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Hydrological, physicochemical and metabolic signatures in groundwater and snowmelt streams in the Japanese Alps

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Highlights

• Morphological, hydrological and physicochemical conditions as well as metabolic activity are assessed across streams

• Comparisons are made between snowmelt and groundwater fed systems

• Snowmelt fed streams experience no flow and storm events

• Groundwater streams had higher environmental stability and higher metabolic activity

• Patterns were similar to other regions of the globe, but there was higher inter-stream variation
Abstract

The unique hydrology and physicochemistry of alpine streams provide an important influence on the structure and function of inhabiting biological communities. A substantial body of research exists on alpine streams across many regions of the globe (e.g. Europe, North and South America and Greenland). To date, however, there have been few studies investigating the environmental conditions present in alpine streams across the Japanese archipelago. The lack of information on alpine streams in Japan is problematic as unique regional climates, e.g. some of the highest levels of snowfall globally, are likely to have repercussions for morphological, hydrological, physicochemical and metabolic signatures, causing them to differ from those observed in other regions. In this study we compare the morphology, hydrology, physicochemistry and metabolic activity of snowmelt and groundwater fed streams in the Kamikochi region of the Japanese Alps. Stream discharge, water chemistry (major ions, silica, dissolved oxygen), water temperature and channel stability were measured over a period of 16 months in 2017–2018. Metabolic activity was determined using Resazurin-Resorufin (Raz-Rru) Smart Tracer and variation in the Raz transformation rate was assessed to understand the effects of hydrology and physicochemistry on ecosystem functioning. Snowmelt streams were characterised by higher variability of water temperature, water chemistry and stream discharge, both, within and between sites. Indeed, two of the snowmelt streams experienced no flow conditions for several periods and also floods. In comparison, water chemistry, water temperature and stream discharge in groundwater fed streams were more temporally stable. Metabolic activity was higher in one groundwater fed stream, attributed to significant growth of macrophytes. These findings indicate that the patterns of morphology, hydrology, physicochemistry and metabolic activity across streams in the
Japanese Alps largely resemble those identified elsewhere, although there were higher levels of inter-stream variation. The diversity and inter-site variation of hydrological and physicochemical conditions are likely responsible for the unique flora and fauna in the streams. This study therefore indicates the potential importance of habitat templates for the aquatic biodiversity hotspot in this region.

**Keywords:** alpine streams, water temperature, habitat templates, metabolic activity
1. Introduction

Alpine stream systems are under threat from climate change (Brown et al. 2007a; Khamis et al. 2014), as well as other anthropogenic stressors such as nutrient enrichment (Hotaling et al. 2017). Particular conservation concern is linked to alpine streams as they support high biodiversity and a large number of endemic and rare species that are absent from other aquatic ecosystems across the globe (Robinson et al. 2010). The combination of high biodiversity and endemism alongside rapid environmental change (Prowse et al. 2006) poses a disproportionate risk of species extinctions and biodiversity loss (Dirnböck et al. 2011).

The unique flora and fauna inhabiting alpine systems is in part generated by the high heterogeneity of environmental conditions both within and between alpine stream systems (Ward 1994; Brown et al. 2007a; Finn & Poff 2011; Jacobsen et al. 2012; Hotaling et al. 2017). In particular, streams fed by different water sources support markedly different biological communities resulting in high beta- and gamma-diversity across these streams (Milner et al. 2010). Each stream type is characterised by the dominant contributing water source and has a unique combination (or signature) of flow regime and water temperature, turbidity and chemistry (often characterised by redox indicators, major ions and dissolved nutrients) (Brown et al. 2003, 2009; Hannah et al. 2007). Environmental conditions in space and through time are more constant in groundwater fed streams whereas intermediate variability in conditions occur in streams which respond to seasonal snowmelt dynamics, but the highest levels of variability are typically found in glacial meltwater fed streams (Brown et al. 2007b; Milner et al. 2010; Khamis et al. 2016). Intermediate habitat templates can be generated by mixtures of water sources (Brown et al. 2009; Milner et al. 2010). Only specialised taxa are able to persist in the harsh conditions of unstable channels fed
entirely by snowmelt (Snook & Milner 2001; Cauvy-Fraunié et al. 2014) in comparison to the more species rich assemblages in groundwater dominated streams (Brown et al. 2006b, 2007b; Khamis et al. 2016).

Our understanding of alpine stream habitats is relatively well developed across many regions of the globe, including; North America (Finn et al. 2006; Windsor et al. 2017), South America (Jacobsen et al. 2014; Cauvy-Fraunié et al. 2015), Africa (Musonge et al. 2020), Greenland (Docherty et al. 2018), Svalbard (Blaen et al. 2014) and Europe (Finn et al. 2013; Khamis et al. 2016). Research in the Japanese archipelago is however more limited, with existing studies focusing on the population dynamics and genetics of individual invertebrate, fish and amphibian species, many of them endemic (Fukumoto et al. 2015; Tojo et al. 2017b, a; Saito et al. 2018). As a result, the habitat template, the suite of environmental conditions influencing biological communities (i.e., morphology, hydrology and physicochemistry), is not as well understood for Japanese alpine streams. Previous studies indicate streams in the Japanese Alps are fed by several water sources common in other alpine systems, including snowmelt, groundwater and to a very limited extent glacial runoff (Yoshimura et al. 2005; Milner et al. 2020). It is unclear, however, how source water contributions affect the physicochemical and hydrological conditions in these streams. Furthermore, it is not known whether the patterns in habitat templates within and between Japanese alpine streams mirror those observed across other alpine regions of the globe.

Many of the alpine regions studied previously have similar hydro-climatological conditions and are positioned within similar climate envelopes. Climatic conditions in the Japanese Alps, however, are relatively unique. As an example catchments in this region receive some of the highest levels of snowfall in the world (Ueda 2014) and potentially these streams may display a unique set of morphological, hydrological,
physicochemical and metabolic signatures, supporting novel habitat templates that are different from other regions of the globe.

Here we assessed the morphological, hydrological, physicochemical and metabolic signatures across six streams fed by two different water sources (snowmelt and groundwater) across the Kamikochi region of the Japanese Alps. Water temperature, discharge and water chemistry were used to characterise the systems, following the important variables identified by the classification system produced by Brown et al. (2009). Metabolic activity was measured in streams to understand how environmental conditions may impact ecological functioning. As the environmental conditions and ecological functioning in these systems are relatively poorly described we proposed three exploratory hypotheses based on studies in other regions of the globe:

H1. Water source will significantly influence morphological, hydrological and physicochemical signatures between the study streams

H2. Metabolic activity will vary across the stream sites and be significantly higher in the groundwater systems

H3. Hydrological, physicochemical and metabolic signatures in groundwater streams will be less variable when compared to snowmelt streams

2. Material and methods

2.1. Study sites

The study was completed in the Kamikochi valley within the Chūbu-Sangaku National Park, Nagano Prefecture, Japan (1,743 km²), a region known as the Hida Mountains or northern Japanese Alps. The National Park is characterised by high mountains with Mt. Tateyama (2,455 m) and Mt. Tsurugi-dake (2,926 m) at the northern reach and Mt. Hotaka-dake (3,190 m) as well as Mt. Norikura (3,026 m) at the southern edge.
Kamikochi valley is approximately 18 km in length, with the average valley floor elevation of 1,500 m. Up to about 12,000 years ago the River Azusa primarily flowed into the Jinzu-gawa River system on the Hida-Takayama southern side of Northern Japan Alps but then, potentially due to a volcanic collapse of Mt. Yake-dake (2,455 m), the old Azusa River became naturally dammed and started to flow north towards Matsumoto (Harayama 2015). This river catchment supports a significant biodiversity hotspot in the Japanese archipelago (Tojo et al., 2017a, b).

Six streams were selected in the study area (Figure 1), all of which are tributaries of the Asuza River. Three were fed predominantly by groundwater (Shimizugawa, Minamisawa and Bentenzawa) and three fed predominantly by snowmelt (Dakesawa, Shirasawa and Tokusawa). The dominant water source of the streams was identified through local knowledge and initial field surveys by identifying groundwater springs and snowpacks. The sources of snowmelt fed stream were >2000 m elevation and considerably longer (3-5 km) in comparison to groundwater fed stream sources which were at ~1500 m.a.s.l elevation and relatively short (< 1 km) (see Figure 1). Mean widths varied from 4 to 10 m with mean depths <30 cm. At each site a stream reach of 10 m was designated as the sample site and reaches were above any bridges to minimise potential anthropogenic influence on streams. Sites were typically within 200 m of the mainstem Azusa River.

2.2. Meteorological data

Meteorological data were obtained from the Shinshu University field station in the Myojin area of Kamikochi (1,527 m.a.s.l; Figure 1). Data were collected at 10-minute intervals using a HD9817T1 temperature sensor (Delta OHM, Padua, Italy), a 34-HT-BP precipitation sensor (Ota Keiki, Tokyo, Japan) and manual snow depth measurements.
2.3. Morphological characterisation of the streams

The morphology of the stream reaches was evaluated at the start of the sampling period (July 2017). Mean width and depth of the stream channels at the sample reaches were calculated from replicate measurements (n = 5) along a single transect in the centre of the sampled reach. Although width and depth varied seasonally, measurements taken during this initial period represent baseflow conditions associated with each water source, as demonstrated in the discharge data. The relative channel stability was also assessed using the bottom component of the Pfankuch channel stability index (Pfankuch 1978). This method uses visual assessment of six attributes (substrate brightness, angularity, consolidation, proportion of stable materials, scouring and amount of clinging vegetation) and assigns them a value on a 1 to 4 scale. The scores are weighted according to their importance, and the sum of the scores is the stability index. Lower values indicate higher channel stability.

2.4. Water temperature

Water temperature was recorded at 30-minute intervals over the sampling period (1st August 2017 to 1st December 2018) using TinyTag dataloggers (TGP-4017; Gemini Data Loggers Ltd., Chichester, UK) deployed 0.1 m above the streambed at the six study sites. The number of recordings varied between sites, however, on average >18000 measurements were made at each site over the sampling period. The minimum number of measurements took place at Shirasawa, with only 5420 records of temperature data due to the replacement logger being lost (after ~4 months). At Bentenzawa groundwater stream the separate temperature logger was exposed to air in December 2017 to April 2018 as the channel width constricted. For the following analyses we have removed this section from the water temperature record. The duration of all measurements was accounted for in further analyses.
2.5. Stream discharge measurement

Discharge (m$^3$ s$^{-1}$) was calculated using stage-discharge curves (Herschy 1993, 2009). We collected water depth at 30-min resolution from 1st August 2017 to 1st December 2018 using several types of logger according to their availability, including: Levellogger Edge 3001 (Solinst, Georgetown, Canada), dipperLog (Heron Instruments Inc., Dundas, Canada) and Level TROLL 500 (In-Situ Inc., Fort Collins, USA). Water depth measurements were not available for Shirasawa due to the loss of a sensor in a large storm event in spring 2018. To generate stage-discharge curves we used a conservative tracer approach. Briefly, we used standard cross-section approaches (Herschy 2009) with a Kenek EU20 (Toyko, Japan) flow-meter in conjunction with a conservative NaCl tracer dilution method (Tazioli 2011). The NaCl trace was measured using a hand-held multi-parameter meter (YSI 6050000, USA). These calibration measurements were completed under different flow conditions (0–1.98 m$^3$ s$^{-1}$) across different seasons (n = 5, July–December 2017), to enable discharge calculations at different times of year. Using the rating curves, we converted water depth measurements into a continuous record of stream discharge across sample sites.

2.6. Water chemistry

Triplicate water samples (125 ml) were collected at the six stream sites during the 2017 sampling season. Total dissolved solids (TDS) and dissolved oxygen (DO) concentrations were measured at sites (mg L$^{-1}$) during the sample period (see Milner et al. 2020 for sampling details). These samples were frozen at -20 ºC within 10 hours of collection. Water samples were filtered (Whatman GF/F 0.7 μm, GE Healthcare, UK) and then analysed for the following ions; Ca$^{2+}$, K$^+$, Mg$^{2+}$, Na$^+$, NH$_4^+$, SiO$_2$, Cl$, NO_2^-$, NO$_3^-$, SO$_4^{2-}$ using a Hitachi U-2000 analyser and standard analytical methods for
these ions (APHA 2005). The concentration (mg L\(^{-1}\)) of each ion was calculated from a calibration curve generated using a calibration standards.

2.7. Metabolic activity

The metabolic activity of five of the streams (Shimizugawa, Bentenzawa, Dakesawa, Shirasawa and Tokusawa) was estimated using Resazurin-Resorufin (Raz-Rru) Smart Tracing (RRST) (Haggerty \textit{et al.} 2008, 2009; Argerich \textit{et al.} 2011; González-Pinzón \textit{et al.} 2012). Unfortunately, a determination was not possible for Minamisawa, as it was not possible to obtain sufficient distance between two sampling sites due to the size of the stream. Across each of the five streams we conducted a single instantaneous Resazurin injection (15–17\textsuperscript{th} April 2018). At two sites, approximately 100 to 150 m apart, 12–16 grab samples were collected to capture the tracer breakthrough curves.

The samples were cooled until analysis within 48 hours. Prior to fluorometric analysis with a GGUN FL30 fluorescent spectrometer (Albillia Sarl, Switzerland) in bench-top mode, all samples were allowed to reach room temperature and were buffered to a pH of ~8.5. The buffer was made by mixing equal volumes of 1\textit{M} NaH\textsubscript{2}PO\textsubscript{4}·H\textsubscript{2}O and 1\textit{M} NaOH (Haggerty \textit{et al.}, 2008) and a sample to buffer ratio of 100:1 was used (Blaen \textit{et al.} 2017). The transformation rate (\(\lambda_{\text{Raz}}\), [s\(^{-1}\)]) of Resazurin (Raz) to Resorufin (Rru), used as a proxy for metabolic activity, was then calculated according to the methods presented in Argerich \textit{et al.} (2011) and Blaen \textit{et al.} (2017):

\[
\lambda_{\text{Raz}} = \frac{1}{\tau} \ln \left( \frac{m_{\text{Raz}}^{\text{up}}}{m_{\text{Raz}}^{\text{up}} + m_{\text{Rru}}^{\text{up}} - m_{\text{Rru}}^{\text{down}} \times Q_{\text{ratio}}} \right)
\]

\[
Q_{\text{ratio}} = \frac{m_{\text{Raz}}^{\text{up}} + m_{\text{Rru}}^{\text{up}}}{m_{\text{Raz}}^{\text{down}} + m_{\text{Rru}}^{\text{down}}}
\]

where \(m\) refers to the zero\textsuperscript{th} temporal moment (the integral of the concentration with respect to time) of Raz and Rru at the upstream (up) and downstream (down) site. The
mean travel time between the two sites ($\tau$) was calculated as the difference between the first temporal moments at the downstream and upstream sites (Schmadel et al. 2016). The ratio of the discharges at the upstream and downstream site was included to compensate for the dilution effect. More detail on the biogeochemistry of the Raz-Rru reaction, and further specifics of this reactive tracer approach are provided in González-Pinzón et al. (2012) and Knapp et al. (2018).

2.8. Statistical analysis

All data exploration and analyses were conducted in R (R Core Team 2021) and data used in this study are available at DOI:10.5281/zenodo.4696775. Data were initially investigated for normality, heteroscedasticity and outliers (Zuur et al. 2010).

Temperature duration curves were constructed for water temperature in each of the streams (cf. Hannah et al. 2009; Khamis et al. 2015). This method provides a graphical representation of the percentage of time that a specific water temperature is equalled or exceeded. To generate these duration curves, we used the ‘HydroTSM’ package (Zambrano-Bigiarini 2020), developed for calculating and visualising flow duration curves. The steeper the curve the more variable the water temperature, with flat lines indicating high thermal stability in the stream. We also calculated the coefficient of variation ($CV = \text{standard deviation/mean}$) to understand temperature variability. Water temperature was also used as an indicator of stream conditions, including free-flow, surface freezing, flow cessation and snow cover (Brown et al. 2006a).

Stream discharge data were used to identify high and low flow events. Storm events were classified as periods when the total stream discharge exceeded the mean annual baseflow by 20% for >24 hours using the ‘hydromad’ package (Andrews et al. 2011). We determined baseflow using one variable recursive digital filtering (Eckhardt 2005).
with a constant of 0.96 using the ‘FlowScreen’ package (Dierauer & Whitfield 2019).

For each identified storm event (N = 18), all of which were detected in a single snowmelt fed stream (Tokusawa), we calculated maximum discharge (m$^3$s$^{-1}$), mean water temperature (ºC) and storm duration (hours). These metrics of the storm events (data were first standardised to one standard deviation and mean centred) were analysed using Principal Components Analysis (PCA) (Abdi & Williams 2010). We use the position of storm events on the first two principal components of the PCA to classify storms into different types (i.e., long duration summer storms). We calculated CV for continuous data to understand variability in stream discharge over the sampling period. Finally, flow cessation was indicated by complete flow cessation, i.e., zero water depth, and supported by water temperature data (see method above).

Water chemistry (dissolved oxygen, total dissolved solids and major ion concentrations) and metabolic activity data were investigated using a series of Generalised Linear Models (GLMs) (Nelder & Baker 2006) and Generalised Linear Mixed Models (GLMMs) (Bolker et al. 2009), using site as a random effect to control for autocorrelation as a result of the sampling regime. Model structure, families and link functions depended on the variable of interest and are reported in the results (also see Table S1). All model assumptions were validated following Zuur et al. (2007) and Thomas et al. (2015), by assessing the residual normality using QQ plots, homogeneity of variance determined by plotting the residuals against fitted values and influential observations using Cook’s leverage distances.

Major ion data were also converted to milliequivalents per litre (meq L$^{-1}$) using the ‘smwrBase’ package (Lorenz 2015). Data for individual ions were then converted to percentage of total major ions (%) using the ‘hydrogeo’ package (English 2017) for Piper diagrams (Piper 1944).
3. Results

3.1. Stream morphology

Streams across the Kamikochi region were highly variable in their physical and chemical characteristics, in relation to both water source contributions and other site-specific factors (Table 1). Physicochemical characteristics were variable across streams but were related to the dominant water source (groundwater or snowmelt). Channel width was not significantly different between groundwater and snowmelt fed streams (Square root transformed Gaussian GLM: $R^2 = 0.95$, $t_{1,4} = 105.5$, $p = 0.08$). There were, however, significant differences in water depth (Gaussian GLM: $R^2 = 0.80$, $t_{1,4} = 20.9$, $p = 0.01$) and the bottom component of the Pfankuch index (Gaussian GLM: $R^2 = 0.65$, $t_{1,4} = 3.21$, $p = 0.033$) between snowmelt and groundwater streams. In general, groundwater streams were characterised by higher channel stability (lower Pfankuch index values) and depth in comparison to snowmelt fed streams (Table 1).

3.2. Water temperature

Variation in water temperature was high across four of the streams, with large diurnal and seasonal fluctuations (Figure 2). Two groundwater fed streams (Shimizugawa and Minamisawa), however, had low temporal variation in water temperature variation over the 16-month study period ($5.38 \pm 0.43$ SD, and $5.29 \pm 0.46$ SD, respectively). The other groundwater (Bentenzawa - $3.55 \pm 4.59$ SD) and snowmelt streams (Dakesawa, Shirasawa and Tokusawa) exhibited markedly higher levels of temperature variation ($6.06 \pm 3.38$ SD, $8.16 \pm 5.47$ SD and $5.24 \pm 5.98$ SD, respectively), and water temperatures ranged from below zero up to nearly 20 °C (Figure 2). Several of the loggers recorded extremely low temperatures, i.e., significantly below freezing (Figure 2a).
Duration curves for water temperature in groundwater streams had extremely shallow slopes, indicating low temporal variability (Figure 2b). Conversely, snowmelt fed streams had characteristic duration curves with steep slopes, indicating greater variation and lower/higher stream temperature. Individual snowmelt streams, however, maintained relatively unique duration curves and exhibited different levels of temporal variability in water temperature. In particular, Dakesawa had a shallower curve with a slope between that of the groundwater streams and other snowmelt fed streams (Figure 2b).

Extreme low water temperature recorded on a number of sensors also hinted at periods of dewatering in several streams. Of particular note were two prolonged winter and spring no flow periods in both Shirasawa and Tokusawa snowmelt fed streams. Tokusawa exhibited low temperatures (mean = -3.34 °C, min = -8.74 °C, max = 0.55 °C) over approximately 3 months, with high variation (CV = 1.82). In comparison, Shirasawa had lower variation (CV = 0.32), however, only a short period of temperatures below 0 °C was recorded before the logger finished recording (December 2017).

### 3.3. Stream discharge

Variation in stream discharge was significant within and across streams observed in the Kamikochi region (Figure 3). Snowmelt streams exhibited higher variability in stream discharge (CV = 0.005–0.02) in comparison to groundwater fed streams (CV = 0.001–0.006). There was, however, substantial variation in the stream discharge between the individual streams and overlap between the two groups of streams (groundwater and snowmelt), indicating site-specific discharge characteristics. Again, Dakesawa appeared less variable than the other snowmelt streams, and Bentenzawa was more variable than the other groundwater streams.
The snowmelt fed stream, Tokusawa, exhibited the highest levels of discharge variation with a total of 18 storm events identified (Figure 3). The mean stream discharge during storm events was $0.12 \pm 0.001 \text{ m}^3 \text{s}^{-1}$ and on average the duration of these events lasted for 132 hours (26–427 hours). These storm events occurred relatively consistently throughout both 2017 and 2018, with no association to specific months or seasons (Figure 4a). Many of the storms occurred shortly after peaks in daily precipitation (Figure 4b). Several storm events, however, occurred when there were low levels of precipitation, especially during spring 2017, but there were substantial snowpack depths and higher spring air temperature (Figure 4b). Furthermore, some discharge peaks occur after precipitation events on high snowpack depths. Storms with different characteristics occurred at different times of the year, and storm events clustered into two groups on the principal components (Figure 4c). These groups were: (1) warm temperature, long duration storm flows that occurred in summer and autumn; and (2) cold temperature, short duration storm flow events that occurred in winter.

### 3.4. Water chemistry

Anion and cation concentrations were highly variable within and across streams (Table 2). Several ions, including NO$_2^-$, NO$_3^-$ and SiO$_2^-$, were below limits of detection at sites at different sampling intervals, and NH$_4^+$ was only detected in 3 of the 83 samples during the sample period (Table S2). Marked variation was found in the commonly detected major ions, with similar patterns across the sites (Figure 5). Concentrations of all major ions at the sites were markedly different, although several sites were more similar to one another, in comparison to others (Figure 5). Once variation between sites was controlled for using random effects in GLMMs (Table 2), many of the major ion concentrations were similar between groundwater and snowmelt
streams. High inter-site variation, indicated by high standard deviations for random
effect terms and the large difference between marginal and conditional $R^2$ values,
masked differences between streams with different water sources (Table 2). Nitrate
(NO$_3^-$) concentrations were significantly different between snowmelt and groundwater
fed streams, with groundwater streams showing higher values (Figure 5).

3.5. Metabolic activity

Transformation rates of Raz to Rru were relatively low across all streams ($0.58\text{--}1.22$
$x 10^{-3}$ s$^{-1}$) (Table 4). The groundwater stream Shimizugawa had a higher Raz to Rru
transformation ($1.22 \times 10^{-3}$ s$^{-1}$) than the other groundwater and the two snowmelt
streams. Indeed, the other groundwater stream, Bentenzawa, showed the lowest
transformation rate ($0.58 \times 10^{-3}$ s$^{-1}$). Variation between the snowmelt streams was
lower ($0.07 \times 10^{-3}$ s$^{-1}$) in comparison to that between the groundwater streams ($0.62 \times$
$10^{-3}$ s$^{-1}$). The transformation rate for the snowmelt stream Dakesawa was not
determined as the peak of the breakthrough-curve at the upstream site was not
captured.

4. Discussion

Studies of alpine streams across the globe have shown relatively consistent patterns
of habitat templates (i.e., morphology, hydrology, physicochemistry) and biota in
relation to water source contributions (groundwater, snowmelt and glacial meltwater),
even across different biogeographical zones (Hannah et al. 2007; Brown et al. 2009).
Here, we show that variation in morphology, hydrology, physicochemistry and
metabolic signatures in streams across the Kamikochi region of Japan predominantly
conform to these patterns. Nevertheless, the high levels of inter-site variation, even
between streams with similar water source dynamics, particularly in those dominated
by snowmelt, appears a unique feature of the systems investigated in this study. However, the relatively small number of streams studied here (n = 6) restricts widely generalisable conclusions past this region of the Japanese Alps. However, if the highly variable conditions observed in these streams are consistent across other alpine stream systems in the Japanese archipelago, then these ecosystems could allow for interesting additional insights in both hydrology and ecology in alpine stream systems.

Environmental conditions in the two groups of streams, groundwater and snowmelt, were generally different from one another thereby supporting H₁ "Water source will significantly influence morphological, hydrological and physicochemical signatures between the study streams". These findings are similar to those from other regions of the globe (e.g., Europe, North America and the Arctic), where groundwater fed streams typically show low temporal variation in discharge, water temperature and water chemistry compared to other systems (Brown et al. 2003, 2006a, 2007b; Milner et al. 2010). Studies from other regions commonly indicate that streams with the same dominant water source are more similar to one another, i.e., groundwater streams are generally all more stable with lower variation and snowmelt streams are similarly more variable (Brown et al. 2007b, 2009; Hannah et al. 2007; Milner et al. 2010; Khamis et al. 2016). The groundwater streams in the Kamikochi region were generally more similar to one another than to snowmelt fed streams. Snowmelt fed streams exhibited large amounts of variation between each stream. In particular, there were significant differences in diurnal and seasonal variation. A large number of high flow events were evident at the snowmelt stream, Tokusawa, in contrast with all other streams. Discharge data, however, were not available for another snowmelt fed stream, Shirasawa, due to sensor loss in 2018 and it is likely high flow events also occurred here based on visual observations. Other findings, however, indicate Shirasawa
exhibited similar albeit less extreme hydrological variation, with temperature loggers detecting flow cessation (low recorded temperatures due to air exposure or freezing; Brown et al. 2006a) at a similar time to Tokusawa. Furthermore, the reason for the sensor loss at the site was a large high flow event, again occurring at a similar time to the series of high flow events detected in Tokusawa between April and November 2018.

Higher rates of metabolic activity are attributed to differences in water sources contributions, specifically the high environmental stability in groundwater dominated streams. As only one groundwater stream had a higher rate of metabolic activity than the snowmelt streams, there was limited support for hypothesis H$_2$ “Metabolic activity varies significantly across sites and will be higher in the groundwater systems”. Although in a study with a greater sample size different patterns may be observed, several potential explanations exist for the patterns observed across the streams in this study. In general, the findings in this study indicate that environmental stability (i.e., stable flow regime, stable sediments and less variable water temperature), as observed in the groundwater stream Shimizugawa, may be favourable for high rates of metabolic activity. Across other groundwater streams the environmental conditions, particularly consistent flow and temperature, enables the persistence of organic matter and production of benthic algae, as well as a high rate of utilisation and decomposition of these resources (Tank et al. 2010). Furthermore, the stability allows for the colonisation of the streams by macroalgae and macrophytes, which have a strong association with increased stream respiration and metabolism (Alnoee et al. 2016). Kurz et al. (2017) modelled Raz transformation rates in vegetated flumes and found that transformation rate in the advection-dispersion dominated mobile zone, where vegetation was a dominant influence, was an order of magnitude higher than in the
transient-storage (immobile) zone. Thus, it is apparent that the abundances of macrophytes, associated epiphytes and the influence of these plants on benthic heterotrophic processes (Logue et al. 2004), influence the levels of metabolic activity in streams. Certainly, Shimizugawa, the groundwater stream with Raz transformation rates orders of magnitude higher than the other streams, had very high macrophyte abundance (see Figure S1). Our understanding of the direct and indirect drivers of stream metabolism in alpine streams with different water sources, however, is not well developed and further research is required to fully determine the drivers of this important ecosystem process.

Groundwater and snowmelt streams were generally dissimilar and groundwater streams were less variable supporting the third hypothesis H₃ “Hydrological, physicochemical and metabolic signatures in groundwater streams will be less variable when compared to snowmelt streams”. Thus, although the climatological conditions present in the Japanese Alps are relatively unique, the dominant patterns across streams (i.e., groundwater streams are more temporally stable) do not markedly differ from other regions across the globe. However, significant variation in water chemistry, water temperature and metabolic activity between the three groundwater streams was found. These differences are likely due to a number of reasons either solely or combined (i) ground water had a variable residence time in subsurface flow paths; (ii) the dominant subsurface flow paths are relatively shallow and the transit time of water through them is highly variable; or (iii) the underlying geology and weathering processes across the three groundwater streams are different. These reasons are supported by the hydrographs for the groundwater streams, which although less variable than the snowmelt streams, still exhibited
delayed flow increases in both summer and winter in response to snow melt discharge and rainfall (see Milner et al. 2020).

Across the stream systems investigated in this study, high inter-site variation was not explained by the dominant water source contribution, particularly for snowmelt streams. Studies from other alpine streams, suggest that even systems dominated by one water source can exhibit differences in hydrology, water chemistry and ecosystem functions, as a result of contributions of other water sources (e.g., interflow and surface runoff; Brown et al. 2009). It is likely, therefore, that some of the variation in hydrology, physicochemistry and metabolic activity between streams with the same dominant water sources (snowmelt and groundwater) may be due to other water source contributions. For example, Dakesawa, considered a snowmelt dominated stream, showed intermediate degrees of variation in water temperature and discharge between that of three groundwater streams and the two other snowmelt fed streams.

The findings of this study provide further evidence of the variability in the habitat templates in alpine stream systems according to water source. The suite of environmental conditions present in any given stream has important implications for the structure and function of these ecosystems (Brown et al. 2003, 2009; Hannah et al. 2007). Here, we showed that stable morphological, hydrological and physicochemical conditions in groundwater fed streams were typically associated with higher rates of metabolic activity. This is likely due to a lower number of high flow events and lower concentrations of suspended sediments (lower turbidity) in the Kamikochi groundwater streams (see Milner et al. 2020), allowing for greater retention of organic detritus and higher algal production, respectively. The lower temporal variation in hydrological and physicochemical conditions in groundwater stream systems potentially enables higher levels of primary productivity, as has been
documented in other stream systems (LaPerriere et al. 1989; Tockner et al. 1997; Klein & Tockner 2000; Rott et al. 2006). Higher primary productivity is also expected to translate to greater levels of secondary productivity, and is a potential explanation for observed differences in macroinvertebrates between stream systems with different water sources both in the Kamikochi streams (Milner et al. 2020), and other alpine stream systems (Brown et al. 2004, 2006b). In a corollary study we showed that a greater macroinvertebrate abundance in groundwater streams may have resulted from higher chlorophyll $a$ concentrations, an indicator of primary productivity (Milner et al. 2020).

**Conclusion**

In summary, snowmelt and groundwater streams in this unique bioregion of the Japanese Alps showed highly variable environmental conditions between them, providing a range of habitat templates for biotic communities. Metabolic activity, an important indicator of ecosystem functioning, varied between streams with different water sources due to environmental stability and its effects on biological communities (e.g., submerged macrophytes). Patterns in environmental conditions across the streams in the Japanese Alps were quite similar to other alpine regions of the globe, but it was clear that the unique climatological conditions in the Japanese Alps did create several noticeable differences, including greater inter-stream variability. This high variability of the environmental conditions within and between stream systems, combined with the unique flora and fauna of this biogeographic region therefore make it an invaluable reserve of biodiversity across the globe.

Further research is now required to advance the understanding of how environmental change may affect these systems into the future. The high variability in flow conditions present in the snowmelt streams indicates that further meteorological change, for
example reduced snowpacks (Sato et al. 2013) and increased frequency of typhoons alongside heavy rainfall events (Tsunematsu et al. 2013), may lead to greater frequencies of extreme high and low flow events. For example, reduced snowpacks may lead to increased intermittency during low flow periods of July and August in snowmelt fed streams. Groundwater fed streams appear more resilient to these alterations, with far lower hydrological variation, yet the impact of extreme events in snowmelt streams may be significant enough to generate changes in aquatic biodiversity. Hence management methods to mitigate potential losses in biodiversity are essential.
5. References


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6. Acknowledgements

Funding was provided by a JSPS Long Term Fellowship L16553 awarded to AM and by Shinshu University for CD. This research was part of the EU 2020 HiFreq project led by the University of Birmingham (Grant Agreement ID: 734317). We thank Professor Keisuke Suzuki (University of Shinshu) and Dr Akihiko Sasaki (Kokushikan University) together with the staff of the Mountain Environmental Science Center of Shinshu for the meteorological observations. Thanks to Masaki Dr Takenaka, Dr Tomoya Suzuki, Dr Koki Yano, and Dr Miquel Vall-Ilossera Camps for assistance with collecting samples.
7. Figures

Figure 1. Sample sites at the six streams within the Kamikochi region of the Japanese Alps.
Figure 2. Water temperature (°C) across the groundwater and snowmelt fed streams. (a) Water temperature time series data. (b) Water temperature duration curves (Minamisawa and Shimizugawa overlap). Following Brown et al. (2006a), temperatures below 0 ºC in (a) are indicative of flow cessation either due to freezing or drying processes. Inset graphs show diurnal variation over two separate weeks in September 2017 and June 2018.
Figure 3. Stream discharge time series and variation estimates. CV = Coefficient of variation. Inset figures demonstrate discharge variation at diurnal timescales for periods selected from the wider time series in each stream. Figures to the right of the time series are flow duration curves for the time series data (x axis indicates % time exceedance for the given discharge value on the y axis).
Figure 4. Storm events and characteristics over the sampling period in a snowmelt fed stream (Tokusawa). (a) Stream discharge time series. (b) Daily precipitation, (c) mean daily air temperature and (d) monthly measured snowpack depth from the Kamikochi Meteorological Station. (e) Storm event characteristics from principal components analysis (n = 18).
Figure 5. Variation in regularly detected major ion concentrations across the six streams in the Kamikochi region of the Japanese Alps. For piper diagrams (a-b) mg L$^{-1}$ data were converted in percentage meq L$^{-1}$ using the ‘smwrBase’ and ‘hydrogeo’ packages in R. Major ions not included in (a-b) are presented in boxplots in (c-e). Colours in (c-e) indicate the dominant water source contribution within each stream (red = groundwater, blue = snowmelt).
8. Tables

Table 1. Coordinates and physical characteristics of sample streams.

<table>
<thead>
<tr>
<th>Site</th>
<th>Decimal degrees</th>
<th>Water source</th>
<th>Mean width (m)</th>
<th>Mean depth (cm)</th>
<th>Pfankuch Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latitude</td>
<td>Longitude</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shimizugawa</td>
<td>36.250482</td>
<td>137.639672</td>
<td>GW</td>
<td>9.4</td>
<td>27.0</td>
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<td>Minamisawa</td>
<td>36.256573</td>
<td>137.643104</td>
<td>GW</td>
<td>7.5</td>
<td>26.4</td>
</tr>
<tr>
<td>Bentenzawa</td>
<td>36.265480</td>
<td>137.690354</td>
<td>GW</td>
<td>4.6</td>
<td>21.8</td>
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<tr>
<td>Tokusawa</td>
<td>36.261673</td>
<td>137.693700</td>
<td>SM</td>
<td>4.2</td>
<td>17.0</td>
</tr>
<tr>
<td>Dakesawa</td>
<td>36.253908</td>
<td>137.634887</td>
<td>SM</td>
<td>4.1</td>
<td>14.7</td>
</tr>
<tr>
<td>Shirasawa</td>
<td>36.248523</td>
<td>137.670093</td>
<td>SM</td>
<td>3.6</td>
<td>11.2</td>
</tr>
</tbody>
</table>
Table 2. Variation in major ion concentrations. Statistical results are derived from a series of GLMMs. Df = Degrees of freedom (numerator and denominator).

<table>
<thead>
<tr>
<th>Ion</th>
<th>Mean (min–max) (mg L⁻¹)</th>
<th>Model results</th>
<th>Random effect (variance ± SD)</th>
<th>Variable</th>
<th>t value*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R²m</td>
<td>R²c</td>
<td>Df</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>1.34 (0.53–2.02)</td>
<td>0.28</td>
<td>0.73</td>
<td>12, 67</td>
<td>0.08 ± 0.28</td>
<td>Water source</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sample time (date)</td>
</tr>
<tr>
<td>K⁺</td>
<td>0.22 (0.01–0.07)</td>
<td>0.22</td>
<td>0.54</td>
<td>12, 67</td>
<td>0.01 ± 0.08</td>
<td>Water source</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td>Sample time (date)</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.35 (0.07–1.29) **</td>
<td>0.03</td>
<td>0.90</td>
<td>12, 64</td>
<td>0.03 ± 0.16</td>
<td>Water source</td>
</tr>
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<td>Sample time (date)</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>4.47 (0.57–8.3)</td>
<td>0.04</td>
<td>0.92</td>
<td>12, 66</td>
<td>5.8 ± 2.41</td>
<td>Water source</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td>Sample time (date)</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>0.3 (0.13–0.78)</td>
<td>0.56</td>
<td>0.71</td>
<td>12, 68</td>
<td>0.002 ± 0.05</td>
<td>Water source</td>
</tr>
<tr>
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<td></td>
<td>Sample time (date)</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>0.001 (0–0.003)</td>
<td>0.38</td>
<td>0.38</td>
<td>12, 44</td>
<td>0 ± 0 ***</td>
<td>Water source</td>
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<td>Sample time (date)</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>0.14 (0.03–0.32) **</td>
<td>0.75</td>
<td>0.77</td>
<td>12, 67</td>
<td>0.0001 ± 0.007</td>
<td>Water source</td>
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<td>Sample time (date)</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>3.54 (1.03–9.36) ****</td>
<td>0.15</td>
<td>0.60</td>
<td>12, 67</td>
<td>1.54 ± 1.24</td>
<td>Water source</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Sample time (date)</td>
</tr>
<tr>
<td>SiO₂</td>
<td>6.28 (3.3–8.6)</td>
<td>0.17</td>
<td>0.66</td>
<td>5, 19</td>
<td>1.19 ± 1.09</td>
<td>Water source</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sample time (date)</td>
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</tbody>
</table>

* Calculated from single term deletions using Satterthwaite’s method
** Outlying values (Cook’s distance > 0.5) were removed from the model (n = 1–2)
*** A singular model, i.e., parameters are on the boundary of the feasible parameter space, as NO₂⁻ was not detected across all sites
**** Non-significant models
Table 3. Raz transformation rates and mean water temperature at injection for four streams in the Kamikochi region of the Japanese Alps.

<table>
<thead>
<tr>
<th>Site</th>
<th>Water source</th>
<th>Raz transformation rate ($10^3$ s$^{-1}$)</th>
<th>Temperature (°C) during injection ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shimizugawa</td>
<td>GW</td>
<td>1.22</td>
<td>5.39 ± 0.08</td>
</tr>
<tr>
<td>Bentenzawa</td>
<td>GW</td>
<td>0.58</td>
<td>5.04 ± 0.09</td>
</tr>
<tr>
<td>Shirasawa</td>
<td>SM</td>
<td>0.88</td>
<td>3.70 ± 0.08</td>
</tr>
<tr>
<td>Tokusawa</td>
<td>SM</td>
<td>0.81</td>
<td>3.25 ± 0.05</td>
</tr>
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</table>