The dark side of rocks: An underestimated high-quality food resource in river ecosystems

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Abstract

1. Algae are generally a high-quality diet source because they provide essential compounds to aquatic consumers. In forested stream ecosystems, the availability of high-quality algae is low compared to terrestrial organic matter, which may constrain the dietary transfer of essential compounds to consumers. However, there could be other overlooked high-quality resource pools that provide essential compounds to consumers in river ecosystems.

2. We conducted a field study along a subalpine river continuum in Austria to identify the nutritional role of a ‘hidden’ food resource for aquatic consumers; the biofilms growing on the underside of rocks (dark biofilms). Dark and light (i.e. upper surface of rocks) biofilms and invertebrates were collected, and their fatty acid (FA) composition was analysed.

3. Compared with light biofilms, dark biofilms contained greater proportions of bacterial FA, long-chain saturated FA (biomarkers of terrestrial plants) and oleic acid (18:1ω9; a fungal biomarker), but a lower proportion of algal FA, especially omega-3 polyunsaturated FA (ω3 PUFA). The ω3 PUFA composition in dark biofilms was strongly correlated with that in light biofilms. Furthermore, the overall FA profiles of dark biofilms were significantly associated with invertebrate FA profiles. Strong correlations were also observed between invertebrates and dark biofilms for bacterial FA and the ω3 PUFA eicosapentaenoic acid (EPA, 20:5ω3).

4. Synthesis. This field study demonstrates that dark biofilms are a high-quality resource pool for invertebrates in river ecosystems that is often overlooked. Similar to light biofilms, dark biofilms provide physiologically important FA and bacterial FA for stream invertebrates. However, these high-quality resources are threatened by increasing human disturbances to river ecosystems. Future research is required to better understand how the quality of both dark and light biofilms changes in response to human disturbance, and how this affects dietary energy transfer to upper trophic consumers, including fish and humans.

Keywords

bacteria, diatoms, fatty acids, food quality, invertebrates, subalpine rivers
1 | INTRODUCTION

A high-quality diet is central for consumer somatic growth, maintenance, reproduction and their ecological interactions within food webs (Paine, 1980; Simpson & Raubenheimer, 2012). At the base of aquatic food webs, algae provide essential compounds, such as amino acids, sterols and polyunsaturated fatty acids (PUFA) to consumers, and thus are considered to be high-quality resources for consumers (Brett et al., 2009). However, in river ecosystems, in particular headwaters, the availability of algae is assumed to be low compared to terrestrial organic matter (Vannote et al., 1980), which may constrain the dietary transfer of essential compounds to consumers. Despite this, animals living in streams and rivers are highly enriched in essential compounds, particularly PUFA (Guo et al., 2017). Most aquatic animals have a limited ability to synthesize these essential compounds and thus must obtain them directly from their diet, originally from basal algae. However, in addition to periphyton and planktonic algae, which have been intensively studied in river ecology (Allan & Castillo, 2007), there may be other overlooked high-quality resource pools that provide essential compounds to consumers.

Biofilms growing on submerged substrata are an important food source for consumers in river ecosystems (Allan & Castillo, 2007; Battin et al., 2016). Biofilms are mostly composed of algae and readily develop on the surface of leaves, woody debris and rocks. In particular, biofilms growing on rocks, also called epilithon, have been found to better support stream food webs than do terrestrial leaves due to their high PUFA content (Guo et al., 2016a; Lau et al., 2009; McInerney et al., 2020). However, in most previous studies, the method to sample these biofilms has not been uniform; a few studies collected biofilms on the upper side of the rock (light-exposed biofilms; in short ‘light biofilms’), whereas most studies do not clarify whether the biofilms were only from the upper side or a combination of the upper side and underside of rocks.

Many invertebrates cling to the underside of rocks, which may be a strategy to avoid predators, high ultraviolet radiation and/or being dislodged by flow (Allan & Castillo, 2007). It is therefore also likely that some invertebrates feed on biofilms from the underside of rocks (dark-exposed biofilms; in short ‘dark biofilms’). Sedentary invertebrates (e.g. cased caddis larvae) are found to spend much of their time on rock surfaces (Hynes, 1970) and may not feed on dark biofilms. In contrast, mobile invertebrates (e.g. mayflies and stoneflies) drift mostly at night, but during the day, they hide in sheltered locations (Brittain & Eikeland, 1988; Hynes, 1970), such as the underside of rocks. Dark biofilms may be an overlooked resource pool for these aquatic consumers. However, little is known about the nutritional composition and thus trophic importance of dark biofilms for aquatic consumers.

Long-chain polyunsaturated fatty acids (LC-PUFA) are an informative and increasingly important indicator of diet quality for aquatic consumers (Brett et al., 2017). Among LC-PUFA, eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) are required for invertebrate development, reproduction and hormone regulation (Stanley-Samuelsen, 1994). Diatoms with high contents of EPA and DHA are considered as high-quality diet for consumers (Brett et al., 2009), while cyanobacteria are usually low-quality diet because they lack these specific PUFA as well as sterols (Martin-Creuzburg et al., 2008). Diatoms have been shown to support higher growth rates and reproduction of consumers (Guo et al., 2016a) and enhance dietary energy transfer efficiency to upper trophic levels (Lau et al., 2013; Müller-Navarra et al., 2000).

Biofilms are a mixture of terrestrial particles, fungi, bacteria and algae, with light biofilms being more autotrophic and dark biofilms more heterotrophic (Allan & Castillo, 2007; Romani et al., 2004), which results in different nutritional quality for consumers. Terrestrial plants are considered low-quality diets for aquatic consumers because they lack LC-PUFA (Brett et al., 2009). However, many terrestrial plants are rich in the short-chain PUFA linoleic acid (LIN, 18:2ω-6) and α-linolenic acid (ALA, 18:3ω-3), which are precursors for LC-PUFA, as well as saturated fatty acids (SAFA), which are generally used for energy storage and to support catabolism (Brett et al., 2017). Initially, as terrestrial leaves decompose in water, their ALA and LIN proportions decrease, but SAFA does not change (Guo et al., 2016a; Hiltunen et al., 2019). Fungi and bacteria are considered less nutritious than algal diets for aquatic consumers because they lack LC-PUFA. Studies on terrestrial and marine fungi reported that 16:0, 18:0 and 18:1ω9 are the most common FA in fungi (Cooney et al., 1993; Stahl & Klug, 1996). Bacteria are characterized by odd-chain FA, for example, 15:0, 17:0 and their branched homologues, as well as vaccenic acid (18:1ω7) (Desvillettes et al., 1997). Therefore, algae within light and dark biofilms are an important determinant of their nutritional quality for consumers.

This study explores the nutritional role of dark biofilms for river consumers. Light and dark biofilms (Figure 1) and invertebrates...
were collected along a river continuum of a subalpine catchment in Austria during three seasons. Our hypotheses were: (1) Dark biofilms contain more FA from bacteria, terrestrial matter and fungi, whereas light biofilms contain more algal FA; (2) The nutritional quality of dark biofilms is associated with corresponding light biofilms, because it is expected that algae on the upper surface of stream cobbles contribute organic matter rich in LC-PUFA to the dark biofilms; (3) Dark biofilms are an additional high-quality resource of LC-PUFA for stream invertebrates, particularly mobile mayflies. Based on many previous studies (Brittain & Eikeland, 1988; Elliott, 1968; Hynes, 1970) and our field observation, mayflies cling to the underside of rocks during the day, and they may feed on dark biofilms in addition to avoiding predators, high ultraviolet radiation and/or being dislodged by flow.

2 | MATERIALS AND METHODS

2.1 | Study streams

This study was conducted in the subalpine River Ybbs catchment, Austria (47°45′N, 15°12′E; drainage basin = 254 km²). This catchment has a temperate climate with distinct seasonal changes, although precipitation is fairly even over the year. Catchment geology is dominated by dolomite and karst. Forestry is the dominant land use in the catchment, and alpine meadows and agricultural areas constitute only a small fraction of the catchment (Guo et al., 2018). Our study streams were selected in the upper catchment, Weiße Ois, with only minor human disturbance in the study reaches or their upstream catchments (Table 1). The substrata of all study streams were cobbles, and all streams had an open canopy.

2.2 | Sample collection

Biofilms and macroinvertebrates were sampled at eight riffles from upstream to downstream in the catchment across three seasons, that is, summer (July), fall (October) and winter (November) in 2017 (Table 1). Three paired samples of light and dark biofilms were collected for FA analyses along a 20-m reach from each riffle. Each paired sample was collected using a quadrant (1.5 m × 1.5 m), and five cobbles (diameters ranging from 8 to 16 cm) within the quadrant were randomly picked. Light and dark biofilms were scraped from the substrate with a toothbrush and kept separate.

Macroinvertebrates clinging to the above cobbles within each quadrant were washed into white trays. Ecdyonurus sp. (Heptageniidae, Ephemeroptera), which is the most abundant algal grazer in the study streams (Kühmayer et al., 2020), was selected for analysis. Although there are four Ecdyonurus species from our study streams, including E. helveticus (Eaton, 1885), E. venosus (Fabricius, 1775), E. dispar (Curtis, 1834) and E. picteti (Meyer-Dur, 1864), more than 95% of the larvae were late instars of E. helveticus (Moog, 2002). All four species have identical feeding habits and are assigned to the same functional feeding groups: 50%
grape and 50% detritivore/gatherer/collector (Moog, 2002). One
collected with at least 10 individuals of Ecdyonurus sp. was col-
collected from each riffle. All samples were immediately placed in
ziplock plastic bags, stored on ice and kept in the dark in a portable
freezer. Samples were brought to the laboratory within 4 hr and
placed in a −80°C freezer until further processing.

At each site, triplicate water samples were collected to deter-
mine the concentrations of dissolved inorganic nutrients, including
nitrate (NO$_3$–N), nitrite (NO$_2$–N) and ammonium nitrogen (NH$_4$–
N), and soluble reactive phosphorus (SRP). Nutrient samples were
stored in a portable dark cooler in the field and filtered through
0.7 μm glass microfiber filters (GF/F; Whatman™, GE Healthcare)
in the laboratory within 4 hr of collection. All nutrient analyses were
completed within 4 days. Additionally, stream temperature and pH
were measured using an HQD portable measuring meter (HQ30D–
Multi/1 Channel, HACH LANGE, Germany; Table 1).

Due to the heavy snow in early November, there was no access
to one of the upstream sites (WO), where biofilms, invertebrates
and water samples were not collected. There was limited access to
the sites FB, TB and WO during winter and invertebrates were not
collected.

2.3 Sample processing

All biofilm and macroinvertebrate samples were freeze-dried
(Virtis Genesis Freeze Dryer). Dry mass from each biofilm sam-
ple (~10 mg) and from each invertebrate sample (5–7 mg) was
used for the lipid extractions. Lipids were extracted and meth-
ylated according to the methods reported in Guo, Kainz, Valdez,
et al. (2016), with nonadecanoic acid (19:0) used as an internal
standard. Fatty acid methyl esters (FAME) were analysed using
a gas chromatograph (THERMO Trace; FID 260°C, Carrier gas:
He: 1 ml/min, Detector gases: H$_2$: 40 ml/min, N$_2$: 45 ml/min, air:
450 ml/min, temperature ramp: 140°C (5 min)–4°C/min–240°C
(20 min) = 50 min) equipped with a temperature-programmable
injector and an autosampler. FAME were separated by a Supelco™
SP-2560 column (100 m, 25 mm i.d., 0.2 μm film thickness), and
FA peaks were identified by comparison of their retention times
with known standards, that is, 37-component FAME mix (Supelco
47885-U) and Bacterial Acid Methyl Ester Mix (Supelco 47080-U),
and quantified with reference to seven-point calibration curves
based on known standard concentrations. FA compositions were
expressed as percentages relative to the total FA (FA%).

Dissolved nutrients (NO$_3$–N, NO$_2$–N, NH$_4$–N and SRP) were an-
alsed using a continuous flow analyser (Alliance instrument GmbH,
5020; Table 1).

2.4 Data analysis

The FA characteristics of dark biofilms were assessed by two steps.
Firstly, the similarity in overall FA profiles between dark and light
biofilms was examined by permutational multivariate analysis of
variance (PERMANOVA) and the test of multivariate homogeneity
of group dispersions (PERMDISP; Anderson & Walsh, 2013).
PERMANOVA was run with rock sides (light and dark) as the fixed
factor and the interaction of sites and seasons as the blocking term
with 1,000 permutations. Then, PERMDISP was applied to test if the
dispersions (variances) of FA profiles between dark and light biofilms
were different.

Secondly, the individual FA composition between dark and light
biofilms was compared by linear mixed-effect models. The terms in
linear mixed models are response variables, and the fixed and ran-
dom factors. In our study, the response variables were individual
FA% (Table 2), with rock sides (light and dark) as the fixed factor
and sites and seasons as the random factors. The protocol for linear
mixed model fit and validation followed by Zuur et al. (2009) and Guo
et al. (2016a). Four models (M1, M2, M3 and M4) were defined: a lin-
ear model using generalized least squares (without random effect) to
investigate the fixed factor effects, and three mixed-effect models,

The relationships of the FA composition between inverte-
brates and dark/light biofilms were explored by Procrustes analysis
and correlations. Procrustes analysis is known as the analysis of
congruence between two multivariate datasets (Peres-Neto &
Jackson, 2001), and is essentially a multivariate way to test the
overall degree of associations in the FA profiles between inver-
tebrates and dark/light biofilms. Procrustes analysis produces an
m$^2$-statistic that can be transformed into an r-statistic ($r$ = square
root of (1-m$^2$)) and used for interpreting simply as the match be-
tween two ordinations (e.g. invertebrate FA and dark biofilm FA;
Peres-Neto & Jackson, 2001). Moreover, correlations of dark ver-
sus light biofilms on invertebrates in terms of individual FA were
estimated by correlation analysis.

All relative FA (%) data of biofilms and invertebrates were arcsine-square-root-transformed for normal distribution approxima-
tion before analysis. All statistical analyses were conducted in the
statistical software R version 3.6.3 (R Core Team, 2020), using the
extension package vegan (Oksanen et al., 2013) for PERMANOVA,
PERMDISP and Procrustes analysis, and lme4 for linear mixed-effect
models (Bates et al., 2014) and Hmisc for correlation analysis (Harrell
et al., 2019).
TABLE 2  Fatty acid compositions (% relative to total FA, mean ± SD) of biofilms growing on the upper (light) and lower (dark) sides of rocks from 8 study streams during summer, fall and winter in 2017 in the River Ybbs catchment, Austria. The differences were evaluated by linear mixed-effect models, with rock side (light and dark) as the fixed factor and sampling site and season as the random factors. FA with average proportions <1% was excluded for statistical analysis, unless it was present in >70% samples. The title raw should be used in bold

| Fatty acids | Dark biofilms (%) | Light biofilms (%) | F-value | p |
|------------|------------------|-------------------|--------|---|
| 14:0       | 4.09 ± 2.66      | 6.51 ± 3.78      | 22.55  | ***|
| 15:0       | 0.20 ± 0.12      | 0.24 ± 0.10      | 9.82   | 0.002|
| 16:0       | 24.57 ± 5.17     | 22.6 ± 4.04      | 6.04   | 0.01|
| 17:0       | 0.28 ± 0.14      | 0.21 ± 0.15      | 17.22  | ***|
| 18:0       | 12.0 ± 5.73      | 6.76 ± 5.10      | 40.21  | ***|
| 20:0       | 0.27 ± 0.13      | 0.22 ± 0.13      | 9.17   | 0.003|
| 21:0       | 0.00 ± 0.01      | 0.00 ± 0.00      |        |    |
| 22:0       | 0.15 ± 0.12      | 0.15 ± 0.09      |        |    |
| 23:0       | 4.27 ± 7.51      | 0.00 ± 0.00      | 35.71  | ***|
| 24:0       | 0.11 ± 0.21      | 0.07 ± 0.17      |        |    |
| SAFA       | 45.99 ± 14.91    | 36.8 ± 9.61      | 22.20  | ***|

Note: SAFA, sum of saturated fatty acids; MUFA, sum of monounsaturated fatty acids; PUFA, sum of polyunsaturated fatty acids; BAFA, bacterial fatty acids, including 15:0, 17:0 and their branched iso- and anteiso-homologues, and 18:1ω7; long-chain SAFA, SAFA with >20 carbon bonds.

***p < 0.001.

3 | RESULTS

3.1 | Fatty acid characteristics of dark biofilms

SAFA were the most abundant FA group in dark biofilms (~46% of the total FA) (Table 2), followed by MUFA (~27%) and PUFA (~26%). Palmitic acid (16:0) (~25%), palmitoleic acid (16:1ω7) (~11%) and EPA (~10.1%) were the principal SAFA, MUFA and PUFA, respectively. EPA was commonly used as a diatom biomarker. The short-chain PUFA, ALA and LIN, biomarkers of most green algae and a number of cyanobacteria, accounted for ~11% of the total FA. Although the proportions of ARA and DHA were very low, <1%, they were present in >70% samples. Additionally, BAFA accounted for ~5% of the total FA in dark biofilms.

The overall FA profiles of dark biofilms were significantly different from those of light biofilms (PERMANOVA, F-value = 14.62, p < 0.001), and there was no difference in dispersion effects (PERMDISP, F-value = 0.35, p = 0.55).

Pronounced differences in individual FA composition between dark and light biofilms were detected by linear mixed-effect models (Table 2, Figure 2). Dark biofilms contained significantly greater proportions of SAFA, MUFA and BAFA compared with light biofilms. Long-chain SAFA (>20 carbon), biomarkers of terrestrial plants and the fungal biomarker 18:1ω9 were more abundant in dark biofilms than in light biofilms. In contrast, dark biofilms contained less algaesynthesized PUFA, especially EPA, ALA, LIN and DHA compared with light biofilms.

3.2 | Relationships between FA compositions of dark and light biofilms

Correlations between dark and light biofilms differed among the FA groups (Figure 3). No significant relationships were found for SAFA
FIGURE 2  Fatty acid compositions (% relative to total FA, mean ± SD) of biofilms growing on the upper side (light) and underside (dark) of rocks. Dark, dark biofilms; light, light biofilms. ***p < 0.001; *p < 0.05

FIGURE 3 Correlations of fatty acid compositions between biofilms growing on the upper side (light) and underside (dark) of rocks. Dark, dark biofilms; light, light biofilms

(correlation coefficient: $r = 0.00, p = 0.97$) and MUFA ($r = 0.19, p = 0.14$) between dark and light biofilms, whereas BAFA in dark biofilms showed significant correlations with that in light biofilms ($r = 0.47, p < 0.001$). Furthermore, no significant correlations were detected for the ω6 PUFA ARA ($r = 0.23, p = 0.08$) and LIN ($r = 0.09, p = 0.48$) between light and dark biofilms. Conversely, strong correlations were found for the ω3 PUFA EPA ($r = 0.57, p < 0.001$), ALA ($r = 0.51, p < 0.001$) and DHA ($r = 0.62, p < 0.001$) between light and dark biofilms.
3.3 Relationships between FA compositions of invertebrates and dark/light biofilms

Results of Procrustes analysis show that there were significant positive correlations of overall FA profiles between invertebrates and dark/light biofilms (dark vs. invertebrates: \( p = 0.028, r = 0.52 \); light versus. invertebrates: \( p = 0.03, r = 0.51 \)).

In terms of individual FA (Figure 4), for dark biofilms, only EPA and BAFA were significantly correlated with invertebrate EPA and BAFA, respectively (EPA: \( r = 0.59, p = 0.03 \); BAFA: \( r = 0.60, p = 0.03 \)). The same trend was observed for light biofilms (EPA: \( r = 0.56, p = 0.045 \); BAFA: \( r = 0.77, p = 0.02 \)). No significant correlations were detected for other FA between biofilms and invertebrates.

4 DISCUSSION

This study highlights the nutritional importance of dark biofilms growing on the underside of rocks for stream invertebrates. We found that dark biofilms were characterized by containing more FA from heterotrophic sources, in contrast to light biofilms containing more algal FA, supporting hypothesis 1. Strong correlations of \( \omega 3 \) PUFA (i.e. EPA, DHA and ALA) and BAFA between dark and light biofilms indicate possible contributions of organic matters from light to dark biofilms, supporting hypothesis 2. Furthermore, the overall FA profiles or important individual FA of invertebrate grazers were significantly associated with the FA of both dark and light biofilms, extending hypothesis 3 that dark biofilms not only provide high-quality algae for invertebrate grazers but also bacteria, similar to light biofilms.

Our results complement prior findings from a nutritional perspective that biofilm structure and function differ between light and dark environments (Romani et al., 2004). Compared with light biofilms, dark biofilms growing on the underside of rocks contained significantly more of the terrestrial plant biomarkers long-chain SAFA (Brett et al., 2017), the fungus biomarker 18:1\( \omega 9 \) (Stahl & Klug, 1996) and bacterial FA (Desvillettes et al., 1997), but less algal-derived PUFA (Torres-Ruiz et al., 2007), especially EPA, ALA and LIN. These three PUFA accounted for ~21% of the total FA in the dark biofilms, in contrast to 32% in the light biofilms, and could potentially contribute to stream food webs. In particular, increased algal EPA is reported to stimulate invertebrate growth and energy transfer to higher trophic consumers (Guo et al., 2018; Guo et al., 2016a). The similar EPA content in dark (10%) compared to light biofilms (15%) may play an important, yet unrecognized dietary role for consumers feeding on the dark biofilms that consequently transfer dietary energy to consumers at higher trophic levels.

Strong correlations in PUFA and BAFA were detected between dark and light biofilms, indicating the possible transfer of organic matter between these biofilms. In river ecosystems, algae and bacteria mainly occur in two forms: benthic (attached to substrata, e.g. rocks) and planktonic (free floating in water column). The equilibrium between these two forms is largely regulated by hydrodynamics (Augspurger et al., 2010; Battin et al., 2016). Our biofilms were collected in riffles, with relatively fast flowing water, which may wash off parts of light biofilms from rock surfaces to the drift. Dark
biofilms might capture these particles, partly resulting in the strong correlations in mostly algae-derived PUFA and BAFA between light and dark biofilms. Additionally, the strong correlations could also be induced by the settlement of planktonic cells of algae and bacteria on dark and light biofilms. However, more details regarding flow regimes (laminar vs. turbulent) and biofilm topography (nascent vs. mature) are required to estimate the settlement of planktonic cells on biofilms (Augsburger et al., 2010).

Our data show that the overall FA profiles of dark and light biofilms were significantly correlated with those of invertebrates, suggesting that both sides of rocks are potential sources of FA for the study grazers. However, compared with dark biofilms, light biofilms are a more nutritious and abundant source for consumers, since the quality of light biofilms, measured by EPA, was significantly higher than that of dark biofilms, and algal quantity of light biofilms is enhanced by high light intensity (Mosisch et al., 2001). Mayfly grazers usually selectively remove nutritious patches by direct consumption, and spend more time in nutritious patches than would be expected under random movement (Kohler, 1984). Accordingly, there is a good chance that mayfly grazers prefer light to dark biofilms. Furthermore, mayfly larvae are known to be nocturnal drifters or more negatively phototropic (Hynes, 1970). They tend to hide in sheltered places during the day, for example, the underside of rocks as we observed in this field study, and likely also feed on dark biofilms in addition to the avoidance of predators and high ultraviolet radiation (Allan & Castillo, 2007). Mayfly larvae venture out of shelter at night (Elliott, 1968), and their drift is greatest at night and significantly affected by algal availability and quality (Brittain & Eikeland, 1988). Therefore, it is likely that light biofilms are the main diet source for mayfly grazers at night, but dark biofilms are the main source during the day.

The observed significant EPA correlations between invertebrates and dark and light biofilms, respectively, imply that dark biofilms may provide physiologically important EPA for invertebrate grazers, similar to light biofilms. EPA plays a critical supporting role for the production of stream invertebrates (Ahlgren et al., 2009; Guo et al., 2016b). The other ω3 PUFA, DHA, was low or absent in biofilms and invertebrates, and its functional role for invertebrate neural development may be fulfilled by EPA (Ahlgren et al., 2009; Stanley-Samuelson, 1994). However, most stream invertebrates have a limited ability to synthesize EPA from dietary precursors, and thus, they must obtain EPA directly from their diet (Torres-Ruiz et al., 2010), originally from basal algae. Even when the algal availability was low, the study grazers Ecdyonurus sp. were found to still preferentially feed on high-quality algae and selectively assimilate EPA over other FA (Kühmayer et al., 2020). In our study, for both dark and light biofilms, the strong correlation was only observed in EPA with invertebrate grazers, not for other algal PUFA, including DHA, ALA, ARA and LIN. This indicates the importance of EPA over other PUFA for invertebrate consumers (Guo et al., 2016a; Kühmayer et al., 2020; Torres-Ruiz et al., 2007), no matter whether EPA was from light or dark biofilms. Moreover, increased human disturbances in aquatic ecosystems, such as warming, eutrophication, riparian degradation and sedimentation (Burdon et al., 2013), are likely to decrease the availability of algal EPA for aquatic consumers (Guo et al., 2015; Hixson & Arts, 2016; Müller-Navarra et al., 2004; Whorley et al., 2019). In streams with high-nutrient inputs and high levels of removed riparian vegetation, algal communities in light biofilms can become dominated by filamentous green algae (deficient in EPA; Mosisch et al., 2001), whereas in dark biofilms, which are shaded and perhaps cooler from subsurface flows, a higher proportion of diatoms (rich in EPA) is expected (Mosisch et al., 2001). Diatoms in dark biofilms are potentially a high-quality diet for aquatic consumers. Therefore, assuming that the feeding pressure on rock biofilms is equal between the up- and downside of rocks, we propose that the observed EPA in dark biofilms, which was overlooked in previous studies, is an additional important dietary source of EPA for invertebrate consumers.

Interestingly, BAFA in dark and light biofilms were also strongly correlated with BAFA in the grazers, suggesting dark biofilms may provide bacteria for stream invertebrates. However, BAFA are not typically found in cell membranes of macroinvertebrates, and the only known role of BAFA in consumers is to be used as an energy source to support catabolism (Hiltunen et al., 2019; McMeans et al., 2015; Taipale et al., 2012). Biofilms could contribute to grazer BAFA through two pathways. Firstly, grazers acquired BAFA concurrently when feeding on high-quality algae. The grazers may feed on various portions of organic matter in biofilms, such as algae, bacteria, fungi and terrestrial matter. However, this is unlikely since the study mayfly, Ecdyonurus sp., is a selective feeder (Kühmayer et al., 2020), and thus strongly discriminates between high-quality algae and low-quality materials. Secondly, when the availability of high-quality algae is low in biofilms, grazers may actively feed more on other resources, such as bacteria or terrestrial particles, and depend less on algae. This compensatory feeding strategy is commonly used by benthic invertebrates to adapt to limited high-quality resources (Goedkoop et al., 2007; Strayer, 1991). Our study streams are pristine, with low nutrient concentrations (Table 1), which probably limits algal growth and consequently leads to low algal availability for grazing invertebrates (Mosisch et al., 2001). The study mayflies could perhaps tolerate bacteria-dominated diets, if mixed with nutritious algae, since bacteria barely support consumer growth and reproduction (Brett et al., 2009; McMeans et al., 2015). Therefore, dark and light biofilms may contribute to grazer BAFA when grazers experience limited availability of high-quality algae.

5 | CONCLUSIONS

This study demonstrates that dark biofilms represent an additional high-quality resource pool for invertebrates in river ecosystems. Although to a lesser extent than light biofilms, dark biofilms provide physiologically important PUFA, especially EPA for consumers, and other FA, such as bacterial FA. Our finding provides a more comprehensive understanding of the dietary energy flow in river ecosystems, in particular across the plant–animal interface. This interface is easily
affected by surrounding environmental changes, directly leading to variations in the availability of high-quality algae for aquatic consumers (Hixson & Arts, 2016; Müller-Navarra et al., 2004; Whorley et al., 2019). Further research is required to better understand how the quality of both dark and light biofilms would change in response to such environmental changes, and how this might affect dietary PUFA transfer to upper trophic consumers, including fish and humans.

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AUTHORS’ CONTRIBUTIONS
F.G. and M.J.K. conceived the ideas and designed methodology; F.G. and H.H. collected the data; F.G. analysed the data; F.G. led the writing of the manuscript. All authors F.G., S.E.B., M.T.B., H.H. and M.J.K. contributed critically to the drafts and gave final approval for publication.

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