort) among the three depths were unequal in each season owing to the loss of wells (Table 1), we tested whether the ratio of the number of fish captured among the three depths differed from that of the number of hyporheic samples using Fisher's exact test. All analyses were performed in R version 3.1.2 (R Core Team 2014).

Results

During the study period, we sampled the streambed surface and hyporheic zone 33 and 57 times, respectively (Table 1). Although the study reach did not dry up completely during the period, the water depth in some of the sampling wells fell to almost zero in summer due to the reduced discharge decreasing the size of the wetted channel. Therefore, the data for wells that were exposed on the bank and >50 cm from the retreating water's edge were removed from the following results to separate seasonal use of the hyporheic zone from the use of temporal refuges from streambed drying. In total, 1,804 *Cobitis shikokuensis* (13–58 mm total length) were captured from the streambed surface, while 102 individuals (10–46 mm total length) were present in 1,147 hyporheic samples (15, 47 and 40 individuals from the 5–10, 15–20 and 20–25 cm samples, respectively). In most hyporheic samples, the number of fish contained was less than four individuals (up to seven individuals).

In both 2009 and 2010, the density of *C. shikokuensis* on the streambed surface started to decrease at the onset of autumn (September–October) and dropped to almost zero when the minimum water temperature fell below approximately 15 °C (late December; Fig. 1b, c). However, there was no obvious concurrent increase in hyporheic density at this time, although fish were present in hyporheic samples during almost every winter month (Fig. 1d). When the maximum water temperature reached approximately 17 °C in spring (March–early April) 2010 and 2011, *C. shikokuensis* individuals were once again found on the streambed. The density of fish peaked in late August–early September both on the streambed surface and in the hyporheic zone (4.14 individuals/m^2 and 1.19 individuals/sample, respectively; Fig. 1c, d).

GLMMs indicated that temporal variations in the densities of fish both on the streambed surface and in the hyporheic zone were significantly explained by month (likelihood ratio tests against a null model, both $P < 0.001$). The multiple comparison of the model for the streambed surface indicated that the fish density was the lowest in early winter–early spring (December–March) and highest in September (Table 2). The multiple comparison in the model for the hyporheic zone showed that the fish density in August was significantly higher than that in months from late autumn to late spring (October–May) and that the fish density in September was also significantly higher than that in winter (December–January; Table 2).

In August, when the hyporheic density of *C. shikokuensis* peaked, small juveniles (≤22 mm total length) were caught in the hyporheic zone, accounting for over half of the individuals captured (Fig. 2). Similarly, >100 small juveniles (≤22 mm total length) were captured from the streambed surface in August and September (Fig. 2). In August, a large mature female with eggs (46 mm total length) was caught from a 20–25-cm hyporheic sample. The total length of the fish captured from the hyporheic zone was smaller than that of those captured from the streambed surface in each month (November 2009, May, August, September and October 2010 and March 2011; Wilcoxon rank-sum test, $P < 0.05$).

The ratio of the number of individuals across the three depths differed significantly from that of the number of hyporheic samples in autumn 2009 and spring 2011 (Fig. 3), when >90% of *C. shikokuensis* individuals were found in the 15–20- and 20–25-cm samples (Fig. 3). The ratios in winter 2009/10 and spring 2010 (as well as in winter 2010/11) also tended to be skewed towards the deep layers and no individuals were found from the 5–10-cm samples of winter, although these differences were not significant, probably due to the small total number of individuals captured in each season (Fig. 3).

Discussion

Our results suggest that, over the course of its life history, *Cobitis shikokuensis* migrates between the streambed surface and the hyporheic zone. The significant increase in the hyporheic density and the presence of a large mature female with eggs in the hyporheic zone in August strongly support our hypothesis that *C. shikokuensis* spawns in this zone. The small-sized cohort that was found in the hyporheic zone in August clearly consisted of age 0+ fish. An artificial breeding study on *C. shikokuensis* has reported that the fertilised eggs hatch after approximately 4 days at 20 °C and that the larvae grow into juveniles (approximately 17 mm total length) at 35 days and have a total length of approximately 25 mm at 56 days after hatching (Ehime Prefectural Chuyo Fisheries Experimental Station 1995). Therefore, based on the size of the age 0+ fish caught in our hyporheic sampling, it seems that these fish spend the first 2 months of their life in the interstices of the benthic and hyporheic zones, although fish development and growth rates are dependent on water temperature. Our results suggest that the interstices of the hyporheic zone act as a spawning and rearing habitat for *C. shikokuensis*.

Our findings did not support our second hypothesis that *C. shikokuensis* overwinters in the hyporheic zone. When the water temperature decreased in autumn (September–November), the density of *C. shikokuensis* on the streambed surface also decreased, supporting the field observations made by Shimizu (2002). However, there were no pronounced concurrent increases in the hyporheic density

Table 2 Post hoc multiple comparisons of generalised linear mixed models with a Poisson error distribution that were used to examine seasonal changes in the density of *Cobitis shikokuensis*

Between-month comparisons		Streambed surface				Hyporheic zone			
		Estimated coefficient	SE	*Z*	*P*	Estimated coefficient	SE	*Z*	*P*
Feb.	vs. Jan.	1.30	1.23	1.06	0.994	0.27	1.00	0.27	1.000
Mar.		3.09	1.02	3.04	0.069	1.37	0.79	1.73	0.811
Apr.		4.42	1.00	4.40	**<0.010**	1.01	0.80	1.26	0.976
May		4.85	1.00	4.84	**<0.010**	2.14	0.76	2.82	0.135
Jun.		5.52	1.00	5.51	**<0.010**	1.34	1.00	1.34	0.963
Jul.		4.50	1.01	4.47	**<0.010**	−21.58	512.00	−0.04	1.000
Aug.		5.21	1.01	5.17	**<0.010**	3.25	0.74	4.42	**<0.010**
Sep.		6.27	1.00	6.26	**<0.010**	2.50	0.78	3.20	**0.046**
Oct.		5.57	1.00	5.57	**<0.010**	1.69	0.75	2.24	0.449
Nov.		4.78	1.00	4.76	**<0.010**	1.78	0.75	2.37	0.361
Dec.		2.89	1.03	2.81	0.132	0.38	0.91	0.42	1.000
Mar.	vs. Feb.	1.79	0.73	2.45	0.301	1.10	0.79	1.39	0.951
Apr.		3.12	0.71	4.38	**<0.010**	0.74	0.80	0.93	0.998
May		3.55	0.71	4.99	**<0.010**	1.87	0.76	2.46	0.303
Jun.		4.22	0.71	5.95	**<0.010**	1.06	1.00	1.07	0.994
Jul.		3.20	0.72	4.48	**<0.010**	−21.85	512.00	−0.04	1.000
Aug.		3.91	0.72	5.46	**<0.010**	2.98	0.74	4.05	**<0.010**
Sep.		4.97	0.71	7.02	**<0.010**	2.23	0.78	2.86	0.123
Oct.		4.27	0.71	6.01	**<0.010**	1.42	0.75	1.88	0.714
Nov.		3.48	0.71	4.87	**<0.010**	1.51	0.75	2.01	0.622
Dec.		1.59	0.75	2.13	0.523	0.11	0.91	0.12	1.000
Apr.	vs. Mar.	1.33	0.20	6.65	**<0.010**	−0.36	0.52	−0.69	1.000
May		1.76	0.19	9.10	**<0.010**	0.77	0.45	1.72	0.817
Jun.		2.43	0.20	12.42	**<0.010**	−0.03	0.79	−0.04	1.000
Jul.		1.41	0.22	6.56	**<0.010**	−22.95	512.00	−0.05	1.000
Aug.		2.12	0.21	9.93	**<0.010**	1.88	0.41	4.61	**<0.010**
Sep.		3.18	0.19	16.74	**<0.010**	1.13	0.49	2.33	0.387
Oct.		2.48	0.18	13.65	**<0.010**	0.32	0.44	0.73	1.000
Nov.		1.69	0.20	8.46	**<0.010**	0.41	0.43	0.95	0.998
Dec.		−0.20	0.29	−0.68	1.000	−0.99	0.68	−1.46	0.931
May	vs. Apr.	0.43	0.12	3.61	**0.011**	1.13	0.47	2.41	0.335
Jun.		1.10	0.12	9.33	**<0.010**	0.32	0.80	0.40	1.000
Jul.		0.08	0.15	0.54	1.000	−22.59	512.00	−0.04	1.000
Aug.		0.78	0.14	5.42	**<0.010**	2.24	0.43	5.21	**<0.010**
Sep.		1.85	0.11	17.10	**<0.010**	1.49	0.50	2.96	0.093
Oct.		1.15	0.10	11.02	**<0.010**	0.68	0.46	1.48	0.926
Nov.		0.36	0.13	2.74	0.157	0.77	0.45	1.69	0.833
Dec.		−1.53	0.25	−6.05	**<0.010**	−0.63	0.69	−0.92	0.998
Jun.	vs. May	0.67	0.10	6.49	**<0.010**	−0.81	0.76	−1.06	0.994
Jul.		−0.35	0.14	−2.54	0.254	−23.72	512.00	−0.05	1.000
Aug.		0.36	0.13	2.67	0.187	1.11	0.34	3.22	**0.043**
Sep.		1.42	0.09	15.39	**<0.010**	0.36	0.43	0.83	0.999
Oct.		0.72	0.09	7.98	**<0.010**	−0.45	0.38	−1.20	0.984
Nov.		−0.07	0.12	−0.60	1.000	−0.36	0.37	−0.97	0.997
Dec.		−1.96	0.25	−7.92	**<0.010**	−1.76	0.64	−2.75	0.159
Jul.	vs. Jun.	−1.02	0.13	−7.83	**<0.010**	−22.92	512.00	−0.05	1.000

Table 2 continued

Between-month comparisons		Streambed surface				Hyporheic zone			
		Estimated coefficient	SE	*Z*	*P*	Estimated coefficient	SE	*Z*	*P*
Aug.		−0.32	0.13	−2.51	0.266	1.92	0.74	2.60	0.227
Sep.		0.75	0.08	9.10	**<0.010**	1.17	0.78	1.49	0.921
Oct.		0.05	0.10	0.50	1.000	0.35	0.75	0.47	1.000
Nov.		−0.74	0.12	−6.23	**<0.010**	0.44	0.75	0.59	1.000
Dec.		−2.63	0.25	−10.62	**<0.010**	−0.96	0.91	−1.05	0.995
Aug.	vs. Jul.	0.70	0.15	4.54	**<0.010**	24.83	512.00	0.05	1.000
Sep.		1.77	0.12	14.56	**<0.010**	24.08	512.00	0.05	1.000
Oct.		1.07	0.13	8.11	**<0.010**	23.27	512.00	0.05	1.000
Nov.		0.28	0.15	1.85	0.730	23.36	512.00	0.05	1.000
Dec.		−1.61	0.26	−6.12	**<0.010**	21.96	512.00	0.04	1.000
Sep.	vs. Aug.	1.07	0.12	9.10	**<0.010**	−0.75	0.39	−1.91	0.692
Oct.		0.37	0.13	2.86	0.117	−1.56	0.33	−4.75	**<0.010**
Nov.		−0.43	0.15	−2.93	0.098	−1.47	0.32	−4.56	**<0.010**
Dec.		−2.32	0.26	−8.85	**<0.010**	−2.87	0.61	−4.69	**<0.010**
Oct.	vs. Sep.	−0.70	0.08	−8.27	**<0.010**	−0.81	0.42	−1.93	0.681
Nov.		−1.49	0.11	−13.59	**<0.010**	−0.72	0.42	−1.74	0.808
Dec.		−3.38	0.24	−13.90	**<0.010**	−2.12	0.67	−3.19	**0.049**
Nov.	vs. Oct.	−0.79	0.10	−7.73	**<0.010**	0.09	0.36	0.25	1.000
Dec.		−2.68	0.24	−11.17	**<0.010**	−1.31	0.63	−2.07	0.579
Dec.	vs. Nov.	−1.89	0.25	−7.47	**<0.010**	−1.40	0.63	−2.22	0.464

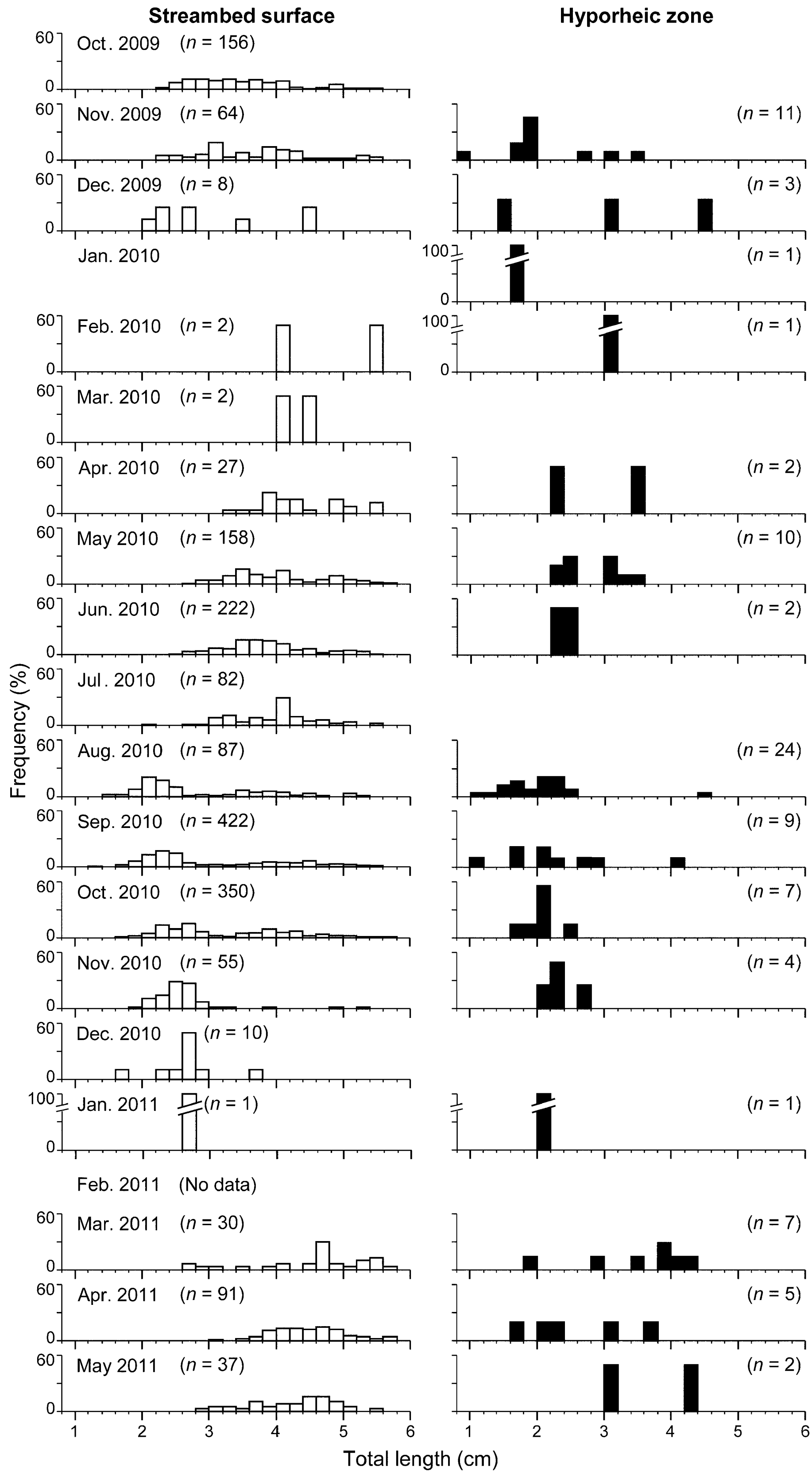
Significant *P* values (<0.05) are shown in *bold*

SE standard error

of *C. shikokuensis* (Fig. 1). It is possible that *C. shikokuensis* emigrated out of the study reach to seek overwintering habitats. However, the disappearance of this species from the streambed surface in autumn is well recognised (Shimizu 2002), and its absence during winter is also common across various habitats in the study river (R. Kawanishi, unpublished data; M. Inoue, personal observation). Therefore, both longitudinal and lateral migrations for overwintering are unlikely. Instead, we suggest that the majority of *C. shikokuensis* burrow into the hyporheic zone during winter to depths greater than those investigated in this study. Two lines of evidence support this idea. First, the vertical distributions of individuals were significantly skewed towards the deeper hyporheic zone both immediately before and after the overwintering season (autumn 2009 and spring 2011; Fig. 3); second, no individuals were present in the 5–10-cm hyporheic samples during winter, whereas some individuals were captured from depths greater than 15 cm (Fig. 3). *Cobitis shikokuensis* may burrow into the hyporheic zone in response to a decline of surface water temperature relative to subsurface temperature. The temperature of water in spring-fed ponds around the study area suggests that groundwater temperature in the aquifer is 18 °C–20 °C, which is seasonally stable (Fujiwara et al. 2014). The actual subsurface water temperatures (15–60 cm below the streambed surface) that we recorded near the study reach were approximately 13 °C–17 °C during winter (December 2011–February 2012), which was up to 5 °C warmer than the surface water (R. Dohi and R. Kawanishi, unpublished data). The period when the streambed-surface density of *C. shikokuensis* gradually decreased (November–December; surface water temperature was 15 °C–20 °C) coincided with the season when surface water temperature became lower than that in the hyporheic zone.

This study demonstrated that the pump sampling method with wells is useful for long-term surveys of the use of the hyporheic zone by fish as well as invertebrates. Unlike other sampling methods, such as freeze-coring (Stocker and Williams 1972) and the use of colonisation chambers (Fraser and Williams 1997), this simple method causes less disturbance to the hyporheic zone and requires less processing time. However, there are some technical challenges that will need to be addressed in future studies. First, it is difficult to estimate the absolute density of organisms in the hyporheic zone using this method, although the relative abundance can be compared between samples (Dole-Olivier et al. 2014). In this study, we collected a larger sample

Fig. 2 Monthly changes in the frequency distribution of the total length of *Cobitis shikokuensis* captured from the streambed surface and the hyporheic zone in the Shigenobu River, southwestern Japan. Some of the fish captured from the hyporheic zone (one or two individuals for some months, but six for October 2009) were not included due to data on their length not being available. Note that sampling methods and effort differed between the streambed surface and the hyporheic zone (see "Materials and methods")



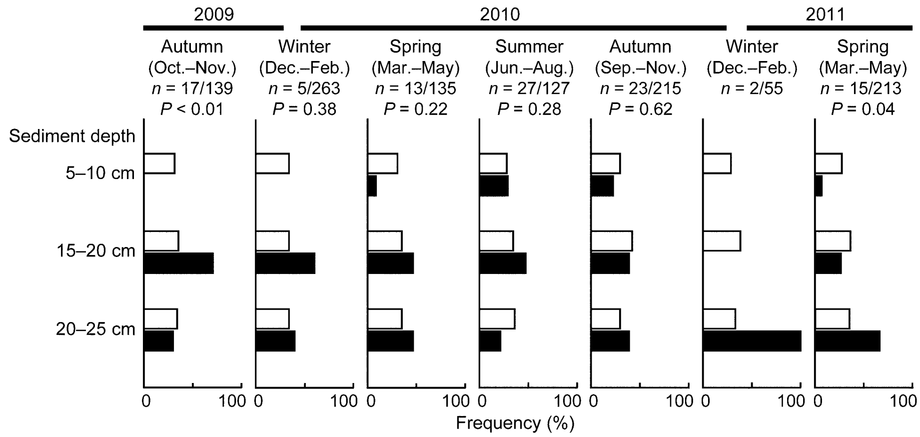


Fig. 3 Seasonal changes in the vertical distribution of *Cobitis shikokuensis* in the Shigenobu River, southwestern Japan. *Open* and *solid bars* indicate the percentages of hyporheic samples taken and *C. shikokuensis* individuals captured at each depth, respectively. The significance of the difference between these ratios was measured using Fisher's exact tests and is shown below the numbers of *C. shikokuensis* individuals/hyporheic samples for each season; the sample size of fish was too small to analyse in winter 2010/2011

volume (15 L) than in most invertebrate studies (4–6 L; Clinton et al. 1996; Stubbington et al. 2015) because there was no prior information on fish abundance and sampling efficiency in the hyporheic zone. However, most hyporheic samples had a low number of fish individuals (typically less than four). This result might suggest that fish far away from the intake hole of the wells are not captured, likely due to the filtering effect of the interstices (Fraser and Williams 1997). Therefore, an optimal sample volume that reflects the actual density of fish should be examined in future studies (e.g., Boulton et al. 2003). The second limitation is associated with sampling depth. Because the sampling well used in this study had a wider bore than that used for sampling invertebrates, it was difficult to drive the well into the hyporheic zone to a depth of >30 cm. However, this problem could be resolved by replacing the polyvinyl chloride material of the well with iron or stainless steel. In a separate trial, we successfully used an iron well with a cone-shaped stainless steel tip to reach a depth of approximately 60 cm (R. Kawanishi et al., unpublished data).

Compared with the distances of longitudinal and lateral migrations by stream fishes (approximately 10^2–10^5 m), vertical migrations are likely to be very short (probably less than a few metres). However, despite the 'short trip', the hyporheic zone would provide various benefits for fishes, including a stable water temperature, weak flow and low predation risk (Boulton et al. 1998; Poole and Berman 2001; Malard et al. 2003), although there may also be some disadvantages (e.g., lower availability of oxygen; Malcolm et al. 2004). Thus, vertical migration appears to be an adaptive strategy for certain fish species. Migration into the hyporheic zone requires a morphology that is well adapted to interstitial environments, such as a small, elongate and/or flexible body. These traits are exhibited by stream fish species belonging to various families, including Anguillidae, Cobitidae, Gobiidae and Galaxiidae, some of which have previously been found to burrow into substrate interstices (Jurajda and Rulík 2001; Davey et al. 2006; Dunn and O'Brien 2006). The use of the hyporheic zone may be more common in small fish including the larvae and juveniles of large species. For example, even juvenile Atlantic salmon can easily burrow into substrate interstices (Heggenes et al. 2013); juveniles of several species, such as a Japanese spined loach *Cobitis* sp. BIWAE type A sensu Nakajima et al. (2012) and Amur catfish *Silurus asotus*, were captured from the hyporheic samples during the present study and subsequent studies (R. Dohi and R. Kawanishi, unpublished data). These findings suggest that vertical connectivity with the hyporheic zone is more important for stream fishes than previously thought.

Human activities have impaired hydrological connectivity by creating barriers in river networks, which threaten various aquatic organisms (Fullerton et al. 2010). For vertical connectivity between the streambed and the hyporheic zone, sedimentation as a result of human activities may be the most serious barrier. Fine sediments are deposited in the interstices and reduce the permeability of the hyporheic zone (Schälchli 1992; Jones et al. 2012), preventing benthic organisms from burrowing into it (Dunn and O'Brien 2006; Vadher et al. 2015) and decreasing both benthic and hyporheic invertebrate densities and diversities (Descloux et al. 2013). Because increased sedimentation is strongly related to changes in land use such as logging and agriculture (Sutherland et al. 2002; Opperman et al. 2005), catchment management, such as the planting of riparian buffer stripes, will be key to maintaining vertical hydrological connectivity. This study clearly indicates that the integrity of vertical hydrological connectivity is crucial not only for invertebrates but also for stream fishes.

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