Fish determine macroinvertebrate food webs and assemblage structure in Greenland subarctic streams

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SUMMARY

1. Climate warming in (sub)arctic regions is expected to increase freshwater fish overwinter survival and dispersal, potentially with strong implications for macroinvertebrate assemblage composition and ecosystem processes. Several studies worldwide have shown large effects of top predators (usually fish) on macroinvertebrates in streams. However, the influence of top predators on trophic diversity, the range of food resources exploited and trophic niche redundancy is less well studied, particularly in cold regions.

2. Using stable isotopes (^{13}C and ^{15}N) and fish gut content analysis, we investigated the effect of top predators on macroinvertebrate food webs in streams in Greenland by comparing adjacent sites with and without fish (*Gasterosteus aculeatus*).

3. Food-web metrics estimated from stable isotope data showed that the presence of fish reduced the diversity of food sources exploited by macroinvertebrates as well as their trophic diversity. In addition, fish presence increased packing and trophic redundancy of macroinvertebrate taxa in the food web, possibly due to behavioural changes in foraging activity. Furthermore, predatory macroinvertebrate taxa were unable to attain the trophic position of fish so that food webs were one trophic level longer in streams with fish. Focusing on macroinvertebrate food webs alone, predatory macroinvertebrates did not change their trophic position in the presence of fish.

4. Filter feeders dominated the macroinvertebrate assemblage when fish were present, while the relative abundance of collector-gatherers was marginally higher in the fishless streams. This pattern was consistent with stronger selectivity for collector-gatherers by fish.

5. Climate-driven fish colonisation in currently fishless Greenland streams may induce a shift in macroinvertebrate food webs, with a reduction in both trophic diversity and the variety of food sources consumed. To some extent, this might counteract a change towards a broader use of food resources by macroinvertebrates that might otherwise be expected at higher temperatures. In addition, a shift in the macroinvertebrate assemblage towards dominance of filter feeders can be expected to promote an increase in periphyton biomass in streams with fish.

Keywords: arctic streams, climate change, fish colonisation, Layman's community-wide metrics, top-down effect

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Introduction

Empirical studies of the role of top predators in stream food webs have shown a top-down effect of predatory fish on the abundance and feeding behaviour of invertebrates (Peckarsky & McIntosh, 1998; McIntosh, Peckarsky & Taylor, 2002; McIntosh et al., 2005; Moulton et al., 2010) and a consequent increase in periphyton biomass (Flecker & Townsend, 1994; Peckarsky & McIntosh, 1998; Nyström, McIntosh & Winterbourn, 2003; Moulton et al., 2010), with important consequences for ecosystem functioning (Townsend, 2003). However, these studies focused, to a large extent, on changes in macroinvertebrate assemblage structure, whereas the effects of fish on food-web properties have scarcely been addressed.

Numerous stream food-web studies have recently been conducted with the main emphasis on structural attributes, such as the presence of certain types of species (e.g. omnivores and cannibals), food chain length and number of trophic levels (for a review, see Thompson, Dunne & Woodward, 2012). Empirical comparative studies under natural conditions remain rare (Thompson et al., 2012), and studies focusing on the role of top predators in shaping food webs in streams are particularly scarce (Woodward & Hildrew, 2001; Woodward et al., 2005; Layer et al., 2011). One such study elucidated the effects of trout (*Salmo trutta*) invasion in New Zealand streams. The native top predators, *Galaxias* spp., were replaced by trout, modifying the transfer of carbon within the trophic webs and creating cascading effects on basal resources (Flecker & Townsend, 1994; Townsend, 2003). In Alaskan arctic streams, trophic webs are longer in streams with fish than in those without fish (Parker & Huryn, 2006, 2013). In Broadstone stream in England, the arrival of a new macroinvertebrate top predator (the dragonfly *Cordulegaster boltonii*) resulted in slight increases in food-web length, trophic web complexity and omnivory, as well as higher trophic species packing within the food web (i.e. species were more closely trophically connected), probably increasing the trophic redundancy of species (Woodward & Hildrew, 2001). More recently, trout invasion in the same study system (Broadstone stream), which co-occurred with decreased acidity, increased food-web length by one trophic position (Layer et al., 2011). This invasion has had strong effects by producing local extinctions (never before observed following addition of a top predator invertebrate), which re-shaped the food web in terms of macroinvertebrate composition and abundance and is expected to reduce food-web persistence over time (Layer et al., 2011).

The arrival of a top predator, such as a fish, is likely to indirectly change feeding links in the food web by altering macroinvertebrate behaviour (Layer et al., 2011). A change in the trophic structure of the macroinvertebrate assemblage, an increase in the trophic redundancy of species and a reduction in the diversity of food resources exploited in the presence of fish would indicate strong top-down effects of fish on macroinvertebrate assemblages. When fish are present, invertebrates reduce (Lancaster, Hildrew & Townsend, 1988; Kohler & McPeek, 1989; Huang & Sih, 1990; Flecker & Townsend, 1994; Peckarsky & McIntosh, 1998) or modify (i.e. a shift towards nocturnal feeding strategies) (Flecker, 1992; Peckarsky & McIntosh, 1998; McIntosh et al., 2002) their foraging activity, use less space for feeding and reduce time spent out of predation refuges (Lancaster et al., 1988; Kohler & McPeek, 1989; Huang & Sih, 1990; Flecker & Townsend, 1994). This change in macroinvertebrate foraging activity is likely to be reflected in the architecture of their food webs in the form of decreased diversity of resources exploited and increased trophic redundancy, although this has not been proved so far.

New knowledge of food-web structure and dynamics of subarctic and arctic stream ecosystems is particularly relevant in the context of global warming, as even small changes in temperature and/or precipitation may have profound ecological consequences (Woodward, Perkins & Brown, 2010b; Woodward et al., 2010a) such as the creation of windows of opportunity for fish colonisation. Studies of trophic dynamics in subarctic and arctic streams that consider both fish and macroinvertebrates are particularly scarce and most have been conducted in streams and rivers in Alaska and Iceland (Parker & Huryn, 2006, 2013; Woodward et al., 2010a). Further north, the distribution of temperate fish species is currently restricted by overwinter starvation (applicable to at least 25 genera from Canada; Shuter & Post, 1990). It is predicted that several fish species may increase their distribution ranges, benefitting from a longer growing season and a reduced overwinter starvation period, thus increasing overwinter survival (ACIA, 2005; Chu, Mandrak & Minns, 2005; Reist et al., 2006). Although freshwater fish diversity is very low in Greenland (six species in total, most occurring in the southern tip of Greenland and only two recorded in western Greenland; Froese & Pauly, 2013), these species could also increase their distribution northwards, as expected for their continental Nearctic counterparts (Shuter & Post, 1990; ACIA, 2005). An increase in temperatures may promote glacier retreat, whereas an increase in precipitation may further enhance run-off and thus create stronger connectivity

between lakes and streams, permitting colonisation of formerly fish-free fresh waters (e.g. Bennike *et al.*, 2008; Milner *et al.*, 2008).

Our aim was to compare macroinvertebrate assemblage composition and food-web properties of the main consumers in a series of streams with and without fish in Greenland. We had two main hypotheses: (i) fish presence will promote more elongated food webs (considering fish and macroinvertebrate consumers), higher macroinvertebrate trophic species packing and a reduction in the diversity of food sources consumed; (ii) fish will cause changes in the structure of macroinvertebrate assemblages, decreasing the relative abundance and biomass of predation-sensitive species, as shown in previous studies.

Methods

In August 2011, three streams with fish and three streams without fish were sampled in the region of Nuuk in Greenland (Fig. 1). Table 1 shows the environmental characteristics of the streams. Many freshwater ecosystems in the region are fishless, mainly due to their biogeographical history related to the most recent glaciation and physical barriers such as small cascades that prevent colonisation by fish from the sea. Some streams and lakes host at least one fish species, namely three-spined stickleback (*Gasterosteus aculeatus*), and some may additionally, or alternatively, host Arctic charr (*Salvelinus alpinus*) and its sea-run morphs (Jeppesen *et al.*, 2001). Our sampling sites with fish contained only sticklebacks. Streams with sticklebacks are visually distinctive because fish occur in high densities and move actively when disturbed. In a simultaneously conducted extensive sampling campaign of upstream lakes, sticklebacks were likewise observed.

The sampled streams had similar physical characteristics (stream reach depth, width, distance to headwaters, slope and altitude) and nutrient concentrations (total and dissolved nitrogen and phosphorous) (Mann–Whitney $P > 0.1$ in all cases; Table 1). Most streams were downstream of lakes and were sampled at a minimum distance of 0.5 km from these. Macroinvertebrate samples were taken in each stream with a sweep net along three parallel 20-m transects by dragging the sweep net continuously and disturbing stream bottom immediately upstream of the net. Fish, if present, were always caught in the sweep net samples. Additional samples of fish, mosses and epilithic periphyton were collected (by scraping rocks) at each site to allow reconstruction of food webs using stable isotopes. Macroinvertebrate and fish samples were preserved in ethanol, and the samples for stable isotope analyses were frozen.

Macroinvertebrate samples were sorted in the laboratory, and individuals were identified to the lowest possible taxonomic level and measured for estimation of biomass according to published equations (Benke *et al.*, 1999; Miyasaka *et al.*, 2008; Newton & Proctor, 2013). Both preserved and fresh (frozen) samples of bulk macroinvertebrates and fish muscle were freeze-dried, weighed (0.5–1.5 mg for animal tissues, 2–3 mg for mosses and periphyton) and analysed for stable isotopes
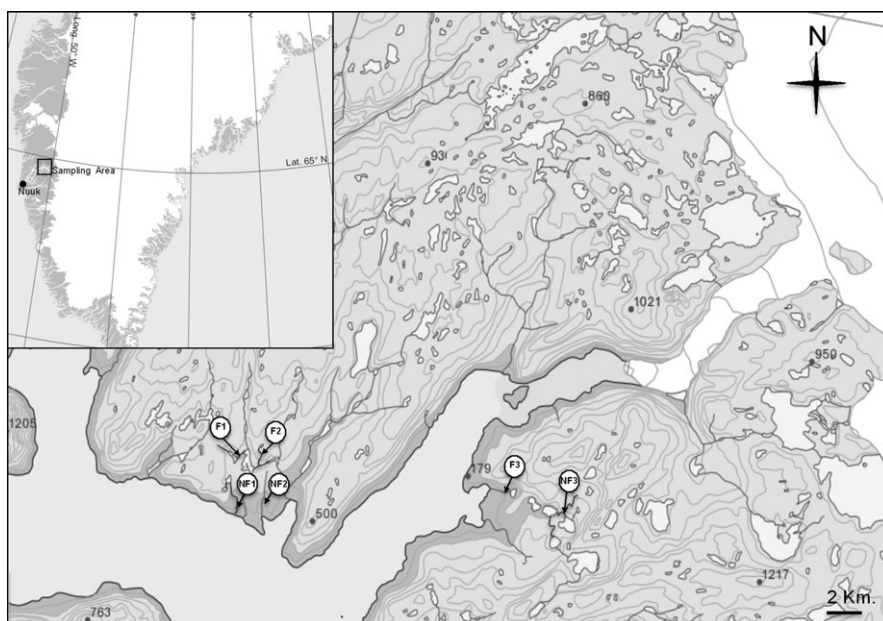

Fig. 1 Approximate locations of the sampled streams in the Nuuk region, Greenland. Streams with fish are denoted as F1, F2 and F3 and streams with no fish as NF1, NF2 and NF3. Maps are taken from the website http://en.nunagis.gl/.

Table 1 Geographical coordinates and environmental characteristics of sampled sites. Streams with fish are named streams F1 to F3 and the ones without fish are named NF1 to NF3. Slope was calculated from 50 m downstream the sampling point to 50 m upstream. There were no significant differences between streams with and without fish for any of these characteristics (Mann–Whitney $P > 0.1$)

Stream site	F1	F2	F3	NF1	NF2	NF3
Latitude (N)	64°45′26.97″	64°45′34.45″	64°46′ 0.77″	64°44′15.16″	64°44′ 37.44″	64°45′50.55″
Longitude (W)	50°25′ 9.74″	50°23′53.31″	50° 7′ 48.61″	50°24′47.02″	50°23′ 11.26″	50° 3′ 51.77″
Water source	Downstream of a lake*	Downstream of a lake*	Downstream of a lake*	Downstream of a lake*	Ground-water fed	Downstream of a lake*
Stream order	2	1	1	2	1	2
Altitude (m)	251	289	19	82	136	190
Distance to source (km)	1.9	1.2	1	1.5	1.3	2.4
Slope (100 m)	3.3	5.4	14.8	11.8	24.2	3.6
Depth range (m)	0.1–0.6	0.1–0.5	0.2–0.5	0.2–0.6	0.2–0.6	0.1–0.5
Width range (m)	0.5–1.8	0.5–2	0.4–1.5	0.9–2.2	0.5–1.5	0.7–1.5
TN (mg/l)	0.4	0.85	0.23	0.2	0.18	0.19
TP (mg/l)	0.006	0.041	0.014	0.003	0.018	0.002
PO_4–P (mg/l)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
NO_{2+3}–N (mg/l)	<0.005	0.03	0.06	0.09	0.04	0.03

*Minimum distance to a lake was 0.5 km.

(δ^{13}C and δ^{15}N) at the UC Davis Stable Isotope Facility, U.S.A. δ^{13}C and δ^{15}N of the ethanol-preserved specimens were corrected to the fresh equivalents for all samples (Sarakinos, Johnson & Vander Zanden, 2002; Ventura & Jeppesen, 2009). When both fresh and preserved macroinvertebrate samples were present for a given taxon, we made our own correction following the same procedure as in Ventura & Jeppesen (2009). In the few cases where only preserved specimens were available, we used the general correction effect from Ventura & Jeppesen (2009) for all macroinvertebrate groups. The effect of preservation was always minor (on average $<-1.0^0/_{00}$ for δ^{13}C and $<0.5^0/_{00}$ for δ^{15}N) and did not differ between invertebrate taxa (Mann–Whitney $P > 0.1$).

For the analysis of fish gut contents, we identified, counted and measured prey items in guts and intestines by dissecting samples ($n = 33$) under a stereomicroscope at $50\times$ magnification. The relative weight of each diet item was estimated on a grid plate where items were placed together, and the volume of each item was calculated (Hyslop, 1980). The diet of fish with and without parasites (Platyhelminthes *Schistocephalus solidus*) was compared (Mann–Whitney *U*-test) because previous evidence from analyses of Greenland sticklebacks has shown differences (Bergersen, 1996). Since we did not find significant differences in the mean volume or size of food items of infested and un-infested individuals (Mann–Whitney $P > 0.1$), these were grouped in the analyses. Yield–effort curves for the number of prey items identified in fish guts were created and evaluated using the package 'rich' (Rossi, 2011) for R software (R Core Team, 2013). The number of guts analysed sufficed

to represent the vast majority of feeding interactions (see Appendix S1).

Macroinvertebrate assemblage structure

Most of the streams sampled were downstream of lakes, but one was groundwater fed. Lake outlets and groundwater-fed streams in West Greenland typically have the highest abundance and diversity of macroinvertebrates, but they also show high variability in absolute abundances (Friberg *et al.*, 2001). Reflecting this fact, our streams exhibited high variability in absolute abundances and biomasses of macroinvertebrates. To assess the role of fish in the trophic structure of the macroinvertebrate assemblage, and given the low taxonomic diversity present (see Appendix S2), we compared the taxonomic diversity and the relative abundances and biomasses of broadly classified macroinvertebrate trophic groups: collector-gatherers, filter feeders, predators and scrapers. For each stream, we used mean relative abundance, biomass and taxonomic diversity values of three sweep net transects.

Food webs

For the comparison of food-web structure, we used Layman's community-wide metrics for food webs obtained from trophic position (based on δ^{15}N isotopic signature) versus δ^{13}C stable isotope bi-plots (Layman *et al.*, 2007). Layman's community-wide metrics were originally created to be implemented using stable isotope signatures of consumers only, as the signature of basal resources is

highly variable in space and time, but primary consumers represent a more integrated isotopic signature reflecting natural variability in the signatures of basal resources across larger space and time scales (Post, 2002; Layman *et al.*, 2007). Thus, comparison of the selected food-web metrics between streams with and without fish was carried out for the consumer food web (i.e. considering fish and macroinvertebrates together), separately for total macroinvertebrates and also separately for macroinvertebrate collector-gatherers and predators (because these had enough taxa in the web to allow estimation). The metrics analysed were total trophic web length (TWL) as a measure of maximum trophic position attained, total area occupied (TA) as a measure of trophic diversity, carbon range (CR) as a measure of diversity of food sources exploited and mean and standard deviation of nearest distance to a neighbour in the stable isotope bi-plot (mean NND and SD NND), representing the trophic redundancy of species because species closely packed in the food web are usually redundant from the trophic point of view (Layman *et al.*, 2007). Use of these metrics has been criticised when isotopic signatures of basal sources are not considered at all (although they are not utilised directly in the food-web metrics calculations) (Hoeinghaus & Zeug, 2008; Layman & Post, 2008). This criticism concerns the possibility that the carbon range and associated metrics of consumers might be influenced if isotopic signatures of basal resources are differentially separated from each other, in terms of $\delta^{13}C$ values, between the compared systems because of dissimilarity in physicochemical and other environmental characteristics (Hoeinghaus & Zeug, 2008; Layman & Post, 2008). In our study, however, the use of such tools is appropriate, as the food sources did not differ in isotopic signature value between the stream types (mean $\pm$ SD of $\delta^{13}C =$ -29.4 ± 1.15 in streams with fish versus $\delta^{13}C =$ -29.1 ± 2.34 in fishless streams, Student's t-test: $T = -0.22$, $P = 0.8$). Isotopic signatures of detritus and fine particulate organic matter were lacking, but there is no reason to expect that these isotopic signatures differ systematically, being always equally further apart from the $\delta^{13}C$ signatures of the other resources in the fishless than in the fish-containing streams. Furthermore, all streams are located in a landscape of similar environmental conditions (Table 1), and these kinds of isotopic signatures typically remain stable across streams of a same region (France, 1995; Finlay, 2001; Rasmussen, 2010). Moreover, in the case of $\delta^{15}N$ signatures, we standardised the values to trophic position (following Post, 2002) to reduce variability. Estimation of metrics was carried out using the package 'Siar' (Parnell *et al.*, 2013) in R software version 3.0.2 (R Core Team, 2013). The relationships between fish body size and trophic position were tested by linear regressions for each stream separately and also by grouping all individuals. We also conducted analyses of fish gut content and macroinvertebrate assemblage data for the purpose of validation.

Fish diet

Fish were measured and weighed to test for potential differences in dietary characteristics due to differences in body size. Fish dietary analysis was undertaken by comparing the relative weight of each food item between the three streams, grouping macroinvertebrate food items according to trophic group broadly categorised as collector-gatherers, filter feeders, scrapers and predator strategists and dividing them into macroinvertebrates and zooplankton. The Ivlev food selectivity index (Ivlev, 1961) was calculated using the relative weight of each macroinvertebrate species or group in the diet against the relative biomass found in the environment. This was carried out for each food item consumed and also grouped by trophic group. The selectivity index could not be calculated for chydorids as they were not retained in the sweep net samples. As chydorids could not be collected in all streams, they were not considered in the food-web metric estimations; they were, however, included in the stable isotope bi-plot of the streams with fish using a bulk sample of well-preserved individuals collected from the anterior part of the guts after correction for preservation effects. The purpose of including them was to validate the fish gut content and the stable isotope reconstruction.

Statistical analyses

Log$_{10}$ transformation was applied (in determination of dietary selectivity and macroinvertebrate assemblage composition) when the statistical assumptions for parametric tests were not met (Shapiro–Wilk test for normal distribution and Levene test for homogeneity of variance). All statistics were conducted at α level = 0.05.

Layman's community-wide metrics and macroinvertebrate taxon diversity were compared between streams with and without fish using Student's t-test (α = 0.05). Differences in fish length between streams were tested using one-way ANOVA. To test for differences in the proportions of food items consumed between streams, a nonparametric Kruskall–Wallis test was applied, followed by Mann–Whitney pairwise comparison tests for significant differences.

Table 2 Summary of main changes in assemblage composition of streams with and without fish

Trophic group	Presence–absence*		Relative abundance (%)		Relative biomass (%)	
	Fish	Fishless	Fish	Fishless	Fish	Fishless
Filter feeders	3	2	**46.6 ± 24.5**	**2.3 ± 3.7**	**39.0 ± 21.9**	**4.1 ± 6.7**
Scraper feeders	1	1	2.5 ± 3.3	0.2 ± 0.3	NM	NM
Collector-gatherer feeders	3	3	*31.8 ± 18.3*	*67.9 ± 13.3*	42.7 ± 24.0	51.6 ± 9.3
Predators	3	3	19.1 ± 10.9	29.2 ± 16.6	*18.3 ± 10.9*	*44.3 ± 14.5*

*Number of streams in which the trophic group is present (i.e. 0, 1, 2 or 3).
Mean and standard deviation values are given (Mean ± SD), significant differences (Student's t-test α = 0.05) are marked in bold, and marginal *P* values are given in bold italics.

The relationship between fish standard length and the proportion of macroinvertebrates (versus microcrustaceans) in the diet was tested using generalised linear models with a binomial error distribution structure (McCullaugh & Nelder, 1989), and the relationship between fish body size and trophic position estimated from SIA was tested by linear regressions. Finally, we tested whether dietary selectivity was different from zero by comparing the selectivity of each item with an artificially created treatment with selectivity values −0.1, 0 and 0.1, representing a range of values considered as neutral selection (Kohler & Ney, 1982), using a Student's t-test and with each stream as a replicate.

Results

Fish and macroinvertebrate assemblages

Fish body size ranged from 19 to 45 mm standard length (mean ± SD standard length of 21 ± 10 mm in stream F3, 33 ± 03 mm in stream F2 and 39 ± 04 mm in stream F1), being significantly smaller in stream F3 than in the rest (F = 9.52, df = 30, *P* < 0.001). Probably due to the small size range studied, fish body size did not cause any change in trophic position (linear regression *P* > 0.1).

Macroinvertebrate taxon diversity did not differ between streams with and without fish (Student's t-test, *P* > 0.1). However, the composition of macroinvertebrate assemblages varied between the streams, and some macroinvertebrate taxa were only present in one stream

type. This was the case for the mayfly *Baetis bundyae* (constituting *c*. 15% of the macroinvertebrate biomass), which was exclusively confined to the fishless streams (see Table S2). In contrast, the clam *Pisidium* sp. and the caddisfly *Agripnia* sp. were only present and constituted a high proportion of the macroinvertebrate biomass in the streams with fish (see Table S2). However, relative abundance and biomass of the remaining macroinvertebrate taxa did not vary significantly between stream types; only annelids had marginally higher relative abundances and biomasses in the fishless streams (Student's t-test *P* = 0.06 for abundance and biomass; see Table S2).

When grouped according to trophic strategy, filter feeders had a higher relative abundance (T = 4.02, *P* = 0.01) and biomass (T = 2.6, *P* = 0.05) in streams with fish (Table 2). The relative abundance of collector-gatherers was marginally higher in the fishless streams (Student's t-test, T = −2.76 *P* = 0.06), and the relative biomass of predatory invertebrates was marginally higher in the fishless streams (T = −2.5, *P* = 0.06) (Table 2). Scraper feeders (Gastropoda: Limnaeidae) were only found in one stream with and one stream without fish and represented a minor proportion of the assemblage (Table 2).

Food webs

There was a large difference in food-web architecture between streams with and without fish (Fig. 2, Table 3). Trophic web length (TWL) was shorter and food source

Fig. 2 Food webs (trophic position vs. dC^{13} bi-plots) in streams with (left panels) and without fish (right panels) in Greenland. Top panel: all streams with and without fish grouped, showing mean and standard deviation of stable isotope signatures. The other panels show food webs for each stream site. In black: fish, in grey: macroinvertebrates, in white: basal resources. Convex hull area represents trophic niche space (macroinvertebrate food-web area is shown as dashed line in the fish-containing streams). The order of the streams in the plot is arbitrary; streams were not paired. Taxa in the food-web plot are abbreviated as follows: Ahy: adult *Hydroporus* sp.; Ap: *Apatania* sp.; Ag: *Agrypnia* sp.; Chi: Chironominae sp.; Chp: Chironomidae pupae; Chy: Chydoridae sp.; Em: Empididae sp.; *G. aculeatus*: *Gasterosteus aculeatus*; Hy: *Hydroporus* sp.; Lp: *Limnophora* sp.; Li: Limnaeidae; Lmn: *Limnephilus* sp.; Ol: Oligochaeta sp.; Or: Ortocladiinae; Ori: Oribatidae; Pi: *Pisidium* sp.; Pr: *Prosimulum* sp.; Ta: Tabanidae; Tan: Tanypodinae; Parasite: *Schistocephalus solidus*.
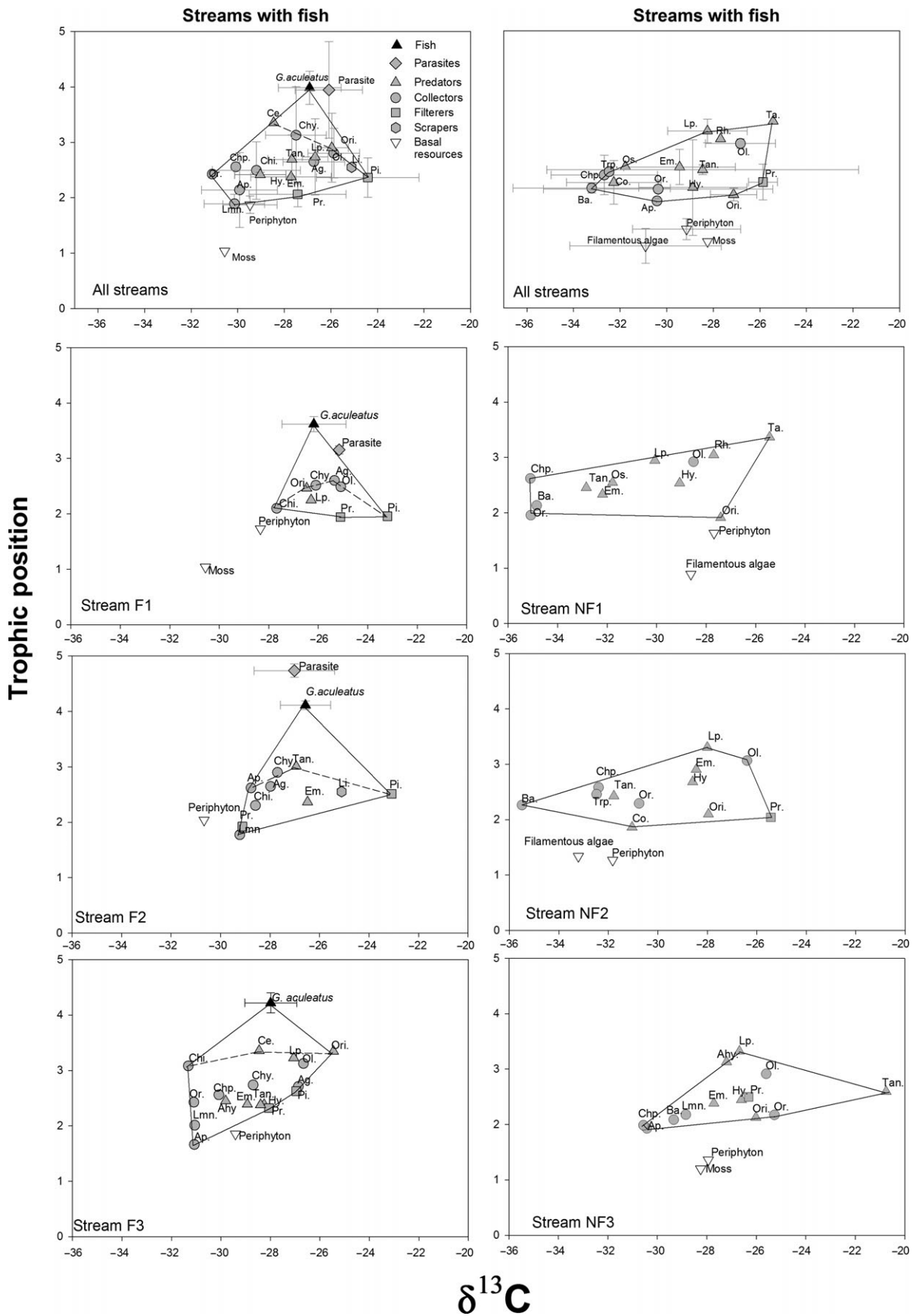
Table 3 Differences in Layman's community-wide metrics for streams with and without fish, considering the food web of macro-invertebrates and fish, the food web for all macroinvertebrates and separately for collector-gatherer and predator macroinvertebrate food webs

	Streams with fish ($n = 3$) (mean ± SD)	Streams without fish ($n = 3$) (mean ± SD)	Student's t-test parameters (T; P)
Total food web			
TWL	**4.2 ± 0.3**	**3.3 ± 0.03**	5.0; **0.03**
TA	1.7 ± 0.3	1.7 ± 0.3	−0.3; 0.8
CR	**−5.5 ± 0.9**	**−9.9 ± 0.2**	8.8; **0.0009**
Mean NND	0.3 ± 0.07	0.4 ± 0.02	−0.6; 0.6
SDNND	0.2 ± 0.01	0.3 ± 0.08	−2; 0.2
All macroinvertebrates			
TWL	3.0 ± 0.4	3.3 ± 0.03	−1.6; 0.3
TA	**1.0 ± 0.2**	**1.7 ± 0.3**	−3.3; **0.03**
CR	**−5.5 ± 0.9**	**−9.9 ± 0.2**	8.8; **0.0009**
Mean NND	0.3 ± 0.07	0.4 ± 0.02	−1.6; 0.2
SD NND	*0.1 ± 0.03*	*0.3 ± 0.08*	−2.5; *0.06*
Collector-gatherer feeders			
TWL	2.7 ± 0.3	2.9 ± 0.06	−0.9; 0.4
TA	0.3 ± 0.5	0.5 ± 0.04	−0.6; 0.62
CR	**−2.7 ± 1.6**	**−7 ± 1.9**	2.9; **0.04**
Mean NND	*0.4 ± 0.1*	*0.8 ± 0.2*	−2.4; *0.07*
SD NND	*0.2 ± 0.1*	*0.9 ± 0.5*	−2.4; *0.07*
Predators			
TWL	2.9 ± 0.5	3.3 ± 0.02	−1.6; 0.26
CR	*−1.6 ± 2.3*	*−6.0 ± 1.9*	*2.5; 0.06*

Mean and standard deviation values are given (mean ± SD), significant differences (Student's t-test α = 0.05) are marked in bold and marginal *P* values are given in bold italics. TWL, trophic web length (maximum trophic position attained); TA, total area (trophic diversity in the web); CR, carbon range (diversity of food sources exploited); Mean nearest neighbour distance (NND, trophic species packing in the web) and standard deviation of nearest neighbour distance (SD NND, evenness of trophic species packing in the web).

diversity, measured as carbon range of consumers (CR), larger in the fishless streams than in the streams with fish (Table 3). In contrast, trophic diversity (measured as food-web convex hull area: TA) and trophic redundancy (measured as packing of taxa in the food web: mean NND and SD NND) did not differ significantly between streams with and without fish.

However, after excluding fish from the analysis, the macroinvertebrate food webs had higher CR and TA values in the fishless streams (Table 3). Additionally, fishless streams had a marginally significantly higher SDNND (Table 3); in other words, there was less even macroinvertebrate taxa packing and less trophic redundancy among macroinvertebrates within fishless food webs as opposed to streams with fish (Table 3). If fish are ignored, TWL did not differ between the stream types.

When considering macroinvertebrate trophic groups separately, CR was higher in the fishless streams for both collector-gatherers and predator strategists, although TWL did not differ for any of these groups (Table 3). Additionally, mean NND and SDNND within the web were marginally lower in streams without fish than in those with fish for collector-gatherer macroinvertebrates (Table 3). Food-web metrics could not be compared for all trophic levels as some were absent or represented by fewer than three taxa (preventing estimation of some food-web metrics) in some streams.

Fish gut content analysis revealed that the most consumed food items (in terms of relative weight) were chydorid cladocerans at all three sites, and in most cases, the proportion of items in the diet did not differ between streams. However, in stream F3 (where fish were smallest), the contribution of chydorids was greater than in the other streams (K–W, $H = 5.89$, $P = 0.05$). The proportion of macroinvertebrates in the diet increased, along with a decreasing proportion of zooplankton in the diet, with fish length (L0–L1 = 4.51, df = 1, $P = 0.03$).

Overall, the results of the fish gut content analyses correspond with those of the stable isotope analyses as fish were one trophic position higher and enriched by less than one unit in $\delta^{13}C$ relative to their main prey (Chydoridae) in terms of relative weight in gut contents (Figs 2 and 3).

Dietary selectivity of fish for the majority of the macroinvertebrate groups did not differ from 0 ($P > 0.05$) (implying that fish consumption reflected natural availability). However, fish exhibited positive dietary selectivity for the collector-gatherers: Chironomidae pupae ($T = -15.4$, $P = 0.0001$), Orthocladiinae chironomids ($T = -14.5$, $P = 0.0001$) and ostracods ($T = -17.3$, $P = 0.03$) and negative selectivity towards filter-feeding macroinvertebrates ($T = 3.65$, $P = 0.02$).

Discussion

In this set of species-poor Greenland streams, fish markedly affected the architecture of the food webs. The food web of macroinvertebrates and fish was more elongated, narrower and had higher macroinvertebrate trophic species packing (implying higher trophic redundancy of species) in the streams with fish. The trophic diversity of macroinvertebrate food webs was, accordingly lower in the streams with fish. These findings support our first hypothesis and are partly in accordance with those of Layer *et al.* (2011) and Parker & Huryn (2013), who revealed that food webs that included fish were at least one trophic position longer than fishless food webs. Furthermore, Woodward & Hildrew (2001) found elongated trophic webs and closer species packing in response to
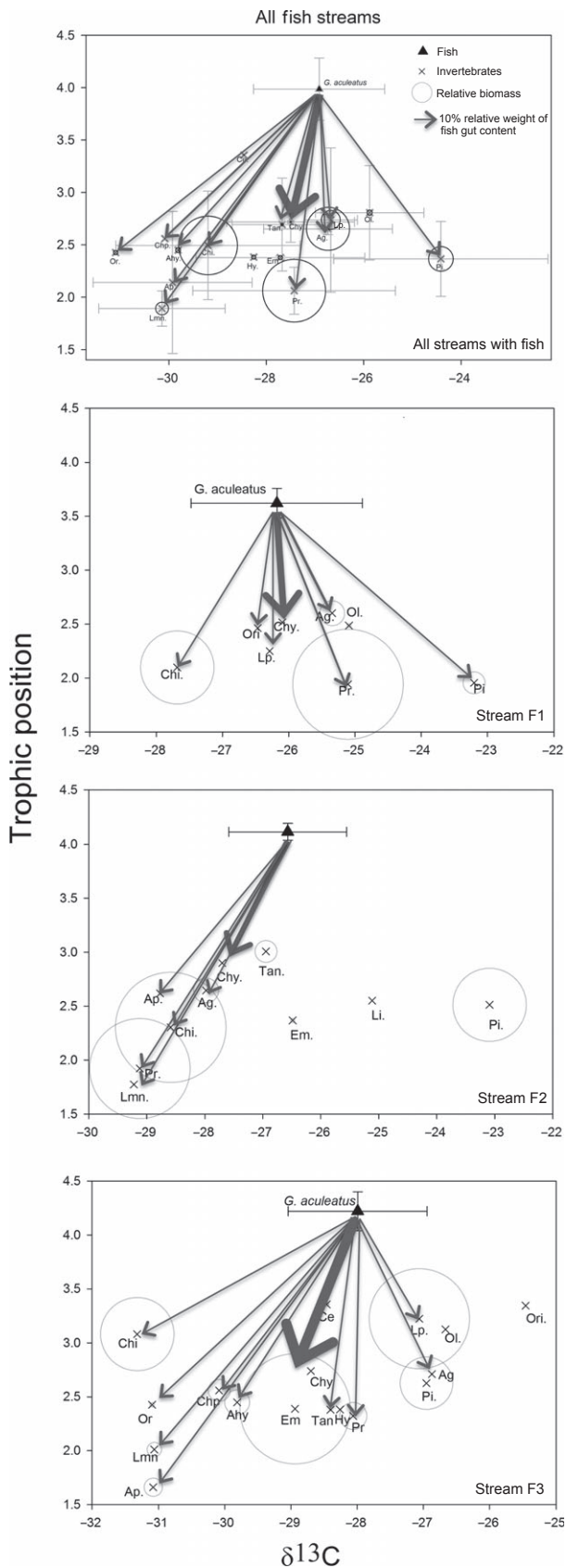
invasion of a top predator, the dragonfly *C. boltonii*. However, the increase in food-web length in their study was lower than our observations (0.5 versus 1 trophic position), probably because their top predator was a macroinvertebrate.

The trophic position of predators is often driven by their body size; predators of similar size usually occupy similar trophic positions regardless of taxonomic identity (Woodward & Hildrew, 2002; Woodward *et al.*, 2005). In some streams, the trophic position occupied by small benthivorous fishes is usually also occupied by large predatory macroinvertebrates (Woodward & Hildrew, 2002; Mantel, Salas & Dudgeon, 2004; Parker & Huryn, 2006; I. González-Bergonzoni unpubl. data). In our study, despite the presence of several predatory macroinvertebrates such as coleopterans (Dyticidae) in the fishless streams, none was able to increase its trophic position in the absence of fish, for example by attaining a larger size in the absence of fish, as seen in other studies (e.g. Hildrew, 2009). However, colonisation of macroinvertebrates in Greenland occurred relatively recently after numerous extinctions of freshwater species during the last Ice Age (Nørrevang & Lundø, 1981). Thus, large macroinvertebrate predators present in continental Arctic regions, such as predatory stonefly and dragonfly species (Parker & Huryn, 2006, 2013), may not yet have colonised the streams of West Greenland.

The dietary habits of Greenland sticklebacks were first described by Bergersen (1996) in three lentic systems; our dietary analysis partly supports his results, showing high selectivity for chironomid pupae and larvae (in our case Orthocladiinae) compared with other macroinvertebrates. However, in contrast to Bergersen (1996), chydorid cladocerans were the dominant food item in our study both in abundance and in volume of gut occupied. This difference may perhaps be attributed to differences in fish size (18–41 mm standard length in our study versus 26–52 mm in Bergersen's study) since we found a declining proportion of zooplankton in the fish diet with increasing fish length.

Fish presence led to a narrower macroinvertebrate food web, revealed by the stable isotope analyses, not only for the food web of macroinvertebrates and fish but also for the food web of macroinvertebrates

separately and also for collector-gatherers and predatory strategists. This means that the diversity of food sources consumed by macroinvertebrates decreased in the presence of fish, which is probably also the main reason for the higher trophic redundancy and lower trophic diversity in the web. This pattern probably reflects constraints on grazer activity caused by the presence of fish predators. Examples of this kind of response in streams are abundant for diverse invertebrate groups (e.g. Trichoptera, Ephemeroptera, Plecoptera, Isopoda) and across different regions (including South and North America, Europe and New Zealand) (Lancaster et al., 1988; Kohler & McPeek, 1989; Huang & Sih, 1990; Flecker, 1992; Flecker & Townsend, 1994; Peckarsky & McIntosh, 1998; Moulton et al., 2010). Although important indirect effects of fish on food webs by promoting behavioural changes in macroinvertebrates have been noted (Layer et al., 2011), an experimental investigation using a design of simplified macroinvertebrate food webs in the presence and absence of fish kairomones was unable to demonstrate such an effect (Layer et al., 2011). To the best of our knowledge, our study is the first to show this kind of response by macroinvertebrates to the presence of fish using stable isotope techniques.

It should be noted that our results may not be representative for more eutrophic and species-rich streams. Although increased productivity might buffer top-down effects of fish, it has also been observed that trophic webs are not scale-invariant; thus, several food-web properties (such as species packing) may depend on the total number of species in the web (Martinez, 1994), although this topic is a subject of debate with evidence for and against (Dunne, 2006). However, we tend to disregard the potential effects of total diversity in our studied food webs, which remained similar in streams with and without fish. Moreover, stream productivity, disturbance regime, size and heterogeneity have been shown to be strong controls of food-web structure (Thompson & Townsend, 2005; Parker & Huryn, 2013), and differences related to fish presence can be difficult to uncover because of variations in these other factors. However, even across large gradients in environmental variables, in some arctic streams, the relationships between food chain length and disturbance regime (negative) and productivity (positive) are dependent on the presence of fish, as relationships with productivity only become significant when fish are present (Parker & Huryn, 2013).

Our second hypothesis was also supported by our findings; the empirical data showed top predators to be a key driver of macroinvertebrate assemblage structure in these Greenland streams, increasing the relative abundance and biomass of filter feeders at the expense of other trophic groups (although differences were marginally significant for collector-gatherers and predators). As fish affect competitive interactions between macroinvertebrates that are differentially sensitive to fish predation (Huang & Sih, 1990; Peckarsky & McIntosh, 1998), it seems probably that such competition may account for the dominance of different groups, as seen previously in streams (Yeung & Dudgeon, 2013). The collector-gatherer feeding strategy implies that individuals move more actively when foraging, rendering them more vulnerable to vision-based fish predation, thus potentially allowing filter feeders such as Prosimulium sp. to become dominant. The presence of fish implies that competition for space between collector-gatherer feeders and filter feeders may be less intense due to higher predation on collector-gatherer species, thereby reducing their use of space (Lancaster et al., 1988; Kohler & McPeek, 1989; Huang & Sih, 1990; Flecker, 1992). This is supported by the lower relative abundance and biomass of filter feeders in our fishless streams and their negative feeding selectivity values, while selection for collector-gatherer macroinvertebrates was neutral or positive.

Macroinvertebrate and fish assemblages in subarctic and arctic streams are particularly vulnerable to the consequences of climate change because warming effects and hydrological disruptions are predicted to be strongest in these locations (ACIA, 2005; Woodward et al., 2010a,b; Friberg et al., 2013). However, given that organisms in high-latitude regions undergo large intra-annual variations in temperature and hydrological conditions, they might adapt to short-term environmental shifts comparatively better than species located at lower latitudes (Woodward et al., 2010b). In many subarctic regions, macroinvertebrate assemblage structure has been shown to be determined by hydrological factors (e.g. affecting stream bed stability) (e.g. Parker & Huryn, 2006, 2013; Friberg et al., 2013), temperature (Friberg et al., 2001, 2013; Woodward et al., 2010a) and the presence of predatory fish (Woodward et al., 2010a; Parker & Huryn, 2013; this study). A temperature increase will probably affect the composition of macroinvertebrate assemblages and increase productivity, fish body size (due to increased food availability), and food-web lengths in subarctic regions, as evidenced by a comparison of geothermal streams that contrast in their temperature regimes in Iceland (Woodward et al., 2010a). Furthermore, lotic systems at high latitudes and altitudes are expected to have increased productivity along with enhanced richness and trait diversity of

macroinvertebrates (including use of a broader diversity of food resources) as well as stronger intensities of trophic interactions as glacial influence declines and temperature increases (Milner, Brown & Hannah, 2009; and references therein). Besides these climate change-driven effects, one of the main expected responses of organisms is a shift in distribution patterns, varying according to the trophic level occupied by the organisms and their dispersal abilities (Milner *et al.*, 2009; Woodward *et al.*, 2010b). Facultative anadromous fish species (characterising the fish fauna of Greenland) are expected to shift their distribution ranges considerably, for example, the distributions of cold-water adapted species such as the arctic charr will probably shrink. However, species with wider temperature tolerances (such as sticklebacks) have been predicted to increase their distribution ranges towards the north (ACIA, 2005; Woodward *et al.*, 2010b), and this will probably occur for many species given the expected lower over-winter mortality rates at the extremes of distribution ranges (ACIA, 2005; Reist *et al.*, 2006). Some of the currently fishless Greenland streams are thus likely to be colonised by fish in the future, which will produce changes in the patterns of macroinvertebrate assemblage composition, probably causing substantial reductions in abundance, or even local extinctions, of the most sensitive species (as seen in systems recently invaded by fish; Layer *et al.*, 2011) and radical re-structuring of food webs, with implications also for energy flow and other ecological processes (Milner *et al.*, 2009; Woodward *et al.*, 2010b; Layer *et al.*, 2011). For instance, fish colonisation may, to some extent, counteract some changes expected to occur in macroinvertebrate assemblages triggered by increased temperature regimes, such as enhanced richness and broader use of food resources (Milner *et al.*, 2009). Additionally, it may strengthen other effects such as local species extinctions, because not only temperature-sensitive species (Milner *et al.*, 2009; Woodward *et al.*, 2010b) but also predation-sensitive species might disappear. We predict that with the arrival of colonising fish in previously fishless streams, the trophic web structure of the macroinvertebrate assemblage may shift towards a higher proportion of filter feeders. In addition, food webs of macroinvertebrates and fish may become longer, but probably with less trophic diversity and less variety in the food sources consumed by the macroinvertebrates (although this may not persist in the long term, when the effects of temperature rise might compensate for the loss). This may reduce abundance and feeding activity of macroinvertebrate grazers, promoting cascading effects that will increase periphyton biomass and primary production as well as increase nutrient uptake rates and retention, as seen in other stream systems (Townsend, 2003).

Acknowledgments

References

ACIA (2005) *Arctic Climate Impact Assesment*. Cambridge University Press, Cambridge.

Benke A.C., Huryn A.D., Smock L.A. & Wallace J.B. (1999) Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the Southeastern United States. *Journal of the North American Benthological Society*, **18**, 308–343.

Bennike O., Sorensen M., Fredskild B., Jacobsen B., Bocher J., Amsinck S. *et al.* (2008) Late Quaternary environmental and cultural changes in the Wollaston Forland Region, Northeast Greenland. *Advances in Ecological Research*, **40**, 45–79.

Bergersen R. (1996) Sticklebacks from Greenland. *Journal of Fish Biology*, **48**, 799–801.

Chu C., Mandrak N.E. & Minns C.K. (2005) Potential impacts of climate change on the distributions of several common and rare freshwater fishes in Canada. *Diversity and Distributions*, **11**, 299–310.

Dunne J.A. (2006) The network structure of food webs. In: *Ecological Networks: Linking Structure to Dynamics in Food Webs*. (Eds Pascual M. & Dunne J.A.), pp. 27–86. Oxford University Press, New York.

Finlay J.C. (2001) Stable-carbon-isotope ratios of river biota: implications for energy flow in lotic food webs. *Ecology*, **82**, 1052–1064.

Flecker A.S. (1992) Fish Predation and The evolution of invertebrate drift periodicity: evidence from Neotropical streams. *Ecology*, **73**, 438–448.

Flecker A.S. & Townsend C.R. (1994) Community-wide consequences of trout introduction in New Zealand streams. *Ecological Applications*, **4**, 798–807.

France R. (1995) Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 651–656.

Friberg N., Bergfur J., Rasmussen J. & Sandin L. (2013) Changing Northern catchments: is altered hydrology, temperature or both going to shape future stream communities and ecosystem processes? *Hydrological Processes*, **27**, 734–740.

Friberg N., Milner A.M., Svendsen L.M., Lindegaard C. & Larsen S.E. (2001) Macroinvertebrate stream communities along regional and physico-chemical gradients in Western Greenland. *Freshwater Biology*, **46**, 1753–1764.

Froese R. & Pauly D. (2013) FishBase (Editors). World Wide Web electronic publication. www.fishbase.org, version (10/2013).

Hildrew A.G. (2009) Sustained research on stream communities: a model system and the comparative approach. *Advances in Ecological Research*, **41**, 175–312.

Hoeinghaus D.J. & Zeug S.C. (2008) Can stable isotope ratios provide for community-wide measures of trophic structure? Comment. *Ecology*, **89**, 2353–2357.

Huang C. & Sih A. (1990) Experimental studies on behaviorally mediated, indirect interactions through a shared predator. *Ecology*, **71**, 1515–1522.

Hyslop E.J. (1980) Stomach contents analysis – a review of methods and their application. *Journal of Fish Biology*, **17**, 411–429.

Ivlev V.W. (1961) *Experimental Ecology of the Feeding of Fishes*. Yale University Press, New Haven.

Jeppesen E., Christoffersen K., Landkildehus F., Lauridsen T., Amsinck S., Riget F. *et al.* (2001) Fish and crustaceans in northeast Greenland lakes with special emphasis on interactions between Arctic charr (*Salvelinus alpinus*), *Lepidurus arcticus* and benthic chydorids. *Hydrobiologia*, **442**, 329–337.

Kohler C.C. & Ney J.J. (1982) A comparison of methods for quantitative analysis of feeding selection of fishes. *Environmental Biology of Fishes*, **7**, 363–368.

Kohler S.L. & McPeek M.A. (1989) Predation risk and the foraging behavior of competing stream insects. *Ecology*, **70**, 1811–1825.

Lancaster J., Hildrew A.G. & Townsend C.R. (1988) Competition for space by predators in streams: field experiments on a net-spinning caddisfly. *Freshwater Biology*, **20**, 185–193.

Layer K., Hildrew A.G., Jenkins G.B., Riede J., Rossiter S.J., Townsend C.R. *et al.* (2011) Long-term dynamics of a well-characterised food web: four decades of acidification and recovery in the Broadstone Stream model system. *Advances in Ecological Research*, **44**, 69–117.

Layman C.A., Arrington D.A., Montaña C.G. & Post D.M. (2007) Can stable isotopes ratios provide for community-wide measures of trophic structure? *Ecology*, **88**, 42–48.

Layman C.A. & Post D.M. (2008) Can stable isotope ratios provide for community-wide measures of trophic structure? Reply. *Ecology*, **89**, 2358–2359.

Mantel S.K., Salas M. & Dudgeon D. (2004) Foodweb structure in a tropical Asian forest stream. *Journal of the North American Benthological Society*, **23**, 728–755.

Martinez N.D. (1994) Scale-dependent constraints on food-web structure. *American Naturalist*, **144**, 935–953.

McCullaugh P. & Nelder J. (1989) *Generalized Linear Models*. Chapman and Hall, Boca Raton.

McIntosh A.R., Greig H.S., Mcmurtrie S.A., Nyström P.E.R. & Winterbourn M.J. (2005) Top-down and bottom-up influences on populations of a stream detritivore. *Freshwater Biology*, **50**, 1206–1218.

McIntosh A.R., Peckarsky B.L. & Taylor B.W. (2002) The influence of predatory fish on mayfly drift: extrapolating from experiments to nature. *Freshwater Biology*, **47**, 1497–1513.

Milner A.M., Brown L.E. & Hannah D.M. (2009) Hydroecological response of river systems to shrinking glaciers. *Hydrological Processes*, **23**, 62–77.

Milner A.M., Robertson A.L., Monaghan K.A., Veal A.J. & Flory E.A. (2008) Colonization and development of an Alaskan stream community over 28 years. *Frontiers in Ecology and the Environment*, **6**, 413–419.

Miyasaka H., Genkai-Kato M., Miyake Y., Kishi D., Katano I., Doi H. *et al.* (2008) Relationships between length and weight of freshwater macroinvertebrates in Japan. *Limnology*, **9**, 75–80.

Moulton T.P., Souza M.L., Silveira R.M.L., Krsulović F.A.M., Silveira M.P., De Assis J.C.F. *et al.* (2010) Patterns of periphyton are determined by cascading trophic relationships in two neotropical streams. *Marine and Freshwater Research*, **61**, 57–64.

Newton J.S. & Proctor H.C. (2013) A fresh look at weight-estimation models for soil mites (Acari). *International Journal of Acarology*, **39**, 72–85.

Nørrevang A. & Lundø J. (1981) *Danmarks Natur-Grønland*. Politikens Forlag, Copenhagen (In Danish).

Nyström P., McIntosh A.R. & Winterbourn M.J. (2003) Top-down and bottom-up processes in grassland and forested streams. *Oecologia*, **136**, 596–608.

Parker S.M. & Huryn A.D. (2006) Food web structure and function in two arctic streams with contrasting disturbance regimes. *Freshwater Biology*, **51**, 1249–1263.

Parker S.M. & Huryn A.D. (2013) Disturbance and productivity as codeterminants of stream food web complexity in the Arctic. *Limnology and Oceanography*, **58**, 2158–2170.

Parnell A., Inger R., Bearhop S. & Jackson A.L. (2013) SIAR: Stable isotope analysis in R.

Peckarsky B.L. & McIntosh A.R. (1998) Fitness and community consequences of avoiding multiple predators. *Oecologia*, **113**, 565–576.

Post D.M. (2002) Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology*, **83**, 703–718.

R Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www. R-project.org/.

Rasmussen J.B. (2010) Estimating terrestrial contribution to stream invertebrates and periphyton using a gradient-based mixing model for δ13C. *Journal of Animal Ecology*, **79**, 393–402.

Reist J.D., Wrona F.J., Prowse T.D., Power M., Dempson J.B., Beamish R.J. *et al.* (2006) General effects of climate change on Arctic fishes and fish populations. *Ambio: A Journal of the Human Environment*, **35**, 370–380.

Rossi J.P. (2011) rich: An R Package to analyse species richness. *Diversity*, **3**, 112–120.

Sarakinos H.C., Johnson M.L. & Vander Zanden M.J. (2002) A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. *Canadian Journal of Zoology*, **80**, 381–387.

Shuter B.J. & Post J.R. (1990) Climate, population viability and the zoography of temperate fishes. *Transactions of the American Fisheries Society*, **119**, 314–336.

Thompson R.M., Dunne J.A. & Woodward G.U.Y. (2012) Freshwater food webs: towards a more fundamental understanding of biodiversity and community dynamics. *Freshwater Biology*, **57**, 1329–1341.

Thompson R.M. & Townsend C.R. (2005) Energy availability, spatial heterogeneity and ecosystem size predict food-web structure in streams. *Oikos*, **108**, 137–148.

Townsend C.R. (2003) Individual, population, community, and ecosystem consequences of a fish invader in New Zealand Streams. *Conservation Biology*, **17**, 38–47.

Ventura M. & Jeppesen E. (2009) Effects of fixation on freshwater invertebrate carbon and nitrogen isotope composition and its arithmetic correction. *Hydrobiologia*, **632**, 297–308.

Woodward G., Dybkjær J.B., Ólafsson J.S., Gíslason G.M., Hannesdóttir E.R. & Friberg N. (2010a) Sentinel systems on the razor's edge: effects of warming on Arctic geothermal stream ecosystems. *Global Change Biology*, **16**, 1979–1991.

Woodward G. & Hildrew A.G. (2001) Invasion of a stream food web by a new top predator. *Journal of Animal Ecology*, **70**, 273–288.

Woodward G. & Hildrew A.G. (2002) Body-size determinants of niche overlap and intraguild predation within a complex food web. *Journal of Animal Ecology*, **71**, 1063–1074.

Woodward G., Perkins D.M. & Brown L.E. (2010b) Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 2093–2106.

Woodward G., Thompson R., Townsend C.R. & Hildrew A.G. (2005) Pattern and process in food webs: evidence from running waters. In: *Aquatic Food Webs an Ecosystem Approach*. (Eds Belgrano A., Scharler U.M., Dunne J. & Ulanowicz R.E.), pp. 51–66. Oxford University Press, New York.

Yeung A.C.Y. & Dudgeon D. (2013) A manipulative study of macroinvertebrate grazers in Hong Kong streams: do snails compete with insects? *Freshwater Biology*, **58**, 2299–2309.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Yield-effort curves for the diversity of prey items found in fish guts.

Appendix S2. Macroinvertebrate assemblage structure in streams with and without fish.

(Manuscript accepted 8 April 2014)