Effects of locality and stone surface structure on the distribution of Collembola inhabiting the stone-ice border on an alpine glacier

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Abstract

Supraglacial zones worldwide serve as suitable habitats for psychrophiles including metazoans. In this study, we tested whether the occurrence and abundance of springtails (Collembola) occurring in water films under stones on the Forni Glacier in the Alps were affected by a) the locality of the stone (i.e. stones located in supraglacial streams, on bare ice, in the forefield, on the medial moraine), b) the distance from the glacier terminus, c) the roughness of the stone surface, and d) the inclination of the stone to the ice. We found that springtails inhabit stones located only on ice and their abundance showed no relation with distance from the glacier terminus. They were also more frequent under stones located on bare ice than under those in the supraglacial streams, medial moraine or glacier forefield. The roughness of stone surface adjacent to ice had a
positive effect on animal counts, while the inclination had no effect. We estimated that the total abundance of springtails under stones on the tongue of Forni may reach up to 13.6 million individuals. Since springtails are important components of ecosystems, estimation of their distribution and abundance in glacial environments may facilitate understanding of supraglacial ecosystems in the Alps.

**Keywords** Arthropods, Forni glacier, glacial biome, springtails, supraglacial zone

**Introduction**

The supraglacial zone of glaciers are exposed to harsh conditions such as low temperatures (oscillating around 0 °C during summer), high doses of UV-radiation and periodic freezing. Despite these conditions, several groups of organisms are strictly adapted to survive and reproduce in these environments (Anesio and Laybourn-Parry 2012; Franzetti et al. 2016; Hodson et al. 2008; Kohshima 1984; Mueller et al. 2001; Sommers et al. 2017). Debris-covered glaciers (i.e. glaciers whose ablation zone is mostly covered by debris) typically host relatively high biodiversity and complex trophic networks, and several studies have been devoted to the biodiversity and ecology of these peculiar ice bodies and to their role as potential warm-stage refugia for cold-adapted organisms (Azzoni et al. 2015; Caccianiga et al. 2011; Gobbi et al. 2010, 2017; Tampucci et al. 2017a, b). In contrast, glaciers without continuous debris cover seem to host different biotic assemblages and simpler trophic webs, mainly connected with a dark-coloured sediment typical of glaciers called ‘cryoconite’ (Hodson et al. 2008).
Cryoconite is a peculiar type of sediment that forms on glaciers by sticking together mineral matter and organisms (i.e. archaea, bacteria, fungi, algae) by extracellular polymeric substances (Anesio et al. 2017). Such polysaccharides are produced by numerous species of cyanobacteria (glacial ecosystem engineers) in response to environmental stress caused by low temperatures and high doses of UV-radiation (Takeuchi et al. 2001). Due to the lower albedo of cryoconite than the surrounding bare ice, sediment melts into ice forming cryoconite holes. These freshwater bodies act as bioreactors, producing organic matter and are considered the most biologically active environments on the glacier surface (Cook et al. 2016).

In general, knowledge on the faunal diversity of both types of glaciers, namely debris-covered and bare, is relatively limited in comparison to other ecosystems on Earth (e.g. De Smet and Van Rompu 1994; Porazinska 2004; Tampucci et al. 2017a, b; Zawierucha et al. 2019b). Supraglacial zones are inhabited by invertebrates, which live mostly in cryoconite holes (mainly Rotifera and Tardigrada (e.g. De Smet and Van Rompu 1994; Porazinska et al. 2004; Zawierucha et al. 2019b)), in mosses directly on the ice surface, so called ‘glacier mice’ (Collembola, Nematoda, Tardigrada (Coulson and Midgley 2012)), and glacier moss gemmae aggregations on glaciers in Uganda (Tardigrada and Rotifera (Uetake et al. 2016; Zawierucha et al. 2018)). Furthermore, some species occur in more than one habitat. For instance, on Himalayan glaciers Glaciella yalensis (Copepoda) and Diamesa sp. (Insecta) live on the surface of ice, but inhabit also small cryoconite holes (Kikuchi 1994; Kohshima 1984). On Patagonian glaciers, insects like the glacier stonefly Andiperla willinki live on the glacier surface, in supraglacial streams and in cryoconite holes, while springtails (Collembola) live directly on ice (Kohshima et al. 2002). Springtails on glaciers seem to be one of the most diverse animal groups
(various species belong to the *Desoria*, *Myopia*, *Pseudisotoma*, and *Gnathisotoma* genera) recorded on glaciers worldwide (e.g. Coulson and Midgley 2012; Fjellberg 2010; Gobbi et al. 2010).

Springtails (Collembola) are small, mostly terrestrial arthropods (up to a few millimetres in length) considered as a cosmopolitan group (Deharveng et al. 2007; Rusek 1998). In terrestrial environments, they feed on detritus, plants, fungi or other small invertebrates, and are extremely diverse in terms of morphology and ecological function (Rusek 1998). Owing to their various functions in the trophic networks, their high densities and biomass, springtails are an important component of many ecosystems in terms of nutrient cycling and energy flow through trophic networks (Rusek 1998).

Many species of springtails are known from alpine and polar regions where they play an important role as decomposers (Hågvar 2010; König et al. 2011; Raso et al. 2014). In glacier forefields, they have a large impact on pedogenesis through the recycling of matter (Hågvar 2010; Kaufmann et al. 2002; König et al. 2011). Previous studies have presented data on the distribution, ecology, and diversity of springtails in the supraglacial environments in North and South America, the Caucasus, Iceland and surface of debris-covered glaciers in the Alps (e.g. Coulson and Midgley 2012; Fjellberg 2010; Gobbi et al. 2010; Kohshima et al. 2002; Makowska et al. 2016). However, there are no specific data and analyses about the estimation of their total abundance and their spatial distribution on glaciers.

In this study, we focused on factors determining the distribution and abundance of springtails on an alpine glacier and their occurrence under supraglacial stones - a type of supraglacial habitat not described so far. Moreover, for the first time, we present a
snapshot of their total abundance for the whole ablation zone of a glacier. Based on observations in the field, we hypothesized that sediment may be flushed from cryoconite holes during intense melt or rain. The washed cryoconite may then be retained under supraglacial stones, thus providing nourishment for cryophilic springtails (Figure 1). The film of water between supraglacial stones and ice may therefore be considered a peculiar ecological niche on glacier surface. Our first aim was to investigate whether the presence of springtails was linked to supraglacial environments (for instance due to easy access to nourishment, a proper temperature regime or the presence of a film of water). To this end, we compared their occurrence under stones located a) in the glacier forefield (stones in contact with the ground), b) the medial moraine (stones in contact with other stones), and c) on the glacier tongue (stones in contact with ice). Second, we examined the changes in springtail abundance along transects at regular distances from the glacier terminus. However, we had no clear expectation on the change in springtail abundance along this transect because of the very poor knowledge of springtail ecology in these environments. We hypothesized that water runoff may have a great impact on springtail abundance because during intense melting of glacier, it may wash them away from stones. We therefore tested whether stones located in temporary supraglacial streams (“bedieres”) are less inhabited by springtails than those located outside bedieres. Moreover, we tested the effect of habitat structure, such as the roughness of stones and inclination of the stone to the ice surface, as factors influencing springtail abundance through protection against removal.
Methods

Study area and sampling design

The Forni Glacier (46°12’30"N, 10°13’50"E) is a valley glacier located in the Central Italian Alps belonging to Ortles-Cevedale Group. It is 10.83 km² in area and its elevation ranges from 2600 to 3670 m a.s.l (Azzoni et al. 2017). Fieldwork was conducted on 24 July 2018. In order to investigate whether the abundance of springtails varied among stones located in the glacier forefield or directly on ice, as well as with the distance from the glacier terminus, we performed sampling along transects parallel to one another and perpendicular to the glacier tongue axis at approximately 50 m of distance to one another. Importantly, this sampling design implies that each stone in a particular transect was approximately at the same altitude and at the same distance from the glacier terminus. In each transect, we counted the number of springtails under 10 stones, at about 1 m to one another, and we recorded parameters such as the roughness of the stone surface, the inclination between the stone and the ice, and the stone surface area adjacent to the ice. The inclination of each stone relative to the surface of the ice was assessed on a 3-point scale (1-flat, up to ~5°; 2-slight slope, between ~5° and ~30°; 3-high slope, more than 30°). The roughness of the stone surface in contact with ice was determined on a 3-point scale (1-smooth, 2-slightly rugged, 3-very rugged, see Figure S1).

Overall, we collected data from 14 transects, three in the forefield and eleven on the glacier. Transects on the glacier included stones on bare ice as well as on the medial and lateral moraines (Figure 2). We also investigated the presence of springtails under 135 randomly chosen stones along the medial moraine to check whether springtails live only
on bare ice (Figure 2). Finally, in order to determine the effect of runoff water on springtail occurrence, we checked the presence of the springtails under 70 randomly chosen stones found directly on ice and 70 randomly chosen stones found in bedieres. Each stone in the transects through the ablation zone on bare ice (n = 100) was turned over and a high-resolution picture was taken (Sony Cyber-shot, DSC-WX 300). In order to determine the surface size and abundance of springtails, a scale (accuracy of 1 millimetre, Figure S2) was included in each picture.

**Measurements of surface, count of springtails and total abundance estimation**

Based on the photo with the scale, we calculated the surface of stones adhering to the ice using ImageJ software (Abramoff et al. 2004), with the assumption that the surface was flat. It was not possible to assess the surface size more accurately based on 2D photos. For 11 stones, we counted the number of springtails in the field. For the other stones, counts were performed on photos of stone and the surface of ice where the stone was located because springtails can remain on both the stone and the ice when the stone is turned out. If the counting of all springtails was not possible due to high densities, in areas where they were not visible due to overlapping, we count them as a single layer without considering the individuals under them, while for the rest of the stones where the animals were visible, we counted them all. Thus, we may have slightly underestimated the total amount of springtails. We estimate the total number of springtails on the Forni Glacier based on the total number of stones located on the ablation tongue and the density of springtails obtained from 98 stones. This procedure was performed through ImageJ software using high-resolution drone orthophotos (pixel...
size 0.08 m × 0.08 m) of the glacier surface: considering the accuracy of the orthophotos, only stones bigger than 15 cm were included in this analysis.

**Statistical analysis**

We tested whether the counts of the springtails under stones are related to a) distance from the glacier terminus, b) roughness of the stone surface in contact with ice, and c) stone inclination with respect to ice using a generalized linear model (GLM) implemented via the MASS package (Venables and Ripley 2002) in R 3.4.2 software (R Core Team 2017). We built a negative binomial model (using a log-link function) with the count of springtails as the response variable, roughness, inclination and the distance from the front of the glacier (every 50 m) as fixed effects and surface of stone (log-transformed) as an offset (global model). Stones on medial and lateral moraines (n = 10) were excluded from the above model. Due to the low number of stones with 3rd degree of roughness (n = 5) and with 3rd degree of inclination (n = 6) we merged the 2nd and 3rd degree of both these variables into one category. We then relied on an information theoretic approach to assess the relative contribution of the different variables to explain the observed springtail distribution. To assess the goodness fit of the models, we used the Akaike Information Criterion (AIC). Models with the best fit (ΔAIC < 2) were averaged by the MuMIn package (Barton 2018) and the conditional average was built from the best-fit models. Assumptions of GLMs were checked separately for each model included in the conditional average.

**Results**
The abundance of springtails under a single stone on ice ranged from 0 to 3,807. The minimum size of the analysed stone surface was 12 cm² and the maximum 1,153 cm² with a mean surface of 185 cm² (Table 2). Density of individuals on the tongue of Forni Glacier covered by sparse debris may thus be up to 155,398 individuals/m². We classified 32 stones as smooth, 73 as slightly rugged, and 5 as very rugged (after merging, 32 as smooth and 78 as rugged). The stones were categorized following their inclination as 72 flat stones, 32 slight-slope and 6 high-slope (after merging, 72 as flat and 38 as inclined). Springtails were more abundant under steep than under flat stones, and under rough than under smooth stones (Figures 3 and 4).

Springtails occurred under 79% of the stones laying directly on ice and only under 13% of the stones in bedieres (Table 1). They also occurred under 79% of stones located on the ablation zone (i.e. the stones in the transects), under 3% of stones in the forefield and 2% of stones in the medial moraine (Table 1).

The information theoretic approach selected six models with ΔAIC < 2 from the best model, which included all variabiles as the global model. The conditional average model, however, indicated that distance from the glacier terminus was not significantly related to the variation in springtail counts (Table 3). Furthermore, the inclination of stone had no significant effect on springtail counts. However, we found a slightly significant difference between smooth and rough stones on springtails counts, with more abundant springtails under rough stones (Table 3).

Using drone images, we estimated that stones larger than 15 cm covered between 633.05 and 780.45 m² of the ablation zone of the Forni Glacier excluding the central and the lateral moraines. This means that this estimate includes only stones scattered on
the surface of the ice. Thus, we estimated that the total springtail number under these stones could be between 10.1 and 13.6 million individuals on the glacier ablation tongue.

**Discussion**

Our results show that springtails on the Forni Glacier inhabit mostly the peculiar environment at the interface between supraglacial stones and ice in high density up to 17,432 individuals per square meter of ablation zone of the glacier (Table 2). On the Forni Glacier, springtails were found much more frequently under stones sparse on the glacier tongue than in the forefield or on the medial moraine, where they occurred under a minority of stones. On the glacier tongue, springtail occurrence and abundance were negatively affected by the presence of flowing water as shown by the much lower occurrence under stones in bedieres. In addition, we found a slightly larger density under stones with a rougher surface, which probably protects the animals against flushing. On the other hand, the stone inclination seemed not to affect springtail numbers, probably because it did not protect them against flowing water and densities seemed not to differ according to distance from the glacier terminus. These results confirm the negative effect of flowing water on the springtails’ ability to establish stable communities in bedieres, even under very rough stones. Positive effects of habitat heterogeneity on animal richness and density have been observed in several ecosystems like forests, grasslands or eutrophic ponds (e.g. Brönmark 1985; Freemark and Merriam 1986; Kindvall 1996). In the specific case of stone-ice habitats on glaciers, stones with rougher surfaces may more effectively protect against gentle flushing by providing numerous micro-crinkles between the ice and stones. In addition, they can potentially
trap cryoconite flowing in water more effectively than smooth stones. The potential scenario described above indicates that habitat (stone) heterogeneity may shape densities of glacial springtails. Contrary to our assumptions, we did not find a significant effect of the inclination of stone toward ice surface on animal counts. The fact that the surface adjacent to the ice is large and rugged can sufficiently determine the ability of animals to mitigate the rinsing, regardless of inclination. We note, however that the effects of these variables, were rather weak, thus suggesting that springtails occur at similar densities under all sparse stones on the ablation tongue of Forni Glacier, provided that they lay on ice and are not in bedieres.

Total abundance of springtails on the ablation area of the Forni Glacier (about 0.7 km²) may reach 13.6 million individuals, with a mean density of 17,432 individuals per m². We suspect that the real number of springtails may be even larger owing to the technical limitations in our estimates. Indeed, stones smaller than 15 cm in width, which were the most abundant in our study area, were not included in our analysis because they could not be distinguished from gravel, sand and finer sparse debris from drone images. Moreover, springtails on Forni inhabit also dirt cones (i.e. wet and fine gravel on the glacier tongue and moraines), which were not included in our estimates (Zawierucha et al. 2019b).

An assessment of arthropods in the High Arctic tundra reported that springtails may reach up to 21,000 and 38,000 ind./m² on two tundra lichen plots in Svalbard (Bengtson et al. 1974). These densities are higher than those reported here on Forni. However, Bengtson et al. (1974) considered both vegetation and the litter layer as well as the upper 3 cm of the soil layer where most arthropods should occur. Thus, these higher
densities are related to more variable habitats than the interface between stones and ice that we considered on Forni Glacier. Being the largest grazers in supraglacial habitats, springtails in the under-stone habitat probably feed on biogenic sediment (cryoconite) and may have a crucial impact on the functioning of supraglacial ecosystems. Most of the known springtail species are detritivorous (Rusek 1998), thus considering their total abundance and their relatively large size in comparison to other glacial invertebrates like tardigrades and rotifers, they can greatly contribute to matter and energy flow, and secondary production in a supraglacial zone. More specific studies are therefore needed to understand the ecological effect of springtails on the functioning of supraglacial ecosystems.

Springtails are widely adapted to live in low temperature, however, they have so far been studied mainly in terrestrial environments. Zettel (1999) presented a review of the phenological, physiological, morphological and behavioural adaptations of springtails that live in cold environments. Sinclair and Sjursen (2001) showed with field experiments conducted in the Antarctic that, in springtails, body glycerol, glucose and trehalose content varied across the seasons, moreover glycerol content was negatively correlated with its supercooling point (letal point). As a typical adaptation to high doses of UV radiation on glaciers, we observed intense black pigmentation in springtails, which most probably protect them from irradiation. Despite numerous adaptations to cold conditions, springtails only occasionally occur in cryoconite holes and in those cases they can be observed at the water’s surface, not in the layer of cryoconite at the bottom of the hole, where most organic matter concentrates (our personal observations). This limitation is probably due to a lack of adaptation of any springtail species to live
directly in water (Deharveng et al. 2007; Scott 1963). We therefore suspect that these individuals have been transported passively to cryoconite holes by wind or water.

The lack of springtails under stones in the forefield and medial moraine, as well as the lack of any effect of distance from the glacier terminus on the animals’ density, suggests that they are exclusive supraglacial dwellers. We can speculate that the lack of these animals under stones in the forefield and on the central moraine may be due to a lack of flushed cryoconite as well as to the higher temperature at the interface between stones and soil in the forefield or between stones and gravel on the moraine (i.e. most probably, these springtails are low temperature specialist), or even to the presence of a film of water at the stone-ice interface, which was absent under stones in the forefield and on the moraine (the ground under stones in the forefield was dry during sampling; our personal observations). Taking into account that the temperature on the ice surface is almost constant and does not exceed 1°C (Zawierucha et al. 2019a) and there is no precise data on organic matter content or on the availability of water under stones, we leave the question open on which of the following possible factors affects the occurrence of these animals in these habitats: presence of organic matter, proper temperature or film of water?

Despite many studies devoted to the functioning of glacial ecosystems and their biota such as bacteria or algae, knowledge about the densities and occurrence of cryophilic arthropods remains largely unexplored. Relations between organisms in a supraglacial zone and their function in energy and matter flow may be a key to understanding ecological processes in high mountains glaciers, for instance their effects on downstream ecosystems. Thus, our study, which revealed high densities of animals,
suggests the important role of springtails in glacial ecosystems. Taking into account the
role of springtails in terrestrial ecosystems, it is crucial to undertake further research on
the ecology and influence of these animals on the functioning of supraglacial
ecosystems, especially in the context of fast-changing alpine glaciers.

Acknowledgements

JB would like to thank Łukasz Ciecierski for constructive comments during the analysis
of the image.

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Table 1. The occurrence of springtails under stones in different localities on the glaciers and in its vicinity. 1 – stones located on bare ice in transect, 2 – stones located on bare ice beside bedieres.

<table>
<thead>
<tr>
<th></th>
<th>Forefield (n=30)</th>
<th>Medial moraine (n=135)</th>
<th>Bare ice¹ (n=100)</th>
<th>Bare ice² (n=70)</th>
<th>Stream (n=70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence</td>
<td>0.03</td>
<td>0.02</td>
<td>0.79</td>
<td>0.79</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Table 2. Values of numeric and factorial variables. Densities on bare ice estimated per area of stone [cm²].

<table>
<thead>
<tr>
<th>Numeric variable</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SE of mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface [cm²] (n = 98)</td>
<td>12</td>
<td>1153</td>
<td>185</td>
<td>19.76</td>
</tr>
<tr>
<td>Collembola counts on bare ice (n = 100)</td>
<td>0</td>
<td>3807</td>
<td>352</td>
<td>68.74</td>
</tr>
<tr>
<td>Collembola densities on bare ice [ind./cm²] (n = 98)</td>
<td>0</td>
<td>15.5</td>
<td>1.9</td>
<td>0.28</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Factor variable</th>
<th>1st degree</th>
<th>2nd degree</th>
<th>3rd degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roughness [n]</td>
<td>32</td>
<td>73</td>
<td>5</td>
</tr>
<tr>
<td>Inclination [n]</td>
<td>72</td>
<td>32</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 3. Results of global GLM ($\chi^2$=36.154, $p<0.001$, $df = 3$) and conditional average (from six models with $\Delta$AIC < 2 in comparison to best model).

<table>
<thead>
<tr>
<th>Explanatory Variables</th>
<th>Estimate</th>
<th>SE</th>
<th>z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.401</td>
<td>0.737</td>
<td>0.544</td>
<td>0.587</td>
</tr>
<tr>
<td>Distance from the glacier terminus</td>
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<td>0.063</td>
<td>-0.829</td>
<td>0.407</td>
</tr>
<tr>
<td>Inclination $2^{nd}$ degree</td>
<td>0.469</td>
<td>0.356</td>
<td>1.317</td>
<td>0.188</td>
</tr>
<tr>
<td>Roughness $2^{nd}$ degree</td>
<td>0.613</td>
<td>0.402</td>
<td>1.526</td>
<td>0.127</td>
</tr>
<tr>
<td><strong>Conditional average</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.247</td>
<td>0.603</td>
<td>0.407</td>
<td>0.6836</td>
</tr>
<tr>
<td>Distance from the glacier terminus</td>
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<td>0.066</td>
<td>0.935</td>
<td>0.3468</td>
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<tr>
<td>Inclination $2^{nd}$ degree</td>
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<td>0.357</td>
<td>1.389</td>
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<tr>
<td>Roughness $2^{nd}$ degree</td>
<td>0.787</td>
<td>0.396</td>
<td>1.961</td>
<td><strong>0.0498</strong></td>
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</table>


Figure 1. Stone-ice border habitat: a – ablation zone of Forni; b – stone on ice; c, d – springtails on bare ice under stone.
Figure 2. Part of the Forni glacier tongue with marked field study. White lines indicate individual transects, the black dotted square indicates the place where stones were checked for presence of the animals in bedieres and beyond, black solid polygon indicate area on medial moraine where presence of the animals where checked.
Figure 3. Springtails densities in relation to the inclination of the stone relative to the ice (two-level scale). Each black dot represents one sample (stone), horizontal distribution of dots is random.

Figure 4. Springtails densities in relation to the roughness of stone surface adjacent to the ice (two-level scale). Each black dot represents one sample (stone), horizontal distribution of dots is random.