Title

Spring-fed streams as flow refugia: Changes in fish assemblages after a rainfall event

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Abstract

Understanding the structure and function of flow refugia in river networks is critical to freshwater conservation in the context of anthropogenic changes in flow regimes. Safety concerns associated with field data collection during floods have largely hindered advances in assessing flow refugia for fishes; however, spring-fed streams can be safely surveyed during rainfall events owing to their stable flow regimes. In this study, we assessed temporal changes in fish assemblages in a lowland, spring-fed tributary in northern Japan after a 48.5 mm rainfall event. A total of seven fish species were collected, and responses to the flood varied among species. Abundance of salmonids (*Oncorhynchus keta* and *O. masou masou*) increased immediately after rainfall but subsequently decreased. Collections of *O. keta* consisted entirely of mature individuals, while *O. masou masou* collections included only juveniles, suggesting that the former uses the tributary for spawning whereas *O. masou masou* may evacuate into the tributary during rainfall events. Benthic species (*Cottus hangiongensis* and *Gymnogobius opperiens*) were only observed in the main stream under ordinary flow conditions, and did not appear in the tributary during the flood, suggesting that other flow refugia are available to these species. Our results suggest that spring-fed tributaries may provide flow refugia for nekton species, whereas substrate preferences may be the major determinant of whether spring-fed tributaries function as flow refugia for benthic species.

KEYWORDS

flow refugia, flow regime, flood disturbance, groundwater, lowland, river ecosystem

1. INTRODUCTION

Flood disturbance is a key factor in the assembly of stream communities (Junk, Bayley, & Spark, 1989; Poff *et al*., 1997; Walton *et al*., 2017). Flooding is one of the most ubiquitous and frequent disturbances in stream ecosystems, and many stream organisms have developed strategies for coping with this disturbance (Lytle & Poff, 2004). For example, stream organisms may avoid the lethal impacts of flooding through strategies such as remaining in their original habitats and enduring the flood, or alternatively, evacuating to locations with relatively mild currents during flood events, and subsequently returning to their usual habitat (Miyake, 2013). These areas of relatively mild currents are called "flow refugia" (Lancaster & Hildrew, 1993a; 1993b). Functionally available flow refugia can be found in shallow bankside areas (Negishi, Inoue, & Nunokawa, 2002; Sueyoshi, Nakano, & Nakamura, 2013), deep pools (Matthews, 1986), complex habitats with woody debris and plant roots (Borchardt, 1993; Palmer, Arensburger, Martin, & Denman, 1996), hyporheic zones (Williams & Hynes, 1974), and tributaries (Koizumi, Kanazawa, & Tanaka, 2013), including spring-fed streams. This indicates that maintaining habitat heterogeneity, including a variety of flow refugia, is important for conserving stream biodiversity (Sedell, Reeves, Hauer, Stanford, & Hawkins, 1990).

Despite the importance of flow refugia, anthropogenic impacts have steadily degraded stream environments and have introduced both temporal and spatial disruptions to flow regimes. Examples include unpredictable and/or massive flood disturbances that result from dam operations (Choi, Yoon, & Woo, 2005; Magilligan & Nislow, 2005) and climate change (Milner, Robertson, McDermott, Klaar, & Brown, 2012; Yin *et al*., 2018); these may interrupt radical strategies of stream organisms. In

addition, flood control measures can lead to habitat homogenization and the disappearance of flow refugia such as shallow aquatic-terrestrial transition zones (Junk *et al*., 1989). In this context, understanding the responses and strategies of stream organisms to flood disturbance, and restoring appropriate flow refugia, are important for the sustainability of stream ecosystems.

While it is important to understand evacuation behavior and flow refugia of aquatic organisms, conducting field investigations during floods is generally unsafe and difficult, particularly in the case of fishes. As a result, information regarding the responses of stream fishes to floods has been primarily derived from artificial flood experiments (e.g., Koizumi *et al*., 2013) and simulation models (e.g., Booker, 2002), and the lack of field-based data has resulted in continued uncertainty regarding the quality and usage of flow refugia. For example, artificial flooding experiments are typically spatially restricted and cannot imitate actual precipitation events in broader stream networks (Miyake, Hiura, Kuhara, & Nakano, 2003; Koizumi *et al*., 2013), and as a result they often overestimate the responses of stream fishes to floods. Field investigations after floods may not capture short-term responses in which species migrate quickly between flow refugia and their usual habitats. Therefore, investigating the dynamics of stream fishes in flow refugia that can be safely accessed during floods may contribute valuable information that can be applied to the conservation and restoration of aquatic habitats.

Spring-fed streams, which are maintained by permanent groundwater discharge, constitute unique ecosystems with stable flow regimes (Mattson, Epler, & Hein, 1995; Lusardi, Bogan, Moyle, & Dahlgren, 2016). Owing to their high stability, spring-fed streams may function as high-quality flow refugia and are suitable for investigating

community dynamics during flood events. However, substantial information gaps remain regarding the functional role of spring-fed streams in the dynamics of stream fishes during floods. Because springs generally provide purified water, spring-fed streams are frequently conserved for human water supply (LaMoreaux & Tanner, 2001). Elucidating the ecological functions of spring-fed streams in providing flow refugia would further highlight their value.

We investigated temporal changes in stream fish assemblages in a spring-fed tributary after a rainfall event. While access to the surrounding non-spring-fed streams was prohibitive at the time, the spring-fed tributary was relatively calm and could be safely accessed for faunal surveys. Here, we report the responses of fishes to the flood event and discuss differences among species. We also assessed the function of spring-fed streams as flow refugia, and propose future perspectives for river network management that will be informative to the conservation of stream biodiversity.

2. METHODS

2.1 Study site

The study was conducted in the Shubuto River basin in Kuromatsunai, Hokkaido, Japan (42.64° N, 140.34° E), which encompasses 367 km^2 and includes both montane and lowland regions. The region is underlain by sandstone and mudstone, including Cenozoic fossil shells and tuff. Mean annual precipitation between 2009 and 2018 was 1615.8 mm, and mean air temperature was 7.5°C, measured at the Kuromatsunai AmeDAS automated weather station, located 4 km northwest of the site. The dominant tree species in riparian zones in the study area are *Salix* species and

Quercus crispula, and dominant understory plants are *Sasa kurilensis* and *Reynoutria sachalinensis*.

Localized areas of groundwater discharge such as flowing wells, seepages, and springs can be found in lowland regions of the Shubuto River system. Among these, significant amounts of groundwater discharge (ca. 0.1 m^3/s) have created a spring-fed stream that connects directly to the Shubuto River. This stream has no artificial structures that inhibit longitudinal connections, and stream fishes can thus migrate between the spring-fed tributary and the main stream. We used this spring-fed tributary as a study site and examined its role as a refugium for stream fishes. We established a study reach in this tributary ca. 500 m upstream of the confluence with the main stream. In addition, to determine whether any species evacuate to the tributary from the main stream, we established a second study reach in the main stream, approximately 500 m downstream from the confluence. Both study reaches drain into the Kuromatsunai Lowland, and stream widths in the tributary and main stream reaches were 4.1 and 12.4 m, respectively (Table 1). The bed of the tributary was primarily sandy and muddy sediments, which are consistently supplied by the source spring; by contrast, the main stream was dominated by cobbles and boulders (Table 1 and Fig. 1). A facility for capturing *Oncorhynchus keta* for egg collection is located upstream of the study reach in the tributary.

2.2 Fish collection

A 40 m long study reach was established in both the tributary and main stream to investigate fish assemblages. We used different methods in each reach to maximize the efficiency of collections. In the tributary, we conducted electrofishing using a

backpack electrofisher (LR-20; Smith-Root Inc., Vancouver, WA, USA); this method is particularly effective for narrow reaches with many obstacles (e.g., vegetation cover). In the main stream reach, we used cast net fishing owing to the stream width and the lack of obstacles. Both methods are particularly effective for collecting fishes inhabiting relatively shallow habitats, and were suitable for our study reaches as neither contained deep-water habitats, such as pools > 50 cm depth.

The upper and lower limits of the tributary study reach were partitioned using fishnets to prevent fishes from entering or exiting the reach during collection. Then three-pass electrofishing was conducted from lower to upper positions to collect as many fishes as possible (cf. Inoue, Sakamoto, & Kikuchi, 2013). Twenty net casts were conducted within the study reach in the main stream.

To cover all phenological groups, sampling was conducted on 24–29 August and 17–20 November 2017, and on 29–30 May and 10–11 October 2018. All investigations were performed under ordinary flow conditions. In addition to these seasonal collections, we conducted collection in the tributary during a rainfall event. On October 6–7, 2018, a temperate low-pressure system (formerly the typhoon Kong-rey) released a total of 48.5 mm of rain at the study site. According to our visual surveys immediately after the rainfall event, water levels in most non-spring-fed streams in the Shubuto River system increased substantially; however, the study tributary did not show dramatic elevations of water level. We conducted fish collection both before (6 October) and after (7, 9, and 10 October) the rainfall event; hereafter, we refer to these collections as “before,” and “after 1,” “after 2,” and “after 3” days, respectively (Fig. 2).

Most females and some males of *O. masou masou* become anadromous after approximately 2 years of growth in the river (Miyazaki, 2017); therefore, small

individuals collected in this area are mainly juveniles and resident males. Because the rainfall event occurred during the species' reproductive period, we determined whether small individuals of *O. masou masou* (standard body length = 60–160 mm) were mature or juvenile based on the presence or absence of nuptial coloration.

2.3 Hydrological monitoring

Water level loggers (HOBO CO-U20L-04, Onset, Bourne, MA, USA) were installed on the streambed in both study reaches using metal tubes and wire. They were programmed to collect hourly measurements of hydraulic pressure. Prior to the onset of the rainfall event, we recorded water levels at monitoring points using a folding scale, and developed linear regression formulae describing the relationship between water levels (cm) and hydraulic pressure (kPa). Based on the regression formulae, hydraulic pressure data recorded by the loggers were transformed into water level data. The water level at each monitoring point as of 1:00 am on 6 October, 2018 was set at 0 cm in both study reaches, and we developed hydrographs of ensuing water level changes over the course of the rainfall event. Hourly rainfall data were obtained from the Kuromatsunai AmeDAS weather station.

3. RESULTS

After the onset of the rainfall event, water levels in the tributary reach declined slightly, but overall variation during the monitoring period was clearly lower than that in the main stream reach (Fig. 2). Water levels in the main stream reflected changes in rainfall intensity and peaked at 55 cm above the initial water level (Fig. 2).

Seven native fish species were collected before and after the rainfall event (Fig. 3). Because the number of individuals did not decrease between the first and third passes for most species and sampling timings, we did not apply mathematical removal methods to estimate abundance (e.g., Hayne 1949; Zippin 1956). Rather, we pooled all individuals collected in each of the three passes and assessed changes in total abundance for each species (Fig. 3).

The abundance of four species (*Oncorhynchus masou masou*, *O. keta*, *Lethenteron* sp. N, and *Tribolodon* sp.) increased after the rainfall event, but subsequently decreased (Fig. 3). That of *O. masou masou* peaked at after 2 days, whereas those of the other decreased after 1 day. We collected multiple individuals of *O. masou masou* (175 individuals), *O. keta* (35 individuals), and *Lethenteron* sp. N (23 individuals), but only one individual of *Tribolodon* sp. (Fig. 3). All *O. masou masou*, *Tribolodon* sp., and *Lethenteron* sp. N were juveniles; by contrast, only mature, reproductive individuals of *O. keta* were collected.

In contrast to the upward convex responses of the aforementioned species, the other three species (*Noemacheilus barbatulus toni*, *Salvelinus leucomaenis leucomaenis*, and *Cottus reinii*) exhibited different patterns (Fig. 3). Total collections of these species were also relatively low (*N. barbatulus toni* = 6 individuals, *S. leucomaenis leucomaenis* = 4 individuals, and *C. reinii* = 1 individual).

The species composed of fish assemblage in the spring-fed tributary estimated by the seasonal collections were completely corresponded to those collected before and after the rainfall event in October 2018 (Table 3). According to the seasonal collections, *C. hangiongensis* and *Gymnogobius opperiens* were absent in the spring-fed tributary

whereas *S. leucomaenis leucomaenis*, *Lethenteron* sp. N, *N. barbatulus toni* were absent in the mainstream (Table 3).

4. DISCUSSION

Lowland, spring-fed streams formed by groundwater discharge are not typically steep or deep channels (Sear, Armitage, & Dawson, 1999), and their flow regimes are generally stable (e.g., Mattson *et al*., 1995; Lusardi *et al*., 2016). In this study, water levels in the spring-fed tributary did not exhibit the same marked increase observed in the main stream during the rainfall event. Rather, they decreased after the onset of rainfall. This may be attributable to water transmission into the *O. keta* egg collection facility, because local fishermen know that this species actively migrates to upstream spawning habitats during floods (Banks, 1969). This was reflected in our results, which indicated that the abundance of *O. keta* peaked immediately after the peak of flooding in the main stream. Thus, for *O. keta*, the tributary likely provides spawning habitat rather than a flow refugium.

Oncorhynchus masou masou occurs throughout the Shubuto River system (Miyazaki, Terui, Senou, & Washitani, 2011; Terui & Miyazaki, 2015), and was one of the most abundant species in our collections from both the tributary and main stream. The collected *O. masou masou* were regarded as juveniles and did not contain matured individual. Because the proportion of anadromous populations of *O. masou masou* is higher in more northerly parts of the Japanese Archipelago (Masuda, Amaoka, Araga, Uyeno, & Yoshino, 1984), the population of this species we observed was substantially occupied with future anadromous individuals and less stream resident ones. The observed absence of mature resident and anadromous individuals may also be

associated with the lack of deep-water habitats, which this species preferentially inhabits, within the study reaches (Edo & Suzuki, 2003). Hence, our results, combined with the biology of *O. masou masou*, suggest that the tributary functions primarily as a refugium for juvenile individuals of the species.

The abundance of *O. masou masou* increased after the rainfall event, but decreased thereafter, suggesting that this species evacuated from the main stream into the tributary. To identify evacuated individuals, it is important to accurately distinguish between tributary residents and evacuated main stream residents. For example, it may be informative to use video cameras to capture migration of fish from the main stream to the tributary during floods. Alternatively, stable isotope signatures may distinguish the origin of collected individuals if food web structure differs between the tributary and the main stream (e.g., Hobson, 1999; Cunjak *et al*., 2005).

Lethenteron sp. N was present in the tributary but absent in the main stream under ordinary flow conditions, suggesting that the tributary is less likely to introduce the species coming from main stream during floods. Nevertheless, this species responded to the rainfall event by increasing in abundance immediately after rainfall and subsequently decreasing. This may reflect a small-scale evacuation event of this species. Because this species normally inhabits areas of weak current with fine sediments (Sugiyama & Goto, 2002), rainfall may induce migration from faster flowing waters in central parts of the channel to slower bankside waters, even under the generally calm flow conditions of spring-fed streams. Because individuals in bankside waters are easily captured by electrofishing, small-scale evacuation may explain the trends we observed; however, testing this hypothesis would require documenting the location where each individual was collected.

Due to the small number of individuals collected, temporal changes in other species were not clear compared to the trends observed for *O. keta*, *O. masou masou*, and *Lethenteron* sp. N. However, species observed only in the tributary, including *S. leucomaenis leucomaenis* and *N. barbatulus toni*, may exhibit small-scale migration after rainfall events, similar to *Lethenteron* sp. N. Particularly, *N. barbatulus toni* is a benthic fish as common with *Lethenteron* sp. N, and thus micro-scale migrations within the tributary is more likely in *N. barbatulus toni* than in *S. leucomaenis leucomaenis*, which is a nekton. Although *S. leucomaenis leucomaenis* generally inhabits cool montane headwaters (Masuda *et al*., 1984), lowland spring-fed streams with stable water temperatures may also provide suitable habitat for the species.

Meanwhile, for the species only found in the main stream (*C. hangiongensis* and *G. opperiens*), spring-fed streams may not be attractive as their flow refugia. These two species are benthic, but their preferred habitats are cobble-boulder dominated riffles (Masuda *et al*., 1984; Miyazaki, 2017), unlike *Lethenteron* sp. N and *N. barbatulus toni*, which prefer fine substrates. The habitat preferences of *C. hangiongensis* and *G. opperiens* suggest that appropriate flow refugia for these species include interstitial spaces in the coarse riverbed of the main stream. Therefore, spring-fed streams with fine sediment substrates would not provide suitable refugia. By contrast, *C. reinii*, a relative of *C. hangiongensis*, tends to inhabit calm waters (Miyazaki, 2017), and was found in the tributary, although in low numbers.

Tribolodon sp. is a nekton and is assumed to migrate easily between the tributary and the main stream. However, this species also prefers interstitial habitats (Katano, Aonuma, & Matsubara, 2001), suggesting it may find suitable flow refugia in both spring-fed streams and interstitial spaces in coarse riverbeds. We detected a small

peak in the abundance of *Tribolodon* sp. immediately after the rainfall event. Additional studies with more individuals may clarify the function of spring-fed streams as refugia for this species.

We previously explored the function of spring-fed streams in providing flow refugia for different fish species during flood events. Nekton species can easily migrate into tributaries, and may use spring-fed tributaries as refugia. In the case of benthic species, species-specific substrate preferences may determine whether spring-fed streams function as flow refugia or not. Because spring-fed streams are often dominated by fine substrates (Sear *et al*., 1999), they provide both suitable habitat and flow refugia for fine-sediment burrowers. Our results, combined with existing information on the biology of the study species, suggest that the functional role of spring-fed streams as flow refugia is not generalizable, but rather is species-specific. Therefore, even if spring-fed tributaries are available within a river system, maintaining the resilience of the system to food disturbance, particularly for benthic species, requires the maintenance of multiple types of flow refugia, such as shallow bankside waters and hyporheic zones.

Because spring-fed streams are valuable habitats that can be safely surveyed during rainfall events, understanding their function as flow refugia may be valuable to biodiversity conservation efforts in river networks and the sustainable use of spring-fed streams. In addition, the stable flow regimes and water temperatures in spring-fed streams (Mattson *et al*., 1995) may also provide refugia for stream fishes experiencing thermal stress during cold or hot spells (e.g., Greer, Carlson, & Thompson, 2019). Examining these functions may further increase our understanding of the role of spring-fed streams in metacommunity stability in river networks through species

interactions (e.g., Unionoida parasites; Akiyama & Iwakuma, 2007). Here, we have provided the first report on the potential function of spring-fed tributaries as flow refugia for stream fishes. Our results will be helpful in the management and conservation of river biodiversity in the face of environmental change.

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TABLE 1 Abiotic characteristics of the study reaches.

	Spring-fed tributary			Main stream		
	Mean	±	SD	Mean	±	SD
Stream width (m)	4.07	±	0.22	12.40	±	1.28
Water depth (cm)	15.02	±	1.68	26.05	±	4.93
Current velocity (cm/s)	12.43	±	3.92	48.54	±	13.07
% fine sediment deposition*	80.63	±	8.46	9.69	±	5.65

*Estimation method based on Sakai *et al.*, 2013.

TABLE 2 Fish assemblages in the study reaches, estimated based on seasonal investigations; 1 = presence, 0 = absence.

Family	Scientific name	Spring-fed tributary	Main stream
Petromyzontidae	*Lethenteron* sp. N	1	0
Salmonidae	*Oncorhynchus masou masou*	1	1
Salmonidae	*Oncorhynchus keta*	1	1
Salmonidae	*Salvelinus leucomaenis leucomaenis*	1	0
Cyprinidae	*Tribolodon* sp.	1	1
Nemacheilidae	*Noemacheilus barbatulus toni*	1	0
Gobiidae	*Gymnogobius opperiens*	0	1
Cottidae	*Cottus reinii*	1	1
Cottidae	*Cottus hangiongensis*	0	1

FIGURE CAPTIONS

FIGURE 1 Landscapes of the study streams. (a) spring-fed tributary, (b) main stream, (c) streambed of the study reach in the tributary, (d) streambed of the study reach in the main stream.

FIGURE 2 Hydrographs for the spring-fed tributary and main stream during 6–10 October, 2018. Red arrows indicate timings of fish collections in the spring-fed tributary.

FIGURE 3 Temporal changes in number of individuals of the collected seven fish species in the study spring-fed tributary.

Fig. 1

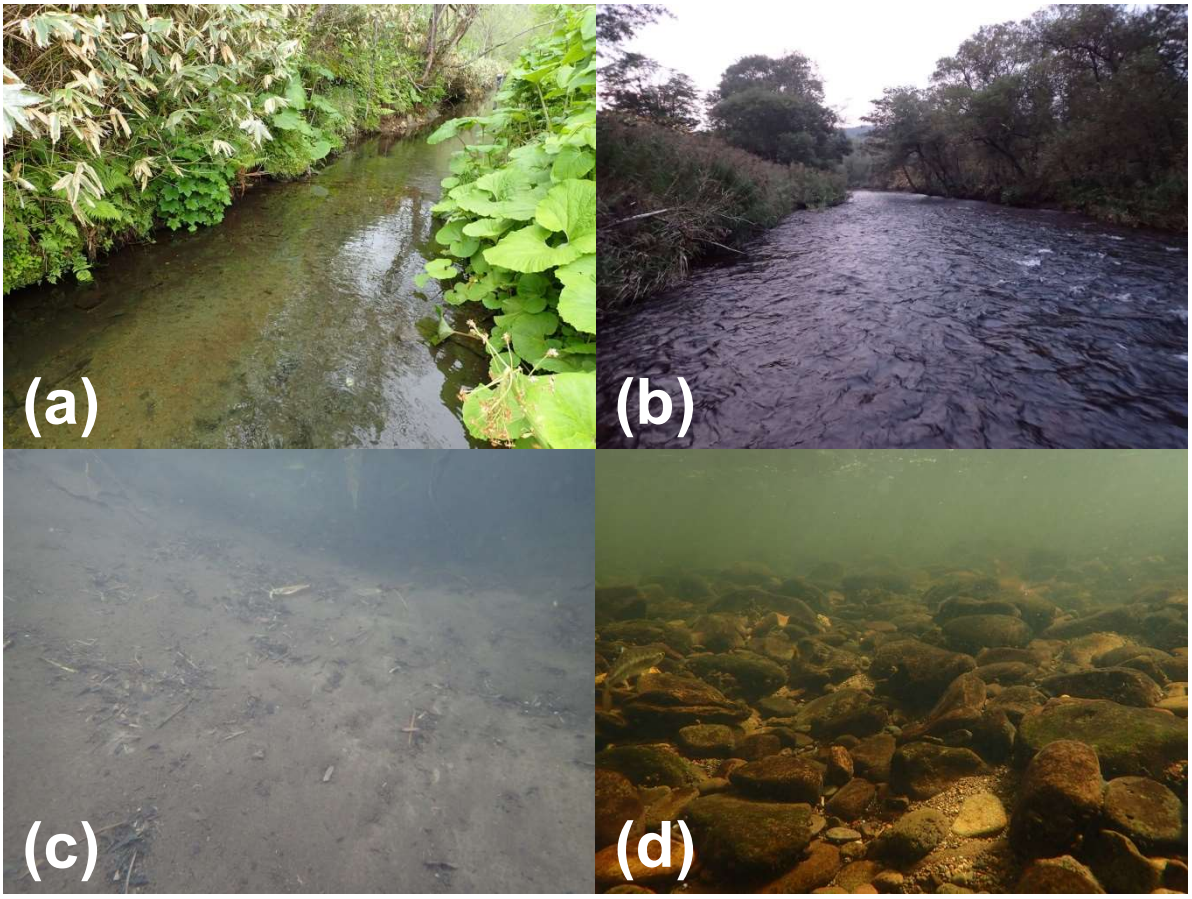


Fig. 2

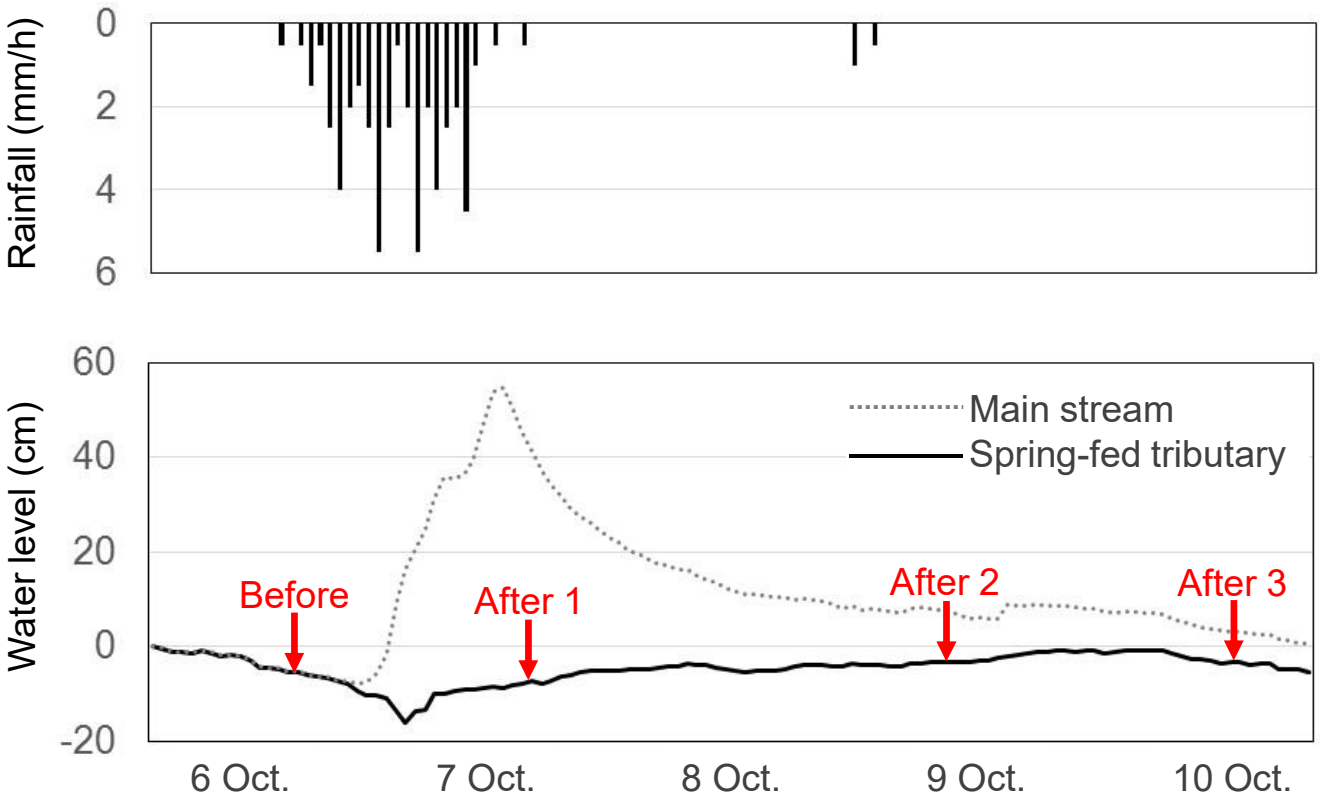
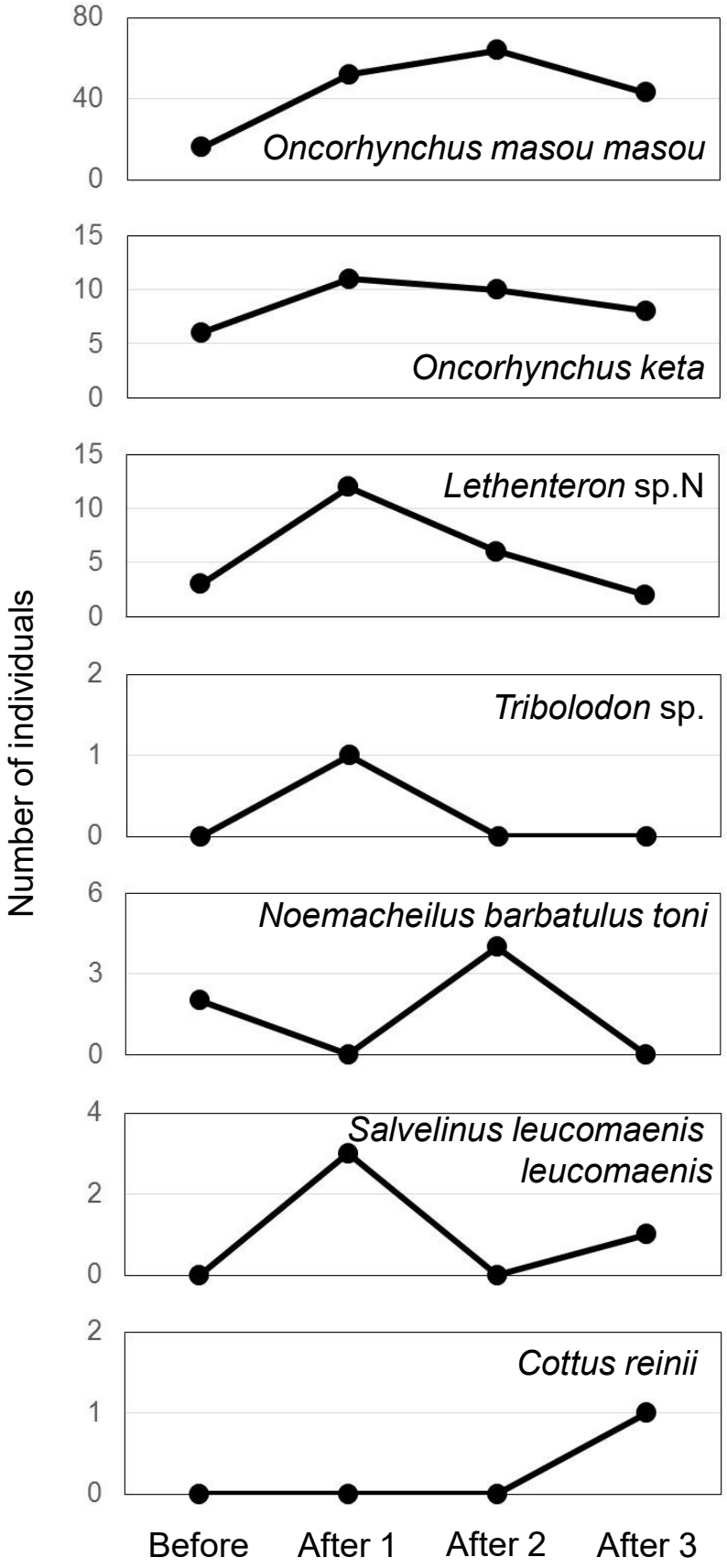

Rainfall (mm/h)
0
2
4
6
Water level (cm)
60
40
20
0
-20
Main stream
Spring-fed tributary
Before
After 1
After 2
After 3
6 Oct.
7 Oct.
8 Oct.
9 Oct.
10 Oct.

Fig. 3


Number of individuals
80
40
0
Oncorhynchus masou masou
15
10
5
0
Oncorhynchus keta
15
10
5
0
Lethenteron sp.N
2
1
0
Tribolodon sp.
6
3
0
Noemacheilus barbatulus toni
4
2
0
Salvelinus leucomaenis leucomaenis
2
1
0
Cottus reinii
Before
After 1
After 2
After 3