Feeding on the roof of the world: the first gut content analysis of very high altitude Plecoptera

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The biology of Plecoptera from high altitude Himalayan freshwaters is almost unknown. Very few and sporadic studies have been devoted to these insects, and exclusively with a taxonomic approach. We provide information on the feeding of *Capnia* nymphs from three lakes localized above 4,800 m a.s.l. (NE Nepal). Nymphs from these high altitude lakes feed mainly on fine detritus, acting as collector-gatherers, with the accidental ingestion of mineral matter, mostly in larger specimens. It is likely that the harsh environmental conditions in our study area create an environment unfavorable to both the allochthonous input of coarse particulate organic matter (such as terrestrial leaves) and the autochthonous input related to aquatic primary productivity, so that these nymphs feed on small organic particles that originates mainly from the catchment.

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1. Introduction

Studies dedicated to the trophic ecology of aquatic insects have become a central element in modern aquatic entomology (Lancaster & Downes 2013). Expanding the knowledge about foraging of aquatic hexapods is crucial for describing auto-ecological aspects of the dominant invertebrate group in inland waters, analyzing trophic webs, and better understanding functional dynamics of freshwater ecosystems (Monakov 2003).

Plecoptera, or stoneflies, constitutes a relatively small order of hemimetabolous insects, particularly adapted to the life in cold waters and for this reason mainly distributed in mountainous systems (Fochetti & Tierno de Figueroa 2008). Among the most orophilous stonefly families, Capniidae includes 17 genera and 315 species with a mainly Holartic distribution (Fochetti & Tierno de Figueroa 2008). According to Merrit and Cummins (1996), members of the family Capniidae are generally considered shredders, mainly feeding on plant fragments and other coarse particulate organic matters (Fleituch 2013, Taylor & Chauvet 2014).

A conspicuous deal of attention has been devoted to the study of various aspects of the limnology of high altitude Himalayan lakes (Pandit 1999), but interestingly, almost no data are available about trophic ecology of the few aquatic insect taxa that inhabit these extreme ecosystems.

Table 1. Main abiotic characteristics of the study lakes*.

	LCN 75*	LCN 128*	LCN 129*
Altitude (m a.s.l.)	4,839	5,090	5,240
Valley	Nojimba	Thyanmoche	Thyanmoche
Map coord. (UTM W GS84)			
latitude	3092496	3089787	3091945
longitude	469790	465110	465940
Lake surface (km^2)	0.42	0.03	0.07
Conductivity ($\mu S\ cm^{-1}$)	41.4	55.6	58.2
Temperature (°C)	8.0	6.0	4.0
pH	7.40	6.92	7.38
N-NH4 ($\mu eq\ l^{-1}$)	0.0	0.0	0.0
N-NO3 ($\mu eq\ l^{-1}$)	3.0	7.0	2.0
SO4 ($\mu eq\ l^{-1}$)	131	334	333
Ca ($\mu eq\ l^{-1}$)	329	395	429
Mg ($\mu eq\ l^{-1}$)	51	87	92
Cl ($\mu eq\ l^{-1}$)	1.0	2.0	1.0
Alk ($\mu eq\ l^{-1}$)	262	161	202
Total P ($\mu g\ P\ l^{-1}$)	6.0	4.0	4.0
Total N ($\mu g\ N\ l^{-1}$)	70	150	70

* These Himalayan lakes do not have a proper name, but are identified by progressive numbers (Lake Cadastre Number, LCN).

Accordingly, the aim of the present study is to provide the first data, as far as we know, about feeding habits of very high altitude stoneflies.

2. Material and methods

During 2008 and 2012 sampling campaigns of the Italian National Research Council (CNR), stonefly nymphs were collected from three high altitude lakes (> 4,800 m a.s.l.) in Sagarmatha National Park, in the North-Eastern region of Nepal. These Himalayan lakes do not have a proper name, but are identified by progressive numbers (Lake Cadastre Number, LCN). They are fed prevalently by perennial snow, glaciers, or direct runoff from precipitation, and are located in basins where large areas are covered by glaciers, rocks, landslides and, in minor amount, alpine prairies (Tartari *et al.* 1998a,b). The main abiotic characteristics at the sampling time are reported in Table 1.

Sampling for chemical and biological analysis was performed using one litre polyethylene bottle at a lake depth of 50 cm, and kicking the lake sediment with a handle-net for a maximum of five minutes along the littoral. Sampling was carried out simultaneously in both occasions, in autumn, after the monsoon period. Chemical samples were then stored in the dark at 4 °C until the laboratory analyses which were performed according to the methods described on the web site: http://www.idrolab.ise.cnr.it/. Biological samples were then fixed with 80% alcohol before being sieved, sorted, and identified to genus level. The extreme paucity of literature regarding Himalayan stoneflies and the fact that all specimens were in the immature stage prevented a deeper identification, but we are confident that the unicity of our study, realized at such elevate altitudes, justifies its interest.

Total length, pronotum width and metathoracic femur length of the sampled nymphs were measured with a Nikon SMZ 1500 stereomicroscope (0.10 mm accuracy). The measurements were standardized by placing each nymph between two slides. Nymphal diet was determined through the gut content observation of the collected specimens. Each specimen was cleared after introducing it in Hertwigs' liquid and heating it in an oven at 65 ºC for 24 hours, following the methodology of Bello and Cabrera (1999), which is used in many studies of nymphal feeding of aquatic insects (e.g. Fenoglio *et al.* 2007, Bo *et al.* 2012). The percentage of the absolute gut content (at 40x) was estimated as the proportion of digestive tract occupied by dietary items. The relative gut content (at 400x) was calculated by measur-

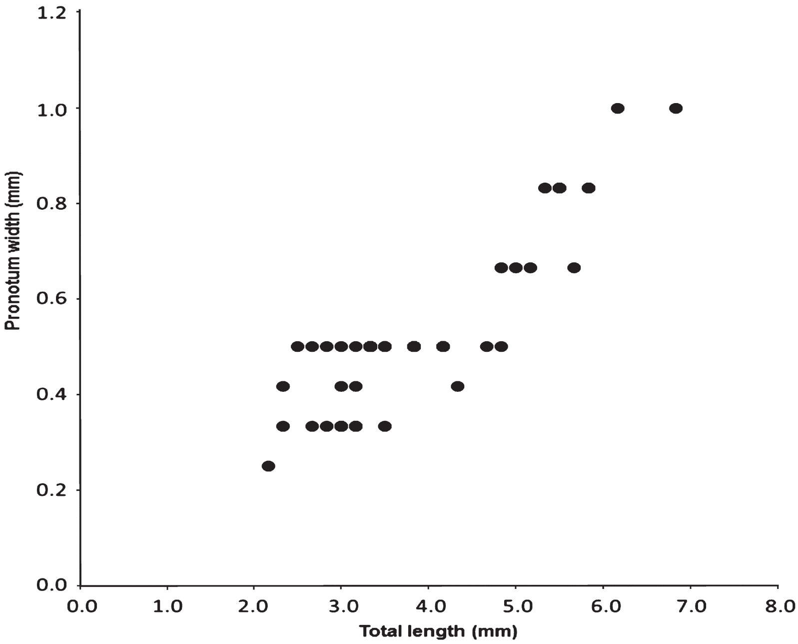

Fig. 1. Relationship between total body length and pronotum width in high altitude *Capnia* nymphs.

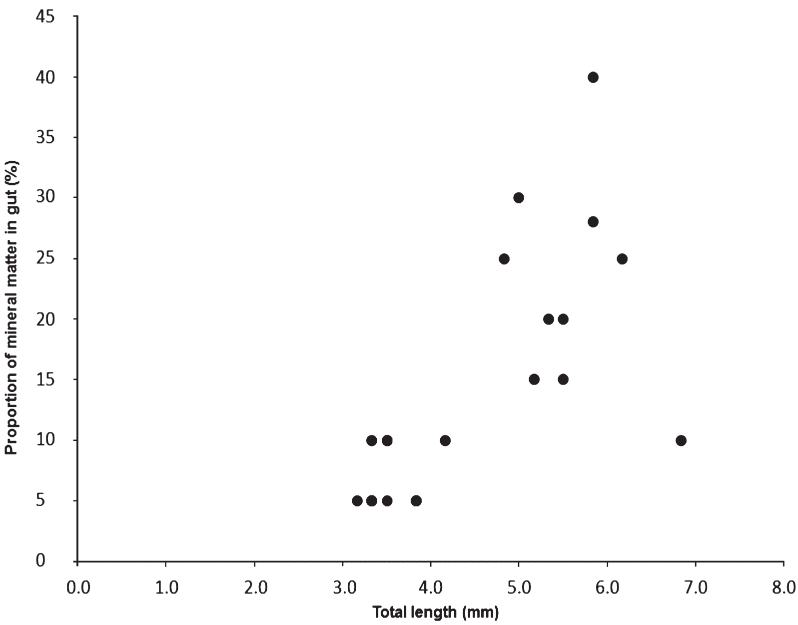

Fig. 2. Relationship between total body length and amount (%) of ingested mineral matter in high altitude *Capnia* nymphs.

ing the area of gut content occupied by each dietary item. Mean, standard deviation, minimum and maximum were calculated. Data were analyzed using PAST software (Hammer *et al.* 2001): Pearson correlation was used for continuous variables (nymph measures) while Spearman test was employed to find a correlation between nymph size and gut content (a categorical variable).

3. Results

All stonefly nymphs belonged to the genus *Capnia*, family Capniidae. A total of 46 stonefly

Table 2. Gut contents of the Capniidae nymphs as proportion (%) of the gut volume filled with food (n = 46) and proportions of different components in the total food volume in cases with food in the gut (n=41, i.e. 5 guts were empty)

	Mean	SD	Minimum	Maximum
% absolute content	43.7	33.4	0.00	90.0
% detritus	81.8	29.7	0.00	100.0
% diatoms	0.41	1.34	0.00	5.00
% pollen	0.24	1.56	0.00	10.0
% CPOM*	0.20	0.90	0.00	5.00
% hyphae	0.05	0.31	0.00	2.00
% mineral matter	7.27	10.3	0.00	40.0

* Coarse particulate organic matter.

nymphs were measured and processed for gut content analyses (n = 30 from Lake LCN 75, n = 11 from Lake LCN 128 and n = 5 from Lake LCN 129). Mean (± S.D.) total length was 3.89 mm (± 0.2 mm).

Among the three morphometric parameters measured, we detected a significant correlation between total length and pronotum ($r = 0.88$; $p < 0.001$; Fig. 1), total length and femur ($r = 0.83$; $p < 0.001$), femur and pronotum measures ($r = 0.75$; $p < 0.001$). For these reasons, we utilized the total length as an indicator parameter of nymph size.

Forty-one nymphs had some content in their guts and fine detritus was the fundamental component in the guts of these high altitude Capniidae nymphs (Table 2). Diatoms, pollen, CPOM (coarse particulate organic matter) and fungi were present in the diet in very low quantities. Mineral matter constituted a considerable percentage of the gut contents, but this was probably due to incidental ingestion. No significant correlation with body size was found for the food items except the amount of mineral matter being positively correlated with total length ($rs = 0.65$; $p < 0.001$; Fig. 2).

4. Discussion

As mentioned above, Capniidae are among the most limnophilous Plecoptera, often inhabiting ponds and lakes. An exceptional example is provided by *Capnia lacustra* Jewett, 1965 which spends its entire life, including the adult stage, in the deep waters of Lake Tahoe, but many other Capniidae species inhabit lentic systems too (Donald & Patriquin 1983). Moreover, Capniidae are "an icon for cold adaptation" (Pescador *et al.* 2000). Brittain (1974) reported that *Capnia atra* Morton, 1896 occurs even in the most extreme lentic habitats in Norway and has the capacity to rapidly colonize even lakes recently created by glacier retreat. For these reasons, it is not surprising that nymphs of this family are able to develop in very high altitude Himalayan lakes. At present, the few and sporadic studies that regarded the presence of these stoneflies in Himalaya (e.g., Kimmins 1946, Kawai 1968, Harper 1977, Zwick & Sivec 1980) were exclusively focused on taxonomic aspects.

Recently, López-Rodríguez and Tierno de Figueroa (2008) reported a greater variation in food resources among Capniidae nymphs than previously known. Moreover, Bo *et al.* (2013) reported that *Capnia bifrons* (Newman, 1838) shifts from being a collector-gatherer to a shredder along its nymphal growth. Our results show that in high altitude Himalayan lakes Capniidae nymphs are mainly collector-gatherers feeding on fine organic detritus. These freshwater systems are located above the tree-line, where allochthonous inputs of organic matter is expected to be extremely low. Moreover, these lakes have cold and ultra-oligotrophic waters, so that even autochthonous inputs (i.e., productivity of macrophytes, phytoplankton and ephilitic algae) are particularly low. For these reasons, we can hypothesize that Capniidae nymphs can only feed on small organic particles mainly derived from the catchment. Nymphs collect fine detritus directly from the substratum, and we can assume that larger nymphs ingest more mineral matter because of the larger dimension of their mouthparts.

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